Final Program and Abstracts

ASM CONFERENCES

3rd ASM Conference on

Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens in Animals, Humans, and the Environment

June 26 – 29, 2012

Aix-en-Provence, France

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ASM CONFERENCES

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ASM Conferences Mission

To identify emerging or underrepresented topics of broad scientific significance.

To facilitate interactive exchange in meetings of 100 to 700 people.

To encourage student and postdoctoral participation.

To recruit individuals in disciplines not already involved in ASM to ASM membership.

To foster interdisciplinary and international exchange and collaboration with other scientific organizations.

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General Information

GENERAL SESSIONS

All general sessions will be held in the Amphitheatre at the Aix-en-Provence Centre de Congres. A name badge is required for entry into all sessions, meals, and social events. ASM staff will be available during the sessions.

POSTER SESSIONS

Poster boards are located in the Milhaud and Forbin Rooms at the Aix-en-Provence Centre de Congres. Posters are divided between three sessions; posters 1 – 43 will be presented in Session A, posters 44 – 86 will be presented in session B, and posters 87 – 130 will be presented in session C.

Posters in Session A should be mounted before the conference opening session on Tuesday, June 26 and should be removed at the conclusion of the day on Tuesday, June 26. Posters in Session B should be mounted before the start of the morning session on Wednesday, June 27 and should be removed at the conclusion of the day on Wednesday, June 27. Posters in Session C should be mounted before the start of the morning session on Thursday, June 28 and should be removed at the conclusion of the day on Thursday, June 28. Please check your assigned number in the abstract index. The same number is used for the presentation and board number. The poster area will be open for informal viewing throughout the conference. Official poster sessions when authors are to stand by the poster board to present the work will be held on Tuesday afternoon (Poster Session A), Wednesday afternoon (Poster Session B), and Thursday Afternoon (Poster Session C).

CERTIFICATE OF ATTENDANCE

Certificates of Attendance can be found in the registration packet received at the registration desk. Certificates of Attendance do not list session information.

CAMERAS AND RECORDINGS

Digital recorders, cameras (including camera phones) and video cameras (including video phones) are prohibited in the poster hall and general sessions. Anyone found photographing, videotaping or recording in the prohibited areas will be asked to surrender their badge immediately and leave the conference. No refund will be provided. This rule is strictly enforced.

CHILD POLICY

Children are not permitted in session rooms, poster sessions, conference meals or social events. Please contact your hotel to arrange for babysitting services in your hotel room.

GUEST REGISTRATION

As noted in the program schedule, certain meals and social events are included in the registration fee for conference participants. Registered participants may also purchase tickets for an accompanying guest (age 16 and older) to attend the welcome reception and walking tour for an additional fee of 25€ and/or the conference dinner for a fee of 50€. Guests are not permitted in the general sessions or poster sessions, lunches or coffee breaks. Non-registered guests are not permitted to attend any part of the conference or social events.

Travel Grants

STUDENT TRAVEL GRANTS

ASM encourages the participation of graduate students and new postdocs at ASM Conferences. To support the cost of attending the conference, ASM has awarded travel grants of $500 to each of the following individuals:

Katerina Albrechtova
Raghavendra Amachawadi
Victor Amadi
Gamonsiri Bhumibhamon
Maria Madalena Centeno
Michelle Chen
Mette Christiansen
Jennie Fischer
Angela Flores Ribeiro
Alima Gharout
Roumi Ghosh
Elena Gómez-Sanz
Patricia Huijbers
Pekka Juntunen
Neena Kanwar
Teresa Ribeiro
Tara Roberts
Dalia Rodrigues
Courage Saba
Maria Seier-Petersen
Natasha Weatherspoon-Griffin

Scientific Program

Tuesday, June 26, 2012

9:00 – 9:45 am Ampitheatre	**Opening Session**
9:00 – 9:05 am	Welcome Remarks ***Jean-Yves Madec**; Anses, Lyon, France*
9:05 – 9:45 am	Opening Lecture: Quinolone Resistance, its Mechanisms, Reservoirs and Epidemiology ***David C. Hooper**; President, American Society for Microbiology, Washington, DC, and Massachusetts Gen. Hosp., Boston, MA*
9:45 – 11:00 am	**Session 1: Exposure to Antimicrobials or Antimicrobial Resistant Bacteria in Animals and Humans**
9:45 – 10:00 am	Serotype Distribution and Antimicrobial Resistance Profiles of *Salmonella spp*., *Escherichia coli*, and *Campylobacter spp*. Isolates Obtained from Conventional, Antimicrobial-Free, and Organic Broiler Chicken Production Systems in Ontario ***Tara E. Roberts;** Department of Population Medicine, University of Guelph, Guelph, ON, CANADA*
10:00 – 10:15 am	Antimicrobial Resistance Diversity in *Salmonella* Typhimurium DT104 from Animals and Humans in Scotland ***Alison Mather**; Wellcome Trust Sanger Institute, Cambridge, UNITED KINGDOM*
10:15 – 10:30 am	Prevalence of Extended-Spectrum β-Lactamases in Humans Living in Municipalities with High or Low Broiler Density ***P. M. Huijbers**; Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, NETHERLANDS*
10:30 – 10:45 am	Occupational Exposures to Antimicrobial Resistant Pathogens Associated with Food Animal Production ***Ellen Silbergeld;** Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD*
10:45 – 11:15 am	**Coffee Break**

11:15 am – 12:15 pm	**Session 2: Experimental Approaches of AMR Selection and Transmission**
11:15 – 11:30 am	Effect of at-Birth Ceftiofur Administration on Commensal *E. coli* esbl ctx-m Genes In Pigs from Portuguese Industrial Herds ***Maria Madalena Centeno;*** *Laboratory of Antimicrobial and Biocide Resistance, CIISA, Faculty of Veterinary Medicine, UTL, Lisbon, PORTUGAL*
11:30 – 11:45 am	Effect of Intervention Strategies on Antimicrobial Susceptibility Profiles and Their Relationship with tetA, tetB, and blaCMY-2 Genes Among *E. coli* Isolates in Feedlot Cattle ***Neena Kanwar;*** *Kansas State University, Manhattan, KS*
11:45 am – 12:00 pm	Epidemiology of a Transferable Copper Resistance Gene, *tcrB*, and its Co-Selection with Macrolide [*erm*(B)] and Tetracycline [*tet*(M)] Resistance Among Fecal Enterococci of Cattle and Swine Diets Supplemented with Varying Concentrations of Copper Sulfate ***Raghavendra Amachawadi****; Kansas State University, Manhattan, KS.*
12:00 – 12:15 pm	The Transcriptional Effect of Sub-Inhibitory Concentrations of Biocides on the Conjugative Transposon Tn916 ***Maria Seier-Petersen****; National Food Institute, The Technical University of Denmark, Kgs. Lyngby, DENMARK*
12:15 – 2:15 pm	**Lunch and Poster Session A**
2:15 – 5:00 pm	**Session 3: Sales and Consumption of Antimicrobials in Animals**
2:15 – 2:45 pm	Keynote Lecture – Quantifying Antimicrobial Consumption in Animals to Assess Antimicrobial Resistance Selection Pressure ***Jeroen Dewulf****; Gent University, Gent, BELGIUM*
2:45 – 3:00 pm	Course Dose: Converting Premix Sales to Pigs and Calves Exposed to Oral Antimicrobials ***Olivier Flechtner****; Swissmedic, Swiss Institute for Therapeutic Products, Bern, SWITZERLAND*

3:00 – 3:15 pm	Antibiotic Prescribing Practice in Cattle in the UK ***Nicola Williams**; University of Liverpool, Neston, UNITED KINGDOM*
3:15 – 3:30 pm	Low Levels of Fluroquinolone Resistance in Bacterial Isolates Causing Human Disease in Australia is Related to Their Use Being Banned in Food Animals ***Peter Collignon**; Canberra Hospital, Woden, AUSTRALIA*
3:30 – 4:00 pm	**Coffee Break**
4:00 – 4:15 pm	Veterinary Antimicrobial Prescriptions for Cattle in France: Description and Labeling Compliance ***Emilie Gay**; Anses, Lyon, FRANCE*
4:15 – 4:30 pm	Fluoroquinolones Selective Pressure Induces the Increase of Multidrug-Resistant *Escherichia Coli* Isolates from Calves ***Maria Madalena Centeno;** Laboratory of Antimicrobial and Biocide Resistance, CIISA, Faculty of Veterinary Medicine, UTL, Lisbon, PORTUGAL*
4:30 – 4:45 pm	Antimicrobial Resistance in Portugal - Sales Figures 2010 ***Teresa Ribeiro**; Direcção-Geral de Alimentação e Veterinária, Lisbon, PORTUGAL*
4:45 – 5:00 pm	Increase in Fluoroquinolone Usage in Poultry Production in France - Impact of an Unplanned Intervention ***Claire Chauvin**; Anses, Ploufragan, FRANCE*
5:15 – 6:45 pm	**Walking Tour of Aix-en-Provence** Leaving in small groups directly after the sessions from the Centre de Congres, English-speaking guides will show you through the center of the town, noting sites of historical significance, architecture, and beauty. The end destination is the Pavillon Vendome, site of the Welcome Reception.
7:00 – 8:30 pm Pavillon Vendome	**Welcome Reception** The reception is hosted by the deputy mayor of Aix-en-Provence who will welcome the conference to the city. Enjoy the gardens of the Pavillon Vendome while sampling local wine and regionally-sourced savories and sweets.

Wednesday, June 27, 2012

9:00 am – 12:00 pm	**Session 4: AMR in the Food Chain/Food Products/ Industrial Processes**
9:00 – 9:30 am	Keynote Lecture - *Escherichia coli* O104:H4 Outbreak Strain Did Not only Spread Disease but CTX-M-15 Extended Spectrum β-lactamase as Well ***Beatriz Guerra**; National Salmonella Reference Laboratory, Federal Institute for Risk Assessment (BfR), Berlin, GERMANY*
9:30 – 9:45 am	Short Review: Effect of Food Structure and Composition on the Efficiency of Bacteriophages for Specific Control of Foodborne Pathogens ***Mai Huong Ly-Chatain**; ISARA-Lyon, Lyon, FRANCE*
9:45 – 10:00 am	Detection of Enterobacteriaceae and *Aeromonas Sobria* Harboring blaCTX-M-2, blaSHV-2, and qnr Variants in Retail Chicken Meat in Brazil ***Mara Nogueira**; Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, BRAZIL*
10:00 – 10:15 am	Foodborne Origins of Antimicrobial Resistant Escherichia coli Causing Extraintestinal Infections ***Amee Manges**; McGill University, Montreal, QC, CANADA*
10:15 – 10:45 am	**Coffee Break**
10:45 – 11:00 am	Presence and Impact of Antibiotic-Resistant Pathogens in the Food Supply in the United States ***Caroline Smith DeWaal**; Center for Science in the Public Interest, Washington, DC*
11:00 – 11:15 am	Accessing the Molecular Basis of Transferable Quinolone Resistance in *Escherichia coli* and *Salmonella* spp from Food-Producing Animals and Products ***Manuela Caniça**; National Institute of Health, Lisbon, PORTUGAL*
11:15 – 11:30 am	Genetic Relationship and Antimicrobial Susceptibility Among *Escherichia coli* Isolates from Humans, Poultry and Street Foods in Ghana ***Courage Saba**; Universidad Complutense de Madrid, Madrid, SPAIN*

11:30 – 11:45 am	Phenotypic and Genetic Characterization of Antimicrobial Resistance in *Salmonella* Serovars Isolated from Retail Meats in Alberta, Canada ***Mueen Aslam**; Agriculture and Agri-Food Canada, Lacombe, AB, CANADA*
11:45 am – 12:00 pm	Antimicrobial Resistance and Virulence Genes of Methicillin-Resistant *Staphylococcus aureus* from Abattoir Pigs and their Horizontal Transfer ***Marie Archambault**; University of Montreal, Faculty of veterinary medicine, Saint-Hyacinthe, QC, CANADA*
12:00 – 2:00 pm	**Lunch and Poster Session B**
2:00 – 5:00 pm	**Session 5: AMR in the Environment**
2:00 – 2:30 pm	Keynote Lecture - The Role of Natural Environments in the Evolution and Dissemination of Resistance in Pathogenic Bacteria ***Jose Luis Martinez**; Centro Nacional de Biotecnologia Madrid, Madrid, SPAIN*
2:30 – 2:45 pm	Accelerated Biodegradation of Veterinary Antibiotics in Agricultural Soil Following Long-Term Exposure, and Isolation of a Sulfonamide-Degrading Microbacterium sp. ***Edward Topp**; Agriculture and Agri-Food Canada, London, ON, CANADA*
2:45 – 3:00 pm	Persistence and Dissemination of the Multiple-Antibiotic-Resistance Plasmid pB10 in Complex Environmental Communities ***Xavier Bellanger** CNRS-UdL, Vandoeuvre, FRANCE*
3:00 – 3:15 pm	High Carriage Rates of Esbl-Producing *Escherichia coli* in Wild Birds from the Remote Mongolian Gobi Desert ***Christa Ewers;** Institute of Hygiene and Infectious Diseases of Animals, Justus-Liebig-Universität Giessen, Giessen, GERMANY*
3:15 – 3:45 pm	**Coffee Break**

3:45 – 4:00 pm	Transfer of Resistance from Pig Manure to Agricultural Fields - A Field Study ***Gamonsiri Bhumibhamon**; IRAS, Utrecht University, Utrecht, NETHERLANDS*
4:00 – 4:15 pm	Multi-Resistant and ESBL-Producing *E. coli* in Discharged Wastewater and Dutch Surface Waters ***Hetty Blaak**; National Institute of Public Health and Environment (RIVM), Bilthoven, NETHERLANDS*
4:15 – 4:30 pm	Beta-Lactam Resistance in Staphylococci Isolated from Captive and Wild Wallabies in South Australia ***Michelle Chen**; Flinders University, Bedford Park, AUSTRALIA*
4:30 – 4:45 pm	Reservoirs of Antibiotic Resistant and Multi-Resistant Environmental Bacteria in the North Italian Adriatic Coasts ***Maria Lleo**; University of Verona, Verona, ITALY*
4:45 – 5:00 pm	Antimicrobial Resistance Among Pathogens and Indicator Organisms from Raw Shrimp Purchased in Canada. ***Nicol Janecko**; Public Health Agency of Canada, Guelph, ON, CANADA*

Thursday, June 28, 2012

9:00 am – 12:00 pm	**Session 6: AMR and Evolutionary Biology, Genome Sequencing**
9:00 – 9:30 am	Keynote Lecture - Ecology or Epidemiology: The Essential Method for Controlling Antimicrobial Resistance in Foodborne Bacteria? ***Harvey M. Scott**; Kansas State University, Manhattan, KS*
9:30 – 9:45 am	ResFinder: Database for Identification of Transferable Antimicrobial Resistance Genes in Whole Genome Sequenced Bacteria ***Susanne Karlsmose**; WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens and EU Reference Laboratory for Antimicrobial Resistance, National Food Institute, Technical University of Denmark, Kgs. Lyngby, DENMARK*

9:45 – 10:00 am	Multiplex Sequencing of Antimicrobial Resistance Genes by PGM ***Shih Feng Tsai***; *National Health Research Institutes, Zhunan, Miaoli, TAIWAN*
10:00 – 10:15 am	Antibiotic and Heavy Metal Resistance Transposons Driving the Evolution of IncHI2 Plasmids from *Salmonella enterica* ***Ruth Hall***; *Univ. of Sydney, Sydney, AUSTRALIA*
10:15 – 10:45 am	**Coffee Break**
10:45 – 11:00 am	Identification and Characterization of the Integrative and Conjugative Element ICE*Pmu1* from Bovine *Pasteurella multocida* which Carries and Transfers 12 Resistance Genes ***Geovana Brenner Michael***; *Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, GERMANY*
11:00 – 11:15 am	Characterization of Methicillin-resistant *Staphylococcus aureus* ***Mette Christiansen***; *National Food Institute, Technical University of Denmark, Kgs. Lyngby, DENMARK*
11:15 – 11:30 am	Host-Directed Antimicrobial Therapeutics for Intracellular Bacterial Pathogens ***Howard Shuman***; *University of Chicago, Chicago, IL*
11:30 – 11:45 am	Longitudinal Analysis of Antibiotic Resistance Gene Quantities in Dairy Cattle ***Randall Singer***; *University of Minnesota, St. Paul, MN*
11:45 am – 12:00 pm	ColE1 Plasmid in Antimicrobial Resistance in Human and Animal Bacteria ***Bruno Gonzalez-Zorn***; *Animal Health Deparment and VISAVET, Universidad Complutense, Madrid, SPAIN*
12:00 – 2:00 pm	**Lunch and Poster Session C**
2:00 – 3:30 pm	**Session 7: Integrated Surveillance Systems**
2:00 – 2:30 pm	Keynote Lecture - How to Prevent the Spread of Multiple-Resistant Gram-Negative Bacteria in Human Medicine? ***Stephan Harbarth***; *University of Geneva Hospitals and Faculty of Medicine, Geneva, SWITZERLAND*

2:30 – 2:45 pm	Farm to Fork? Deliberations on the Where, What, When, Who, Why of Resistance Monitoring ***Mary Torrence**; USDA ARS, Beltsville, MD*
2:45 – 3:00 pm	European Antimicrobial Susceptibility Surveillance in Animals (EASSA) Programme: Results on Enteric Bacteria from Healthy Broiler Chickens at Processing ***Shabbir Simjee**; EASSA Study Group, Brussels, BELGIUM*
3:00 – 3:15 pm	Integrated Surveillance For Antimicrobial Resistance - Pilot Study in Kenya ***Awa Aidara-Kane**; World Health Organization, Geneva, SWITZERLAND*
3:15 – 3:30 pm	OIE Activities on Surveillance and Monitoring of Antimicrobial Use and Antimicrobial Resistance ***Elisabeth Erlacher-Vindel;** OIE-World Organisation for Animal Health, Paris, FRANCE*
3:30 – 4:00 pm	**Coffee Break**
4:00 – 5:00 pm	**Session 8: AMR in Salmonella and Campylobacter**
4:00 – 4:15 pm	Multiresistant *Salmonella* Typhimurium and Typhimurium-like Strains in England and Wales - An Increasing Problem in Humans and Food Animals ***Katie Hopkins**; Health Protection Agency Microbiology Services, London, UNITED KINGDOM*
4:15 – 4:30 pm	Antimicrobial Resistance of *Campylobacter* species and Risk Factors for Ciprofloxacin-Resistant *Campylobacter jejuni* from Human Patients in Saskatchewan (1999-2006) ***Simon Otto**; Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, CANADA*
4:30 – 4:45 pm	Increasing Resistance to Ciprofloxacin in Multidrug Resistant *Salmonella* Typhimurium in Mexico ***Mussaret Zaidi**; Hospital General O'Horan/Hosp. Regional de Alta Especialidad, Merida, MEXICO*

4:45 – 5:00 pm

VIM-1 Carbapenemase Carrying *Escherichia coli* and *Salmonella* enterica from Livestock Farms
***Jennie Fischer**; Federal Institute for Risk Assessment, Berlin, GERMANY*

8:00 – 11:00 pm
La Bastide du Cours Restaurant

Conference Dinner
Located on the main avenue (the Cours Mirabeau) in the center of town and within walking distance of the conference center and all conference hotels, the restaurant Bastide du Cours is the private venue for the official conference dinner. All conference participants are invited to join together for a convivial evening of French wine and food on the covered terrace overlooking the Cours Mirabeau, as well as in the warm and inviting dining rooms inside the restaurant. The dinner is included in the registration fee for participants.

Friday, June 29, 2012

9:00 am – 12:00 pm

Session 9: Comparative Molecular Epidemiology of AMR in Animals and Humans

9:00 – 9:30 am

Keynote Lecture - ESBL and AmpC-producing *Escherichia coli* from Livestock and Companion Animals, and their Putative Impact on Public Health
***Christa Ewers**; Institute for Hygiene and Infectious Diseases of Animals, Justus-Liebig-Universität Giessen, Giessen, GERMANY*

9:30 – 9:45 am

Quantifying the Proportion Of Antibiotic-Resistant Extraintestinal Infections of Food-Animal Origin: A Progress Report
***Lance B. Price**; Translational Genomics Research Institute North, Flagstaff, AZ*

9:45 – 10:00 am

High Frequency of the *vga*(A) Gene Among mrsa st398 from Pigs and Humans in Portugal
***Constança Pomba**; Faculty of Veterinary Medicine, Lisbon, PORTUGAL*

10:00 – 10:15 am

Dogs of Nomadic Pastoralists in Northern Kenya are Reservoirs of Plasmid-Mediated Cephalosporin- and Quinolone-Resistant *Escherichia coli* Including Pandemic Clone B2-O25-ST131

Katerina Albrechtova; *Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, CZECH REPUBLIC*

10:15 – 10:45 am **Coffee Break**

10:45 – 11:00 am

E. coli Producing Plasmid-Mediated AmpC Isolated from Broilers and Humans in Sweden Carry the bla_{CMY-2} Gene on an IncK Plasmid

Stefan Börjesson; *NATIONAL VETERINARY INSTITUTE (SVA), Uppsala, SWEDEN*

11:00 – 11:15 am

Animal and Human MRSA Harbouring the New mecC: The Challenge of Screening, Detection and Confirmation

Frederic Laurent*; French National Reference Centre for Staphylococci, Hospices Civils de Lyon, Inserm U851, Lyon, FRANCE*

11:15 – 11:30 am

Similar SHV-12-harbouring Plasmids Circulating in *Escherichia coli* Isolates from Both Human and Animal Food Origin in the South Of Spain.

Lorena López-Cerero*; University Hospital Virgen Macarena, Seville, SPAIN*

11:30 – 11:45 am

Multi-Drug Resistance IncHI1 Plasmids Carrying blaCTX-M-1 from Equine *Escherichia coli* from the Czech Republic

Monika Dolejska*; Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, CZECH REPUBLIC*

11:45 am – 12:00 pm

Closing Remarks

Jean-Yves Madec*; Anses, Lyon, France*

Speaker Abstracts

S1:1

SEROTYPE DISTRIBUTION AND ANTIMICROBIAL RESISTANCE PROFILES OF SALMONELLA SPP., ESCHERICHIA COLI, AND CAMPYLOBACTER SPP. ISOLATES OBTAINED FROM CONVENTIONAL, ANTIMICROBIAL-FREE, AND ORGANIC BROILER CHICKEN PRODUCTION SYSTEMS IN ONTARIO

T. E. Roberts[1], *M. T. Guerin*[1], *R. Reid-Smith*[2], *S. A. McEwen*[1], *J. M. Sargeant*[3], *A. Agunos*[2], *D. Léger*[2]; [1]*Department of Population Medicine, University of Guelph, Guelph, ON, CANADA,* [2]*Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, CANADA,* [3]*Centre for Public Health and Zoonoses, University of Guelph, Guelph, ON, CANADA.*

Antimicrobial use in broiler chicken production has been identified as a risk factor in the development of antimicrobial resistant pathogens that can be transferred to humans via the food chain. Antimicrobial-free and organic production are recent industry approaches to antimicrobial resistance reduction. However, data are lacking in Ontario that compare the level of antimicrobial resistance in foodborne pathogens among the different broiler production systems. The objective of this study was to determine the serotype distribution of *Salmonella* spp. and the antimicrobial resistance profiles of *Salmonella*, generic *E. coli*, and *Campylobacter* isolates according to production type. Sixty-three conventional, 34 antimicrobial-free, and six organically-raised flocks distributed throughout Ontario, Canada were enrolled and sampled between August 2010 and March 2012. Within the last 7 days of the growing period, samples collected included: dust on feeders, drinkers, and walls; boot socks worn to collect particulate matter from the floor; and pooled fecal samples. Samples were submitted to the Animal Health Laboratory for bacterial culture using standard techniques; positive isolates were serotyped (*Salmonella* only) and tested for susceptibility to 15 antimicrobials using Sensititre™. At the isolate level, the three most commonly identified *Salmonella* serotypes obtained from conventional flocks (n=175) were Kentucky (52.0%), Heidelberg (12.0%), and Enteritidis (11.4%); compared to Kentucky (54.6%), Heidelberg (26.1%), and Schwarzengrund (11.4%) from antimicrobial-free flocks (n=88); and Kentucky (70.0%), Typhimurium (20.0%), and I:8,20:i:- (10%) from organic flocks (n=10). No resistance of *Salmonella* isolates to ciprofloxacin, azithromycin, chloramphenicol, nalidixic acid, kanamycin, or gentamicin or of *E. coli* isolates to ciprofloxacin, from any production type was found. Statistical differences (Fisher's Exact test; $\alpha = 0.05$) existed in the percentages of *Salmonella* isolates resistant to streptomycin (36.0% vs. 12.7% vs. 40.0%, $P < 0.001$) and tetracycline (36.8% vs. 19.0% vs. 70.0%, $P = 0.001$), and *E. coli* isolates resistant to streptomycin (50.5% vs. 22.4% vs. 6.3%, $P < 0.001$), trimethoprim-sulfamethoxazole (25.6% vs. 1.2% vs. 3.1%, $P < 0.001$), and tetracycline (65.8% vs. 31.2% vs. 43.8%, $P < 0.001$) from conventional, antimicrobial-free, and organic systems, respectively. Among *Campylobacter* isolates, only resistance to tetracycline was identified. In conclusion, these results suggest that differences exist in the serotype distribution and antimicrobial resistance profiles of *Salmonella* spp., generic *E. coli*, and *Campylobacter* spp. among flocks raised under conventional, antimicrobial-free, and organic production systems. This study also highlights the need for continued surveillance of antimicrobial resistant bacteria at the broiler farm level.

S1:2

ANTIMICROBIAL RESISTANCE DIVERSITY IN SALMONELLA TYPHIMURIUM DT104 FROM ANIMALS AND HUMANS IN SCOTLAND

A. Mather[1], *S. Reid*[2], *D. Haydon*[3], *D. Mellor*[3], *M. Fookes*[1], *L. Matthews*[3], *R. Reeve*[3], *M. Denwood*[3], *T. Connor*[1], *R. Reid-Smith*[4], *P. Boerlin*[5], *D. Brown*[6], *J. Coia*[6], *L. Browning*[7], *D. Maskell*[8], *N. Thomson*[1]; [1]*Wellcome Trust Sanger Institute, Cambridge, UNITED KINGDOM,* [2]*The Royal Veterinary College, North Mymms, Hatfield, UNITED KINGDOM,* [3]*University of Glasgow, Glasgow, UNITED KINGDOM,* [4]*Public Health Agency of Canada, Guelph, ON, CANADA,* [5]*University of Guelph, Guelph, ON, CANADA,* [6]*Scottish Salmonella Shigella and Clostridium difficile Reference Laboratory, Glasgow, UNITED KINGDOM,* [7]*Health Protection Scotland, Glasgow, UNITED KINGDOM,* [8]*University of Cambridge, Cambridge, UNITED KINGDOM.*

Antimicrobial resistance (AMR) is a major threat to the health and welfare of both humans and animals. Whilst the use of any antimicrobial agent can select for resistance, the relative contributions from use in different populations to the overall resistance burden remain poorly understood. Here, data on Salmonella Typhimurium DT104 isolated between 1990 and 2004 in Scotland were used to investigate the patterns and diversity of resistance, taking an ecological perspective. Multidrug resistant DT104 was one of the most common types of Salmonella isolated from animals and humans in the UK and in other parts of the world, peaking in the mid 1990s. Salmonella is a reportable pathogen in the UK; between 1990 and 2004, there were 5,200 isolates of DT104 from humans and animals forwarded to the Scottish Salmonella Shigella and Clostridium difficile Reference Laboratory through this ongoing passive surveillance programme. Phenotypic susceptibility testing data were used to examine the resistance phenotypes, or profiles, of these isolates. Whole genome sequencing was used to investigate the resistance determinants in a subset of 148 isolates of DT104. Ecological diversity metrics, which included all weightings of numbers of unique profiles and of abundance, were used to investigate the diversity of resistance profiles. The diversity of phenotypic resistance profiles was greater in the human isolates, which was supported by the sequencing data. These results are consistent with the possibility that there is more varied selection pressure for resistance in humans than animals in Scotland, and, on the premise that the population with the lesser diversity is unlikely to be the principle source of resistance diversity, that there are sources of resistance other than the local animals, such as imported food, that are contributing to the diversity of resistance observed in the human bacterial population. Whilst these conclusions relate to S. Typhimurium DT104 in Scotland, the focus on diversity, rather than prevalence, provides a different perspective on the issue of antimicrobial resistance, and its emergence, that can be applied to other organisms in other settings to attain a greater understanding of the ecology of resistance.

S1:3

PREVALENCE OF EXTENDED-SPECTRUM Β-LACTAMASES IN HUMANS LIVING IN MUNICIPALITIES WITH HIGH OR LOW BROILER DENSITY

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Introduction: In The Netherlands, the prevalence of extended-spectrum β-lactamase (ESBL)-producing bacteria causing infections in hospitals is increasing. Little information, however, is available about prevalence in the

general Dutch population. ESBL-producing bacteria have been found on Dutch broiler farms. Among people working and/or living on these farms, 11.3% was ESBL-positive, with no difference in prevalence between persons working in close contact with broilers, and persons living in the farm residence. This might indicate that humans living in areas with high broiler densities could be at risk for ESBL-carriage. The aim of this cross-sectional study was to determine the prevalence of, and identify risk factors for, carriage of ESBL-producing *Enterobacteriacea* in people living in municipalities with either a high or low broiler density. **Method:** A random sample of 3949 adults (≥18 yr), stratified according to age and gender, was taken from 8 Dutch municipalities across 4 provinces. Per province, the municipality with the highest number of broilers per km^2, and a municipality with a similar number of inhabitants, but without broilers, were selected. Each person was asked to fill in a questionnaire about life-style, eating habits, travelling, and health characteristics, and to take a rectal swab. Isolates were selected after growth in (non)-selective LB broth and on McConkey agar supplemented with 1µg/ml cefotaxime. β-Lactam resistance was examined by disk-diffusion tests and bacterial species were determined. ESBL genes were characterized by PCR and sequence analysis. **Results:** In total, 1025 persons responded (26.0%), with a mean age of 53.5 (range 18-89) years. Overall prevalence of ESBL-carriage was 5.1% (52/1025; 95% CI 3.8-6.6%). The percentage of ESBL-positive persons was lower in municipalities with high broiler densities (3.6%) than with low broiler densities (6.7%) (P<0.05). Univariable analyses showed that travel abroad, and contact with pets and/or livestock might be associated with ESBL-carriage. Most ESBL-positive samples were found in *Escherichia coli* (n=42), followed by *Klebsiella pneumoniae* (n=3) and *Citrobacter Freundii* (n=1). Major ESBL-genotypes were CTX-M-1 (n=18) and CTX-M-15 (n=16). **Conclusion:** Prevalence of carriage of ESBL-producing bacteria was 5.1% among adults in The Netherlands, and was not associated with living in areas with high broiler density. Univariable analyses suggest that travel abroad, and contact with pets and/or livestock could be potential risk factors for carriage. Currently, further analysis into risk factors is being conducted.

S1:4

OCCUPATIONAL EXPOSURES TO ANTIMICROBIAL RESISTANT PATHOGENS ASSOCIATED WITH FOOD ANIMAL PRODUCTION

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Use of antimicrobials as animal feed additives is associated with increased prevalence of antimicrobial resistant (AMR) pathogens in food. However, risks for workers in contact with food animals have not been as widely studied or addressed. We studied workers in confined animal houses and in slaughter and processing plants to assess risks of exposure in the workplace using cross sectional designs as well as secondary analysis of a subset of the US Agricultural Health Study (AHS). Exposure was determined by nasal swab sampling or stool collection as well as serology. In all studies prevalence among workers was compared to referents. These studies were approved by the Johns Hopkins Bloomberg School of Public Health Institutional Review Board. Results. Exposures: Broiler house workers were more likely to be exposed to C. jejuni as measured by serum antibodies. Stool cultures indicated t substantially increased risk of carrying gentamicin-resistant E. coli (32X community referents) and risks of carrying multidrug resistant E coli. Hog slaughter and processing workers were at substantially increased risk of

exposure to multidrug resistant S .aureus. In the AHS study, Iowa farmers reporting work with swine or poultry had higher levels of anti-C. jejuni antibodies. Health outcomes: Broiler house workers were more likely to report gastrointestinal disease and symptoms consistent with inflammatory peripheral neuropathy. In the AHS subjects, farmers with animal contact were more likely to report symptoms consistent with inflammatory peripheral neuropathy. In these farmers we also found increased prevalence of seropositivity for antiganglioside autoantibodies, which are biomarkers of inflammatory peripheral neuropathies. Discussion: These are among the first studies of occupational exposures to zoonotic pathogens and AMR pathogens in association with food animal production (farm and slaughter/processing plants). The results are consistent with other reports on S aureus among slaughter/processing workers. These are the first studies to examine peripheral neuropathy as an outcome of occupational exposure to C. jejuni. Exposures of slaughter/processing workers to zoonotic pathogens, including S. aureus, are of concern, given high rates of lacerating injuries reported in this industry in the US. The results support consideration of exposure to antimicrobial resistant zoonotic pathogens as an occupational health risk and monitoring for both exposure and disease among workers at risk. Support: This research was supported by grants from the Center for a Livable Future, Johns Hopkins, the Pew Charitable Trusts, the Heinz Foundation, and the National Institute for Occupational Safety and Health - CDC.

S2:1

EFFECT OF AT-BIRTH CEFTIOFUR ADMINISTRATION ON COMMENSAL E. COLI ESBL CTX-M GENES IN PIGS FROM PORTUGUESE INDUSTRIAL HERDS

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Introduction: Pork is the most consumed meat in Europe and EU data indicates that third-generation cephalosporin consumption in pigs has increased. The common off-label use of ceftiofur in pig production as a preventive measure is of great concern. The extent to which this use in food-producing animals poses a risk to public health is still unknown. Objective: This study aims to evaluate the influence of a high-dose administration of ceftiofur as a preventive measure against neonatal infections on third generation cephalosporin (3GC) resistance in commensal E. coli isolates from faecal samples of healthy piglets from 3 Portuguese industrial farms. **Methods:** For each farm 10 litters were randomly chosen and 7 piglets from each litter were tagged. Each piglet (n=209) was swab sampled at birth [before the ceftiofur (XNL) administration of 20mg/piglet] and at weaning (n=194). After enrichment in buffered peptone water, 100µl of bacterial suspension was inoculated on MacConkey agar supplemented with 1.5µg/ml of cefotaxime (CTX). E. coli strains resistant to CTX were identified by gadA PCR. Also the blaCTX-M-1Group gene cluster was identified by PCR. Logistic regression was used to assess the association between XNL use, 3GC resistance and time of sampling (SAS v9.2 software). **Results:** Farm 1 increased CTX resistant E. coli isolates from birth (72.5%, CI95% 60.2 - 82.2%) to weaning (93.4%, CI95% 83.3 - 97.9%) while farms 2 and 3 decreased prevalence [68.6% (CI95% 56.2 - 78.9%) to 53.8% (CI95% 41.1 - 66.1%) and 81.4% (CI95% 70 - 89.4%) to 19.1% (CI95% 10.9 - 30.8%), respectively]. The increase observed in farm 1 was influenced by the administration of XNL (p=0.0038) as was

the decrease registered in farm 3 (p<0.001). Farm 2 was not influenced by the use of XNL (p=0.0804). At birth, farm 1 had a prevalence of 71% (CI95% 58.7 - 81%) of E. coli strains harbouring the blaCTX-M-1Group genes; farm 2 had 68.6% (CI95% 56.2 - 78.9%) and farm 3 had 81.4% (CI95% 70 - 89.4%). At weaning farm 1 showed an increased prevalence of E. coli strains with blaCTX-M-1Group genes (91.8%, CI95% 81.2 - 96.9%), while farms 2 and 3 decreased their prevalence (53.8%, CI95% 41.1 - 66.1%, and 14.7%, CI95% 7.7 - 25.8%, respectively). **Conclusion:** There was an influence of preventive ceftiofur administration (four times the therapeutic approved dose of 5mg/kg) on 3CG resistance in commensal E. coli isolates from faecal samples of healthy piglets from 3 Portuguese industrial farms. The blaCTX-M-1Group gene cluster presence at birth and weaning decreased in farms 2 and 3 while ESBL genes increased in farm 1 after XNL use. Two possible explanations for these facts arise: i) the population of resistant E. coli in the pig gut diminished due to high-level exposure; ii) biosecurity and management differences among farms accounted for the differences observed. Risk assessment is urgently needed in order to attribute the impact of 3GC use in pig production on human health.

S2:2

EFFECT OF INTERVENTION STRATEGIES ON ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND THEIR RELATIONSHIP WITH TETA, TETB, AND BLACMY-2 GENES AMONG E. COLI ISOLATES IN FEEDLOT CATTLE

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Antimicrobials can selectively favor resistance in bacteria. Further, these resistant bacteria may spread to humans causing public health risks. Ceftiofur is widely used in animals; ceftriaxone, a related human drug is used to treat salmonellosis in children. The objective of this study was to investigate the effects of two intervention strategies (i.e., feeding of preventive doses of chlortetracycline following ceftiofur (Excede®) treatment and mixing of ceftiofur-treated with untreated animals at a ratio of 1:10) on antimicrobial susceptibility profiles and examine their relationships with the tetA, tetB, and blaCMY-2 genes among the E. coli isolated from feces. A field trial was conducted on 88 steers. Steers were randomly allocated to 8 pens of 11 steers each. All steers in 4 pens were given ceftiofur on day 0; 2 of these pens received three 5-day regimens (with a one day break in between) of chlortetracycline (CTC) in feed starting at day 4. In the remaining 4 pens, ceftiofur was given to only 1 steer among the 11 pen mates (mix). Among these 4 pens, CTC was likewise given to all animals in just 2 pens. Fecal samples were collected every other day to 26 days. E. coli isolates (n=1050) were isolated from day 0, 4, 12, and 26 fecal samples. Antimicrobial susceptibility profiles were determined using microbroth dilution technique. PCR assay was used to detect tet resistance and blaCMY-2 genes. Mixed logistic regression models using 3-way full factorial design (CTC, mix, day) revealed that both ceftiofur and CTC treatments increased tetA and tetB gene copies; however, there was a differential selection favoring tetA over tetB (P<0.001). Moreover, tetA was associated with higher levels of multidrug resistance (median=6, 95%CI= 4-8) verses tetB which was more associated with the lower-level resistance phenotypes (median=3, 95%CI= 3-4). Furthermore, tetA and tetB genes were negatively associated with one another.

S2:3

EPIDEMIOLOGYOF A TRANSFERABLE COPPER RESISTANCE GENE, *TCR*B, AND ITS CO-SELECTION WITH MACROLIDE [*ERM*(B)] AND TETRACYCLINE [*TET*(M)] RESISTANCE AMONG FECAL ENTEROCOCCI OF CATTLE AND SWINE DIETS SUPPLEMENTED WITH VARYINGCONCENTRATIONS OF COPPER SULFATE

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Elevated concentrations of copper sulfate are often used for growth promotion in both swine and cattle diets. *Enterococcus* spp., gut commensals, may acquire resistance to copper via a transferable copper resistance (*tcrB*) gene carried on a plasmid. In Europe, the plasmid also carries genes for macrolide [*erm*(B)] and glycopeptide (*vanA*) resistance. We conducted studies in cattle and swine to determine the relationship between copper supplementation and the fecal prevalence of *tcrB*-positive enterococci as well as its potential co-selection for macrolide and tetracycline resistance. The cattle study consisted of 261 crossbred yearling heifers, which were assigned randomly to a 2×2 factorial arrangement of treatments of dietary copper and a linseed supplement. The swine study consisted of 240 weaned piglets, which were assigned randomly to 6 treatment groups. The pens were assigned in an incomplete factorial design, to an incomplete 2×2×2 factorial arrangement of treatments of copper, tylosin, and chlortetracycline. The enterococci were isolated from the fecal samples and speciation was done by multiplex PCR and superoxide dismutase gene (*sodA*) sequence analysis. Overall, prevalences of *tcrB*-positive enterococci were 14.5% (372/2592) and 3.9% (22/576) in swine and cattle, respectively ($P < 0.05$). The *tcrB*-positive isolates belonged to either *Enterococcus faecium* or *E. faecalis;* the majority were *E. faecium*. All *tcrB*-positive isolates also contained both *erm*(B) and *tet*(M) genes; however, none of them harbored the *vanA* gene. The mean MICs ofcopper for *tcrB*-negative and *tcrB*-positive enterococci were 6 and 18 mM, respectively ($P < 0.001$). The overall prevalence of *erm*(B) and *tet*(M) genes among enterococcal isolates of cattle were 57.1 % and 66.1%, respectively; in contrast, fully 100% of the swine isolates tested were positive for both *erm*(B) and *tet*(M) genes. Conjugation assay demonstrated the co-transfer of *tcrB* along with *erm*(B) and *tet*(M) genes both between and within *E. faecium* and *E. faecalis* donor and recipient strains. Our results demonstrate that the epidemiology of antimicrobial and metal resistance differs between swine and cattle enterococcal isolates. The higher occurrence of *tcrB*-positive enterococci in heifers and piglets fed elevated copper compared to normal copper suggests that supplementation of copper in both cattle and swine diets selected for resistant strains. The genetic link between copper resistance with other antibiotic resistance determinants explains the genome plasticity of *Enterococcus* sp. as well as the potential importance of elevated copper supplementation in aiding in the spread and persistence of antibiotic resistance. Further molecular epidemiological studies have been undertaken to investigate the role of plasmids and predominant clones in dissemination and persistence of resistance determinants in enterococci.

S2:4

THE TRANSCRIPTIONAL EFFECT OF SUB-INHIBITORY CONCENTRATIONS OF BIOCIDES ON THE CONJUGATIVE TRANSPOSON TN916

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Background: Large amounts of biocides are used as disinfectants to reduce and control bac-

terial growth in hospitals and the food industry. It has been suggested that bacterial exposure to biocides may induce DNA transfer between bacteria. The conjugative transposons of the Tn*916*-family are mobile genetic elements of clinical relevance due to their involvement in the spread of multiple antibiotic resistances between many different bacteria. The proposed regulatory system of Tn*916* involves transcriptional attenuation upstream of the tetracycline resistance gene *tet*(M), which is de-repressed by the presence of tetracycline. Transcription of this region is believed also to result in the transcription of mobility genes. **Objective:** The aim of this study was to investigate the effect of sub-inhibitory concentrations of commonly used biocides; hydrogen peroxide (HP), ethanol (ETOH) and chlorhexidine digluconate (CHX) on the transcription of the region upstream of *tet*(M) in Tn*916*. **Methods:** Investigation of transcription was conducted using *Bacillus subtilis* carrying β-glucuronidase (*gus*A) reporter constructs. After two hours of growth biocides were added at sub-inhibitory concentrations corresponding to MIC/4. Samples were collected before (2.0h) and after addition of the compounds (2.5h, 3.0h, 3.5h and 4.0h). Tetracycline (10µg/ml) was included as a positive control compound. The enzyme activity of each sample was normalized to the corresponding control sample and analyzed using two-sided t-test. **Results:** The addition of tetracycline resulted in a significant increase in transcription of the upstream region of *tet*(M) (p-value < 0.05). A significant increase in transcription was also observed in sample 3.5h and 4.0h with HP. However, no significant increase in transcription was found when adding ETOH, but a transcription increase tendency might be suggested (p-value = 0.06) for the 4h sample. CHX had a significant negative effect on transcription of the upstream region in the 2.5h sample. **Conclusion:** Improper use of HP and ETOH is likely to increase transcription of Tn*916* mobility genes and therefore could induce the spread of Tn*916*-like elements and their resistance genes, while CHX might reduce transfer. The effect of HP, ETOH and CHX will be further investigated in conjugation experiments using exposure times allowing the highest differences in transcription to be investigated.

S3:1

COURSE DOSE: CONVERTING PREMIX SALES TO PIGS AND CALVES EXPOSED TO ORAL ANTIMICROBIALS

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Use of antimicrobials contributes to resistance in bacteria of relevance for both human and animal health. Since 2004 Swissmedic collects and publishes data on sales of veterinary antibiotics but, as these data do not take factors like potency or species repartition into account, a way has to be found to estimate the populations exposed. In 2011, premixes represent 65 % of the total antimicrobials sold. Being applied orally to groups of animals with varying feed intake, they pose a particular risk. We propose to convert sales data of premixes to number of animals potentially treated using recommended dosage, duration, weight at treatment and an estimation of species repartition. Parameters are obtained using marketing authorisations, PSURs and direct contacts with MA holders. Population exposition is estimated using number of animals slaughtered and live populations. The results show that 44.1 tons of antimicrobials sold during 2010 were contained in premixes. The highest tonnage was represented by sulfonamides (46%) and tetracyclines (29%). The sales of premixes represented 4.3 mio potential treatments in pigs, and 0.72 mio in calves. The latter are most likely treated with amoxicillin (56%), followed by triple combinations (sulfonamide, tetracycline, macrolide; 63%). In pigs, most treatments are likely made with colistin (79%), followed by amoxicillin (23%) and triple combinations (20%). The overall incidence of oral group treatments was 1.5 annual treatments for both calves and pigs. We conclude that calcu-

lating course doses using MAH data is a valuable tool to convert sales figures in potential treatments. In the absence of prescription data at national level, this method may be used as a surrogate in producing results necessary in the context of national monitoring programs and for regulatory or legislative action.

S3:2

ANTIBIOTIC PRESCRIBING PRACTICE IN CATTLE IN THE UK.

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Introduction: To maintain antibiotic efficacy we need to understand how antibiotics are used. Currently, there are no objective data describing antibiotic usage in UK farm animal practice. **Methods:** A random cross-section of vets (n=1438) working with farm animals within the United Kingdom were sent a questionnaire to gather information regarding antibiotic prescribing practices including; practice policies, clinician demographics, antibiotic choice and factors involved in decision making regarding the prescription of antibiotics. Additionally, four clinical scenarios explored prescribing practices including; acute *E.coli* mastitis, post-calving endometritis, groups of coughing calves, and introduction of new calves. Responses from clinical scenarios were explored using multivariable logistic regression models with the outcomes of; fluoroquinolones, 3rd/4th generation cephalosporins and macrolides prescribed as 1st choice in any of the scenarios. **Results**: In total, 255 vets contributed to this study. The majority (95.3%) reported being able to dispense antibiotics at their own discretion, with only 7 (2.8%) respondents reporting that their practice had a written policy on antibiotic prescribing. The most important factors involved in prescribing decisions were clinical signs, site of infection, antibiotic sensitivity, with equal ranking of culture results and withdrawal period. The majority of vets (>90%) reported using both fluoroquinolones and 3rd/4th generation cephalosporins within the last year. In total, 133 (52.2%) vets stated fluoroquinolones, 37 (14.5%) stated 3rd/4th generation cephalosporins and 109 (42.7%) stated macrolides as 1st choice antibiotics across all scenarios. Multivariable modelling showed that vets that work with dairy cattle (OR 8.9, 95% CI 2.0, 40.5) or those who work with sheep (OR 2.4, 95% CI 1.1, 5.3) were more likely to prescribe fluoroquinolones as a first choice. There were also significant differences in prescribing of fluoroquinolones, cephalosporins and macrolides, depending upon which antibiotic use guidelines or sources of information vets used for prescribing. **Conclusions:** This study provides novel data on vet prescribing practices within the UK. It highlights clinical preferences regarding antibiotic prescribing choices among farm animal vets. A number of factors were found to be associated with decisions to prescribe fluoroquinolones, 3rd/4th generation cephalosporins, and macrolides. Although the reported associations between the information source and prescribing choices may not be directly causal, it may suggest that vets decisions may be partially influenced by their perception of the content or advice they state they access. Further investigation is needed to advance our understanding of prescribing practices, particularly among classes of antibiotics which hold such significance to public health.

S3:3

LOW LEVELS OF FLUROQUINOLONE RESISTANCE IN BACTERIAL ISOLATES CAUSING HUMAN DISEASE IN AUSTRALIA IS RELATED TO THEIR USE BEING BANNED IIN FOOD ANIMALS

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E. coli is the commonest bacterial pathogen infecting people. It causes very common infections such as in the urinary tract, but also blood-stream infections in thousands of people in Australia every year and millions world-

wide. E.coli is not readily transmissible from person to person. E. coli are acquired by all of us every day from foods In Australia fluoroquinolone are banned in food production animals (including any off-label use). There are only low levels of fluoroquinolone resistance seen in E. coli isolates causing community acquired infections (~5%), in contrast to nearly all other countries, and where fluoroquinolone resistance rates can be 50% or more. This is despite over 30 years of human fluoroquinolone use and the relatively high overall use of antibiotics per capita in Australia. In contrast to the rest of the world, we also see almost no fluoroquinolone resistance in food borne infections with salmonella and campylobacter acquired domestically. In the Australian domestic poultry industry, third generation cephalosporins are not used, unlike most other countries where meat chickens are injected with 3rd generation cephalosporins (and receive fluoroquinolones via water). Resistance to 3rd generation cephalosporins is not seen in bacteria found in foods derived from poultry domestically. This is the likely reason there are such very low rates of third generation cephalosporin resistance in community isolates of E. coli in Australia (<3%), which is in stark contrast to almost everywhere else in the world. There is now ample evidence that show that antibiotic resistance to "critically important" antibiotics in people, such as third generation cephalosporins and fluoroquinolones, is related to the use of these drugs in food animals. The same factors that drive MRSA, multi-resistant E.coli and C. difficile infections in people (increased use of any antibiotics but particularly fluoroquinolones, cephalosporins and poor infection control) are the same factors that drive the development of these bacteria in food animals and their spread. Thus the evidence is overwhelming that if you use antibiotics, particularly "critically important" or "last line" antibiotics in food animals, this results in resistance in bacteria such as E. coli, in food animals and then these resistant bacteria are acquired by people and these cause serious and life threatening disease.

S3:4

VETERINARY ANTIMICROBIAL PRESCRIPTIONS FOR CATTLE IN FRANCE: DESCRIPTION AND LABELING COMPLIANCE

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Linked with the great concern about antimicrobial resistance, recommendations and prudent use guidelines about antibiotic use in animals are more and more numerous. To better target these recommendations and assess their implementation and efficiency, it is necessary to monitor antimicrobial use. A survey was conducted in 2006-2007 to gather information about veterinary antimicrobial prescriptions for cattle. The objectives were to qualitatively describe antimicrobial use and to characterise compliance to label requirements. The study design consisted in a retrospective cross-sectional survey over one whole year targeting all veterinarians dealing with cattle in France. One twelfth were surveyed each month via mailed questionnaires about the last two antibiotic prescriptions. A descriptive analysis of the distribution of the antibiotics used, the disease context and the extra-label use was conducted. The determinants of non-compliance to label requirements were identified using a polytomous logistic regression analysis. The response rate was 25%, 1,200 veterinarians were enrolled and gave information about 2,345 prescriptions for cattle including 3,047 treatments. The main diseases leading to antimicrobial use were udder disorders (36%), locomotor disorders (14%) and respiratory, reproduction or digestive troubles (11% each). Penicillins were the most used antimicrobial class (45%), followed by aminosides (37%), fluoroquinolones (24%), cephalosporins (19% with 15% for 3rd and 4th generations) and tetracyclines (14%). For 13% of the treatments, there was no agreement between the declared disease and the label indications. The compliance of the therapeutic scheme prescribed to label requirements, considering dose, administration rhythm and duration of the treatment,

was satisfactory for 53%, 31% of the treatments were overdosed, and 16% underdosed. Both underdose and overdose were more likely to occur among beef cattle than among dairy cattle. Underdose was also more likely to occur for reproduction troubles, and overdose for abdominal disorders. Overdose was less likely to occur for respiratory troubles, for preventive and first line treatments, and for therapy for a group of animals. Several aspects of antimicrobial use in catlle raise concern: the wide use of critically important antimicrobials and the extra-label use. It is needed to explore these aspects more precisely to detect if there is a real need or if it can be considered as practices to avoid.

S3:5

FLUOROQUINOLONES SELECTIVE PRESSURE INDUCES THE INCREASE OF MULTIDRUG-RESISTANT ESCHERICHIA COLI ISOLATES FROM CALVES

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Introduction: Few studies clearly demonstrate that the use of antimicrobial agents in veterinary medicine affects the antimicrobial resistance in bacteria isolated from food-producing animals. **Objective:** In this cohort study the influence of enrofloxacin administration in milk (50 mg/L) and the susceptibility of E. coli isolates from faecal samples of healthy calves (n=106) from a Portuguese farm were evaluated. **Methods:** Each calf was sampled at 2 (T0, before enrofloxacin administration), 6 (T1, after 3 administrations of 5 consecutive days with a 5 day interval each) and 10 weeks of age (T2). Between February and May 2010 237 E. coli isolates were obtained and identified by the gadA PCR. Antimicrobial susceptibility testing was done by the disk diffusion method with: enrofloxacin (5 µg), ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), ceftiofur (30 µg), tetracycline (30 µg) and trimethoprim/sulfamethoxazole (1.25/23.7 µg). The ESBL-producing isolates were detected by the double disk diffusion test. Strains for the study of resistance to third generation cephalosporin resistance were selected when two of the following three criteria were complied: i) growth in MacConkey agar supplemented with 2 µg/ml ; ii) resistance to ceftiofur detected by disk diffusion; iii) positive synergy between ceftiofur and amoxicillin/clavulanic disks. The blaCTX-M-1Group, blaCTX-M-9Group and blaTEM genes were identified by PCR and blaTEM genes were sequenced. Results were interpreted according to clinical M31-A3 breakpoints (CLSI, 2008). Data was analysed by the logistic regression model (SAS v9.2 software). **Results:** Overall E. coli resistance to ampicillin was 69. 3% (T0), 79.8% (T1) and 89.5 (T2), to amoxicillin/clavulanic acid 0.0%, 2.5% and 1.6%, to ceftiofur 27.8%, 3.8% and 22.8%, to enrofloxacin 35.6%, 98.7% and 71.9%, to tetracycline 78.2%, 94.9% and 94.7% and to trimethoprim/sulfamethoxazole 64.4%, 77.2% and 70.2%. There was a highly significant association ($p<0.001$) between fluoroquinolone exposure and E. coli resistance towards ampicillin, ceftiofur and enrofloxacin. There was a highly significant increase ($p<0.001$) in multidrug-resistant E. coli isolates (resistance to three or more different antimicrobial classes) from T0, T1 and T2. ESBL-producing isolates were 29.7% (T0) (n=17 blaCTX-M-1Group, n=12 blaCTX-M-9Group, n=1 blaTEM-52), 2.53% (T1) (n=2 blaCTX-M-9Group) and 19.3% (T2) (n=5 blaCTX-M-1Group, n=6 blaCTX-M-9Group). **Conclusion:** This study demonstrates that calves' off-label exposure to fluoroquinolones influences the emergence of fluoroquinolone and multidrug-resistance among E. coli isolates. Further scientific evidence-based studies are warranted to evaluate the public health risk.

S3:6

ANTIMICROBIAL RESISTANCE IN PORTUGAL - SALES FIGURES 2010

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Antimicrobial resistance has increased worldwide in bacterial pathogens leading to treatment failures in human and animal infectious diseases. Resistance against antimicrobials by pathogenic bacteria is a major concern in the anti-infective therapy of both humans and animals. The review of Portuguese surveillance data on the sale and use of veterinary antibiotics and on antimicrobial resistance in bacteria of non-human origin, began in 2008. The Portuguese monitoring of sales of antimicrobial agents used in veterinary medicine is coordinated by the DGV in collaboration with ESVAC - European Surveillance of Veterinary Antimicrobial Consumption**,** on a voluntary basis. This surveillance program has been funded by the Portuguese Ministry of Agriculture in 2011, reporting to data of 2010. The results of sales of antimicrobials used in veterinary medicine by class, in 2010 shows that approximately 80%of the total amount sold concerns 4 antimicrobial classes (tetracyclines, penicillin, polymyxines, and macrolides). Tetracyclines alone represent around 43% of the overall sales, while penicillin around 20% polymyxines 8% and macrolides 8%. The quantitative importance of the use of the molecules should be relativized to the real dosage of the medicine in which they are contained. Nevertheless, the results were also studied taking into account the total body weight of animals potentially treated by antimicrobials, that for Portugal in 2010 was consider to be 2029495,9 t. In this way the sales of active veterinary antimicrobial compounds relative to the body weight of potentially treated animals in 2010 were Aminoglycosides 0,001mg/kg b.w.; Penicillins 0,017 mg/kg b.w.; Macrolides 0,007 mg/kg b.w.; Phenicols 0,0007 mg/kg b.w.; Polymyxins 0,008 mg/kg b.w.; Quinolones 0,003 mg/kg b.w.; Sulphonamides + trimethoprim 0,006 mg/kg b.w.; Tetracyclines 0,0377 mg/kg b.w; Cephalosporins 0,0004 mg/kg b.w. and other classes 0,007 mg/kg b.w. The researchers and risk assessors regularly and systematically examine the factors that can lead to the presence of antimicrobial resistant bacteria in food and animals and providing scientific advice relevant to decision making. The factors are multiple and many of them still unknown, and not necessarily related to the availability of antimicrobials for the treatment of diseases It will, however, interesting to ascertain as soon as data are available where the active substances more available are those found in emerging antimicrobial resistance of bacteria isolated from animal species. If this parallelism is not found, it would be interesting to search for environmental factors, cross-resistance between drugs and bad practice on the use these molecules may be included in the priority list of combat and control in European legislation.

