Whole-genome sequencing data-based modelling for the investigation of an outbreak of community-associated methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit in Hong Kong

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Abstract

Background: We describe a nosocomial outbreak of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) ST59-SCC*mec* type V in a neonatal intensive care unit (NICU) in Hong Kong.

Methods: In-depth epidemiological analysis was performed by whole genome sequencing (WGS) of the CA-MRSA isolates collected from patients and environment during weekly surveillance, and healthcare workers from the later phase of the outbreak. Case-control analysis was performed to analyse potential risk factors for the outbreak.

Results: The outbreak occurred from September 2017 to February 2018 involving 15 neonates and 1 healthcare worker. WGS analysis revealed complicated transmission dynamics between patients, healthcare worker, and environmental, from an unrecognized source introduced into the NICU within 6 months before the outbreak. In addition to enforcement of directly observed hand hygiene, environmental disinfection, cohort nursing of colonized and infected patients, together with contact tracing for secondary patients, medical, nursing, and supporting staff were segregated where one team would care for CA-MRSA confirmed/CA-MRSA exposed patients, and the other for newly admitted patients in the NICU only. Case-control analysis revealed use of cephalosporins [Odds ratio: 49.84 (3.10 – 801.46), p=0.006] and length of hospitalization [Odds ratio: 1.02 (1.00 – 1.04), p=0.013] as significant risk factors for nosocomial acquisition of CA-MRSA in NICU using multivariate analysis.

Conclusion: WGS facilitates the understanding of transmission dynamics of an outbreak, providing insights for outbreak prevention.

Introduction

Since the identification of community-associated *Staphylococcus aureus* (CA-MRSA) among the indigenous populations lacking history of healthcare contacts in western Australia in the early 1980s, global dissemination of CA-MRSA had occurred in both community and healthcare settings with nosocomial outbreaks [1]. In view of the virulence of CA-MRSA and a higher clinical attack rate of developing invasive infections after colonization, it is important to enhance infection control practices through early recognition of CA-MRSA in colonized or infected patients, and implementation of contact precautions with single room isolation, contact tracing of potential secondary cases, environmental disinfection, and hand hygiene practice to prevent the spread of CA-MRSA in the healthcare settings. With the advance in molecular diagnostics, whole genome sequencing and modelling have been used to understand the molecular epidemiology and transmission dynamics in outbreak settings [2, 3].

In contrast to the general and geriatric medical units where healthcare-associated MRSA (HA-MRSA) is endemic, HA-MRSA and CA-MRSA are not yet endemic in our neonatal and pediatric populations in Hong Kong. Therefore, we report the investigation of nosocomial outbreak of CA-MRSA in a neonatal intensive care unit in Hong Kong, using whole genome sequencing-based typing to understanding the transmission dynamics of CA-MRSA between patients, healthcare workers, and the hospital environment.

Methods

Epidemiological investigation of the outbreak

An outbreak of CA-MRSA was identified in a neonatal intensive care unit (NICU) in Queen Mary Hospital, an acute care university-affiliated teaching hospital with 1700 beds in the Hong Kong West Healthcare Network. This 16-bed NICU has two 6-bed cubicles and two two-bed rooms, located within ward K10N, where another 44 beds are designated for babies with low birth weight or requiring special care, including babies discharged from the NICU. The medical, nursing, and supporting staff are responsible for patients in the NICU as well as those requiring special care in ward K10N. We defined CA-MRSA as a strain of MRSA demonstrating the presence of the Panton-Valentine leukocidin (PVL) gene and staphylococcal cassette chromosome (SCC) *mec* type IV or type V as previously described [4-8]. An outbreak was defined as three or more epidemiologically-linked patients colonized or infected with CA-MRSA in the same unit since the diagnosis of the first case as previously described [9, 10]. The infection control team coordinated the epidemiological investigation and enhanced infection control measures to control the spread of CA-MRSA in the NICU.

Contact tracing and screening were conducted to identify all potential asymptomatic carriers exposed to the CA-MRSA-positive cases as previously described [11].

Nasal swabs were taken for culture from all patients. A case was defined as a patient colonized or infected with CA-MRSA after 48 hours of hospitalization, while a control was defined as CA-MRSA-negative patient who had stayed in the same ward for 3 days of more during the outbreak period. Case-control analysis was

performed to identify the risk factors for the nosocomial acquisition of CA-MRSA in this outbreak.

Patient with newly diagnosed CA-MRSA colonization or infection would be transferred to a single room with implementation of contact precautions. Cohort nursing of cases would be arranged if the single room was not fully occupied. Environmental disinfection with terminal cleansing would be performed by trained supporting staff for the entire cubicle or room, wherever a CA-MRSA patient was identified, using chlorine dioxide solution 125 ppm (Tristel Solutions Ltd., NY).

Screening of healthcare workers for CA-MRSA colonization was offered on a voluntary basis. Nasal swabs were collected by infection control nurses. Staff with CA-MRSA colonization would be counseled by a designated infection control nurse in a confidential manner. Decolonization with intranasal mupirocin twice daily and 4% chlorhexidine bathing daily for 5 days would be provided. Repeated CA-MRSA screenings were performed to document the clearance of CA-MRSA colonization one week post-decolonization for two consecutive weeks.

Environmental surveillance for CA-MRSA during the outbreak

Weekly environmental surveillance cultures were taken by infection control nurses to assess the extent of contamination by CA-MRSA as previously described [10]. Briefly, patient immediate areas such as incubators for babies, resuscitaires, ventilators, infusion pumps, designated stethoscopes, phototherapy lamps, touchscreen monitors, bedside computer keyboards, and bedside tables were sampled. Equipment in the communal areas were also sampled, including file cabinets, milk fridges, milk preparation benches, trolleys, sinks, baby scales, portable ultrasound

equipment, computer keyboards and telephones at the nursing station, and breastfeeding pumps.

Environmental samples were collected using Polywipe sponge swabs of 5 cm x 10 cm (Medical Wire & Equipment, UK) as previously described [12]. These swabs are sterile pre-moistened thin flexible sponges tailor-made for sampling environmental surfaces. Each sampled sponge swab was placed in an individual sterile plastic bag, which would be sealed and labeled for further processing in the microbiology laboratory.

Laboratory investigation

Swab specimens collected from the study subjects were delivered to the laboratory immediately for inoculation on MRSA chromID culture media (bioMérieux), and then incubated aerobically at 35°C for 48 hours. For each sponge swab, 2 ml sterile normal saline (0.85% saline) was added into the sterile plastic bag. The sponge was squeezed repeatedly for proper mixing. Then, 100 µl suspension from the bag would be inoculation on a MRSA chromID culture media and processed as described above. Colonies were confirmed to be *Staphylococcus aureus* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany). The Kirby–Bauer disk diffusion method was used to determine the antimicrobial susceptibility of the *S. aureus* and results interpreted according to the CLSI [13-15].

The SCC*mec* types were determined by PCR as previously described [5, 6]. SCC*mec* types were designated according to the ccr type and *mec* class combinations [7, 8].

