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Changes in nasopharyngeal carriage and serotype distribution of antibiotic-resistant Streptococcus pneumoniae before and after introduction of 7-valent pneumococcal conjugate vaccine, Hong Kong

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Abstract

This study assessed the changes in serotype distribution and antibiotic resistance of Streptococcus pneumoniae isolates in children before and after introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in Hong Kong. Nasopharyngeal specimens were collected from 1978 and 2221 children (ages, 2 to 6 years) attending day care centers or kindergartens in period 1 (1999-2000) and period 2 (2009-2010), respectively. Carriage of PCV7 serotypes decreased from 12.8% to 8.6% (P<0.01). The relative contribution of PCV7 serotypes 14 and 18C had decreased while that for non-PCV7 serotypes 19A, 6A, 6C, 23A and 15B had increased. In period 2, PCV7 penetration rate (at least one dose) for children aged 2, 3, 4 and 5 years were 43%, 35.7%, 26.7% and 20.4%, respectively. In multivariate analysis, PCV7 use was the only independent variable associated with fewer PCV7 serotypes carriage (OR 0.5, *P*=0.001). In period 2, high rates of dual penicillin/erythromycin nonsusceptibility were found in serotypes 6B (77.3%), 14 (100%), 19F (100%), 23F (78%), 19A (75%), 6A (87.8%), 6C (59.3%) and 23A (78.9%).

169 words

1. **Introduction**

Streptococcus pneumoniae is a major cause of childhood infections (Ho et al., 2006b; Ho et al., 2006a; Ho et al., 2007). In western countries, the introduction of the 7-valent pneumococcal conjugate vaccine (PCV-7) has led to drastic decline in the incidence of invasive pneumococcal disease (IPD) caused by serotypes included in the vaccine (Dagan, 2009a; Pilishvili et al., 2010). The vaccine also protects against carriage of those serotypes in the vaccinated children (Hanquet et al., 2010; Huang et al., 2009). Consequently, there is reduced transmission of these serotypes by vaccinated children; resulting an extension of protection to unvaccinated population (i.e. herd immunity) (Centers for Disease Control and Prevention, 2005). However, these benefits have been partly offset by an increase in the incidence of diseases caused by serotypes not included in the vaccine; the magnitude of such replacement remained low relative to the reduction in PCV7 diseases (Pilishvili et al., 2010). In February 2010, the Food and Drug Administration in the United States approved a 13-valent pneumococcal vaccine (PCV13) which provides coverage for the seven serotypes common to PCV7 and six additional serotypes (1, 3, 5, 6A, 7F and 19A) (Centers for Disease Control and Prevention, 2010b). In the United States, PCV13 has been recommended to replace PCV7 in the childhood immunization program from February 2010 onward (Centers for Disease Control and Prevention, 2010b). The policy change is expected to further reduce IPD in the United States (Centers for Disease Control and Prevention, 2010a).

In Hong Kong, PCV7 has been available in the market since October 2005. The 10-valent pneumococcal conjugate vaccine (PCV10) and PCV13 were later marketed in August 2009 and May 2010, respectively (Ho et al., 2011). In Asia, Hong Kong is one of the first cities to introduce PCV7 into the childhood immunization program and this was implemented since September 2009 (Centre for Health Protection, 2011a). The immunization schedule consists of a standard three-dose primary series (at 2, 4 and 6 months of age) and a booster

dose at 12-15 months. Before its introduction into the childhood immunization program, PCV7 was available as a self-financed item and its uptake in the vaccine target population had been low (Ho et al., 2011). In our locality, the effect of the introduction of PCV7 to the market and before its full implementation through the childhood immunization program upon IPD was recently reported by our group (Ho et al., 2011). In children <5 years, our data showed that the proportion of PCV7 serotypes had declined from 89.5% in 1995-2001 to 65.7% in 2007-2009 (Ho et al., 2011). The current study was conducted to assess the changes in serotype distribution and antibiotic resistance of nasopharyngeal isolates prior to and during the early introduction of PCV7 in Hong Kong.

2. Materials and method

2.1 Study design and data collection

Nasopharyngeal specimens were collected from children between 2 and 6 years of age who attend day care centers (DCC) or kindergartens (KG) in Hong Kong during September 2009 to April 2010 (period 2). A similarly designed study was conducted during December 1999 and June 2000 (period 1) (Chiu et al., 2001). The same recruitment process and sampling strategy was used in this and the previous study (Chiu et al., 2001). In brief, Hong Kong is divided into 18 school districts, and the sample size of each district was calculated according to the number of DCC and KG places in each district. Ages of children who attend DCC and KG range from 2 to 6 years and 3 to 6 years, respectively. Normally, all children attend 5 days per week for 7 to 9 hours a day in DCC and for 3 to 4 hours a day in a KG. Out of the total of 147,516 DCC and KG places, DCC accounted for 30% while KG accounted for 70%. This ratio was used to calculate the number of children to be recruited from DCC and KG from each district to make the targeted study population of about 2000. Parental consent

was obtained by the research staff. The protocol is approved by the Institutional Review Board at the Hong Kong West Cluster/University of Hong Kong.

