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Short Report

Increase in incidence of invasive pneumococcal disease caused by serotype 3 in children eight years after the introduction of the pneumococcal conjugate vaccine in Hong Kong

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KEYWORDS

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Abstract

This study used several datasets of reported and serotyped invasive pneumococcal disease (IPD) cases to estimate vaccine and non-vaccine type incidence in Hong Kong children. Incidence was analyzed by four time periods to indicate pre-PCV (period 1, 1995-2004), private market only (period 2, 2006-2009), and following early (period 3, 2010-2014, mixed use of 7-, 10- and 13-valent vaccines) and more than five years (period 4, 2015-2017, 13-valent vaccine only) of routine implementation (since September 2009). IPD incidence decreased by 85% and 35% in aged <2 years and aged 2 to <5 years, respectively, from period 1 to period 4. This was due to a 97% reduction in the serotypes covered by 7-valent vaccine. In period 4, 59% of the disease was caused by serotype 3 and was largely attributed to an *ermB* positive, novel ST6011 clone. The finding corroborates an increasing body of evidence that the efficacy of the 13-valent vaccine against infection by this serotype is low.

Hong Kong is one of the first Asian cities to implement pneumococcal conjugate vaccine (PCV) in the childhood immunization program (CIP). The 7-, 10- and 13-valent pneumococcal conjugate vaccines (PCV7, PCV10 and PCV13) were sequentially introduced. Since September 2009, all children were immunized using a 3-dose primary series at 2, 4 and 6 months of age and a booster dose at age 12-15 months.[1-3] PCV7 was used during September 2009-September 2010, and was replaced by PCV10 from October 2010 to November 2011, and PCV13 from December 2011 onwards.[2,3] A one-off catch-up program was arranged in 2009 for children <2 years of age.[1] Before routine implementation, PCV7 has been available in the local private market since July 2005. PCV10 and PCV13 were marketed in August 2009 and July 2010, respectively.[1] In addition to the seven serotypes included in PCV7 (4, 6B, 9V, 14, 18C, 19F and 23F), PCV10 contains serotypes 1, 5, 7F, and PCV13 contains 1, 3, 5, 6A, 7F and 19A. A survey conducted prior to the addition of PCV to the CIP found that 23% of children aged <5 years had received at least one dose of PCV7 in 2009.[1,2] Usage of PCV10 and PCV13 prior to their implementation in the CIP was very low. The vaccine update among children of the targeted age groups was very high (>97%) following their routine use.

In this study, we described the impact of PCV implementation on the incidence of invasive pneumococcal disease (IPD) among young children. A case of IPD was defined by the isolation (from January 2015 onward, culture and/or PCR detection) of *Streptococcus pneumoniae* in blood and/or other normally sterile sites.[2,4,5] IPD and serotype data from several sources were used.[2,4-6] Firstly, previously published data on IPD incidence before the availability of PCV in 1995-2004 was used as the baseline.[5] The raw data was used to recalculate the age-specific incidence to allow comparison. The incidence attributed to serotypes according to PCV7, PCV13-

nonPCV7 and non-PCV13 groups was predicted by using the serotype information available for the subset of isolates collected in 1995-2001 in the same age groups.[6] Secondly, data for 2006 to 2014 were those collected by a working group which was set up in December 2005 to coordinate a territory-wide surveillance for IPD.[2,4] Clinical laboratories providing service to hospitalized patients were invited to forward pneumococcal isolates recovered from a normally sterile site for centralized laboratory testing.[2] The annual IPD figures were adjusted by the number of participating laboratories and their inpatient service coverage (50% in 2006, 70% in 2007, 90% in 2008-2009 and 100% in 2010-2014).[2] Thirdly, data for 2015-2017 were obtained from the notified database at the Centre for Health Protection, Department of Health, following a mandatory requirement to report all IPD from January 2015 onwards. All the isolates included in this study were checked and only one isolate from each patient was included.