Whole genome sequencing and bioinformatics analyses

The isolates were analyzed further by genome sequencing using an Illumina MiSeq Platform (Illumina, California, USA) at the Genome Research Center of the University of Hong Kong at >150-fold coverage. A commercial software package (CLC Genomics Workbench 9.01) was used for *de novo* assembly and further improved using a Sanger pipeline [16]. The BLAST Ring Image Generator was used to align and compare the genomic diversity [17, 18]. The genome of a CA-MRSA strain HKU96 (ST59, SCC*mec* V, PVL positive), collected from the wound of a patient in our hospital in 2010, was sequenced in the same run and used as an unrelated control (designated as strain C). Online databases, including the SCC*mec*Finder, ResFinder 3.0, the CARD (comprehensive antibiotic resistance database) v3.0.0 database and Virulence-Finder 1.5 were used to identify and annotate SCC*mec* cassette, acquired resistance genes, chromosomal mutations-associated with resistance and virulence determinants, respectively [19-22]. Parsnp was used for core-genome alignment and variant calls [23]. MEGA 7 and BEAST 1.10 were employed for the construction of phylogenetic trees [24, 25]. A recently described method TransPhylo was used for automated inference of person-to-person transmission events from the genomic data. The method involves a reversible jump Monte-Carlo Markov Chain (MCMC) algorithm and is suitable for investigation of partially sampled and ongoing outbreak [26]. A consensus transmission tree was constructed with the dated phylogeny from BEAST and a colored phylogeny was used to illustrate the transmission scenario as previously described [26].

Mean mutation rate and the 95% Highest Posterior Density (HPD) interval was calculated using Tracer v1.7.1 through deciphering the log file from BEAST [27]. Snippy v3.0 (https://github.com/tseemann/snippy) was used to identify indels

(insertions/deletions) using strain C as the reference genome at a minimum threshold of 30 ×coverage. All indels were summarized and annotated using in-house scripts (Supplementary file, Table S1). Rearrangements and inversions were identified after whole genome alignment using Mauvae v2.4.0 and confirmed using MUMmer4 [28, 29]. Recombination events were further predicted on the core genome alignment using Gubbins [30].

To place our CA-MRSA isolates into the context of published genomes, all *S. aureus* genomes (*n* = 10,197, last accessed on 3 December 2018) available at the GenBank repository (https://www.ncbi.nlm.nih.gov/genome/genomes/154) were downloaded and the sequence type of each assigned using MLST v2.7 (https://github.com/tseemann/mlst). Genomes assigned to ST59 and its single locus variant (n = 39) together with our CA-MRSA genomes (n = 20) were further analyzed for variant sites using ParSNP v1.1.2 [23]. Afterwards, the SNPs were employed to construct a Maximum Likelihood phylogenetic tree with 1,000 times bootstrap using IQ-TREE v1.5.4 and the GTR+I+G substitution model [31]. The phylogenetic tree was visualized using FigTree v1.4.3 as in our previous work [32]. Metadata of all genomes were retrieved from Genbank using in-house python scripts.

Statistical Analysis

Chi-square test, Student t test, and logistic regression were used where appropriate. SPSS, version 20 (IBM), and XLSTAT were used to perform the statistical analyses. A level of significance at 5% was adopted.

Results

Epidemiological investigation and outbreak control

On 4 September 2017, CA-MRSA was cultured from a pus swab collected from the right palm in a 31-day old neonate (patient 1, P1, index case) with prematurity of 33 weeks of gestation who was 1610 gm at birth. Terminal disinfection of P1's bed in the 6-bed cubicle was performed using chlorine dioxide solution 125 ppm (Tristel Solutions Ltd., NY). The 10 neonates staying in the same cubicle with the index case (contacts) were screened, and one additional patient (P2) was identified to be CA-MRSA carrier (Figure 1). These two cases were cohorted in the same cubicle with contact precautions, while hand hygiene among healthcare workers and environmental cleaning were enforced. All known CA-MRSA patients were discharged by 9 October 2018, but on 27 October 2017, a third neonate (P3) was noted to have CA-MRSA isolated from blood, endotracheal aspirates, and tip of intravenous catheter. Further contact tracing of the 5 neonates staying in the same cubicle of P3 revealed two more neonates with CA-MRSA colonization (P4 and P5), and a hospital outbreak in the NICU was declared.

To control further nosocomial transmissions of CA-MRSA in the NICU, phase 2 infection control measures including universal admission screening, weekly surveillance for all hospitalized patients, weekly environmental culture, and directly observed hand hygiene once every 2 hours for all healthcare workers were implemented [33, 34], in addition to the phase 1 infection control measures described above. While no further cases of CA-MRSA were identified by universal admission screening, weekly surveillance culture revealed 10 more CA-MRSA carriers (P6 to P15) during the outbreak period (Figure 1), where five of them (P11 to P15) were admitted to the NICU after phase 2 infection control measures were implemented.

In response to the ongoing nosocomial transmission especially among the newly admitted patients, phase 3 infection control measures were initiated with the support from hospital administration. Healthcare workers (medical, nursing, and supporting staff) were segregated into two teams, one team is responsible for the care of CA-MRSA positive/CA-MRSA neonates, while the other for newly admitted CA-MRSA negative neonates confirmed by admission screening. Segregation of bedside ultrasonography equipment was made possible by urgent purchase of a new one for the newly admitted neonates.

A total of 482 nasal swabs were collected from 144 patients (~3.3 screening specimens per patient). Except for two CA-MRSA patients (P1 and P3) who were detected from clinical specimens, 13 CA-MRSA cases were diagnosed by active surveillance culture. The remaining 131 patients without CA-MRSA on screening culture were served as control. Case-control analysis revealed that use of cephalosporins during hospitalization and length of hospitalization were significant risk factors for nosocomial acquisition of CA-MRSA in the NICU using multivariate analysis (Table 1).

Three sessions of educational talk was held at the bedside to explain the rationale of staff screening. Of the 163 healthcare workers opted for screening, 6 (3.7%) were colonized with MRSA, and 1 (0.6%) was confirmed to be CA-MRSA colonization (Table 2). All healthcare workers with known MRSA and CA-MRSA carriage received decolonization therapy.

Environmental surveillance for CA-MRSA during the outbreak

231 Upon identification of the fifth case of CA-MRSA, environmental cultures
232 were collected from the patient immediate and communal areas before terminal
233 disinfection of the NICU during phase 2 and 3 of infection control measures. Two
234 (1.7%) of the 121 environmental samples collected at baseline were contaminated
235 with CA-MRSA, one was collected from the window bench and the other from the
236 baby scales. Repeated environmental cultures of communal areas at weekly interval
237 involving 198 specimens revealed only one item (scale for weighing babies) positive
238 with CA-MRSA.