As in the previous study (Chiu et al., 2001), a standardized questionnaire was used to obtain the following information from parents of participating children: household size, overcrowding (≤5.5 m/person in accordance with the guideline of the Hong Kong Housing Authority), number and age of siblings, participant demographics, medical history, presence of symptoms of upper respiratory tract infection (URTI) symptoms at the time of sampling, recent use of antibiotics (past 3 months), vaccination history, physician visits (past 3 months) and prior hospitalization (past 1 year).

2.2 Specimen collection and microbiological methods

A calcium alginate-tipped swab on a flexible aluminum wire (TRANSWAB per nasal; Medical Wire and Equipment Co. Ltd, Corsham, Wilts, England) was used. The specimens were brought back to the laboratory immediately for processing. For selective isolation of S. pneumoniae, swabs were inoculated onto 5% horse blood agar supplemented with gentamicin (2 μg/ml) and incubated in 5% CO2 for 16 to 24 hours. All isolates were identified by colony morphology, Gram stain, optochin susceptibility, and bile solubility. Isolates with atypical optocin/bile solubility test results were confirmed by a slide co-agglutination test (Phadebact Pneumococcus Test, Remel).

Susceptibility of the isolates to antibiotics was determined by Etest (AB Biodisk, Solna, Sweden) or the disc diffusion method. The D-test was used to detect inducible resistance to clindamycin. Quality control strains were included on each day of testing. All results were interpreted according to the Clinical Laboratory Standards Institute (Clincal and Laboratory Standard Institute,). Pneumococcal isolates were serotyped by PCR assays and/or the quellung reactions. The isolates were initially tested by a sequential multiplex PCR

approach. Strains that could not be serotyped by the PCR assays and those that required further testing to the serotype level were then tested by the quellung method with sera of various reactivities from the Statens Seruminstitut (Copenhagen, Denmark) (Ho et al., 2004; Pai et al., 2006). The serotypes distribution for the isolates collected in period 1 has been previously reported but the method do not allow some subtypes to be distinguished (Ho et al., 2004). Hence, all the isolates (n=163) that could not be identified the serotype level and those that were designated an untypeable at that time were retrieved and tested again by PCR methods (Pai et al., 2006). PCR assays were used to identify the newly described 6C and 6D serotypes (Ho et al., 2010). All serogroup 6 isolates, including those collected in period 1 were tested by PCR assays that could distinguish the 4 subtypes (6A-6D) of serogroup 6 isolates. All isolates were tested in a uniform manner.

2.3 Statistical analysis

Data from the two similarly designed studies was pooled and compared. The sampling periods, 1999-2000 (period 1) and 2009-2010 (period 2), were intended to indicate time windows before and after the registration and marketing of PCV7 in Hong Kong, respectively. The serotype coverage for the 7-valent, 10-valent and 13-valent PCVs for carriage was calculated as the proportion of all isolates included in the vaccine formulations, without taking into account the potential serogroup cross-protection. In view of the demonstrated efficacy of PCV7 for serotype 6A, additional coverage due to this cross protection was also calculated. The Chi square or Student's *t* test was used to compare participant demographics, pneumococcal carriage rates and serotype frequencies between the two collection periods. Potential risk factors for carriage of PCV7 were studied by univariate analysis. Variables that were significant in the univariate analysis were further analyzed by logistic regression using the forward-conditional method. The correlation between PCV7 penetration and reduction in

carriage of PCV7 serotypes by age groups was analyzed by the Correlate Bivariate analysis. A P value of <0.05 was considered to indicate statistical significance. All statistic analysis was performed by the SPSS statistic package.

3. Results

3.1 Demographics

There were 1978 and 2211 child participants in period 1 and period 2, respectively. As shown in Table 1, there are some differences in the characteristics of the children recruited in the two periods. The average age for period 2 children was significantly younger than that for period 1 children. Four variables were less common among period 2 children: recent antibiotic use, overcrowding, URTI at time of sampling and physician visits. However, about twice as many children had history of prior hospitalization in period 2 than in period 1. The two groups of children had similar household size and number of siblings.

As PCV7 has only been available in Hong Kong after October 2005, no children had been vaccinated with PCV7 in period 1. Among children recruited in period 2, the PCV7 vaccine penetration (at least one dose) was 28.1% (622/2211). PCV7 vaccination was significantly more likely in younger children than in older children. The age-stratified PCV7 penetration rate was 20.4% (149/731) among children aged 5 years, 26.7% (203/759) among children aged 4 years, 35.7% (196/549) among children aged 3 years and 43% (74/172) among children aged 2 years (P <0.001, chi square for trend). The age at first vaccination could be obtained from 366 children. Among these children, 20.5% (75/366) were vaccinated at age ≤1 year, 49.7% (182/366) at age 2 years and 29.8% (109/366) at age 3-5 years. At the time of sampling, 80.7% (502/622) of the vaccinated children could be considered age appropriately vaccinated for PCV7. In period 2, 26.1% (578/2211) children had received

influenza vaccination in past year. Information about influenza vaccination was not obtained for period 1.