Susceptibility of the isolates was determined by Etest (penicillin) or disc diffusion method (erythromycin) and results interpreted according to the CLSI.[7] The serotypes of the isolates were determined by multiplex PCR (covering 35 serotypes and including all PCV13 serotypes) and the Quellung reaction.[2,3] Multilocus sequence tying (MLST) was performed using the protocol published at https://pubmlst.org/spneumoniae/. Target specific PCR was used to detect erythromycin resistance determinants.[8]

Age-stratified population figures for the study period were obtained from the Census and Statistic Department of the Hong Kong Government. For calculation of the mean annual age-specific rates, the mean annual number of IPD cases was divided by the total population in each age band then expressed as number per 100,000 persons at specified ages per year.[5] Due to the relatively small annual number of cases,

89 the incidence rates were groups into four periods to indicate the burden before
90 availability of PCV (period 1, 1995-2004), availability in the private market (period 2,
91 2006-2009, PCV7 only), and following early (period 3, 2010-2014, mixed use of
92 PCV7, PCV10 and PCV13) and more than 5 years (period 4, 2015-2017, PCV13 only)
93 of implementation in the CIP. Poisson distribution was used to construct the 95%
94 confidence intervals and to compare the incidence rates across different periods
95 (supplementary file, Table S1). A *P* value of <0.05 was considered to indicate
96 statistical significance. A software package (OpenEpi, version 3.01) was used for all
97 statistical analysis.

98 Considering the whole period, the total number of episodes from children aged
99 <2 years and 2 to <5 years were 100 and 219, respectively. The numbers confirmed
100 by culture and PCR were 299 and 20, respectively. The IPD incidence in children
101 aged <5 years decreased by 53% (Figure 1). The reduction was more pronounced in
102 children aged <2 years (85%) than in children aged 2 to <5 years (35%). Stratification
103 revealed that disease caused by PCV7 serotypes decreased by 97% (Figure 2). IPD
104 incidence of non-PCV13 serotypes remained unchanged while that for PCV13-
105 nonPCV7 increased by eight fold from 0.7 to 6.0 per 100,000 persons per year.

106 The increase in PCV13-nonPCV7 disease was largely attributed to an increase
107 in disease caused by serotype 3 (Figure 2). In the whole period, the total number of
108 serotype 3 disease was 68, of which 52 cases were confirmed by cultures and 16 cases
109 were confirmed by PCR. These included one case in period 1, two cases in period 2,
110 23 cases in period 3 and 42 cases in period 4. All cases in period 1 to 3 were
111 confirmed by culture. Of the 42 cases in period 4, 26 cases were confirmed by
112 cultures and 16 cases were confirmed by PCR. When both culture and PCR confirmed
113 cases were considered, serotype 3 caused 59% of the disease in period 4. The

incidence of serotype 3 IPD in aged <5 years for cases confirmed by culture alone and both methods was 3.1 and 5.0 per 100,000 persons per year, respectively. Both incidences were significantly higher than those observed in period 1 to 3 (culture alone, P <0.001 and both methods, P <0.001, respectively). Incidence of IPD caused by serotype 3 among children aged 2 to <5 years was higher than children aged <2 years (period 4, 7.3 versus 1.5 per 100,000 persons per year, respectively, P <0.001). In the collection, other serotypes causing PCV13-nonPCV7 disease included serotype 1, 6A, 7F and 19A. No rising trend was observed for these serotypes.

Susceptibility data was available for 265 isolates. Among all serotypes (Figure 3), rates of penicillin resistance (meningitis breakpoint) had decreased significantly from 47% in period 1 to 14% in period 4 (P < 0.001). Erythromycin resistance rates remained high in all four periods (69%-85%, P = 0.226).