S3:7

INCREASE IN FLUOROQUINOLONE USAGE IN POULTRY PRODUCTION IN FRANCE - IMPACT OF AN UNPLANNED INTERVENTION

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Antimicrobial usage in poultry production was monthly monitored from 2003 to 2008 in France through collection of official paper forms recording animals treatment history. Percentages of flocks treated, number of treatments and amount of antimicrobials used were calculated per antimicrobial compound, on a random sample of 10% of chicken and turkey broiler flocks slaughtered in Brittany. Time series regularly computed displayed in 2007 an abrupt increase in the fluoroquinolones usage graphical representation. Statistical tools (rupture tests, segmented regression analysis) were thus applied to 1/ assess significance of the change observed 2/ quantify and char-

acterize the increase (month of occurrence, estimation of intercept and slope change) 3/ check for an associated evolution of treatments characteristics (i.e. age at treatment and treatment duration) 4/ explore different hypothesis to explain the change (e.g. occurrence of an unplanned sanitary/health change that would have required more treatments or generic products arrival on the market). Results confirmed occurrence of a significant increase in fluoroquinolone frequency of usage, which occurred on the same month in 2007 in chicken and turkey productions. No significant evolution could be observed on treatment characteristics (age at treatment or treatment duration). Event time was consistent with arrival of fluoroquinolones generics products on the French veterinary pharmaceutical market, as recorded through the monitoring program. No similar pattern could be observed on flocks mortality time series, number of laboratory examinations performed (e.g. *Escherichia coli* isolation) and *E. coli* antimicrobial resistance results, to support the concurrent hypothesis of a primary increase in health problems. Fluoroquinolone generics arrival on the veterinary pharmaceutical market may explain the increase that was recorded through the French monitoring program of antimicrobial use in poultry production.

S4:2

SHORT REVIEW : EFFECT OF FOOD STRUCTURE AND COMPOSITION ON THE EFFICIENCY OF BACTERIOPHAGES FOR SPECIFIC CONTROL OF FOODBORNE PATHOGENS

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Despite technological advances and good manufacturing practices, the number of foodborne diseases and intoxication has continued to increase in recent years. Moreover, with the globalization of the food market and the evolution of food consumption modes (e. g. consumption of fresh products or ready to eat products) food preservation technologies must increase the product's shelf life while also ensuring its nutritional and organoleptic quality. In this context, there is a need for better methods to prevent contamination and promising novel approaches should be considered. In recent years, the use of bacteriophages as antimicrobial agents controlling pathogenic bacteria appeared as a new promising alternative strategy to the addition of food preservatives or food processes such as pasteurization. The bacteriophages are considered as "intelligent" antimicrobial for their specific. The presentation is a summary of the current status on the use of bacteriophages against pathogens in food products, the advantages and disadvantages of phage use over traditional methods. Factors which influence the effectiveness of the use of bacteriophages such as bacteriophages/bacteria ratio, environmental conditions (pH, aw, temperature ...) and the structure of the food matrix will be presented. Finally, the perspectives of this strategy will be identified. One of them consists in the incidence of food matrix structure and composition on the efficiency of bacteriophages to control pathogenic bacteria.

S4:3

DETECTION OF ENTEROBACTERIACEAE AND AEROMONAS SOBRIA HARBORING BLACTX-M-2, BLASHV-2, AND QNR VARIANTS IN RETAIL CHICKEN MEAT IN BRAZIL.

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Bacterial antimicrobial resistance is a global public health problem, regarded as consequence of the extensive use of antimicrobials in human and veterinary medicine, and in animal food production. Bacteria resistant to antimicrobials important for human therapy have been increasingly isolated from food animals and meats, and surveillance of antimicrobial

resistance in bacteria of animal origin and animal derived food products has been gaining importance as an action to better control the spread of antimicrobial resistance through the food chain. During an ongoing study to investigate the presence of antimicrobial resistant Gram-negative bacteria in retail chicken meat in Brazil, we detected three strains of K. pneumoniae and six E. coli resistant to third generation cephalosporins and quinolones, harboring the qnrB-like, qnrS, blaCTX-M-2 and blaSHV-2 genes. Also, one P. mirabilis and one Citrobacter diversus presenting resistance to third generation cephalosporins and harboring the blaCTX-M-2 gene, one Aeromonas sobria resistant to quinolones and simultaneously harboring the qnrA-like, qnrS-like genes were detected. Chicken meat was purchased from grocery stores in São José do Rio Preto, São Paulo, Brazil. For bacterial isolation meat samples were mixed with tetrathionate broth (TT) added with 5% brilliant green in sterile plastic bags, and incubated at 37°C for 24h. After this, 100 µL of TT broth was transferred into MacConkey agar plates, incubated for 24h hours at 37°C. Colonies with distinct morphology were collected. Identification and antimicrobial susceptibility testing was performed using the Vitek 2 system. Additionally, susceptibility to ceftriaxone, ceftiofur, nalidixic acid, enrofloxacin, levofloxacin and moxifloxacin was determined by the disc diffusion method. Interpretation was performed according to CLSI and EUCAST criteria. Detection and identification of qnrA, qnrB, qnrS, blaCTX-M and blaSHV genes was performed by PCR and sequencing using previously published primers and protocols. The presence in chicken meat of Enterobacteriaceae and Aeromonas carrying genes for ESBLs such as blaCTX-M-2 and blaSHV-2, and plasmid mediated quinolone resistance genes such as qnr variants is concerning because these bacteria may colonize the human gut after exposure through the food chain, cause infections of difficult treatment or become a reservoir for resistance genes that can be horizontally transferred among commensal and pathogenic bacteria. We believe that occurrence of such resistant bacteria in chicken meat is a result of the selective pressure caused by the extensive use of enrofloxacin and ceftiofur in poultry production in Brazil. Advance in the spread of such resistance genes is to be expected and monitored, since it may compromise even further the usefulness of quinolones and cephalosporins for treating serious infections by Gram-negative pathogens.

S4:4

FOODBORNE ORIGINS OF ANTIMICROBIAL RESISTANT ESCHERICHIA COLI CAUSING EXTRAINTESTINAL INFECTIONS

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Most human extraintestinal Escherichia coli infections, including those involving antimicrobial-resistant strains, are caused by a limited number of distinctive E. coli lineages, termed extraintestinal pathogenic E. coli (ExPEC), that have the ability to exit their intestinal tract reservoir and cause disease at an extraintestinal site. Evidence suggests that many of the ExPEC strains encountered in human infections may have a food animal source and may be transmitted to humans via the food supply. Molecular epidemiology studies of ExPEC have revealed the existence of several potential non-human reservoirs for ExPEC, including food animals and retail meat products, sewage and other environmental sources, and companion animals. The food animal reservoirs for antimicrobial-resistant ExPEC lineages that account for a large fraction of human infections are reviewed here. Indistinguishable ESBL-positive E. coli O25:H4-B2-ST131 strains have been recovered from human and chicken sources, and from poultry farms. Multidrug-resistant E. coli O11/O17/O77:K52:H18-D-ST69, also termed clonal group A (CgA), has been linked to non-human reservoirs, primarily pork and chicken and possibly beef. In an experimental study, CgA E. coli from human

infections and retail chicken meat were equally able to cause UTI in a mouse model, suggesting that food animal-source CgA E. coli are just as pathogenic as human-derived CgA. In one study from The Netherlands, CTX-M-1-producing ST10 isolates were identified in human blood cultures and poultry cecal samples, and TEM-52-producing ST10 isolates were recovered from human urine and poultry cecal samples. In a second such study, ESBL-producing E. coli ST10 isolates were identified in chicken meat, other types of meat, rectal swabs from healthy humans, and human blood cultures. These studies also linked ESBL-producing ST117 E. coli to the same human and chicken reservoirs. In Canada, multidrug-resistant ST10 isolates were recovered from human clinical samples, cecal samples from chicken and pigs, and retail chicken and pork meat, whereas closely related O114:H4-ST117 isolates were identified in a human UTI case and retail chicken meat. Evidence of food animal reservoirs for other important ExPEC groups such as O1/O2/O18:K1:H7-B2-ST95, O15:K52:H1-D-ST393, O6:K2:H1-B2-ST73, (various serotypes)-D-ST405, and O75:K+:H5-B2-ST14 is limited. Certain prominent ExPEC groups occur in non-human reservoirs, including food animals and retail meat products. The extent of this phenomenon, its importance to human health, and the strength of the evidence varies by both source and E. coli group. Identification of potential reservoirs for these increasingly multidrug-resistant and clinically important E. coli could help to curb their transmission, or, at least, reduce the extent of antimicrobial resistance associated with human ExPEC infections.

S4:5

PRESENCE AND IMPACT OF ANTIBIOTIC-RESISTANT PATHOGENS IN THE FOOD SUPPLY IN THE UNITED STATES

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Hypothesis: Antibiotic-resistant pathogens present in the food supply are posing a public health burden. ***Experimental Methods and Data*:** The National Antimicrobial Resistance Monitoring System in the U.S. documented that in 2010, 43% of *Salmonella* serotypes present in retail samples of ground beef and 34% present in retail ground turkey were resistant to at least three antimicrobial classes. CSPI analyzed foodborne outbreak data from literature reviews and Internet searches to determine if antimicrobial-resistant pathogens were also associated with human foodborne outbreaks. ***Results:*** CSPI documented 38 outbreaks between 1973 and 2011, causing 20,064 illnesses and 27 deaths in which the bacteria identified were resistant to at least one antimicrobial. Thirty-one (82%) of the total outbreaks were due to *Salmonella*, with *S.* Typhimurium the most frequently identified bacterial pathogen (15 outbreaks or 40%), followed by *S.* Newport (9 outbreaks or 24%). *S.* Heidelberg was associated with two outbreaks; *S.* Havana, *S.* Stanley, *S.* Dublin, *S.* 4,5,12:i-, and *S.* Hadar were each associated with one outbreak. *E. coli* was responsible for 5 (13%) of the outbreaks. Outbreaks were most common in dairy products (12 outbreaks or 32%) and ground beef (10 outbreaks or 26%). Ground turkey appeared as a vehicle for the first time with two outbreaks in 2011. Resistance patterns were determined for 34 outbreaks. Bacteria were resistant to 15 different antibiotics and to one class of antibiotics. Of these, nine were classified by the World Health Organization as critically important in human medicine. The number of outbreaks increased in recent years, with 45% occurring from 2000 to 2011. ***Conclusion****:* The human health consequences of antibiotic-resistant pathogens are illnesses that may be of longer duration, more often lead to bloodstream infections, require increased hospitalization, and result in increased mortality. These pathogens are appearing in the U.S. food supply and are increasingly associated with foodborne outbreaks.

S4:6

ACCESSING THE MOLECULAR BASIS OF TRANSFERABLE QUINOLONE RESISTANCE IN *ESCHERICHIA COLI* AND *SALMONELLA* SPP FROM FOOD-PRODUCING ANIMALS AND PRODUCTS

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Background: *Salmonella* and *Escherichia coli* resistant to quinolones frequently arise in animals, being easily transferred to humans through the food chain, which can ultimately lead to the development of untreatable infectious diseases. The aim of the present study was to investigate the presence of PMQR determinants among *Salmonella* spp and *E. coli* from food-producing animals and derivative food products. ***Methods:*** *Salmonella* spp (n=183) and *E. coli* (n=182) isolates were collected from food-producing animals (n=274) and derivative food products (n=91). Antimicrobial susceptibility testing was performed by standard disk diffusion method, according to the CA-SFM veterinary guidelines. PCR and sequencing were used to detect PMQR- (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6')-Ib-cr*, and *qepA*) and β-lactamase-encoding genes (bla_{TEM}, bla_{SHV}, bla_{OXA} and *ampC*) and to examine the QRDR of *gyrA*, *gyrB*, *parC* and *parE* genes in PMQR positive isolates. Plasmid characterization was accessed by conjugation followed by replicon-typing. Genetic relatedness of PMQR positive *E. coli* was examined by MLST and *Salmonella* isolates were serotyped according to the Kauffmann-White scheme. Mobile genetic elements were also investigated through PCR mapping assays. ***Results:*** Overall, 4.7% (17/365) harbored Qnr-encoding genes from *qrnB* and *qnrS* families, specifically *qnrB2* (n=3), *qrnB19* (n=3), and *qnrS1* (n=11). All but one isolate presented at least one mutation in QRDR region of genes *gyrA*, *parC* or *parE* genes. 35.3% of Qnr-producing isolates presented resistance to β-lactam antibiotics that were justified by the presence of β-lactamases from TEM (TEM-1, n=10; and TEM-135, n=1) and SHV (SHV-108, n=1) families in QnrB19- and QnrS1-harbouring isolates. All but one Qnr-producing isolates were positively typed by replicon-typing, varying among IncN (n=2), IncFIB (n=11), IncFIC (n=3), IncI1 (n=2), IncHI2 (n=5), IncY (n=1) and IncL/M (n=3) and were, mostly, genetic unrelated. Qnr genes were detected nearby several mobile elements like IS*Ecl2*, IS*26* and ISCR*1*. ***Conclusions:*** This study illustrated the existence of Qnr-producing *E. coli* and *Salmonella* from food-producing animals, associated to specific mobile elements that can mediate their transference between species and among distinct settings. Epidemiology of PMQR mechanisms and the dissemination of plasmids carrying Qnr-encoding genes in veterinary isolates can compromise the efficacy of fluroquinolone treatments in both animals and humans.

S4:7

GENETIC RELATIONSHIP AND ANTIMICROBIAL SUSCEPTIBILITY AMONG ESCHERICHIA COLI ISOLATES FROM HUMANS, POULTRY AND STREET FOODS IN GHANA

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Introduction: Reports from WHO indicate that majority of those who suffer annually from foodborne related diseases are from developing countries, especially from sub-Saharan Africa. However, there is little information in some Sub-Saharan African countries to help curb these preventable foodborne related diseases. **Purpose:** The purpose of this research was to characterize Escherichia coli isolated from human, poultry and street foods and check their genetic relatedness by PFGE. To determine their pathotype and anti-

biotic susceptibility patterns. **Methods:** Food samples were bought from 25 (49 samples) Street food vendors and 30 poultry cloacal Swabs were taken from 5 different commercial poultry farms from Tamale, the Northern Regional Capital. Fecal samples from 91 patients submitted to the laboratory section of the Tamale Teaching Hospital were also analyzed for this research. E. coli was isolated on MacConkey agar plates and verified with PCR by amplifying the beta-glucuronidase genes. The PFGE was done by using XbaI digested DNA whiles the phylogenetic groups were derived by a triplex PCR. **Results:** Eighty-nine, thirty and fifteen E. coli were identified from Human, Poultry and Street foods respectively. Forty-six percent (46%) of the human isolates belonged to group A, 33% to B1, 8% to B2 and 13% to D. Most of the strains from food (87%) belonged to group A while 13% belonged to group B1. The E. coli strains from poultry belonged to group A (53%), group B1 (33%), group D (10%), and group B2 (3%). From all the isolates only two human isolates were resistant to third generation cephalosporins. Both isolates possessed blaTEM-1 and blaCTX-M-15. While one isolate further bore a dfrA1 gene, the other isolate possessed additionally, a qnrS1 gene. **Conclusions:** These results showed that there was fecal contamination of some street foods in the Northern Region of Ghana. Food vendors in the region must be educated rigorously on basic principles of food safety. Most of the E. coli strains isolated from human, poultry and food belonged to the commensal or intra-intestinal phylogenetic group A. There was a risk of transmission of virulent strains (group D and B2) of E. coli to humans through food and poultry products if proper handling, slaughtering, and sanitary procedures are not observed by food vendors and slaughterhouses or during egg collection and processing for human consumption. Some isolates of E. coli from the three origins were possibly related genetically hence a possible circulation of genetically related E. coli strains among human, poultry and food.

S4:8

PHENOTYPIC AND GENETIC CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA* SEROVARS ISOLATED FROM RETAIL MEATS IN ALBERTA, CANADA

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Emergence of multidrug-resistant strains of *Salmonella* in meat has been largely attributed to the use of antimicrobials in livestock production. Such strains can transfer to meat including poultry meat during slaughter and processing. The objectives of this study were to: determine the prevalence of *Salmonella* serovars, antimicrobial resistance (AMR) and resistance genes in *Salmonella* from retail meat purchased in Alberta, Canada. In addition, this study assessed associations between phenotypic resistance and the presence of genetic resistance determinants by meat type using simple logistic regression models where each predictor and the outcome were looked at individually. Following protocols of Canadian Integrated Program for Antimicrobial Resistance Surveillance, samples were collected weekly over one year period (May 2007-April 2008) from stores in 19 census divisions in Alberta. A total of 564 raw samples consisting of chicken (n=206), turkey (n=91), ground beef (n=134) and pork (n=133) were purchased. *Salmonella* were recovered from chicken (40% of samples), turkey (27%) and pork (2%) but not from ground beef. A total of 21, 8, and 3 serovars were recovered from chicken, turkey and pork, respectively. *Salmonella* Hadar was

most prevalent in chicken whereas *S*. Heidelberg was the most prevalent serovar in turkey meat. Overall 29% (32/110) of isolates were susceptible to all 15 antimicrobials. Resistance to ciprofloxacin, amikacin and nalidixic acid was not observed. Multidrug-resistance (≥2 antimicrobials) was found in 56% of isolates. Resistance to amoxicillin-clavulanic acid (AMC), ceftiofur (TIO), and ceftriaxone (CRO) was found in about 21% of chicken and 25% of turkey isolates. Resistance to either of tetracycline (TET), streptomycin (STR) or ampicillin (AMP) was associated with *S*. Hadar. Resistance to either of TET, AMP, AMC, TIO, CRO or cefoxitin was associated with *S*. Heidelberg. The most common resistance genes were *strA/B* (42% isolates), *tet*(A) (28%), bla_{CMY-2} (21%) and bla_{TEM} (17%). The bla_{CMY-2} and bla_{TEM} genes were associated with *S*. Heidelberg; *tet*(A) and *strA/B* with *S*. Hadar and *tet*(B) gene with *S*. Kentucky. The *strA/B* genes were not associated with *S*. Heidelberg. In conclusion, the prevalence of *Salmonella* was higher in poultry meats relative to other meats with some serovars showing higher prevalence of resistance to certain antimicrobials. In general, statistical associations observed among many of the pair-wise combinations of resistance genes of interest in this study suggests potential linkage due to presence on the same genetic elements.

S4:9

ANTIMICROBIAL RESISTANCE AND VIRULENCE GENES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM ABATTOIR PIGS AND THEIR HORIZONTAL TRANSFER

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Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) found in food producing animals is a major public health concern. Transmission to humans has been reported and LA-MRSA represents a reservoir of antimicrobial resistance genes. Data on antimicrobial resistance in LA-MRSA in Canada are scarce and little is known on their virulence genes. This study was first conducted to determine antimicrobial resistance and virulence genes of MRSA from abattoir pigs in Canada. We then hypothesized that some of these genes could be horizontally transferred to MRSA of human origin and thus enhance its antimicrobial resistance and possibly its virulence. A total of 107 MRSA strains were recovered from a previous study performed on pigs from two slaughterhouses located in the province of Quebec, Canada. Antimicrobial susceptibilities were determined by broth microdilutions. All MRSA isolates were susceptible to ciprofloxacin, gatifloxacin, gentamicin, levofloxacin, linezolid, quinupristin/dalfopristin, rifampin, streptomycin, trimethoprim/sulfamethoxazole and vancomycin. Antimicrobial resistance was observed toward clindamycin (29%), daptomycin (0.9%), erythromycin (29%) and tetracycline (98.1%). A subset of MRSA strains of porcine origin (n=24) was selected for further characterization using a StaphyType array of CLONDIAG. Most strains were ST398-MRSA-V and harboured several antimicrobial resistance genes such as *erm*(A), *tet*(K), and *tet*(M), and virulence genes encoding for staphylococcal leukocidins, hemolysins, proteases, capsule/biofilm, adhesion, immunoevasion, and superantigens. Transferability of these genes to MRSA of human origin was assessed using filter mating. Microarray results demonstrated that transconjugant T68 which was obtained from donor MRSA-81 of porcine origin with a recipient MRSA of human origin had received 21 genes including antimicrobial resistances

genes (*erm*(A), *tet*(M) and *qac*C), and virulence genes encoding for proteases, adhesion, immunoevasion, and for staphylococcal superantigen-like proteins. Together, these results indicate that MRSA strains originating from healthy pig carriers can harbour virulence and antimicrobial resistant genes and have the potential to transfer some of their genes to MRSA of human origin.

S5:2

ACCELERATED BIODEGRADATION OF VETERINARY ANTIBIOTICS IN AGRICULTURAL SOIL FOLLOWING LONG-TERM EXPOSURE, AND ISOLATION OF A SULFONAMIDE-DEGRADING MICROBACTERIUM SP.

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The use of antibiotics as growth promoting agents in livestock production contributes to the increasingly worrisome development of antibiotic resistance. In order to evaluate the long term impacts of antibiotic exposure on soil microbial populations, a series of field plots were established in 1999 that have since received annual applications of a mixture of sulfamethazine, tylosin and chlortetracycline at concentrations (0, 0.1, 1.0 and 10 mg/kg soil) bracketing that which would result from an annual application of manure from medicated swine. Following ten annual applications, the fate of the drugs in the soil was evaluated. Residues of sulfamethazine and tylosin, but not chlortetracycline were removed much more rapidly in soil with a history of exposure to the drugs than in untreated control soil. Residues of ^{14}C-sulfamethazine were rapidly and thoroughly mineralized to $^{14}CO_2$ in the historically treated soils, but not at all in the untreated soil. Sulfamethazine-degrading enrichment cultures yielded a *Microbacterium* sp. isolate. The bacterium degraded the sulfonamide antibiotics sulfamethazine, sulfapyridine and sulfamethoxazole but not sulfisoxazole. Plate counts of 'total' soil bacteria in the presence and absence of sulfamethazine detected no treatment effect on the abundance of bacteria resistant to this antibiotic. Overall, these results indicate that soil bacteria adapt to long term exposure to some veterinary antibiotics resulting in sharply reduced persistence. Accelerated biodegradation of antibiotics in matrices exposed to agricultural, wastewater, or pharmaceutical manufacturing effluents would attenuate environmental exposure to antibiotics, and merits investigation in the context of assessing potential risks of antibiotic resistance development in environmental matrices.

S5:3

PERSISTENCE AND DISSEMINATION OF THE MULTIPLE-ANTIBIOTIC-RESISTANCE PLASMID PB10 IN COMPLEX ENVIRONMENTAL COMMUNITIES

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Antibiotic resistance gene dissemination has long been recognized to be mediated by mobile genetic elements such as plasmids. Nevertheless, studying the dissemination of such elements in complex environmental matrices remains difficult because suitable molecular tools are not yet available to back up standard culture-dependent approaches. Recently, we developed a quantitative PCR approach to monitor the fate of particular plasmids in the vastness of environmental microbial communities maintained in microcosms. Basically, this molecular approach relies on the use of two sets of highly specific PCR primer/probe designs allowing the relative quantification of the bacterial host and plasmid DNAs used to inoculate the microcosms. The dissemination of the plasmid appears then as an increasing plasmid to donor ratio in total DNA extracted from the metagenome of microbial communities. This

approach was used to monitor the dissemination of the model plasmid pB10 in microbial communities sampled in various environments (wastewater treatment sludge, sediments, manure, etc.) and maintained in microcosms under different conditions. In wastewater treatment sludge, pB10 did not persist because of an apparent loss of the donor bacteria. If the persistence of the donor bacteria in wastewater sludge was noticeably increased by the lack of aeration or by amending antibiotics at subinhibitory concentrations, it did not stimulate the dissemination of pB10. The origin and the composition of the microbial community associated to given environments seemed to prevail over the persistence of the donor bacteria to promote the dissemination of pB10, which apparently occurred with an increased efficiency in spatially organized communities. Additionally, recent experiments showed that the eukaryotic microbial fraction of the communities also influence the dissemination of pB10, bringing out the role of protozoa predation in gene transfer. Altogether, our results tend to suggest that plasmid dissemination is controlled by a complex network of parameters, among which the influence of plasmids from the same family already present in the communities will be discussed.

S5:4

HIGH CARRIAGE RATES OF ESBL-PRODUCING ESCHERICHIA COLI IN WILD BIRDS FROM THE REMOTE MONGOLIAN GOBI DESERT

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Reports about the occurrence of Extended-spectrum beta-Lactamases (ESBL)-producing E. coli in the feces of wild birds in remote, uninhabited parts of the world have raised many questions towards a possible link to the global antimicrobial resistance situation. We therefore screened avian fecal E. coli isolates (i) from remote areas in Mongolia and (ii) from an intensively agriculturally used area in Germany for ESBL production and performed genotypic characterization of such strains in a second step. For that, cloacal swabs (Germany n=171, Mongolia n=110), mostly taken from juvenile birds, were screened. Among 65 German and 43 Mongolian E. coli isolates phenotypic ESBL-production was confirmed in 13.8% (n=9) and 11.6 % (n=5), respectively. Avian ESBL-producing E. coli from the different locales generally differed in their phylogenetic background and types of ESBL enzymes, which might be simply due to the fact that the investigated hosts represent separate populations which are not connected by migration. Although CTX-M was identified as predominant ESBL type, blaCTX-M-1 was present in all German strains, whereas 80% of the Mongolian strains harbored a gene of the blaCTX-M-9-group. Structure analysis based on MLST data was performed and strains belonging to all ancestral groups could be identified (Mongolia: A n=1, ABD n=3, B2 n=1; Germany: A n=5, AxB1 n=1, B1 n=1, D n=1, B2 n=1). Genotyping of virulence associated genes led to the frequent detection of genes associated with extraintestinal pathogenicity whereas genes associated with intestinal pathogenic E. coli such as Shiga-toxin genes stx1 /2 were not detected. Sequence types (STs) determined for the Mongolian isolates, including ST167 (ancestral group A; n=1) and ST648 (hybrid group ABD; n=2) are well known representatives of ESBL-E. coli in the medical field. Also among the German isolates we identified phylotypes, such as ST12 (ancestral group B2, n=1) and ST744 (ancestral group A, n=4) which have already been observed in human clinical samples. Macrorestriction patterns generated

by PFGE revealed a clonal relatedness of Mongolian strains to animal and human clinical isolates from Europe, indicating the possible global and interspecies dissemination of multiresistant strains. Irrespective of their origin of sampling we detected comparable high fecal carriage rates of ESBL-E. coli in wild avian hosts. These findings underline, that there are still unknown factors apart from the influence of the civilized world which might have an influence on the spread of multiresistant strains into the environment, even in the absence of antimicrobial selection pressure. A better understanding of this phenomenon is crucial for any control programs that aim to restrict the spread of resistances in microorganisms.

S5:5

TRANSFER OF RESISTANCE FROM PIG MANURE TO AGRICULTURAL FIELDS - A FIELD STUDY

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The usage of antibiotics in veterinary farming leads to increased antimicrobial resistance in the animal bacterial flora. If manure is used for soil fertilization, this resistance might spread to the environment, namely agricultural soil and adjacent ditches. Microcosm studies have shown that manure indeed enhances the level of resistance genes in agricultural soils above the background level. We conducted a field study in agricultural soils fertilized with pig slurry, in order to confirm effects of manure application under normal agricultural practice. Four farms were chosen, together with reference sites located in close proximity, but without manure application. The influence of manure application could clearly be shown for the tetracycline and sulfonamide resistance genes tet(M) and sul(2), in accordance with the application of these antibiotics at the farms, and the presence of antibiotic residues in manure and soil. Effects were significant both for short-time (4 weeks) and long-time (1 year) impact. In contrast, no increase in resistance was visible in isolates of oligotrophic bacteria, showing that resistance transfer to these soil bacteria did not occur on a large scale. In analyses of resistance in ditches adjacent to agricultural fields, the effects depended on the gene identity. While the diversity of resistance genes was generally higher in ditches located in regions with intensive pig farming (characterized by a high intensity of antibiotic usage in the Netherlands), studies at one particular location showed that some antibiotic resistance genes might be associated with run-off from manured fields, while others seem to reflect different sources. In conclusion, we showed that an increase of tetracycline- and sulfonamide resistance was detected at field scale on farms working in accordance with normal agricultural practice.

S5:6

MULTI-RESISTANT AND ESBL-PRODUCING *E. COLI* IN DISCHARGED WASTEWATER AND DUTCH SURFACE WATERS

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Background: Bacteria that have acquired resistance in humans and animals enter the environment, e.g. through discharge of untreated or partially treated wastewater from hospitals, farms and slaughterhouses, sewage overflows during heavy rainfall, or run off of animal droppings and manure. People may get exposed to these bacteria for example when recreating in contaminated surface water. The aim of the current research was to determine the prevalence of multidrug-resistant and ESBL-producing *E. coli* in discharged wastewater and Dutch surface waters. **Methods:** *Escherichia coli* was isolated from surface water (large rivers Meuse, Rhine and New Meuse, as well as three official recreation waters) and from wastewater. Wastewater was derived from two hospitals and a nursing home

discharging to the public sewer network, from wastewater treatment plants (WWTPs) receiving this sewage, as well as from wastewater from an international airport. Isolates were screened for their susceptibility to 8 to 9 antimicrobials. Wastewater and recreation waters were additionally screened for the specific presence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* using selective medium. **Results:** In untreated wastewater from health care centers, 9 - 19% of *E. coli* were ESBL-producers, compared with 3% in airport wastewater. By contrast, influents of regional WWTPs contained 0.1% and 1% ESBL-producing *E. coli*, and effluents 0.3% and 0.8%. The concentration of ESBL-producing *E. coli* in discharged wastewaters ranged from $7x10^2$ - $5,4x10^3$ colony forming units (cfu) per liter. Taking into account the capacities of the individual WWTPs, this translates into approximately 10^{10} ESBL-producing *E. coli* discharged to surface water per WWTP per day. Fourteen percent of all ESBL-producing wastewater isolates were resistant to all 9 antimicrobials tested. In large rivers, 32 - 48% of *E. coli* were resistant to one or more antimicrobials and 10% of all *E. coli* were resistant to 5 or more antimicrobials. In the river Meuse, ESBL-producing *E. coli* were detected in 4 out of 13 4-weekly samples. In these 4 samples, percentages ESBL-producing *E.coli* ranged from 13-22% of all *E. coli* with concentrations ranging from $1x10^4$ to $1x10^5$ cfu/l. In all three official recreation waters, ESBL-producing *E. coli* were detected at multiple sampling time-points (3 to 5 out of 5) . The percentage of ESBL-producing *E. coli* in these waters was 0.03 to 0.83%, and concentrations ranged from 1.3 to 146 cfu/l. **Discussion and Conclusions:** Multi-drugresistent and ESBL-producing *E. coli* were detected in discharged wastewater and surface waters. Also in official recreation waters ESBL-producing *E. coli* were detected, albeit in lower concentrations than in the large rivers. The risk of human exposure to these AMR and ESBL-producing bacteria needs to be evaluated.

S5:7

BETA-LACTAM RESISTANCE IN STAPHYLOCOCCI ISOLATED FROM CAPTIVE AND WILD WALLABIES IN SOUTH AUSTRALIA

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Background: The majority of studies conducted to assess the antibiotic susceptibility of staphylococci recovered from domestic pets, livestock or wildlife are from animals brought into veterinary clinics for treatment. This represents a bias in the reported prevalence rates of antibiotic resistant staphylococci in animals. Few studies have examined the presence of antibiotic resistant staphylococci in captive vs wild animals from different localities with varying levels of human contact. The objective of this study was to assess the antibiotic susceptibility of commensal staphylococci species isolated from healthy captive and wild wallabies from various localities in South Australia. **Methods:** One nasal swab per animal was obtained from 118 wallabies (68 captive, 50 wild) during routine health examinations from June 2008 to October 2010. Species included: Yellow footed rock wallaby (*Petrogale xanthopus*; n=28), Black-flanked rock-wallaby (*Petrogale lateralis*; n=66) and Tammar wallaby (*Macropus eugenii*; n=24) Nasal swabs were cultured on Staphylococcus medium number 110 and oxacillin resistance screening agar. Isolates typical of staphylococci were subject to Gram staining and coagulase tests. Staphylococci isolates were tested for their antibiotic susceptibility to a panel of 10 agents from 7 families using the disc diffusion method. **Results:** Staphylococci were isolated from 87 wallabies (Yellow footed rock wallaby, n=24, 86%; Black-flanked rock-wallaby, n=50, 69%; and Tammar wallaby, n=13, 52%). A total of 104 out of 174 Gram positive cocci isolates showed

phenotypic beta-lactam resistance, of which 83 (20 and 63 isolates from wild and captive population respectively) exhibited phenotypic oxacillin resistance and 27 isolates were found to be multidrug resistant. From 68 captive wallabies, 80 out of 119 (66%) staphylococci isolates were found to be resistant towards at least one beta-lactam antibiotic. These 80 isolates arose from 43 (63%) captive wallabies. In contrast, 24 out of 55 (44%) staphylococci isolates recovered from 50 wild wallabies were resistant towards at least one beta-lactam antibiotic. These 24 isolates were isolated from 17 (34%) wild wallabies. **Discussion and Conclusions:** The proportion of antibiotic resistant staphylococci recovered is expected to be directly related to the amount of human contact encountered. In line with this, it was found that 60% (104 out of 174) of isolates were resistant towards at least one beta-lactam with 80% (83 out of 104) of these isolates exhibiting oxacillin resistance. Of the oxacillin resistant isolates, 24% (20 out of 83) were from wild populations and 76% (63 out of 83) were from captive populations. Given the emergence and importance of community acquired methicillin resistant staphylococci it is critical that we are able to limit the potential for the development of antibiotic resistant bacterial reservoirs in wildlife.

S5:8

RESERVOIRS OF ANTIBIOTIC RESISTANT AND MULTI-RESISTANT ENVIRONMENTAL BACTERIA IN THE NORTH ITALIAN ADRIATIC COASTS

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Among the main coastal areas used for fish farming in Italy there is the North Adriatic region near the foces of large rivers and the area close to Gargano. Several marine autochthonous species, such as *Vibrio parahaemolyticus*, *Vibrio anguillarum*, *Photobacterium damselae* and *Aeromonas salmonicida* among others, are known to induce severe infections in aquaculture livestock. To treat or avoid these infections, antibiotics are increasingly used in fish farms. In Italy, the use of antibiotics amoxycillin, tetracycline and oxytetracycline, flumequine, trimethoprim and the association of trimethoprin with sulfadiazide is allowed by the current legislation. Marine bacteria exposed to antibiotics within the fish farming environment can acquire antimicrobial resistance by mobile genetic elements and horizontal gene transfer. A collection of 872 autochthonous marine bacterial strains was obtained from water, sediment, biofilm and fish samples withdrawn from four different fish farms located in northern and southern Italian Adriatic coast during different sampling campaigns. The bacterial isolates included mostly *Vibrio* strains but some other isolates were presumptively identified as *Aeromonas spp* and *Photobacterium*. All the strains were tested for their susceptibility to oxytetracycline/tetracycline (TET), flumequine (FL), trimethoprim (TIM) and the association trimethoprim-sulfadiazine (TIM+SUL). Resistance to only tetracycline was the most frequent event (147 strains, 17%) followed by resistance to the association trimethoprim-sulfadiazine (7%). Only few strains (17 out of 872, ca 2%) were resistant to only trimethoprim while as concern flumequine only 0.3% of the strains resulted resistant to this quinolone. Comparing strains isolated from coastal areas and fish farms, it has been revealed a significant higher incidence (4% versus 10%) of multi-resistant strains among those from aquaculture centers. Averaged MICs for multiresistant strains were 32-128 ug/ml for TET, 64 ug/ml for TIM and 2-8 ug/ml for FLU. Of the 115 multiresistant strains isolated, about 25% carried the integrase gene of the class1 integron, a mobile genetic element circulating also in clinical settings. Important differences in antibiotic resistance (AR) incidence were detected in the four fish farms: it is possible that the different types of

environmental areas, the specific farm management system and the use more or less frequent of antibiotics have influenced the emergence of AR bacteria. Although a high incidence of antibiotic resistance was found in sediments and biofilm, in most cases is the water coming out from the fish farm, the source where we obtained the highest percentage of resistant bacteria. The AR and multiresistant strains isolated constitute an environmental reservoir directly involved in the seafood chain and migh represent both a risk for human health and a public health concern.

S5:9

ANTIMICROBIAL RESISTANCE AMONG PATHOGENS AND INDICATOR ORGANISMS FROM RAW SHRIMP PURCHASED IN CANADA.

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Shrimp aquaculture has been a steadily growing industry in which antimicrobials are used for the treatment and prevention of disease; however, regulations and indications for use differ among the producing/importing countries. The potential for antimicrobial resistance and transferrable resistance genes to emerge in aquatic zoonotic and indicator/reservoir bacteria poses a concern to public health. Since 2008 the retail component of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) has incorporated a pilot retail surveillance project of domestic and imported raw fish and shellfish. The objective of this study was to describe the prevalence of antimicrobial resistance and resistance determinants of pathogens (Salmonella and Vibrio) and indicator organisms (E. coli and Aeromonas) cultured from retail raw shrimp. Epidemiological information, including the product of origin was captured for each sample. Each bacterium was recovered using standard isolation protocols and MICs were determined by broth microdilution using the Sensititre™ automated microbiology system. An aquatic and the CMV2AGNF panels were used for Vibrio and Aeromonas, and the National Antimicrobial Montioring System (NARMS) susceptibility panel plates, CMV1AGNF and CMV2AGNF, were used for Salmonella and E. coli. In addition, Salmonella were serotyped and phagetyped. Specifically selected E. coli isolates were also subject to testing for a panel of β-lactamase, carbapenamase and quinolone resistance genes. The origin of raw shrimp available in Canada was distributed among 13 countries, with the region of Southeast Asia a predominant supplier. Twelve serotypes of Salmonella were recovered from 3% of 689 shrimp and originated from five countries in the Southeast Asia (Bangladesh, India, Indonesia, Thailand, Vietnam). Resistance was rare in Salmonella; only one isolate was resistant (to sulfisoxazole). Fifty percent of samples were positive for Vibrio spp. with 30% of isolates tested on the CMV2AGNF plate showing resistance to amoxicillin clavulanic acid and cefoxitin. No resistance to 3rd generation cephalosporins was observed. Generic E. coli was recovered from 37% of shrimp; resistance to ciprofloxacin and nalidixic acid was found in 4% and 7% of E. coli, respectively. E. coli isolates resistant to ≥2 antimicrobials were found in 21% of shrimp. Plasmid-mediated β-lactamase resistance genes (blaCMY, blaCTX-M, blaOXA-1, blaPSE-1, blaSHV, blaTEM) and quinolone resistance genes (qnrB, qnrS) were found in E. coli, most with phenotypic multi-drug resistance patterns and all from the Southeast Asian region. As in other food animal commodities, awareness of the use of antimicrobials in the aquaculture industries and presence of pathogens the raw food products present important intervention points for the prevention of dissemination of resistance in the human food chain.

S6:1

ECOLOGY OR EPIDEMIOLOGY: THE ESSENTIAL METHOD FOR CONTROLLING ANTIMICROBIAL RESISTANCE IN FOODBORNE BACTERIA?

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The fields of molecular microbiology and evolutionary biology are clearly essential for explaining, respectively: 1) how bacteria can resist antimicrobials, and 2) how they have come to do so. While each of these two disciplines varies in the scope and scale of its considerations and arguments, explanations for resistance are often phrased in a language that invokes either the cellular mechanisms or adaptations that help to determine resistance in individual bacteria. Ecology and epidemiology, on the other hand, are disciplines for which the 'population' is foremost in consideration and both the language and methods therefore apply mostly to the group, and not the individual. While these latter two disciplines are numeric by nature, epidemiology tends to have as its focus the categorized status of the individuals that comprise its populations, whereas ecology tends to feature quantitative endpoints such as counts and densities. These differences are reflected in the language (or currency) of each discipline: 1) incidence or prevalence for epidemiology, and 2) concentration, carrying capacity, and thresholds for ecology. Geoffrey Rose (1985) illustrated the public health conundrum of the epidemiologist: explaining the causes of cases of disease versus explaining the causes of incidence of disease in a population. The former is clearly most useful for counseling an individual patient regarding risk factors they might avoid, the latter is useful for planning public health interventions at the population level to shift the overall burden of disease. Typically, the ecologist is focused more on the population outcomes and disease burden, albeit often in a more dynamic (time-dependent) manner than the epidemiologist.

An illustrative example of how the varying languages, methods, and interpretations of epidemiology and ecology can both complement and contradict one another is provided by examining the potential role of pre-harvest food safety interventions in reducing the on-farm burden of antimicrobial-resistant enteric bacteria in cattle. Using simulations of fecal flora under both equilibrium and disequilibrium populations, I will demonstrate across a wide range of bacterial concentrations how the use of relative change in prevalence (efficacy) of resistance can act to grossly over- or under-estimate the perceived effectiveness of the intervention in question. Using the same data, I will illustrate how a singular focus on $\log_{10}$ quantitative reductions of resistant bacteria (more the language of microbial ecology) may likewise be misleading. For example, interventions measured within either high or low concentrations of bacteria will be poorly represented using prevalence change alone. Instead, an approach to measuring intervention effectiveness by combining key aspects of both microbial ecology and epidemiology is justified and warranted.

S6:2

RESFINDER: DATABASE FOR IDENTIFICATION OF TRANSFERABLE ANTIMICROBIAL RESISTANCE GENES IN WHOLE GENOME SEQUENCED BACTERIA

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Background: Identification of antimicrobial resistance genes is important for understanding the underlying mechanisms and the epidemiology of antimicrobial resistance. As the costs of Whole Genome Sequencing (WGS) continue to decline, it becomes increasingly available in routine diagnostic laboratories and is anticipated to substitute traditional methods for resistance gene identification. However, the current challenge is how to extract the relevant information from the large amount of generated data. **Methods:** We developed a web-based method (ResFinder) that uses BLAST for identification of transferable antimicrobial resistance genes in whole genome data (an additional feature currently under development is resistance mediated by mutations in for instance housekeeping genes). As input, the method can use both pre-assembled complete or partial genomes and short sequence reads from four different sequencing platforms. **Results:** The method was evaluated on 1,862 Genbank files containing 1,411 different resistance genes. In all cases the method found the genes with a 100% match. The method was further tested on four to five isolates of five different species, where resistance phenotypes were also available. Almost complete agreement between *in silico* predictions and phenotypic testing was found. Furthermore, the method was evaluated on whole genome sequenced chromosomes and plasmids of 30 isolates. Seven of the 30 isolates were annotated to have antimicrobial resistance, and in all seven cases, these annotations were compatible with the ResFinder results. The rest of the isolates had not been tested for acquired antimicrobial resistance. **Conclusions:** The method provides a convenient way of identifying transferable antimicrobial resistance genes in completely sequenced isolates and is publically available online at www.cbs.dtu.dk/services/ResFinder. ResFinder is a further step in our development of bioinformatics tools for analyzing WGS data, which are specifically designed to be easy to use - also for investigators with limited bioinformatics experience. An online tool allowing identification of multi locus sequence types based on all currently available schemes was recently published (Larsen *et al*., 2012). Additional features under development include options for identification of virulence genes, species identification and phylogenetic analysis based on SNPs and pan-genome analysis.

S6:3

MULTIPLEX SEQUENCING OF ANTIMICROBIAL RESISTANCE GENES BY PGM

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Whole-genome shotgun sequencing is a powerful approach for obtaining complete genetic information of a bacterial pathogen. The constant improvement of sequencing technology offers different choices of platforms and makes possible comparative genomics analysis of multiple clinical isolates within a short period of time. Recently, semiconductor chip technology has been applied to develop a benchtop next-generation sequencer, the Ion PGM™. To establish protocols for long-term study of the evolution of antimicrobial resistant bacterial infection, we have conducted a pilot study demonstrating the possibility of multiplex sequencing of drug-resistant isolates on the PGM using the 316 chip. A total of 16 isolates were processed for plasmid DNA extraction and each was barcoded before semiconductor sequencing with the PGM. The generated sequences were compared to the public sequence databases and correlated with the antimicrobial phenotypes and molecular test results of known antimicrobial genes. In most cases, the genetic determinants for the antimicrobial resistance were correctly identified. We conclude that it is possible to collect antimicrobial resistance information through this approach and it should facilitate the rapid annotation of resistance information for newly sequenced strains of zoonotic bacteria and foodborne pathogens.

S6:4

ANTIBIOTIC AND HEAVY METAL RESISTANCE TRANSPOSONS DRIVING THE EVOLUTION OF INCHI2 PLASMIDS FROM SALMONELLA ENTERICA

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Conjugative plasmids play an important role in spreading and maintaining antibiotic resistance in human and animal pathogens. Some larger plasmids, such as IncHI2 plasmids, also carry determinants of resistance to heavy metals that may be advantageous in the environment or in animal rearing situations where heavy metals such as copper have been used as growth promoters. IncHI2 plasmids carrying antibiotic resistance genes appear to play an important role in resistance dissemination, and have been detected in several species of Gram negative bacteria and recovered from human clinical and animal-derived samples. However, in order to understand the epidemiology of resistance when it involves the spread of resistance plasmids, and to determine the relationship of plasmids from the two environments, discriminatory assays are needed. These need to be underpinned by detailed information on variation within the plasmid family. IncHI2 plasmids conferring resistance to multiple antibiotics recovered from Australian Salmonella enterica serovar Typhimurium isolates from cattle, and serovar Infantis isolates from chickens and domestic pets. The plasmids were recovered and their structures of were analysed using restriction mapping, an enhanced set of mapping PCRs, cloning and DNA sequencing. Plasmids from bovine, chicken, canine and feline isolates were related. All carried Tn10 and a mercuric ion and antibiotic resistance transposon related to Tn1696, with the 2 transposons located in the same positions in all cases. Resistance regions were in the same locations in two sequenced IncHI2 plasmids, R478 and pK29. Compared to R478, the original sequenced IncHI2 plasmid, two plasmids from bovine S. enterica were found to be largely the same but lack two segments that are present and close to one another in R478. These segments include determinants of resistance to arsenate and arsenite, and to copper and silver ions, respectively and the two plasmids did not confer resistance to arsenate or arsenite. The sequence of this region in one of the Australian plasmids revealed two previously unidentified transposons, one of 7.2 kb carrying an ars region and one of 32.4 kb carrying genes for resistance to copper and silver and transposition genes related to those of Tn7. The transposons are 9.4 kb apart in R478. PCRs were developed to detect this ancestral configuration. We conclude that IncHI2 plasmids have gained resistance to both heavy metals and to antibiotics via the acquisition of transposons. As more than one lineage, based on the presence of resistance transposons in different locations, is disseminating antibiotic resistance, assays that distinguish the lineages will assist in epidemiological studies.

S6:5

IDENTIFICATION AND CHARACTERIZATION OF THE INTEGRATIVE AND CONJUGATIVE ELEMENT ICE*PMU1* FROM BOVINE *PASTEURELLA MULTOCIDA* WHICH CARRIES AND TRANSFERS 12 RESISTANCE GENES

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Background: Multiresistant *Pasteurella multocida* isolates from bovine respiratory tract infections have been identified during recent years. These isolates were mostly plasmid-free, but exhibited resistance to many classes of commonly used antimicrobial agents. The aims of this study were to (i) identify the resistance genes present and (ii) to determine their

localisation, organisation and transferability. **Materials and Methods:** One representative *P. multocida* isolate was subjected to whole genome sequencing. Predicted coding sequences were compared with the SWISSProt/EMBL databases. PCR assays for the resistance genes and their linkage were developed. Conjugation by filter mating was conducted with *P. multocida*, *Mannheimia haemolytica* and *Escherichia coli* recipients. Transconjugants were tested for acquired resistance properties by determination of the minimum inhibitory concentrations (MICs). **Results:** An integrative and conjugative element, designated ICE*Pmu1* of 82,214 bp in size was found to be integrated into a chromosomal tRNALeu gene. This ICE harbored twelve resistance genes which confer resistance to streptomycin/spectinomycin (*aadA25*), streptomycin (*strA* and *strB*), gentamicin (*aadB2*), kanamycin/neomycin (*aphA1*), tetracycline [*tetR-tet*(H)], chloramphenicol/florfenicol (*floR*), sulfonamides (*sul2*), tilmicosin/clindamycin [*erm*(42)], tilmicosin/tulathromycin [*msr*(E)- *mph*(E)] or penicillins (bla_{OXA-2}). These resistance genes were organized in two regions of approximately 15.7 and 9.8 kb. Based on the sequences obtained, it is likely that plasmids, gene cassettes and insertion sequences (IS*26*, IS*Apl1* and IS*CR21*) have played a role in the development of these resistance gene regions. The ICE was transferred by conjugation into *P. multocida*, *M. haemolytica* and *E. coli*. PCR assays confirmed the presence and the organization of the resistance genes and also detected the intermediate circular form of the ICE which occurs only after excision from the donor genome and prior to integration into the recipient genome. MIC testing confirmed that the ICE*Pmu1*-associated resistance genes are expressed in different hosts. **Conclusions:** This is to the best of our knowledge the first description of an ICE in *P. multocida*. The observation underlines the risk of simultaneous acquisition of multiple resistance genes by *P. multocida* via a single horizontal gene transfer event. The spread of ICE*Pmu1* will limit dramatically the therapeutic options in bovine respiratory disease.

S6:6

CHARACTERIZATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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Livestock-associated Methicillin-resistant Staphylococcus aureus (LA-MRSA) Sequence Type 398 (ST398) constitute a large MRSA reservoir outside the hospital settings worldwide. The primary host for ST398 is pigs; however the sequence type has a broad host-spectrum and possesses the potential for cross-species transmission. The ability to spread from animals to humans and cause human infections makes this commensal a natural target for a comprehensive characterization. By using transposon mutagenesis, today's high-throughput sequencing technologies can be utilized in a combination with various functional assays and thereby identifying important bacterial characteristics. The aim of this study was to construct a transposon mutant library consisting of a high-complexity mutant pool. The mutant library will be used as a screening tool for identification of essential and/or advantageous genes under specific physiological conditions. A mariner transposon mutant library was generated in the genome sequenced MRSA ST398 SO385 isolate. The complexity of the mutant pool was verified by Linker PCR using a transposon insert specific forward primer and a linker specific reverse primer. Linker PCR was performed on the complete mutant pool and a number of randomly selected individual colonies. Plasmid cures were tested on selective plates. The mutant library was screened in In Vitro Organ Cultures (IVOCs) where the gDNA from input pools pre-inoculation and output pools post-inoculation were extracted. The gDNA will be sequenced using Transposon Directed Insertion-site Sequencing

(TraDIS) on an Illumina sequencing platform. The mutant composition in the input pools will be compared to mutants composing the output pools and via negative selection pinpointing genes that are either essential and/or advantageous in the physiological environment.

S6:7

HOST-DIRECTED ANTIMICROBIAL THERAPEUTICS FOR INTRACELLULAR BACTERIAL PATHOGENS

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Intracellular bacterial pathogens take advantage of host cell functions for survival and replication. We wish to test the hypothesis that interference with specific host cell functions can limit the ability of intracellular bacterial pathogens to cause disease. In order to do this we have taken two complementary approaches to interfere with host cell functions. The first approach is to screen libraries of small molecules with known, well-characterized mammalian targets. The other approach is to use shRNA constructs, delivered on lentiviral vectors to decrease the expression of specific human genes. We found that several commercially available FDA-approved compounds can limit intracellular replication of intracellular pathogens with varying lifestyles. These include: *L. pneumophila, C. burnetti, R. conorii* and *B. abortus*. Intracellular growth was monitored either by taking advantage of specific strains which express fluorescent proteins or by an indirect immunofluorescence assay, in the case of *R. conorii*. Examples of small molecules that limit the replication of more than one species include: Gleevec (*L. pneumophila* and *C. burnetii*); Trifluoperazine (*C. burnetii*, *R. conorii*, *B. abortus*); and Tamoxifen (*C. burnetii* and *B. abortus*). The mechanism(s) by which the compounds limit bacterial growth is under investigation. We have produced a small collection of cell lines derived from HEK293T and THP-1 that express shRNA that reduce the expression of specific targeted human genes. We are currently measuring the intracellular growth of the pathogens in these strains and constructing cell lines that represent a library consisting of 70,000 shRNA that cover the entire set of expressed genes of the human genome. Identification of specific shRNA that result in decreased intracellular replication will be detected using flow cytometry. The identity of the shRNA species will be based on individual bar-codes linked to each shRNA construct. Summary: Small molecules that target host cell functions and shRNA constructs that decrease specific genes can render cells less able to support intracellular growth of several bacterial pathogens. This suggests that targeting host cell functions may be a novel path to the development of antimicrobial therapeutics.

S6:8

LONGITUDINAL ANALYSIS OF ANTIBIOTIC RESISTANCE GENE QUANTITIES IN DAIRY CATTLE

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Most investigations of antibiotic resistance have relied on cultivation of bacterial isolates. Because most bacteria in a sample are non-cultivable, these studies may miss the potential reservoir of resistance that resides among the entire bacterial population of a sample. An alternative to cultivation-based methods is the use of molecular methods to quantify known resistance genes in community bacterial DNA that has been extracted in a cultivation independent manner. The objectives of this study were to use community DNA extraction and qPCR to measure the quantities of six antibiotic resistance genes in the feces of dairy cattle collected over 2.5 years. From September 2001 through March 2004, dairy cattle on four farms in Illinois were sampled. Each farm was visited nine times at approximately three month intervals. The four farms varied in size and management practices, including antibiotic usage. On each visit, fecal samples were collected from the same animals in the cohort,

and several calves aged 0-3 months were added to the cohort. Six resistance genes representing four antibiotic classes commonly used in animal agriculture were selected: *mefA*, *ermB*, *tetM*, *tetA*, *flo*, and bla_{CMY-2}. Mixed effects linear regression models were used with log gene quantities being predicted by age, farm, season, and sampling trip. A random intercept for animal and a random slope to account for repeated measurements within animal were included. Over the 2.5 years of the study, 455 fecal samples collected from 81 animals were analyzed. The mean log quantity of genes per gram of sample over time was consistently highest for *mefA* followed by *tetM* and *ermB*. The other three gene quantities were 1 - 3 orders of magnitude lower. Age was significantly associated with gene quantity for *tetM*, *ermB*, *tetA*, *flo*, and bla_{CMY-2} quantities, with animals less than 6 months of age having on average 0.70 - 2.34 more log gene copies than older animals. There was a significantly increasing trend in *mefA*, *tetA*, and bla_{CMY-2} log quantities over time. There was a seasonal trend for *tetM*, *tetA*, *flo*, and bla_{CMY-2}, with quantities lower in the winter than in the autumn. Three of these four genes (*tetA*, *flo*, bla_{CMY-2}) are often co-located on plasmids in *E. coli* isolated from cattle. The combination of qPCR with community DNA may be preferable to cultivation-based methods for evaluating the effect of selection pressures on bacterial populations. For genes that are widely dispersed and present in very high quantities, such as *mefA*, *tetM* and *erm*B, the quantitative measure provided by qPCR may be better able to identify differences among populations. For emerging resistances mediated by relatively rare genes such as bla_{CMY-2}, qPCR appears to be more sensitive than cultivation-based methods. In addition, the quantitative nature of qPCR makes it more suitable than cultivation based methods for monitoring trends in resistance over time.

S6:9

COLE1 PLASMID IN ANTIMICROBIAL RESISTANCE IN HUMAN AND ANIMAL BACTERIA

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We have recently shown that acquired multiresistance to antibiotics in *Pasteurella multocida* is mediated by coexistence of small plasmids. One of these plasmids, pB1000, has been also identified in clinical isolates of *Haemophilus influenzae* from Europe, USA, and Australia. pB1000 and further small ColE1 superfamily plasmids can stably cohabit in the same bacterium, giving rise to multiresistance phenotypes in *Pasteurellaceae*. We show here that this elegant strategy is not unique to *Pasteurellaceae* and is in contrast with the more common multiresistance acquisition mediated by large conjugative plasmids. The evolution of antimicrobial resistance and multiresistance mediated by small plasmids has been analyzed. Strains bearing several small cohabiting plasmids were evolved in vitro under different conditions of antimicrobial pressure, mimicking antibiotic therapy. To analyze the evolution of plasmids and strains, antimicrobial resistance levels and plasmid copy number by Q-PCR were determined at different time points. Interestingly, antimicrobial pressure induced an important increase in plasmid copy number. The rise affected all plasmid types in the cell, irrespectively of the antibiotic used, giving rise to an increase in the antimicrobial resistance levels. Further, when antimicrobial pressure was removed, strains recovered their original plasmid copy number and resistance levels. This novel phenomenon implies that antibiotics can orchestrate plasmid copy number and antimicrobial resistance levels. Finally, ColE1 plasmids have been sought in human and animal pathogens, and we show that these elements are widely-spread in clinical isolates of different origins.