3.2 Serotype distribution

A total of 730 isolates were included in the study, comprising 383 isolates collected in period 1 and 347 isolates in period 2. PCV7 serotypes included 443 isolates, PCV13-nonPCV7 serotypes included 90 isolates and non-PCV13 serotypes included 207 isolates. Table 2 showed the serotype distribution of the isolates according to these PCV categories for the two collection periods. In both collection periods, the three most prevalent serotypes were 6B, 19F and 23F. The prevalence of seven serotypes had significantly changed during the two time periods. The two serotypes that had become less common in period 2 were 14 (8.9% vs. 2.6%, $P<0.01$) and 18C (2.1% vs. 0.6%, $P=0.049$). The five serotypes that had significantly increased in prevalence were 19A (0% vs. 2.3%, P<0.001), 6A (3.4% vs. 11.8%, $P<0.001$), 6C (1.8% vs. 7.8%, $P<0.001$), 23A (1.6% vs. 5.5%, $P=0.003$) and 15B (0.3% vs. 2.6%, $P=0.007$).

3.3 Changes in pneumococcal conjugate vaccine coverage over time

Table 2 also showed that the serotype coverage of PCV7 had significantly decreased from 66.1% in period 1 to 54.8% in period 2 ($P = 0.001$). The reduction in the serotype coverage of PCV7 was greater among isolates obtained from younger children. The serotype coverage of PCV7 in period 1/period 2 for children of different ages was as follows: 2 year (94.1%/41.3%), 3 years (69.5%/56.2%), 4 years (69.1%/57.4%) and 5 years (59.8%/54%), respectively. Due to the absence of serotypes 1, 5 and 7F, serotype coverage of PCV7 and PCV10 was the same. The serotype coverage of PCV13 was similar in period 1 and period 2, being 72.6% and 70.6% for all ages, respectively. In period 1, the difference in serotype

coverage of PCV7 (66.1%) and PCV13 (72.6%) approached but did not reach statistical significance (P=0.05). In period 2, serotype coverage of PCV13 was 15.9% higher than that of PCV7 (P<0.001, 95% confidence interval for difference, 8.7 – 22.8%). However, if complete cross protection against 6A was assumed, the difference in serotype coverage of PCV13 and PCV7 became insignificant (4%, 95% CI -2.9% to 10.9%).

3.4 Changes in pneumococcal carriage over time

Overall, the nasopharyngeal carriage rate in period 2 was 15.7% (347/2221) which was lower than that in period 1 (19.4%, 383/1978, P < 0.01). However, the carriage rate of penicillin-nonsusceptibile (Pen-NS) *S. pneumoniae* in the two periods remained unchanged (11.3% vs. 11.1%, P = 0.8). Table 3 showed that the carriage rate of PCV7 isolates had significantly decreased from 12.8% (253/1978) in period 1 to 8.6% (190/2211) in period 2 (P <0.001). The decline in PCV7 carriage was most remarkable among children aged 2 years (from 27.1% to 7.0%) and 3 years (from 22.7% to 9.1%). The changes among children aged 4 years and 5 years were only modest. After stratification by age, the magnitude of reduction in PCV7 carriage was found to correlate positively with the PCV7 penetration rate (correlate bivariate analysis, Pearson correlation = 0.99, P = 0.01). The carriage rate for PCV13-nonPCV10 types showed a nearly 100% increase from 1.3% (25/1978) to 2.5% (55/2211) (P < 0.001). Increases in PCV13-nonPCV10 serotypes were greatest in children aged 2 years but the difference was not statistically significant. The prevalence of non-PCV13 carriage remain unchanged over the two time periods (5.3% [105/1978] vs. 4.6% [102/2211], P = 0.5).

To further understand how availability of PCV7 might have affected pneumococcal carriage, the data for children enrolled in period 2 was subjected to univariate and multivariate analyses. Univariate analysis showed that overcrowding had positive correlation with PCV7 carriage (odds ratio [OR] 1.8, 95% CI 1.03-3.2; P=0.04) while a history of PCV7

vaccination had negative correlation (OR 0.5, 95% CI 0.3-0.8; *P*=0.001). The PCV7 carriage rate in children who were age appropriately vaccinated for PCV7 was significantly lower than those who were not (4.8% vs. 9.7%, respectively, $P < 0.001$). No statistically significant association between PCV7 carriage and the other child characteristics (recent antibiotic use, prior hospitalization, respiratory symptoms at time of sampling, having young siblings aged ≤5 years and recent physician visit) were found. In the logistic regression, a history of PCV7 vaccination was the only variable significantly associated with a lower chance of PCV7 carriage (OR 0.5, 95% CI 0.3-0.7; *P*=0.001). On the other hand, the carriage rates for PCV13-nonPCV10 (2.2% vs. 2.6%, $P = 0.6$, respectively) and non-PCV13 (5.2% vs. 4.4%, $P = 0.5$, respectively) isolates were similar among children who were and were not age appropriately vaccinated for PCV7.