239

240 *Whole-genome sequencing of the CA-MRSA isolates*

241 A total of 19 isolates (15 patient isolates, one healthcare worker isolate and 3
242 environmental isolates) were tested by whole-genome sequencing. All the isolates
243 belonged to spa t441 and ST59. Blast search revealed that the outbreak isolates were
244 most closely related to strain M013 (Genbank accession CP003166) which is
245 representative of a ST59-SCC*mec* type V CA-MRSA clone reported in Taiwan [35].
246 Therefore, strain M013 was included as a reference to facilitate orientation and
247 mapping of the *de novo* contigs from the 19 isolates into draft genomes. Circular plot
248 of the draft genomes showed a very high degree of shared identity among the
249 outbreak isolates and with the unrelated control (Figure 2).

250 A phylogenetic tree based on core SNPs identified the 19 isolates to a highly
251 related cluster (Figure 3). The numbers of SNP difference in pairwise comparison
252 among the outbreak isolates ranged from 2 to 17, compared with 153 to 163 SNPs
253 between the isolates and the unrelated control. All the isolates shared an identical
254 profile of virulence genes. These include genes encode exoenzyme (*aur*),

255 staphylococcal complement inhibitor (*scn*), enterotoxins (*seb*, *sek*, *seq*), Panton-
256 Valentine leukocidin (*pvl*), beta-hemolysin (*hlb*) and gamma hemolysin (*hlgABC*). In
257 the isolates, *pvl* was found in a phi596PVL-like prophage while the enterotoxin genes
258 were found within pathogenicity island SaP1. With the exception of one isolate (P10),
259 the outbreak isolates shared an identical profile of acquired resistance genes which
260 correlate with the observed phenotypic resistance to streptomycin (*ant(6)-Ia*, *aph(3')-*
261 *III*), β-lactam (*mecA, blaZ*), erythromycin (*ermB*), chloramphenicol (*cat*) and
262 tetracycline (*tetK*). In strain P10, susceptibility to chloramphenicol correlated with the
263 loss of a *cat*-carrying, pC223-like plasmid (GenBank accession AY355285.1). The
264 outbreak and control isolates has the SCC*mec* type V cassette and can be further
265 designated as subtype Vb. The profiles of chromosomal mutations that may be
266 associated with resistance phenotypes were identical in all the isolates. These included
267 substitution in two putative fosfomycin resistance genes, *murA* (E291D and T396N)
268 and *glpT* (A100V). No mutations were found in the following chromosomal genes
269 included in the CARD database: *mepR*, *mgrA*, *arlR*, *arlS*, *cls*, 16s rRNA, *rpoB*, *gyrA*,
270 *gyrB, parC, parE, pgsA, EF-Tu, fusA, fusE, mprF, walF, UhpT, menA,* and 23S
271 rRNA.

272 *Inference of transmission tree and phylogenetic construction of the ST59 populations*

273 Figure 4 showed the colored phylogeny and consensus transmission tree for
274 this outbreak. It shows that the outbreak started after an un-sampled unidentified
275 source that introduced the clone to the unit, initially affecting P2 and P5. Afterwards,
276 the inference indicted several small clusters of onward transmission. These included a
277 smaller P5 related cluster involving two patients (P6 and P8) and contamination of an
278 environment (S1). The larger P2-related cluster followed a more complex route of
279 transmission, from P2 to three patients (P1, P3 and P10) and two environmental

surfaces (W and S2). After that, transmission occurred from P3 to three other patients (P4, P11 and P12), and P11 to two further patients (P13 and P14). The colonized healthcare worker (HCW) was inferred to have acquired the CA-MRSA from P10. The environmental surface (W) was linked to the transmission to 3 patients (P7, P9 and P15).

The mutation rate in the outbreak CA-MRSA isolates was estimated to be 3.8 $\times 10^{-6}$ per site per year (95% HPD interval, 8.9×10^{-7} to 7.2×10^{-6}). Alignment against the control strain revealed a total of 41 indels in the isolates (Supplementary file, Figure S1 and Table S1). These included 15 deletions (involving 1 to 6 bases), insertions (involving 1 to 56 bases) and 7 complex ones (with double base substitutions and combination of deletion and substitution). To explore the ancestral origin of the outbreak CA-MRSA isolates, the genomes were analyzed against all completed and draft ST59 or its single locus variants genomes deposited in the GenBank (supplementary file, Figure S2). SNP calling and phylogenetic analysis revealed that our isolates were linked to a phylogenetic cluster with ST59/SCC*mec* type V isolates originating from Taiwan (2000 to 2005), mainland China (2012-2016) and Europe (2009-2014).

Discussion

This is the first nosocomial outbreak of CA-MRSA ever reported in Hong Kong. This outbreak occurred in NICU where MRSA and CA-MRSA is not endemic. However, the spread of CA-MRSA could not be readily controlled because our usual proactive infection control measures including directly observed hand hygiene before meal and medication for hospitalized adult population [9, 33, 36-38], as well antimicrobial stewardship [39] could not be applied in this group of patients. These neonates stayed in the NICU for prolonged periods, requiring intensive patient care by healthcare workers, and have received multiple courses of antimicrobial therapy. As illustrated in our case-control analysis, length of stay in the NICU with a mean of 60 days, and use of cephalosporins during hospitalization were significantly associated with CA-MRSA colonization or infection by multivariate analysis. Given the limited number of single rooms in the NICU, the risk of nosocomial transmission of CA-MRSA would have been increased due to cohort nursing of colonized or infected patients in NICU [40]. The CA-MRSA outbreak lasted for 6 months from September 2017 to February 2018. Our outbreak persisted for a longer time when compared with previously reported outbreaks in neonatal settings [41, 42]. Despite the enforcement of hand hygiene practice and environmental disinfection, the nosocomial transmission of CA-MRSA was not controlled until segregation of medical, nursing, and supporting staff into two teams to look after CA-MRSA confirmed/exposed patients, and newly admitted patients in the NICU to further eliminate transmission from the affected to the unaffected patients.

Screening of the healthcare workers for CA-MRSA colonization was also performed on a voluntary basis in a confidential manner. The clinical evidence for healthcare workers screening in CA-MRSA outbreak is lacking. Most of the evidence

for healthcare workers screening comes from outbreak reports where the outbreak of MRSA was under control following the introduction of staff screening as part of the infection control measures [43]. Although one (0.6%) of our healthcare workers was confirmed to be CA-MRSA colonization, the epidemiological analysis could not differentiate if the healthcare was a source or victim in the outbreak.