S7:2

FARM TO FORK? DELIBERATIONS ON THE WHERE, WHAT, WHEN, WHO, WHY OF RESISTANCE MONITORING

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With continuing concerns about rising levels of antimicrobial resistance in certain bacteria and the negative impact that this could have on human and animal health, there is a need for a sound monitoring system in the U.S. Even with the general acknowledgement that such a system is desperately needed, a "standard" scientific framework for an epidemiologically-designed monitoring system remains elusive. Aside from the obvious budgetary concerns, the epidemiologic challenge is designing an antimicrobial resistance monitoring system that will be suitable for assessing temporal trends and for providing data that address the key goals of the program with minimal bias. This presentation will challenge participants to more closely examine the objectives and goals, epidemiologic approaches, and sampling strategies for an animal antimicrobial resistance monitoring system that are being currently discussed in the U.S. and internationally. Some of the key questions that must be addressed prior to implementing a monitoring system include: What points in the production system best measure the potential public health risk associated with antimicrobial resistant bacteria? What types of samples should be collected to best reflect this potential risk? Is slaughter house sampling a nexus for animal and public health risks? How should sample sizes be estimated, and if budget is a limiting factor, how should the sample sizes be adjusted? Which bacteria should be monitored? USDA, in collaboration with FDA, conducted an antimicrobial resistance monitoring pilot study for 4 months with university and USDA-ARS microbiologists and epidemiologists. This collaborative effort collected samples from beef and dairy cattle and poultry in different geographic regions and stages of production. After bacterial isolation was performed at collaborating institutions, antimicrobial resistance testing was completed at FDA's Center for Veterinary Medicine laboratory. Descriptive data will be presented. These data are being used to evaluate the addition of on farm and slaughter plant sampling to FDA's National Antimicrobial Resistance Monitoring System. Final discussions will include the challenge of developing an epidemiologic-based monitoring system in the U.S. while balancing scientific and political barriers and incentives.

S7:3

EUROPEAN ANTIMICROBIAL SUSCEPTIBILITY SURVEILLANCE IN ANIMALS (EASSA) PROGRAMME: RESULTS ON ENTERIC BACTERIA FROM HEALTHY BROILER CHICKENS AT PROCESSING

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Intro.: The potential transfer of antimicrobial resistance from enteric bacteria of food animals to humans is a concern. National surveillances of susceptibility in enteric bacteria are common but international surveys are rare. EASSA is the first program studying the antimicrobial susceptibility of zoonotic and commensal bacteria isolated from healthy poultry, pigs, and cattle across Europe. The survey includes standardized sampling and bacterial isolation procedures, and MIC determination to panels of human-use antibiotics (ABs). Here, we report the results for chickens. **Methods:** Caecal samples were randomly collected in 2002-2006 at various abattoirs across France, Germany, Netherlands, Spain, United Kingdom. Non-repetitive samples (usually 300/country) were sent to national laboratories for isolation, using uniform protocol, of *Escherichia coli, Enterococcus* spp., *Campylobacter* spp., and *Salmonella* spp. Agar dilution testing

following CLSI guidelines was conducted in a central lab. Clinical resistance was based on CLSI breakpoints (M100-S21; M45-A2); decreased susceptibility (DS) on epidemiological cut-off values (ECVs) defined by EFSA, i.e., MICs exceeding the wild-type MIC distribution (>ECV) but not the clinical breakpoint, was determined for at most 4 ABs. **Results:** Mean resistance (%) for *E. coli* (n=1562) was: ampicillin (A) 53.1; cefotaxime (CTX) 1.9; ciprofloxacin (CIP) 5.6; chloramphenicol (CA) 14.4; gentamicin (G) 3.5; tetracycline (TE) 67.3 and trimethoprim/sulfamethoxazole (TS) 48.2. No resistance to cefepime (CP) and 0.2% to colistin (CO) were observed. DS was 31.5% (CIP), 1.9% (CTX), 0.5% (A) and 0.3% (G). For *Salmonella* (n=182), mean resistance (%) was 12.6 (A), 0 (CP), 1.1 (CTX), 0 (CIP), 4.4 (CA), 27.5 (CO), 1.6 (G), 36.3 (nalidixic acid), 12.2% (sulfisoxazole), 13.2 (TE), and 4.9 (TS). DS was 35.2% for CIP, and absent for A, CTX and G. The low prevalence of *Salmonella* precluded a valid comparison among the countries. For *C. jejuni* (n =372), resistance rates to erythromycin (E), drug of first choice for *Campylobacter* infections, and G were < 2%, for CIP 32.5% and for TE 49.6%. DS to CIP was negligible; to E 2.7%. For *C. coli* (n =366), a much less frequent human pathogen, resistance was markedly higher for all ABs tested. All enterococci were susceptible to linezolid. Clinical resistance of *E. faecium* (n=657), the major *Enterococcus*, to A, G and vancomycin was < 3%, but resistance to quinupristin/dalfopristin was 31%; DS amounted to 26%. Recovery of *E. faecalis* was minimal (n=13). **Conclusion:** This pan-EU survey, based on standardized methods, shows that the prevalence of resistance among enteric bacteria in chickens varies for 'older' drugs and between countries. The results indicate a generally low prevalence of resistance to ABs that are commonly used to treat foodborne disease in humans. Using decreased susceptibility criteria, more variation was observed.

S7:4

INTEGRATED SURVEILLANCE FOR ANTIMICROBIAL RESISTANCE - PILOT STUDY IN KENYA

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Antimicrobial resistant foodborne pathogens including *Salmonella enterica, E. coli* and *Campylobacter* spp have been reported with increasing frequency as causes of food-borne illness in Kenya, and these infections also pose a public health problem of global significance. The objective of the study was to determine the prevalence and characteristics of antimicrobial resistant *Salmonella* spp and *Campylobacter* spp as important foodborne pathogens and investigated *E. coli* as important as possible reservoirs of antimicrobial resistance genes in farm animals brought for slaughter, retail meats sold to public and in children presenting with diarrhoea at nearby health centres. From a total of 3540 rectal swabs and meat samples, 2930 bacterial isolates from ; consisting of 2496 *E. coli* (45% from cattle, 22% from poultry, 5% from pigs, 28% from retail meat samples); 108 Salmonella spp (52 from pigs, 34 from cattle and 28 from poultry) were processed. A total of 320 (52% contamination) *Campylobacter* spp (87% were *C. jejuni*) from poultry were also examined. In addition 632 rectal swabs and stool samples from children with diarrhea from 3 hospitals and clinics around Nairobi and one in Kajiado 60km south of Nairobi, were processed. Susceptibility testing against 10 commonly used antibiotics showed that *E. coli* isolates from poultry had highest prevalence of resistance, most commonly to ampicillin (39%), cotrimoxazole (50%) and tetracycline (40%), and this was followed closely by resistance among the isolates from pigs. In general resistance in *E. coli* from

children with diarrhea showed the highest levels of resistance to commonly available antibiotics including ampicillin (62%), cotrimoxazole (48%), and tetracycline (37%), while 25% and 23% respectively were resistant to streptomycin and co-amoxiclav. Prevalence of resistance in *E. coli* against ampicillin and tetracycline were similar among poultry and pigs, and just slightly lower than levels observed among *E. coli* isolates from children. Salmonella isolates from cattle and chickens showed similar patterns of resistance as those seen among *E. coli* isolates, while Campylobater spp were only resistant to tetracycline (98%). Although rates of resistance to commonly used drugs especially ampicillin, co-amoxiclav, co-trimoxazole and streptomycin among isolates from animals was slightly lower than that seen in *E. coli* from children, the rates are significantly higher than those observed in similar studies in Kenya. We recommend implementation of a systematic National surveillance program for antimicrobial resistance in order to monitor effectiveness of currently available antimicrobials to inform policies aimed at curbing emergence and spread of resistance.

S8:1

MULTIRESISTANT *SALMONELLA* TYPHIMURIUM AND TYPHIURIUM-LIKE STRAINS IN ENGLAND AND WALES - AN INCREASING PROBLEM IN HUMANS AND FOOD ANIMALS

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BACKGROUND: Recent years have seen a decline in the level of antibiotic resistance in *Salmonella* Typhimurium, mainly due to a reduction in penta-resistant Typhimurium DT104. This has been counteracted in part by an increase in Typhimurium DT193 and its monophasic variant *S.* 4,[5],12:i:, both types expressing resistance to ampicillin, streptomycin, sulphonamides and tetracyclines (R-type ASSuT), first noted in foodborne infections and pigs but now also in cattle and poultry. In order to gain a clearer picture of the prevalence, diversity and antibiotic susceptibility of *S.* 4,[5],12:i:- in England and Wales, we characterized "Typhimurium-like" ("*S*Tm-like") DT193 and serologically-defined *S.* 4,[5],12:i:- received by the HPA *Salmonella* Reference Unit in 2010. **METHODS:** 609 "*S*Tm-like" DT193 isolates (identified as O:4, H:i by referral laboratory) and 142 serologically-defined *S.* 4,[5],12:i:- isolates were screened for the presence of *fljB* and *hin*. Susceptibility to 18 antibiotics was determined using national breakpoints. *S.* 4,[5],12:i:- isolates were also phage-typed and typed by multiple-locus variable-number tandem repeat analysis (MLVA). All isolates originated from humans, animals and food products. **RESULTS:** 76% of "*S*Tm-like" DT193 were *fljB-/hin-*, of which 70% were R-type ASSuT. Other common resistance patterns were ASSu (11% of isolates) and T (4% of isolates); 2% were fully susceptible. R-type ASSuT was also common among *S.* 4,[5],12:i:- of phage types DT120, U311and U323. Such isolates were also *fljB-/hin-* and formed part of the same MLVA clonal complex as *fljB-/hin-* DT193. Two *fljB-/hin-* DT193 isolates exhibited decreased susceptibility to ciprofloxacin (MICs 0.25-1 mg/L); five were resistant to cefotaxime (MICs >1 mg/L), of which two carried a CIT-type AmpC and three a group 1 CTX-M. **CONCLUSIONS:** Prevalence of R-type ASSuT among *S.* 4,[5],12:i:- is extremely high in England and Wales, as noted in other European countries. This study provides evidence that the emergence of *S.* 4,[5],12:i:- DT193 R-type ASSuT has contributed significantly to the increase in DT193 R-type ASSuT over the last decade. Identification of genetically-related *fljB-/hin-* R-type ASSuT isolates of other phage types suggests *fljB-/hin-* DT193 may be diversifying by undergoing phage type conversion. Although the occurrence of resistance to ciprofloxacin and cefotaxime was relatively low, monitoring should continue as severe infections with *S.* 4,[5],12:i:- have been reported.

S8:2

ANTIMICROBIAL RESISTANCE OF CAMPYLOBACTER SPECIES AND RISK FACTORS FOR CIPROFLOXACIN-RESISTANT *CAMPYLOBACTER JEJUNI* FROM HUMAN PATIENTS IN SASKATCHEWAN (1999-2006)

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Background: Ciprofloxacin (CIP) resistance in *Campylobacter jejuni* from animals, food and humans is a global public health concern as it may increase the burden of illness of human campylobacteriosis. There is a paucity of antimicrobial resistance information for cases of human *Campylobacter* infection in Canada. Saskatchewan is the only province in Canada to have tested a provincial collection of human *Campylobacter* species isolates obtained by passive surveillance. The objectives of this study were to describe the antimicrobial resistance of this provincial collection of isolates and to determine risk factors for infection with CIP-resistant *C. jejuni*. We hypothesized that: 1) there would be differences in resistance between *C. jejuni* and *C. coli*; and 2) socio-economic and agricultural risk factors would be associated with CIP-resistant *C. jejuni* infection**. Methods:** Speciation and minimum inhibitory concentrations (MICs) to eight antimicrobials were determined by E-test for 1378 human *Campylobacter* species isolates at the Saskatchewan Disease Control Laboratory from 1999 to 2006. Susceptible-intermediate-resistant categorization was completed using Canadian Integrated Program for Antimicrobial Resistance Surveillance *Campylobacter* MIC breakpoints. Logistic regression models compared the differences in resistance between *C. jejuni* (n=1200) and *C. coli* (n=129). Multilevel logistic regression models were used to identify significant risk factors (P≤0.05) using individual, contextual socio-economic and agricultural density factors. **Results:** *Campylobacter coli* had significantly higher CIP (15.5%), erythromycin (13.2%) and multidrug (2.3%) resistance compared to *C. jejuni* (9.4%, 0.5% and 0% respectively). The odds of having a CIP-resistant compared to susceptible infection were increased with: 1) winter infection compared to any other season; 2) being between 30-50 years of age compared to younger and older people; 3) residing in an urban compared to rural region; and 4) residing in a region with moderately high poultry density (40-60 birds/km^2) based on a continuous quadratic. **Discussion and Conclusions:** Human clinical *C. jejuni* isolates from Saskatchewan demonstrated resistance to multiple antimicrobials, but had significantly less fluoroquinolone, macrolide and multidrug resistance than *C. coli* isolates. People infected with *C. jejuni* in the winter or between 30-50 years of age may be more likely to be infected with a CIP-resistant strain during foreign travel. Residents living in an area of moderate poultry density had a higher risk of CIP-resistant infection, but this may represent other unmeasured risk factors as opposed to a direct link between poultry and resistant infection. Further study is required to understand these contextual socio-economic and agricultural risk factors for resistant infection.

S8:3

INCREASING RESISTANCE TO CIPROFLOXACIN IN MULTIDRUG RESISTANT SALMONELLA TYPHIMURIUM IN MEXICO

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Background: Nontyphoidal *Salmonella* is associated with a high diarrhea burden worldwide. Antimicrobial-resistant *Salmonella* has progressively risen during the last decades and is associated with increased hospitalization, systemic infections and mortality. We report six-year trends in *Salmonella* infections at a major referral hospital in southeast Mexico. **Methods:** The Hospital General O'Horan is a tertiary care university hospital that admits approximately 400 children annually for gastroenteritis. Active surveillance is conducted at the Oral Rehydration Unit and the Pediatric Emergency Services. All *Salmonella* isolates are routinely tested for susceptibility to 12 antimicrobials by disk diffusion; susceptibility to ciprofloxacin, azithromycin, ceftriaxone and furazolidone is performed by agar dilution. Methods and breakpoints are those recommended by the CLSI guidelines. Breakpoints for resistance to azithromycin and furazolidone are 64 µg/ml and 8 µg/ml, respectively. **Results:** From 2005 to 2011, 2358 children under 10 years of age were admitted for gastroenteritis. *Salmonella* gastroenteritis decreased from 17.7% in 2005 to 11.3% in 2011 (p=0.001). The top five serotypes remained constant throughout the study period; Typhimurium was consistently the most frequently isolated serotype (19-21%), followed by Agona (9%), Muenster (6-11%), Muenchen (6-7%) and Enteritidis (5-7%). Resistance to azithromycin and furazolidone remained stable, fluctuating between 2.6% to 7.5%, and 24% to 35%, respectively. Resistance to ampicillin decreased from 34% in 2005-2006 to 18% in 2010-2011 (p<0.001), and trimethoprim-sulfamethoxazole decreased from 39% to 27% (p=0.014). Ceftriaxone resistance, mainly present in serovar Typhimurium, decreased from 20% to 9% (p=0.009). Ceftriaxone resistance was also detected in serovars Anatum and Agona. In contrast, the percentage of isolates with ciprofloxacin MICs between 0.25 µg/ml and 1 µg/ml rose from 14% in 2005-2006 to 23% in 2010-2011. A ciprofloxacin non-susceptible (MIC=2 µg/ml) strain of *S.* Typhimurium emerged for the first time in 2011; the strain was also resistant to other clinically important antibiotics such as extended-spectrum cephalosporins, ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, azithromycin, gentamicin and furazolidone. **Conclusions:** *Salmonella* gastroenteritis, and the resistance of this pathogen to ampicillin, trimethoprim-sulfamethoxazole and ceftriaxone, has decreased over the last six years in southeast Mexico. Low-level resistance to ciprofloxacin is increasing, and non-susceptibility to this antimicrobial has been detected in MDR, cephalosporin resistant *S.* Typhimurium. Continuous monitoring of this newly emerging threat is warranted.

S8:4

VIM-1 CARBAPENEMASE CARRYING ESCHERICHIA COLI AND SALMONELLA ENTERICA FROM LIVESTOCK FARMS

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Carbapenems are critically important antimicrobials considered as drugs of last choice in clinical settings. During the last few years, the prevalence of resistance to these antimicrobial agents in Enterobacteriaceae has increased worldwide. Till recently, the presence of carbapenem non-susceptible Enterobacteria seemed to be mainly restricted to hospitals. However, carbapenemase-encoding genes were also detected in the community and environment, and very recently in livestock farms. The objective of the present study was the characterization of E. coli and Salmonella isolates producing carbapenemases collected in German livestock farms (swine and poultry) within a national ESBL-surveillance project (www.RESET-Verbund.de). The isolates were tested for their susceptibility to several antimicrobials including 17 b-lactams/b-lactamase inhibitors. The identification of ESBLs, AmpC and/or carbapenemases encoding genes was done by PCR/sequencing. The isolates were analysed

by MLST, XbaI-PFGE and S1-nuclease-PFGE plasmid-profiling. The bla-genes were mapped by Southern-blot hybridization. Mating and transformation experiments were carried out as well. Plasmids were characterized by replicon PCR-typing. Two E. coli and three Salmonella enterica isolated in three farms were resistant to 3rd generation cephalosporins, and showed zone diameters between 20-25 mm for imipenem, meropenem, and ertapenem. The isolates were positive for the blaACC-1 AmpC encoding gene, and the blaVIM-1 carbapenemase-gene. This gene was located in a class 1 integron together with accC4 (amikacin-kanamycin resistance, not detected phenotypically) and aadA1 (streptomycin-spectinomycin resistance). In the E. coli isolates, blaVIM-1, blaACC-1, and strA/B (streptomycin resistance) were located on a non-selftransferable 220 kb HI2 plasmid. In the Salmonella isolates, blaVIM-1 blaACC-1, strA/B, as well as catA (chloramphenicol) and a non-characterized gene conferring trimethoprim-resistance, were carried by a non-selftranferable 300 kb HI2 plasmid. These isolates are the first carbapenemase producing E. coli and Salmonella isolated from livestock described so far. The epidemiological importance of carbapemenase producers in livestock could be underestimated, as there are no data available about the prevalence of carbapenemase encoding genes in commensal or zoonotic bacteria. The transmission of these isolates via food in the community or hospital settings is of Public Health concern and deserves further surveillance.

S9:2

QUANTIFYING THE PROPORTION OF ANTIBIOTIC-RESISTANT EXTRAINTESTINAL INFECTIONS OF FOOD-ANIMAL ORIGIN: A PROGRESS REPORT

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Background: Although best known for diarrheal disease outbreaks, E. coli also causes millions of urinary tract infections (UTIs) and up to 40,000 deaths due to bloodstream infections (BSIs). While these infections are often community-acquired, the dominant sources of antibiotic-resistant extraintestinal pathogenic E. coli are largely unknown. There is increasing evidence linking antibiotic-resistant UTIs and BSIs with Escherichia coli from food animals; however, an accurate proportion of extraintestinal infections caused by foodborne exposures has yet to be measured. **Hypothesis:** We hypothesize that a quantifiable proportion of UTIs and BSIs are caused by foodborne E. coli. **Methods:** We are conducting a one-year study on E. coli from retail pork and poultry and from UTIs and BSIs in Flagstaff, AZ. Twice per month we are purchasing all available brands of pork, chicken, and turkey from all major grocery stores in Flagstaff, which are cultured for E. coli. Over the same period, we are collecting UTI and BSI E. coli isolates from outpatient clinics and the sole local hospital (Flagstaff Medical Center). We are characterizing all Isolates by antimicrobial susceptibility testing, followed by whole genome sequencing. We will infer the proportion of human UTIs and BSIs attributable to foodborne exposures by assessing the overlap in antibiotic-resistance genes, mobile resistance elements, strain identity, and temporal correlations among foodborne and human pathogenic E. coli. **Results:** We developed a custom sample tracking and data management system to evaluate temporal trends in real time and initiated sample collection in January 2012. We are collecting 400 to 500 foodborne and human E. coli isolates. We observe substantial overlap in phenotypic resistance profiles between foodborne and human E. coli isolates. We are in the process of evaluating phylogenetic relatedness by whole genome sequencing and anticipate sequencing approximately 5,000 E. coli genomes by the conclusion of the study. **Discussion:** Retail meat and poultry may be an important source of human exposure to antibiotic-resistant extraintestinal pathogenic

E. coli in the community. Quantifying the proportion of antibiotic-resistant extraintestinal infections arising from food animals will have important implications with regard to how and which antibiotics are utilized in food-animal production.

S9:3

HIGH FREQUENCY OF THE *VGA*(A) GENE AMONG MRSA ST398 FROM PIGS AND HUMANS IN PORTUGAL

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Introduction: The *vga* genes code for ABC transporters, which confer resistance to streptogramin A antibiotics, lincosamides and pleuromutilins. These genes have been previously described among methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 398 to be located on small or large plasmids. **Objective:** The aim of this study was to screen for the presence of *vga* genes among clindamycin-resistant MRSA ST398 from infected and colonized pigs, pig herds and humans in close contact with pigs. **Methods:** A total of twenty-eight MRSA ST398 isolates from various sources were included in the study: pigs with exsudative epidermitis (*n*=11), colonized pigs (*n*=6), dust samples from breeding pig herds (*n*=9) and humans in contact with pigs (*n*=2). Minimum inhibitory concentrations of clindamycin and erythromycin were determined by broth microdilution. The presence of the *vga*(A), *vga*(B) and *vga*(C) genes was screened by PCR. Plasmid DNA was prepared using standard protocols. Plasmids were transferred into *S. aureus* RN4220 by electrotransformation with subsequent selection of the transformants on clindamycin- (2 mg/L) or tiamulin-containing (10 mg/L) plates. Two of the plasmids had been previously sequenced and were designated pCPS32 and pCPS49. **Results:** Twenty-four MRSA ST398 isolates showed resistance to clindamycin (MIC of > 2 mg/L) and susceptibility to erythromycin (MIC of ≤ 0.5 mg/L). Eleven isolates were from infected pigs, 7 from dust samples, 5 from colonized pigs and only one from a colonized human in contact with pigs. All isolates harboured the *vga*(A) gene, except for two isolates from the environment. One isolate had the *vga*(C) gene and the other did not harbour any of the tested *vga* genes. In addition, one isolate resistant to clindamycin and erythromycin was also *vga*(A)-positive. Plasmid analysis showed the presence of at least two plasmids of sizes below 10 kb in all isolates. Transformants were obtained in 9 isolates: 8 carrying a 5.7 kb plasmid containing the *vga*(A) gene (including pCPS32) and one strain carrying a 5.3 kb plasmid (pCPS49) holding the *vga*(C) gene. **Conclusion:** This study identified a high number of *vga*(A) gene carriers among clindamycin-resistant MRSA ST398 isolates from Portugal. Additionally, this study confirmed that *vga* genes do not only occur on large plasmids, such as p6847J or pKKS825, but also on small resistance plasmids, such as pCPS32 and pCPS49, in MRSA ST398. Mobilization and recombination of such small plasmids with larger plasmids may occur and therefore enhance the dissemination of *vga* genes. The previous suggestion that exchange of *vga*(A)-carrying plasmid has occurred between humans and pigs in Portugal and the high frequency of these genes in pig-related Portuguese MRSA ST398 strains increases the concern for food safety and requires further surveillance.

S9:4

DOGS OF NOMADIC PASTORALISTS IN NORTHERN KENYA ARE RESERVOIRS OF PLASMID-MEDIATED CEPHALOSPORIN- AND QUINOLONE-RESISTANT *ESCHERICHIA COLI* INCLUDING PANDEMIC CLONE B2-O25-ST131

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Resistance in *Escherichia coli* colonizing gastrointestinal tracts of dogs, cats, camels and their owners in Northern Kenya was investigated with an emphasis on extended-spectrum beta-lactamases (ESBLs). *E. coli* and other coliform bacteria were tested for susceptibility to antimicrobials and screened for presence of corresponding resistance genes. Resistance-carrying plasmids were characterized by restriction fragment length polymorphism, group of incompatibility and conjugation experiments. ESBL-producing isolates were compared by their pulsed-field gel electrophoresis (PFGE) profiles. Among all isolates tested, resistance to beta-lactam antibiotics was the most frequent. Seventeen percent of all obtained isolates produced CTX-M-15 beta-lactamase; the pandemic clone B2-O25-ST131 producing CTX-M-15 gene was also detected. Six different types of large conjugative plasmids carrying $bla_{\text{CTX-M-15}}$ gene were distinguished; all of them carried additional resistance genes. Identical CTX-M-15-producing *E. coli* was detected in animals and humans living in the same area. The study suggests that gastrointestinal microflora of domestic carnivores in low-resource settings can form large reservoir of antimicrobial resistance. Although probably never treated by antibiotics, these animals are capable to disseminate resistant bacteria across large areas.
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S9:5

E. COLI PRODUCING PLASMID-MEDIATED AMPC ISOLATED FROM BROILERS AND HUMANS IN SWEDEN CARRY THE BLA$_{\text{CMY-2}}$ GENE ON AN INCK PLASMID

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Occurrence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBL) and plasmidmediated AmpC (pAmpC) is increasing worldwide. It has been suggested that the largest driving force to this trend is the use of cephalosporins. In Swedish broiler production there is a limited prescription of antimicrobials and cephalosporins are not used. It was therefore surprising when 34% of broilers were shown to carry ESBL/pAmpC producing *E. coli* in the Swedish Veterinary Antimicrobial Resistance Monitoring during 2010. Two genes were identified $bla_{\text{CTX-M1}}$ and $bla_{\text{CMY-2}}$with the $bla_{\text{CMY-2}}$ dominating. The aim of the study was to establish if occurrence of ESBL and pAmpC in Swedish broilers is due to a limited clonal spread and if broilers may be a source for genes, plasmids or isolates in human clinical settings. 32 *E. coli* isolates (22 $bla_{\text{CMY-2}}$ and 10 $bla_{\text{CTX-M1}}$) from broilers collected spring 2010 were included in the study. All isolates were characterised by PFGE, MLST and antimicrobial susceptibility. The plasmids carrying the genes were identified using conjugation and transformation followed by PCR plasmid replicon typing. A collection of human clinical *E. coli* isolates producing AmpC (n=78) from 2009-spring 2010 were screened for occurrence of $bla_{\text{CMY-2}}$ and plasmid replicon types identified in broiler isolates. If positive for $bla_{\text{CMY-2}}$ and same replicon types identified in isolates from broilers, the human isolates were subjected to PFGE, transformation and

plasmid replicon typing. Isolates from broilers showed an overall high genetic diversity, but clustering of a few specific isolates was observed. Of the bla_{CMY-2} isolates 41% were resistant to β-lactams only and 2 isolates were multiresistant (resistant ≥3 antimicrobial classes). All bla_{CTX-M1} isolates were resistant to sulfamethoxazole and tetracycline and 3 were streptomycin resistant. The $bla_{CTX-M-1}$ was carried on an incI1 plasmid and the bla_{CMY-2} on an incK plasmid. The bla_{CTX-M1}+incI1 plasmid also carried resistance to sulfamethoxazole and tetracycline, while the bla_{CMY-2}+incK plasmid only transferred resistance to β-lactams. In the screening of human clinical isolates 21 were positive for both bla_{CMY-2} and incK and 19 of these carried bla_{CMY-2} on the incK plasmid. All transformants of human isolates showed resistance to β-lactams only. None of the human clinical isolates were identical or clustered with the broiler isolates when compared using PFGE. The spread in Swedish broilers is therefore not due to a single or few clones. However, when studying the plasmids the picture is more homogenous with only two types of plasmids incK with bla_{CMY-2} and incI1 with bla_{CTX-M1}. There are no indications of clonal spread of bla_{CMY-2} isolates from broiler to human clinical settings, but 24% of the investigated AmpC producing human clinical isolates carried the bla_{CMY-2} on an incK plasmid indicating that broiler may be a source for bla_{CMY-2}.

S9:6

ANIMAL AND HUMAN MRSA HARBOURING THE NEW MECC: THE CHALLENGE OF SCREENING, DETECTION AND CONFIRMATION

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A new *mec*A variant, named *mec*A-LGA251 and definitively renamed *mec*C, showing less than 70% homology with the classical *mec*A gene, has been recently described in methicillin-resistant (MR) Staphylococcus aureus (MRSA) isolates from human and animal. We investigated the performance of different phenotypic and genotypic methods routinely used in microbiological labs to screen, detect, and confirm the presence of isolates harboring such a methicillin-resistance (MR) mechanism. A large collection including 111 *mec*C-positive isolates collected in UK, Scotland, Denmark and France were tested. Four chromogenic MRSA selective media (MRSA Select (bio-Rad), ChromID MRSA (bioMérieux), BBL CHROMagar MRSA II (Becton Dickinson), Brilliance MRSA 2 (Oxoid)) were tested for screening. Antimicrobial susceptibility tests (AST) included MIC for FOX and OXA using BMD, AST-P581 (Vitek), PMIC/ID-60 (Phoenix), Pos MIC Panel Type 31 (Microscan). Immunological detection of additionnal PBP were performed using Clearview Exact PBP2a (Alere) and PBP2a agglutination (Oxoid). Finally, molecular tests, including ”homebrew” *mec*A PCR, BD GeneOhm StaphSR assay (BD), Xpert MRSA/MSSA SSTI and nasal (Cepheid), NucliSENS EasyQ MRSA (bioM) and DNA microarray StaphyType (Alere), were performed. The 111 isolates belonged to CC130 (n= 92, agr 3, 16 spa-types), CC1943 (n=14, agr 4, 4 spa-types) and CC425 (n=5, agr 2, 4 spa-types). All were MR but susceptible to all the other antibiotics tested. Data highlighted a highly variable sensitivity for

the various selective media as well AST tested : surprisingly some MRSA were misdetected as MSSA. Clearview Exact PBP2a test, performed after cefoxitin induction (disc), were the only method allowing the confirmation of expression of additionnal PBP in all isolates. None of the homebrew *mec*A PCR or commercial molecular kits currently available was able to identify these isolates. Using DNA microarrays (n=37), assignment to the specific clones known to be positive for *mec*C gene were achieved and data revealed the seldom presence of some toxins and virulence genes : tst (n=7), egc (n=9), edinB (n=8), sec (n=3), sel (n=3). The data presented demonstrate that i) the ability of commercial methods used to screen, identify or confirm *mec*C-positive isolates is highly variable, ii) *mec*C-positive isolates may be missed depending on the technical algorithms used. At the moment, the only ways to definitively confirm the methicillin-resistance in *mec*C-positive isolates are the use of specific *mec*C PCR or Clearview Exact PBP2a after cefoxitin induction. Table 1 : Data obtained for a collection of 111 European MRSA isolates harboring *mec*C gene.

S9:7

SIMILAR SHV-12-HARBOURING PLASMIDS CIRCULATING IN ESCHERICHIA COLI ISOLATES FROM BOTH HUMAN AND ANIMAL FOOD ORIGIN IN THE SOUTH OF SPAIN.

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Introduction: ince 2000, the prevalence of human infections caused by CTX-M-producing *E coli* is increasing worldwide. In our area, besides this increase in CTX-M producing strains (particularly CTX-M-9 group) causing human infections, a high prevalence of SHV-12 producing *E. coli* strains has also been observed along these years. Moreover, the majority of poultry raw meet samples obtained from local markets were colonized with SHV-12-producing *E. coli*. This enzyme has mainly been found in *E. coli* isolates belonging to phylogroup A in both clinical and food-producing animal origins. In this study, the genetic relatedness, and plasmid characteristics of SHV-12-producing phylogroup A *E. coli*, isolated in our area from human clinical samples and raw meat, were analyzed. **aterial and methods:** Phylogroup A SHV-12-producing *E. coli* isolates included clinical isolates recovered in two different periods (n=11, 2007 and n=7, 2011) and food isolates from poultry raw meat samples isolated in two different studies (n=25, 2007 and n=14, 2010). Conjugation experiments were carried out with *E. coli* J53AziR and electroporation with *E. coli* DH10. Plasmids were extracted using by the Kieser´s method. A total of 56 transconjugants/transformants harbouring only one plasmid were selected for further analysis. Molecular typing was carried out by pulsed-field gel electrophoresis (PFGE). A dendrogram was produced by UPGMA algorithm based on the Dice similarity coefficient with a 1.0% band position tolerance. Major plasmid incompatibility groups of transconjugants and transformants were determined by the PCR-based inc/rep typing method according to Carattoli´s scheme. Inc I1 plasmids were subtyped by plasmid Multi Locus Sequence Typing (pMLST) using pMLST typing webbase (http://pubmlst.org/plasmid). **Results:** Most of the SHV-12-producing isolates were not genetically related (less than 85% similarity), but two pairs with identical PFGE patterns were found. Fifty (89%) plasmids were assigned to Inc I1 family (94,4% of clinical strains and 86,8% of meat strains). Other plasmid families were: Inc F group (7%), Inc N (1,8%). One plasmid was not typable. Four different ST were observed among Inc I1 plasmids: ST3 (46%) ST26 (38%), ST26 like (6%), ST28 (2%) and ST29 (2%). ST3 plasmids were found both in clinical (8) and food (15) isolates, as well as ST26 plasmids (5 clinical and 14 food isolates). **Discussion:** Our findings suggest that the spread of SHV-12 in

our area is not mainly caused by the dissemination of specific *E. coli* lineages, but to the dissemination of two ST plasmids associated to colonized poultry meat.

S9:8

MULTI-DRUG RESISTANCE INCHI1 PLASMIDS CARRYING BLACTX-M-1 FROM EQUINE ESCHERICHIA COLI FROM THE CZECH REPUBLIC

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Plasmids of incompatibility group HI1 are important vectors of antibiotic resistance in Salmonella Typhi and S. Paratyphi A, the major causal agents of enteric fever. We have previously described IncHI1 plasmids carrying blaCTX-M-1 gene in Escherichia coli isolates disseminated in an equine clinic in the Czech Republic giving the first evidence of extended-spectrum producing beta-lactamases associated with IncHI1 plasmids. The aim of this study was to perform complete sequencing of two blaCTX-M-1 harbouring IncHI1 plasmids and compare them with IncHI1 plasmids described in S. Typhi. Complete nucleotide sequencing of one 220 kb IncHI1 (pEQ1) and a fused 285 kb IncHI1/X1plasmid (pEQ2) was performed by the 454-Genome Sequencer FLX procedure on a library constructed on plasmid DNA purified from the E. coli transconjugants. Open reading frames (ORFs) were predicted and annotated using the Artemis software (Wellcome Trust Sanger Institute). Comparison with six fully sequenced IncHI1 plasmids revealed high DNA sequence similarity from. Both the plasmids share IncHI1 associated backbone, which showed 99% nucleotide similarity with other IncHI1 plasmids including pR27. The pEQ1 and pEQ2 sequence contains multiple antibiotic resistance gene elements and mercury resistance cluster inserted into the IncHI1 backbone. Differences in organizations of AR gene clusters in respect to other IncHI1 plasmids can be attributed to the independent insertion of transposable elements and their further rearrangements. Integration of IncX1 plasmid harbouring qnrS1 and blaTEM-1 genes was observed in the surroundings of the CTX-M-1 module in pEQ2. Both the plasmids pEQ1 and pEQ2 contain 9 kb module that is potentially involved in carbohydrate uptake and assimilation and could be linked to virulence. In both the plasmids the gene blaCTX-M-1 was followed by macrolide resistance cluster and surrounded by IS26 elements. The similar organization of the CTX-M-1 region was observed in several IncI1 and IncN plasmids in human and porcine E. coli isolates in Germany. The finding of the same gene arrangements in the direct genetic neighborhood of blaCTX-M-1 in plasmids of different incompatibility groups suggest the exchange of large blaCTX-M-1-containg modules between different plasmid backbones. Our data show successful dissemination of S. Typhi and S. Paratyphi A IncHI1 plasmid variants to E. coli from animals. Integration of the element containing genes for carbohydrate transport and metabolism could explain successful dissemination of these IncHI1 plasmids in E. coli in horses. Our results show that these plasmids are rapidly evolving toward larger antimicrobial gene content by acquisition of resistance determinants and plasmid fusion with other resistance plasmids. This study was funded by FEMS Advanced Fellowship Grant 2011, Czech Science Foundation P502/10/P083 and CEITEC CZ.1.05/1.1.00/02.0068.

Poster Abstracts

1A

IMPACT OF USE OF ANTIBIOTICS ON PATHOGENIC BACTERIA SENSIBILITY OF LAYER AND BROILER CHICKEN IN NGAOUNDÉRÉ TOWN - CAMEROON.

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The non-controlled use of antibiotics in poultry farming leads to resistant germs selection with disastrous consequences as the upsurge of the infections, the increase of mortality and the decrease of productivity. The aim of this work was to evaluate the impact of the use of antibiotics on pathogenic bacteria sensibility in poultry farming. Thus, we made an investigation firstly to appreciate the types and the quantities of antibiotics used, and secondly to make some withdrawals in every farms. Thereafter, we isolated the pathogenic bacteria by the streaking method on non-selective media. The antibiotic sensitivity test was determined by the agar diffusion method. Results showed twelve antibiotics most commonly used in poultry farming are oxytetracyclin (6000g/month), sulphamides (4000g/month) and furaltadone (2600g/month), followed by erythromycin (1000g/month), norfloxacin (615g/month), fluméquin (500g/month), colistin (470g/month), penicillin (80g/month), streptomycin (80g/month), tylosin (60g/month), neomycin (25g/month) and ampicillin (20g/month). These antibiotics cost 1 200 000 CFA francs (~2,634 US $) roughly per month. Twenty strains of pathogenic bacteria were identified as belonging to genus: Aeromonas (1 strain/20), Bordetella (1/20), Cedecea (1/20), Citrobacter (1/20), Proteus (5/20), Pseudomonas (1/20), Salmonella (9/20) and Vibrio (1/20). The bacteria identified, presented multiresistance to the 11 antibiotics tested, ampicillin (90% of resistance), erythromycin (100%), penicillin (100%), cefixim (60%), tetracyclin (45%), chloramphenicol (45%), amoxicillin (40%), streptomycin (40%), nitrofurantoïn (40%), oxolonic acid (25%) and sulphamides (20%). Key words: antibiotics, resistance, broiler chicken, layer chicken, Ngaoundere, Cameroon.

2A

EVALUATION OF PROBIOTIC POTENTIAL AND PARTIAL CHARACTERIZATION OF ANTIBACTERIAL COMPOUNDS PRODUCED BY LACTIC ACID BACTERIA ISOLATED FROM CHICKEN INTESTINE.

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During our previous survey in the town of Ngaoundere (Cameroon, Central Africa) and its surroundings, we have noticed that over 83% of farmers practiced self-medication and used a wide range of antibiotics. Furthermore, the multiresistance observed in the majority of pathogenic bacteria (Salmonella, E. coli, Proteus ...) is a real public health problem that should be given special attention. In order to offer an alternative to limit the spread of antibiotic resistance, we intended to search for lactic acid bacteria with high potential probiotic for chicken. Six strains among hundred isolated by microbiological techniques, have shown great probiotic potentialities. Putative probiotics, codified LAB1 to LAB6, were tested for acid tolerance, bile salt tolerance, high salt tolerance and antibacterial activity against certain pathogenic bacteria (Salmonella choleraesuis, Salmonella sp., Salmonella arizonae, Proteus mirabilis, Citrobacter diverticus, Pseudomonas cepacia, Cedecea lapagei). The antibacterial activity observed was the result of organic acid produced and bacteriocin-like compounds. These potential probiotic strains could be used to substitute antibiotic growth

promoter in poultry farm. However, it would be wise to identify them at molecular level, to master their formulation with food and, assess their impact on zootechnical parameters such as weight gain and food consumption index.
Key words: lactic acid bacteria, bacteriocin, probiotic, antibacterial activity, chicken, Ngaoundere, Cameroon

3A

STUDY PROFILES ANTIMICROBIAL RESISTANCE IN YERSINIA ENTEROCOLITICA ISOLATED OBJECTS OF VETERINARY-SANITARY CONTROL

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Studied the biological properties of strains of Yersinia enterocolitica isolated from swabs of carrots and potatoes, from the corpses of house mice (Mus musculus) and shrews (Soricomorpha, Soricidae) and human feces with diarrhea. The study of sensitivity to 54 antimicrobial various pharmacological groups: inhibitors of cell wall synthesis (penicillins, cephalosporins), inhibitors of protein synthesis (aminoglycosides, tetracyclines, chloramphenicol, macrolides, lincosamides), inhibitors of transcription and nucleic acid synthesis (fluoroquinolones rifampin), drugs acting on the cytoplasmic membrane (nitrofurans, azoles). The investigated strains of Y. Enterocolitica were resistant to antibiotics of the groups azoles, nitrofurans, lincosamides, were sensitivity on the effect of drugs from the group of benzylpenicillin, cephalosporins, fluoroquinolones. According to the results study of morphological and enzymatic properties of cultures, study of the profile antimicrobial resistance, compiled passport strains of Yersinia enterocolitica, with a view to further using them for standardization of diagnostic tests for yersiniosis.

4A

MYCOFLORA, MYCOTOXINS, BACTERIOLOGICAL ANALYSIS AND MOLECULAR ASSAY OF SOME BACTERIAL SPECIES FROM COFFEE BEANS IN SAUDI ARABIA

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The mycoflora analysis of some coffee beans in Saudi Arabia showed a wide range of fungal contamination in 31 samples collected from different markets in El-Riyadh. Thirty four species belonging to 16 genera and 28 species belonging to 18 genera were isolated from coffee beans on glucose and cellulose Czapek's agar medium at 25°C from seed-plate method. Aspergillus niger and A. flavus were the most prevalent species, but Penicilliun oxalicum was isolated in moderate occurrence, while 12 genera comprised 16 species and 8 genera comprised 10 species were isolated on the same types of media at 25°C from seed suspension method. A. niger was the most common species, while A. flavus and P. funiculosum were isolated in moderate occurrence. A. niger, also was the most prevalent on 20% sucrose-Czapek's agar medium at 25°C, but the genus Eurotium (3 spp) appeared in moderate occurrence. Five fungal species belonging to four genera were isolated on starch yeast extract agar medium at 45°C. A. fumigatus and A.niger were the most prevalent thermo tolerant species, while three species of thermophilic fungi were of low or rare incidence. Thin layer chromatographic analysis of chloroform extracts of 31 coffee beans samples revealed that 20 samples were free from mycotoxins, while 11 samples were contaminate with aflatoxins B1, B2, G1 and G2 of concentrations ranged from 110-600 ug/kg, but 6 samples were contaminate with sterigmatocystin ranged from 60-600 ug/kg. Screening of the characteristic mycotoxins of 25 fungal isolates revealed that 17 of them produced, aflatoxin B1 at 450 ug/kg, ochratoxin A at 600 ug/kg, ochratoxin B at 400 ug/kg, and sterigmatocystin 280 ug/kg

from Aspergillus species, while three isolates of Penicillium produced penicillic acid (ranged from 720-240 ug/kg) and one isolate of Trichoderma produced Trichodermine at 360 ug/kg. The bacteriological analysis of the coffee bean samples indicated that Bacillus cereus was detected in six samples at levels (2x10 cfu/g), E. coli in two samples (14x10 and 89x10 cfu/g), Feacal coliform was detected in one sample only, while Staphylococcus appeared in 29 samples (55x103 cfu/g). All samples were free from Salmonella. PCR assay for detection of some bacterial species revealed that all samples were negative for Yersinia enterocolitica, Campylobacter and Listeria monocytogenes, while the results of B. cereus and Salmonella were similar to the results obtained using cultural method.

5A

THE STUDY OF PROPERTIES OF NANOPARTICLES BAKTERIOTSIDNYH AG, ZN, CU, MN, FE AGAINST PASTEURELLA MULTOCIDA AND STAPHYLOCOCCUS AUREUS

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The effect of colloidal nanoparticles (NP) of metals Ag (31,5 ± 0,9 nm), Zn, Cu, Mn, Fe (110,0 ± 10,0 nm) obtained by the condensation method, the rate of biomass accumulation gramnegativ and grampozitiv microorganisms on models of Pasteurella multocida and Staphylococcus aureus. Evaluated the bacteriostatic effekt in the concentration range of metal NP 200, 80, 40, 4, 0.4, 0.04, 0.004, 0.0004 micrograms of ml (ug/ml) in the culture medium. It is established that the Ag NP exhibit a bacteriostatic effect on Pasteurella multocida in all the studied concentration - from 50% at a concentration of 200 ug/ml to 74% at concentrations of 0.004, 0.0004 ug/ml against Staphylococcus aureus 50% bacteriostatic effect found in the concentration ug/ml and the concentration of Ag 0,04, 0,004, 0,0004 ug/ml in culture medium hindered the accumulation of biomass at 62-63% compared with controls. Mn NP hindered the accumulation of biomass Pasteurella multocida by 49.2% compared with the control only at a concentration of 0.0004 ug/ml, but with respect to Staphylococcus aureus 50,8% bacteriostatic effect is detected at a concentration of 40 ug/ml, and traced the gain efficiency (53.2 - 63.9%) in a gradient of concentrations of 4 - 0.0004 ug/ml. Cu NP at concentrations of 0.4, 0.04, 0.004 ug/ml hindered the accumulation of biomass Pasteurella multocida by 54.3%, 52.2%, 48.3%, respectively, and Staphylococcus aureus - 0,4, 0,04, 0.004, 0.0004 ug/ml hindered the accumulation of biomass by 45.6%, 46.4%, 46.4%, 46.8%, respectively. Zn NP impeded the accumulation of biomass Pasteurella multocida by 32.4% compared with the control only at a concentration of 0.4 ug/ml, and against Staphylococcus aureus at concentrations of 4, 0.4, and 0.04 ug/ml were recorded decrease in the accumulation biomass by 30%, 29% and 28.6% respectively. NP of Fe did not show significant bacteriostatic effect against Pasteurella multocida, but against Staphylococcus aureus - recorded a bacteriostatic effect at concentrations of 0.4, 0.04 ug/ml in 35.5% and 40.7% respectively.

6A

PLASMID-MEDIATED QUINOLONE RESISTANCE GENES ARE WIDELY SPREAD IN TROUT AQUACULTURES

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Background: Aquaculture is currently one of the fastest growing food production sectors and is associated with 50% of the fish consumedworldwide. The occurrence of food safety threats, as resistance to antibiotics (AB^R) relevant to the treatment of human infections (e.g. fluoroquinolones), is of concern to public health. The goal of this study was to analyze the presence of plasmid-mediated quinolone resistance (PMQR) genes in different Gram negative bacteria collected from trout aquacultures and to evaluate the role of this niche as a reservoir/vehicle of these genes. **Methods:** Two trout aquacultures (TR-A/TR-B) were studied (Portugal; winter and summer; 2010-2011). Samples were collected from water/sediments located upstream (n=11) and downstream (n=11) trout farms, water/sediments from juvenile/adult fish ponds (n=13) and feed (n=5). They were analysed for *Salmonella* (ISO method), *Aeromonas* (selective medium with/without 5µg/mL ampicilin) and other Gram negative/oxidase negative bacilli (selective medium with 0.125µg/mL ciprofloxacin) after an enrichment step. Genes encoding resistance to fluoroquinolones [*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, *aac(6')-Ib-cr*, *oqxAB*] were searched by PCR/sequencing. Species were identified by ID32GN/PCR/16SrRNA-sequencing. AB^R was studied by agar diffusion/Etest (CLSI/EUCAST). Clonality was assessed by MLST in specific *Escherichia coli* isolates. **Results**: PMQR genes were detected in 9% (n=13/145) of the isolates analysed, which were susceptible to ciprofloxacin (MIC 0,008-1 µg/mL). *qnrS2* (n=2; adult fish tank or downstream river water/TR-A and *aac(6')-Ib-cr* (n=2; upstream water/TR-A, output sediment/TR-B) genes were identified in *Aeromonas* spp (n=4/57; 3 *A. hydrophila)*. *qnrS3*, *qnrB* variants and *oqxAB* were observed among other Gram negative bacilli (n=9/70; 8 samples). The *qnrS3* gene was detected in multidrug-resistant (MDR) *E. coli* (n=3; ST641/ST661; downstream river water/TR-A, juvenile/adult ponds water or sediment/TR-B) and *Citrobacter* sp. (n=1; adult pond sediment/TR-B). Different *qnrB* variants were detected in MDR *E. coli* (n=1; ST1049; downstream river water/TR-A), *Citrobacter freundii* (n=2; feed/TR-A, output sediment/TR-B) and MDR *Klebsiella pneumoniae* (n=1; downstream river sediment/TR-A). The recently described *oqxAB* was detected in 2 MDR *Klebsiella* spp (downstream river sediment/TR-A, feed/TR-B), one of them also carrying *qnrB*. PMQR genes were not detected in *Salmonella* (n=18; 13 samples; both TR/summer). **Conclusion**: Diverse PMQR genes are carried by Gram negative bacterial species of trout aquaculture and surrounding environments demonstrating the importance of this setting as reservoir of resistance genes relevant in human medicine.

7A

PLATELETS ENHANCE BIOFILM FORMATION AND ANTIBIOTIC RESISTANCE OF ENDOCARDITIS-INDUCING STREPTOCOCCI

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Commensal or pathogenic bacteria can colonize and accumulate on the interface to form biofilms, which are characterized by polymicrobial aggregates in the form of mats or floes and are typically encased in an extracellular matrix that is mostly produced by the organisms themselves. The most severe clinical problems caused by bacterial biofilms are the persistence of infection and the resistance to conventional antibiotic therapy. A typical example of biofilm-associated disease is found in infective endocarditis (IE) for humans or dogs. In human, IE is induced most frequently by staphylococci or oral commensal streptococci. Oral streptococci, like *Streptococcus*

mutans or *S. gordonii,* form biofilm through the production of exopolysaccharides on the tooth surface, but the mechanisms and contribution of host factors in biofilm formation on the injured heart valves is unclear. We found that platelets are essential for *in vitro* biofilm formation by *S. mutans* or *S. gordonii* grown in human plasma. The biofilms were composed of bacterial floes embedded with platelet aggregates in layers, and a similar architecture was also detected *in situ* on the injured valves of a rat model of experimental endocarditis. Similar to planktonic cells, the streptococci in biofilms were also able to induce platelet aggregation, which facilitates multilayer biofilm formation. Entrapping of platelets directly enhances the resistance of streptococcal biofilms to clindamycin. Prophylactic antibiotics or aspirin can reduce but not prevent or abolish biofilm formation on injured heart valves. Therefore, the platelet is a host factor for commensal streptococci in the circulation to consolidate biofilm formation and protect bacteria against antibiotics.

8A

ASSESSMENT OF ANTIBIOTIC ACTIVITY ON BACTERIAL STRAINS ISOLATED FROM URINE AT BUTARE UNIVERSITY TEACHING HOSPITAL (BUTH) LABORATORY

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Infection of the urinary tract is one of the most common infectious diseases, which affects all age groups of people including men, women and children. Bacteria isolated from urine are becoming resistant to antimicrobials worldwide. It is estimated that 20% or more of the female population suffers from some form of UTIs in their lifetime. Although antibiotics are the first treatment choice for urinary tract infections, antibiotic-resistant strains of the most common cause of UTIs, are increasing worldwide. The purpose of this study was to determine the causative agents of UTIs and their susceptibility patterns to antibiotics at BUTH laboratory. A retrospective study was carried out on bacteria isolated from the urine of patients at the BUTH laboratory between January 2006 and December 2010. Bacterial susceptibility testing was performed by disk diffusion method. Data were collected from registers of antibiogram of the bacteriology service of BUTH. A total of 1611 pathogens have been found. The most commonly isolated bacteria were *Escherichia coli* (876 strains), *Klebsiella Species* (190 strains), *Coagulase negative Staphylococcus* (114 strains), *Streptococcus species* (97 strains), *Proteus species* (90 strains) and *staphylococcus aureus* (86 strains). Most of isolates were resistant to Aminopenicillins (Ampicillin and Amoxicillin) and to Trimethoprim- Sulfamethoxazole (TMP-SMZ). Nitrofurantoin, Amoxicillin, TMP-SMZ, and Naldixic acid should no longer be used as first-line treatment of uncomplicated UTIs in Rwanda. Strains were rarely resistant to more expensive antibiotics (Imipenem and Cefotaxime). The most effective antibiotic to almost all isolates was Imipinem, which is not commonly used in treatment of UTIs in Rwanda. The rate of Amoxicillin and Trimethoprim-Sulfamethoxazole resistance to Enterobacteriaceae implies that another antibiotic should be used for empirical treatment. Imipenem could be included as a reasonable alternative for the therapy of UTIs in Rwanda.

9A

PREVALENCE OF RESISTANCE TO THIRD GENERATION CEPHALOSPORIN IN *E. COLI* ISOLATED FROM LAYERS IN FRANCE

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Several studies have reported the high prevalence of resistance to third generation cephalosporin (3GC) in *E. coli* from broilers in different countries but little is known about prevalence in the egg production sector. It was thus decided to use fecal samples collected in French pullet and laying hen farms in the

frame of the national *Salmonella* official control to evaluate the prevalence of 3GC resistant *E. coli.* From February to May 2011, 300 fecal samples were collected from 300 different flocks in 71 French departments. The fecal samples were sent to departmental veterinary laboratories. One *E. coli* isolate obtained on non selective media was randomly collected and sent to our laboratory. Upon arrival, the species identification was checked by PCR. A standardized inoculum of each isolate was deposited on Mueller Hinton agar containing cefotaxime (CTX) (1 mg/L) according to the CLSI method for determination of MIC. The isolates with a MIC of CTX higher than 1 mg/L were further analyzed. The susceptibility and pulse field gel electrophoresis profiles were determined. The presence of plasmids was checked by conjugation on agar media supplemented with rifampin and CTX or cefoxitin (FOX) and susceptibility of transconjugants was studied. 3CG resistance genes of the isolates were detected by use of Clondiag microarrays (Biocentric). From the 300 fecal samples, 293 viable *E. coli* could be analyzed. Among them, 22 (7.51% [4.49-10.54]) isolates could grow on CTX supplemented agar. Resistant isolates were more frequently obtained from young birds and isolation probability decreased with weeks of age (P=0.0002 ; OR=0.94 [0.91-0.97]). Seventeen of the 22 resistant isolates produced extended-spectrum beta-lactamase (ESBL) as disk diffusion assay showed susceptibility to FOX and resistance to 3GC with synergy between amoxicillin-clavulanic acid and 3GC. All but one were resistant to tetracycline and a few ones showed additional resistances to aminoglycosides or trimethoprime-sulphonamides (SXT). Their PFGE profiles were various. Conjugation experiments revealed that the resistance genes for cephalosporins, and most often tetracycline or SXT could be transferred to recipient cells. The 3GC resistance genes belonged to the $bla_{CTX\text{-}M1}$ group for all of the 17 strains. The five resistant non ESBL isolates were obtained from pullets. They exhibited additional resistances to tetracycline, and for four of them to aminoglycosides. Resistance to FOX could be transferred to recipient cells, along with tetracycline resistance for two isolates. Four isolates harbored a gene of the bla_{CMY2} group. In conclusion, 7.5% of the *E. coli* randomly isolated from pullets and layers feces were resistant to 3GC due to beta-lactamase genes belonging to $bla_{CTX\text{-}M1}$ (mainly) or bla_{CMY2} groups. These genes could be transferred by conjugation, most often together with tetracycline resistance gene.

10A

INVESTIGATION OF ANTIMICROBIAL ACTION OF THE NEW SYNTHESIZED MODIFIED COMPOUNDS.

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Antibiotic resistance is one of the most important problem in the world. Therefore, there exists an urgent need to develop novel therapeutic agents to overcome antibiotic resistance. Almost 200 compounds presented by samples what were sintezed by us in a samples of different chemical groups (acridine, phenazine, acridone, thioxanthenone, triazinobenzothiazine, quinoline and triazine). The synthesized compounds were tested for their *in vitro* growth inhibiting activity against a range of Gram-positive and Gram-negative bacteria (Erysipelothrix rhusiopathiae, Klebsiella spp, Salmonella cholerae suis, Pasteurella multocida, Streptococcus suis, Staphylococcus aureus, Escherichia coli). Each compound sample was solubilised in DMF or DMSO (1 mg/mL) and investigated by qualitative and quantitative screening using the disk diffusion and/or microdilution methods. All compounds were preliminary tested at concentration of 100 µg/ml. Experimental results showed that 150 test agents inhibited the visible growth of different types of microorganisms. The wide spectrum of inhibition were showed by 30 compounds, notably 8 compounds suppressed all types of bacteria, 10 compounds suppressed 6 and 12 compounds suppressed 5 types of bacteria.