In period 1, a history of recent use of antibiotics was significantly associated with carriage of penicillin-nonsusceptible *S. pneumoniae* (Odds ratio [OR] 1.9, 95% confidence interval [CI], 1.2-2.9, *P*=0.003) but not with erythromycin-nonsuceptible *S. pneumoniae* (*P*=0.2). In period 2, on the contrary, recent use of antimicrobial agents was significantly associated erythromycin-nonsuceptible *S. pneumoniae* (OR 4.7, 95% CI, 1.7-14.0) but not with penicillin-nonsusceptible *S. pneumoniae* (*P*=0.2).

3.5 Changes in antimicrobial susceptibility over time

Table 4 summarized the serotype-specific antimicrobial susceptibility rates of the isolates for the two collection periods. Overall, it showed that the rates of penicillin nonsusceptibility (meningitis breakpoint, 58.2% vs. 70.6%, for period 1 vs. period 2, respectively, *P*<0.001), erythromycin nonsusceptibility (77.0% vs. 86.7%, for period 1 vs. period 2, respectively, *P*<0.001), and dual penicillin/erythromycin nonsusceptibility (52.5% to 70.3%, for period 1 vs. period 2, respectively, *P*<0.001) had significantly increased over

time. There was no significant change in nonsusceptibility rate for levofloxacin (0% vs. 0.3%) over time. The capsular serotype of the only isolate with resistance to levofloxacin (MIC 16 μg/ml) was 19F.

Stratification by serotype showed that the penicillin and dual penicillin/erythromycin nonsusceptibility rates for serotypes 19F, 14, 6A and 23A had significantly increased over the two time periods. The changes for serotype 6A were most dramatic. The erythromycin nonsusceptibility rates for these serotypes were similar in the two time periods. In period 1, five serotypes (6B, 19F, 23F, 14 and 6C) were often nonsusceptible to both penicillin and erythromycin with rates of 42.9% to 86.5%. In period 2, these serotypes continued to exhibit high rates of dual penicillin/erythromycin nonsusceptibility (59.3% to 100%). Besides these, an additional three serotypes (19A, 6A and 23A) were found to exhibit high rates of dual penicillin/erythromycin nonsusceptibility (75% to 87.8%). No serotype 19A was found in period 1. Six (75%) of the eight serotype 19A isolates found in period 2 were nonsusceptible to both penicillin and erythromycin.

PCV7 covered significantly fewer dual penicillin/erythromycin-nonsusceptible isolates collected in period 2 than in period 1 (66% vs. 93.5%, respectively, P<0.001). Although PCV13 covered more isolates, the serotype coverage for dual penicillin/erythromycin-nonsusceptible isolates in period 2 was still lower than that for period 1 (83.1% vs. 94.0%, respectively, P<0.001).

Figure 1 showed a scatter plot of the serotype-specific prevalence with the penicillin-nonsusceptibility rates. Curve fitting and linear regression revealed that the two variables had significant associations for both time periods. The correlation coefficient was higher in period 1 (R-square = 0.74, P<0.001) than in period 2 (R-square = 0.43, P<0.001). Weaker but still statistically significant correlations were obtained for erythromycin nonsusceptibility. The

finding showed that antimicrobial resistance could be an important determinant of the serotype prevalence.

4. Discussion

This study assessed the changes in incidence of pneumococcal carriage, antimicrobial resistance rates and serotype distribution of nasopharyngeal isolates prior to and during early introduction of PCV7 in Hong Kong. The carriage of PCV7 serotypes was found to have declined significantly, especially among younger children in whom the vaccine penetration is higher. This was partially off-set by a slight increase in the non-PCV7 serotypes. The net effect was that children were less likely to carry pneumococci in the latter period. This occurred at a time when the vaccine update was still low (20.4% to 43%) among children aged 2 to 5 years. Consequently, fewer isolates recovered in period 2 were included in the PCV7 formulation than in period 1. These observations were in agreement with experiences in places where PCV7 was made readily available earlier (Dagan, 2009b; Huang et al., 2009; Sa-Leao et al., 2009). However, not all the seven PCV7 serotypes had decreased and overall carriage of types included in PCV7 was still high. The types that had declined significantly were serotype 14 and 18C. This may be explained by variations in the vaccine efficacy for the different serotypes (Whitney et al., 2006). In the Kaiser PCV7 efficacy study, the geometric mean concentration of pneumococcal antibodies was found to be highest for serotype 14, followed by serotype 18C and 6B (Black et al., 2000). The antibody level concentrations for the remaining serotypes were similar (Black et al., 2000). Since the vaccine uptake was low, it might take longer for the other serotypes to decrease. While conjugate vaccines confer protection against carriage of types included in the vaccines and occasionally other types in the same serogroups, they provide no protection against serogroups that are not included in the vaccine. Therefore, conjugate vaccine use might be