In an attempt to understand the routes of transmission, we obtained the genomic data from the CA-MRSA isolates in this cluster and analyzed the patterns of shared nucleotide variation using the TransPhylo algorithm. Since the culture sampling is incomplete, it is possible that some patients, staff and visitors with CA-MRSA carriage may remain undetected. In addition, differences in the genomic sequences among the isolates are influenced by time gaps between transmission events and detection of CA-MRSA carriage. Therefore, an inference method is required for reconstruction of transmission events. Transphylo takes these confounding issues into account and transmission events are inferred by analyzing the time-labeled genomic data with a reversible jump MCMC algorithm, particularly considering the observation of cases and a branching process with constant reproduction number throughout the outbreak [26]. Therefore, the output is useful in providing a most likely scenario for the transmission events. The finding (Figure 4) not only dismissed the healthcare worker as the source but illustrated the acquisition of CA-MRSA from patient 10 (P10), it also revealed that the outbreak started after an un-sampled, unrecognized CA-MRSA patient, introducing the clone to the NICU within six months before the recognition of this outbreak. The environmental surface (W) was also linked to the transmission of CA-MRSA to 3 patients (P7, P9 and P15). With this information, the healthcare worker could be reassured as an unlikely source of this outbreak. Decolonization could be given to the concerned healthcare worker to

minimize risk of manifestation to invasive CA-MRSA infections [44], as this outbreak strain contains virulence genes encoding exoenzyme, staphylococcal complement inhibitor, enterotoxins, Panton-Valentine leucocidin, beta-hemolysin and gamma hemolysin as shown in our analysis. Admission screening should be considered for early recognition of asymptomatic CA-MRSA for outbreak prevention [45], and environmental surveillance and disinfection should be enforced as previously described [10].

The CA-MRSA outbreak isolates were attributed to the ST59/SCC*mec* V clone. This clone has previously been reported to be prevalent in Hong Kong, mainland China and Taiwan [5, 6, 46]. Genomic comparison and phylogenic analysis indicated that the isolates have likely evolved from ancestral CA-MRSA isolates in Taiwan in the early 2000s. Animal and *in vitro* experiments have demonstrated that the ST59/SCC*mec* V clone is more virulent than the ST59/SCC*mec* IV variant [46]. The virulent nature of this clone together with the detection in a vulnerable population further prompted us to offer decolonization to all positive babies and healthcare workers. The genomes of the ST59/SCC*mec* V outbreak isolates have remained relatively stable over the sampling timeframe. The observed evoluntionary changes were largely limited to a relatively small numbers of SNP and indels. The mutation rate in the ST59 populations were similar to those previously reported for ST22 and ST239 MRSA populations [47]. Although three substitutions in two putative fosfomycin resistance genes were identified in the genomes, all of our outbreak isolates were phenotypically susceptible to fosfomycin. According to Fu et al, the three substitutions that we observed in the genomes were not associated with fosfomycin resistance [48]. Use of whole genome sequencing technique provides in-depth epidemiological analysis of an outbreak in a clinical unit, but could also be

applied to investigation of protracted outbreaks involving patients from different inpatient and outpatient departments [2]. However, the technological requirement and cost may prohibit routine use of whole genome sequencing in outbreak investigation at this moment.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval was not required because the infection control measures are the standard patient care to control and prevent hospital outbreak.

Informed consent No informed consent was required since this was a retrospective analysis of data.

References

[1] Otter JA, French GL (2011) Community-associated meticillin-resistant Staphylococcus aureus strains as a cause of healthcare-associated infection. J Hosp Infect 79 (3):189-193

[2] Kossow A, Kampmeier S, Schaumburg F, Knaack D, Moellers M, Mellmann A (2018) Whole genome sequencing reveals a prolonged and spatially spread nosocomial outbreak of PVL positive MRSA (USA300). J Hosp Infect

[3] Sassi M, Felden B, Revest M, Tattevin P, Augagneur Y, Donnio PY (2017) An outbreak in intravenous drug users due to USA300 Latin-American variant community-acquired methicillin-resistant Staphylococcus aureus in France as early as 2007. Eur J Clin Microbiol Infect Dis 36 (12):2495-2501

[4] Cheng VC, Lau YK, Lee KL, Yiu KH, Chan KH, Ho PL, Yuen KY (2009) Fatal co-infection with swine origin influenza virus A/H1N1 and community-acquired methicillin-resistant Staphylococcus aureus. J Infect 59 (5):366-370

[5] Ho PL, Cheung C, Mak GC, Tse CW, Ng TK, Cheung CH, Que TL, Lam R, Lai RW, Yung RW, Yuen KY (2007) Molecular epidemiology and household transmission of community-associated methicillin-resistant Staphylococcus aureus in Hong Kong. Diagn Microbiol Infect Dis 57 (2):145-151

[6] Ho PL, Chuang SK, Choi YF, Lee RA, Lit AC, Ng TK, Que TL, Shek KC, Tong HK, Tse CW, Tung WK, Yung RW, Hong Kong CAMsn (2008) Community-associated methicillin-resistant and methicillin-sensitive Staphylococcus aureus: skin and soft tissue infections in Hong Kong. Diagn Microbiol Infect Dis 61 (3):245-250

[7] Ho PL, Lo PY, Chow KH, Lau EH, Lai EL, Cheng VC, Kao RY (2010) Vancomycin MIC creep in MRSA isolates from 1997 to 2008 in a healthcare region in Hong Kong. J Infect 60 (2):140-145

[8] Ho PL, Chiu SS, Chan MY, Gan Y, Chow KH, Lai EL, Lau YL (2012) Molecular epidemiology and nasal carriage of Staphylococcus aureus and methicillin-resistant S. aureus among young children attending day care centers and kindergartens in Hong Kong. J Infect 64 (5):500-506

[9] Cheng VC, Tai JW, Wong LM, Ching RH, Ng MM, Ho SK, Lee DW, Li WS, Lee WM, Sridhar S, Wong SC, Ho PL, Yuen KY (2015) Effect of proactive infection control measures on benchmarked rate of hospital outbreaks: An analysis of public hospitals in Hong Kong over 5 years. Am J Infect Control 43 (9):965-970

[10] Cheng VCC, Wong SC, Chen JHK, So SYC, Wong SCY, Ho PL, Yuen KY (2018) Control of multidrug-resistant Acinetobacter baumannii in Hong Kong: Role of environmental surveillance in communal areas after a hospital outbreak. Am J Infect Control 46 (1):60-66

[11] Cheng VC, Tai JW, Ng ML, Chan JF, Wong SC, Li IW, Chung HP, Lo WK, Yuen KY, Ho PL (2012) Extensive contact tracing and screening to control the spread of vancomycin-resistant Enterococcus faecium ST414 in Hong Kong. Chin Med J (Engl) 125 (19):3450-3457

[12] Cheng VCC, Chen JHK, Wong SCY, Leung SSM, So SYC, Lung DC, Lee WM, Trendell-Smith NJ, Chan WM, Ng D, To L, Lie AKW, Yuen KY (2016) Hospital Outbreak of Pulmonary and Cutaneous Zygomycosis due to Contaminated Linen Items From Substandard Laundry. Clin Infect Dis 62 (6):714-721