11A

EUROPEAN ANTIMICROBIAL SUSCEPTIBILITY SURVEILLANCE IN ANIMALS (EASSA): RESULTS ON ENTERIC BACTERIA FROM HEALTHY CATTLE ACROSS THE EU

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Background: EASSA is a pan-European surveillance dedicated to the collection and antimicrobial susceptibility testing of zoonotic and commensal bacteria isolated from healthy food animals (poultry, pigs, cattle). Susceptibility to human-use antibiotics (ABs) was determined for zoonotic bacteria *Salmonella*, *Campylobacter jejuni* and *C. coli* as well as for commensal bacteria *Escherichia coli*, *Enterococcus faecium* and *E. faecalis* isolated from beef cattle at slaughter in 2002-2006. **Methods:** Colon content was randomly sampled in France, Germany, Ireland, Italy and United Kingdom at several abattoirs per country. Each herd was sampled once. Isolation was performed by standard methods, and susceptibility by agar dilution (CLSI) against a panel of ABs in a central laboratory. Clinical resistance (CLSI, M100-S21 or M45-A2) was assessed per drug/organism/country; decreased susceptibility (DS) was based on epidemiological cut-off values as defined by EFSA (2007, 2008). **Results:** In total, 418 *C. jejuni* and 166 *C. coli* strains were recovered. Mean resistance rates (%) for *C. jejuni* were: ciprofloxacin (CIP) 18.7; erythromycin (E) 1.0; gentamicin (G) 0; nalidixic acid 24.2; tetracycline (T) 34.2. DS to CIP was negligible and was 4.5% for E. In the case of *C. coli*, a much less frequent pathogen for humans, resistance rates were clearly higher for all 5 ABs tested. Only 57 *Salmonella* isolates were recovered. This indicates that cattle are not a major reservoir and allows only limited conclusions on susceptibility. Neither clinical resistance nor DS was noted for both CIP and cefotaxime (CTX; except 4.8% DS in France). Resistance rates (%) for *E. coli* (n=1396) were: ampicillin (A) 6.2; CIP 1.0; chloramphenicol 3.2; G 1.4; T 12.3; and trimethoprim/sulfamethoxazole 5.7. Resistance to cefepime or CTX was absent; for colistin, resistance was < 0.1%. DS was 2.0% for CIP and 0.4% for CTX. None of the *E. coli* and *Salmonella* isolates was identified as ESBL. For *Enterococcus* spp., 290 isolates were identified as *E. faecium* and 84 as *E. faecalis*. All enterococci, except one isolate, were susceptible to linezolid. For *E. faecium,* resistance for A, G and vancomycin did not exceed 1%; resistance to quinupristin/dalfopristin (Q/D) was 18.6%; and DS was 34.8%. For *E. faecalis* no resistance to any of the ABs tested was observed, except intrinsic Q/D resistance. **Conclusions:** This EU survey, using standardized methods, shows that antimicrobial resistance among enteric zoonotic and commensal bacteria isolated from cattle at slaughter is variable. For *E. coli,* clinical resistance rates were low, albeit variable, and decreased susceptibility to medically important antibiotics such as CTX or CIP was very low. Negligible resistance to erythromycin, drug of choice for campylobacteriosis in humans, was observed for *C. jejuni. E. faecium* showed no or very low resistance rates for ampicillin, gentamicin, linezolid and vancomycin.

12A

PATHOLOGY OF CAMEL TUBERCULOSIS, MOLECULAR CHARACTERIZATION OF IT'S CAUSES AND ZOONOTIC IMPLICATIONS IN HERDERS

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A cross sectional study was conducted on 906 apparently healthy camels slaughtered at Akaki and Metehara abattoirs and on 120 (60 tuberculosis (TB) suspected humans and 60 camel herders) between September 2009 and April 2010 in Ethiopia to investigate the pathology of camel tuberculosis (TB) and characterize its causative agents as well as to assess public

health importance of the disease in owners using postmortem examination, mycobacteriological culturing, and multiplex polymerase chain reaction (PCR), region of difference-4 (RD4)-based PCR and spoligotyping and questionnaire survey. The prevalence of camel TB was 10.04% (91/906) on the basis of pathology and it was significantly higher in females (x2 = 4.789; P = 0.029). The tropism of TB lesions was significantly different among the lymph nodes (x2 = 22.697; P = 0.002) and lung lobes (x2 = 17.901; P = 0.006). Mycobacterial growth was observed in 34% (31/91) of camels with grossly suspicious TB lesions. Upon further molecular characterization using multiplex PCR, 68% (21/31) of the colonies showed a positive signal for the genus Mycobacterium, of which two were confirmed Mycobacterium bovis (M. bovis) by RD4 deletion typing. Further characterization of the two M. bovis at strains level revealed that one of the strains was SB0133 while the other strain was new and had not been reported to the M. bovis database prior to this study. Hence, it has now been reported to the database, and designated as SB1953. On the other hand, 6 of the 8 human isolates subjected to RD9 deletion typing were confirmed to be M. tuberculosis. In connection, the questionnaire survey result has shown that 95 % of the respondents drink raw milk and 86.7 % of them eat both raw and cooked meat. In conclusion, the results of the present study have shown that the majority of camel TB lesions are caused by mycobacteria other than Mycobacterium tuberculosis complex. And hence further identification and characterization of these species and antibiotic resistant test for human isolates would be useful towards the efforts made to control TB in camels and herders. Furthermore, multidisciplinary approach (veterinary and public health) should be initiated in the areas.

13A

ABSENCE OF TETRACYCLINE RESISTANCE IN CAMPYLOBACTER ISOLATES FROM FINNISH FINISHING PIGS TREATED WITH CHLORTETRACYCLINE

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Aims: Tetracycline resistance is commonly reported in Campylobacter, especially in porcine C. coli isolates. We studied MICs for tetracycline, ciprofloxacin and erythromycin and presence of a tetracycline resistance gene tet(O) in Campylobacter isolated from finishing pigs medicated with chlortetracycline. **Materials and methods:** Rectal swabs were collected from ear-tagged pigs from two finishing farms (herd 1 and 2) where chlortetracycline was administered orally to treat respiratory tract infections. Samples were collected on the day before treatment, on the 6th or 7th day of a ten-day treatment and 22-24 days after the treatment. On herd 1 all pigs received chlortetracycline and 40 of them were sampled. On herd 2, all pigs except those in two pens were treated with chlortetracycline, and 20 treated and 20 untreated pigs were sampled. Samples were cultivated on mCCDA and additionally enriched in Bolton broth. Two presumptive Campylobacter isolates per pig were identified as C. coli or C. jejuni by species-specific PCR. Presence of tet(O) gene was detected by PCR. MICs for ciprofloxacin, erythromycin and tetracycline were investigated by the broth microdilution method. **Results:** A total of 258 C. coli and one C. jejuni were isolated from herds 1 and 2. Before treatment, 85% of the pigs carried Campylobacter, while 32% and 53% of the pigs were Campylobacter-positive during and after treatment, respectively. Among untreated pigs, 85% and 84% carried Campylobacter during and after the treatment of other pigs, respectively. MICs were determined for 242 isolates. The highest tetracycline MIC among these was 1 µg/ml and the MIC50 and MIC90 for tetracycline were 0.125 and 0.25 to

0.5 µg/ml, respectively, at all sampling stages. Presence of tet(O) gene was studied for 259 isolates and none of those carried the gene. The ciprofloxacin MIC50 and MIC90 values were 0.125 and 4 to ≥ 16 µg/ml, respectively, and the MIC50 and MIC90 for erythromycin were 1 to 2 and 2 to 8 µg/ml, respectively, at all sampling stages. **Conclusions:** Chlortetracycline treatment temporarily reduced the prevalence of Campylobacter in pigs. The treatment did not select isolates carrying tet(O) or MICs higher than the epidemiological cut-off value (ECOFF ≤ 2 µg/ml). The MIC values for ciprofloxacin and erythromycin also remained at the same level before, during and after the treatment.

14A

THE EFFECT OF ENROFLOXACIN USE IN CALVES ON THE SELECTION OF PLASMID-MEDIATED QUINOLONE RESISTANCE IN COMMENSAL E. COLI

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Introduction: The current knowledge about the presence and frequency of plasmid-mediated quinolone resistance (PMQR) genes in commensal *Escherichia coli* strains from cattle is scarce. **Objectives:** i) evaluate PMQR genes prevalence, namely *qnr* genes (*A, B, C, D* and *S*), *aac(6')-Ib-cr* gene and *qepA* gene encoding an efflux pump; and ii) determine Minimum Inhibitory Concentrations (MIC) of nalidixic acid (NAL), ciprofloxacin (CIP) and levofloxacin (LEV) of *E. coli* isolates from healthy calves under *in vivo* enrofloxacin (ENR) selective pressure. **Methods:** 237 *E. coli* isolates from faecal samples of healthy calves were obtained at 3 sampling moments. At T0, T1 (six weeks after ENR administration) and T2 (10 weeks after ENR administration), 101, 79 and 57 *E. coli* were isolated, respectively. Antimicrobial susceptibility testing was performed using MICs determination by microdilution and results were interpreted using EURL-AR and EUCAST epidemiological cut-off values (NAL R>16 µg/mL; CIP R>0,064 µg/mL; LEV: R>0.25 µg/mL). The genotypic characterization of PMQR was performed by PCR and sequencing. Logistic regression was used to assess the association between ENR use and resistance to NAL, CIP and LEV and time of sampling. **Results:** NAL resistant *E. coli* isolates at T0, T1 and T2 were, respectively: 52.5% (n=53), 100% (n=79) and 82.5% (n=47). CIP resistant isolates in T0, T1 and T2 were, respectively: 52.5% (n=53), 100% (n=79) and 89.5% (n=51). LEV resistant isolates in T0, T1 and T2 were, respectively: 46.5% (n=47), 100% (n=79) and 87.7% (n= 50). From the 237 *E. coli* isolates tested: 11.8% (n=28) harboured *qnr* genes *(qnrB2* n=4, *qnrD* n=11 and *qnrS1* n=13) and 0.8% (n=2) were found positive for the *aac(6')-Ib-cr* gene. The analysis of PMQR genes in *E. coli* at the different sampling times showed that: at T0 *qnr* genes were detected in 3% of the isolates (all found to be *qnrS1*) and 2% carried the *aac(6')-Ib-cr* gene; at T1 15.2% of the isolates carried *qnr* genes (10.1% *qnrD* and 5.1% *qnrS1*); and at T2 22.8% of the isolates were found positive for *qnr* genes (7% *qnrB2*, 5.3% *qnrD* and 10.5% *qnrS1*). An increase was observed, even though not found statistically significant (p=0.987), in the prevalence of NAL, CIP and LEV resistant isolates from T0 to T1 after ENR selective pressure, and a significant decrease was observed from T1 to T2 (p<0.05). However, a significant increased frequency of PMQR genes (p<0.05) was observed along the longitudinal study. **Conclusion:** The increased frequency of PMQR genes along the longitudinal study under the ENR selective

pressure is a concerning fact. PQMR may provide a reduced susceptibility to quinolones that may allow mutational events to occur, leading to high-level quinolone resistance. This is to our knowledge the first report on PMQR *qnrD* gene in *E. coli* isolates from cattle.

15A

GUT MICROBIOTA OF ZOO ANIMALS CARRYING ANTIBIOTIC RESISTANCE GENES

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The presence of antibiotic resistance in pathogens has been studied for many years; however, commensal bacterial communities, particularly from the gastrointestinal tract of humans and animals, could be acting as reservoir for the maintenance and spread of antibiotic resistance genes. These genes could ultimately be acquired by pathogenic and opportunistic bacteria leading to less effective treatment of infections. In this study, the aim was to isolate and identify antibiotic resistant commensal gut bacteria from exotic (zoo) animals and to determine their antibiotic resistance profiles. Seventeen faecal samples from mammals housed at Burger Zoo (Arnhem- The Netherlands), were tested in different media containing 2 to 4 different antibiotics. A total of 34 and 18 antibiotic resistant bacteria from chimpanzees, gorillas, giraffes, elephants, siamang, tigers, dusky leaf monkeys and warthogs were isolated in aerobic and anaerobic conditions, respectively, from 12 of the 17 tested faecal samples. *Escherichia coli* was by far the most frequently isolated bacterial species , but also antibiotic resistant strains such as *Proteus penneri, Parabacteroides merdae and Phascolarctobacterium faecium* were identified during this study. Screening for resistance to β-lactam and tetracycline antibiotics contributed for almost 90% of the resistant bacteria. Thirteen isolates showed multidrug resistance phenotypes, the most common being against: Ampicillin, imipenem, chloramphenicol, streptomycin and tetracycline. Out of 52 isolates, 26 carried plasmids. Transferability of antibiotic resistance through plasmids could be demonstrated for three isolates. In conclusion, the results obtained in this study confirm the presence of antibiotic resistant commensal bacteria in the gut of non-human mammals, which could be responsible for the dissemination of resistance genes.

16A

TETRAZOLIUM/FORMAZAN TEST AS AN EFFICIENT METHOD TO DETERMINE FUNGAL CHITOSAN ANTIMICROBIAL ACTIVITY

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Chitosan natural polysaccharide was extracted from Aspergillus niger mycelia and characterized with 89.2% deacetylation degree, a molecular weight of 2.4 × 104 Da and 96.0% solubility in 1% acetic acid. The antimicrobial activity of fungal chitosan was evaluated against two foodborne pathogens, Salmonella Typhimurium and Staphylococcus aureus, using the standard antimicrobial assays and by using 2,3,5,-triphenyltetrazolium chloride (TTC) as chromogenic marker for qualitative and quantitative determination of antibacterial potentiality. The TTC (0.5 % w/v) was added, at concentration of 10%, to cultured broth, containing chitosan with different concentrations then the formed formazan was separated. The formation of red formazan was considered as a qualitative indication, whereas measurement the color intensity of resuspended formazan provided a quantitative evidence for antibacterial agent strength. Regarding the rapidity, technical simplicity and cost-effectiveness, TTC assay, could be recommended as an efficient alternative method for determination of chitosan antimicrobial activity and could be suggested for general evaluation of antimicrobial agents.

17A

PREVALENCE AND CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM COMMERCIAL PORK PROCESSING PLANTS IN CANADA

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In the past several years studies from Europe and Canada have suggested a higher prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs and pig farmers. Evidence from Europe has shown that MRSA strains found primarily in pigs are now a leading cause of community-associated MRSA infections in humans. The aim of this study was to determine the prevalence of MRSA during the swine slaughter process in order to better understand the epidemiology of MRSA in pigs from Canada and contamination of retail pork meat with MRSA. A total of 2,641 samples were collected at different points during slaughter and processing at three commercial pork plants designated as A, B and C in Alberta, Canada. Samples sources were; nasal swabs after bleeding (NSAB), nasal swabs after scalding (plant B) or skinning (plant A, C) (NSAS/S), carcass swabs after pasteurization (plant B) or washing (plant A, C; CSAP/W) and retail pork products (RP). MRSA isolation, detection and confirmation was carried out using standard cultural, phenotypic and molecular methods. PCR was used to detect *mec*A gene. Overall MRSA prevalence in all three plants was 24.8%. The MRSA prevalence was 37.5% (330/879), 12.7% (112/882) and 24.2% (213/880) in plant A, B and C, respectively. The MRSA prevalence was highest in NSAB samples (plant A: 77.3%, plant B: 34.7% and plant C: 74.1%), followed by NSAS/S samples (plant A: 48.9%, plant B: 14.1% and plant C: 22.3%). The MRSA prevalence in CSAP/W samples was 20.9%, 1.8% and 0% in plant A, B and C, respectively. In RP samples the MRSA prevalence was very low (≤ 2.1%). The *spa* typing has been performed on 258 isolates from NSAB and NSAS/S. The majority of MRSA isolates from all three plants belonged to pig-associated *spa* types t034 (plant A, 75.6%; plant B, 66.7%; plant C, 73.0%), t002 (plant A, 12.2%; plant B, 33.3%; plant C, 17.3%), and t011 (plant A, 11.1%). The *spa* types t1094, t111, t2971, t4030, t6408 and t777 were also found in <1% of MRSA from plant C. Compared with recent studies of MRSA in pigs on-farm in Canada the results to date show that a higher than expected percentage of incoming pigs carrying MRSA. The results also suggest a substantial reduction in MRSA prevalence through the slaughter process to retail pork products.

18A

EUROPEAN ANTIMICROBIAL SUSCEPTIBILITY SURVEILLANCE IN ANIMALS (EASSA): RESULTS ON ENTERIC BACTERIA FROM HEALTHY PIGS IN THE EU.

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Introduction. The potential transfer of antimicrobial resistance from enteric bacteria to humans raises concern. Several countries have national surveillances of zoonotic and commensal bacteria, but international surveys are rare. EASSA is the first on going programme monitoring the antimicrobial susceptibility of these bacteria from healthy poultry, pigs, and cattle across Europe. Uniform abattoir sampling was applied and susceptibility to human-use antibiotics (ABs) was determined in a central laboratory. Here, we report the results for fattening pigs. **Methods.** Colon samples were randomly collected in 2002-2006 by meat inspectors at several abattoirs/country in Denmark, France, Germany, The Netherlands and Spain. Each herd was sampled once. Samples (usually 300/country) were sent to national

laboratories for isolation of *Escherichia coli*, *Enterococcus* spp., *Salmonella* spp. and *Campylobacter* spp. following standard methods. As the isolation rate of *Salmonella* was low, additional isolates were obtained from existing national collections. Agar dilution testing following CLSI guidelines was conducted. Clinical resistance (CR) was assessed based on CLSI breakpoints (M100-S21; M45-A2), decreased susceptibility (DS) was based on epidemiological cut-off values as defined by EFSA. **Results.** For *E. coli* (n=1543), mean CR rates (%) were: ampicillin (A) 32.2; chloramphenicol 16.5; colistin 0.4; gentamicin (G) 1.5; tetracycline (TE) 87.1; trimethoprim/sulfamethoxazole 40.1. DS was for ciprofloxacin (CIP) and cefotaxime (CTX) 4.0 and 0.5%, respectively; CR to these medically important compounds was very low with 0.4 and 0.2%, respectively. For *Salmonella* (n=420), the low prevalence and the variety of different serotypes limited country comparisons. CR was absent for CIP and 0.2% for CTX (1 isolate); DS was 2.9 and 1.7%, respectively. For *C. coli* (n=1193), a minor human pathogen as compared to *C. jejuni*, erythromycin and CIP resistance were 14.8 and 54.6%, respectively. Whilst CR for G was only 1.4%, CR for TE was 77.3%. DS to CIP was absent. Low numbers of *C. jejuni* (n = 20) precluded evaluation of the ABs susceptibility. Among enterococci, *E. faecium* was the most frequently recovered (n=730) and only a few isolates displayed resistance to A, G or vancomycin. However, quinupristin/dalfopristin (Q/D) CR (32.1%) and DS (29.5%) were high. *E. faecalis* (n=163) was intrinsically resistant to Q/D. All enterococci were susceptible to linezolid. **Conclusion.** This survey, based on uniform methodology, shows that antibacterial resistance among enteric organisms in pigs varies between compounds, organisms and countries. Wide variation was apparent with older ABs, but clinical resistance to most new compounds commonly used to treat food-borne disease in humans was usually either absent or very low. Except for Q/D, decreased susceptibility to critically important antibiotics was always low.

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VERTICAL TRANSMISSION OF MONOPHASIC SALMONELLA TYPHIMURIUM IN PIGS

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Introduction: As one of the most common foodborne diseases to this day according to the World Health Organization, salmonellosis continues to be an important subject of study, and one particular serovar of Salmonella enterica O 4,[5],12:i:- has become an emergent and rapidly disseminated zoonotic bacteria among food-animals, companion animals and humans worldwide. Little is known on the dynamics of this monophasic Salmonella Typhimurium variant in the pig reservoir. **Objectives:** The aim of this study was to determine the likelihood of vertical transmission of monophasic S. Typhimurium from sow to piglet. **Methods:** At one industrial pig herd, 10 litters were randomly chosen. Sows and 7 piglets from each respective litter were sampled at birth. Salmonella spp. was isolated according to the protocol described in ISO 6579:2002 Annex D and serotyped based on the Kauffmann-White-Le Minor scheme. Confirmation of genus was done through invA PCR, and the serotyping results were validated through PCR identification of a 1000 bp IS200 fragment and absence of the second phase flagellar antigen fljB as recommended by the EFSA. Susceptibility testing was performed against 13 antimicrobials through the disk diffusion and broth microdilution methods according to CLSI guidelines. Clinical breakpoints were applied to categorized isolates. All isolates were screened for blaTEM, blaSHV, blaOXA-1, aadA, tetA, tetB, floR, sulI, dfrIa antimicrobial resistance genes through PCR. Clonality was assessed by Pulse Field Gel Electrophoresis (PFGE) with XbaI restriction according to the Pulsenet protocol. The definition of a PFGE cluster was based on a similarity cut-off value of 80%

using the unweighted pair group method (UPGMA). **Results:** Three out of the 10 families were positive to monophasic S. Typhimurium. In family A all animals were positive (mother and 7 piglets). In family B only the sow and 3 out of 7 piglets were Salmonella positive. All except one piglet were found carriers in family C. All monophasic S. Typhimurium isolates had a similar resistance pattern amoxicillin-streptomycin-tetracyclin. Exception made for 2 isolates also resistant to florfenicol, 1 to amoxicillin/clavulanate, and 1 simultaneously resistant to both antimicrobials. All Salmonella isolates from the 3 families harbored the blaTEM and tetB genes. Analysis obtained by PFGE revealed that all isolates had profiles belonging to the same cluster and family B isolates were indistinguishable. **Conclusion:** This study shows that monophasic S. Typhimurium may be transmitted from sow-to-piglet at birth in industrial pig herds. This route of transfer may contribute to the persistence and dissemination of the monophasic S. Typhimurium in the pig reservoir. Further studies are needed to fully assess the dynamics of the monophasic S. Typhimurium during the entire productive pig life cycle and its impact on food-safety and human and animal health.

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SOW-TO-PIGLETS TRANSMISSION OF ESCHERICHIA COLI BLACTX-M-1 AND BLACTX-M-32 GENES: EVIDENCE OF IN VIVO MUTATION?

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Introduction: Genes encoding CTX-M ESBLs are often located in mobile episomes and associated with IS sequences that enhance their mobility and dissemination. Furthermore, CTX-M-32 differs from CTX-M-1 by a single Asp240-Gly substitution responsible for its increased level of resistance to ceftazidime. **Objective:** This study aims to characterize CTX-M-producing E. coli strains isolated from sows and respective piglets. **Methods:** Three sows (A, B and C) and their litters were randomly selected from an industrial pig herd. From each litter 7 piglets were randomly chosen. All piglets and sows (n=24) were sampled at the time of birth. Rectal faecal samples were obtained from sows and swab samples from piglets. After enrichment in buffered peptone water, 100µl of bacterial suspension was inoculated on MacConkey agar supplemented with 1.5µg/ml of cefotaxime (CTX). E. coli strains resistant to CTX were identified by gadA PCR. The blaCTX-M-1Group genes were identified by PCR in all E. coli isolates and the entire genes were sequenced. Clonality was assessed by Pulse Field Gel Electrophoresis (PFGE) with XbaI restriction according to the Pulsenet protocol. The definition of a PFGE cluster was based on a similarity cut-off value of 80% using the unweighted pair group method (UPGMA). **Results:** In family A the E. coli isolates from the sow and one piglet harboured the blaCTX-M-1 gene, while the other 3 piglets had the blaCTX-M-32 genes (3 were ESBL negative). The E. coli isolates from sow B and 2 piglets had the blaCTX-M-32 gene, and 3 piglets the blaCTX-M-1 gene (2 were ESBL negative). E. coli CTX-M-producer isolates from families A and B were clonally unrelated. In family C, we found one mother-piglet E. coli pair of indistinguishable isolates harbouring the blaCTX-M-1 gene and blaCTX-M-32 gene, respectively. At the same time 5 piglets had blaCTX-M-32 gene but only 2 were clonally related and different from all the others (one piglet was ESBL negative). **Conclusion:** In this study blaCTX-M-1Group sequences and PFGE profiles of E. coli commensal isolates between sows and corresponding piglets at birth were compared. The majority of isolates were unrelated suggesting the existence of several E. coli lineages in either the sow's intestinal microbiota or in the environment. The blaCTX-M-1 and blaCTX-M-32 genes have been described as possessing identical genetic surroundings with common IS elements, such as ISEcp1. This may facilitate

the intra-species transmission of episomes and may explain identical ESBL genes in diverse E. coli clones. Yet, one mother-piglet E. coli pair of indistinguishable isolates was detected harbouring blaCTX-M genes with one nucleotide difference. This may suggest in vivo gene mutation during E. coli colonization of the piglet gut. Further plasmid characterization is needed to fully understand the microevolution of ESBL in the pig reservoir.

21A

THE CPXR/CPXA TWO-COMPONENT SYSTEM, A GLOBAL REGULATOR FOR BACTERIAL RESISTANCE TO ANTIMICROBIAL PEPTIDES

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Background: The emergence of multi-drug resistant bacteria has led to an urgent demand for novel therapies to prevent and combat infections. The innovative study of bacterial resistance to cationic antimicrobial peptides (CAMPs) addresses this issue because they function differently than conventional antibiotics to inhibit or prevent bacterial growth and infection. Research in this field has been limited due to the sensitivity of CAMPs to laboratory conditions, unavailability of effective genetic tools, and the lack of a general consensus regarding their mechanisms of action. Identification of novel genetic circuits required for bacterial resistance to CAMPs will have a significant impact in this field of study because it will provide a clear, detailed foundation to decipher how, from a physiological standpoint, bacteria adapt and confer resistance to antimicrobial substances, thus providing a basis for the development of novel drug therapies to combat bacterial infections. **Hypothesis:** The CpxR/CpxA two-component system, which has been traditionally known to respond to periplasmic stress, facilitates Escherichia coli resistance to antimicrobial peptides by activating target genes. **Experimental Methods:** A large scale screen was conducted using the Keio collection of E. coli mutants to identify gene loci required for resistance to a model CAMP protamine. Each mutant strain was individually challenged with protamine. Mutants exhibiting an increase in susceptibility, when compared to the isogenic wild-type, were selected as candidates and further characterized. **Results:** The screen, and subsequent analyses, identified several mutant strains that were susceptible to protamine. Analysis of these loci implicated the Twin-Arginine Translocation (Tat) system as well as CpxR-dependent aroK, degP, dsbA, and yqjA. Various biochemical assays were conducted for several loci candidates and identified a new member of the CpxR regulon, amiC, as well as the functionally similar amiA, both of which are required for resistance to protamine and are transported by the Tat system. In addition, the marRAB operon has been previously implicated in resistance to CAMPs, in part, by up-regulating the AcrAB/TolC efflux pump. Here, we demonstrate that Mar-dependent resistance is mediated by the CpxR/CpxA system because it regulates the mar operon. **Conclusions:** Cumulatively, the data presented identifies multiple CpxR-dependent loci that contribute to bacterial resistance to CAMPs. Further, our data identifies a novel genetic circuitry in which the CpxR/CpxA system serves as a master regulator to mediate E. coli resistance to CAMPs.

22A

FLUOROQUINOLONES RESISTANT CAMPYLOBACTER: UPCOMING CAUSE OF PAEDIATRIC DIARRHEA DURING SUMMER IN INDIA

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Introduction: In developed countries Campylobacter species are a leading cause of zoonotic foodborne gastroenteritis. But in developing countries like India *Campylobacter jejuni*

and *Campylobacter coli* are recently gaining importance as pathogenic organism for diarrhea especially during hot and humid seasons. Ingestion of contaminated food or water is the main cause of acquiring infection. Campylobacter enteritis also has an established correlation with subsequent Guillain Barre´ syndrome (GBS). Fluroquinolones are drug of choice whenever antibiotics are needed to treat diarrhea. Changing antimicrobial resistance pattern is an important public health issue. **Objectives:** To explore the importance of *Campylobacter spp.* in paediatric diarrhea and determine the antimicrobial resistance patterns of isolated strains. **Method:** 200 cases (age≤12years) of acute diarrhea admitted in a tertiary-care hospital were investigated for entero-pathogenic organisms by using routine culture method and enzyme-immunoassay for Campylobacter. All samples were cultured onto charcoal cefoperazone deoxycholate agar (CCDA) directly and also after enrichment under microaerophilic environment using Anoxomat™ Mark II, AN2OP system for Campylobacter isolation. The typical colonies were identified by Modified Gram's staining, oxidase test and speciated using different biochemical tests and antimicrobial susceptibility was assessed by disk diffusion method. **Results:** Among 200 stool specimens cultured 15(7.5%) were positive for *Campylobacter spp.* and all the isolates were *C. jejuni*. 41 samples (20.5%) became positive for Campylobacter specific antigen by ProSpecT™ Campylobacter Microplate Assay® (Oxoid Ltd, UK) including 14 culture positives. Polymicrobial infection was common (33.33%). The frequency of isolation (64.28%) was higher during June to August. 100% resistance to Nalidixic acid and 86.66% to Ciprofloxacin were detected. The frequency of resistance against erythromycin was 20%, gentamicin 13.3%, both amoxycillin and chloramphenicol 6.6%. All strains were sensitive to amikacin and cefotaxime. Eight (53.3%) of the isolates were multiresistant, being resistant to 3 or more antimicrobial agents. **Conclusions:** *C. jejuni* contributed to diarrhea in 21% of cases. Contamination of food and water may be the cause of high transmission during rainy seasons. Increased resistance to quinolones, macrolide and multidrug resistance warrant reconsideration of their use as drugs of choice in patients with severe gastroenteritis when Campylobacter is the presumed cause.

23A

HIGH LEVELS OF EXTENDED SPECTRUM BETA-LACTAMASES PRODUCTION BY DIARRHEAGENIC E. COLI IN NEW DELHI, INDIA

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Background: Rapidly increasing antimicrobial resistance is a constant global threat. Extended Spectrum Beta lactamases that render organisms resistant to first, second and third generation cephalosporins and monobactams worsen the situation. The diarrheagenic Escherichia coli (DEC) are an important cause of gastroenteric diseases in children. In addition to fluid and electrolyte replacement, antimicrobial treatment is recommended for children with more severe disease. The third generation cephalosporins are often resorted to if resistance to other commonly used antibiotics is met. **Objectives:** To identify the role of diarrheagenic E. coli in children and determine their antimicrobial susceptibility pattern. **Methods:** 347 children of age less than 5 years, presenting with acute diarrhea were included in the study. E. coli identified on the basis of cultural characteristics and biochemical reactions were subjected to serotyping by the slide agglutination test with specific antisera (Denka Seiken Co., Ltd., Tokyo, Japan). The pathogenic strains identified were subjected to antimicrobial susceptibility testing and ESBL identification by the VITEK® 2 system, using the AST GN-25 cards and disk diffusion methods in accordance with the CLSI guidelines. **Results:** Of the 347 stool specimens,

pathogenic organisms were isolated in 156 (44.9%) patients. These included 87 (25.1%) DEC, 36 (10.4%) Shigella species, 13 (3.7%) Vibrio cholera, 11 (3.25%) Giardia, 5 (1.4%) Ascaris lumbricoides and 4 (4.6%) Entamoeba hisolytica. Enteropathogenic E. coli was the most frequent among the diarrhea causing E. coli (48, 55.1%), followed by Enterotoxigenic E. coli (29, 33.3%), Enteroaggregative E. coli (9, 10.3%) and Enterohemorrhagic E. coli (1, 1.1%). E. coli showed high levels of resistance to the third generation cephalosporins (68 strains, 78.1%). Out of them 56 strains (64.3% of all DEC) were found to be ESBL producers. High resistance levels were also shown by E. coli for ampicillin (90.8%), cotrimoxazole (75.8%) and ciprofloxacin (72.4%). Single strain showed carbapenem resistance and none were resistant to tigecycline. The data will be discussed in detail. **Conclusion:** DEC were found as the most frequent bacterial cause of diarrhea in children less than 5 years of age, followed by Shigella species and Vibrio cholera. It was also found that significantly high percentages of DEC are ESBL producers, posing a major hurdle in adequate treatment.

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CHARACTERIZATION OF SALMONELLA PHAGES ISOLATED FROM CHICKEN FECES IN KOREA

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Salmonella is responsible for human salmonellosis which is one of three dominant foodborne disease in Korea. Of the Salmonella serovars, S. Typhimurium(ST) and S. Enteritidis(SE) are responsible for the most Salmonella outbreaks implemented in contaminated food consumption. In recent decade, widespread of antimicrobial-resistant Salmonella in foods, food processing environments, and farming area, has been reported. In this study, we isolated virulent bacterophages (Φwksl1 and Φwksl6) from sewage samples in chicken farm in Korea and they can specifically infect Salmonella sp. including S. Typhimurium and S. Enteritidis. Morphological observation was performed by transmission electron microscopy (TEM) and both phages were classified as the family siphoviridae. The host range of the mixture of both phages showed that all the tested SE (n=32) and ST (n=36) strains were clearly controlled (100%) regardless of drug resistance. Tested strains have been isolated from foods (e.g. pork, beef, poultry etc.) and farming area from 2002 to 2010, and most of them were resistant to multiple antibiotics. In addition, other serotypes of Salmonella (e.g. S. Montevideo, S. Infantis etc.) were also controlled by the phages. In a demonstration exam for the phage effect on chicken meat and liquid egg, phage application reduced the number of Salmonella by at least 2-log in a day. Reduction effect of SE clone that produces extended spectrum beta-lactamase CTX-M-15 by Φwksl1 was also confirmed in vitro. For the safety evaluation for the phages, single-dose oral toxicity test was performed in mice (Balb/c) resulting in no clinical observations on gastrointestinal tract without diarrheal symptoms. These findings may suggest that phage would be a useful agent for food protection against SE or ST contamination in various foods and an effective tool to control multidrug resistant Salmonella.

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ANTIMICROBIAL RESISTANT

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Outbreak last year induced by consumption of contaminated cucumber in Europe was a big global issue. This incidence was caused

by widespread of super-bacteria originated from vegetables. Since South Korea is not an exception, in this study vegetables such as lettuce and seasame leaves which frequently consumed by Korean people were investigated. Fresh-cut vegetables and fruits were also included. Comparison between food-borne *Enterococcus* recovered from organic farms in 2010 and conventional farms in 2011 was performed. We obtained 21 *E. faecium* and 105 *E. faecalis* from organic vegetables, 83 *E. faecium* and 77 *E. faecalis* from conventional vegetables in 2010 and 2011, respectively. All the strains were tested for susceptibility to antimicrobial agents by the disc diffusion test (13 antimicrobial agents) and agar dilution method recommended by CLSI guidelines. *E. faecium* isolates from 2010 and 2011 showed resistance to Penicillin, Erythromycin, Ciprofloxacin, Tetracycline and Rifampin. No significant different resistant rates to all the antimicrobial agents tested in this study were observed. But more multi-drug resistant *Enterococcus* was isolated from conventional farms. Four strains isolated from organic farms showed resistance to four antimicrobial agents. However, twenty isolates obtained from conventional farms were resistant to four or more antimicrobial agents tested. Especially, three isolates from conventional farms showed resistance phenotypes to nine antimicrobials. And they also had vanA gene. This result may suggests that the effect of vegetables producing environmental differences between organic farms and conventional farms can cause a different appearance rate of MDR strain. Therefore, appropriate management of vegetable producing environment is important to protect public health.

26A

PHENOTYPICCHARACTERISATION OF ANTIBIOTICS RESISTANCE MECHANISMS IN GRAM NEGATIVE RODS ISOLATED FROM THE COMMUNITY

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Purpose: Antibiotic resistance is now a major public health threat. The dissemination of resistance among Gram negative bacteria is increasingly important. The aim of this survey is to characterize the phenotype of resistance to different family of antibiotics and to evaluate the prevalence of resistance to these antibiotics among Gram negative bacilli recovered from community. **Methods:** Strains of Gram negative bacilli were collected from tow laboratories of medical analyses in the region of Béjaïa (Algeria) between October, 2010 and Mai, 2011. These strains were tested toward fluoroquinolones, β-lactams and aminoglycosides. The phenotypes of resistance were determined by the screening of production of: ESBL, cephalosporinase and oxacillinase enzymes by DD-test for β-lactamines and according to the sensitivity to three molecules of quinolones family and three aminoglycosides. **Results:** During our study, 423 Gram negative bacilli were collected from medical laboratories analysis. 401 strains were *Enterobacteriaceae*, 18 strains were *Pseudomonas aeruginosa* and only 4 strains were *Acinetobacter baumannii.* Rates of antibiotics resistance in *Enterobacteriaceae* were 26.43% to nalidixic acid, 15.96 % to ciprofloxacin, 8.47 % to the third generation of cephalosporins, 5.73% to the fourth generation of cephalosporins, 7.48% to gentamycin and none to carbapenem. No resistance is observed in *Pseudomonas aeruginosa* to ceftazidim, to aztreonam, to the fourth generation of cephalosporins, carbapenem and colistin. A rate of 11.11% is observed to ciprofloxacin and gentamycin. Finally, 5.55% are resistant to tobramycin and amikacin. Only two strains of *Acinetobacter baumannii* were resistance to imipenem. **Conclusion:** Increasing importance antibiotics resistance among Gram negative rods in our region gives causes for great concerns. **Key words:** Resistance to antibiotics, Gram negative bacilli, community, mechanism of resistance.

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PRELIMINARY CHARACTERIZATION OF BIOACTIVES FROM MIRACLE FRUIT (SYNSEPALUM DULCIFICUM) LEAVES AGAINST KLEBSIELLA PNUEMONIAE

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Medical progress in the prevention and treatment of infectious diseases are now at risk with the emerging multi-drug resistant (MDR) pathogens such as *Klebsiella pnuemoniae.* The current investigation aims to characterize the bioactive compounds from *Synsepalum dulcificum* leaves against *Klebsiella pneumoniae*. Liquid/liquid separation, open-column chromatography and thin layer chromatography was the method used in purification. Bioautography was the method for screening the antibacterial activity of compounds present on TLC plate was conducted for bioassay against the test organism. Characterization of the potent fraction was done using infrared spectroscopy and nuclear magnetic resonance spectroscopy. Based on results F3 (135 ml toluene: 15 ml acetone) and F4 (120 ml toluene: 30 ml acetone) fractions showed antibacterial activity in the bioautogram after it was stained with MTT. Both fractions F3 & F4 have five positives (+++++ zone of inhibition is highly visible) meaning the compounds present in these fractions are effective against the test organism. Based on spectroscopic results, F3 contains both aromatic and aliphatic functions, with main absorptions of greater and below 3000 cm^{-1} . During NMR analysis,the sample was found to be impure thus the structure of the compounds cannot be elucidated yet. However, distinct signals are still evident due to the ^{1}H NMR signals at 7.40-7.43 ppm, 7.53-7.55 ppm, and 8.03-8.05 ppm. The signals in this region are for the aromatic benzene.The signal at 0.74-2.37 ppm indicate aliphatic methyls (CH3), methylenes (CH2), and methyne (CH) protons.The ^{13}C spectrum also corroborated the signals of the ^{1}H NMR. The peaks at 128.4 and 129.7 ppm indicate aromatic carbons. The peaks of 64.0 ppm and 69.2 ppm indicate methylene or methyne carbons which are directly attached to an oxygen or nitrogen. The result on bioassay proves that *Synsepalum dulcificum* leaves are potential source of antibiotics against *K. pnuemoniae*.

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ANALYSIS OF EXTENDED-SPECTRUM Β-LACTAMASE (ESBL) GENE CARRYING PLASMIDS FROM *ESCHERICHIA COLI* ISOLATES COLLECTED IN THE GE*RM*-VET MONITORING PROGRAM IN GERMANY

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Background: The aims of this study were (i) to detect extended-spectrum β-lactamase (ESBL) genes among *Escherichia coli* isolates from defined disease conditions of companion and farm animals and (ii) to determine the localisation and organisation of these genes. **Materials and Methods:** In total, 1378 *E. coli* isolates from the national resistance monitoring program GE*RM*-Vet collected in 2006/2007 were included in this study. ESBL-producers were identified by phenotypic confirmatory tests. Plasmids were transferred and typed by PCR-based replicon typing. ESBL genes were detected by PCR and Southern blot hybridisation. Sequencing was done by primer walking. Multilocus sequence typing was performed for all ESBL-producers. **Results:** Twenty-seven ESBL-producing *E. coli* isolates were identified. They originated from gastrointestinal (n=11) and urogenital (n=1) tract infections from swine, gastrointestinal (n=11) and urogenital (n=1) tract infections

from cattle, septicaemia from poultry (n=1) and equine urogenital tract infection (n=1). Five different ESBL genes were detected. The $bla_{CTX\text{-}M\text{-}1}$ genes were present on IncN (n=16), IncF (n=3), IncI1 (n=2) or multireplicon (n=1) plasmids. The single $bla_{CTX\text{-}M\text{-}3}$ or $bla_{CTX\text{-}M\text{-}15}$ genes were located on an IncN or IncF plasmid, respectively. A multireplicon and an IncHI1 plasmid carried $bla_{CTX\text{-}M\text{-}2}$. The $bla_{TEM\text{-}52c}$ gene was identified on an IncI1 plasmid. The $bla_{CTX\text{-}M\text{-}1}$ group genes were located within similar genetic contexts with differences due to insertion sequences. The same regions directly flanking $bla_{CTX\text{-}M\text{-}1}$ with a truncated IS*Ecp1* upstream and a Φ*rf477* followed by Φ*urx* downstream was seen in 17 plasmids. In one plasmid the $bla_{CTX\text{-}M\text{-}1}$ gene was immediately followed by an IS*26*; in another, the truncation of the upstream IS*Ecp1* occurred at a novel position. Three plasmids showed a complete IS*Ecp1* upstream of $bla_{CTX\text{-}M\text{-}1}$ and a Φ*rf477* downstream. The $bla_{CTX\text{-}M\text{-}3}$ gene was associated upstream with an IS*Ecp1* disrupted by a novel IS*91*-like insertion sequence and a Φ*rf477* downstream. The $bla_{CTX\text{-}M\text{-}15}$ gene was flanked by IS*Ecp1* and a Φ*rf477*. The $bla_{CTX\text{-}M\text{-}2}$ genes were embedded within complex class 1 integrons, whereas the $bla_{TEM\text{-}52c}$ gene was located within Tn*2*. The *E. coli* isolates belonged to various sequence types with ST10 (n=7), ST167 (n=4) and ST100 (n=3) most commonly identified. **Discussion and Conclusions:** This study showed that $bla_{CTX\text{-}M\text{-}1}$ is the predominant ESBL gene among animal *E. coli* isolates in Germany and that similar genetic environments occur in different plasmids. Very often insertion sequences or transposons seem to be involved in the ongoing alterations of the ESBL gene regions. Moreover, a wide variety of *E. coli* sequence types harboured ESBL genes, indicating plasmid transfer rather than clonal spread.

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SIMILARITY BETWEEN ANTIMICROBIAL RESISTANCE PROFILES OF LA-MRSA ISOLATES FROM DIFFERENT ANIMAL SPECIES, ENVIRONMENT AND HUMANS ON THE SAME FARMS.

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Antimicrobial resistance is an emerging problem in bacterial isolates from animals due to an increase in antibiotic use. Many reports have established that livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has emerged in a wide variety of animal species. The aim of this study was to investigate the antimicrobial resistance of MRSA isolates collected from different animal species, environment, farmers, household members and veterinarians on 2 pig, 2 poultry-pig and 2 dairy cow-pig farms in Belgium. After sampling, all swab specimens were further processed as described in Pletinckx et al., 2011. Susceptibility to 16 antimicrobial agents was tested by disk diffusion method based on the CLSI guidelines (CLSI, 2010) with use of Neo-sensitabs. Antimicrobial susceptibility testing on the selection of MRSA isolates (n=453) originating from pig and non-pig origin revealed the presence of 53 different antibiotic profiles. All MRSA isolates tested showed 100% resistance to tetracycline. Also a high resistance rate to trimethoprim (76%), lincomycin (66%) and ciprofloxacin (57%) was found. Against erythromycin, tylosin, gentamicin, kanamycin and tobramycin a

resistance rate varying between 28-37% was found in the selection of MRSA isolates, while for chloramphenicol, quinupristin/dalfopristin and sulphonamide this was between 9-17%. Furthermore, MRSA isolates showed a low resistance against fucidin (2.5%) and rifampicin (0.06%). No resistance was observed against linezolid and mupirocin, which are not in veterinary use but important antimicrobials for the treatment of human MRSA infections. A high similarity (>70%) was seen between antibiotic profiles from pigs and pig barn environment, dogs, rats/mice, farmers, family members (direct contact) but also with antibiotic profiles from broilers (indirect contact). Less similarity (≤55%) in antibiotic profiles was seen between pigs and purchased gilts, cattle and herd veterinarians. Although a high diversity was seen in antimicrobial resistance profiles among the MRSA isolates tested per farm, similarity was seen between different origins suggesting MRSA transfer from pigs. The high resistance rate of LA-MRSA isolates from animal origin to several antimicrobials implements the importance of continual monitoring and prudent use of antimicrobials in livestock. Pletinckx et al., Infect. Genet. Evol., 2011 CLSI, 2010

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COMPARISON BETWEEN LIVESTOCK AND COMMUNITY ASSOCIATED MRSA IN EUROPE

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Background: Community associa:ed (CA)-MRSA has been in the latest years a major issue in Public Health, showing increasing prevalence worldwide, including Europe and representing a challenge to infection control. Meanwhile, Livestock associated (LA)-MRSA emerged and have been found widespread among livestock populations being considered an emerging threat to Public Health, due to occupational transmission to humans. As part of the European Project CONCORD which focused on the study of CA and LA-MRSA populations in Europe, we have collected strains from human and animal origin in different countries in order to shed light on the origin and evolution of CA and LA-MRSA. **Methods:** A total of 180 CA-MRSA isolates from 16 countries and 586 LA-MRSA (486 from pigs and 100 from cattle) from nine countries in Europe were collected. Species identification was confirmed by PCR and/or biochemical reactions. Isolates were typed by *spa* typing and MLST. Clonal complexes (CC) were defined based on the Ridom *spa* server data and/or confirmatory MLST. PVL was tested for all human isolates. All human isolates and a sample of LA-MRSA were tested for antimicrobial susceptibility. SCC*mec* typing was performed in all CA-MRSA and in 135 selected LA-MRSA. Additional subtyping was performed for type IV elements. In addition, *ccrC* was sequenced in a subset of isolates from both populations harbouring SCC*mec* type V. The presence of *czrC* was tested by PCR in LA-MRSA **Results:** The most prevalent genetic lineages among CA-MRSA were CC8 (40%), followed by CC80 (28%) and CC59 (15%) Among the LA-MRSA 94% of the isolates belonged to CC398, while only 6% belonged to other CC (1, 5, 9 and 97). CC398, CC97 and CC1 were identified in CA and LA-MRSA isolates, but in different proportions. In addition, we found that some MRSA isolates collected from humans and animals belonged to the same clones (ST398-VII; ST5-IVa), but harboring different *spa* types. The distribution of SCC*mec* showed a higher prevalence of type IV elements in CA-MRSA, whereas the type Vc (C2&5) was predominant in LA-MRSA. Sequencing of *ccrC* alleles demonstrated similarities between LA-MRSA SCC*mec* and CA-MRSA and methicillin-resistant coagulase-negative staphylococci (MRCoNS) from humans. **Discussion and conclusions:** These results suggest that dissemination of MRSA between the farm animals and humans

can occur, but is not very frequent. Moreover, the results indicate that CoNS may be acting as donors of SCC*mec* to LA-MRSA.

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INTEGRATED SALMONELLA SURVEILLANCE IN ITALY: THE ENTER-NET AND ENTER-VET NETWORKS

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Salmonella has long been recognised as an important zoonotic pathogen of health and economic significance in animals and humans. In Italy the medical and veterinary integrated surveillance of salmonellosis is represented by two networks named EnterNet and EnterVet which collect human and veterinary isolates respectively. The two networks use harmonised methods for typing and share their databases. Here we report the distribution of Salmonella serotypes reported to the Enter-net and Enter-vet during 2007-2010 with a particular focus on antimicrobial multiresistant strains. Among human isolates, S.Typhimurium (ST) represents the most frequent serovar. During the last 2 years the number of human cases associated to the monophasic variant of ST (STM) increased and in 2010 it accounted for about 20% of all human serovars. More than 50% of ST human strains were resistant to ampicillin, sulfonamide, streptomycin and tetracycline, (R-type ASSuT), whereas the strains resistant also to chloramphenicol (R-type ACSSuT) were decreasing and accounted for 11.3% in 2010. Among STM the prevalence of strains with R-type ASSuT reached 87%. As far as veterinary isolates are concerned, up to 2009 ST was the most common serovar, followed by STM, whereas in 2010 an opposite situation was observed and STM became the first serovar, followed by ST. For these two serovars a high level of antimicrobial resistance was recorded and, in 2010, 60.2% and 88.2% of ST and STM strains were multiresistant. In 2010 more than 70.5% of STM veterinary isolates showed the R-type ASSuT. The same profile was common also among ST isolates (16.1% in 2010), but for this last serovar the most frequent R-type was ACSSuT, that accounted for 21.4% in 2010. Results of phagetyping and pulsed field gel electrophoresis (PFGE) show that among multiresistant ST different clones are circulating both in humans and animals, whereas R-ASSuT STM of human and veterinary origin seem to be highly clonal: more than 60% strains belong to phagetype DT193 and U311 and STYMXB.0079 and STYMXB.0131 are the prevalent PFGE patterns. The Italian surveillance integrated network for Salmonella represents an important database for the study of Salmonella infection epidemiology. Epidemiological data together with serotyping, phagetyping and molecular typing of Salmonella isolates from human and animal sources provide further information for a better estimate of risk factor for human infections.

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EFFECTS OF INDOLE ON DRUG RESISTANCE AND VIRULENCE OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM REVEALED BY GENOME-WIDE ANALYSES

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Background: Many Gram-positive and Gram-negative bacteria produce large quantities of indole as an intercellular signal in microbial communities. Indole demonstrated to affect gene expression in *Escherichia coli* as an intra-species signaling molecule. In contrast to *E. coli*, *Salmonella* does not produce indole because it does not harbor *tnaA*, which encodes the enzyme responsible for tryptophan metabolism. Our previous study demonstrated that *E.*

coli-conditioned medium and indole induce expression of the AcrAB multidrug efflux pump in *Salmonella enterica* serovar Typhimurium for inter-species communication; however, the global effect of indole on genes in *Salmonella* remains unknown. In this study, we sought to resolve the effect of indole on gene expression and phenotypes in the *S. enterica* serovar Typhimurium. **Methods:** To understand the complete picture of genes regulated by indole, we performed DNA microarray analysis of genes in the *S. enterica* serovar Typhimurium strain ATCC 14028s affected by indole. Predicted *Salmonella* phenotypes affected by indole based on the microarray data were also examined in this study. **Results:** Indole induced expression of genes related to efflux-mediated multidrug resistance, including *ramA* and *acrAB*, and repressed those related to host cell invasion encoded in the *Salmonella* pathogenicity island 1, and flagella production. Reduction of invasive activity and motility of *Salmonella* by indole was also observed phenotypically. **Conclusion:** Our results suggest that indole is an important signaling molecule for inter-species communication to control drug resistance and virulence of *S. enterica*.

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FIRST DETECTION AND MOLECULAR CHARACTERIZATION OF METHICILLIN-RESISTANT- *STAPHYLOCOCCUS AUREUS* (MRSA) ST398 AND *S. PSEUDINTERMEDIUS* (MRSP) ST68 IN DISEASED HORSES IN SPAIN

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Background: MRSA and MRSP are emerging pathogens of different animal species. Pigs are the major host for MRSA ST398 with a worldwide distribution. MRSP is mainly isolated from dogs, with MRSP ST71 and ST68 as the predominant lineages detected in Europe and North America, respectively. No data is available on their presence in horses in Spain. The objective of this study was to identify and characterize MRSA and MRSP strains obtained from diseased horses in Spain. **Material and Methods:** five MR coagulase positive staphylococci (MRCoPS) from clinical samples were included. Strains corresponded to all MRCoPS received from horses in a veterinary hospital between 2005 and 2011 and were recovered from 5 animals with different pathologies. Strains were recovered in 2005 (1 strain), 2008 (1), 2010 (1), and 2011 (2). Molecular identification and presence of *mec*A gene were confirmed by PCR and/or PCR-RFLP. Typing by *spa-* , *agr-*, SCC*mec*- and MLST was undergone in all strains. Susceptibility to 17 antimicrobial agents was determined by disc-diffusion. Presence of antimicrobial resistance genes and potential physical linkages when suspected, were analyzed by PCR. The presence of 18 enterotoxin genes, in addition to *lukS/F-PV, lukE/D, lukM, eta, etb, tst* and *cna* virulence genes for MRSA and *lukS/F-I, siet, expA, expB, si-ent* and sec_{canine} genes for MRSP were investigated by PCR. **Results:** Four strains were identified as MRSA and one as MRSP. All MRSA were typed as t011(*spa*)-I(*agr*)-ST398(MLST)-SCC*mec*IVa and the MRSP as t06-IV-ST68-SCC*mec*V_T(5C2&5). All strains were multidrug resistant (MDR). Resistance to the following families of antimicrobials and corresponding resistance genes detected in MRSA strains were as follows (number of isolates): β-lactams/*mec*A and *blaZ* (4), tetracycline/*tet*(K) (4), aminoglycosides/*aacA-aphD* (4) and *str* (1) and thrimethoprim/*dfrK* (3) and *dfrG* (2). Moreover,those detected in MRSP strain were: β-lactams/*mec*A and *blaZ*, tetracycline/*tet*(K), macrolides-lincosamides/*erm*(B), aminoglycosides/*aacA-aphD, aphA3* and *aadE*, thrimethoprim/*dfrG*. In addition, MRSP strain was resistant to fluoroquinolones and also harbored the *sat4* gene within the resistance-gene-cluster *aadE-sat4-aphA3* and was physically linked to *erm*(B). All MRSA ST398 were positive for the *cna* gene whereas toxin genes *lukS/F-I*, *siet*, and *si-ent* were present in our MRSP strain. **Conclu-**

sions: MRSA ST398 and MRSP ST68 can be present in horses as etiological agents of infection. All strains were MDR - especially our MRSP - what implies limited therapeutic options for treatment. This is the first description of MRSA ST398 and MRSP ST68 in horses in Spainand highlights the emergence of these MDR bacteria among different animal species.

34A

ANTIMICROBIAL SUSCEPTIBILITY IN *SALMONELLA* ISOLATES FROM YELLOW-LEGGED GULLS (*LARUS MICHAHELLIS*) FEEDING IN A REFUSE DUMP.

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Salmonella is one of the leading causes of zoonotic bacterial enteric infections worldwide. Due to their scavenging feeding habits, some seagull species have often been reported as carriers of zoonotic enteropathogens, including *Salmonella*. Moreover, because of their great mobility, they may act as effective spreaders of disease through faecal contamination of the environment. To assess the potential role as a reservoir of *Salmonella* and of antimicrobial resistance, 100 adult yellow-legged gulls feeding at a refuse dump close to an urban area in Spain were captured and cloacal swab samples were obtained. *Salmonella* isolation was performed by standard culture methods. Twenty *Salmonella* strains isolated from 17 yellow-legged gulls were tested for their antimicrobial susceptibility to a panel of 18 antimicrobials commonly used in human or veterinary medicine. The disc diffusion method with Neo-Sensitabs™ (Rosco Diagnostica) discs was used. A 17% of *Salmonella* prevalence was detected and a high diversity of serovars were isolated, including some of public health importance such as Typhimurium, Hadar, Montevideo and Rissen. Up to three different serotypes were isolated from some gulls. Half of the isolates showed resistance to at least one antimicrobial. The main resistances found were to ceftiofur, followed by some other β-lactams, streptomycin, nalidixic acid, tetracycline and nitrofurantoin. Multiresistance was detected in 2 strains belonging to serovars Hadar and Kapemba, with resistances to 5 and 8 drugs, respectively. Yellow-legged gulls from the studied area are carriers of resistant and multidrug resistant *Salmonella* serovars. The colony is near urban areas and beaches, and thus may contaminate them. The risk of spilling over to humans should be of public health concern.