expected to contribute to the observed increase in non-PCV7 types (19A, 6A, 6C, 23A and 15B). Nonetheless, we were unable to demonstrate any association between PCV7 use and carriage of these serotypes.

In our locality, similar temporal changes have also been demonstrated among isolates causing invasive disease in children (Ho et al., 2011). Among children aged <5 years, the frequencies of serotype 14 and 18C had decreased from 36% to 15.7% and from 4.7% to 0%, respectively, before (1995-2001) and shortly after (2005-2009) introduction of PCV7 to the Hong Kong market (Ho et al., 2011). Over the same time periods, invasive disease caused by serotype 19A had increased from 0% to 12.9% and serotype 6A increased from 2.3% to 7.1% (Ho et al., 2011). Despite marked increases in prevalence of serotypes 6C, 15B and 23A among isolates causing nasopharyngeal colonization, such serotypes were rarely found among our invasive isolates. Although almost all children become colonized with pneumococci during the first few years of life, only a small faction of the colonization proceed to invasive disease. In pneumococci, the capsular polysaccharide is assumed to be the most important factor that determine the capacity of specific strains to cause invasive diseases (Melin et al., 2010). According to one report, the invasive capacity of different pneumococcal serotypes was found to vary by as many as 50 folds (highest for 3, 7F, 18C, 19A, 22F and 33F; while 6C, 15B and 23A were among the serotypes with the lowest invasive capacity) (Yildirim et al., 2010). This could explain why increasing carriage of serotypes 6C, 15B and 23A was not associated with corresponding changes in invasive disease.

Besides pneumococcal conjugate vaccine use, excessive antibiotic use is another well-established factor exerting pressure on pneumococcal ecology; with incorrect usage leading to an increase in multidrug-resistant strains population. In Hong Kong, antimicrobials (such as oral amoxicillin-clavulanate, cephalosporins and macrolides) are frequently prescribed to

children with febrile illnesses (Lam et al., 2009). Although the frequencies of recent antibiotic uses in period 2 were slightly lower than those in period 1 (Chiu et al., 2001), the 30.6% among participating children and 27% among their household members still constituted substantial consumption. Such high antibiotic uses partly reflect widespread misconceptions about antibiotics among the laypersons in our community (Centre for Health Protection, 2011b). In a territory-wide telephone survey conducted in November 2010, 66.9% of 1569 respondents believed that antibiotics are effective in treating viral infections (Centre for Health Protection, 2011b). Thus, the observed association between penicillin nonsusceptibility and serotype prevalence is not surprising. Heavy antibiotic use could exert pressure through selection of resistance in previously minor serotypes and subsequent clonal expansion (Huang et al., 2009). Alternatively, pre-existing resistant clones could undergo capsular switching and disseminate as non-vaccine serotypes (Ansaldi et al., 2011; Moore et al., 2008). Our finding that a history of recent antibiotic use was associated with different types of antibiotic-resistant *S. pneumoniae* in the two time periods corroborated previous studies that the relationship between exposure to antibiotics and the spread of resistant pneumococci is complex (Huang et al., 2009). Since details of the recent antibiotic use were not collected, we were unable to conduct analysis according to antibiotic class, duration of use and dosage. Unless antibiotic usage are curtailed, the overall antimicrobial resistance among pneumococci would remain high (Dagan, 2009b; Sa-Leao et al., 2009).

The observed increases in prevalence of non-PCV7 serotypes such as 19A, 6C, 23A and 15B were broadly in line with secular trends in the developed countries following the introduction of PCV7 (Hanage et al., 2005; Huang et al., 2009). In Asian countries, emergence of the non-PCV7 serotype 19A has been associated with expansion of multidrug-resistant ST320 isolates and could be unrelated to PCV7 use (Choi et al., 2008; Ho et al., 2011; Shin et al., 2011). Nonetheless, marked variations in the prevalence of serotype 19A