Susceptibility was available for the 52 serotype 3 isolates from the 52 culture-confirmed cases. All except one isolate were penicillin-sensitive at meningitis breakpoint. The only non-susceptible isolate has penicillin MIC of 0.12 μg/ml. Erythromycin resistance rate was 0% in period 1, 0% in period 2, 74% in period 3 and 100% in period 4 (P < 0.01). Of the 52 isolates, 29 isolates could be successfully retrieved for molecular analysis. These included two isolates from period 1 and 2, and 27 isolates from period 3 and 4. The two isolates from period 1 and 2 were of ST180 while the 27 isolates from period 3 and 4 comprised seven different types: ST6011 (n=19), ST180 (n=3), ST1262 (n=1), ST505 (n=1), ST6013 (n=1), ST6014 (n=1) and ST6015 (n=1). All ST6011 serotype 3 isolates were positive for the *ermB* gene.

This study extends our previous observations on the changes in serotypes and antimicrobial susceptibility of invasive pneumococci before and after the introduction of PCV.[6] The results showed that overall IPD declined and was due mainly to the

elimination of PCV7 serotypes. At the same time, there was an increase in serotype 3 disease, especially among older children. Most of the children with serotype 3 disease had necrotizing pneumonia and empyema. As noted in previous reports,[9] we observed that culture is not a sensitive method for confirming serotype 3 disease in lung tissue and pleural fluid. Therefore, the low incidence of serotype 3 disease in period 1 and 2 is limited by the lack of PCR detection. While serotype 3 is covered by PCV13, little to no efficacy on IPD and nasopharyngeal colonization has been observed.[10-12]For this serotype, the serum IgG concentration required for protection was found to be very high comparing to those for the PCV7 serotypes.[10] Following vaccination, such a high concentration is rarely achieved, thus providing an explanation for the poor efficacy in clinical studies.[10,12] The current study suggests that PCV13 may provide some short-term protection against this serotype, thereby the lower incidence of serotype 3 disease in children aged <2 years than older children. Our serotype 3 isolates are macrolide-resistant and mostly belong to a novel, pneumococcal lineage (ST6011). As only about half of the serotype 3 isolates from period 3 and 4 was retrievable for molecular analysis, caution is required in the interpretation of the proportion of serotype 3 disease attributed to this lineage. In the MLST database (last accessed on 25 July 2018), only seven isolates were of this ST. These included six isolates (two serotype 3 from blood cultures and four serotype 15A from carriage) deposited by our group and other investigators in Hong Kong, and one serotype 15B/C carriage isolate from a nearby area in mainland China. In our locality, serotype 15A is an infrequent cause of IPD (<5% in period 4) in children. Nonetheless, it raises the possibility of capsular switching and vaccine escape in this ST6011 lineage. Further genomic investigations of this lineage are being conducted.

In conclusion, this study showed that IPD by previously prevalent PCV7 serotypes have largely been eliminated following the implementation of PCV in children for eight years. A macrolide-resistant, novel clone was mainly responsible for the recent increase in serotype 3 disease among older children. Future vaccines should address the lack of efficacy against serotype 3 disease.