35A

INVESTIGATION OF PLASMID-BORNE ESBL GENES AND CO-LOCATED RESISTANCE GENES IN *ESCHERICHIA COLI* STRAINS FROM FOOD-PRODUCING ANIMALS COLLECTED IN THE GE*RM*-VET PROGRAM

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Background: Food-producing animals are an important reservoir of antimicrobial-resistant zoonotic bacterial pathogens. The aims of this study were to identify the types of ESBL genes and their location on plasmids among *Escherichia coli* isolates collected in the national resistance monitoring program GE*RM*-Vet during the year 2008. **Materials and Methods:** A total of 6/429 *E. coli* from poultry and 23/92 *E. coli* from calves were identified as ESBL-producers by phenotypic tests. All ESBL-producing isolates from poultry and eleven unrelated ESBL-producing isolates from calves were characterized by XbaI macrorestriction analysis (PFGE), screened for subtypes of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes

by PCR with subsequent sequence analysis and tested for susceptibility to 28 antimicrobial agents by broth microdilution and/or disk diffusion. Transformation and conjugation experiments were performed and transformants or transconjugants were analysed for the respective ESBL gene by PCR and sequence analysis of amplicons. Plasmids were characterized by S1 nuclease PFGE, PCR-based replicon typing and restriction analysis. **Results:** XbaI PFGE patterns showed no clonal relationship among the 17 isolates. Almost all calf strains belonged to the phylogenetic group A. The poultry isolates were distributed among groups A, B1 and B2. ESBL genes were located on plasmids of 35-230 kb in five poultry and all eleven bovine isolates. The gene $bla_{CTX\text{-}M\text{-}1}$ was the predominant ESBL gene. In four poultry isolates, $bla_{CTX\text{-}M\text{-}1}$ genes were located on IncI1 plasmids while the single $bla_{TEM\text{-}52}$ gene was on an IncI1/$IncF_{repB}$ plasmid. The seven bovine $bla_{CTX\text{-}M\text{-}1}$-carrying plasmids belonged to different incompatibility groups (IncN, $IncF_{repB}$, IncB/O, IncI1). The remaining four bovine isolates carried $bla_{CTX\text{-}M\text{-}2}$ (IncHI1), $bla_{CTX\text{-}M\text{-}14}$ (IncI1) or $bla_{CTX\text{-}M\text{-}15}$ (IncI1, $IncF_{repB}$/IncFIB). Unrelated restriction patterns were seen among plasmids of the same incompatibility group and/or of similar size. The most commonly co-located resistance genes were *sul2* and *dfrA17*, which encode resistance to sulfonamides and trimethoprim, respectively.**Conclusions:** Most ESBL genes identified in this study were located on plasmids which facilitates their dissemination across species and genus borders. The presence of additional resistance genes on the ESBL-carrying plasmids indicates that co-selection and persistence of ESBL genes may also occur under the selective pressure of non-β-lactam antimicrobial agents, such as sulfonamides and trimethoprim, which are commonly used in veterinary therapy.

36A

ELEVATED MINIMUM INHIBITORY CONCENTRATIONS OF TILDIPIROSIN AND GAMITHROMYCIN AMONG BOVINE *PASTEURELLA MULTOCIDA* AND *MANNHEIMIA HAEMOLYTICA* THAT CARRY THE GENES *ERM* (42) AND/OR *MSR*(E)-*MPH*(E)

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Background: Macrolides play an important role in the treatment of bovine respiratory disease (BRD). Two new macrolides have been approved for the treatment of BRD in 2011: the 15-membered macrolide gamithromycin and the 16-membered macrolide tildipirosin. The aim of this study was to determine whether the recently identified ICE*Pmu1*-associated macrolide resistance genes *erm*(42) and *msr*(E)- *mph*(E) have an effect on minimum inhibitory concentrations (MICs) of gamithromycin and tildipirosin. **Materials and Methods:** The genes *erm*(42) and *msr*(E)-*mph*(E) were cloned separately and expressed in the *Pasteurella multocida* recipient strain B130. These clones and the recipient strain were tested comparatively for their MICs. In addition, naturally occurring *P. multocida* (n=32) and *Mannheimia haemolytica* isolates (n=22) from BRD cases which carry the genes *erm*(42) and/or *msr*(E)- *mph*(E) were tested for their MIC values of gamithromycin and tildipirosin. **Results:** In the *P. multocida* B130 clone carrying erm(42), the MIC of tildipirosin increased 128-fold to 32 mg/L while that of gamithromycin increased only 16-fold to 4 mg/L. In the *P. multocida* B130 clone carrying *msr*(E)-*mph*(E), an opposite observation was made: the MIC of tildipirosin increased only 8-fold to 2 mg/L while that of gamithromycin increased 256-fold to 64 mg/L. *P. multocida* field isolates that carried all three genes showed MIC

values of 16-64 mg/L for gamithromycin and 16-32 mg/L for tildipirosin while similar MIC values of 32-64 mg/L for both macrolides were seen among the *M. haemolytica* field isolates carrying all three resistance genes. The ten *P. multocida* isolates that carried only *erm*(42) exhibited low MICs of 2-4 mg/L for gamithromycin but had higher MICs of 16-32 mg/L for tildipirosin. The single *M. haemolytica* that harboured only erm(42) showed MIC values of 4 mg/L and 32 mg/L for gamithromycin and tildipirosin, respectively. The two *P. multocida* isolates that carried only *msr*(E)- *mph*(E) exhibited a high MIC of 32 mg/L for gamithromycin and a low MIC of 2 mg/L for tildipirosin. **Conclusions:** The analysis of *P. multocida* and *M. haemolytica* field isolates from BRD cases confirmed the results obtained with the cloned *erm*(42) and *msr*(E)-*mph*(E) amplicons. Pronounced increases in the gamithromycin MIC values were seen in the presence of *msr*(E)-*mph*(E) whereas distinct increases in the tildipirosin MICs were detected in the presence of *erm*(42). Isolates that carry all three genes showed elevated MICs to both new macrolides.

37A

RESISTANCE PHENO- AND GENOTYPES OF MRSA ISOLATES FROM BROILER CHICKENS AT SLAUGHTER AND FROM ABATTOIR WORKERS

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Background: Livestock-associated MRSA can readily cross species barriers and also colonize or cause infections in persons with occupational exposure to livestock. In the present study, MRSA from broiler chickens and from abattoir workers, who worked at the respective poultry slaughterhouses, were comparatively investigated for their multilocus sequence types, *spa* types, resistance pheno- and genotypes. **Materials and Methods:** A total of 47 MRSA isolates (28 from broiler chickens and 19 from humans) obtained from four different slaughterhouses were included in this study. The chicken isolates from each slaughterhouse were from animals of one to four different flocks. Multilocus sequence typing and *spa* typing followed standard procedures. Resistance phenotypes were determined by broth microdilution according to CLSI recommendations. Resistance genotypes were determined using a *S. aureus*-specific DNA microarray and specific PCR assays for additional resistance genes. **Results:** In part different resistance pheno- and genotypes were observed among broiler chickens and abattoir workers at each of the four slaughterhouses. Regardless of their flock origin, 23 of the 28 avian MRSA represented ST398/t011 while two isolates were ST398/t108 and three isolates were ST9/t1430. A slightly higher heterogeneity was seen among the isolates of the abattoir workers with three isolates representing ST9/t1430, two isolates ST1453/t4652, one isolate ST1454/t238, one isolate ST398/t034 and the remaining 12 isolates ST398/t011. A comparison of the resistance patterns revealed that all ST398, ST1453 and ST1454 isolates and some of the ST9 isolates from chickens and humans showed resistance to 4 - 9 classes of antimicrobial agents and carried a wide range of resistance genes known to occur in staphylococci. While the resistance pheno- and genotypes of the chicken isolates of the same flock were closely related, they usually differed from the corresponding resistance pheno- and genotypes

of the isolates from the workers at the respective abattoir. However, the chicken and human MRSA ST9/t1430 from slaughterhouse one were only resistant to beta-lactams and fluoroquinolones and carried only the genes *blaZ* and *mecA*. **Conclusions:** The apparent homogeneity of MRSA isolates from the same flock suggests exchange of isolates between the respective animals. The apparent heterogeneity of MRSA from abattoir workers might reflect the occupational contact with animals from numerous chicken flocks.

38A

PREVALENCE OF LIVESTOCK-ASSOCIATED MRSA ON DUTCH BROILER FARMS AND PEOPLE LIVING AND/OR WORKING ON THESE FARMS

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Objective: Although the prevalence of MRSA on pig and veal calf farms has been extensively studied, to date accurate estimates on the prevalence of MRSA on Dutch broiler farms are lacking. The objectives of our study were to estimate the prevalence of MRSA-positive broiler farms and the prevalence of MRSA-carriage of broiler farmers, their family members and employees and to identify and quantify risk factors. **Material and Methods**: Fifty broiler farms were sampled. All broiler houses on these farms were sampled by taking 5 dust samples. In addition, on each farm a randomly selected flock was sampled by taking throat samples from 60 broilers (pooled to 5 samples). Dust samples were also taken from the farm residence. Persons were sampled by taking a nose swab. Farmers filled in a questionnaire on farm management. Samples were cultured using pre-enrichment and selective enrichment. Detection of *mec*A was done by PCR. All MRSA isolates were *spa*-typed. **Results and discussion**: Investigation of 250 pooled throat samples of broilers and 755 dust samples resulted in 4 farms where MRSA-positive samples were present (8.0%). Of 145 persons living and/or working on these farms, 8 tested MRSA-positive (5.5%). This concerned 4/47 farmers, 3/89 family members and 1/9 employees. On MRSA-positive farms 5/18 dust samples of the residence were MRSA positive, whereas 0/215 samples on MRSA-negative farms. All MRSA isolates belonged to CC398. *Spa*-types found were t011, t034, t108, t899 and t3015. Living and/or working on MRSA-positive farms was a risk for MRSA-carriage; 66.7% of people on positive farms were MRSA-positive vs. 1.5% on negative farms ($P<0.0001$). Due to the low number of positive farms and persons and high similarity in farm management, it was impossible to draw statistically valid conclusions on other risk factors. For broiler farming, both farm and human MRSA-prevalence seems much lower than for pig or veal farming. However, MRSA-carriage of people living and/or working on broiler farms is increased compared to the general Dutch population (5.5% vs. <0.1%).

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CHRONOLOGICAL CHANGE OF RESISTANCE TO B-LACTAMS IN SALMONELLA ENTERICA SEROVAR INFANTIS ISOLATED FROM BROILERS IN JAPAN

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Nontyphoidal Salmonella enterica serovars are a major cause of bacterial food-borne diseases world-wide and the emergence and spread of the antimicrobial resistance pose a threat to public health. Since the late 1990s, Salmonella enterica serovar Infantis (S. Infantis) has been

the commonest serovar of Salmonella species isolated from both broiler flocks and retail chicken meat in Japan. During 2004 and 2006, extended-spectrum cephalosporins-resistant S. Infantis isolates producing ESBL TEM-52 were recovered from broilers in Japan for the first time. In this study, epidemiologic surveillance was conducted in southern Japan to determine the chronological shift of antimicrobial resistance phenotypes and characterize the β-lactamase genes and the plasmids harboring these genes in S. Infantis isolates from broilers. Between January, 2007 and December, 2008, a total of 1,472 fecal samples were collected and examined at the Laboratory of Veterinary Public Health, Kagoshima University, Japan. In 93 (6.3%) S. Infantis isolates recovered, 33 (35.5%) isolates showed resistance to cefotaxime, an extended-spectrum cephalosporin (ESC), conferred by TEM-20, TEM-52 and CTX-M-25 extended-spectrum β-lactamases (ESBLs). The percentage of CTX-resistance was higher than that (24%) of the isolates during 2004 to 2006. In addition to ESC-resistance, eight (8.6%) isolates exhibited resistance to cefoxitin mediated by CMY-2 AmpC β-lactamase. Plasmid analysis and polymerase chain reaction replicon typing revealed the blaTEM-20 and blaCMY-2 genes were associated with IncP plasmids, blaTEM-52 was linked with non-typable plasmids and blaCTX-M-25 was carried by IncA/C plasmid. Non- β-lactam resistance to streptomycin, sulfamethoxazole and oxytetracycline encoded by the aadA1, sul1 and tet(A) genes, respectively, was found in 86 (92.5%) isolates. Resistance to kanamycin and ofloxacin was exhibited in 12 (12.9%) and 11 (11.8%) isolates, respectively, the former was mediated by aphA1-Iab. These data indicate that S. Infantis isolates producing ESBLs and AmpC β-lactamase have increasingly spread among broiler farms in Japan. At the best of our knowledge, the blaCMY-2, blaTEM-20 and blaCTX-M-25 genes harbored by S. Infantis isolates from broilers have not previously reported in Japan.

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DIVERSITY OF B-LACTAMASE-ENCODING GENES IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM FOOD-PRODUCING, COMPANION AND ZOO ANIMALS IN PORTUGAL

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Background: A rapid development of plasmid-mediated resistance to extended-spectrum cephalosporins has been observed in *Enterobacteriaceae* worldwide, predominantly due to the dissemination of extended-spectrum β-lactamases (ESBL) and plasmid-mediated AmpC β-lactamases (PMAβ). The aim of the present study was to evaluate the extension of ESBL- and PMAβ-producing *E. coli* strains isolated from different animal origins in Portugal. **Materials and Methods:** For surveillance purposes, 376 *E. coli* isolates identified at National Laboratory of Veterinary Research (2009-2011) were submitted to antimicrobial susceptibility testing: 123, 51 and 202 were isolated from food-producing, companion and zoo animals, respectively. Minimum Inhibitory Concentrations (MIC) of 11 antimicrobials for all isolates was determined through agar dilution method. Susceptibility towards cefoxitin was determined through disk diffusion method. Breakpoints were interpreted accordingly to EUCAST epidemiological cut-off values. 'Non-wild type' (NWT) isolates for cefotaxime (MIC>0.25mg/L) and/or cefoxitin (<19mm) were screened for the presence of ESBL (bla_{TEM}, bla_{OXA}, bla_{SHV}, bla_{CTX}) and PMAβ encoding genes, using PCR method. Sequencing was applied to fully identify β-lactamases. **Results:** Seventeen isolates (4.5%) were 'NWT' strains for cefotaxime, being 5 (29.4%) from companion animals, 4 (23.5%) from food-producing

animals and 8 (47.1%) from zoo animals. We identified $bla_{CTX\text{-}M\text{-}14}$ (n=1) in a dog and $bla_{CTX\text{-}M\text{-}15\text{-}type}$ genes (n=9) in 6 zoo animals and 3 in food-producing animals. We also identified $bla_{CMY\text{-}type}$ genes (n=3) in 'NWT' isolates for cefoxitin, one from each animal category. Other β-lactamase encoding genes were identified: bla_{OXA} in 5 strains (29.4%) isolated from dolphins, bla_{TEM} in 7 strains (41.2%) isolated from 3 companion animals, 2 food-producing and 2 zoo animals, and bla_{SHV} identified in one isolate (5.9%) from a zoo animal; 13 β-lactamase-producing isolates (76.5%) were multidrug resistant. **Conclusion:** Among 'NWT' *E. coli* isolates for cefotaxime, we identified an important diversity of ESBL encoding genes, belonging to different families, being $bla_{CTX\text{-}M\text{-}15\text{-}type}$ gene the predominant. The spread of ESBL-producing bacteria among species from different origins, such as food-producing, companion and zoo animals, is a concern at public health level. Thus, it should be a priority to monitor and identify the reservoirs of antimicrobial resistance, contributing to a single health for all.

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PLASMID-MEDIATED QUINOLONE RESISTANCE GENES IN ENTEROBACTERIACEAE BACTERIA FROM ROOKS (*CORVUS FRUGILEGUS*) COMMONLY WINTERING THROUGHOUT EUROPE

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This study concerned the occurrence of Enterobacteriaceae bacteria with plasmid-mediated quinolone resistance (PMQR) genes in rooks (*Corvus frugilegus*, medium-sized corvid birds) wintering in continental Europe during winter 2010/2011. Samples of fresh rook faeces were taken by cotton swabs at nine roosting places in eight European countries. Samples were transported in Amies transport medium to one laboratory and placed in buffered peptone water. The samples from buffered peptone water were enriched in MacConkey broth and subcultivated onto MacConkey agar (MCA) supplemented with ciprofloxacin (0.06 mg L^{-1}) to isolate fluoroquinolone-resistant Enterobacteriaceae bacteria. DNA was isolated from smears of bacterial colonies growing on MCA and tested by PCR for PMQR genes *aac(6´)-Ib*, *qepA*, *qnrA, qnrB, qnrC, qnrD, qnrS*, and *oqxAB*. All the PCR products were further analysed by sequencing. Ciprofloxacin-resistant Enterobacteriaceae bacteria were isolated from 37% (392 positive/1073 examined) of samples. Frequencies of samples with ciprofloxacin-resistant isolates ranged significantly from 3% to 92% in different countries. The *qnrS1* gene was found in 154 samples and *qnrS2* in 2 samples. The gene *aac(6´)-Ib-cr* was found in 16 samples. Thirteen samples were positive for *qnrB* genes in variants *qnrB6* (1 sample), *qnrB18* (1), *qnrB19* (9), *qnrB29* (1) and *qnrB49* (new variant) (1). Both the *qnrD* and *oqxAB* genes were detected in 6 samples. The genes *qnrA*, *qnrC*, *qepA* were not found. Wintering omnivorous rooks in Europe were commonly colonized by Enterobacteriaceae bacteria with PMQR genes. Rooks may disseminate these epidemiologically important bacteria over long distances and pose a risk for environmental contamination. This study was funded by the project 'CEITEC - Central European Institute of Technology' (CZ.1.05/1.1.00/02.0068) from the European Regional Development Fund.

42A

ANTIMICROBIAL RESISTANCE AND PRESENCE OF CLASS 1 INTEGRONS IN SALMONELLA SEROVARS ISOLATED FROM CLINICAL CASES OF ANIMALS AND HUMANS IN NORTH DAKOTA, USA AND KAMPALA, UGANDA

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Salmonellae are one of the leading causes of food borne illness worldwide and have been used as an indicator organism for studying antimicrobial resistance (AMR) trends. The objective of this study was to characterise AMR patterns of Salmonella isolates from animals and humans in North Dakota (ND), US and Kampala Uganda and determine the association between the observed AMR and presence of class 1 and 2 integrons. Salmonella isolates were collected from the Veterinary Diagnostic Laboratory (VDL) at North Dakota State University and the North Dakota Department of Health, respectively from 2003-2008. Samples were also retrieved from archives at the Microbiology Department, Faculty of Veterinary Medicine at Makerere University in Kampala, Uganda. AMR profiles were determined using a panel of 15 antimicrobials. Screening for the class 1 and 2 integrons was done using PCR with primers specific for the int1 and int2. Out of 359 Salmonella isolates tested 24.79% were resistant to ≥5 antimicrobials while 36.2% were resistant to at least 2. Pan susceptible isolates were mostly (65.05%) from human isolates. The most common multidrug resistant (MDR) phenotype among theisolates was the classic ACSSuT penta-resistance at 29.06% (50/172). The highest resistance frequency was seen against Tetracycline (39.6%) and Streptomycin (34.7 %) while 5.2% (17) of the isolates were resistant to Nalidixic acid and 56 (15.7%) to Ceftiofur. A total of 20.7% (57/276) of the ND samples tested positive for presence of class 1 integrons and was significantly associated ($p< 0.05$) with AMR to Ampicillin, Kanamycin, Tetracycline and Sulfisoxazole. Of all Ugandan Salmonella isolates tested (94.4% 68/72) were resistant to ≥2 antimicrobials. The highest resistance was observed against Sulfisoxazole and Trimethoprim-Sulphamethoxazole and 45.8% of human and 46.2% of cattle isolates tested positive for presence of class 1 integrons. Presence of class 1 integron was significantly associated ($p< 0.05$) with AMR to Tetracycline and Amoxicillin. DNA sequencing of the class 1 integron variable regions identified several resistance genes including aadA1, dfrA7, and dfrA5 genes. The data indicated high AMR among antimicrobials widely used in veterinary and human medicine with some Salmonella isolates exhibiting multidrug resistance. Also, AMR was observed against drugs whose veterinary use is restricted, implying possible horizontal transmission. These results signal serious implications for treatment of salmonellosis in both public and animal health.

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DISTRIBUTION OF EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANCE DETERMINANTS OF *SALMONELLA ENTERICA* AND *ESCHERICHIA COLI* ISOLATED FROM BROILERS IN SOUTHERN JAPAN

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A study was conducted to elucidate the distribution, diversity and transferability of extend-

ed-spectrum cephalosporin (ESC)-resistance determinants harbored by *Escherichia coli* and *Salmonella enterica* isolated from same samples derived from broiler flocks at slaughter in southern Japan. Resistance to ESCs was mediated by β-lactamase genes bla_{CTX-M}-types 2 (6.5%), 14 (6.5%) and 15 (10.8%); bla_{SHV}-types 2 (2.2%) and 12 (6.5%); and bla_{CMY}-types 1 (8.7%) and 2 (73.9%) associated with plasmids belonging to incompatibility groups IncI1, IncFIB, IncFIC, IncK, IncB/O, and IncY were detected in 46 *E. coli* isolates. Chromosomal location of bla_{CTX-M}-types 2 or 15, or bla_{CMY-2} was confirmed in seven *E. coli* isolates by Southern blot analysis. These resistances were not successfully transferred. β-lactamase genes bla_{TEM-52} (86.7%), $bla_{CTX-M-2}$ (2.2%), and bla_{CMY-2} (13.3%) carried by conjugative untypable or IncP plasmids were detected in 45 *S. enterica* isolates. Multilocus sequence typing (MLST) analysis of *E. coli* identified same combinations of sequence types (STs), incompatibility groups, and β-lactamase genes in different flocks. Moreover, *Salmonella* serovars Infantis and Manhattan isolates showing same plasmid profile and antimicrobial resistance pattern were detected in different flocks, suggesting the existence of common source of contamination. Indistinguishable Restriction fragment length polymorphism (RFLP) fingerprints of plasmids conferring ESC resistance were observed in isolates belonging to different STs, signifying transmission of ESC-resistance determinants between different *E. coli* STs. RFLP analysis revealed inter-serovar transmission of 40-kb bla_{TEM-52}-encoding plasmids between isolates of *S.* Infantis and *S.* Manhattan. These observations indicate that a wide variety of β-lactamase genes conferring ESC resistance is distributed, and the transmission of the resistance determinants has been occurring among *E. coli* and *S. enterica* in broiler flocks in southern Japan.

44B

EVALUATION OF ANTIMICROBIAL RESISTANCE IN STRAINS OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICAL MASTITIS IN CATTLE ON DISTRITO FEDERAL, BRAZIL

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Clinical and subclinical mastitis in cattle have a negative effect on animal breeding including low productivity and economic losses.Staphylococcus aureus infections are hardly treated due to lack of microbiological analysis such as antimicrobial resistance (AMR) profile. The consequence is that increasing AMR also threatens agrobusiness, as bacterial diseases in animals become more difficult to treat. The aim of this research was to evaluate the AMR profile in strains of Staphylococcus aureus isolated from clinical mastitis. Twenty-seven samples previously identified as Staphylococcus aureus from fourty-seven animals, collected in 2010 and 2011, were submitted to antibiogram tests in Mueller Hinton agar. The following antimicrobials were used in order to identify the AMR profile: Amoxicilin-clavulanic-acid (30 µg), Ampicilin (10 µg), Bacitracin (10 UI), Cephalexin (30 µg), Cephazolin (30 µg), Ceftiofur (30 µg), Enrofloxacin (5 µg), Licomicin (2 µg), Gentamicin (10 µg) and Spiramycin (20 µg). The result of AMR analysis showed that the most sensitive antibiotics were Bacitracin (92,5%), Cephalexin (88,8%), Cephazolin (88,8%), Ceftiofur (96,2%), Enrofloxacin (70,3%), Gentamicin (88,8%), Lincomicin (81,4%), Neomicin (92,5%), Tetracycline (74%) and Tobramicin (92,5%). The most resistant antimicrobial was Penicilin (77,7%). Other antibiotics, such as Amoxicilin-clavulanic-acid (40,74%), Ampicilin (44,4%), and Spiramycin (40,7%) showed moderated resistance. These results demonstrated that we can still treat these infections by using a large number of antibiotics, however the use of a

few antibiotics demonstrate increasing AMR. Therefore this research indicates that the correct treatment can be achieved once you make a microbiological analysis that points out the most suitable antibiotic.

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METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATES FROM DOGS

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Objectives: Results of the recent studies has shown that *Staphylococcus pseudintermedius* rather than *S. intermedius* is the predominant pathogenic *Staphylococcus* species causing skin and soft tissue infections in dogs. The objectives of this study were to determine the prevalence of *S. pseudintermedius* among isolates from skin infections from dogs and determine the prevalence of methicillin-resistance of *S. pseudintermedius* isolates. **Methods:** A total of 41 isolates from dogs with canine pyoderma were used in this study. Phenotypic identification was performed by the Microbact 12 S API Staph system. *S. pseudintermedius* isolates were molecularly identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the *pta* gene, encoding the enzyme phosphoacetyltranferase. *Mbo*I enzyme was used in RFLP to detect *S. pseudintermedius* isolates harboring this restriction site. For all isolates identified as *S. pseudintermedius*, methicillin-resistance (mecA) was investigated. **Results:** Out of 41 Staphylococcal isolates from skin infections of dogs, 35 (85.4%) isolates were phenotypically identified as *S. intermedius*, 4 (9.8%) as *S. aureus*, and 2 (4.8%) as different species (*S. chromogenes* and *S. capitis*). 33 (80,4%) out of 41 were molecularly identified as *S. pseudintermedius*. The mecA gene was identified in 33.3% (11/33) isolates. **Conclusion:** Staphylococcal isolates from dogs with canine pyoderma identified conventionally as *S. intermedius* should be re-investigated with appropriate methods, since they could in fact be *S. pseudintermedius*. Results of the present study confirmed that *S. pseudintermedius* is the predominant pathogenic *Staphylococcus* species in dogs. The high rate (i.e. one third of the isolates) of methicillin resistance of *S. pseudintermedius* isolates from dogs constitutes a risk for public health considering the pathogen's zoonotic potential and the risk of transmission from these companion animals to their owners.

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ANTIBIOTIC RESISTANCE IN *E. COLI* OBTAINED FROM WILD BIRDS IN THE NETHERLANDS

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Introduction: The role of wild life in the spread of antibiotic resistant bacteria in the environment is unclear. In addition to our national monitoring system on antimicrobial resistance in food-producing animals we performed a pilot study on antimicrobial resistance in *E. coli* obtained from wild birds. Furthermore, we screened these birds for the presence of Extended Spectrum Beta-Lactamase (ESBL) producing *E. coli* by selective culturing. **Methods:** Carcases of wild birds (mostly ducks, swans, gulls and waders) were send to the Central Veterinary Institute in Lelystad for diagnostic purposes (avian influenza, botulism or suspected of poisoning). From October 2011 until February 2012 cloaca swabs were collected and cultured on MacConkey agar and in Luria Bertani broth with 1 mg/L cefotaxime (LB+). After incubation 1 µl LB+ was cultured on MacConkey with 1 mg/L cefotaxime. If growth appeared, one typical *E. coli* colony from the non-selective and from the selective plate were pure cultured and stored.

Susceptibility to a panel antimicrobials was tested by broth microdilution according to ISO 20776-1:2006 using EUCAST cut-off values for interpretation. In addition all cefotaxime resistant *E. coli* collected were tested with micro-array (AMR-ve 05, Alere Technologies) for detection of antibiotic resistance genes. Subsequently, all positive array signals for ESBL genes were confirmed with PCR and sequencing. **Results:** From a total of 165 cloaca samples originating from 40 different bird species 133 *E. coli* were obtained on non-selective plates. These isolates showed no or relative low percentages of resistance to all antibiotics tested: ampicilline (9.0%), cefotaxime (1.5%), ceftazidime (0.8%), chloramphenicol (0.8%), florfenicol (0.0%), tetracycline (9.0%), nalidixic acid (5.3%), ciprofloxacin (5.3%), sulfamethoxazole (6.0%), trimethoprim (6.0%), gentamicin (0.8%), kanamycin (1.5%) and streptomycin (7.5%). Twenty-two cefotaxime (= 13.3 %) resistant *E. coli* were collected on selective plates. PCR and sequencing revealed the presence of different ESBL/AmpC genes: variants of $bla_{\text{CTX-M}}$ (n = 17), $bla_{\text{TEM-52c}}$ (n = 1) and $bla_{\text{CMY-2}}$ (n = 4). **Conclusions:** The susceptibility of predominant *E. coli* showed low resistances to most antibiotics tested. However, a relative high prevalence of ESBL-producing *E. coli* was found harbouring different ESBL-genes. These findings suggest a unsuspected wide spread of ESBL genes in (migrating) wild birds in The Netherlands.

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CHICKEN BROILER MEAT AS A SOURCE OF QUINOLONE-RESISTANT AND EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI* ISOLATES, AN EXPERIENCE FROM THE CZECH REPUBLIC

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The aim of our study was to characterise a risk of transmission of fluoroquinolone and cephalosporin resistant *E. coli* and extraintestinal pathogenic *E. coli* (ExPEC) isolates from chicken broilers to humans at a national level regarding the total consumption of quinolones and cephalosporins in the country in the period monitored. In a prospective study of 319 chicken broilers from 3 slaughterhouses in the Czech Republic during 2008, a total of 115 *E. coli* isolates were characterized regarding their antimicrobial resistance. Moreover, polymerase chain reaction-based assays to define ExPEC associated traits were performed in resistant strains. ExPEC was defined by detection of ≥ 2 of *iutA, cvaC, kpsII, iss, tsh, papC*, i*beA,* and *felA* genes. Consumption of antimicrobial drugs in poultry in the Czech Republic was analysed in 2007 and 2008. Antibiotic resistance to one or more antibiotics was detected in 82 % isolates. Resistance to nalidixic acid and ciprofloxacin was predominant, being found in 69 % and 27 % isolates, respectively. Eleven and two of 94 antibiotic-resistant *E. coli* isolates carried the *intI1* and *intI2* genes, respectively. Plasmid-mediated quinolone resistance genes were detected in 4 out of 94 resistant isolates. Among these 94 isolates, 45% carried two or more virulence genes and hence should be considered as ExPEC isolates. Flumequine, enrofloxacin and difloxacin were used in poultry in the Czech Republic in a total amount of 972 kg in 2008. Chicken broilers may be an important vehicle for community-wide dissemination of fluoroquinolone resistant *E. coli* and ExPEC. High prevalence of chicken broilers with ciprofloxacin-resistant *E. coli* isolates was linked to consumption of quinolones in poultry. A withdrawal of fluoroquinolones from use in chicken production should be seriously considered in the Czech Republic and European Union as well. This

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SURVEILLANCE OF EXTENDED SPECTRUM BETA-LACTAMASE AND AMPC-BETALACTAMASE PRODUCING *E. COLI* IN BROILERS, PIGS, TURKEYS AND CATTLE DURING 2007-2011

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Introduction: ESBL/AmpC-producing isolates are increasingly found in human clinical isolates. It is suggested that this could partly be explained by transfer of these isolates via the food-chain. To understand more of the epidemiology of these isolates in food-producing samples, the objective of this study was to characterise ESBL/AmpC beta-lactamase producing *E. coli* obtained from Dutch broilers, pigs, turkeys, calves and dairy cows collected in the years 2007-2011. **Methods:** Faecal samples from turkeys were collected in 2011 at farm level. Isolates from broilers, veal calves, dairy cows and pigs were collected during 2007-2011 as part of the national monitoring program on antibiotic resistance in food-producing animals. Indole positive *E. coli*-like colonies were non-selectively isolated at random from MacConkey agar plates. Minimum Inhibitory Concentration (MIC) for cefotaxime was determined by broth micro dilution method using Sensititre system. All isolates resistant to cefotaxime according to the EFSA epidemiologically cut off value (MIC > 0.25 mg/L) were further analysed for the presence of ESBL/AmpC genes by micro array, PCR and sequencing. Furthermore, when presenting the data at the conference the results will be compared to results from a monitoring program using selective methods to detect cefotaxime resistance. **Results:** The highest prevalence of cefotaxime resistant *E. coli* was found in broilers (8-21%). In dairy cows the prevalence was the lowest (0% -0.8%). In isolates of pigs, turkeys and veal calves prevalence varied between 2 and 11 %. Most predominant plasmid-mediated ESBL/AmpC genes found in broilers were $bla_{\text{CTX-M-1,-2,}}$ $bla_{\text{SHV-12}}$ (not found before 2009), $bla_{\text{CMY-2}}$ and $bla_{\text{TEM-52}}$, in pigs and veal calves $bla_{\text{CTX-M-1}}$ was most predominant and in dairy cows $bla_{\text{CTX-M-2}}$. Two turkey isolates, derived from 2 different farms, showed resistance to cefotaxime, but no plasmid-mediated ESBL/AmpC genes were found. Cefotaxime resistance was based on mutations in the *ampC* promoter/attenuator region and was not plasmid-mediated. This was also found in some isolates derived from veal calves, dairy cows and broilers.**Conclusion:** Since 2007, prevalence of Dutch ESBL/AmpC producing *E. coli* over the years is stable in all animal species with the highest prevalence found in *E. coli* from broilers. In broilers, $bla_{\text{CTX-M-1}}$ is still most predominant, but after 2009 $bla_{\text{SHV-12}}$ is gradually increasing, this might indicate a change in spread of ESBL/AmpC-producing isolates in broilers.

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EVALUATION OF ANTIMICROBIAL RESISTANCE IN *ESCHERICHIA COLI* ISOLATED FROM HEALTHY SWINE ON DISTRITO FEDERAL, BRAZIL

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The growth of exports of Brazilian swine products has increased the concern about the health of these animals, as affections like diarrhea and colitis promote developmental problems and sometimes it can cause the animal's death. Escherichia coli is among the most common isolated agents causing these infections, and healthy swine may harbor the occurrence of the multi-resistant E. coli strains. The main objective of this research was to identify the

antimicrobial resistance (AMR) profile in strains of E. coli of healthy swine in Distrito Federal, Brazil. A total of 127 strains of E. coli were isolated from 109 swine and biochemically tested, observed for hemolysis on blood agar and were submitted to antibiogram test in Mueller Hinton agar. The following antimicrobials were used in order to identify the AMR profile: Amikacin (30µg), Ampicilin (10µg), Cephalexin (30µg), Chloramphenicol (30µg), Doxycyclin (30µg), Enrofloxacin (5µg), Streptomycin (10µg), Gentamicin (10µg), Lincomicin (2µg), Neomicin (30µg), Norfloxacin (10µg), Sulfametoxazol + Trimetoprim (25µg), Sulfonamide (300µg) and Tetracycline (30µg). The result of AMR analysis showed that the most resistant antibiotics were Lincomycin (100%), Sulfonamide (74.8%), Tetracycline (70.1%), Doxycyclin (66.1%) and Ampicillin (51.2%). Furthermore results also indicated that antimicrobials with higher sensitivity were Norfloxacin (82.7%), Gentamicin (75.6%) and Sulfamethoxazole + Trimethoprim (63%). Other antibiotics presented moderated sensitivity for Enrofloxacin (58,3%), Chloramphenicol (56,7%), Streptomycin (53,5%), Amicacyn (46,5%) e Neomicyn (39,4%). All samples were resistant to one antibiotic at least, 47 (37%) showed resistance to half of antibiotics tested and just 4 (3,2%) samples were resistant to all antibiotics. These results demonstrated that even a healthy swine could carry E. coli strains with high numbers of antimicrobial resistance.

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CO-RESISTANCE GENES AND PHYLOGENETIC GROUPS OF CTX-M, IRT AND AMPC HYPERPRODUCERS *E. COLI* STRAINS FROM DISEASED CALVES IN PORTUGAL

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Introduction: The extensive use of antimicrobials in food-producing animals has led to the emergence of resistant pathogenic bacteria. *E. coli* may be grouped into four major ancestral phylogenetic groups: highly virulent extraintestinal B2 and D lineages and low extraintestinal virulence A and B1. **Objective:** This study aims at determining antimicrobial co-resistance genes and the phylogenetic background of CTX-M, IRT and AmpC hyperproducers *Escherichia coli* strains from diseased calves. **Methods:** Thirty-two strains previously isolated in another study, were fully characterized, with 17 being CTX-M extended spectrum beta-lactamase (ESBL)-producers and 6 harboring inhibitor resistant TEM (IRT) genes. The remaining 9 strains were AmpC hyperproducers (3 with *ampC* promotor mutations only, 2 with *ampC* gene mutations and 4 with both *ampC* promotor and gene mutations). Fourteen strains harbored $bla_{CTX\text{-}M\text{-}32}$ and only 3 were $bla_{CTX\text{-}M\text{-}14}$. IRT-positive strains included 3 $bla_{IRT\text{-}2}$ and 3 $bla_{IRT\text{-}5}$ genes. Susceptibility testing had been previously performed against several antimicrobials through both the disk diffusion and the broth microdilution methods according to CLSI guidelines. Clinical breakpoints were applied to categorize isolates (CLSI M31-A3 and SFM 2010). All isolates were screened for the *aadA*, *tetA*, *tetB*, *floR*, *sulI*, *dfrIa* antimicrobial resistance genes through PCR. Phylogenetic group were also assigned by PCR. **Results:** The majority of CTX-M-producing *E. coli* strains belonged to the phylogenetic group A (n=10), five were D and only one was B2. All these strains were resistant to streptomycin (STR), 16 were resistant to tetracycline (TET), 14 strains were resistant to sulfamethoxazole/trimethoprim (SXT) and only 2 were resistant to florfenicol (FFC). Among the *E. coli* strains harboring IRT genes, five strains were group A and only one was B1. All strains were resistant to TET and STR and 5 of them were also resistant to SXT. Phylogenetic analysis of AmpC hyperproducers *E. coli* strains revealed 4 strains belonging to group A, 3 to D and 2 to B2. STR resistance was present in all strains, while only 6 were resistant to SXT and FFC. From the 32 strains resistant to STR, 30 harbored the *aadA*

resistance gene. The *floR* gene was present in all FFC resistant *E. coli*. Both *tetA* (*n*=13) and *tetB* (*n*=11) genes were present. SXT resistance was explained in 23 strains by the existence of both *dfrIa* and *sul1* genes. **Conclusion:** The combination of beta-lactam resistance with resistance to other antimicrobial classes poses a threat to successful treatment of *E. coli*-related animal diseases. Furthermore, multi-resistant strains are dispersed among ancestral virulence phylogenetic groups. The presence of group B2 and D in these multi-drug-resistant strains is worrying, since it may contribute to the persistence and dissemination of these strains in food-producing animals and eventually in humans.

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TROPICAL BEACH WATERS HARBOR RESISTANT BACTERIAL INDICATORS: *ESCHERICHIA COLI* PRESENTED SIMILAR ANTIBIOTIC RESISTANCE PATTERNS TO UROPATHOGENIC ISOLATES.

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Coastal environment is among the major sources of livelihood. It also serves as an attractive site for recreational activities. We monitored the quality of the coastal waters of Southern Grenada using the MPN over six years. The antibiotic susceptibility profiles of dominant marine bacterial indicators were utilized to determine whether bathing in the coastal waters is a potential of expose to antibiotic-resistant bacterial indicators. *Escherichia coli* strains isolated from the urine samples at the local clinic were identified and assayed for antibiotic-resistance to compare the resistance patterns observed in marine and clinical isolates. Twenty nine clinical isolates from urine were identified as *E. coli,* standardized using the McFarland assay and analyzed using the Kirby-Bauer assay and reference pure cultures against 11 antibiotics. The marine indicators were identified using biochemical tests on 116 bacterial isolates from 255 water samples, which were collected from four coastal sites between November 2008 and November 2010 (Amadi *et al*., 2011). Marine *E. coli* and *Klebsiella pneumoniae* strains were tested against 12 antibiotics and *Enterococcus faecalis* strains against six antibiotics. *E. coli* strains were resistant to amoxicillin/clavulanate, ampicillin, cefoxitin, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, and tetracycline (Amadi *et al*., 2011). Our results showed that 32% of clinical *E. coli* strains were resistant to three and more drugs and only 5% to a single antibiotic while 7% of isolated marine bacteria were resistant to multiple antibiotics, and 42% to a single antibiotic. The likelihood of exposure to antibiotic-resistant bacterial indicators including *E. coli*, *K. pneumoniae,* and *E. faecalis* for the four popular tropical beaches were modeled based on results of the MPN, compliance, and Kirby-Bauer. The exposure model predicted highest likelihood of exposure of 9% for ampicillin and tetracycline-resistant *E. coli* in Black Sand Beach (BSB); of 11 to 20% for ampicillin-resistant *K. pneumoniae* in Prickly Bay (PB), Grand Anse Beach (GAB), and True Blue Bay (TBB); and of 4% for *E. faecalis* in GAB. In accordance with the McNemar, F-test and T-tests analysis, resistance patterns of strains of BSB marine *E. coli* against ciprofloxacin, cephalothin, amoxicillin/clavulanate and gentamicin were not statistically different from the patterns observed in the clinical strains of *E. coli* isolated from patient's urine samples between November 2008 and November 2010. The resistance pattern for amoxicillin/clavulanate observed in marine strains of *E. coli* isolated from GAB, PB and TBB (2%) differed from the clinical isolates (32%). V. A. Amadi, D. E. Lennon, A. A. Qureshi, D. L. Jungkind, and S. V. Kotelnikova. Occurrence

of antibiotic-resistant Indicators in paradise coastal water of Grenada, West Indies. FEMS 2011, Geneva, Switzerland, June 26 -30, 2011.

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COMMUNITY DISSEMINATION OF UROPATHOGENIC ESCHERICHIA COLI PRODUCING EXTENDED-SPECTRUM Β-LACTAMASES.

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Introduction: Extended-spectrum β-lactamase (ESBL)-producing Escherichia coli is an increasingly important group of community pathogens worldwide. These organisms are frequently resistant to many of the antimicrobial agents usually recommended for the treatment of infections caused by E coli, such as penicillins, cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole. We investigated the occurrence of ESBL producing E. coli isolated from patients with community acquired urinary tract infection (UTI) and the clonality among ESBL-producing isolates. **Material and methods:** 490 E. coli strains isolated from urine were collected at private laboratories in the region of Bejaia (Algeria). Susceptibility testing was performed as recommended by the French Society of Microbiology (CA-SFM). ESBL production was determined by the synergy test between clavulanic acid and ceftazidime, cefotaxime, cefepime and aztreonam. Characterization of ESBL genes (TEM, SHV and CTX-M) and plasmid resistance genes to quinolones is performed by PCR. The different genes were determined by sequencing. MICs were determined by E-TEST. Molecular typing of strains was done by RAPD. The replicons carrying the ESBLs and the total plasmid content of the strains have been characterized by PCR replicon typing. **Results:** A total of 13 strains were found resistant by producing ESBL enzymes. Expressed ESBLs are CTX-M-15 (09 strains) and CTX-M-3 (04 strains). blaTEM-1-like and blaOXA-1-like genes were found in all strains. The qnrS1 gene was found associated with the gene blaCTX-M-3 in 04 strains. Molecular typing by RAPD showed multiple profiles. Analysis of plasmid incompatibility groups revealed that Inc FII 1K was the most common types associated with CTX-M genes. **Conclusion:** This study shows the spread of ESBL-producing E coli. Should this resistance mechanism spread further in E coli, community-borne ESBL may become a public health concern over the next few years. **Keywords:** ESBLs, E. coli, PCR-based replicon typing, urinary tract infection, community.

53B

AEROMONAS SPP. FROM ORNAMENTAL FISH AS POTENTIAL SOURCE OF PLASMID-MEDIATED QUINOLONE RESISTANCE GENES

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Background: The occurrence of antibiotic resistance genes in bacteria in the water environment around the world is an increasing concern. The ornamental fish producers tend to administer antibiotics in a non-systematic and uncontrolled manner, thus making the selection and spread of antibiotic-resistant bacteria possible. **Objectives:** To assess the potential risk, the aeromonads from imported ornamental fish and koi carp were tested for susceptibility to antimicrobial agents and presence antibiotic resistance genes, the class 1 and 2 integrase genes and plasmid-mediated quinolone resistance (PMQR) genes. Methods: 80 Aeromonas spp. isolates from 115 tropical ornamental fish of 49 species (the consignments from 8 countries) and 72 Aeromonas spp. isolates recovered from koi carp coming from randomly chosen farms (n = 7) were screened for

susceptibility to chloramphenicol, ciprofloxacin, florfenicol, oxolinic acid, oxytetracycline and trimethoprim by agar dilution method. The presence of resistance genes, integrons and PMQR genes was proved by conventional PCR and DNA sequencing. **Results:** Sixty-seven of 80 Aeromonas spp. isolates originated from tropical ornamental fish and 36 of 72 Aeromonas spp. isolates from koi carp were resistant to at least one antimicrobial agent, respectively. The PMQR genes (qnrS2 or/and aac-Ib-cr) were detected with a relatively high frequency in ornamental fish isolates (24 %) and also in koi carp isolates (22 %). Class 1 integrons with 8 gene cassettes (aadA1, aadA2, aadA5, dhfr1, dhfr12, dhfr17, sat , estX.) and IncU plasmids were detected in ornamental fish isolates. **Conclusion:** The study demonstrates that imported ornamental fish and koi carp can be colonised with antimicrobial resistant bacteria that serve as an important source of PMQR genes. This study was supported by Project QH71057 (NAZV).

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VACCINE STRAIN OF SALMONELLA IN A CAENORHABDITIS ELEGANS-BASED MODEL OF ENTERIC INFECTION.

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Dietary substances that ameliorate pathology from enteric infections have potential to supplement or replace antibiotic use in animal agriculture. A means to cheaply and safely screen feed additive prototypes is desirable to accelerate the process of finding such substances. We report on a C. elegans infection model using a BSL 1 level vaccine strain of Salmonella enterica that none the less is quite pathogenic to C. elegans under our assay conditions. This provides for us a means to rapidly screen feed supplement and additive prototypes that may reduce damage from enteric infections. Furthermore, the fact that the Salmonella strain used is a BSL 1 organism improves biological safety and logistics related to an assay using an important and significant Salmonella species from the standpoint of public and animal health and hygiene.

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THE EFFECT OF CHLORTETRACYCLINE ON GASTROINTESTINAL MICROBIOTA IN GROWING SWINE

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Antimicrobials are used in all stages of pork production in the United States. The effect of antimicrobial use on gastrointestinal microbiota of food animals is of increasing concern as bacteria accumulate resistance to multiple antimicrobials. Only a small fraction, less than one percent, of microorganisms are believed to be culturable making characterization of the swine gastrointestinal microbiome difficult. The objectives of this study were to determine: the effect of chlortetracycline on the gastrointestinal microbiota of swine using pyrosequencing; and to examine the competitive effect of fecal bacteria from chlortetracycline medicated and unmedicated swine on multidrug resistant (MDR) *E. coli* that possessed 0, 2, 6 or 8 plasmids. Four freshly weaned pigs were provided a grower ration of 80% corn and 20% soybean meal for 21 d; on d 21 the diet of two pigs was supplemented with 50 g/ton chlortetracycline. Fecal material was collected from each pig on days 0, 14, 23, 28, 35, 42 and 49 for bTEAFP pyrosequencing. On d 42 fecal growth studies were performed with five MDR *E. coli* strains. Each strain was inoculated into anoxic fecal cultures from swine fed unmedicated and medicated feed. Each treatment group was enumerated at 0, 6, and 24 h. Genotypic characterization of *E. coli* (n=3) from both treatment groups at each time point was performed to determine plasmid stability in a competitive culture. Strain 155 maintained IncA/C, a 165 kb plasmid and the *eaeA* virulence gene, but 100% (n=18) of the isolates examined lost IncFIB, a 90 kb plasmid

and the *hlyA* virulence gene. The genotypes of the other strains remained largely unchanged. Unifrac analysis of pyrosequencing data showed no significant difference in bacterial diversity based on diet ($p < 0.05$). The most abundant phyla in both treatment groups were: Firmicutes, Bacteroidetes, Proteobacteria and Spirochaetes. In the fecal competition studies, there was no significant difference ($p < 0.05$) in population of each plasmid bearing *E. coli* strain between their respective treatment groups. However, the plasmid free strain was eliminated from both treatment groups by 24 h. Unifrac analysis showed that chlortetracycline treatment did not significantly alter the phylogenetic profile of the swine gastrointestinal microbiota under the conditions of this study and did not provide a competitive advantage to the *E. coli* strains used in the short–term competition study. More in depth studies of multi–plasmid bacterial strains in natural habitats are needed to understand the mechanisms of plasmid persistence.

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DISTRIBUTION OF β-LACTAMASE GENES IN ESCHERICHIA COLI FROM CANADIAN FOOD ANIMALS, AND THE EMERGENCE OF BLACTX-M

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The use of β-lactam antimicrobials in the food animal industry exerts selective pressure contributing to increased resistance in Gram-negative bacteria. Susceptibility to these antimicrobials is routinely monitored in *Escherichia coli* isolates by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). A large portion of the *E. coli* isolates collected in 2010 (805/1726) were screened by PCR for the most common β-lactamase genes found in Canada. These isolates were from cecal samples at the abattoir level and from retail meats, from bovine, porcine and chicken sources. Among the 334 ampicillin-resistant isolates (>=16 µg/mL), 51% carried a bla_{TEM} gene (n=172), 36% carried bla_{CMY-2} (n=121), and 3% carried bla_{SHV} (n=10). No bla_{CTX-M}, bla_{OXA-1}, bla_{KPC}, bla_{VIM}, bla_{IMP}, or bla_{NDM} genes were detected. The bla_{CMY-2} gene was largely associated with chicken isolates (83%), which is in keeping with previous results obtained from 2009 isolates. Porcine (51%) and chicken (43%) isolates accounted for most of the bla_{TEM} genes detected. Interestingly, the bla_{SHV}-positive isolates were all from retail sources, mainly chicken, and were almost exclusively obtained from one region of Canada. As bla_{SHV} was not detected in abattoir samples, it is possible that these retail meats had different countries of origin or were cross-contaminated at the processing level. Although there were no bla_{CTX-M} genes found in the 2010 *E. coli* isolates, their prevalence in Europe and other regions prompted further investigation of more recent isolates. *Escherichia coli* and *Salmonella* CIPARS isolates from 2011 were screened for β-lactam resistance using automated broth micro-dilution, and those which were ceftiofur and ceftriaxone resistant but cefoxitin susceptible were tested by PCR for bla_{CTX-M}. To date, six isolates (2 *E. coli* and 4 *Salmonella*) were found to be bla_{CTX-M} positive; all isolates are related to poultry. All *Salmonella* bla_{CTX-M} genes were of the CTX-M-1 variant, and included serovars Ouakam, Senftenberg and Bredeney. *Escherichia coli* bla_{CTX-M} variants included CTX-M-1 and CTX-M-8. Although the numbers are still very low, it is a preliminary indication that bla_{CTX-M} may be starting to emerge in bacteria from Canadian food products since 2011, and may be most likely associated with poultry-related sources.

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PHENOTYPIC CHARACTERISATION OF ANTIBIOTICS RESISTANCE MECHANISMS OF *ESCHERICHIA COLI* IN MUNICIPAL WASTEWATER TREATMENT PLANT (BEJAIA, ALGERIA)

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Purpose: Bacteria resistant to antibiotics have been detected in environmental compartments such as surface water, ground water, sediments and soils. The main sources of dispersion of these resistances are wastewater treatment plants (WWTP). The aim of this study was to characterize the phenotype of resistance to different family of antibiotics and to evaluate the prevalence of resistance to these antibiotics among *Escherichia coli* strains isolated from municipal wastewater treatment plant (Bejaia, ALGERIA). **Methods:** Antimicrobial resistance of *E.coli* isolates was investigated in municipal WWTP based on the activated sludge process. The sensitivity of isolates tested against 17 antibiotics, including 8 β-lactams, 4 aminoglycosides and 5 belonging to other families of antibiotics. The phenotypes of resistance were determined by the screening of production of enzymes. **Results:** The 58 isolates have high rates résistantces to most β-lactams tested, 15.51% of total isolates (9 / 58) are resistant to 7 of 8 antibiotics tested. Imipenem is the only molecule to be effective. 56 strains produced ESBLs. Resistance to aminoglycosides tested are more moderate with rate of 36.19%, 25.03% and 7% for kanamycin, gentamicin and amikacin, respectively, tobramycin is the only antibiotic that belongs to this family to have lost all activity on these strains. Resistance to other antibiotics is variable, the rate of resistance to nalidixic acid was highest (96.55%) and fosfomycin is most effective with a rate of 100% inhibition. **Conclusion:** Due to the introduction and/or selection of resistant bacteria in water environment, changes in natural ecosystems of receivers can be expected, it is necessary to study the diversity of antibiotics genes resistance in aquatic environments. **Key words:** Antibiotics; Resistance; *E. coli*; wastewater, wastewater treatment plant.

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RIBOSOMAL MUTATIONS IN ERYTHROMYCIN RESISTANT *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI* ISOLATED FROM ANIMALS AND RAW MEATS IN KOREA

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Background: *Campylobacter* species are one of the major foodborne pathogens in humans worldwide. Macrolide antimicrobials, the drug for treating *Campylobacter* infection, have been widely used in the veterinary field. Studies on the macrolide antimicrobial resistance of *Campylobacter* spp. in animals and their products are available for many countries, but a few studies are conducted in Korea. Thus, the aims of this study were to determine the incidence of erythromycin resistance and to investigate the mechanisms of macrolide antimicrobial resistance of *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) isolated from animals and raw meats. **Methods:** A total of 250 *C. jejuni* and 184 *C. coli* isolates were retrieved from animals and raw meats during 2004-2010. Minimum inhibition concentrations (MICs) to erythromycin were determined by broth dilution method using the *Campylobacter* MIC plate. To assess the contribution of mutations, domain V of the 23S rRNA gene, ribosomal protein L4 and L22 genes (*rplD* and *rplV*, respectively) were examined by polymerase chain reaction and sequencing. **Results:** Of the 434 *Campylobacter* isolates, 4.4% (11/250) of *C. jejuni* and 26.1% of *C. coli* (48/184) were resistant to erythromycin. Erythromycin resistance was much higher in *C. coli than in C. jejuni*, particularly in *C. coli* isolated from pigs (37.3%, 31/83) and chickens

(21.1%, 8/38). However, no resistance to erythromycin was observed in cattle. All erythromycin resistant *C. coli* (MICs 16 - >64 µg/ml) and *C. jejuni* (MICs 32 - >64 µg/ml) isolates were exhibited an AΦG transition at 2075 of the 23S rRNA of except six isolates which showing the intermediate resistance to erythromycin (MIC 32 µg/ml). Sequence analysis of ribosomal proteins L4 and L22 showed the several amino acid substitutions in erythromycin resistant *Campylobacter* isolates. However, patterns of mutation of L4 and L22 were found to be similar between high and low level of erythromycin resistant groups. **Conclusion:** This study showed that macrolide resistance was much higher in *C. coli* from pigs and chickens than those from cattle in Korea. This result suggests that more prudent use of critically important antimicrobials such as macrolides in swine and poultry production is necessary. Mutation in the 23S rRNA was found to be mainly responsible for erythromycin resistance in *Campylobacter* isolates. However, role of the amino acid substitutions in the L4 and L22 to the macrolide resistance remains unknown and needs further evaluations.

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EMERGENCE OF CTX-M-15 EXTENDED-SPECTRUM Β-LACTAMASE-PRODUCING *ESCHERICHIA COLI* FROM CHICKEN

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The CTX-M family of extended-spectrum β-lactamase (ESBL) has emerged as the most prevalent ESBL type, mainly detected in clinical isolates of *E. coli* and *Klebsiella* spp. To assess the prevalence and genotypes of extended-spectrum β-lactamases (ESBLs) in Korea, we evaluated *E. coli* isolates from chicken. The samples were randomly collected from 6 traditional markets. ESBL production was confirmed phenotypically according to CLSI criteria for ESBL screening and disk confirmation tests. Searches for bla_{TEM}, bla_{SHV}, bla_{CTX-M} genes were performed by PCR amplification, and the genotypes of ESBLs were determined by direct nucleotide sequence analysis of the amplified products. Of two ESBL-producing *E. coli*, one *E. coli* isolate harbored $bla_{CTX-M-15}$ and belonged to the phylogenetic group B_1. *E. coli* isolate was proved to transfer the ESBL phenotype by conjugation. The presence of the $bla_{CTX-M-15}$ gene in the transconjugant was confirmed by PCR. To the best of our knowledge, this is the first report of foodborne *E. coli* isolate producing CTX-M-15 ESBL in Korea. To prevent and control the dissemination of the resistance gene, regular monitoring of CTX-M ESBL-producing *E. coli* should be performed on food origins as well as human by the National Antimicrobial Resistance Management Program in Korea.