were observed. According to a multi-national surveillance (Shin et al., 2011), the prevalence of serotype 19A among clinical isolates in 2008-2009 was high in India (13.0%), Japan (11.1%), South Korea (9.9%), Malaysia (9.1%), and Saudi Arabia (9.0%), but low in Hong Kong (3.1%), Taiwan (4.8%), Thailand (5.2%), the Philippines (1.7%), and Vietnam (0.4%). It is recognized that antibody induced by the 6B component in PCV7 may provide cross protection against the vaccine-related serotype 6A (Whitney et al., 2006). However, we found that the prevalence of serotype 6A among nasopharyngeal isolates has increased and were associated with emergence of dual penicillin/erythromycin nonsusceptibility in this serotype. We have excluded potential confounding with other highly similarly subtypes by using methods that could differentiate 6A from the other closely related 6C and 6D types to analyze all the isolates. Since the strains were antibiotic-resistant, the finding may be partly explainable by selection pressure exerted by heavy antibiotic usage. In southern Israel, increases in multidrug-resistant serotype 6A among Bedouin children have been associated with expansion of ST457 prior to the introduction of PCV7 (Porat et al., 2010). Future studies to investigate the bacterial population structure underlying the observed increases in the five serotypes (6A, 6C, 19A, 23A and 15B) are warranted.

In conclusion, this study showed that the proportion of several serotypes among pneumococci carried by children in Hong Kong have changed following the availability of PCV7 and to a lesser extent, PCV10 as self-financed items. Although PCV10 was also available in the market, no children enrolled in period 2 reported prior PCV10 immunization indicating its usage at that time was very low. Despite some favorable reduction in the vaccine-related serotypes, there was little change in the carriage of antimicrobial-resistant pneumococci and the rates of penicillin and erythromycin resistance among the carried isolates have even increased. The change in vaccine coverage over time and the observed increased in serotype 6A and 19A in both invasive and nasopharyngeal isolates indicate that

PCV13 could provide substantial benefits over PCV7 (Ho et al., 2011). Hence, Hong Kong has announced to switch to PCV13 in the childhood immunization program in the last quarter of 2011. Such change in vaccine policy is expected to impact upon the future evolution of pneumococcal serotypes in this locality. Future studies should monitor how the emerging serotypes will evolve and attention should be paid to non-PCV13 serotypes with higher invasive capacity. Our finding also calls for greater attention to judicial use of antibiotics (Lam et al., 2009).

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References

1. Ansaldi F, Canepa P, de Florentiis D, Bandettini R, Durando P, Icardi G (2011) Increasing incidence of Streptococcus pneumoniae serotype 19A and emergence of two vaccine escape recombinant ST695 strains in Liguria, Italy, 7 years after implementation of the 7-valent conjugated vaccine. Clin Vaccine Immunol 18: 343-345.

2. Black S, Shinefield H, Fireman B, Lewis E, Ray P, Hansen JR, Elvin L, Ensor KM, Hackell J, Siber G, Malinoski F, Madore D, Chang I, Kohberger R, Watson W, Austrian R, Edwards K (2000) Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. Pediatr Infect Dis J 19: 187-195.

3. Centers for Disease Control and Prevention (2005) Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease--United States, 1998-2003. MMWR Morb Mortal Wkly Rep 54: 893-897.

4. Centers for Disease Control and Prevention (2010a) Invasive pneumococcal disease in young children before licensure of 13-valent pneumococcal conjugate vaccine - United States, 2007. MMWR Morb Mortal Wkly Rep 59: 253-257.

5. Centers for Disease Control and Prevention (2010b) Licensure of a 13-valent pneumococcal conjugate vaccine (PCV13) and recommendations for use among children - Advisory Committee on Immunization Practices (ACIP), 2010. MMWR Morb Mortal Wkly Rep 59: 258-261.

6. Centre for Health Protection HK (2011a) Inclusion of Pneumococcal Vaccine in Childhood Immunisation Programme . Last accessed 25 June 2011.

7. Centre for Health Protection HK (2011b) Safe use of antibiotics save lives. Last accessed 25 June 2011.

8. Chiu SS, Ho PL, Chow FK, Yuen KY, Lau YL (2001) Nasopharyngeal carriage of antimicrobial-resistant Streptococcus pneumoniae among young children attending 79 kindergartens and day care centers in Hong Kong. Antimicrob Agents Chemother 45: 2765-2770.

9. Choi EH, Kim SH, Eun BW, Kim SJ, Kim NH, Lee J, Lee HJ (2008) Streptococcus pneumoniae serotype 19A in children, South Korea. Emerg Infect Dis 14: 275-281.

10. Clincal and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing: Twentieth informational supplement M100-S20. Wayne, Pa, National Committee for Clinical Laboratory Standards, 2010.

Ref Type: Generic

11. Dagan R (2009a) New insights on pneumococcal disease: what we have learned over the past decade. Vaccine 27 Suppl 3: C3-C5.

12. Dagan R (2009b) Serotype replacement in perspective. Vaccine 27 Suppl 3: C22-C24.

13. Hanage WP, Kaijalainen TH, Syrjanen RK, Auranen K, Leinonen M, Makela PH, Spratt BG (2005) Invasiveness of serotypes and clones of *Streptococcus pneumoniae* among children in Finland. Infect Immun 73: 431-435.