[13] Cheng VC, Li IW, Wu AK, Tang BS, Ng KH, To KK, Tse H, Que TL, Ho PL, Yuen KY (2008) Effect of antibiotics on the bacterial load of meticillin-resistant Staphylococcus aureus colonisation in anterior nares. J Hosp Infect 70 (1):27-34
[14] Cheng VC, Chan JF, Lau EH, Yam WC, Ho SK, Yau MC, Tse EY, Wong AC, Tai JW, Fan ST, Ho PL, Yuen KY (2011) Studying the transmission dynamics of meticillin-resistant Staphylococcus aureus in Hong Kong using spa typing. J Hosp Infect 79 (3):206-210
[15] Cheng VC, Tai JW, Wong ZS, Chen JH, Pan KB, Hai Y, Ng WC, Chow DM, Yau MC, Chan JF, Wong SC, Tse H, Chan SS, Tsui KL, Chan FH, Ho PL, Yuen KY (2013) Transmission of methicillin-resistant Staphylococcus aureus in the long term care facilities in Hong Kong. BMC Infect Dis 13:205
[16] Page AJ, De Silva N, Hunt M, Quail MA, Parkhill J, Harris SR, Otto TD, Keane JA (2016) Robust high-throughput prokaryote de novo assembly and improvement pipeline for Illumina data. Microb Genom 2 (8):e000083
[17] Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA (2011) BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402
[18] Ho PL, Wang Y, Liu MC, Lai EL, Law PY, Cao H, Chow KH (2018) IncX3 Epidemic Plasmid Carrying blaNDM-5 in Escherichia coli from Swine in Multiple Geographic Areas in China. Antimicrob Agents Chemother 62 (3)
[19] Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM (2014) Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. J Clin Microbiol 52 (5):1501-1510
[20] Kaya H, Hasman H, Larsen J, Stegger M, Johannesen TB, Allesoe RL, Lemvigh CK, Aarestrup FM, Lund O, Larsen AR (2018) SCCmecFinder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome mec in Staphylococcus aureus Using Whole-Genome Sequence Data. mSphere 3 (1)
[21] Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV (2012) Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67 (11):2640-2644
[22] Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Pawlowski AC, Johnson TA, Brinkman FS, Wright GD, McArthur AG (2017) CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res 45 (D1):D566-D573
[23] Treangen TJ, Ondov BD, Koren S, Phillippy AM (2014) The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol 15 (11):524
[24] Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol 4 (1):vey016
[25] Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33 (7):1870-1874
[26] Didelot X, Fraser C, Gardy J, Colijn C (2017) Genomic Infectious Disease Epidemiology in Partially Sampled and Ongoing Outbreaks. Mol Biol Evol 34 (4):997-1007

[27] Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Syst Biol 67 (5):901-904
[28] Darling AC, Mau B, Blattner FR, Perna NT (2004) Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 14 (7):1394-1403
[29] Marcais G, Delcher AL, Phillippy AM, Coston R, Salzberg SL, Zimin A (2018) MUMmer4: A fast and versatile genome alignment system. PLoS Comput Biol 14 (1):e1005944
[30] Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR (2015) Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res 43 (3):e15
[31] Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32 (1):268-274
[32] Wang Y, Tong MK, Chow KH, Cheng VC, Tse CW, Wu AK, Lai RW, Luk WK, Tsang DN, Ho PL (2018) Occurrence of Highly Conjugative IncX3 Epidemic Plasmid Carrying bla NDM in Enterobacteriaceae Isolates in Geographically Widespread Areas. Front Microbiol 9:2272
[33] Cheng VC, Wu AK, Cheung CH, Lau SK, Woo PC, Chan KH, Li KS, Ip IK, Dunn EL, Lee RA, Yam LY, Yuen KY (2007) Outbreak of human metapneumovirus infection in psychiatric inpatients: implications for directly observed use of alcohol hand rub in prevention of nosocomial outbreaks. J Hosp Infect 67 (4):336-343
[34] Cheng VCC, Wong SC, Wong SCY, Yuen KY (2018) Directly observed hand hygiene - from healthcare workers to patients. J Hosp Infect
[35] Huang TW, Chen FJ, Miu WC, Liao TL, Lin AC, Huang IW, Wu KM, Tsai SF, Chen YT, Lauderdale TL (2012) Complete genome sequence of Staphylococcus aureus M013, a pvl-positive, ST59-SCCmec type V strain isolated in Taiwan. J Bacteriol 194 (5):1256-1257
[36] Cheng VC, Chen JH, Poon RW, Lee WM, So SY, Wong SC, Chau PH, Yip CC, Wong SS, Chan JF, Hung IF, Ho PL, Yuen KY (2015) Control of hospital endemicity of multiple-drug-resistant Acinetobacter baumannii ST457 with directly observed hand hygiene. Eur J Clin Microbiol Infect Dis 34 (4):713-718
[37] Cheng VC, Tai JW, Li WS, Chau PH, So SY, Wong LM, Ching RH, Ng MM, Ho SK, Lee DW, Lee WM, Wong SC, Yuen KY (2016) Implementation of directly observed patient hand hygiene for hospitalized patients by hand hygiene ambassadors in Hong Kong. Am J Infect Control 44 (6):621-624
[38] Cheng VC, Tai JW, Chau PH, Lai CK, Chuang VW, So SY, Wong SC, Chen JH, Ho PL, Tsang DN, Yuen KY (2016) Successful control of emerging vancomycin-resistant enterococci by territory-wide implementation of directly observed hand hygiene in patients in Hong Kong. Am J Infect Control 44 (10):1168-1171
[39] Cheng VC, To KK, Li IW, Tang BS, Chan JF, Kwan S, Mak R, Tai J, Ching P, Ho PL, Seto WH (2009) Antimicrobial stewardship program directed at broad-spectrum intravenous antibiotics prescription in a tertiary hospital. Eur J Clin Microbiol Infect Dis 28 (12):1447-1456

[40] Jernigan JA, Titus MG, Groschel DH, Getchell-White S, Farr BM (1996) Effectiveness of contact isolation during a hospital outbreak of methicillin-resistant Staphylococcus aureus. Am J Epidemiol 143 (5):496-504
[41] Sax H, Posfay-Barbe K, Harbarth S, Francois P, Touveneau S, Pessoa-Silva CL, Schrenzel J, Dharan S, Gervaix A, Pittet D (2006) Control of a cluster of community-associated, methicillin-resistant Staphylococcus aureus in neonatology. J Hosp Infect 63 (1):93-100
[42] Giuffre M, Cipolla D, Bonura C, Geraci DM, Aleo A, Di Noto S, Nociforo F, Corsello G, Mammina C (2012) Epidemic spread of ST1-MRSA-IVa in a neonatal intensive care unit, Italy. BMC Pediatr 12:64
[43] Hawkins G, Stewart S, Blatchford O, Reilly J (2011) Should healthcare workers be screened routinely for meticillin-resistant Staphylococcus aureus? A review of the evidence. J Hosp Infect 77 (4):285-289
[44] Orendi JM, Coetzee N, Ellington MJ, Boakes E, Cookson BD, Hardy KJ, Hawkey PM, Kearns AM (2010) Community and nosocomial transmission of Panton-Valentine leucocidin-positive community-associated meticillin-resistant Staphylococcus aureus: implications for healthcare. J Hosp Infect 75 (4):258-264
[45] Pierce R, Lessler J, Popoola VO, Milstone AM (2017) Meticillin-resistant Staphylococcus aureus (MRSA) acquisition risk in an endemic neonatal intensive care unit with an active surveillance culture and decolonization programme. J Hosp Infect 95 (1):91-97
[46] Feng Y, Chen HL, Chen CJ, Chen CL, Chiu CH (2017) Genome comparisons of two Taiwanese community-associated methicillin-resistant Staphylococcus aureus ST59 clones support the multi-origin theory of CA-MRSA. Infect Genet Evol 54:60-65
[47] Hsu LY, Harris SR, Chlebowicz MA, Lindsay JA, Koh TH, Krishnan P, Tan TY, Hon PY, Grubb WB, Bentley SD, Parkhill J, Peacock SJ, Holden MT (2015) Evolutionary dynamics of methicillin-resistant Staphylococcus aureus within a healthcare system. Genome Biol 16:81
[48] Fu Z, Ma Y, Chen C, Guo Y, Hu F, Liu Y, Xu X, Wang M (2015) Prevalence of Fosfomycin Resistance and Mutations in murA, glpT, and uhpT in Methicillin-Resistant Staphylococcus aureus Strains Isolated from Blood and Cerebrospinal Fluid Samples. Front Microbiol 6:1544