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PREVALENCE AND CHARACTERIZATION OF CTX-M Β-LACTAMASE IN *ESCHERICHIA COLI* ISOLATED FROM CATTLE, FARM ENVIRONMENT, AND FARMERS IN KOREA

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Background: Extended-spectrum β-lactamase (ESBL)-mediated resistance is of considerable importance in both human and veterinary medicine. Since 2000, *Escherichia coli* producing CTX-M type ESBLs have been increasingly reported worldwide in the human population. However, only a few studies have been conducted to examine the CTX-M β-lactamase in isolates from animals, farm environment, and farmers. Therefore, in this study the prevalence of CTX-M β-lactamases in cattle, their farm environment, and farmers was determined and characterization of CTX-M β-lactamase-producing *E. coli* was done. **Methods:** *E. coli* in 1536 samples including cattle feces (n = 379), milk (n = 559), farm

environmental samples (n = 512), and swabs from farmers' hands (n = 43) and nose (n = 43) were screened for ESBL production using MacConkey agar with 2 mg/L cefotaxime and confirmed by double disc synergy test. ESBL genes were identified by PCR and sequencing. Conjugation experiments, plasmid purification, replicon typing, and bla_{CTX-M} gene environment analysis were performed to further characterize these isolates. **Results:** *E. coli* from 84 (5.7%) of the 1536 samples examined demonstrated ESBL production. All the 84 ESBL-producing *E. coli* isolates carried bla_{CTX-M} genes belonging to members of CTX-M-1 (n = 35) and CTX-M-9 (n = 49) families. The bla_{CTX-M} genes were identified most commonly in *E. coli* isolated from feces (n = 29), teats (n = 25), milk (n = 14), and floor samples (n = 5), whereas $bla_{CTX-M-14}$ was detected in an *E. coli* isolated from a sample collected from farmer's hands. The most predominant CTX-M type identified was CTX-M-14 (n = 49) followed by CTX-M-32 (n = 26) and CTX-M-15 (n = 6). Transfer of cefotaxime resistance phenotype was demonstrated from 61 bla_{CTX-M}-positive *E. coli* isolates to the recipient *E. coli* J53 by conjugation. The bla_{CTX-M} genes were located on a large conjugative plasmid of approximately 90 - 120 Kb and were associated to insertion sequence IS*Ecp1* upstream of the bla_{CTX-M} genes. The horizontal dissemination of $bla_{CTX-M-14}$ and $bla_{CTX-M-32}$ genes was mostly mediated by IncF or IncI1-IΦ conjugative plasmids, respectively. **Conclusions:** This study revealed the spread of CTX-M β-lactamase-producing *E. coli* in cattle and their farm environment as well as in cattle farmer in Korea. To our knowledge, this is the first report of molecular characterization of CTX-M β-lactamase-producing *E. coli* from cattle, farm environment, and humans in Korea.

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ANTIMICROBIAL RESISTANCE BACTERIA IN HUMANS AND COMPANION ANIMALS OF SOUTH KOREA

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Antimicrobial resistance amongst companion animals, particularly household pets, is a important area because of both animal welfare and public health issues. Recent attention has been paid to the emergence of methicillin-resistant staphylococci. Some of the variations in reported resistance rates likely involve the presence of different MRSA clones in different geographic regions and between different animal species. Most MRSA strains isolated from Korean veterinary hospitals were identified as ST 72-SCC*mec* IVc-t324 in comparing with the predominant clones found in human hospitals and the general community were ST72-SCC*mec* IVa-t324 and ST72-SCC*mec* IVA-t324. Although MRSA receives the most attention, other staphylococci may be of equal or greater clinical concern in companion animals: *S. pseudintermedius* and *S. epidermidis*. Like MRSA, MRSP possesses the *mecA* gene encoded on SCC*mec* complex and MDR among MRSP is very common. MRSE isolates originated from Korean veterinary hospitals showed relatively diverse genotypes and SCC*mec* IVa was the most predominant subtype. Enterococci are commonly found in the gastrointestinal tract of many animal species. In human medicine, vancomycin resistant enterococci (VRE) are a tremendous concern but VRE are rare in companion in worldwide as well as in Korea. Recently, other MDR enterococci are of greater importance. Ampicillin-resistant CC17 *E. faecium*, an important strain in humans, was identified in a surveillance study of dogs in Korea. This strain is of concern because of its typical resistance to ampicillin, potentiated penicillins, first generation cephalosporins, potentiated sulfonamides and fluoroquinolones, the main first line treatments for UTI in pets. *Escherichia coli* is an important pathogen and common compo-

nent of the intestinal microflora. Of particular concern is extended spectrum beta-lactamases (ESBLs), which hydrolyze a broad range of beta-lactam antimicrobials. The most common genotypes of ESBLs in Korea were CTX-M-14 and CTX-M-24. Further, another concerned clone, ST131, in dogs could be sources of UTI in humans. In Korea, the most dominant ST in canine UTI was ST 405 (14.3%) and ST 961 (12.2%), and ST 131 was 6.1%. Emergence and dissemination of AMR in companion animals will undoubtedly continue to be a challenge in veterinary medicine, from both patient health and public health standpoint. More organized surveillance is required to better understand the scope of the problem.

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UNTREATED WATER FOR HUMAN CONSUMPTION AS A RESERVOIR OF CLINICALLY RELEVANT ANTIBIOTIC RESISTANCE GENES AND ESCHERICHIA COLI CLONES

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Objectives: Recent surveys have highlighted the role of environment as a reservoir of blaCTX-M or qnr genes as well as of epidemic Escherichia coli clones. Nevertheless, the role of water used for human consumption in the transmission of clinical relevant bacteria/genes remains scarcely explored. We investigated the contribution of untreated waters used for human consumption for the spread of clinically relevant antibiotic resistance genes and E. coli clones. **Methods:** Sixty-seven water samples (fountains, boreholes, water wells, natural springs) were collected from residential, forest or agricultural areas (North/Centre Portugal, 2006-08) and, after enrichment, were plated on MacConkey agar with/without cefotaxime, ceftazidime or imipenem (2mg/L). Different morphotypes of Gram negative bacilli were selected and E. coli isolates were identified by PCR. Genes encoding ESBL (blaTEM/SHV/CTX-M), carbapenemases (blaVIM/IMP/SPM/GIM/SIM/KPC/OXA-48), or other clinically relevant plasmid-mediated genes encoding resistance to fluoroquinolones [qnrA, qnrB, qnrS, qnrD, aac(6')-Ib-cr] or aminoglycosides (armA, rmtB) were searched by PCR and sequencing. Presence and characterization of class 1, 2 or 3 integrons were performed by PCR and sequencing. E. coli phylogenetic groups were identified by a multiplex PCR and specific epidemic E. coli clones (ST131, ST69, ST95, ST393) were searched by PCR and/or MLST. **Results:** We identified 253 Gram negative bacilli corresponding to different morphotypes. The qnrB genes (qnrB10, qnrB18, other variants) were frequently detected and identified in 8% (n=20/253) of the isolates. They were mostly recovered from wells (n=10), natural springs (n=8) and fountains (n=2) at agricultural areas with domestic animal production, and mainly in the North (95%, 19/20) (2006;2008). intI1 genes were also found (1%, 3/253; fountains, Centre region, 2008), although class 1 integrons variable regions were empty. ESBL and carbapenamase genes as well as other qnr, aac(6')-Ib-cr, armA, rmtB, and intI2/intI3 were not detected. E. coli was identified in 2% (5/253) of the isolates analyzed and mainly belonged to A (n=3; fumC11; fumC290; fumC377), but also to B2 (n=1; fumC24) or B1 (n=1; fumC23) phylogroups. The epidemic O25b-ST131 clone was not detected, but a B2-new ST E. coli clone (n=1; natural spring) was identified. **Conclusion:** This study constitutes the first description of qnrB genes and B2 E. coli clones among bacteria from water used for human consumption, highlighting the natural non-treated waters as potential reservoirs and/or vehicles of these clinically relevant antibiotic resistance genes/clones that can be spread to human by water ingestion.

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BULK TANK MILK SAMPLING TO MONITOR TRENDS IN ANTIMICROBIAL RESISTANCE ON DAIRY FARMS - A PILOT-STUDY

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In Switzerland a steady increase in the use of cephalosporins has been noticed for the treatment of mastitis during lactation. This use could have an influence on resistance in indicator and zoonotic bacteria in the environment of dairy farms - a setting that is not yet well covered by the existing monitoring of antimicrobial resistance. The aim of this pilot-study was to describe antimicrobial susceptibility patterns of certain indicator and zoonotic bacteria recovered from BTM and to assess the feasibility of this BTM sampling for the routine monitoring of trends in antimicrobial resistance on dairy farms. In Switzerland BTM samples are routinely collected twice a month from all dairy farms and subsequently subjected to quality testing in a single laboratory. In November 2011, 200 BTM samples were randomly collected at this laboratory and subsequently cultured for Escherichia coli (E. coli), Enterococcus faecalis (E. faecalis), Enterococcus faecium (E. faecium), Methicillin sensible Staphylococcus aureus (MSSA) and Methicillin resistant Staphylococcus aureus (MRSA). One isolate per sample and bacterial species was tested for antimicrobial susceptibility to selected antimicrobial agents by the minimal inhibitory concentration technique. Epidemiological cut-off values were used to categorise isolates as susceptible or resistant. E. coli were detected in 18 of 200 BTM samples and 19 samples were positive for E. faecalis. 33 MSSA and 2 MRSA were isolated. 66.7% of the E. coli isolates were fully susceptible to all tested antimicrobials. Resistance was most often found against ampicillin (27.8%), streptomycin (27.8%) and sulfamethoxazol (16.3%). 3 isolates (16.7%) were resistant to more than 4 antimicrobials. No resistance was found against cefotaxime or ceftazidime. All E. faecalis isolates were resistant to neomycin, 78.9% were resistant to tetracycline and 42.1% were resistant to bacitracin and streptomycin. 26.3% were resistant to erythromycin and 15.8% resistant to gentamicin. No resistance against vancomycin was found. 54.4% of all MSSA isolates were fully susceptible to all tested antimicrobials, 2 (6.1%) were resistant to more than 4 antimicrobials. Resistance against penicillin (27.3%) and trimethoprim (18.2%) was most often found. The 2 MRSA isolates were both resistant against oxacillin, penicillin, gentamicin, kanamycin, tetracycline and trimethoprim. It is to our knowledge the first time that MRSA could be detected in BTM in Switzerland. So far no extended β-lactamase producing E. coli were found. More selective culture methods could be used in future. Resistance to antimicrobials that are often used in veterinary medicine were commonly found. The sampling of BTM turned out to be easy and cost effective. However to be able to find trends of resistance or newly emerging resistances with a certain confidence, there should be tested far more BTM samples.

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ESBL-PRODUCING ENTEROBACTERIACEAE ON VEGETABLES, IN SOIL AND IRRIGATION WATER

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Background: The environment represents a reservoir of antibiotic resistant bacteria (ABR): it harbors environmental bacteria with natural antibiotic resistance, and receives ABR bacteria excreted by humans and animals through sewage and animal manure. The presence of these bacteria in the environment may pose a risk for public health. One possible route of exposure to ABR bacteria in the environment

is the consumption of vegetables that have been grown on contaminated soil or irrigated with contaminated water. The aim of the current study was to determine the prevalence of ESBL-producing bacteria on fresh produce, soil and irrigation water. **Methods:** Iceberg lettuce, soil and water were sampled at three iceberg lettuce producing companies. In addition, vegetables (iceberg lettuce, endive, chicory, blanched sellery, radish, bunched carrots, spring onion and mushrooms) were obtained from supermarkets. Cefotaxime-resistant (CTX-R) enterobacteriaceae were isolated from vegetables and environmental samples using chromogenic medium for the detection of *E. coli* and coliforms, supplemented with CTX. ESBL-production was determined using disk diffusion tests and ESBL-genes were sequenced. Isolates were identified using the API20 system and partial 16S rDNA sequencing. **Results:** In soil, surface water and on fresh iceberg lettuce, the most prevalent CTX-R enterobacteriaceae species were *Rahnella aquatilis* (39% of all isolates) and *Serratia fonticola* (55% of all isolates). *Enterobacter cloacae* and *Pantoea agglomerans* were each detected on 1 out of 75 crops. CTX-R *R. aquatilis* was isolated from all 8 types of supermarket vegetables and constituted 90% of all isolates. Other CTX-R species detected were *Enterobacter* spp. (radish, mushrooms), *Citrobacter freundii* (blanched sellery), *Citrobacter koseri* (mushrooms) and *Ewingella Americana* (mushrooms). Most CTX-R *R. aquatilis* and *S. fonticola* isolates and the *P. agglomerans* isolate were confirmed ESBL-producers. The ESBL genes were identified as RAHN-1 and RAHN-2 (*R. aquatilis*) and FONA-1, FONA-2, FONA-3, and FONA-5 (*S. fonticola*). Most of the other isolates had phenotypes compatible with derepressed AmpC-production. **Discussion and Conclusions:** On vegetables, in soil and irrigation water, ESBL-producing environmental bacteria were present, and more rarely, AmpC-producing opportunistic pathogens. So far, no anthropogenic ESBL genes were detected.

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ANTIMICROBIAL SUSCEPTIBILITY OF COMMENSAL *ENTEROCOCCUS FAECIUM* AND ENTEROCOCCUS FAECALIS FROM HEALTHY CATTLE, PIGS AND CHICKENS IN EIGHT EUROPEAN COUNTRIES OVER A FIVE YEAR PERIOD (EASSA PROGRAM).

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Background: The European Antimicrobial Susceptibility Surveillance in Animals (EASSA) is the first ongoing program monitoring antimicrobial susceptibility of zoonotic and commensal bacteria from food-producing animals at slaughter. The survey is based on uniform sampling and bacterial isolation procedures performed in local laboratories across Europe, and MIC determination to panels of human-use antimicrobials in a central laboratory. Herewith, the complete susceptibility results of *E. faecium* and *E. faecalis* isolated in 2002-2006 are reported for the first time. **Methods:** Colon or caecal content from healthy beef cattle, fattening pigs and broiler chickens was randomly sampled in 8 EU countries (5 countries per host; 4 or more slaughterhouses per country; 1 sample per herd/flock). Isolation and phenotypic identification of enterococci was performed using standard biochemical methods; MALDI-ToF and PCR were applied to confirm strain identity where necessary. MICs of 5 antibiotics were determined by means of agar dilution (CLSI, M31-A3) in a central laboratory. Results were interpreted using clinical breakpoints (CLSI, M100-S21) and Epidemiological Cut-off Values (ECVs) as defined by EFSA (2008) to determine decreased susceptibility, i.e. MIC values exceeding the wild-type MIC distribution (> ECV) but not the clinical breakpoint. **Results:** In total, 1677 *E. faecium* (cattle 290, pigs 730, chicken 657) and 260 *E. faecalis*

(cattle 84, pigs 163, chickens 13) isolates were recovered. Overall little or no resistance to ampicillin was observed for *E. faecium* strains (on average 0.7-2.7% across host species) and none for *E. faecalis* strains. For gentamicin on average less than 1% resistance was observed for *E. faecium* strains of all three hosts; resistance of *E. faecalis* strains varied from 0 (cattle), 8.0 (pigs) to 15.4% (chickens). The resistance prevalence of *E. faecium* to quinupristin/dalfopristin was much higher, on average between 18.6 to 32.1% and intrinsic resistance was confirmed for *E. faecalis* (67.4-100%). Out of all 1937 *E. faecium* or *E. faecalis* strains, only one (bovine) *E. faecium* isolate was resistant to linezolid. Vancomycin resistance amounted to 0.6 to 2.1% for *E. faecium*; 22 VRE strains (1.3%) were detected. All *E. faecalis* strains were fully vancomycin-susceptible. Decreased susceptibility was only apparent for quinupristin/dalfopristin (26-35% for *E. faecium*), whereas negligible for the other antibiotics. **Conclusions**: This pan-European survey appeared to be an interesting tool to monitor variability in occurrence and antimicrobial susceptibility of commensal enterococci strains isolated from food-producing animals at slaughter. Prevalence of clinical resistance to critically important molecules in human medicine was absent to low, except for quinupristin/dalfopristin, to which a higher prevalence of resistance was observed.

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COLISTIN RESISTANCE IN SALMONELLA AND ESCHERICHIA COLI: A STATISTICAL APPROACH

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Colistin is being re-discovered in multidrug-resistance era. Therapeutic failures with that compound are rarely reported and gene mutations altering cell membrane might not be the only factor effecting resistance. Statistical methods were applied to reveal possible colistin interactions in a dataset comprising MIC values of 14 antimicrobials tested in Salmonella (N=6337) and Escherichia coli (N=2934). Based on EUCAST cut-off values of colistin (≤ 2 mg/L) for E. coli and Salmonella, we observed 14.9% None Wild-Type (NWT) Salmonella isolates (representing 44 serovars of 12 serogroups) and 1.1% NWT E. coli isolates. Confidence interval-based analysis revealed highest prevalence (44.7÷49.4%) in Salmonella O:9 (S. Dublin 68.0%; S. Enteritidis 48.0%). It ranged from 0.5% to 4.1% in, respectively, Salmonella O:8 and O:4, except S. Brandenburg (O:4; 32.1%). Comparable colistin resistance levels were found in isolates being resistant to single (44.3÷49.3%) and 8-9 (27.0÷53.7%) antimicrobial classes but they were significantly lower in resistance profiles comprising 2-7 classes. The findings were confirmed with Colistin resistance index (RCol) demonstrating conditional probability of an isolate being colistin resistant and belonging to: Salmonella serogroup O:9 and resistance profile of 1-3 classes (RCol=0.67), any serovar of serogroup O:9 (RCol=0.44), and resistance profile of 8-9 classes (RCol=0.1). The index for any other conditional category was RCol£0.03. Correlation between MIC values of colistin and other antimicrobials were measured using Spearmans rank correlation coefficient. Positive correlation at the highest significance tested (P<0.0001) was found with ampicillin (0.42), phenicols (0.46-0.67), and tetracycline (0.56) in Salmonella O:9 resistant to 1-3 classes. Negative association was noted with nalidixic acid (-0.63), ciprofloxacin (-0.59), gentamicin (-0.38), and sulfamethoxazole (-0.54). Less significant correlations were found in few other categories but not in serogroup O:4, including S. Brandenburg, S. Typhimurium, or S. Dublin and E. coli resistant to 1-7 classes. In several extensively resistant E. coli (8-9 classes) negative correlation with cephalosporins (-0.71) and positive one with

gentamycin (0.67) was revealed. We conclude that colistin resistance related mainly with Salmonella O:9 serovars, but also seen in a few serovars from other serological groups, may result from LPS structure and not necessarily gene mutations. The outer membrane alternations due to other antimicrobials resistance and multiresistance may account for colistin MIC shifts. The observed negative correlations with some antimicrobials might pose therapeutic implications in multidrug-resistant infections. Our results indicate areas for further research on resistance mechanism and possible clinical implications.

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QUINOLONE RESISTANCE MECHANISMS IN SALMONELLA KENTUCKY WITH HIGH-LEVEL CIPROFLOXACIN RESISTANCE

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Salmonella Kentucky has recently gained epidemiological importance in several countries in Europe, Africa and the Middle East. The virulent strain was traced back to poultry infections in North African countries. Strains of both human and animal origin show resistance to critically important antimicrobials - cephalosporins and quinolones. The serovar was ranked 11th in humans in Poland. Previously we have described an emergence of multiresistant S. Kentucky in turkey. S. Kentucky isolates (N=27) contributing to clonal spread in poultry production, were selected for identification of quinolone resistance mechanisms. Broth microdilution method revealed high level ciprofloxacin resistance (MIC≥8mg/L) in 26 isolates, while a single isolate showed wild-type minimal inhibitory concentration for nalidixic acid (MIC<=4mg/L). Such high MICCip has been observed in our laboratory extremely rare (4 out of aprox. 4000 Salmonella tested over the last 8 years) and therefore detailed investigation of molecular background was justified. Quinolone Resistance Determining Regions (QRDR) of the genes encoding for gyrase subunits A and B (gyrA, gyrB) and topoisomerase IV subunits C and E (parC, parE) were amplified and sequenced. All but two feed isolates (2095/2010 and 2181/2010) showed two mutations in gyrA (Ser83Phe, Asp87Tyr). In parC Ser80Ile mutation in all but feed isolates were detected. Additional Tyr57Ser mutation was recorded in all isolates seems not affect quinolone MIC. No mutations were found in gyrB and parE. All isolates were negative for known Plasmid Mediated Quinolone Resistance (PMQR) determinants (qnrA, qnrB, qnrC, qnrD, qnrS, qepA). Single turkey isolate (1643/2010) was found positive for aac(6')-Ib gene and ciprofloxacin resistant variant (Trp102Arg, Asp179Tyr) harbouring also Ser117Leu was confirmed with amplicon sequencing. The same isolate harboured CTX-M with both resistance mechanisms located on plasmid. Herewith we confirm the genetic background of high level ciprofloxacin resistance in S. Kentucky isolated along the food chain in Poland. Compared to the clone spreading in several European countries and the US, all but feed isolates were Ser80Ile parC mutants. Interestingly, Tyr57Ser mutation seems not reflect quinolone MIC values since it was observed in wild type isolate. QRDR mutations do not exclude the presence of PMQR determinants, although they are neither detectable with phenotypic tests, nor clinically relevant in highly resistant isolates. Still, such isolates might be considered as vectors of transmissible resistance mechanisms. The finding of different quinolone resistance mechanisms associated with determinants of other critically important antimicrobials such as cephalosporins, is of clinical relevance in presumably virulent S. Kentucky isolates.

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SPECIES DIVERSITY AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF STAPHYLOCOCCUS OF ANIMAL FARM ORIGIN IN NKONKOBE MUNICIPALITY, SOUTH AFRICA

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The occurrence, antibiotic susceptibility profile and assessment of antibiotic resistance genes in *Staphylococcus* species isolated from healthy animals in Nkonkobe Municipality as well as the prevalence of associated antibiotic resistance genes were investigated using phenotypic and molecular (PCR dependent) methods. A total of 120 Staphylococcus species were isolated from 150 samples from the animals and consisted of *Staphylococcus haemolyticus* (30%) and *Staphylococcus aureus* (23.3%) from pig; *Staphylococcus capitis* (15%) from goat; *Staphylococcus heamolyticus* (5%); and *Staphylococcus xylosus* (15%) from cattle and other Staphylococci (11%) from dead chicken and pigs. About 23.3% of these isolates were coagulase positive and 76.7% were coagulase negative. Between 75-100% of the isolates were resistant to Penicillin G, tetracycline, sulphamethaxole and nalidixic acid; about 38 % were methicillin resistant consisting of 12.6% methicillin resistant *Staphylococcus aureus* (MRSA) from pig and 12% vancomycin resistant. Also, 12% of the isolates were erythromycin resistant while 40.2 % were resistant to ceftazidime. The antibiotic resistance genes *vanA, VanB, eryA, eryB, eryC* were absent in all the phenotypically resistant isolates, but *mecA* gene and *mph* genes were detected. The high phenotypic antibiotic resistance and the presence of some associated resistance genes is a potential threat to public health and suggests the animals to be important reservoirs of antibiotic resistance determinants in the environment. **Keywords:** Staphylococcus species, generic identification, specie-specific identification, antibiotic resistance, resistance genes, *mecA* gene

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THREE-YEARS MONITORING OF MICROBIOLOGICAL RESISTANCE IN INDICATOR ESCHERICHIA COLI FROM SLAUGHTERED ANIMALS

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Monitoring of antimicrobial resistance of indicator bacteria is valuable tool for screening of impact of antimicrobial usage and assessment of changes in drug efficacy. Previously (2nd ASM Conference, Toronto, 2010) we presented countrywide resistance monitoring program in indicator E. coli isolated from animals at slaughter based on EFSA recommendation (2008). Here we present the results of the project, including temporal trends over the last three years. Minimal Inhibitory Concentrations of 14 compounds was tested in approx. 850 isolates per year and interpreted according to epidemiological cut-off values. Prevalence of microbiological resistance and its trends was assessed with 95% interval-based approach verified with contingency tables. Ampicillin resistance was stable in E. coli from most sources; low in cattle (≈10%), moderate in pigs and laying hens (≈30%), and high in broilers (≈70%). In turkey it increased ($P \leq 0.01$) up to 74.8%. Cephalosporin resistance was sparse reaching occasionally 10.0% in broiler isolates. Resistance to gentamycin was also low (<10%) but in turkey it increased ($P \leq 0.001$) up to 19,3%. Kanamycin values were slightly higher and temporal trends varied: increased ($P \leq 0.01$) in turkey up to 20.5% and decreased ($P \leq 0.05$) in broilers and layers (down to 9.4% and 2.6%, respectively). No significant changes were observed in streptomycin resistance, with high prevalence level in pigs, broilers and turkeys (40÷55%), and lower in cattle and lying hens (10÷15%). Resistance to phenicols remained stable reaching the highest levels in broiler and turkey isolates (<20%). Quinolone resistance was low in cattle and pigs (<10%), but in layers, turkey and broilers it reached, respectively, up to 40, 60, and 80%. However, the 3 years

trends have not been found significant. The higher values for ciprofloxacin than nalidixic acid resistance indicated the role of plasmid mediated quinolone resistance mechanisms. Source-dependent levels of resistance observed in tetracycline and folate pathway inhibitors were stable, with the exception of increasing ($P \leq 0.05$) sulfamethoxazole resistance in turkey isolates reaching the highest value of 60.2%. The successful implementation of national antimicrobial resistance monitoring in E. coli originating from animals at slaughter gave possibility to asses species-related level of resistance as an effect of different management practices in various animal production sectors. The temporal trends noted within isolates from a given source might reflect current changes in antimicrobial usage that leads to an increase or decrease as observed in aminoglycoside resistance in poultry isolates. The level of resistance mostly noted in poultry should be sincerely considered by policy makers, farmers and professionals for joint preventive action against the threat of post-antibiotic era.

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INTEGRONS INTO THE DISCHARGED WATER FROM SLAUGHTERHOUSES

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The spread of antibiotic-resistant bacteria (ARB) is a growing problem and a public health issue. Livestock are a recognized reservoir for ARB. Wastewater treatment plants (WWTPs) receiving ARB-containing slaughterhouse effluents are considered to be hotspots for antibiotic resistance dissemination by horizontal gene transfer. Resistant integrons (RIs) are genetic elements that acquire, exchange and express antibiotic-resistance genes embedded in gene cassettes (GCs). More than 130 GCs conferring resistance to antibiotics have been described in RIs and 3 classes of RIS are involved in antibiotic resistance. This study intends to evaluate the dynamics of class 1 RIs from slaughterhouse effluents to treated effluents of three different slaughterhouse WWTPs. Three beef production slaughterhouse WWTPs using activated sludge process were selected in France. Representative 24h flow proportionate samples of influents and effluents were sampled and concentrated in triplicate using cellulose ester filters (0.45 µm pore size). The total DNA was extracted and the class 1 RIs were quantified using qPCR and normalized to the 16S RNA-encoding DNA gene copy number to obtain the relative abundance of class 1 RIs, so as to estimate the quantity of RIs per bacteria. In regard to the literature, the concentration of class 1 RIs in the three slaughterhouse effluents was high with values comprised between 10^9 and 10^{10} copies.L^{-1}. The three WWTPs allowed a reduction rate of 2 log of these concentrations in the treated effluents. However, with values comprised between 0.016 and 0.083, the class 1 RIs relative abundances in the slaughterhouse effluents and their treated WWTP effluents were low compare to values previously recovered from urban WWTPs. Nevertheless, the effluents treatments reduced the relative abundances of class 1 RIs from the water fraction by a factor 2 and 5 for the 2 sites where their relative abundance were the highest (0.040 and 0.083). To conclude, beef slaughterhouses are not main generators of bacteria harboring class 1 RIs. Actually, while the slaughterhouse WWTP processes reduced the concentration and the proportion of bacteria harboring class 1 RIs, the high bacterial load of these effluents resulted in the release in the environment of around 10^{13} copies per day of RIs.

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CARRIAGE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *ESCHERICHIA COLI* BY HORSES IN THE COMMUNITY

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Aims: To determine the duration of carriage of multi-drug resistant (MDR) *E. coli* in horses in the community. **Methods:** Thirty horses were sampled over 5 months (225 samples), including 15 recently discharged from a tertiary referral equine hospital. Samples were cultured for antimicrobial resistant and extended-spectrum β-lactamase (ESBL) producing *E. coli*. *E. coli* isolates were confirmed by *uidA* PCR and ESBL-producing isolates were screened for bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{AmpC} and sequencing. Multivariable, multilevel logistic regression was used to determine risk factors for carriage of MDR *E. coli*. Kaplan-Meier plots were used to determine median durations of carriage. Differences in duration of carriage were evaluated using the Log-rank test. **Results:** Most horses demonstrated faecal carriage of antimicrobial resistant *E. coli* on the majority of sampling occasions (80.8%), with similar shedding patterns between the recently hospitalised and non-hospitalised horses. However, MDR (34.3% of samples positive) and ESBL-producing *E. coli* (7.2% of samples positive) shedding was more intermittent. The median duration of community carriage was 22 days for ESBL-producing *E. coli* and 61 days for carriage of MDR *E. coli* and this was significantly shorter than for any antimicrobial-resistant *E. coli* (p=0.001). Amongst the 40 ESBL-producing *E. coli* a high prevalence of bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes were identified. The $bla_{CTX-M-1}$ and bla_{CMY-2} genes were only identified in isolates from community horses, and $bla_{CTX-M-9}$ genes were only identified in the recently hospitalised horses. Sequencing of representative isolates from the CTX-M groups 1, 2 and 9 revealed them as $bla_{CTX-M-15}$, $bla_{CTX-M-2}$ and $bla_{CTX-M-14}$, respectively. The results of the macro-restriction pulsed-field gel electrophoresis suggested that a proportionally greater diversity of ESBL-producing *E. coli* strains were recovered from the five non-hospitalised horses compared to those recently hospitalised. Multivariable analysis identified that hospitalisation within the previous 28 days significantly increased the risk for carriage of ESBL-producing *E. coli*. **Conclusions:** This study highlights the persistence of carriage of antimicrobial-resistant *E. coli* by horses, even in the absence of factors such as antimicrobial therapy. Continued shedding of ESBL-producing *E. coli* by animals discharged from hospital has important implications for the dissemination of ESBL-producing bacteria into the wider community, both to other horses and, potentially, to those people involved in their care.

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OCCURRENCE OF ESBL/PAMPC-PRODUCING *E. COLI* AND SALMONELLA IN BEEF AND PORK IMPORTED INTO SWEDEN

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The presence of *Enterobacteriaceae* producing extended spectrum β-lactamases (ESBL) or plasmid-encoded AmpC β-lactamases (pAmpC) is increasingly being reported in humans and in animals worldwide. Due to the occurrence in food-producing animals it has been suggested that food can be a possible link between the two populations. The current study aimed to examine samples of beef and pork imported into Sweden as potential sources of ESBL/pAmpC-producing *E. coli* and salmonella. The prevalence of these bacteria was investigated in 178 and 119 samples of imported meat from cattle and pigs, respectively. Samples were collected at retail stores from January 2010 to June 2011. Suspected resistant *E. coli* were isolated from meat after enrichment in broth with 1 µg/ml cefotaxime and culture on CHROMagar™ESBL or CHROMagar Orientation with 1 µg/ml cefotaxime. ESBL/AmpC-production was verified by Etest. Susceptibility to 14 antibiotics was assessed by broth microdilution using VetMIC GN-mo.

ESBL/pAmpC genes were detected using multiplex PCR and specific gene variants were determined by sequencing using BigDye® v1.1. A selection of isolates was subjected to MLST. Plasmids were characterised using transformation or conjugation with subsequent replicon typing PCR. Beef samples from Ireland and South America did not contain ESBL-producing *E. coli*, whereas these bacteria were found in 8 % of beef samples imported from other EU-countries. ESBL-producing *E. coli* were found in 2 % of Danish pork samples, 7 % of German pork samples and 13 % of pork samples imported from other EU-countries. *E. coli* producing pAmpC was found in one sample tested, from Polish pork. The dominating ESBL gene both among beef and pork associated isolates was $bla_{\text{CTX-M-1}}$. The most prevalent ESBL gene among clinical *E. coli* in Sweden, $bla_{\text{CTX-M-15}}$, was found in 1 % of the bacteria isolated from meat, two samples from Dutch and Austrian beef. The $bla_{\text{CTX-M-15}}$ gene was identified on plasmids belonging to the IncI1 group and the isolates carrying $bla_{\text{CTX-M-15}}$ were typed as ST10. The only pAmpC gene detected was $bla_{\text{CMY-2}}$. 78 % and 88 % of the ESBL/pAmpC-producing *E. coli* isolated from beef and pork, respectively, were resistant to three or more antibiotic classes. Resistance to sulphametoxazole, trimethoprim and tetracycline was the most common trait. *Salmonella* was found in two samples tested, from German and Italian pork. Both *Salmonella* were resistant to ampicillin, tetracycline and sulphametoxazole. Neither isolate was resistant to third generation cephalosporins or fluoroquinolones. In conclusion, ESBL/pAmpC-producing *E. coli* were found in samples of imported beef and pork available on the Swedish market, with the highest prevalence in pork. Further studies are needed, including a more detailed comparison of ESBL/pAmpC genes and *E. coli* isolates from meat and Swedish patients, to assess the potential public health risk of these bacteria in food.

73B

CHARACTERISATION OF E. COLI ANTIBIOTIC RESISTANCE MECHANISMS AND PLASMIDS RECOVERED FROM US DAIRY CATTLE EXPOSED TO ANTIBIOTIC AGENTS

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The acquisition and maintenance of bacterial antibiotic resistance genes by commensal bacteria in food animals and in the environment is a growing global problem. Not only can this introduce resistant bacteria into the food chain, but these ecological niches provide a reservoir from which antibiotic resistance genes can be amplified and disseminated. In the United States E. coli resistant to antibiotics commonly used in animals (eg. ceftiofur and tetracycline) are identified frequently. Resistance is predominantly mediated by genes (eg. blaCMY-2, tetA, tetB) located on plasmids, however the contribution of plasmids to the stability and dissemination of these resistance genes, within and between ecological niches and different strains of bacteria is not clear. In this study we aimed to determine the diversity of E. coli resistance genes obtained from cattle exposed to different antibiotic treatments; and to characterise the common resistance plasmid types selected under these conditions. Steers were randomly assigned into pens and fed either chlortetracycline or an inert feed carrier; within these two groups cattle were then assigned to receive either ceftiofur or no ceftiofur. Fecal samples were collected on Day 0, 4, 12 and 26 and E. coli isolated (n=1050). Minimum inhibitory concentrations of β-lactams and tetracycline were determined and PCR/sequencing used to detect resistance genes and plasmid replicon types in each of the E. coli isolated. IncA/C (n=84) and IncI (n=116) plasmids were identified from E. coli collected from groups treated with ceftiofur. β-lactamase

gene blaCMY-2 was associated with plasmid replicon IncA/C (n=76), however was also found in isolates containing IncIA plasmids (n=26) and in isolates containing neither plasmid type (n=43). The presence of blaCMY-2 was also associated with tetA alone or tetA and tetC in groups treated with ceftiofur. tetB was found more frequently with replicon type IncI both in the presence and absence of a blaCMY-2 gene. While common resistance genes such as blaCMY-2 (in association with IncA/C plasmids), tetA and tetB were most prevalent within this study, resistance was also mediated by other determinants such as tetC, blaCMY-2 associated with IncI plasmids, and by chromosomal mutations in the ampC promoter region. How these different resistance genes and their plasmid vectors interact and how they contribute to the spread and persistence of antibiotic resistance in this environment is yet to be determined.

74B

EXTENDED SPECTRUM BETA-LACTAMASE PRODUCTION IN THREE STRAINS OF ESCHERICHIA COLI ISOLATED FROM FECES OF HEALTHY CHICKEN USED FOR MEAT PRODUCTION IN ABIDJAN CÔTE D'IVOIRE

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Background: Escherichia coli can colonize multiple niches, including human hosts, animals and plants. The pressure generated by the use of increased antibiotics in livestock may caused the selection of resistant commensally bacteria. The transfer of their gene resistances to pathogenic bacteria to humans via the food chain (foods and organic fertilizers) increases the antibiotic resistance and the potential treatment failure. **Objective:** To detect the production of beta lactamases broad spectrum of Escherichia coli strains from digestive carriage of healthy chickens by using molecular detection and antibiotic resistance tests. **Materials and methods:** A total of 243 Escherichia coli strains isolated from cloacae swabs of healthy chickens in 51 semi-industrial farms in the nearby of Abidjan from March 2011 to March 2012. The isolation and the identification were made by conventional bacteriological techniques. Susceptibility testing of the strains was performed as recommended by the Antibiogram Committee of the French Society of Microbiology using an automated reading OSIRIS (Biorad). Production of beta-lactamases broad spectrum was demonstrated by using double disk diffusion method. The following antibiotics were tested: amoxicillin, amoxicillin & clavulanic acid, chloramphenicol, cefotaxime, ceftriaxone, cefepime, ceftazidime, tetracycline, nalidixic acid, ciprofloxacin, amikacin, gentamicin, tobramycin, cotrimoxazole. PCR was done only on the strains producing broad spectrum of beta lactamases. DNA extraction was performed by phenol-chloroform method, and the PCR of the genes blaTEM, blaSHV, blaCTXM1, blaPER, blaGES and blaVEB, qnrA, qnrB and qnrS were done. **Results:** The antibiogram showed that the most important resistance rates were 98.4%, 95.9% and 54.7% respectively with tetracycline, cotrimoxazole and ciprofloxacin. The lowest resistance rates were 2.5% and 2.9% respectively for the cefepime and amikacin. Three strains (1.2%) of 243 produced beta-lactamase broad spectrum. One strain has showed positive for gene for qnrB and blaSHV and three has showed positive genes for blaVeb, blaPer, blaGes. **Conclusion:** The existence of Escherichia coli strains producing extended spectrum beta-lactamase in chicken feces is a health risk for food production and for environmental propagation. Keywords: resistance genes, antibiotics, Escherichia coli, chicken.

75B

EFFECTS OF NATURAL MUTATIONS IN THE *RAMRA* LOCUS ON INVASIVENESS OF EPIDEMIC FLUOROQUINOLONE-RESISTANT *SALMONELLA* TYPHIMURIUM ISOLATES

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Background. Fluoroquinolone (FQ) resistance is increasing worldwide among *Salmonella* spp. Among the mechanisms involved, increased efflux via the tri-partite AcrAB-TolC efflux system is mainly modulated through control of expression via the *ramRA* regulatory locus gene products. Interestingly, in some reference strains these have also been experimentally shown to regulate cell invasion-related genes of the type III secretion system-1 (T3SS-1). In this study we investigated whether natural mutations occurring in the *ramRA* locus of FQ-resistant *S. enterica* serovar Typhimurium (*S*. Typhimurium) epidemic isolates resulted in the same effects. **Methods.** qRT-PCR and cell invasion assays were used to address the question above in three clinical FQ-resistant *S.* Typhimurium isolates representative of the DT104 and DT204 epidemic isolates. For comparison three control reference quinolone-susceptible strains were included. **Results.** As previously shown, the investigated mutations altering the RamR repressor or its DNA binding site increased expression of efflux genes dependently on *ramA*. However, the decreased expression of T3SS-1 genes previously reported was not always observed and seemed to be dependent on the genetic background of the FQ-resistant isolate. As well a *ramA*-dependent decreased invasion of intestinal epithelial cells was observed for only one particular clinical *ramR* mutant. **Conclusion.** *ramRA* mutations occurring in clinical FQ-resistant *S.* Typhimurium isolates may negatively modulate their invasiveness but this is strain-dependent.

76B

ESCHERICHIA COLI RESISTANT TO CEPHALOSPORINS AND FLUOROQUINOLONES IN ZOO ANIMALS

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The occurrence of antimicrobial-resistant commensal *Escherichia coli* was studied in animals housed at the Ostrava Zoological Garden, Czech Republic. Our investigation was focused on the cefotaxime- and ciprofloxacin-resistant isolates. A total of 160 fecal samples from 132 mammal, bird and reptile species were obtained during 4 visits over a period of one year. All samples were cultivated on MacConkey agar (MCA) supplemented with cefotaxime (2 mg/L) and MCA with ciprofloxacin (0.05 mg/L). *E. coli* isolates were tested for susceptibility to 12 antibiotics and screened for the presence of *bla* genes and plasmid-mediated quinolone resistance (PMQR) genes, integrons, and gene cassettes. Epidemiological relatedness of isolates harboring *bla* and/or PMQR genes was assessed using macrorestriction profile analysis by pulsed-field gel electrophoresis. Resistance-carrying plasmids were characterized by restriction fragment length polymorphism, incompatibility group, conjugation, and transformation experiments. The prevalence of cefotaxime-resistant and ciprofloxacin-resistant isolates was 38% and 57%, respectively. The *bla* genes were found in 88% of cefotaxime-resistant *E. coli* and PMQR genes in 16.5% of ciprofloxacin-resistant *E. coli* isolates. The most frequently detected resistance genes were $bla_{\text{CTX-M-1}}$, *sul2*, and *tetA*, frequently located on the same plasmids. Two thirds of *qnrS1* genes were located on conjugative plasmids. The macrorestriction profiles of *E. coli* isolates were highly variable, while the

plasmids were rather uniform. These results suggest limited interspecific transfer of bacterial clones, but frequent horizontal spread of resistance genes. The low variability of plasmids indicates their common source, possibly food. Since so many isolates carried the genes *tetA* and *sul2*, we suspect as possible major source of antibiotic resistance the chickens prophylactically treated with doxycycline and rabbits treated with trimethoprim-sulfonamides that were fed to a significant proportion of zoo animals. Co-selection concerning tetracycline, sulfonamides, cefotaxime, and ciprofloxacin could explain the origin of this type of multiresistance. This study was funded by the project 'CEITEC - Central European Institute of Technology' (CZ.1.05/1.1.00/02.0068) from the European Regional Development Fund.

77B

UP-REGULATION OF MULTIDRUG EFFLUX PUMPS DURING THE BACTERIAL STRESS RESPONSE TO HOST PHYSIOLOGICAL CONDITIONS

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Drug efflux represents an important mechanism for antibiotic and multidrug resistance in bacteria. Different from other resistance determinants which genes usually are acquired by adaptive mutations or horizontal transfer, efflux genes often are chromosomally encoded and they constitute from 6% to 18% of all transporters in bacterial genomes. Since over-expression of these genes almost always causes drug resistance, their up-regulation and roles in bacterial stress response to host environment is of great interest to understand the emergence of drug resistance during the processes of bacterial infection and treatment of infectious diseases. To explore this, we examined expression of all 20 efflux genes encoded on *E. coli* genome under two host relevant conditions: the oxygen deprived anaerobic condition and anaerobic combined with amino acids starvation. We found that two RND multidrug efflux pumps, MdtEF and CusCFBA, are significantly up-regulated under these two host relevant conditions respectively. Consistent with the increased expression of these efflux systems, Δ*mdtEF* renders a declined tolerance to several drugs in an *E. coli* drug sensitive KAM3 strain under anaerobic growth conditions, and Δ*cusCFBA* caused decreased tolerance to heavy metals copper and silver under the condition of oxygen and amino acids limitation. Since up-regulation of these efflux systems was independent of antibiotics pre-exposure, we speculate that these two systems contribute to the adaptation of *E. coli* to the anaerobic, and anaerobic and amino acids starvation stresses encountered in human host. Growth and physiological studies revealed that multidrug efflux pump MdtEF protects *E. coli* from nitrosative stresses by expelling indole nitrosative metabolic by-products and the CusCFBA system promotes bacterial survival by protecting the Fe-S cluster containing enzymes in anaerobic respiration and branched amino acids biosynthesis from copper toxicity under the condition of oxygen and amino acid limitation. Taken together, these studies strongly suggest that up-regulation of multidrug efflux pumps contributes to bacterial stress responses to host environments and this up-regulation may represent an important source of drug resistance during the process of bacterial infection and treatment of infectious diseases.

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FIRST IDENTIFICATION OF BACTERIA FROM ANIMALS TIGECYCLINE RESISTANT.

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Introduction: Tigecycline is the first of a new tetracycline class, the glycylcyclines. Its use is

restricted to human Hospitals for the treatment of complicated infections caused by resistant microbes. To date, the emergence of human isolates resistant to tigecycline has been limited. In *Klebsiella pneumoniae* the tigecycline resistance mechanism identified to date is restricted to overexpression of AcrAB-TolC, an RND efflux pump. The aim of this study was to characterize the genetic basis of tigecycline resistance in two multiresistant *K. pneumoniae* isolated from two dogs in Madrid. **Methods:** PFGE was performed to analyze the genetic relation between the isolates. MICs with and without PAβN (nonspecific efflux pump inhibitor) were performed and interpreted following the Clinical Laboratory Standars Institute guidelines to assess the implication of an efflux pump. The operon *acrAB* and its regulome were sequenced and analyzed. Real-Time Quantitative PCR was performed to determine expression levels of the operon *acrAB*, in our resistant strains compared to a susceptible control (ATCC 10031). Plasmid profiles of resistant strains were analysed by S1-PFGE and, plasmid were transformed into a laboratory *E. coli.* **Results:** PFGE revealed that the strains were not clonal. The MICs to tigecycline in presence of PAβN medium were lower than in medium lacking PAβN, showing that an efflux pump was responsable for resistant to tigecycline in our strains. The sequencing of the operon and its regulome did not reveal any significant mutation. Analysis of the RT qPCR results showed that the expression levels of resistant and susceptible strains were not significantly different. Therefore AcrAB is not overexpressed and is not responsable for the tigecycline resistance in our strains. **Conclusions**: This study describes for the first time the identification of two animal isolates resistant to tigecycline, which opens new ways of spreading for bacteria resistant to these antibiotics. Besides, we have desmostrated that the AcrAB efflux pump, the only known efflux pump responsable for tigecycline resistance in *Klebsiella,* is not involved in tigecycline resistant in these isolates. Our results suggests that we have here a new mechanism of resistance to tigecycline in *Klebsiella*. Furthermore this poses new and intriguing question about the origin of these resistant isolates in companion animals.

79B

EXTENDED-SPECTRUM-B-LACTAMASE AND AMPC-PRODUCING *ENTEROBACTERIACEAE* IN FECAL SAMPLES OF DOGS AND CATS

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Introduction: Extended-spectrum-β-lactamase (ESBL) producing *Enterobacteriaceae* with resistance to extended-spectrum cephalosporins, like cefotaxime, have been isolated from different reservoirs. Intestinal carriage of ESBLs in companion animals has not been studied in The Netherlands. This knowledge is important to identify the potential source of clinical isolates and to determine the role of companion animals in the transmission of ESBL to humans. The aim of this pilot study was to determine the presence of cefotaxime-resistant *Enterobacteriaceae* in the gut of healthy dogs, and dogs and cats with diarrhea. **Methods:** Faecal samples from healthy dogs (n=20) and cats (n= 20) were collected from different parts of The Netherlands. Samples from dogs (n=20) and cats (n=20) with diarrhea were obtained from the Veterinary Microbiological Diagnostic Center (VMDC) of the Faculty of Veterinary Medicine. Each sample was inoculated onto MacConkey agar supplemented with 1 mg/L cefotaxime (MacConkey+) and in 1 mL LB-medium supplemented with 1 mg/L cefotaxime for enrichment. After overnight incubation 10 µL was plated onto MacConkey+. The species of 5 colonies/sample were biochemically identified. ESBL/AmpC-production of 130 isolates was determined by combination disc tests. A selection of isolates (n=46) were confirmed with the

Check-Points tube array Check-MDR CT103 and with sequence analysis of the chromosomal *ampC* promoter region. **Results:** After direct plating 11/20 healthy dogs were positive. Nine samples showed growth of *E. coli* and 2 samples of non-*Enterobacteriaceae* species. No healthy cats were positive. Enrichment showed the same results. Of the dogs with diarrhea 9/20 were positive. Nine with *E. coli* and one with a non-*Enterobacteriaceae* species. After enrichment, 3 more dogs were positive for *E. coli* and one for Pseudomonas. 6/20 cats were positive, 4 samples with *E. coli*, one sample with *E. coli* and *P. mirabilis* and one with non-*Enterobacteriaceae* species. Seventy two *E. coli* strains displayed an ESBL phenotype, 54 *E. coli* and one *P. mirabilis* displayed and AmpC phenotype and four samples displayed inconclusive results. The array identified the following ESBL genes: bla_{CTX-M} group 1, 9 and 15, bla_{SHV-12}-group, bla_{TEM-11}-group, and bla_{TEM-52}-group. bla_{CTX-M} group 1 was predominant (n=12). Among the AmpC phenotype the plasmid mediated or bla_{CMY-2}-group was identified in *E. coli* and *P. mirabilis*. Also *E. coli* strains with mutations in the chromosomal *ampC* gene promoter were found. Seven animals were co-colonized with an *E. coli* with or bla_{CMY-2}-group and *E. coli* with either bla_{CTX-M} group 1, bla_{SHV-12}-group, bla_{TEM-11}-group or bla_{TEM-52}-group. **Conclusion:** This pilot study shows a high prevalence of cefotaxime-resistant *E. coli* in dogs and cats with diarrhea and in healthy dogs. Close contact between companion animals and humans may provide a potential risk for transfer of ESBLs.

80B

OCCURRENCE OF CTX-M-15-PRODUCING KLEBSIELLA PNEUMONIAE OF SEQUENCE TYPE ST274 AMONG PETS IN FRANCE, AND EMERGENCE OF A NEW PLASMID TYPE

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Background: Although the occurrence of extended-spectrum ß-lactamase (ESBL) producing Enterobacteriaceae in humans is now becoming alarming, the emergence of ESBLs in animals raises some important concern too. CTX-M-15 is considered as the main ESBL determinant worldwide. Whereas ESBL-producing enterobacterial isolates have been often reported in animals, the occurrence of CTX-M-15 seems to be very limited. Our study aimed to evaluate the colonization rate for ESBL-positive or carbapenemase-producing Enterobacteriaceae of domestic animals living in France. **Materials:** Rectal swab samples (n=90) were recovered from pets and sheeps during a one-year period in 2011 at the Veterinary School of Maisons-Alfort, suburb of Paris, France. Those swabs were resuspended in sterile water and then plated onto Drigaslki agar plates containing either ceftazidime (2 µg/ml) or imipenem (2 µg/ ml). In addition, a total of 105 enterobacterial isolates recovered from pet clinical samples (all urines) were included. For comparison, a total of 20 CTX-M-15-producing K. pneumoniae clinical isolates recovered at the Bicêtre hospital, France, were analyzed. Multilocus sequence typing (MLST) to identify the genetic backround of the strains. PCR experiments and DNA sequencing were used to identify the ß-lactamase genes. Clonality was assessed by DiversiLab analysis (bioMérieux). Plasmid typing was performed by PCR-based Replicon Typing. **Results:** A total of 30 isolates exhibiting an ESBL phenotype were recovered from this screening, being from dogs (n=19), cats (n=7), sheeps (n=4) and domestic goose (n=1). A total of 21 isolates were recovered from the rectal screening approach and 9 from urines. There were 14 K. pneumoniae, 13 E. coli, 2 Klebsiella oxytoca, and one Escherichia fergusonii. No imipenem-non susceptible enterobacterial isolate was recovered during this screening. Only four types of ESBLs were identified, being CTX-M-15 (n=18), CTX-M-1 (n=10), CTX-M-14 (n=1), and TEM-52 (n=1). Noticeably, the 14 K. pneumoniae isolates harboring the blaCTX-

M-15 gene all belonged to the same ST274, corresponding to four clonal strains. By contrast, no ST274 was identified among the human isolates, those latter corresponding to 15 distinct ST types. Nine CTX-M-1-producing E. coli were identified, belonging to five different STs, but with ST124 (n=5) being the major one. The same and newly identified plasmid type was shown to carry the blaCTX-M-15 gene in all animal isolates, and was identified in 4 out of the 20 human isolates. **Conclusion:** This study indicates that pets are often colonized or infected by ST274 K. pneumoniae producing CTX-M-15. However, those isolates are different from those identified in humans, thus excluding a direct transmission. Very interestingly, a single CTX-M-15-encoding plasmid was identified in all animal isolates, and was also identified in some human isolates.

81B

CHARACTERIZATION OF HUMAN AND ANIMAL CLINICAL ISOLATES FROM SERBIA CARRYING 16S RIBOSOMAL RNA AMINOGLYCOSIDE RESISTANCE METHYLTRANSFERASES

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Introduction: The 16S rRNA methyltransferases have emerged in Gram-negative pathogenic bacteria as an acquired resistance mechanism conferring high-level resistance to most clinically relevant aminoglycosides. Since 2003, seven different 16S rRNA methyltransferases have been described: armA, rmtA-E and npmA. The aim of this study was to detect and characterize the presence of these genes in bacteria from human and animal clinical isolates in Serbia recovered during 2010 and 2011. **Methods:** PCR screening for the known methyltransferase genes was performed and products were sequenced. The MICs (Minimal Inhibitory Concentrations) were performed and interpreted following the Clinical Laboratory Standards Institute guidelines. PCR mapping for Tn1548 was performed on the armA-positive samples. PFGE was performed in the Klebsiella isolates and the transferability of aminoglycoside resistance was assessed by conjugation and transformation. Plasmid profiles of the transconjugants and transformants were analysed by Replicon Typing. PCRs and sequencing was performed in order to elucidate the β-lactam and fluoroquinolone resistance determinants. **Results:** Ten Enterobacteriaceae (eight Klebsiella pneumoniae, one Proteus mirabilis and one Acinetobacter baumanii) and one Pseudomonas aeruginosa isolated from humans were positive for the armA gene. The rmtB gene was identified in Aeromonas hydrophila from a fish. The MICs showed they were all high-level resistant to all 4, 6-disubstituted deoxystreptamine aminoglycosides and most of them concomitantly resist to ampicillin, tetracycline, chloramphenicol, ceftazidime, sulfamethoxazole, and fluoroquinolones. Four of the K. pneumoniae isolates showed by PFGE to be genetically related, and their transconjugants bore the pCTX-M3 plasmid that includes Tn1548, blaCTX-M3 and blaTEM-1. The other four K. pneumonie showed to be clonal and bore the blaCTX-M3 gene as well as armA but not in an IncL/M plasmid like pCTX-M3. All eight K. pneumoniae isolates bore further the blaSHV-12 gene and one of them possessed also qnrB. **Conclusions:** This is the first report of aminoglycoside resitance methyltransferases in Serbia and also the first time that a methyltransferase gene is found in a fish isolate. The spread of multirresistant isolates bearing human and animal isolates is crucial to identify the emergence of resistant isolates and preserve the effectiveness of this important class of antibiotics.

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COLE1 PLASMID IN ANTIMICROBIAL RESISTANCE IN HUMAN AND ANIMAL BACTERIA

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We have recently shown that acquired multiresistance to antibiotics in *Pasteurella multocida* is mediated by coexistence of small plasmids. One of these plasmids, pB1000, has been also identified in clinical isolates of *Haemophilus influenzae* from Europe, USA, and Australia. pB1000 and further small ColE1 superfamily plasmids can stably cohabit in the same bacterium, giving rise to multiresistance phenotypes in *Pasteurellaceae*. We show here that this elegant strategy is not unique to *Pasteurellaceae* and is in contrast with the more common multiresistance acquisition mediated by large conjugative plasmids. The evolution of antimicrobial resistance and multiresistance mediated by small plasmids has been analyzed. Strains bearing several small cohabiting plasmids were evolved in vitro under different conditions of antimicrobial pressure, mimicking antibiotic therapy. To analyze the evolution of plasmids and strains, antimicrobial resistance levels and plasmid copy number by Q-PCR were determined at different time points. Interestingly, antimicrobial pressure induced an important increase in plasmid copy number. The rise affected all plasmid types in the cell, irrespectively of the antibiotic used, giving rise to an increase in the antimicrobial resistance levels. Further, when antimicrobial pressure was removed, strains recovered their original plasmid copy number and resistance levels. This novel phenomenon implies that antibiotics can orchestrate plasmid copy number and antimicrobial resistance levels. Finally, ColE1 plasmids have been sought in human and animal pathogens, and we show that these elements are widely-spread in clinical isolates of different origins.

83B

ESBL-PRODUCING SALMONELLA DETECTION BASED ON CHECK-KPC ESBL ARRAY

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Antimicrobial resistance surveillance programs especially focus nowadays on detection and identification of Extended Spectrum Beta-Lactamases (ESBL) producing bacteria. This surveillance is based on the ESBL-gene detection using PCR, followed sometimes by sequencing to retrieve β-lactamase sequence identifications. Check-KPC ESBL micro-array method developed by Check-Points B.V. (Wageningen, the Netherlands) using Clondiag (Jena, Germany) technology allows to target blaKPC, groups of blaCTX-M and delineates ESBL from non ESBL for blaTEM and blaSHV. The goal of our study was to evaluate the performance this commercial DNA microarray format, Check KPC ESBL, to detect β-lactam resistance genes in Salmonella and to determine the feasibility of a routine use on surveillance purposes. Nineteen ESBL producing Salmonella strains previously confirmed by PCR and sequencing and collected through the French Salmonella network (ANSES, Maisons-Alfort, France) between 2005 and 2009 were tested with the Check-KPC method at least twice. Thereafter, feasibility of this new micro-array method was evaluated for a routine use, on a prospective collection of suspected ESBL carrying Salmonella isolates from non human sources. Thirty-seven isolates collected between January and June 2010 were selected for displaying a resistance-type to at least one cephalosporin. Both methods, Check-KPC microarray versus PCR and sequencing, were performed on this collection. Check-KPC ESBL applied to the retrospective collection proved to be reliable with a sensitivity of 98.5% and a specificity of 98.6%. Analysis of the prospective collection

data, showed comparable performances with a sensitivity of 100% and a specificity of 99.1% and also an important reduction of cost (up to 40%), handling time (50%) and reporting time (24-48h versus 2 weeks). Rapid identification of ESBL carrying Salmonella is an important value to improve risk management. In this perspective, tools such as Check-KPC ESBL appear to be fully suited to surveillance and molecular resistance investigation purposes.