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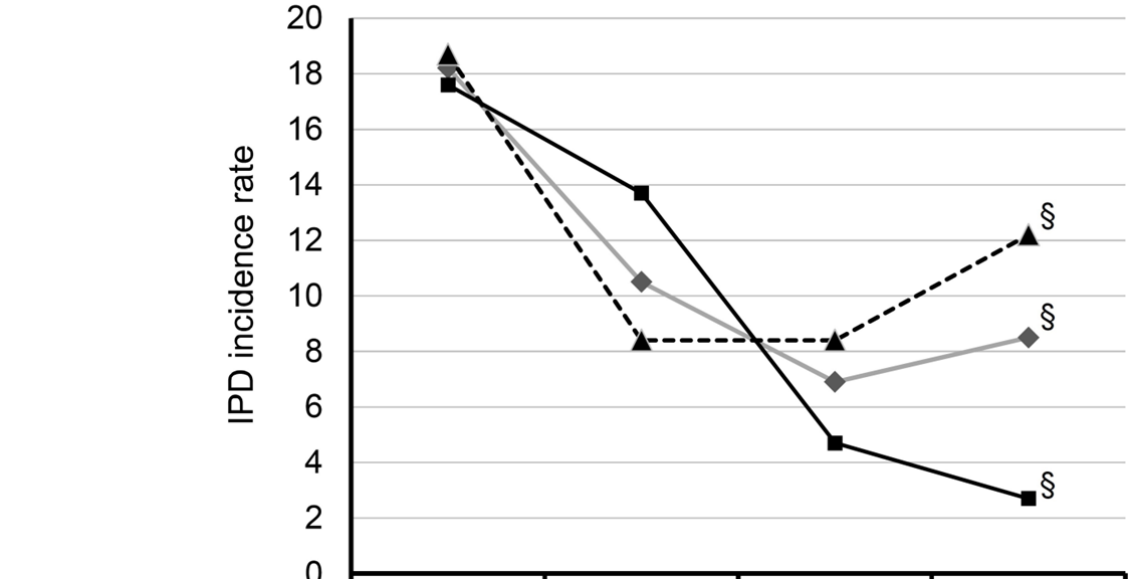
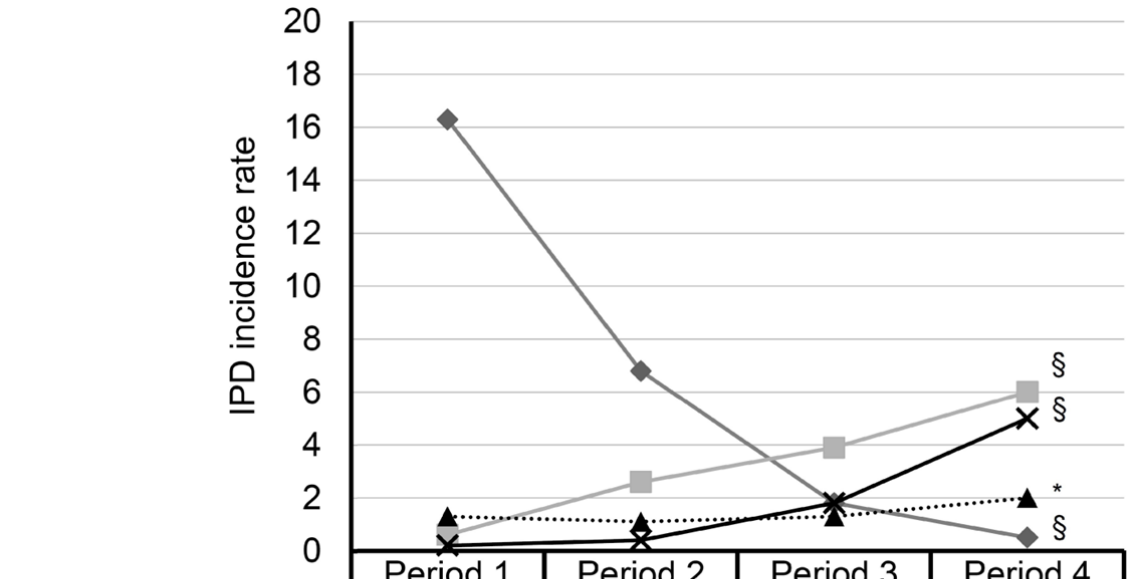
Figure 1. Incidence rate of IPD in Hong Kong children according to age groups. Since September 2009, all children were immunized using a 3-dose primary series at 2, 4 and 6 months of age and a booster dose at age 12-15 months. The incidence rates (as 100,000 per persons per year) were grouped into four periods to indicate the burden before availability of PCV (period 1, 1995-2004), availability in the private market (period 2, 2006-2009), and following early (period 3, 2010-2014) and more than 5 years (period 4, 2015-2017) of implementation in the childhood immunization program. Differences in the rates in the time periods were assessed by chi-square for trend. §P <0.001