Figure legend

Figure 1. Timeline and measures to control the outbreak of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in the neonatal intensive care unit (NICU)

Note. The diagonal boxes represent the period of hospitalization of patient 1 (P1) to patient 15 (P15) in the NICU. @ denote the time of collection of clinical specimens culture positive for CA-MRSA. P1: CA-MRSA isolated from pus swab collected from the right palm; P3: CA-MRSA isolated from blood culture, endotracheal aspirate, and catheter tip of intravenous catheter.

denote the time of collection of screening specimens culture positive for CA-MRSA.

DOHH, directly observed hand hygiene - delivery of alcohol-based hand rub to all healthcare workers

Figure 2. Whole-genome sequencing analysis of the clinical and environmental isolates of community-associated methicillin-resistant *Staphylococcus aureus*

Note. Circular plot of genome diversity between the 19 CA-MRSA isolates from the outbreak and the unrelated control. The circular maps were generated with the BLAST Ring IMAGE Generator and each genome was colored according to the pairwise identity against the control strain C in the following order (inner to outer circles): C, P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, W, S1, S2, P11, P12, P13, P14, P15 and HCW.

588 Figure 3. Maximum likelihood phylogenetic tree of 20 ST59 SCC*mec* type V CA-MRSA
589 isolates based on total core genome SNPs.

590 Note. A control strain (C) unrelated to the present outbreak was included as an outgroup. P1
591 to P15 represent patient isolates from patient 1 to patient 15, whereas HCW represents the
592 isolate from the healthcare worker. The environmental isolates were collected from window
593 bench (W), and baby scale (S1) at baseline period, and baby scale (S2) on repeated
594 environmental culture.

595

596 Figure 4. Phylogeny and consensus transmission tree for CA-MRSA outbreak.

597 (A) A best-case scenario, colored phylogeny of the 19 CA-MRSA isolates from the current
598 study. In the phylogeny, each branch was colored by a separate color for each host (P1 to
599 P15, HCW) or environmental source (S1, S2, W). Both sampled and the potential existence
600 of an un-sampled individual is included in the phylogeny. Each section of the colored tree
601 represents the genomic evolution happening within a distinct host. Changes of colors on
602 branches correspond to transmission events from one host to another. The transmission
603 events are further indicated by red stars.

604 (B) A consensus transmission tree for the current CA-MRSA outbreak. Each horizontal line
605 represents an isolate. Vertical arrows represent transmission from one host (or environment)
606 to another. The red circles indicate the specimen collection date for each host and
607 environmental source.

608 Table 1. Epidemiological characteristics of patients with and without community-associated methicillin-resistant *Staphylococcus*
609 *aureus* (CA-MRSA) during the outbreak period

Characteristics	Patients with CA-MRSA colonization or infection (n=15)	Patients without CA-MRSA colonization or infection (n=131)[a]	Bivariable analysis		Multivariable analysis [b]	
			Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Week of gestation at birth (mean ± SD)	30.9 ± 4.36	35.2 ± 3.89	0.77 (0.66 – 0.89) [c]	< 0.001		
Birth weight in gm (mean ± SD)	1505 ± 781	2258 ± 884	0.99 (0.98 – 1.00) [c]	< 0.001		
Male sex (%)	5 (33.3%)	76 (58.0%)	0.36 (0.12 – 1.12)	0.068		
Presence of						
Arterial catheterization (%)	7 (46.7%)	19 (14.5%)	5.16 (1.68 – 15.89)	0.002		
Central venous catheter (%)	13 (86.7%)	40 (30.5%)	14.79 (3.19 – 68.60)	<0.001		
Supportive therapies or procedures						
Transfusion of packed cell or plasma (%)	7 (46.7%)	16 (12.2%)	6.29 (2.01 – 19.69)	0.001		
Total parental nutrition (%)	13 (86.7%)	49 (37.4%)	10.88 (2.36 – 50.24)	<.0001		
Mechanical ventilation (%)	4 (26.7%)	15 (11.5%)	2.81 (0.79 – 9.96)	0.097		
Use of broad spectrum antibiotics during hospitalization						
Cephalosporins	15 (100%)	40 (30.5%)	70.03 (4.09 – 1199.22)[d]	0.003	49.84 (3.10 – 801.46)	0.006
Carbapenems	4 (26.7%)	3 (2.3%)	15.52 (3.08 – 78.30)	0.002		
Length of hospitalization, day (mean ± SD)	59.7 ± 31.3	26.3 ± 27.0	1.02 (1.01 – 1.05) [c]	<0.001	1.02 (1.00 – 1.04)	0.013

610 CPAP, Continuous positive airway pressure; [a] Controls were CA-MRSA-negative patients who had stayed in the same ward for 3
611 days of more during the outbreak period; [b] final model was selected by backward selection procedure with the full model including all
612 the variables examined in the bivariable analysis. The Hosmer-Lemeshow test p value = 0.981; [c] calculated by logistic regression; [d]
613 Haldane correction applied to avoid zero error.