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IMPACT OF THIRD GENERATION CEPHALOSPORIN (3GC) ADMINISTRATION IN HATCHERY ON THE PREVALENCE OF 3GC-RESISTANT *E. COLI* IN INTESTINAL FLORA OF BROILERS

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In many European countries, a dramatic increase of the prevalence of extended-spectrum cephalosporin (3GC)-resistant *E. coli* in broilers is observed (1). A possible link between off-label use of 3GC in hatcheries and increase of 3GC resistance in *E. coli* is suspected. This study aimed at assessing the prevalence and the percentages of 3GC resistant fecal *E. coli* in flocks of broilers injected or not injected *in ovo* with ceftiofur (TIO). Thirty flocks of free range broilers originating from two hatcheries were included in the study. Fifteen flocks were treated with TIO by injection *in ovo* at 18 days of incubation. These treated flocks and fifteen untreated flocks from the same hatcheries were monitored up to 77 days of life. Each flock was sampled five times, from the first day of life (D1), before arrival on the farm, and two, seven, 41 and 77 days after. Fecal samples were collected from transport box papers at D1 and from boot swabs collected in the farm thereafter. All the 150 collected fecal samples were inoculated onto MacConkey agar (MC) and MacConkey agar supplemented with 1 $mg.L^{-1}$ of cefotaxime (MCC). A maximum of five randomly chosen isolates per sample were collected on MC for further study. Thus, standardized inoculum of 359 *E. coli* (PCR identification) obtained on MC were deposited on Mueller Hinton agar to check if the minimal inhibitory concentration of cefotaxime was superior to 2 $mg.L^{-1}$ (EUCAST breakpoint). On MCC, *E. coli* resistant to 3GC were detected in 123 samples out of 150. All the 30 flocks, either TIO treated or not treated, were positive for 3GC-resistant *E. coli* on at least three out of five sampling days. Even apparently negative flocks at hatch became positive for 3GC-resistant *E. coli* on day 2 and/or day 7. Analysis of *E. coli* isolated on MC showed a significant increase of the proportion of 3GC-resistant *E. coli* between D1 and Day 7 in treated birds (25.0% resistant *E. coli* on D1, 93.9% on Day7, $p<0.01$).The overall percentage of 3GC-resistant *E. coli* among the isolates collected from MC was significantly higher in treated flocks than in untreated flocks (49.3% vs 16.0%, $p<0.01$). These preliminary results confirm the frequent presence of 3GC-resistant *E. coli* in broilers, especially during the first days of life. The off-label use of 3GC *in ovo* injection in hatchery is associated with higher percentages of the proportion of 3GC-resistant *E. coli* in intestinal flora of broilers.

References: Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals1, EFSA Journal 2011;9(8):2322

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ANTIMICROBIAL RESISTANCE OF *SALMONELLA* HEIDELBERG ISOLATED FROM POULTRY IN ALBERTA (1996-2010)

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Background: Antimicrobial resistance (AMR) in *Salmonella* Heidelberg (SH) is an increasing

concern for public health. There are few data on SH resistance from poultry in Alberta, Canada. The objective of this study was to investigate the AMR of SH isolates from poultry in Alberta collected by passive surveillance. We hypothesized that: 1) there would be significant temporal trends in resistance of SH in Alberta poultry over time; and 2) there would be differences in resistance between poultry commodities in the province. **Methods:** Antimicrobial susceptibility was tested for 951 SH isolates of poultry origin obtained by the Food Safety and Animal Health Division of Alberta Agriculture and Rural Development from 1996-2010. Minimum inhibitory concentrations (MIC) were determined for 18 antimicrobials by Sensititre (AVIAN1F plate, TREK Diagnostic Systems) and annual and overall prevalences of resistance were determined using accepted MIC breakpoints provided by TREK. Logistic regression models were used to determine differences in resistance prevalence by year and between different poultry commodities (broiler chickens, broiler breeders, layers including layer breeders, meat turkeys and turkey breeders). **Results:** Ceftiofur (TIO) resistance varied significantly by year (annual range 0-10.5%) and was significantly higher in meat turkeys compared to layer and broiler breeder chickens. The amoxicillin (AMX) resistance model contained a significant interaction between commodity and year (annual range 0-42.6%). Chicken AMX resistance was higher than for turkeys at the beginning and end of the study, with the opposite relationship in the middle of the study. Gentamicin (GEN) resistance decreased significantly from the beginning to the end of the study period (annual range 0-33.3%) and was significantly higher in broiler breeder chickens and meat turkeys compared to layer chickens. The tetracycline (TET) resistance model contained a significant interaction between commodity and year (annual range 0-39.6%). Chicken TET resistance remained low with a small increase in the middle of the study period. Meat turkey TET resistance increased throughout the study and was higher than chicken resistance. All turkey breeder isolates were resistant to TET. There were no SH isolates resistant to enrofloxacin (ENR). **Discussion and Conclusions:** This study provides AMR data for SH isolates from the Alberta poultry industry. Resistance to some antimicrobial classes important for human health (TIO, ENR, GEN) remained less than 10% for most of the study period. There were significant temporal trends in resistance, as well as significant differences between poultry commodities.

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ARMA METHYLTRANSFERASE IN KLEBSIELLA PNEUMONIAE ISOLATES FROM PETS IN SPAIN

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Introduction: Methylation of the aminoacyl site of bacterial 16S rRNA confers high-level resistance to clinically important aminoglycosides. Seven 16S rRNA methyltransferase genes (armA, rmtA-E, npmA), have been identified to date among Gram-negative pathogenic bacteria, in most cases from human clinical isolates. There was no report of a 16S rRNA methyltransferase in bacteria isolated from pets until 2011, when RmtB has been found in several Enterobacteriacea collected from pets in China. The aim of this study was to characterize an outbreak of seven Klebsiella pneumonie strains isolated from pets in Madrid, which showed high-level resistance to aminoglycosides. **Methods:** PCR screening of the seven isolates for the known methylase genes was performed. The MICs (Minimal Inhibitory Concentrations), were performed and interpreted following the Clinical Laboratory Standards Institute guidelines. Transfer of armA to a laboratory E. coli strain was carried out by transformation using a plasmid DNA extraction. In order to elucidate the β-lactams

and fluoroquinolones resistance determinants, Multiplex PCR assays were performed. Plasmid profiles of the wild-type strains and the transformants were analysed by S1-PFGE method and Replicon Typing. PCR mapping for Tn1548 was performed using plasmid extractions as templates. **Results:** armA was identified in the seven K. pneumoniae isolates. The MICs showed high-level resistance to all 4,6-disubstituted deoxystreptamines aminoglycosides, as well as to ampicillin, ceftazidime, sulfamethoxazole, tetracycline, chloramphenicol and fluoroquinolones. Multiplex PCR assays and sequencing revealed presence of blaDHA-1 and qnrB4 in the seven isolates. In five strains armA was co-located with blaDHA-1 and qnrB4 in the same plasmid, named pB1025, while in the other two strains, armA and blaDHA-1 were located on pB1025' without qnrB4. Plasmid profiles showed that both pB1025 and pB1025' are IncR plasmids of approximately 50 kb in size. Tn1548 mapping showed that genetic environment of armA together with blaDHA-1 in these strains is identical to that in pKP048 recently reported from human isolates in China. **Conclusions:** This study describes the occurrence of ArmA methyltransferase in K. pneumoniae isolated from pets in Spain, in association with the resistance genes blaDHA-1 and qnrB4. This is the first time armA gene is detected in bacteria from pets, and the second identification of a 16S rRNA methyltransferase from pets at the global level. Further monitoring of emerging resistance genes in bacteria isolated from pets is essential to minimize their spread between humans and animals.

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MULTILOCUS SEQUENCE TYPING FOR CHARACTERIZATION OF POTENTIAL RISK ESBLS-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM PIGS, INCLUDING STRAINS OF NEW SINGLETONS ST2528, ST2524 AND ST2525

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Infections caused by *Escherichia coli* harboring extended-spectrum beta-lactamases (ESBL) have a tremendous impact on public health, because of treatment complications. ESBL-producing *E. coli* are increasingly reported in healthy food-producing animals that can spread to humans either by direct contact or, more importantly, through the food chain. Here we describe a molecular survey aimed at determining the population structure and dynamics of ESBL-producing *E. coli* strains recovered from healthy pigs slaughtered for human consumption in Portugal. For this purpose, a total of 71 faecal samples from pigs were collected (2008 to 2009) in different geographical regions of Portugal. Susceptibility to 16 antibiotics was tested by disk-diffusion method in all recovered isolates and ESBL detection was carried out by double-disk test. PCR and sequencing methods characterized bla_{ESBL} genes responsible for the ESBL-phenotype. In addition, we used multilocus sequence typing (MLST) to identify the genetic lineages of all ESBL-producing *E. coli* strains, which

were characterized by sequencing the internal fragments of 7 housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, *recA*); the MLST database was used to determine allelic profiles and for sequence type (ST) and clonal complex (CC) assignment. Among the 35 ESBL-producing strains, MLST analysis revealed 9 different STs under 6 CCs and 9 singletons STs. The CC10 and CC155 were the most common CCs, with 4 and 11 isolates, respectively. Two other isolates were assigned to the CC101. Moreover, 5 strains were included in 3 new STs; 3 of them were identified in a new allele for the *fumC* gene that originated the new ST2528; in addition, 2 isolates were registered as ST2524 and ST2525 through new combination of alleles. Through the MLST database we found that ST656 (CC10) and ST8 (CC165) have a higher homology to ST2524 and ST2525, respectively. However, by the definition of CCs, ST2524 and ST2525 most likely belong to CC10 and CC165, respectively. Our data shows the presence of ESBL producing *E. coli* isolates in pigs slaughtered for human consumption and raises important questions in the potential risk factors to public health due to the transmission of bacteria carrying resistance through the food chain, and spreading resistance to other bacteria of human clinical significance. A great heterogeneity of MLST types was observed, among which CC10, CC155 and CC101 have already been associated with human clinical isolates.

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EFFECT OF RAMR MUTATIONS ON EFFLUX GENES EXPRESSION AND ON FLUOROQUINOLONE SUSCEPTIBILITY IN SALMONELLA ENTERICA SEROTYPE KENTUCKY ST198

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Background. Efflux is a mechanism that has been previously reported to increase sometime fluoroquinolone (FQ) resistance levels in clinical isolates of *Salmonella enterica* mainly of serotype Typhimurium. In this study efflux genes were investigated in the emerging FQ-resistant epidemic *S. enterica* serotype Kentucky ST198 clone. **Methods.** Among a representative panel of thirty serotype Kentucky strains from Egypt or east Africa with decreased FQ susceptibility, three strains overproducing the AcrAB-TolC efflux system were detected and studied. Two FQ-resistant strains with basal expression level of AcrAB-TolC and the susceptible reference strain 98K were used as control. Genetic relatedness was determined by *Xba*I-pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The *ramRA*, *soxRS, marOR* loci and *acrR*, *acrS* genes were sequenced. Wild type strains, *ramR* mutants and *ramR* mutants complemented with a wild-type *ramR* gene were analysed (i) by qRT-PCR for gene expression of regulatory and efflux genes and (ii) by MIC determinations of quinolones, FQ and florfenicol as other substrate of AcrAB-TolC. **Results.** All serotype Kentucky strains studied were X1-ST198, excepted the 98K strain which was X4-ST198. The three strains overproducing AcrAB-TolC presented different mutations in the *ramR* gene in comparison to the two FQ-resistant and the susceptible reference strain 98K with a basal expression level of AcrAB-TolC. All other efflux regulatory genes were not affected. The three detected mutations (deletion of 91 bp, insertion of 1 bp or 4 bp) resulted in frame shift of the *ramR* gene. As confirmed by complementation, all three mutations were responsible for increased expression of *ramA* and *acrAB*. Increased expression of *tolC* and *acrEF* genes was observed in 2 out of the 3 strains. All three mutations were shown to increase two-fold the MICs of FQ and florfenicol. **Conclusion.** Various novel *ramR* mutations, responsible for increased efflux, were detected in the emerging epidemic serotype Kentucky ST198 clone. However, *ramR* mutations seem to be sporadic

as previously reported for other FQ-resistant strains of serotypes Typhimurium or Schwarzengrund and contribute only to a little extent to the decreased FQ susceptibility.

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INFLUX OF ENTEROCOCCUS CARRYING ANTIBIOTIC, COPPER AND VIRULENCE RESISTANCE GENES IN ANTIBIOTIC-FREE TROUT AQUACULTURES BY RIVER WATER AND FEED

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Objective: Potential risks of aquacultures for the public health include the development of antibiotic (AB) resistant or virulence (VIR) bacteria/genes reservoir that can reach human through food chain. Little is known about the role of contaminated river water or feed entering aquacultures in the spread of ABR/VIR, as well as of non-AB compounds carried by feed in their selection. Our goal was to assess the occurrence and flux of AB, VIR and copper (Cu) resistance genes among Enterococccus from two AB free trout aquacultures (TRA) receiving water from secondary rivers. **Methods:** Two Portuguese TRA producing 5-40 tons of trout/year (mainly for human consumption), without AB exposure and supplied by feed containing Cu were studied (2010-2011). Samples were collected from river water/sediments located upstream (n=11) and downstream (n=6) of the TRA, water/sediments from fish tanks (n=18) and feed (n=5). They were enriched in peptone water and plated in Slanetz-Bartley media with and without AB. Susceptibility to 12 AB was studied by disc diffusion (CLSI). Identification and search of ABR , VIR and CuR genes were done by PCR. **Results:** Enterococcus were detected in 93% samples (114 E.faecalis, 79 E.hirae, 68 E.faecium, 18 E.casseliflavus; 5 E.gallinarum; 1 E.durans, 10 Enterococcus spp). Resistance to tetracyclines (61%; tetM-93%, tetL-55%, tetS-1%), erythromycin (33%; ermB-97%), HLR-streptomycin (19%, aadE-38%), ciprofloxacin (11%), nitrofurantoin (8%), chloramphenicol (7%) or HLR-gentamicin (7%, aac6-aph2-55%) was detected. Resistance to ≥2AB was found in 90% of the samples, including water/sediments collected upstream TRA and feed. The putative virulence genes gel (41%), asa (31%), esp (14%) or cyl (7%) were found in different species and 98% of the E. faecium carried acm. No significant differences were detected among species, ABR rates and VIR genes distribution among isolates collected upstream, inside and downstream of aquacultures ($p>0,05$). The CuR genes (tcrB or cueO) were detected in similar rates in isolates collected upstream (16-17%; E. faecalis and E.hirae), fish tanks (12-13%; E. faecalis, E.hirae. E.faecium, other Enterococcus spp) and feed (3%-18%. E. faecium, other Enterococcus spp). Isolates carrying CuR genes were more resistant to tetracyclines, erythromycin and nitrofurantoin ($p<0,05$). The vanA/B; ermA/C; tetO/K; cfr, aac(6)-Ib/Ic/Id genes weren't found. **Conclusion:** Fish raised in free-AB TRA are in contact with Enterococcus resistant to AB used in human medicine and carrying VIR factors with possible clinical relevance. There was no evidence of selection or amplification of ABR/CuR/ VIR genes in the TRA analysed or a significant impact of the TRA in the downstream environment. Upstream river water and feed seem to be the most likely factors contributing to the presence of these genes in fish tanks.

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OCCURRENCE OF PUTATIVE ENTEROPATHOGEN BACTERIA AND PATTERNS OF ANTIMICROBIAL RESISTANCE OF ESCHERICHIA COLI ISOLATED FROM NATURAL STREAM WATER, AGUASCALIENTES, MEXICO.

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Water contamination in Mexico is one of the major problems. In the last decades, the natural stream water had been used as vehicle for wastewater with or without previous treatment, leading to possible water outbreaks. In this study, we evaluated natural stream water from San Pedro's River, the major riverbed and main pluvial collector of Aguascalientes State, Mexico; relevant to automotive sector, agricultural area, livestock industry, irrigation and recreational use. Currently, the River is being contaminated by the influx of wastewater from several industries and sectors, highlighting agricultural activities and livestock industry. We hypothesize that this environment could harbor high levels of microorganisms and this selective pressure encourages the presence of pathogenic bacteria and the emerging of antimicrobial agents' resistant bacteria that could create potential risk to public health and environment. Total and fecal coliforms were determinated as indicator of fecal pollution in the riverbed. All coliforms were exceeded the maximum permissible limits established by the World Health Organization and Mexican norms by five magnitude orders. Physico-chemical parameters were also determined. It was demonstrated the presence of the pathogens: *Escherichia coli*, *Salmonella*, *Shigella*, *Proteus mirabilis*, *Proteus vulgaris*, *Morganella*, *Providencia*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Enterobacter*; which represent a main risk since are able to spread in the environment and cause disease. The antimicrobial resistance phenotype was tested in 152 *Escherichia coli* isolated by the disk diffusion test for twelve antimicrobial agents (amikacin, carbenicillin, ampicillin, caphalothin, cefotaxime, ceftriaxone, chloramphenicol, gentamicin, nitrofurantoin, netilmicin, pefloxacin, trimethoprim-sulfamethoxazole, and levofloxacin). 51,32% (78/152) isolates were presented resistance for at least one antimicrobial agent, 34,87% isolates (53/152), presented resistance for at least two antimicrobial agent, and, 20,39% (31/152) were considered multi-resistant since presented resistance to three or more antimicrobial agents. The main antimicrobial resistance was presented for ampicillin (38,82%, 59 strains), followed by trimethoprim-sulfamethoxazole (27,63%, 42 isolates), chloramphenicol (21,71% 33 strains), and cephalotine (17,11%, 22 isolates). The lowest rates of resistance (1,32%, 2 strains) were presented for cephotaxime, netilmicin, and amikacin. Interestingly, 4,61% (7/152) of the isolates were presented resistance to levofloxacine, and 7,24% (11/152) presented resistance to pefloxacine. The results showed the deficient water quality and lacking of adequate wastewater treatment leading and negative impact in the aquatic environment that serves as reservoir of antimicrobial resistant strains.

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LOW RATE OF COLONIZATION BY EXTENDED-SPECTRUM PRODUCING ENTEROBACTERIACEAE IN DAIRY CATTLE IN FRANCE

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Background: The occurrence of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae in animals is increasingly reported, and several studies showed that food-producing animals might be reservoirs of ESBL producers. Our study aimed to evalu-

ate the colonization rate by ESBL-positive or carbapenem-resistant Enterobacteriaceae of dairy cattle in France. **Materials:** Rectal swab samples (n=200) were recovered from cows during a one-year period in 2011 in different farms located in the suburb of Paris, France. Three farms were studied, and one farm was studied twice with a time interval of 6 months. Samples were pre-cultured in buffered peptone water and incubated 18h at 37°C. Cultures were inoculated by streaking 100 µl of the suspensions onto Drigaslki agar plates containing either ceftazidime (2 µg/ ml) or imipenem (2 µg/ ml). Multilocus sequence typing (MLST) to identify the genetic backround of the strains. PCR experiments and DNA sequencing were used to identify the ß-lactamase genes. Plasmid typing was performed by PCR-based Replicon Typing (PBRT). **Results:** Only 4 isolates exhibiting an ESBL phenotype were recovered from this screening, being all Escherichia coli isolates. They were from two different farms. Two isolates expressed TEM-52, had been recovered from the same farm, and were clonally-related, belonging to sequence type ST359. One isolate was an ST1421 and produced CTX-M-27, whereas the last isolate was an ST244 and produced CTX-M-32. The plasmids carrying the ESBL encoding genes were not typeable by PBRT, except the blaCTX-M-32-positive plasmid that was typed as an IncF plasmid. No imipenem-non susceptible enterobacterial isolate was recovered during this screening, however carbapenem-resistant Acinetobacter genomospecies 15TU expressing the carbapenem-hydrolyzing class D beta-lactamase OXA-23 were obtained (published study). **Conclusion:** Our study revealed a low rate of ESBL-producing enterobacterial isolates in dairy cattle. Only E. coli isolates were identified as ESBL producers, and they belonged to three distinct ST types rarely identified in humans. Interestingly, different types of ESBLs were identified, all of them being quite frequently identified in human isolates.

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FREQUENCY AND MECHANISMS OF FLUROQUINOLONE RESISTANCE AMONG ESBL-PRODUCING ESCHERICHIA COLI FROM COMPANION ANIMALS AND HORSES

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ESBL-producing bacteria often show additional resistance to fluoroquinolones (FQ), which serve as first-line antimicrobials in a number of infections both in medical and veterinary practice. Frequent mechanisms underlying FQ resistance (FQR) in Escherichia coli are stepwise mutations of the chromosomally encoded determinants GyrA and ParC, whereas plasmid-encoded factors, such as AacIb-cr and Qnr are supposed to be of minor relevance. In the present study 1343 consecutively collected ESBL-E. coli, mainly from companion animals (753/334 dogs/cats) and horses (153) were screened for their FQR and the underlying mechanisms. All strains had a clinical background and were isolated from urinary/genitary tract (350/155), respiratory tract (146), and soft tissue infections (293) as well as from enteritis (340) and other infections (154). MIC data referring to FQR were confirmed in 68.8% of the strains while 4.7% and 26.5% had an intermediate and sensitive phenotype, respectively. Irrespective of the FQR phenotype, a high proportion (>85%) of ESBL producers harboured the aacIb-cr gene variant, while genes qnrA, qnrB, and qnrS were present in no more than 7% of the strains with an even higher proportion among FQ-sensitive isolates. An initial view on the number and type of mutations determined by sequence analysis of gyrA and parC genes in the phylogenetic background of nearly 500 multilocus sequence typed strains

partially indicates a co-evolutionary process of bacterial core genome and gyrA/parC mutational changes. Predicted amino acid substitutions basically resembled those known from human FQR E. coli isolates. Together with the previous observation of shared sequence types and ESBL enzymes, these findings support the idea of a transmission of multiresistant strains between humans and animals. Moreover, the high proportion of clinical FQR ESBL-E. coli has major implications on therapeutic options which are left for the treatment of infections in companion animals and horses.

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COMPLETE NUCLEOTIDE SEQUENCE ANALYSIS OF MULTIDRUG-RESISTANCE INCA/C PLASMID PR55 FROM KLEBSIELLA PNEUMONIAE ISOLATED IN 1969

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Objectives: To determine the complete nucleotide sequence of the multidrug-resistant IncA/C plasmid pR55 from a clinical Klebsiella pneumoniae strain that was isolated from urinary tract infection in 1969 in a French hospital and compare it with those of contemporary emerging IncA/C plasmids. **Methods:** The plasmid was purified and sequenced using a 454 sequencing approach. After draft assembly, additional PCRs and walking reads were performed for gap closure. Sequence comparisons and multiple alignments with other IncA/C plasmids were done using BLAST algorithm and CLUSTAL W, respectively. **Results:** Plasmid pR55 (170,810 bp) revealed a shared plasmid backbone (> 99% nucleotide identity) with current members of the IncA/C2 multidrug resistance plasmid family widely disseminating antibiotic resistance genes. Nevertheless, two specific multidrug resistance gene arrays probably acquired from other genetic elements were identified inserted at conserved hotspot insertion sites in the IncA/C backbone. A novel transposon named Tn6187 showed an atypical mixed transposon configuration composed of two mercury resistance operons and two transposition modules that are related to Tn21 and Tn1696, respectively, and an In0-type integron. **Conclusions:** IncA/C2 multidrug resistance plasmids possess a broad host-range and have been largely implicated in the dissemination of antibiotic resistance among Enterobacteriaceae from humans and animals. This typical IncA/C2 genetic scaffold appears to carry various multidrug resistance gene arrays and is now also a successful vehicle for spreading of AmpC-like cephalosporinase and metallo-β-lactamase genes such as blaCMY and blaNDM, respectively.

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LONGITUDINAL COLONIZATION OF NASAL COAGULASE POSITIVE STAPHYLOCOCCI (COPS) AMONG DOG-OWNING HOUSEHOLD MEMBERS INVOLVED IN PREVIOUSCASES OF SUSPECTED INTERSPECIES TRANSMISSION.

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Background: Close contact to dogs seems to be an increased factor to acquire nasal CoPS. The objective of this study was to investigate the dynamic of nasal colonization by *S. aureus* (SA) and *S. pseudintermedius* (SP) among healthy dog-owning household members involved in previous cases of suspected interspecies transmission (Abstract S2:3, ASM, Washington, September 2011). **Material and Methods:** Members (humans and dogs) of 7 households (H1 to H7) with recent description of suspected interspecies transmission and coexisting individuals were included. Three types of transmission were considered: a) **direct** transmission when an identical SA or SP strain was concomitantly isolated in both human and dog from a shared household, b) suspected **indirect zoonotic** transmission when SP was isolated from humans, and c) suspected **indi-**

rect anthropozoonotic transmission when SA, commonly found in humans, was isolated from dogs. Tested households [transmission type and CoPS isolated in index cases]: $H1_d$, $H2_d$, and $H3_d$ [**direct** transmission of methicillin-susceptible SA (MSSA) ST1654, ST121 and ST45, respectively]; $H4_d$ [**direct** transmission of methicillin-susceptible SP (MSSP) ST142]; $H5_{iz}$ and $H6_{iz}$ [**indirect zoonotic** transmission of MSSP ST100 and ST21, respectively]; and $H7_{ia}$ [**indirect anthropozoonotic** transmission of MSSA ST121]. Eighteen owners and 11 dogs were analyzed once every 2-3 months for one year with a total of 115 tested samples for CoPS recovery. Obtained isolates were identified by PCR and/or PCR-RFLP. *spa* typing was performed in all SA isolates and MLST in all SP. All isolates were tested for *mec*A gene by PCR. **Results**: In households $H1_d$, $H2_d$ and $H3_d$, identical MSSA to those of index cases were detected in humans in all samplings, while only the $H3_d$ dog was permanently colonized. The $H2_d$ dog was, instead, frequently colonized by a MSSP not present in the index sampling. In $H4_d$, owner and dog were permanently colonized by the former MSSP strain throughout the sampling year. As for $H5_{iz}$, human was permanently colonized by MSSP along the whole sampling period while coexisting dogs were intermittently positive for MSSP. $H6_{iz}$: neither the human with former MSSP nor the coexisting dog was positive for MSSP in the subsequent samplings. The $H7_{ia}$ dog, positive for MSSA in the index sampling, resulted only positive for a MSSP strain in the following tested samples. The $H7_{ia}$ owner was always CoPS negative. In most households, various MSSA lineages were detected in different individuals in several samplings, stressing the intermittent presence of MSSA t1451 (ST398) in few human or animal isolates in 2 households. **Conclusions:** Humans in contact with dogs can be colonised by SP, in addition to SA, for prolonged periods of time. In general, dogs were occasionally colonized by SA and variably colonized by SP. High diversity of lineages was present in both CoPS species. Methicillin-resistant isolates were not detected.

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DETERMINATION OF GENETIC VARIANCE IN QUINOLONE RESISTANCE SALMONELLA TYPHI IN QRDR REGION OF *GYRA*, *GYRB PARC* AND *PARE* BY DENATURED HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (DHPLC).

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Aim: Fluroquinolone are the drug of choice in the treatment of enteric fever caused by *Salmonella* Typhi The predominant mechanism of resistance of fluroquinolone resistance is mediated by mutation in quinolone-resistance determining region (QRDR). The present paper evaluates DHPLC for detection of mutations in *gyr*A, *gyr*B, parC and *par*E. **Method**: A Total of 106 isolates of *S.*Typhi with varied susceptibility to Ciprofloxacin (Ci) were studied. This included isolates with Ci MIC <0.064µg/ml (N=13), Ci MIC ≥0.064 - 1µg/ml (N=39), and Ci MIC >1µg/ml (N=54). In addition isolates with previously characterised mutations were included as controls. Wild type control DNA was prepared from *S.*Typhi (Ty2). The DNA of test isolates was amplified by PCR. The PCR products were mixed with wild type and run on DHPLC using suitable chromatographic conditions. Control isolates were also run under the same conditions. Chromatograms for control and PCR product were compared against each other and to the wild type. The different elusion patterns to the wild type or same elusion pattern with shifted retention time under the same conditions were considered to indicate the presence of mutation. To further confirmation DNA sequencing was performed for those samples that show different elusion pattern against wild type. **Result:** Using DHPLC mutations in *gyr*A, *gyr*B, *par*C were readily detected by comparison with control chro-

matograms. Sequencing confirmed the *gyr*A, *gyr*B, *par*C predicted mutations as detected by DHPLC. No mutation were detected in *par*E. **Conclusion**: DHPLC is very effective in detection of mutation and help in reduction of samples for final sequencing. It is also cost effective and time saving technique.

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CARRIAGE RATE, METHICILLIN RESISTANCE, VIRULENCE TRAITS AND GENETIC LINEAGES OF NASAL *STAPHYLOCOCCUS PSEUDINTERMEDIUS* FROM HEALTHY DOGS IN TUNISIA

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Background: *S. pseudintermedius* is a recently re-classified species which mainly colonizes dogs; however, it is also a potential pathogen responsible for numerous clinical infections in these animals. Methicillin-resistant *S. pseudintermedius* (MRSP) is emerging in different regions (Europe, North America, Asia); nevertheless, no data is available on its prevalence in dogs in Africa. The objective of this study was to investigate the carriage rate, resistance mechanisms, virulence traits and genetic lineages of nasal *S. pseudintermedius* of healthy dogs in Tunisia. **Material and Methods:** Nasal swabs of 100 healthy dogs were obtained from a Veterinary Institute and from several veterinary clinics that receive animals from all Tunisia for routine control visit for a period of six months in 2011. Samples were inoculated onto Baird Parker for *S. pseudintermedius* recovery. Isolates were identified by biochemical methods, *S. (pseud)intermedius* specific *nuc* PCR and PCR-RFLP of *pta* gene. Susceptibility to 18 antimicrobials was determined by disc-diffusion and involved resistance genes were investigated by PCR. MLST was performed in one representative strain per common antimicrobial resistance profile. The presence of *lukS/F-I*, *siet*, *si-ent*, sec_{canine}, *expA*, *lukS/F-PV, lukE/D, lukM, eta, etb, tst,* haemolysin toxin genes and eighteen staphylococcal enterotoxin genes were investigated by PCR. **Results:** *S. pseudintermedius* was recovered in 55 of the 100 tested samples (55%). All 55 *S. pseudintermedius* isolates (one/positive sample) were susceptible to methicillin (MSSP) but showed resistance to the following antimicrobials (% resistant isolates/gene detected): penicillin (56.4/*blaZ*), tetracycline (40/*tet*M), trimethoprim-sulfamethoxazole (23.7), fusidic acid (9), kanamycin (3.7/*aph*(3´)-Ia), erythromycin-clindamycin (1.8/*erm*B), streptomycin (1.8/*ant*(6)-*Ia*), chloramphenicol (1.8) and ciprofloxacin (1.8). MLST was conducted in 11 representative MSSP isolates showing 5 different sequence-types (STs): ST20, ST44, ST69, ST70 (2 isolates), ST78, ST100, ST160, ST161 (2 isolates) and ST162, the last 3 revealing novel alleles or allele combinations. Virulence genes carried by MSSP isolates were as follows (number of isolates): *lukS/F-I* (54), *expA* (3), *si-ent* (54), sec_{canine} (1), *siet* (55), *sea* (3), *sec* (6), *sek* (2), *seb* (2), *sed* (30), *sei* (3), *ser* (5), *sej* (16), and hlg_{v} (21). **Conclusions.** MSSP is very common among healthy Tunisian dogs but not MRSP. The nares of healthy companion animals (dogs) could be a reservoir of antimicrobial resistant and virulent MSSP, highlighting the presence of the recently described exfoliating gene *expA* and several enterotoxin genes, with potential implications in public health. This is the first description of *S. pseudintermedius* in Tunisia.

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FLUROQUINOLONE RESISTANCE IN *SALMONELLA* TYPHI: MECHANISM OF RESISTANCE AND THERAPEUTIC OPTIONS

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Aim: Increasing resistance of *Salmonella* Typhi to quinolone is a big therapeutic challenge. 75 isolates of *Salmonella* Typhi with high level fluoroquinolone resistance isolated during 2006 to 2011, were studied for mechanism of fluoroquinolone resistance (FR) and MIC to newer fluroquinolones, azithromycin, ceftriaxone and penems. **Method:-** Antibiotic susceptibility was performed as per CLSI guidelines to Nalidixic acid (NA), Ciprofloxacin (CF), Chloramphenicol (C), Co-trimoxazole (Co), Ampicillin (Am) and Tetracycline (T). MIC was performed for ciprofloxacin, Levofloxacin, Gatifloxacin and ofloxacin, Azithromycin, ceftriaxone Faropenum and Ertapenem by E-test. Isolates with CF MIC of ≤1 2 and ≥4µg/ml were defined as sensitive, intermediate and resistance respectively. All isolates were screened for mutation in *gyr*A, *gyr*B *par*C and *par*E by denaturing high performance liquid chromatography (DHPLC). PCR was performed for plasmid mediated quinolone resistance (PMQR) genes *qnr*A, *qnr*B, *qnr*S, *aac(6')-lb-cr*,, *qep*A. **Result:-** A total of 75 ciprofloxacin resistant isolates were detected from 2006-2011. Fluoroquinolone resistance increased from 2004 (1.9%) and (13.4%) in 2011. MIC of CF ranged from 2->32µg/ml. The predominant phenotype was NACF (43/75, 57.3%) Other phenotypes were NACFCoT (21/75,28%), NACFCCoT (1/75,1.3%) NACFCoAT (3/75,4%), NACFCo (6/75,8%) NACFCCoTA (1/75, 1.3%).The predominant mechanism of fluroquinolone resistance was a double mutations in *gyr*A and a single mutation in *par*C. The predominant mutation detected were 83 SerΦhe and at 87 Aspartic acid ΦAsparagine in *gyr*A gene and at 80 SerΦso in *par*C gene. Other mutations observed in *gyr*A at 83&87 SerΦTyro. PMQR *aac(6')-lb-cr*,, *qep*A were not detected, however one isolate with the ciprofloxacin MIC 4 µg/ml was positive for *qnr*S gene in addition to mutations in *gyr*A and *par*C. MIC of fluoroquinolone resistant *S.*Typhi were 8-24 µg/ml for ofloxacin, 1-4 µg/ml for gatifloxacin, 2-6 µg/ml for levofloxacin, 0.006-0.016 µg/ml for Ertapenem, 0.125-0.5 µg/ml for Faropenum, 1.5 -12 µg/ml for Azithromycin, 0.023 µg/ml -0.125 µg/ml for ceftriaxzone . **Conclusion: -** Resistance to fluoroquinolone is high and these isolates are also resistance to co-trimoxazole and tetracycline. The predominant mechanism of resistance was chromosomal mutation in quinolone-resistance determining region (QRDR). The therapeutic option includes Chloramphenicol, Azithromycin, Ceftriaxzone and penems.

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A MICROBIAL MAT AS A POTENTIAL RESERVOIR OF ARCHAEAL AMINOGLYCOSIDE RESISTANCE GENES

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The most common mechanism of resistance to aminoglycoside antibiotics is enzymatic inactivation. Since 2003, target modification has emerged as a mechanism conferring broad spectrum, high-level resistance with the appearance of 16S rRNA methyltransferases. The aim of this study was to identify reservoirs of genes that mediate resistance to aminoglycosides in natural environments. We constructed metagenomic libraries in *E. coli* with DNA from a hypersaline microbial mat from Puerto Rico, a largely undisturbed site. We identified a gene encoding a new environmental 16S rRNA methyltransferase, *nemA*. The deduced protein, NemA, contains 27% identity to the methyltransferase from the aminoglycoside producer, *Micromonospora inyonensis*, and 28% identity with RmtC of *Proteus mirabilis*. The primary sequence similarity and the high level of resistance for 4,6-disubstituted 2-deoxystreptamines (4,6-DOS) aminoglycosides (>512 $\mu g.ml^{-1}$) conferred by *nemA* suggest that it is a new member of the G1405 16S rRNA methyltransferase family. In phylogenetic analysis, *nemA* affiliated with genes in clade,

containing many pathogens suggesting that this gene could have been transferred from or to an animal pathogen. Genes flanking *nemA* have similarity to archaeal genes, including a putative translation factor, pelota, which is found in Archaea but not in bacteria. Other putative methyltransferases in the same family as *nemA* are near archaeal genes on other metagenomic DNA and in a thermophilic Archaea genome. The data suggest that we isolated a gene that originated in Archaea that confers high level of aminoglycoside resistance in *E. coli*. This is the first report of a functional member of this methyltransferase family found outside pathogenic or aminoglycoside-producing bacteria.

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Introduction: Edema disease is a common and often fatal disease in newly weaned piglets. In our study, 61 Escherichia coli strains, isolated from 92 piglets showing clinical signs of edema disease from 13 provinces in northern Vietnam, were positive for both the VT2e toxin and the F18 fimbrial adhesion factor. Edema disease infection is an acute disease and treatment is too late for pigs with clinical signs. Consequently, antibiotic administration is applied to the treat the remaining piglets of the litter. Therefore, we decided to determine the antibiotic resistance profiles of the isolated edema disease causing E. coli strains. We restricted our study to the common drugs used in the treatment of animals in Vietnam. **Methodology:** We determined the antibiotic resistance of these isolates by the disk diffusion as described in the National Committee for Clinical Laboratory Standards (2002). The inhibition zones were interpreted according to National Committee for Clinical Laboratory Standards (2002) and the recommendation of the Comité de l' Antibiogramme de la Société Francaise de Microbiologie (CASFM). For colistin resistance, an agar dilution with colistin sulfate powder from Sigma was used (Jones et al., 2005, J. Clin. Microbiol. 43, 925). The reference strain E. coli ATCC 259299 was used to test each lot of antibiotics. The following antibiotics were purchased from bioMérieux (tetracycline-30 µg, amoxicillin-clavulanic acid-30 µg, kanamycin-30 µg, gentamicin-10 µg, streptomycin-10 units, trimethoprim-sulfamethoxazole-25 µg, norfloxacin-10 µg), from Bio-Rad (cefoperazone-30 µg, Ceftiofur-30 µg, and spectinomycin100 µg), and from Oxoid (enrofloxacin-5 µg). **Results:** The majority of the isolates showed resistance to spectinomycin (67%), amoxicillin-clavunalic acid (69%), streptomycin (70%), trimethoprim-sulfamethoxazole (98%), and tetracycline (100%). Relatively high frequencies of resistance were observed for cefoperazone (46%), kanamycin (43%) and enrofloxacin (41%). Most isolates were multiple antibiotic resistant to three (trimethoprim-sulfamethoxazole, tetracycline and streptomycin) or five antibiotics (trimethoprim-sulfamethoxazole, tetracycline, streptomycin, spectinomycin and amoxicillin-clavulanic acid). The most frequent multiple drugs resistant combination was tetracycline, trimethoprim-sulfamethoxazole, streptomycin, amoxicillin-clavulanic acid/spectinomycin. Only ceftiofur (7%) was still active against most of these F18+/Stx2e+ E. coli isolates. This latter antibiotic is specifically licensed for use in veterinary medicine. **Conclusion:** High prevalence of resistance was observed to the common drugs of tetracycline, streptomycin, trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, and spectinomycin. Multiple resistances were widely distributed with 84% of isolates resistant to five antibiotics.

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GENOTYPIC CHARACTERISTICS OF *SALMONELLA* SER. ENTERITIDIS SUBMITTED TO ^{60}CO IRRADIATION.

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Salmonella ser. Enteritidis and eggs or egg products have been associated as a major vehicle for human cases. Most serotypes of Salmonella contaminate shell eggs on the exterior shell surface, gaining entry to the interior contents by cracks in the shell or by other circumstances that can lead to eggshell penetration or its ability to be present in internal egg contents due to trans ovarian deposition of the organisms in egg contents as a result of the infected reproductive tissues of laying hens. To extend the shelf life, food irradiation has been identified as alternative technology enables to eliminate pathogens without affecting egg quality, although knowledge is limited about the effect of this methodology on genotypic characteristics of *S*. Enteritidis The goal of this study was to determine the effects of ^{60}Co gamma irradiation on the genotypic characteristics of three from twenty *S*.ser. Enteritidis strains inoculated into *SPF* eggs. Those strains presented resistance to fluoroquinolone and / or 3th generation cephalosporin and to Φr-radiation up to 3.0 kGy (strain 3305), 5 kGy (s.3597) and 10.0 kGy (s.92). For the detection of resistant genes (bla_{CTX}, *gyr*A, *gyr*B) and virulence genes located on plasmids and chromosome (*sly*A, *pho*P/Q, *stn*, *spv*C) and in pathogenicity islands, SPI1 (*inv*E/A, *org*A, *sip*A, *hil*A), SPI3 (*mgt*C) and SPI4 (*sii*E) all genes from original strains (N.3) and their subcultures (N.12) were searched for by PCR. The results show deletion of genes in the strains after irradiation being more visible in strain 3597. Strain 3305 showed the highest stability however it was found that the subculture of strain 92, expressed more genes as compared to the original culture. Regarding resistance genes except the strain 92 and a subculture resistant to 1 kGy showed strains were negative for bla_{CTX} and the genes *gyr*A and *gyr*B were detected in all 92 subcultures until doses 10 kGy and the strain 3597 showed those genes just until 1kGy. From the total *S*.Enteritidis strains analyzed, except the strain 3597 the strains 92 and 3305 and its subcultures were positive for genes from SPI1. Irradiation induced changes in the profiles of genes located on plasmids and chromosome and in SPI3 and SPI4. To examine the genetic diversity of the strains and it´s subcultures we used PFGE performed according the PulseNet protocol. Macrorestriction patterns were compared using the BioNumerics Fingerprinting software (Version 5.0). The unweighted-pair group method using average linkages (UPGMA) was used to construct a dendrogram of all isolates Assessment of these strains by PFGE, indicating that the changes detected at the DNA level magnitude are not can be detected by PFGE. Given the results, it is important evaluate the rate at which Salmonella can repair damaged DNA and produce strains phenotypically unrecognizable as survival strategies which can represent an important public health threat.

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OCCURRENCE OF EXTENDED-SPECTRUM BETA-LACTAMASES IN *SALMONELLA ENTERICA* STRAINS ISOLATED FROM BROILERS AND FOOD OF ANIMAL ORIGIN IN PORTUGAL

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Background: *Salmonella enterica* is a zoonotic bacteria transmitted through the food chain and isolates harbouring extended-spectrum β-lactamases (ESBLs) have emerged worldwide during the last decade, with the CTX-M group being particularly important. The aim

of the present study was to determine the antimicrobial susceptibility of *S. enterica* strains isolated from broilers and food of animal origin and to characterize ESBLs producers. **Materials and Methods:** On the scope of the national antimicrobial resistance surveillance programme on *Salmonella*, a total of 283 strains isolated from broilers (n=100) and food of animal origin (n=183), were received at the National Laboratory of Veterinary Research in 2011. The minimum inhibitory concentration (MIC) of 11 antimicrobials (nalidixic acid, ciprofloxacin, ampicillin, cefotaxime, chloramphenicol, florfenicol, streptomycin, gentamicin, tetracycline, sulphamethoxazole and trimethoprim) for all isolates was determined by agar dilution method. Susceptibility towards cefoxitin was determined through disk diffusion method. Breakpoints were interpreted accordingly to EUCAST epidemiological cutoff values. 'Non-wild type' ('NWT') isolates for cefotaxime (MIC>0.5mg/L) and cefoxitin (<19mm) were screened for the presence of ESBL- (bla_{TEM}, bla_{OXA}, bla_{SHV}, bla_{CTX}) and PMAβ-encoding genes, using PCR method. Sequencing was applied to fully identify β-lactamases. **Results:** Among broilers, we identified 62% of 'NWT' isolates for ciprofloxacin, 57% for nalidixic acid and 28% for sulphamethoxazole, whereas in isolates from food of animal origin, 71%, 63% and 56% were 'NWT' isolates for tetracycline, sulphamethoxazole and ampicillin, respectively. Among all, 5/283 (1.8%) strains presented 'NWT' MICs for cefotaxime and were multidrug resistant: 2 *Salmonella* Havana isolated from **broilers** and 3 *Salmonella S.* 4,[5],12:i:- isolated **from food of animal origin** (swine); these isolates had one $bla_{CTX\text{-}M\text{-}type}$ gene, and 2 from food of animal origin presented 1 $bla_{TEM\text{-}type}$ gene and 1 $bla_{SHV\text{-}type}$ gene, respectively; they were 'wild type' for cefoxitin and no PMAβ-encoding gene was detected. **Conclusion:** To our knowledge, this is the first time in Portugal that ESBL-encoding genes, particularly from $bla_{CTX\text{-}M}$ family, were detected in isolates of *Salmonella* Havana, a very common serotype isolated from our broiler population. It should also be emphasised that 3rd generation cephalosporins are not allowed in the national poultry production, contrary to the large animal production, which may explain the detection of ESBL-encoding genes in our strains from swine origin. Horizontal gene transfer may be responsible for the coresistance of strains to non-β-lactam antibiotics. This study shows that national animal health monitoring systems play an important role and should be improved in an international level.

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DYNAMICS, CHARACTERISTICS AND PREVALENCE OF ESBL/AMPC PRODUCING *ESCHERICHIA COLI* IN VEAL CALVES IN THE NETHERLANDS

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Introduction: Resistance to 3rd/4th generation cephalosporins has been reported in many food-producing animals. Detailed information regarding resistance in veal calves is limited. This study comprises three approaches conducted to describe the prevalence, molecular characteristics and dynamics of ESBL/AmpC producing *E. coli* in veal calves in the Netherlands. **Methods:** Three sets of faecal samples were included. 1) Samples collected for surveillance studies from 1997 to 2010. 2) Samples from 10 individual calves per batch were obtained from 100 batches at slaughter. 3) At 3 farms, faecal samples from individual calves were collected on arrival and after 3, 6, 8 and 10 weeks upon arrival. For all samples ESBL/AmpC producing *E. coli* were isolated by inoculating faeces in 1 ml LB-enrichment broth and subsequently on MacConkey agar plates. Both media contained 1 mg/L cefotaxime. Molecular characterization was performed by PCR- and sequence analysis, Identibac

AMR-ve micro-array analysis and PCR-Based Replicon Typing. Furthermore, disk diffusion assays were performed. **Results:** Until 2004 the AmpC phenotype was predominant, and shifted to ESBL phenotype from 2005 on. The majority of AmpC phenotype was caused by chromosomal promotor mutations of the *ampC* gene. Only few *bla*CMY$_{-2}$ genes were found in 2005 to 2010. The ESBL phenotype was mainly caused by *bla*CTX-M enzymes. At slaughter 67% of the batches harboured cefotaxime resistant *E. coli*,of which 78% harboured *bla*CTX-M variants, mainly caused by *bla*CTX-M$_{-1}$, $_{-14}$ or $_{-15}$. IncI1 and incF were the predominant plasmid types. The longitudinal study showed that in all 3 farms 20% of the calves carried cefotaxime resistant *E. coli* upon arrival. These harboured mainly *bla*CTX-M group 1 variants. At week 3, farm 1 was completely negative, farm 2 and 3 were almost completely negative for group 1 variants, however both showed a high prevalence of *bla*CTX-M$_{-14}$, which was not observed upon arrival. This high prevalence of *bla*CTX-M$_{-14}$ also faded to values near or below detection at week 10 in farm 2 and 3. **Conclusion:** Resistance to 3rd/4th generation cefalosporins in *E. coli* of Dutch veal calves is mainly caused by *bla*CTX-M$_{-1}$, $_{-14}$ and $_{-15}$ enzymes and chromosomal promotor mutations of the *ampC* gene. Based on 3 farms, the prevalence upon arrival is relatively high, but decreases near or below detection within 10 weeks.

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DETECTION OF ACHROMOBACTER XYLOSOXIDANS IN HOSPITAL ENVIRONMENT

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Achromobacter xylosoxidans is an important emerging pathogen in Cystic Fibrosis patients. It is also increasingly isolated in different samples from patients in Intensive Care Units (wounds and lungs especially) in our University Hospital (Dijon, France). This species is innately resistant to aminoglycosides, aztreonam and cefotaxime. Acquired resistance to ceftazidime and carbapenems is frequent, some clinical isolates being resistant to all available antibiotics. The aim of this study was to search for the presence of Achromobacter xylosoxidans in hospital environment (surfaces, toilets, shower wastes, wash-hand basin, shower) in different departments (ICUs, Hematology, Pediatric outpatients, Pneumology Department and Nephrology). For this purpose we have developed a selective medium. Genotypic analysis of environmental isolates has been performed by PFGE and compared with clinical isolates. Antibiograms have been performed by disk-diffusion method. **Results:** We have isolated 25 strains of Achromobacter xylosoxidans, mostly in wash-hand basins and shower wastes. Identification was performed by conventional methods (Api20NE) and/or mass spectrometry. Environmental isolates from different departments were genotypically different. In a few cases we found environmental isolates with a profile identical to the profile of clinical isolates. This might suggest a patient contamination by environmental Achromobacter xylosoxidans through medical procedures. The environmental isolates were mainly susceptible to ceftazidime and carbapenems, with the exception of two isolates highly resistant to ceftazidime and imipenem which produced the metallo beta-lactamase IMP-19. **Conclusion:** We have detected some reservoirs of Achromobacter xylosoxidans in hospital environment. The selective medium which we have developed improves the detection of the isolates. Achromobacter xylosoxidans is mostly resistant to quaternary ammonium compounds present in many products used for hospital disinfection procedures. In some cases the use of bleaching water should be recommended.

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ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROTOXIGENIC *S. AUREUS* ISOLATED FROM CHEESE

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Staphylococcus aureus is the etiological agent in numerous infections in humans and livestock. This pathogen can also produce enterotoxins responsible for staphylococcal food poisoning (SPF), one of the leading causes of food-borne outbreaks in industrialized countries. In this study, 60 *S. aureus* strains, isolated from dairy products between 2008 and 2012, were selected for their ability to produce enterotoxins (SEA to SED) in cheese. Those strains were first phenotyped using the disk diffusion method as recommended by the Antibiogram Committee of the French Society of Microbiology; 16 antimicrobials of veterinary and human interest, representing 10 different classes of antibiotics, were tested. Most of the strains were resistant to penicillinG, while fewer, approximately 15%, displayed reduced susceptibility to Cefoxitine. Nevertheless, PCR detection of *mecA* or *mecC* genes confirmed the absence of MRSA in the collection. No strain was resistant to fluoroquinolones. Only three strains were multidrug resistant. Overall, resistance rates observed in this study seem to be considerably lower than the one observed for human clinical *S. aureus* strains. Molecular characterization such as *spa*-typing, PVL and *mecA*/*C* detection are in progress and will allow us, in the second step of this project, to determine if this population of strains is related to the one associated to dairy animal clinical mastitis.

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THE GERMAN NATIONAL ANTIBIOTIC RESISTANCE MONITORING (GERM-VET): RESULTS FROM 800 FISH-PATHOGENIC BACTERIA OVER A PERIOD OF FIVE YEARS

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Objectives: Since 2005 the BVL investigates fish-pathogenic bacteria collected from all over Germany concerning their MIC values to monitor the development of resistances. So far, more than 800 isolates (Aeromonas spp., Pseudomonas spp., Vibrio spp. and Yersinia ruckeri) were investigated. **Methods:** The isolates collected on the basis of a statistically valid sampling plan were investigated by using the broth microdilution method according to CLSI document M49-A. The determined MIC values were correlated with the MIC50- and MIC90-values for classification. **Results:** On the one hand, our results showed a broad range of the MIC results (e.g. Aeromonas spp. for tetracycline: MIC50 = 0.25 mg/L, MIC90 = 8 mg/L). On the other hand, we found high MIC90-values e. g. Aeromonas spp. for enrofloxacin (2 mg/L), chloramphenicol (4 mg/L) and trimethoprim/sulfamethoxazole (8 mg/L). For Pseudomonas aeruginosa many high MIC90-values of several antimicrobial agents were identified except enrofloxacin with a MIC90-value of 1 mg/L. The data determined for Vibrio spp and Yersinia ruckeri showed low MIC50- and MIC90-values. **Discussion and conclusions:** In Germany, only a fixed combination of trimethoprim and sulfonamide is approved for therapy in fish. This combination showed high MIC90-values, which are a sign of a decreased efficacy. Furthermore several other antimicrobial agents were concerned, which may be related to the rededication of certain antimicrobial agents appropriate to the German law for veterinary medicinal products. Further investigation of the resistance level of fish pathogenic bacteria in Germany is neces-

sary to implement risk management measures at an early stage.

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MONOPHASIC VARIANT OF *SALMONELLA* TYPHIMURIUM BEARING AN ESBL DETECTED IN A FRENCH POULTRY FARM

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For the past few years, all over Europe is described the emergence of a monophasic variant of *S*. Typhimurium (1,4,[5],12:i:-), characterized by a chromosomally encoded resistance to ampicillin (A), streptomycin (S), sulphonamides (Su) and tetracyclines (T). It rapidly became amongst the most prevalent serovars in humans in numerous countries worldwide and has been associated with a wide range of animal species such as to poultry, pigs, cattle, horses, mice, snakes, turtles In September 2011, an ESBL *Salmonella* 4,5,12:i:- was detected in a layers farm from south of France. The identification of this monophasic variant of Typhimurium isolate was confirmed by glass slide agglutination serotyping following the Kauffmann-White Scheme, deletion of *fljB*-encoded second phase H antigen was confirmed by PCR as recommended by EFSA. The antibiogram performed by Disk diffusion as recommended by CLSI confirmed the resistance phenotype ASSuT of the emerging European clone by this phenotype was supplemented by an ESBL phenotype. Molecular characterization confirmed the presence of $bla_{\text{CTX-M-1}}$ beta-lactamase carried by an IncI1 plasmid. A recent publication (Rodrigues et al, JAC 2011) described this kind of strains in the pig, bovine and sheep channels in Germany, but, to our knowledge, it is the first description in poultry in France.This strain represents the combination of 2 emerging dangers for Public health: monophasic variant of *Salmonella* Typhimurium and CTX-M EBSL. Constant monitoring and increased vigilance is required now to detect any diffusion phenomena along the food chain of this kind of strains, which pathogenicity to humans can't be denied.

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PREVALENCE OF CTX-M PRODUCING E. COLI STRAINS IN A FRENCH RIVER: OCCURRENCE IN BIOFILM AND FAUNA (INVERTEBRATES AND PISCES)

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Recent emergence of CTX-M producing E. coli strains in clinical infections is a major health concern. The fate of such strains released from waste water treatment plants in the fresh water environment remains to be elucidated. We have investigated the prevalence of CTX-M producing E. coli strains in the Ouche watershed near Dijon by sampling epilithon, invertebrates (Gammarus pulex) and several species of pisces in the river at several sites along a 40 km transect. The occurrence of CTX-M E.coli was monitored by isolation on selective TBX Agar supplemented with cefotaxim and confirmation by antibiotic succeptibility testing of the strains. First results demonstrate that BLSE E. Coli occurs in the fresh water environment in biofilm, shellfishes and pisces.

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THE FOODBORNE LINK FOR COMMUNITY-ACQUIRED CLOSTRIDIUM DIFFICILE INFECTIONS

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There has been a dramatic increase in the incidence of community- acquired *Clostridium difficile* infections (CDI). Community -acquired CDI is defined as a clinical case of *Cl difficile* whereby the infected person had not recently visited a clinical setting or

taken antibiotics. It has been speculated that community-acquired CDI is contracted via zoonotic routes or the environment. However, it has also been proposed that *Cl difficile* is a foodborne pathogen. The following provides supporting evidence for a foodborne link to Community-Acquired CDI. Ribotyping, PCR, and PFGE profiles of different *Cl difficile* isolates derived from animal, environmental (water and soil), in addition to clinical cases, were typed. It was found that the same strains (ribotype 078) linked to Community-Acquired CDI could be matched with those recovered from pigs. Also, gradient plates were used to study CD 078 and CD027 growth with several microclimatic factors, such as PH, temperature, NaCl, and bile salt. The growth of *Cl difficile* on agar plates was restricted to pH >8 with inhibition being observed at neutral or acidic conditions. Yet, *Cl difficile* was found to proliferate on ground beef (pH 5.8). It was also found that Cl difficile underwent germination, growth, and sporulation in meat juice medium. Further studies illustrated the Cl *difficile* could undergo germination, growth and sporulation in fish extract medium. However, the initiation of germination can be measured spectrophotometrically by following the decrease in the absorbance at 600nm. The results were shown that the germination rate of CD 078 in meat juice medium was 0.067 OD per minute and in fish juice medium was 0.15 OD per minutes. In addition, the germination rate of CD027 in fish juice media was 0.22OD/min. Also, there is no effect for meat juice medium on the spore germination of CD 027. However, the results were shown that the sporulation yield was so high for CD078 and CD 027 when they grew in meat and fish juice media. The collectively, the results confirm that Cl *difficile* can proliferate on foods commonly linked to the pathogen. Given that Cl *difficile* spores can survive the cooking process there is a strong possibility that susceptible groups can acquire the pathogen via foodborne transmission.