14. Hanquet G, Kissling E, Fenoll A, George R, Lepoutre A, Lernout T, Tarrago D, Varon E, Verhaegen J (2010) Pneumococcal serotypes in children in 4 European countries. Emerg Infect Dis 16: 1428-1439.

15. Ho PL, Ang I, Chow KH, Lai EL, Chiu SS (2010) 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 68: 439-444.

16. Ho PL, Chiu SS, Ang I, Lau YL (2011) Serotypes and antimicrobial susceptibilities of invasive Streptococcus pneumoniae before and after introduction of 7-valent pneumococcal conjugate vaccine, Hong Kong, 1995-2009. Vaccine 29: 3270-3275.

17. Ho PL, Chiu SS, Cheung CH, Lee R, Tsai TF, Lau YL (2006a) Invasive pneumococcal disease burden in Hong Kong children. Pediatr Infect Dis J 25: 454-455.

18. Ho PL, Chiu SS, Chow FK, Mak GC, Lau YL (2007) Pediatric hospitalization for pneumococcal diseases preventable by 7-valent pneumococcal conjugate vaccine in Hong Kong. Vaccine 25: 6837-6841.

19. Ho PL, Lam KF, Chow FK, Lau YL, Wong SS, Cheng SL, Chiu SS (2004) Serotype distribution and antimicrobial resistance patterns of nasopharyngeal and invasive *Streptococcus pneumoniae* isolates in Hong Kong children. Vaccine 22: 3334-3339.

20. Ho PL, Que TL, Ng TK, Chiu SS, Yung RW, Tsang KW (2006b) Clinical outcomes of bacteremic pneumococcal infections in an area with high resistance. Eur J Clin Microbiol Infect Dis 25: 323-327.

21. Huang SS, Hinrichsen VL, Stevenson AE, Rifas-Shiman SL, Kleinman K, Pelton SI, Lipsitch M, Hanage WP, Lee GM, Finkelstein JA (2009) Continued impact of pneumococcal conjugate vaccine on carriage in young children. Pediatrics 124: e1-11.

22. Lam TP, Ho PL, Lam KF, Choi K, Yung R (2009) Use of antibiotics by primary care doctors in Hong Kong. Asia Pac Fam Med 8: 5.

23. Melin M, Trzcinski K, Meri S, Kayhty H, Vakevainen M (2010) The capsular serotype of Streptococcus pneumoniae is more important than the genetic background for resistance to complement. Infect Immun 78: 5262-5270.

24. Moore MR, Gertz RE, Jr., Woodbury RL, Barkocy-Gallagher GA, Schaffner W, Lexau C, Gershman K, Reingold A, Farley M, Harrison LH, Hadler JL, Bennett NM, Thomas AR, Mcgee L, Pilishvili T, Brueggemann AB, Whitney CG, Jorgensen JH, Beall B (2008) Population snapshot of emergent Streptococcus pneumoniae serotype 19A in the United States, 2005. J Infect Dis 197: 1016-1027.

25. Pai R, Gertz RE, Beall B (2006) Sequential multiplex PCR approach for determining capsular serotypes of Streptococcus pneumoniae isolates. J Clin Microbiol 44: 124-131.

26. Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, Reingold A, Thomas A, Schaffner W, Craig AS, Smith PJ, Beall BW, Whitney CG, Moore MR (2010) Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis 201: 32-41.

27. Porat N, Amit U, Givon-Lavi N, Leibovitz E, Dagan R (2010) Increasing importance of multidrug-resistant serotype 6A Streptococcus pneumoniae clones in acute otitis media in southern Israel. Pediatr Infect Dis J 29: 126-130.

28. Sa-Leao R, Nunes S, Brito-Avo A, Frazao N, Simoes AS, Crisostomo MI, Paulo AC, Saldanha J, Santos-Sanches I, de Lencastre H (2009) Changes in pneumococcal serotypes and antibiotypes carried by vaccinated and unvaccinated day-care centre attendees in Portugal, a country with widespread use of the seven-valent pneumococcal conjugate vaccine. Clin Microbiol Infect 15: 1002-1007.

29. Shin J, Baek JY, Kim SH, Song JH, Ko KS (2011) Predominance of ST320 among Streptococcus pneumoniae serotype 19A isolates from 10 Asian countries. J Antimicrob Chemother 66: 1001-1004.

30. Whitney CG, Pilishvili T, Farley MM, Schaffner W, Craig AS, Lynfield R, Nyquist AC, Gershman KA, Vazquez M, Bennett NM, Reingold A, Thomas A, Glode MP, Zell ER, Jorgensen JH, Beall B, Schuchat A (2006) Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched case-control study. Lancet 368: 1495-1502.