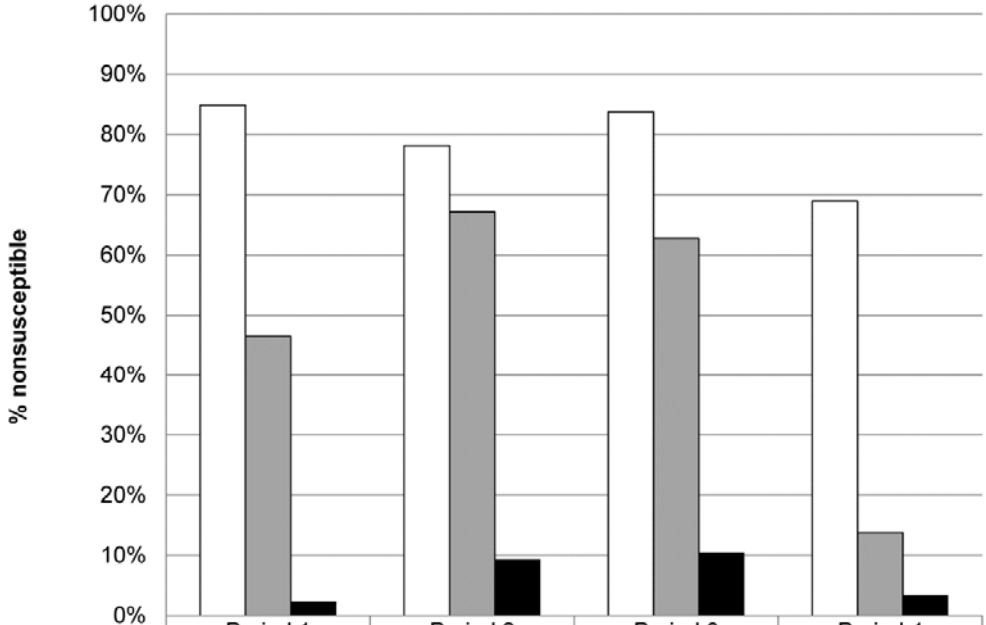
	Period 1	Period 2	Period 3	Period 4
Total	18.2	10.5	6.9	8.5
<2 years	17.6	13.7	4.7	2.7
2-4 years	18.7	8.4	8.4	12.2

Figure 2. Incidence rate of IPD in Hong Kong children according to serotype groups. Since September 2009, all children were immunized using a 3-dose primary series at 2, 4 and 6 months of age and a booster dose at age 12-15 months. The incidence rates (as 100,000 per persons per year) were grouped into four periods to indicate the burden before availability of PCV (period 1, 1995-2004), availability in the private market (period 2, 2006-2009), and following early (period 3, 2010-2014) and more than 5 years (period 4, 2015-2017) of implementation in the childhood immunization program. Differences in the rates in the time periods were assessed by chi-square for trend. §P <0.001, *P =0.369



	Period 1	Period 2	Period 3	Period 4
PCV7	16.3	6.8	1.8	0.5
PCV13-nonPCV7	0.6	2.6	3.9	6.0
Non-PCV13	1.3	1.1	1.3	2.0
Serotype 3	0.2	0.4	1.8	5.0

Figure 3. Antimicrobial resistance rates for IPD isolates in different time periods in Hong Kong. Since September 2009, all children were immunized using a 3-dose primary series at 2, 4 and 6 months of age and a booster dose at age 12-15 months. The time periods indicate a time before availability of PCV (period 1, 1995-2004), availability in the private market only (period 2, 2006-2009), and following early (period 3, 2010-2014) and more than 5 years (period 4, 2015-2017) of implementation in the childhood immunization program.