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VIRULENCE POTENTIAL OF EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING *ESCHERICHIA COLI* IN CATTLE

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Background: Extended-Spectrum Beta-Lactamases (ESBLs), mediating resistance to most beta-lactams used in human and veterinary medicine, are frequently isolated in *Escherichia coli* from cattle, especially due to the spread of CTX-M-type enzymes. In parallel, cattle are also recognized as the main reservoir of Enterohaemorrhagic *E coli* (EHEC). Contrary to multiple reports where ESBLs were found in Extra-Intestinal Pathogenic *E. coli* (ExPEC), such as the highly virulent O25b:H4-ST131 clone, the association of ESBLs with Shiga toxin-producing *E. coli* (STEC) or EHEC was rarely investigated. Our aim was thus to determine whether ESBL genes may have invaded the sub-population of EHEC strains in cattle, and to characterize the phylogroups, serotypes and the main virulence factors of those strains. **Material and Methods:** A total of 204 ESBL-producing *E. coli* isolates from diarrheic cattle was included in this study. Antimicrobial susceptibility was tested by disc diffusion. Where necessary, ESBL genes were sequenced, and ESBL plasmids transferred to *E. coli* recipients and rep-typed. The distribution of the CTX-M groups and phylogroups (A, B1, B2, D), as well as the five major EHEC serogroups that are pathogenic to humans (O26, O111, O103, O145, O157), were investigated by PCR. Virulence factors (VFs) genes were identified by PCR and commercial DNA array (Alere, France). **Results:** In this large collection, only one O111:H8 Stx1-producing *E. coli* was identified, which harboured the $bla_{\text{CTX-M-15}}$ gene on an untypeable plasmid. Two other isolates were *eae*-positive but *stx*-negative, of which one belonged to one of the five main EHEC serotypes (O26:H11). ESBL genes were mostly of

the CTX-M-1 (65.7%) and CTX-M-9 (27.0%) groups whereas those of the CTX-M-2 and TEM_{ESBL} groups were much less represented (3.9% and 3.4%, respectively). ESBL isolates mainly belonged to phylogroup A (55.4%), and to a lesser extent to phylogroups D (25.5%) and B1 (15.6%), whereas B2 strains were quasi-absent (1/204). The number of VFs was significantly higher in phylogroup B1 than in phylogroups A ($p = 0.04$) and D ($p = 0.02$). Almost all of the VFs detected were found in CTX-M-1 isolates whereas only 64.3% and 33.3% of them were found in CTX-M-9 and CTX-M-2 isolates, respectively. **Conclusion:** These results indicate that the widespread dissemination of the bla_{CTX-M} genes within the cattle *E. coli* population still spared the subpopulation of EHEC/STEC isolates, even if such strains can be sporadically isolated and if their prevalence may expand in the future. Contrary to other reports on non-ESBL *E. coli* isolates from domestic animals, B1 was not the main phylogroup identified. However, phylogroup B1 was found to be the most virulent one, suggesting a host-specific distribution of virulence determinants among phylogenetic groups.

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USING NALIDIXIC ACID -SUSCEPTIBILITY AND -BORDERLINE RESISTANCE TO IDENTIFY PLASMID-MEDIATED QUINOLONE RESISTANCE (PMQR) IN *SALMONELLA* IN TAIWAN

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Background. Fluoroquinolones (FQ) are a main treatment choice for *Salmonella* infections. *Salmonella* isolates with ciprofloxacin (CIP) minimal inhibitory concentration (MIC) in the 0.12-2 µg/mL range have been considered to have reduced susceptibility to FQ (FQ-RS). Two major mechanisms account for FQ-RS in *Salmonella*. Those due to single mutation in the drug target typically also display high-level nalidixic acid resistance (NA-R). In contrast, those due to plasmid-mediated quinolone resistance (PMQR) often display borderline NA -resistance (NA-BR, MIC 32 ug/mL) or -susceptibility (NA-S, MIC ≤ 16 µg/mL). In our national surveillance of clinical *Salmonella* isolates in Taiwan, an increasing proportion of isolates displayed the NA-S/CIP-RS phenotype. The present study was performed to investigate the prevalence of PMQR in those *Salmonella* isolates and their serotype distribution. **Methods.** Isolates were collected from >20 hospitals yearlong between 2005 and 2009. All isolates were serotyped, and MICs were determined by broth microdilution method following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Multiplex PCR was used to detect the presence of PMQR determinants [*qnrA, qnrS, qnrB, qnrC, qnrD*; *aac(6')-Ib-cr*, *qepA*] on all NA-S/FQ-RS, NA-BR/FQ-RS, and NA-R/FQ-R isolates. Due to the large number of NA-R/FQ-RS isolates, we randomly selected some for PMQR detection. Sequencing was performed on selected PCR products to confirm specificity and determine variants. **Results.** A total of 11907 non-duplicate isolates from 2005 to 2009 were tested, and CIP-R ranged 1.7 to 3.1% while FQ-RS ranged 23.6 to 26.2% in those years. Isolates with NA-S/FQ-RS phenotype increased from 0.2% (n = 4) in 2005 to 5.5 % (n = 104) in 2009. PMQR was detected in 79.4% (251/316) and 90.7% (39/43) of the NA-S/FQ-RS and NA-BR/FQ-RS isolates, respectively, but in only 4.6% (10/217) and 0.7% (1/135) of the NA-R/FQ-RS and NA-R/FQ-R isolates, respectively. There were 13 isolates with ≥ 2 PMQR determinants. The most common PMQR determinant was *qnrS* (n = 240), followed by

qnrB (n = 47), *aac-Ib-cr* (n = 13), *qnrD* (n = 13), and *qnrA* (n = 1). PMQR-positive isolates were found in serotypes Typhimurium (n = 223), Montevideo (n = 28), Enteritidis (n = 14), and 14 other serotypes. **Discussion and Conclusions**. Our results indicated that PMQR is increasing in Taiwan *Salmonella* isolates. Detection of PMQR is important because horizontal transfer of PMQR can occur and PMQR facilitates the selection of chromosomal mutants in the presence of quinolones to result in emergence of higher level FQ resistance. Because NA-R may not detect all mechanisms of FQ-R, CLSI lowered the CIP breakpoints for *Salmonella* in 2012. Until a suitable marker is found, in *Salmonella* strains with CIP MIC in the 0.12-2 µg/mL range, NA-susceptibility or borderline resistance is a helpful indicator for detecting PMQR positive strains.

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CARRIAGE OF EXTENDED-SPECTRUM B-LACTAMASE- AND AMPC-PRODUCING ENTEROBACTERIACEAE IN HEALTHY DOGS IN FRANCE

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Background: Resistance to extended-spectrum cephalosporins (ESCs) has broadly disseminated in veterinary medicine, principally due to the production of Extended-Spectrum β-Lactamases (ESBLs). These enzymes are mainly plasmidic and often associated with resistance to non beta-lactams, thus reducing the therapeutic options for the veterinarians. The aim of this study was to estimate the prevalence of fecal carriage of resistance to ESCs in healthy dogs in France, and to characterize the collected strains. **Material and Methods:** Between February and December 2011, feces of 368 healthy dogs were plated on selective agar (ChromID ESBL, Biomérieux). Presumptive ESC-resistant colonies were identified by API20E galleries. Antimicrobial susceptibility was tested by disc diffusion. ESBL and AmpC phenotypes were determined by the double-synergy test and cefoxitin resistance, respectively. Genes responsible for these phenotypes, as well as phylogenetic groups, were assessed by PCR and sequencing. The presence of the B2-O25:H4- ST131 clone was determined by PCR. **Results:** In all, 71 samples (19.3%) were positive on selective medium. Four samples presented two different isolates. Species identification revealed one *Raoultella spp*, one *Salmonella enterica*, one *Klebsiella pneumoniae* and 72 *E. coli*. ESBL phenotype was observed for 47 strains (63%), mainly mediated by CTX-M-1 group enzymes (80%, including six CTX-M-15). Two ST131 clones, carrying a CTX-M-15 enzyme and a CTX-M-9 group enzyme, were identified. AmpC phenotype was observed in 23 strains (31%), principally due to the bla_{CMY-2} gene. Finally, two strains presented both a bla_{ESBL} and the bla_{CMY-2} genes. Phylogroups were the following: A=21, B1=18, B2=4, D=27, whereas two were undetermined. Associated resistances were numerous, mainly to tetracyclines (64%), quinolones (52%) and fluoroquinolones (42%). **Conclusion:** Our study showed that carriage of resistance to ESCs in healthy French dogs is high (19.3%). Resistance is largely due to the dissemination of ESBL enzymes but, interestingly, plasmidic AmpC account for 31% of those phenotypes. Resistance to non beta-lactams was also frequent. The detailed analysis of the clones and plasmids (conjugation, reptyping) is ongoing and will help understanding the epidemiology of these resistance traits. Yet, these results are worrying since healthy pets are a reservoir of ESBL and AmpC genes possibly transmissible to humans.

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CHARACTERIZATION OF CTX-M-15 CARRYING ESCHERICHIA COLI AND SALMONELLA FROM LIVESTOCK

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Over the last years, the number of extended-spectrum beta-lactamases (ESBL)- and AmpC producing bacteria has increased worldwide. CTX-M-15 is the most prevalent ESBL produced in Enterobacteria from human origin, probably related to the spread of the E. coli clone O25:H4-ST131-B2 (carrying IncF plasmids with blaCTX-M-15 and blaOXA-1). The blaCTX-M-15 genes were rarely found among livestock bacteria. However, the number of E. coli isolates harbouring this gene seems to increase. To ascertain a potential connection between isolates of human and animal origin and along with the German EHEC O104:H4-ST678-B1 blaCTX-M-15 outbreak in 2011, we started to look for the presence of isolates carrying a blaCTX-M-15 among German non-human strains isolated between 2003-2012. The isolates from the National Reference Laboratories for Antimicrobial Resistance, Salmonella and Escherichia coli Collections (including isolates collected from routine and national surveillance programs) as well as isolates collected within the national ESBL-surveillance project RESET (www.RESET-Verbund.de) which showed resistance to 3rd generation cephalosporins were tested for the presence of ESBLs-, and AmpC-encoding genes. CTX-M-15 positive strains were analysed by PFGE (XbaI, S1-nuclease) and MLST. The E. coli strains were tested by phylogenetic grouping. Transformation experiments were done and plasmids were characterized by rep-PCR typing. Twelve isolates, 10 E. coli (5 from cattle, 4 from swine, 1 from poultry), 1 Salmonella serovar Typhimurium (S. Typhimurium, horse) and 1 S. Bredeney (swine) carried a blaCTX-M-15 gene. The E. coli isolates yielded six XbaI-PFGE profiles. Seven E. coli isolates (serotype ONT:HNM or O8:HNM) showed the same or highly related XbaI-PFGE patterns, belonged to phylogenetic group A and were ST410 (CC23). Transformation of potential ESBL-plasmids from these isolates failed. The E. coli R261 isolate (swine, phylogroup A) also carried a blaOXA gene, and showed a very different XbaI-PFGE-pattern to the ST410 isolates. MLST and serotype of this isolate are under analysis. blaCTX-M-15 was located on an IncI1 80 kb plasmid in the E. coli isolates 11E00604 (cattle, O8:H7-ST196-B1) and 11E00851 (cattle, Or:H4-ST117-D). In the Salmonella isolates, the blaCTX-M-15 genes were located on an IncI1 90 kb and an IncHI2 320 kb plasmid. The increasing number of detected CTX-M-15 encoding genes in German E. coli isolated from livestock could be related to the spread of a clone belonging to ST410, and differs from those found in the two human isolates used as controls (ST131 and EHEC outbreak). However IncI1 plasmids seem to play an important role in the spread of this gene. The possibility of an exchange of isolates between livestock and humans is a major Public Health concern and deserves surveillance.

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PRESENCE OF RESISTANCE GENES IN MRSA ST398 FROM DIFFERENT PIG FARMS

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) of sequence type (ST) 398 strains carry often additional plasmid-borne antimicrobial resistance genes, including the novel trimethoprim resistance gene *dfrK* and the ABC transporter genes *vga*(A) or *vga*(C). The aim of this study was to identify the distribution of resistance genes in MRSA ST398 isolates from infected and colonized pigs, dust samples from pig sheds and humans in close contact with pigs collected from different pig farms. **Methods:** A total of 33 MRSA ST398 isolates from pigs with exsudative epidermitis (n=11), colonized pigs (n=6), dust samples from breeding pig sheds (n=14) and humans in contact with pigs (n=2) obtained from 18 pig farms was included in the study. Minimum inhibitory concentrations (MICs) were determined by broth micro- or

macrodilution. Presence of the genes *aacA-aphD*, *aadD*, *dfrK*, *erm*(A), *erm*(B), *erm*(C), *fexA*, *tet*(K), *tet*(L), *tet*(M), *vga*(A), *vga*(B), *vga*(C) and *vga*(E) as well as the presence of the transposons Tn*558* and Tn*559* was tested by PCR. Plasmid DNA was prepared using standard protocols. **Results:** All 33 isolates were tetracycline-resistant with 24 carrying *tet*(M)+*tet*(K), three isolates *tet*(M)+*tet*(L) and six isolates solely *tet*(M). All 22 isolates with high trimethoprim MICs harboured the gene *dfrK*. In 19 isolates the *dfrK*-harbouring transposon Tn*559* was detected. PCR analysis confirmed the linkage of *dfrK* and *tet*(L) in the three *tet*(L)-harbouring isolates. One isolate was resistant to chloramphenicol and florfenicol, and carried the gene *fexA* on a complete transposon Tn*558*. A single isolate was gentamicin- and apramycin-resistant and harboured the recently described gene *apmA*. High MICs to gentamicin and kanamycin were observed in one isolate, in which the genes *aacA-aphD* and *aadD* were detected. The *erm*(C) gene was detected in the three isolates, which showed resistance to erythromycin and clindamycin. These isolates were also tiamulin-resistant. Resistance to clindamycin and tiamulin, but susceptibility to erythromycin was seen in 24 isolates. One of these isolates had the gene *vga*(C) and all remaining tiamulin-resistant isolates (n=26) harboured the gene *vga*(A) Plasmid analysis revealed that isolates from the same farm had the same plasmid profile. **Conclusion:** Analysis of the susceptibility data revealed an unexpected diversity of resistance patterns. In total, seven different resistance patterns were seen with four of them being present in only single isolates. Although MRSA ST398 is able to acquire resistance genes, isolates from the same farm seemed to be very similar in their resistance pheno- and genotypes. The farm-specific similarities among the resistance properties may be the result of either the spread of a single MRSA ST398 strain among animals of the same farm and/or the consequence of a specific selective pressure imposed by the farm-specific use of antimicrobials.

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INCI1/ST3 PLASMIDS CONTRIBUTE TO THE DISSEMINATION OF THE *BLA*$_{CTX-M-1}$ GENE IN *ESCHERICHIA COLI* FROM SEVERAL ANIMAL SPECIES IN FRANCE

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Background: Extended-Spectrum Beta-Lactamases (ESBLs) are widespread enzymes in animals and humans. In animals, the *bla*$_{CTX-M-1}$ gene is a frequently reported ESBL gene. Plasmids play a key role in the spread of ESBL genes and certain combinations displayed an epidemiological success. In France, the same *bla*$_{CTX-M-1}$-carrying IncI1/ST3 plasmid was previously reported in *Salmonella enterica* from humans, poultry and cattle, and in *Escherichia coli* from poultry. The aim of this study was now to characterize *bla*$_{CTX-M-1}$-carrying IncI1 plasmids from other animal species in France. **Material and Methods:** Eight *E. coli* isolates that were resistant to ceftiofur were recovered from 2006 to 2010 from clinical specimens of various hosts, including a cat, four dogs, two horses and a goat. Antimicrobial susceptibility was tested by disc diffusion. ESBL production was determined by the double-synergy test. The clonality of the isolates was assessed by Pulsed Field Gel Electrophoresis (PFGE). Resistance genes were characterized by PCR and sequencing. Plasmid DNA was analyzed using PCR-based Replicon Typing and plasmid sub-typing schemes, Restriction Fragment Length Polymorphism (RFLP), S1 nuclease-PFGE and Southern hybridization. **Results:** All isolates harboured the *bla*$_{CTX-M-1}$ gene preceded by the IS*Ecp1* element. Three isolates additionally produced TEM-1 and one isolate produced OXA-1. Resistance to non beta-lactams varied, but with constant resistance to tetracyclines and sulphonamides. All isolates were genetically unrelated and belonged to phylogenetic groups A (n=2), B1 (n=2), B2 (n=1) or D (n=3). The *bla*$_{CTX-M-1}$ gene was identified in all isolates on an IncI1/ST3 plasmid of 112 to 120 kb. RFLP profiles of the *bla*$_{CTX-M-1}$/IncI1/ST3 plasmids

from pets were identical to that found previously in *S. enterica* in humans, poultry and cattle. The $bla_{CTX-M-1}$/IncI1/ST3 plasmids from horses and the goat were indistinguishable albeit slightly different from those from pets. **Conclusion:** We demonstrate that IncI1/ST3 plasmids contribute to the dissemination of the $bla_{CTX-M-1}$ gene in *E. coli* from a wide range of animal species in France, irrespective of the nature of *E. coli* clones and animal host. This ESBL plasmid may have spread successfully and extensively into the animal population. Alternately, IncI1 plasmids, which are highly prevalent in animals, may have acquired the $bla_{CTX-M-1}$ gene independently within different hosts.

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LARGE CONJUGATIVE ENTEROCOCCAL PLASMIDS FROM DIFFERENT SOURCES CARRY ANTIBIOTIC RESISTANCE GENES AND A DIVERSITY OF MERA (MERCURY) GENES SPREAD AMONG GRAM-POSITIVE BACTERIA

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Objectives: Diverse environmental stressors (e.g. metals) can participate in the selection and maintenance of antibiotic resistance (ABR) strains and/or genetic elements. Mercury (Hg) is present in environment, in part associated with anthropogenic contamination. The occurrence of mer operon among Enterococcus was scarcely analyzed, although it has been linked to mobile genetic elements with ABR. Our goal was to assess the occurrence of known HgR genes among Enterococcus spp from several sources, and to evaluate their genetic context. **Methods:** We analyzed 231 E. faecalis-Efl, 252 E. faecium-Efm, and 205 Enterococcus spp-Ep from hospitalized (H, n=47), healthy humans (HV, n=68), poultry (P, n=155), piggeries environment/swine PE, n=220), aquacultures (A, n=167) and sewage (S, n=52) (Portugal; 1997-2011). Genes linked to ABR (vanA, tetM, tetL, ermB, aac6"-aph2, blaZ), HgR (4 merA sequences) and Tn6009 were searched (PCR). Mating assays, clonal relatedness (PFGE/MLST) and analysis of the plasmid carrying HgR genes (S1-PFGE, rep typing, hybridization) were done. **Results:** Genes encoding HgR (merA1-2%; merA2-3%,; merA3-0,5%) were identified in 16 Efm, 2 Efl, and 2 Ep, and linked to 8 PFGE types (10 Efm, 1 Efl) from PE/H/HV (9%) and S (8%). Representative isolates corresponded to Efm CC17 (ST132, ST393 and ST431; PE and H), Efm CC5 (ST185; PE), STnew (S) and to Efl ST159 (H). The merA3 and tetM were located at chromosome (Efl-H) while other Hg/ABR genes were detected on plasmids: i) merA1+merA2 (185Kb, 190Kb;Efm-2PE); ii) merA1+merA2+tetM (250kb, 270Kb; Efm-1S, 1PE); iii) merA1+merA2+tetM+ermB (200kb;Efm-1HV); iv) merA1+merA2+merA3+ermB+vanA (75kb; Efl-1H). ABR (erythromycin, tetracycline, ampicillin, HLR-streptomycin, HLR-gentamicin) was co-transferred with HgR genes at different rates. The repA-pLG1 was only identified in Efm plasmids tested which carry only HgR (n=1) or HgR+ABR genes (n=2). The merA were not linked to Tn6009 or plasmids carrying blaZ as described. **Conclusion:** This work represents the first study addressing the diversity of merA genes among Enterococcus from different ecological niches. Their co-location with ABR genes on widespread large pLG-like conjugative plasmids of E. faecium may favoured their persistence under different selective pressures linked to antibiotic use or environmental Hg contamination. The diversity of merA genes also suggests frequent lateral gene transfer between bacteria sharing common ecological settings.

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WIDE SPREAD OCCURRENCE OF PLASMID MEDIATED FLUOROQUINOLONE RESISTANCE (PMQR) IN COMMENSAL FLORA FROM THE GUT OF NEONATES

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Introduction: Antibiotics target both pathogenic bacteria and normal commensal flora. Current strategies to monitor the presence of antibiotic resistant bacteria mainly examine resistance in pathogenic organisms. Emergence of resistance amongst the commensal flora is a serious threat to the community. There is a paucity of data regarding the dynamics of antibiotic resistance in the commensals in the absence of antibiotic pressure. Fluoroquinolones have been listed as the priority drug by WHO and are used widely used in acquired infections. Aim To study the fluoroquinolone resistance in the commensal flora in absence of antibiotic selection pressure with special reference to PMQR. **Material & Methods:** This study was hospital based and followed up at home involving 75 vaginally delivered neonates. All of them were antibiotic naive, breast fed and healthy (Age: 1-60 days). The mothers did not receive any antibiotics during their pregnancy. Stool samples were collected on Day (D) 1, D21 and D60 of birth. Break points for nalidixic acid and ciprofloxacin were as per CLSI guidelines. ESBL was tested by double disc diffusion method. AmpC detection was done by disc antagonism test. Stool samples of 28 randomly selected neonates were subjected to molecular characterization of qnr genes (A, B, S), qepA and aac-6-Ibr. DHPLC was performed for screening of gyraseA mutation in these isolates and were sequence analyzed. **Result:** Out of 267 gram negative isolates from 75 neonates, the predominant species was E.coli (87%). Nalidixic acid resistance was ~55% on all the 3 occasions whereas resistance to ciprofloxacin increased up to 3 folds from D1 to D60 which was 8% to 29.2% respectively. The co-occurrence of resistance to 3rd generation cephalosporins (3GC) and fluoroquinolone was 9% (D1) 18.2% (D2) and 31% (D3). Stool samples of 28 neonates yielded 91 Enterobacteriaceae isolates. Out of 91 isolates 22 had qnr gene. QnrA was isolated in 13 samples, qnrB in 3 isolates and qnrS in 10 isolates. QepA was seen in 20 isolates, aac-6-Ibr in 15 isolates, 13 isolates had multiple PMQR genes. Forty eight isolates had mutation at 83 positions in gyrase gene, 18 had mutation at 87 positions, 2 had a mutation at 154 and 171 position and 2 had mutation at 200 position of amino acid sequence. **Conclusion:** This study indicates the tremendous load of antibiotic resistance present in the community in the absence of antibiotic selection pressure. This is the first report from India showing the high load of PMQR with mutation in gyrase A gene in the commensal flora. Isolates carrying resistance to both Fluoroquinolones and 3GC can further pose a threat to the community by limiting the treatment options. **Acknowledgement:** This study was funded by Indian Council of Medical Research, New Delhi Govt. of India.

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MONITORING OF ANTIMICROBIAL RESISTANCE IN COMMENSAL E. COLI WITH PUBLIC HEALTH RELEVANCE - STRATEGIES AND FIRST RESULTS

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A systematic monitoring of resistance to antimicrobials in zoonotic bacteria and commensals was established in Germany. The overall objective is to estimate the prevalence of resistance to antimicrobial substances relevant for public health along the food chain, to detect new resistance patterns and changes in the prevalence rate of antimicrobial resistance,

and to identify factors with major impact on the related risks. The sampling strategy includes monitoring of all major food production chains. As regards poultry, laying hens for table egg production, broilers of Gallus gallus and turkeys are considered each as a separate population. For cattle, beef cattle, veal calves and dairy cows are dealt separately. Regarding pigs, only fattening pigs ready for slaughter are included. As regards foodstuffs, raw meat derived from cattle, pigs, broilers and turkeys will be covered separately. Faecal samples or food samples are collected within this active monitoring program. Samples are used to estimate both, the prevalence of the microorganism at the specific level, and antimicrobial resistance among the isolates collected. Data are analysed on the basis of epidemiological cut off values reflecting optimum sensitivity for detection of acquired resistance. In the years 2009 and 2010, around 3800 E. coli isolates were collected from primary production, at slaughterhouses and at retail. While the majority (60%) of isolates from laying hens was susceptible to all antimicrobials tested, most isolates from broilers, turkeys, chicken meat and turkey meat showed resistance to at least one (85% to 94%) but frequently even to several antimicrobial classes (73% to 89%). Resistance rate in dairy cows was lowest (16%), whereas resistance in veal calves, veal and pork ranged between 43% and 92%. Besides resistance to antimicrobial classes that have been extensively used in the veterinary field for a long time (e.g. sulphonamides and tetracyclines) resistance to (fluoro)quinolones and 3rd generation cephalosporins was observed. In 2010, an increase for resistance to 3rd generation cephalosporins was identified in E. coli isolates in those populations (laying hens, broilers, veal calves, turkey meat) covered in both years. In contrast, resistance rates for (fluoro)quinolones remained on the same level as in 2009. Resistance to (fluoro)quinolones and cephalosporins in E. coli isolates observed is of special concern because they are critically important antimicrobials in human antimicrobial therapy. The increase of resistance to 3rd generation cephalosporins, which is an indicator for spread of ESBLs, requires immediate action. Together with continuous monitoring of antimicrobial usage this monitoring system for antimicrobial resistance provides the scientific background for the implementation of appropriate management strategies.

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GENETIC LINEAGES, ANTIMICROBIAL RESISTANCE AND VIRULENCE TRAITS OF METHICILLIN-SUSCEPTIBLE *STAPHYLOCOCCUS PSEUDINTERMEDIUS* (MSSP) FROM HOUSEHOLD AND POUND DOGS

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Background: *S. pseudintermedius* is a normal inhabitant of skin and mucosa of dogs but it is also an opportunistic pathogen. Scarce data is available on the molecular characteristics of MSSP given that most studies focus on methicillin-resistant isolates. The objective of this study was to identify the genetic lineages, antimicrobial resistance and virulence profile of MSSP strains obtained from healthy dogs in La Rioja (Spain). **Material and Methods:** 34 MSSP strains obtained from healthy household dogs (18 isolates) and healthy pound dogs (16) between 2009 and 2011 were included. MLST and *spa* typing were performed in all strains. Clonal relatedness was assessed by *SmaI*-PFGE. Susceptibility to 17 antimicrobials was determined by disc-diffusion and involved resistance genes were investigated by PCR. The presence of the following groups of virulence genes was tested by PCR (number of virulence genes tested): bi-component leukocidins (4), exfoliatins (6), haemolysins (5), and pyrogenic-toxin-superantigens (21). **Results:** Twenty-seven distinct sequence-types (STs) were identified revealing the presence of 15 novel STs (10 showed novel allele combinations whereas 5 novel alleles). The 12 already described STs detected were as follows: ST7

(2 strains), ST42 (2), ST77 (2), ST17, ST20, ST29, ST33, ST69, and ST141 from household dogs and ST160, ST20, ST44 and ST41 from pound dogs. Five of the 15 novel STs were detected in 6 strains from household dogs whereas 10 novel STs were observed in 12 strains from pound dogs; what implies that 33% and 75% of strains from household and pound dogs, respectively, presented novel STs. The elevated diversity observed by MLST was corroborated by a high diversity of band patterns by *SmaI*-PFGE. Only 4 strains were *spa* typeable presenting the t02, t05 and t43, in addition to a novel *spa* type. Resistance to the following antimicrobials and involved resistance genes were as follows (% strains): penicillin/*blaZ* (74), tetracycline/*tet*(K) and/or *tet*(M) (47), erythromycin-clindamycin/*erm*(B) (21), gentamicin/*aacA-aphD* (3), kanamycin/*aphA3* (21), streptomycin/*aadE* (21), cloramphenicol/cat_{pC221} (24) and cotrimoxazol/*dfr*(G) or *dfr*(D) (12). Seven multidrug resistant (MDR) strains (21%) harbored the resistance-gene-cluster *aadE-sat4-aphA3*. All SP presented the leukocidin genes *lukS-I* and *lukF-I*. Exfoliatin genes detected were (% strains): *siet* (100), *expA* (9) and *expB* (9) whereas Enterotoxin genes observed were as follows: *si-ent* (100), sec_{canine} (6), *sel* (6), *sek* (6), and *seh* (3). **Conclusions:** High diversity of lineages was detected among our MSSP strains of dog origin. Elevated number of novel STs was detected, especially among those recovered from pound dogs. High variety of antimicrobial resistance genes was observed with 21% of MDR strains. Presence of virulence determinants was evidenced and the detection of the novel exfoliatin genes *expA* and *expB* is of relevance.

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OCCURRENCE OF ANTIBIOTIC-RESISTANT BACTERIA IN BIOFILMS COLLECTED DURING DRINKING WATER PRODUCTION PROCESS

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Antibiotic-resistant bacteria are mainly selected in human and animals under therapeutic treatment and are transferred into the Environment mostly by the wastewater effluents and run-off on pasture. Water has a preponderant role in the circulation of bacteria and their associated-resistance genes, and could also constitute a return pathway to human *via* drinking water. Despite the treatment of drinking water, bacteria are present in the circulating water and can notably form biofilms which are the site of potential genes transfers and consequently can contribute to the dissemination of antibiotic-resistance genes. This study focused on biofilms collected in: a drinking water treatment plant, which is supplied by an aquifer highly vulnerable to microbial contamination (karstic hydrosystems), and in drinking water distribution system in Le Havre (France). We determined *E. coli* (fecal bacteria) and *Pseudomonas* (ubiquitous bacteria) densities and stored the isolates from three tanks: (i) the tank after sand bed filtration, (ii) the chlorination tank and (iii) the drinking water tank. For these isolates, we determined antibiotic-resistance and adherence ability. In the tank after sand bed filtration, a few *E. coli* were isolated (8 $CFU.cm^{-2}$) in a biofilm which also contained *Pseudomonas* (10^{1} $CFU.cm^{-2}$). *E. coli* isolates were sensitive whereas 90% of *Pseudomonas* were resistant to antibiotics from 1 to 7. Chlorination eliminated the *E. coli* and allowed a high decrease in the *Pseudomonas* density ($<10^{-2}$ $CFU.cm^{-2}$). Indeed, in the chlorination tank none *Pseudomonas* was isolated from biofilms collected whereas in the drinking water tank, 14 *Pseudomonas* were isolated for one biofilm, indicating a colonization of *Pseudomonas* in the distribution system but probably at a very weak level. Surprisingly, these 14 *Pseudomonas* isolates harbor a « hypersensitivity » phenotype to all antibiotics tested, including their natural resistance (β-lactams). Sequencing of the 16S rRNA gene and phylogenetic analysis suggest that these isolates constitute a new species of *Pseudomonas*.

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CHARACTERIZATION OF THE FIRST EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING NONTYPHOIDAL SALMONELLA STRAIN ISOLATED IN PORTUGAL

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Background: Extended-spectrum beta-lactamases (ESBLs)-producing Salmonella constitute an emerging threat worldwide though their distribution is uneven in different geographical regions. The goal of our study is to characterize the first ESBL-producing Salmonella identified in Portugal, including bla genetic environment. **Methods:** Susceptibility to antibiotics and beta-lactamase production was tested by disk diffusion methods and/or E-test. Clonal identification was performed by MLST. Screening of genes encoding resistance to antibiotics or biocides found in the animal setting (feed, disinfectants and environmental pollution) was performed by PCR and sequencing. Transferability of bla and characterization of integron and plasmid backbones was performed by PCR, RFLP, hybridization (I-CeuI/S1 nuclease gels) and/or sequencing. **Results:** A CTX-M-9-producing S. Bovismorbificans isolate belonging to ST142 was identified from a 4-years-old boy outpatient with a gastrointestinal infection (February 2011). It was resistant to ampicillin (MIC>256 mg/L), cephalotin (MIC>256 mg/L) and cefotaxime (MIC=4 mg/L), but not to ceftazidime (MIC=0,5 mg/L), cefepime (MIC=1 mg/L), aztreonam (MIC=1 mg/L) and carbapenems (MIC=0,008-0,38 mg/L). Resistance to gentamicin, kanamycin, tobramycin, streptomycin and sulfamethoxazole was also detected. The blaCTX-M-9 gene was located within an In60 variant carrying an atypical gene cassette array (aadB-aadA2) and lacking IS3000. This genetic platform was located on a transferable 240Kb-IncHI2 plasmid (rep 99% homologous with that of the prototype R478 plasmid), carrying also arsB (arsenic resistance) and terF (tellurium resistance), but lacking merA (mercury resistance), silA (silver/copper resistance) and copD (copper resistance), previously described in R478 plasmid. **Conclusion:** This study corresponds to the first description of an ESBL-producing Salmonella strain in Portugal and of a CTX-M-9 enzyme in the rare serotype S. Bovismorbificans. The emergence of blaCTX-M-9 in a similar genetic environment (In60 variant / IncHI2) than that circulating in other Salmonella serotypes and Escherichia coli from humans/animals in Europe suggests a common reservoir and requires surveillance to contain further dissemination.

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SUSCEPTIBILITY TO ANTIMICROBIALS OF *FLAVOBACTERIUM PSYCHROPHILUM* STRAINS RECOVERED FROM OUTBREAKS OCCURRED IN CHILEAN SALMON FARMS

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Flavobacterium psychrophilum is the causal agent of rainbow trout fry syndrome (RTFS), which is actually considered the most important bacterial disease in Chilean freshwater salmonid farming, being mainly controlled by the use of antimicrobial agents. The present study was undertaken to gain information of the susceptibility to antimicrobials of *F. psychrophilum* strains recovered from RTFS outbreaks developed in Chilean salmon farming during

the 2010-2011. The enzyme profiles of 72 Chilean strains were determined by using API-ZYM strips (bioMerieux) and confirmed to be *F. psychrophilum* by 16S rRNA polymerase chain reaction. Susceptibility patterns to 12 antibacterial agents and Minimum Inhibitory Concentration (MIC) values of 5 antimicrobials were tested on diluted (1:5) Mueller-Hinton agar by using an agar disk diffusion method and an agar dilution method, respectively. The majority of *F. psychrophilum* strains were susceptible to florfenicol, oxytetracycline, kanamycin, gentamicin, flumequine and enrofloxacin, but an important number was resistant to oxolinic acid, trimethoprim-sulfamethoxazole, streptomycin and furazolidone. *F. psychrophilum* strains exhibited high levels of susceptibility to florfenicol (MIC_{90}: 1 µg/mL), flumequine and enrofloxacin (MIC_{90}: 4 µg/mL), whereas MIC_{90} values of oxytetracycline and oxolinic acid were remarkable higher (8 and 16 µg/mL, respectively). Otherwise, no differences in antimicrobial susceptibility patterns as well as susceptibility levels between strains isolated in 2010 and 2011 were observed. These results suggest that florfenicol and oxytetracycline would be efficient to treat this pathogen in Chilean salmonid farming but the increasing incidence of resistance to oxolinic acid prompt the necessity to know the molecular mechanisms involved in this resistance and how this species could develop resistance to other fluoroquinolones. This study was supported by INNOVA-CORFO (grant 09MCSS-6704).

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MRSA IN THE FOOD PRODUCTION CHAIN DURING 2010 - IS THERE A LINK BETWEEN SPA-TYPE AND RESISTANCE PHENOTYPE

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During 2010 the national monitoring for zoonotic agents in Germany was conducted to determine the prevalence of MRSA along the food chain. Altogether, 589 isolates from several food production chains were sent to the National Reference Laboratory for Staphylococci at the Federal Institute for Risk Assessment (BfR) for MRSA confirmation and further typing. The majority (N=521) of these isolates were collected at all production levels in the turkey food chain. The remaining 68 isolates were collected at primary production in veal and dairy farms. Isolates were confirmed as MRSA by PCR (Poulsen et al. 2003), spa- (Shopsin et al. 1999) and SCCmec-typed (Zhang et al. 2005). Resistance profiles to a panel of 19 antimicrobials representing 17 substance classes were determined for all isolates by broth microdilution according to CLSI standards. Evaluation of resistance complies with epidemiological cut-offs defined by EUCAST at the time of submission. Isolates with an MIC above the cut-offs were considered as resistant, otherwise as susceptible. Overall, 20 different spa-types were identified. Spa-typing revealed mainly types related to the livestock associated clonal complex CC398 (47% t011 and 37% t034). However, 11% (N=62) of isolates belonged to non-CC398 associated spa-types, and were, with one exception, collected in the turkey production chain. Among those isolates, mainly the types t002 (N=37), and t1430 (N=22) were found. Spa-type t1430 belongs to MLST ST9, which is associated with pig farming. Spa-type t002 is related to MLST ST5 that has also been found in community and hospital acquired MRSA. As expected, resistance phenotyping revealed nearly all isolates (>99%) to be resistant to the tested ß-lactams and tetracycline. High resistance levels were also found for clindamycin (85%), erythromycin (78%), trimethoprim (69%), quinupristin/dalfopristin (62%), and tiamulin (54%). However, a closer look at the data revealed interesting distribution patterns among the spa-type groups “t011”, “t034”, and “non-CC398”. Resistance to gentamicin was mostly found in isolates of spa-type t011 (44%; “t034” 4%; “non-CC398” 8%). Isolates with spa-type t034 were more often resistant to quinupristin/dalfopristin and tiamulin than other spa-types (96%; “t011” 36%; “non-CC398” 55%), while

"non-CC398" associated isolates were highly resistant to ciprofloxacin compared to the most prevalent CC398 spa-types (98%; "t011" 19%; "t034" 28%). Further investigation is required to elucidate the underlying mechanisms for this distribution.

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CIPROFLOXACIN AND NALIDIXIC-ACID RESISTANCE IN PUTATIVE ENTEROHAEMORRHAGIC *E. COLI* ISOLATED FROM HOSPITAL SEWAGE

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Prevalence of shiga-toxin producing *Escherichia coli* (STEC) or enterohaemorrhagic *E. coli* (EHEC) is a major concern worldwide. Antibiotic therapy of suspected STEC infections still remains controversial and is not recommended due to the fact that it could increase the risk of haemolytic uraemic syndrome (HUS) by bacterial lysis, thereby liberating shiga toxin. The aim of this study was to isolate and characterize EHEC/STEC from hospital sewage, and determine their antimicrobial susceptibility patterns to infer possible trend of infection management in respective hospitals. Samples were obtained from three major hospitals in Dhaka, Bangladesh. All samples were screened for the presence of presumptive EHEC by selective enrichment followed by plating on sorbirtol MacConkey agar supplemented with cefixime and potassium tellurite (CT-SMAC). Forty non-sorbitol-fermenting (NSF) bacteria were isolated and further tested for confirmation of *E. coli* by classical biochemical methods. Of these, 13 (32.5%) were found to be *E. coli*, which were screened for true-positive sorbitol non-fermentors in carbohydrate fermentation broth containing sorbitol. This attempt yielded only four (10%) NSF *E. coli* additionally tested negative for rhamnose and cellobiose. MUG (4-methyl umbelliferyl β-D-glucuronide)-assisted fluorescence assay detected production of β-glucuronidase in three of the isolates. The MUG-negative isolate was considered to be STEC serotype O157:H7. Results were compared to phenotypic characteristics of *E. coli* reference strains, NCTC 12079 and ATCC 8739 included as positive and negative EHEC controls, respectively, in each experimental step. Antimicrobial susceptibility was performed by disc diffusion method with five carefully chosen antibiotics conventionally used in local hospitals to tackle bacterial infections. Diameter of inhibition zone was interpreted according to Clinical and Laboratory Standards Institute (CLSI), USA. The results revealed resistance of all four strains to ciprofloxacin (5 µg) and nalidixic acid (30 µg), while sensitivity to meropenem (10 µg) and imipenem (10 µg). The only β-glucuronidase-negative *E. coli* strain was also resistant to neomycin (30 µg). These findings suggest that hospital sewage in Dhaka potentially contains serotypes of STEC. It is also possible to conclude that aminoglycoside and fluoroquinolone antibiotics are frequently administered to treat enteric diseases in certain city hospitals without adequate knowledge of shiga-toxin producing *E. coli*. Investigation of virulence-associated genes in the isolated strains is being currently performed to further confirm detection in this study.

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URBAN RED FOXES (VULPES VULPES) AND THEIR POSSIBLE ROLE IN THE TRANSMISSION OF 3RD GENERATION Β-LACTAM RESISTANT E. COLI TO THE ENVIRONMENT

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Recent studies about the occurrence of antimicrobial resistant (AMR) bacteria in various rural wildlife species, in particular birds and boars, have pointed towards the necessity to include wildlife as an important issue in terms of unraveling transmission pathways and ongoing sources of such AMR strains. In this context the critical importance of wild animals has been demonstrated by the identification of human pandemic ST131-O25:H4 *Escherichia coli* in a feral rat from Berlin only recently. It was against this background that we decided to screen another urban wildlife species, namely the urban Red Fox (*Vulpes vulpes*) for *E. coli* resistant to 3rd generation cephalosporins. Although the sampling of animals was more or less randomly, depending on the number of foxes sent for rabies diagnostic, we assessed urban red foxes as suitable host for AMR screening as they (i) represent one of the most frequent predators in the Berlin city area; (ii) are on top of the food chain, probably accumulating multiresistant bacteria from their prey; (iii) have easy access to the sewage system. Overall, 300 fecal samples from red foxes were collected within a two years period. Screening for *E. coli* with resistance to 3rd generation cephalosporins was performed on chromogenic agar, containing 1µg/ml cefotaxim. Resistant strains with typical *E. coli* morphology were further identified and their resistance was specified via confirmatory test as given by the CLSI (CLSI M31-A3). The proportions of ESBL and AmpC producing isolates were almost identical. Phylogenetic investigations via pulsed-field gel electrophoresis (PFGE) and Multilocus Sequence Typing (MLST) showed a) that AMR strains from urban red foxes in the Berlin city area are very diverse, precluding the existence of a certain wildlife clone; b) several strains are closely related to those that are common in humans and companion animals. Our data, although very preliminary, hint towards the suitability of red foxes as proper wildlife species to assess the existence and diversity of AMR bacteria in their urban environment, including humans and domestic hosts. At this point, the transmission of AMR bacteria between humans, companion animals and urban wildlife in city areas cannot be ruled out. Needless to say, that this hypothesis still needs to be proven, but wild animals should definitely be included in future studies to further unravel the ongoing global spread of multiresistant bacteria.

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A NOVEL QUINOLONE RESISTANCE PHENOMENON IN *SALMONELLA ENTERICA SEROVAR* TYPHI: NALIDIXIC ACID SUSCEPTIBLE ISOLATES WITH REDUCED SUSCEPTIBILITY TO FLUOROQUINOLONES -1ST REPORT FROM INDIA

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Background & Objectives: Fluoroquinolones are commonly used for management of enteric fever and resistance *has* until recently been attributed to point mutations in DNA gyrase.. The detection of resistance caused by mutations is simple, as a first mutation causes resistance to nalidixic acid, a first generation quinolone and is associated with decreased ciprofloxacin susceptibility (DCS) (MIC ≥ 0.125 µg/ml).Accumulating data indicate that patients infected with isolates having DCS are less likely to respond to fluoroquinolone therapy . CLSI recommends use of Nalidixic acid (NA) disc test for screening isolates with DCS. **Methods:** Antibiotic susceptibility of *Salmonella* Typhi isolated between 2001 and 2011 was performed by disc-diffusion. MDR resistance was defined as resistance to ampicilin, chloramphenicol and co-trimoxazole . MIC was determined by E-test for nalidixic acid and ciprofloxacin. A subset of 47 NAS isolates with DCS were characterized using 1 and 5 µg ciprofloxacin disc, screened for mutations at QRDR of gyr A, B and *parC* and plasmid-mediated quinolone resistance by multiplex amplification of qnrA, B and S, aac (6')-Ib-cr6 and qepA **Results:** 304 isolates of Salmonella

Typhi were isolated between 2001 and 2003 , nalidixic acid resistance (NAR) increased from 56.9 to 88.9%. NAR isolates had DCS (MIC 0.125 -1 µg/ml). All nalidixic acid susceptible (NAS) isolates, except three, had MIC range 0.023- 0.064. Between 2004-2011, NAR was predominat phenotype, however the proportion of NAS isolates with DCS increased from 50% in 2004 to 84% in 2011. These strains were not detected by NA disc screening test. , did not show any mutations in *gyrA* and *parC* and were negative for *PMQR* genes. These isolates with non classical quinolone resistance phenotpype were frequently associated with MDR, and a mutation at codon 464 of *gyr*B genes . Their detection could be maximized by screening using CIP 1 µg disc and ciprofloxacin MIC. **Conclusions:** *Salmonella* Typhi isolates with reduced DCS but NAS have emerged and on rise in India. This threatens the value of NA disc test. Their association with MDR narrows therapeutic options.

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EVALUATION OF AZITHROMYCIN (AZM), CEFTRIAXONE (CRO), AND FLUOROQUINOLONE (FQ) RESISTANCE SELECTION IN *SALMONELLA* CHOLERAESUIS, *S.* PARATYPHI, AND *S.* TYPHIMURIUM

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Background: *Salmonella* species, including *Salmonella enterica* subspecies *enterica* serovars Choleraesuis, Paratyphi, and Typhimurium (*S.* Choleraesuis, *S.* Paratyphi, *S.* Typhimurium) are a leading cause of bacterial gastroenteritis. Unfortunately, antimicrobial resistance in salmonellae is increasing, and the optimal antimicrobial therapy for salmonellosis that will prevent further resistance is unknown. The mutant prevention concentration (MPC) is a novel parameter that allows analysis of resistance induction. Antimicrobial concentrations above the MPC restrict resistance, whereas concentrations in the mutant selection window (MSW; the range of concentrations between the MIC and MPC) select resistant subpopulations. We measured MICs and MPCs of AZM, CRO, ciprofloxacin (CIP), levofloxacin (LVX), and moxifloxacin (MXF) against *S.* Choleraesuis, *S.* Paratyphi, and *S.* Typhimurium and used human pharmacokinetic data to predict the risk of resistance selection with each antimicrobial. **Methods:** MPCs were determined by plating 10^{10} colony-forming units (CFU) on antimicrobial-containing agar and measuring the lowest concentration to inhibit growth. FQ-susceptible isolates of *S.* Choleraesuis (ATCC 10708), *S.* Paratyphi (ATCC 9150), and *S.* Typhimurium (ATCC 14028) were tested. Free (unbound) pharmacokinetic parameters (relevant human dosage in parentheses) were evaluated for AZM (C_{max} 72.7 mg/L, $T_{1/2}$ 68 h, $AUC_{0\text{-}24}$ 704 mg · h/L (500 mg PO q24h)), CRO (23 mg/L, 8 h, 135 mg · h/L (1 gm IV 24h)), CIP (2.1 mg/L, 4 h, 19.2 mg · h/L (500 mg PO q12h)), LVX (3.9 mg/L, 7 h, 41.3 mg · h/L (500 mg PO q24h)) and MXF (2.7 mg/L, 12 h, 28.8 mg · h/L (400 mg q24h)). **Results:** MIC / MPC (mg/L) of AZM, CRO, CIP, LVX, and MXF for *S.* Choleraesuis were 16 / >256, 0.0625 / 0.5, 0.015625 / 0.0625, 0.03125 / 0.125, and 0.0625 / 0.5, respectively. MIC / MPC of AZM, CRO, CIP, LVX, and MXF for *S.* Paratyphi were 32 / >256, 0.125 / 2, 0.015625 / 0.25, 0.0625 / 0.5, and 0.125 / 1, respectively. MIC / MPC of AZM, CRO, CIP, LVX, and MXF for *S.* Typhimurium were 16 / >256, 0.125 / 1, 0.03125 / 0.25, 0.0625 / 0.25, and 0.125 / 0.5, respectively. All FQ, as well as CRO, attain concentrations above the MPC for the entire dosage interval, except MXF against *S.* Paratyphi (% of the dosage interval that concentrations fall in the MSW (T_{MSW}) = 28.3%). All FQ achieve AUC/MPC values (> 25-30) that predict a lack of resistance selection, save MXF against *S.* Paratyphi (AUC/MPC = 28.8). AZM fails to achieve concentrations above the MPC. **Conclusions:** CIP, LEV, and CRO are predicted to prevent further resistance in susceptible *S.* Choleraesuis, *S.* Paratyphi, and *S.* Typhimurium. MXF may cause further FQ resistance in *S.* Paratyphi only. AZM is pre-

dicted to select resistant mutants. As resistance in salmonellae increases, avoidance of AZM is warranted, as is judicious use of MXF. Our results indicate a need for novel therapies, especially as resistance to AZM, CRO, and FQ develops further.

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SMALL SCALE POULTRY FARMING AND ZOONOTIC TRANSMISSION OF ANTIBIOTIC RESISTANCE IN RURAL ECUADOR

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Introduction: Poultry farming is being promoted for international development because poultry are inexpensive, an efficient source of protein, have little cultural or religious beliefs attached to them, and grow quickly. While poultry farming offers a promising economic development strategy, the potential risk of development of antibiotic resistant bacteria is of concern. Previous studies have reported shared resistant strains between humans and poultry abattoir workers in industrialized settings, but few studies have addressed this issue in community settings. In developing countries the potential for zoonotic transmission may also be elevated due to inadequate water, sanitation, and hygiene conditions. **Methods:** We collected fecal samples from humans (n= 741), chickens (n=294) and environmental media (n=530) in communities with active small-scale poultry farming operations in northern coastal Ecuador between June 2011-February 2012. Most villages had backyard farms with coops close to households, and one community had a larger cooperative facility set apart from the village center. We took advantage of a natural experiment that occurred during the study when the latter village converted to backyard operations between sampling periods. Environmental samples were collected from domestic (household drinking water and household cooking and eating surfaces), outdoor (soil outside households), and coop (soil outside coop and coop surfaces) locations. We isolated E.coli from all samples and carried out disc diffusion testing to assess phenotypic resistance to a suite of 12 antibiotics. Isolates were considered multidrug resistant (MDR) if they were resistant to more than five antibiotics (80th percentile value). **Results:** We observed high rates of MDR (67-88%) and resistance to fluoroquinolones (enrofloxacin and ciprofloxacin; 50-62%) in coop samples, suggesting that resistance occurs in these villages at rates comparable to industrial farming operations. In the village with a collective farming operation, MDR in human samples was significantly elevated during the period when the village was engaged in backyard farming (94% of samples MDR) as compared to farming in a communal facility (28% of samples MDR; $p<0.0001$). MDR and fluoroquinolone resistant E.coli were also isolated from the domestic environment at higher rates during the time of backyard farming than communal operations, offering a potential mechanism for zoonotic transmission of resistant organisms. **Discussion:** Small-scale poultry operations are associated with high levels of resistant bacteria in this community setting, and proximity of the farm to the household is associated with elevated rates of resistance in both environmental and human samples. These results suggest that it would be safer to promote poultry farming in centralized facilities rather than in backyard operations in community settings.

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CANADIAN INTEGRATED PROGRAM FOR ANTIMICROBIAL RESISTANCE SURVEILLANCE: RETAIL FOOD PROGRAM 2003-2011

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The retail sampling component of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was initiated in 2003. As the point in the food pathway immediately pre-consumer, retail food is a logical sampling point in antimicrobial resistance (AMR) surveillance. The CIPARS Retail component monitors resistance in bacteria isolated from retail food, primarily meat, to assess consumer exposure to resistant bacteria. Raw ground beef, chicken legs and wings, pork chops and ground turkey are routinely collected in randomly selected census divisions, weighted by population, in 7 provinces (British Columbia, Saskatchewan, Ontario, and Québec, sampled as individual provinces; and Nova Scotia, New Brunswick and Prince Edward Island, sampled as a region). Cultured bacteria, comprising *Campylobacter*, *Salmonella*, *Enterococcus* and generic *E. coli*, are tested for antimicrobial susceptibility using the Sensititre® broth microdilution system and the US National Antimicrobial Resistance Monitoring System susceptibility plate formats. Not all of these bacterial species are cultured and tested from all samples or with sufficient numbers to meet a target sample size. For example only generic *E. coli* is has been cultured and tested consistently in beef and pork during the first 9 years of CIPARS Retail operation because of the low prevalence of *Campylobacter* and *Salmonella* in these commodities, and suspension of routine *Enterococcus* testing in 2010. Turkey was added as a regularly sampled commodity in 2011, although time-limited pilot projects were conducted prior to that. The most recent temporal (2003-2011) resistance data for select antimicrobials, including Category I antimicrobials (Very High Importance to Human Medicine as categorized by Health Canada), such as ciprofloxacin and ceftiofur, stratified by region, will be presented for the commodity-bacteria pairs. Recent findings of public health significance include and an upward trend, after 2006, in the prevalence of ceftiofur/ceftriaxone resistance in *Salmonella* isolated from chicken in Ontario and Québec: and a statistically significant increase, since 2009, in the prevalence of ciprofloxacin resistant *Campylobacter* from chicken in western Canada (British Columbia, Saskatchewan).

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ESBL-PRODUCING E. COLI IN THE FAECES OF NORWAY RATS (RATTUS NORVEGICUS): A RELEVANT ISSUE FOR PUBLIC HEALTH?

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In recent years, few studies have described the occurrence of ESBL-producing Escherichia coli in the faeces of urban rats from Europe, Africa and Asia. However, extensive genotypic and phylogenetic characterization of such strains, in particular with regard to their zoonotic potential, has only been addressed exceptionally so far. Therefore, we screened 56 urban Norway rats (Rattus norvegicus, n=9 sewage system, n=47 house/garden), provided by professional pest control in Berlin (Germany), for intestinal E. coli (both ESBL and non-ESBL), determined their ESBL phenotype, and finally performed detailed characterization of ESBL gene, phylogenetic background/clonal relatedness (MLST, EcoR typing, PFGE), and virulence associated genes (VAGs). E. coli could be isolated from 80% of the animals (n=45). Up to four isolates per animal (n=120 isolates) underwent PFGE typing to exclude duplicate strains, and subsequent analysis was performed on 42 unique E. coli strains. Of these, eight were ESBL producers, while 34 showed a negative result in the confirmatory test (non-ESBL). Sixteen percent of all rats (n=9) were identified as carriers of ESBL-E. coli and blaCTX-M-1 was the predominant ESBL-encoding gene (n=6). Among animals caught directly from the sewage system, the

ESBL carriage rate was even higher (33%). A difference in the overall pattern of virulence associated genes with respect to their ESBL phenotype could not be observed. Thus, the presence of strains with high numbers (> 20) of genes, resembling a virulence pattern which is also typical for human extraintestinal pathogenic E. coli (ExPEC), was not linked with the ESBL status but rather with the phylogenetic background of a strain. Multilocus sequence types (STs) determined for the rodent ESBL-E. coli, including ST410 and ST90 have previously also been found in ESBL-E. coli from humans, pets, and livestock Initial comparative analysis of rodent ESBL isolates with fifty human clinical strains, sampled in the same time and local, raise the question as to whether urban rats might act as a reservoir for ESBL-E. coli or constitute a common source of environmental pollution with multiresistant strains which in turn would represent a serious issue of public health.

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ANTIMICROBIAL RESISTANCE OF SHIGA TOXIN GENE (*STX*)-POSITIVE AND INITIMIN GENE (*EAE*)-POSITIVE *ESCHERICHIA COLI* ISOLATES FROM WASTEWATERS OF AN URBAN WASTEWATER TREATMENT PLANT (WWTP) COLLECTING SLAUGHTERHOUSE EFFLUENTS

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Introduction & Objectives: Enterohemorrhagic *Escherichia coli* (EHEC) strains are responsible for severe clinical symptoms in humans. Typical EHEC strains carry *stx* and *eae* genes. The objective of this study was to determine the antimicrobial resistance of *stx*- and *eae*-positive *E. coli* strains, previously isolated from wastewaters of an urban WWTP, collecting slaughterhouse effluents. **Material & Methods:** The antimicrobial resistance of 143 *stx*- and/or *eae*-positive isolates was investigated by disk diffusion method. These isolates were obtained from the input effluents from the slaughterhouse (55), and the city (41), and from the output treated effluent (47 isolates, among which four *stx2*- and *eae*-positive *E. coli* O157:H7 isolates). **Results:** Concerning the animal isolates, 71% were resistant, mainly to tetracycline. Concerning the human isolates, 24% were resistant, mainly to ampicillin and tetracycline. However, we have detected an ESBL-producing *E. coli* in the input effluent from the city. This isolate carried the $bla_{\text{CTX-M-14}}$ and was positive for the *eae*-β*1* gene. Concerning the output effluent, 28% of the isolates were resistant. The four *stx2*- and *eae*-positive *E. coli* O157:H7 isolates were susceptible to all tested antibiotics. **Conclusions:** Our results suggest that the profile of antimicrobial resistance of *stx*- and *eae*-positive *E. coli* seemed to be different between human and animal origin. In addition, the isolates showing high virulence for humans were susceptible to all tested antibiotics.

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