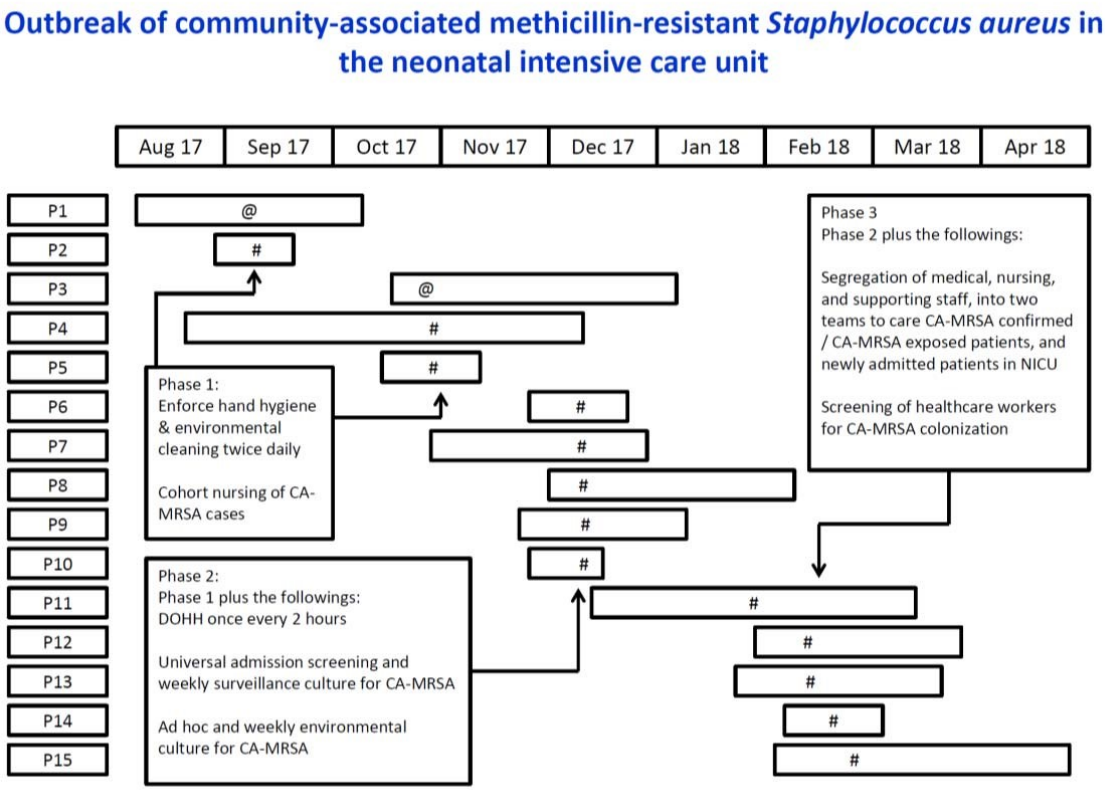
Table 2. Screening of healthcare workers with exposure to neonatal intensive care unit during the outbreak of community-associated methicillin-resistant *Staphylococcus aureus* [a]

Professional category of healthcare workers with exposure to NICU	Number of staff in the category	Number (percentage) of staff receiving voluntary MRSA and CA-MRSA screening
Pediatrician	39	34 (87.2%)
Pediatric surgeon	7	6 (85.7%)
Pediatric ophthalmologist	3	1 (33.3%)
Pediatric radiologist	4	0
Pediatric nurse [b]	141	78 (55.3%)
Physiotherapist	15	7 (46.7%)
Occupational therapist	2	1 (50.0%)
Supporting staff [c]	40	36 (90.0%)
Total	251	163 (64.9%)

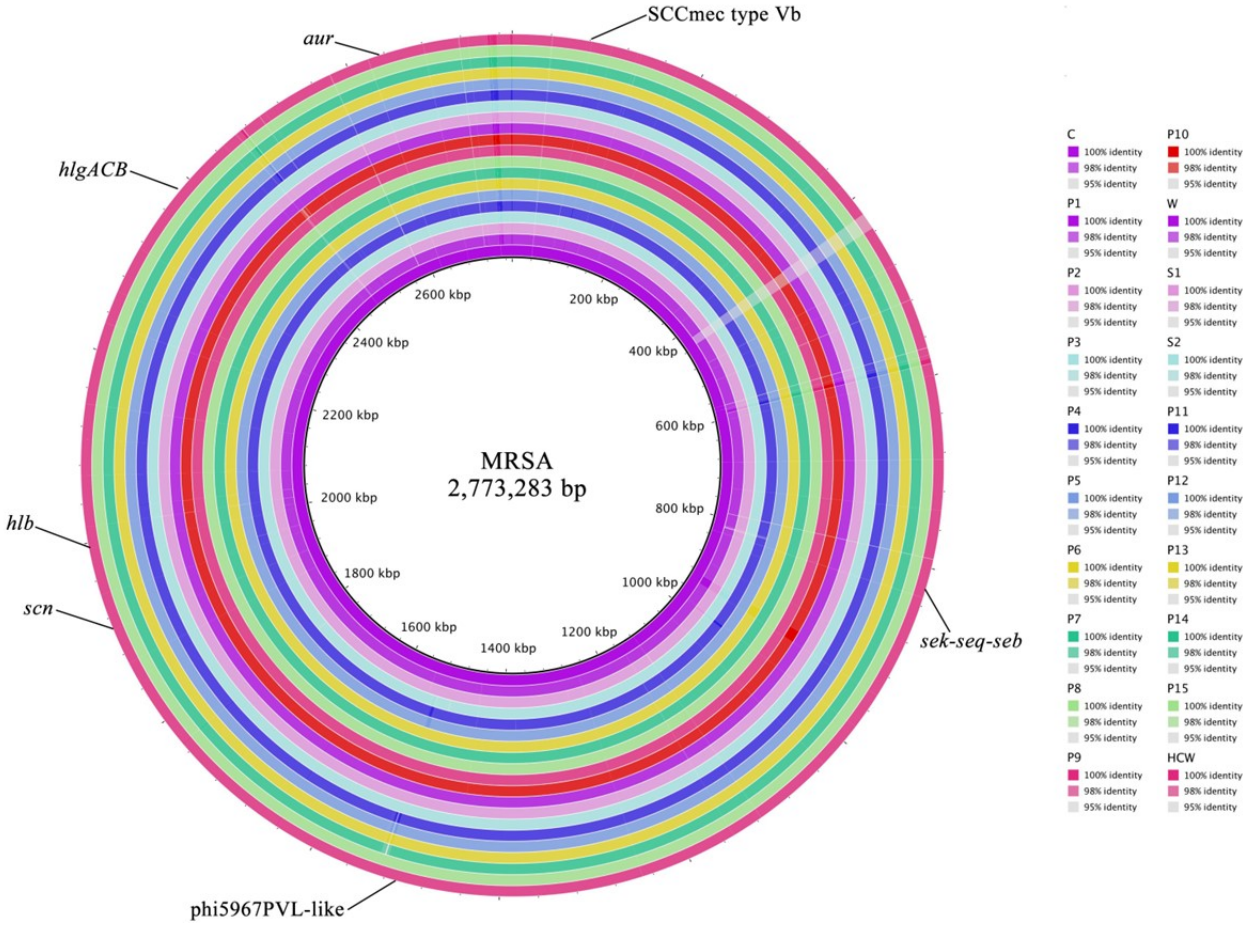
MRSA, methicillin-resistant *Staphylococcus aureus*; CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus*; NICU, neonatal intensive care unit.

[a] Of 163 healthcare workers who opted for screening, 6 (3.7%) were MRSA carrier, where 1 of whom (0.6%) was confirmed to be CA-MRSA. The professional category of MRSA and CA-MRSA-positive healthcare workers was not disclosed for confidentiality; [b] Pediatric nurses were rotating between pediatric intensive care unit and neonatal intensive care unit so both groups of nursing staff were offered voluntary screening; [c] supporting staff including patient care, clerical, and cleansing staff also rotated between the pediatric intensive care unit and neonatal intensive care unit.