	Period 1	Period 2	Period 3	Period 4
Erythromycin	84.9%	78.1%	83.7%	69.0%
Penicillin (meningitis)	46.5%	67.2%	62.8%	13.8%
Penicillin (nonmeningitis)	2.3%	9.4%	10.5%	3.4%

References

1. Ho P L, Chiu SS, Chan MY, Ang I, Chow KH, and Lau YL. Changes in nasopharyngeal carriage and serotype distribution of antibiotic-resistant *Streptococcus pneumoniae* before and after the introduction of 7-valent pneumococcal conjugate vaccine in Hong Kong. Diagn Microbiol Infect Dis 2011;71(4):327-334.
2. Ho P L, Chiu SS, Ang I, and Lau YL. Serotypes and antimicrobial susceptibilities of invasive *Streptococcus pneumoniae* before and after introduction of 7-valent pneumococcal conjugate vaccine, Hong Kong, 1995-2009. Vaccine 2011;29(17):3270-3275.
3. Ho P L, Chiu SS, Law PY, Chan EL, Lai EL, and Chow KH. Increase in the nasopharyngeal carriage of non-vaccine serogroup 15 *Streptococcus pneumoniae* after introduction of children pneumococcal conjugate vaccination in Hong Kong. Diagn Microbiol Infect Dis 2015;81(2):145-148.
4. Ho P L, Que TL, Ng TK, Chiu SS, Yung RW, and Tsang KW. Clinical outcomes of bacteremic pneumococcal infections in an area with high resistance. Eur J Clin Microbiol Infect Dis 2006;25(5):323-327.
5. Ho P L, Chiu SS, Cheung CH, Lee R, Tsai TF, and Lau YL. Invasive pneumococcal disease burden in Hong Kong children. Pediatr Infect Dis J 2006;25(5):454-455.
6. Ho P L, Lam KF, Chow FK, Lau YL, Wong SS, Cheng SL, and Chiu SS. Serotype distribution and antimicrobial resistance patterns of nasopharyngeal and invasive *Streptococcus pneumoniae* isolates in Hong Kong children. Vaccine 2004;22(25-26):3334-3339.
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 28th ed. CLSI M100. CLSI, Wayne, PA 2018.
8. Ho P L, Ang I, Chow KH, Lai EL, and Chiu SS. The prevalence and characteristics of *Streptococcus pneumoniae* isolates expressing serotypes 6C and 6D in Hong Kong

prior to the introduction of the 7-valent pneumococcal conjugate vaccine. Diagn Microbiol Infect Dis 2010;68(4):439-444.

9. Selva L, Ciruela P, Esteva C, de Sevilla MF, Codina G, Hernandez S, Moraga F, Garcia-Garcia JJ, Planes A et al. Serotype 3 is a common serotype causing invasive pneumococcal disease in children less than 5 years old, as identified by real-time PCR. Eur J Clin Microbiol Infect Dis 2012;31(7):1487-1495.
10. Andrews N J, Waight PA, Burbidge P, Pearce E, Roalfe L, Zancolli M, Slack M, Ladhani SN, Miller E et al. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. Lancet Infect Dis 2014;14(9):839-846.
11. Dagan R, Patterson S, Juergens C, Greenberg D, Givon-Lavi N, Porat N, Gurtman A, Gruber WC, and Scott DA. Comparative immunogenicity and efficacy of 13-valent and 7-valent pneumococcal conjugate vaccines in reducing nasopharyngeal colonization: a randomized double-blind trial. Clin Infect Dis 2013;57(7):952-962.
12. Vila-Corcoles A and Ochoa-Gondar O. Pneumococcal conjugate vaccination: correlates of protection. Lancet Infect Dis 2014;14(9):784-786.
13. Lo S W, Gladstone RA, van Tonder AJ, Hawkins PA, Kwambana-Adams B, Cornick JE, Madhi SA, Nzenze SA, du PM et al. Global Distribution of Invasive Serotype 35D Streptococcus pneumoniae Isolates following Introduction of 13-Valent Pneumococcal Conjugate Vaccine. J Clin Microbiol 2018;56(7):e00228-18.