Figure 1.


Outbreak of community-associated methicillin-resistant Staphylococcus aureus in the neonatal intensive care unit
Aug 17
Sep 17
Oct 17
Nov 17
Dec 17
Jan 18
Feb 18
Mar 18
Apr 18
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
P15
Phase 1:
Enforce hand hygiene & environmental cleaning twice daily
Cohort nursing of CA-MRSA cases
Phase 2:
Phase 1 plus the followings:
DOHH once every 2 hours
Universal admission screening and weekly surveillance culture for CA-MRSA
Ad hoc and weekly environmental culture for CA-MRSA
Phase 3
Phase 2 plus the followings:
Segregation of medical, nursing, and supporting staff, into two teams to care CA-MRSA confirmed / CA-MRSA exposed patients, and newly admitted patients in NICU
Screening of healthcare workers for CA-MRSA colonization

627 **Figure 2.**



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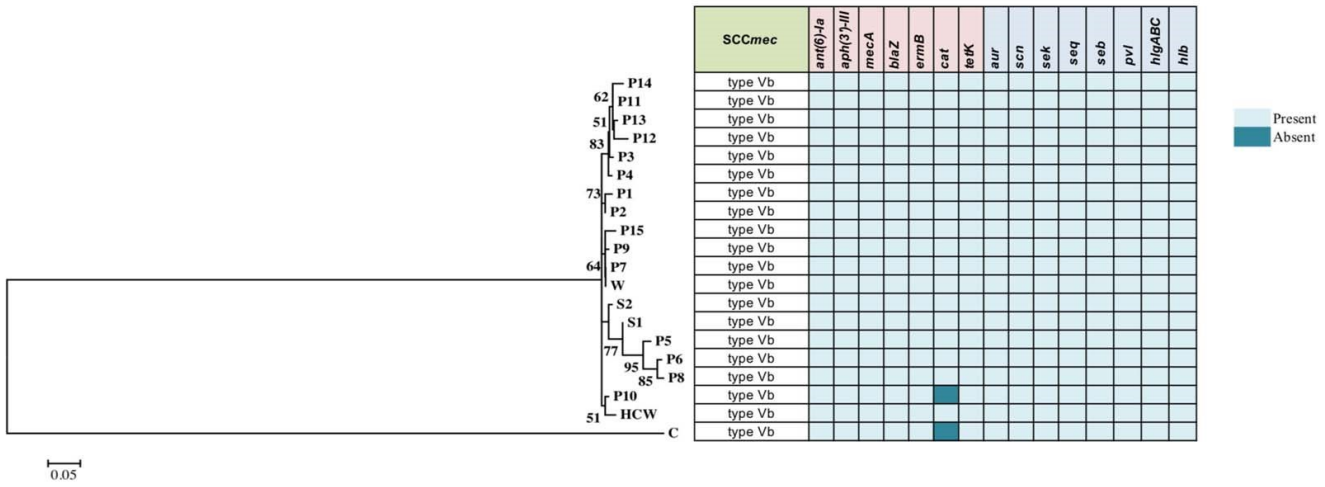
629 **Figure 3.**



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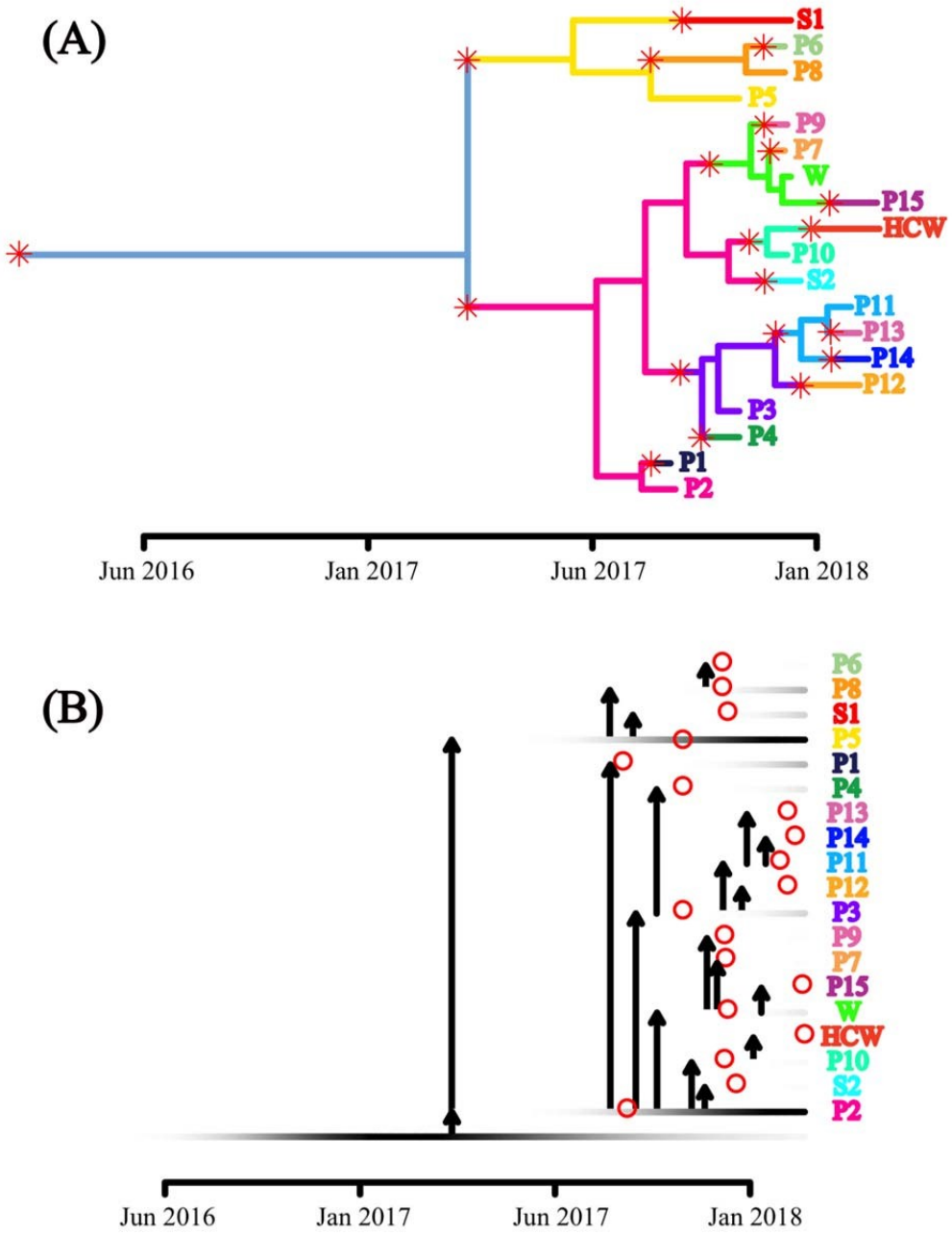
631 **Figure 4.**



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