31. Yildirim I, Hanage WP, Lipsitch M, Shea KM, Stevenson A, Finkelstein J, Huang SS, Lee GM, Kleinman K, Pelton SI (2010) Serotype specific invasive capacity and persistent reduction in invasive pneumococcal disease. Vaccine 29: 283-288.

Table 1. Characteristics of children according to time periods, Hong Kong

	% (No/total)		
	Period 1 (n=1978)	Period 2 (n=2211)	*P*
Age, mean ± SD (yr)	4.3 ± 0.8	3.9 ± 0.9	<0.001
Household size, mean ± SD	4.5 ± 1.3	4.4 ± 1.2	0.5
Recent antibiotic use by			
subject	50.1 (883/1747)	30.6 (677/2211)	<0.001
household members	34.7 (616/1777)	27.0 (596/2211)	<0.001
Overcrowding	14.0 (277/1972)	4.8 (105/2183)	<0.001
Prior hospitalization	3.2 (63/1978)	7.5 (165/2211)	<0.001
Respiratory symptoms at time of sampling	25.6 (507/1978)	9.7 (214/2211)	<0.001
Has young siblings (<=5y)	27.6 (546/1978)	27.8 (614/2211)	0.9
Recent physician visit			
At least once	77.6 (1535/1978)	62.7 (1386/2211)	<0.01
Multiple (≥3 times)	23.7 (468/1931)	13.1 (289/2211)	<0.01

The time periods were 1999-2000 (period 1) and 2009-2010 (period 2). The data for period 1 was extracted from a previous work (Chiu et al., 2001) and those for period 2 were collected in the present study. Unless specified, the figures were number (%) of children. The number of participating institutes for period 1 and period 2 were 79 and 191, respectively.

Table 2. Comparison of the serotype distribution of nasopharyngeal isolates of *Streptococcus pneumoniae* collected in two time periods, Hong Kong

Serotype[a]	No. (%)		
	Period 1	Period 2	*P*
Total	383 (100)	347 (100)	-
PCV7			
4	0 (0)	0 (0)	-
6B	88 (23.0)	75 (21.6)	0.7
19F	69 (18.0)	62 (17.9)	0.9
23F	52 (13.6)	41 (11.8)	0.5
14	34 (8.9)	9 (2.6)	<0.01
18C	9 (2.3)	2 (0.6)	0.049
9V	1 (0.3)	1 (0.3)	0.9
PCV13-nonPCV7			
3	12 (3.1)	6 (1.7)	0.2
19A	0 (0)	8 (2.3)	<0.001
6A	13 (3.4)	41 (11.8)	<0.001
Non-PCV13			
6C	7 (1.8)	27 (7.8)	<0.001
23A	6 (1.6)	19 (5.5)	0.003
15B	1 (0.3)	9 (2.6)	0.007
Others[b]	91 (23.78)	47 ((13.5)	-
Vaccine coverage			
PCV7	253 (66.1)	190 (54.8)	0.001
PCV10	253 (66.1)	190 (54.8)	0.001
PCV13	278 (72.6)	245 (70.6)	0.6

[a] No PCV10-nonPCV7 serotypes (1, 5 and 7F) were found in both periods. If serotype cross protection for 6B and 6A was assumed, the vaccine coverage for PCV7 and PCV10 in period 1/period 2 was both 69.5%/66.6%, respectively.

[b] For period 1, these included 7C (n=2), 8 (n=1), 13 (n=3), 11A (n=12), 15C (n=7), 15F (n=6), 19B (n=3), 22F (n=1), 28A/F (n=1), 29 (n=1), 33B/C/D (n=2), 34 (n=13), 35AC/42 (n=5), 35B (n=4), 35F (n=2) and nontypeable (n=28). For period 2, these included 8 (n=1), 10A (n=2), 11B/F (n=1), 11D (n=2), 15C (n=23), 15F (n=6), 22F (n=1), 23B (n=1), 29 (n=1), 33B/C/D (n=1), 34 (n=11) 35AC/42 (n=2), 35B (n=2), 35F (n=1), 37 (n=1) and 7C (n=1).

Table 3. Proportions of children carrying pneumococci within each sampling period, according to age

	% children with carriage			
	Period 1	Period 2	Difference (95% CI)	*P* value
PCV7 types				
2y	27.1	7.0	-20.1 (-9.3, -32.9)	<0.001
3y	22.7	9.1	-13.6 (-8.4, -19.2)	<0.001
4y	13.9	9.7	-4.2 (-0.5, -8.0)	0.02
5y	9.1	7.4	-	0.19
All ages	12.8	8.6	-4.2 (-2.3, -6.1)	<0.01
PCV13-nonPCV10				
2y	1.7	7.0	-	0.19
3y	2.4	1.6	-	0.43
4y	1.2	2.5	-	0.12
5y	1.0	2.1	-	0.05
All ages	1.3	2.5	1.2 (0.4, 2.1)	<0.01
Non-PCV13				
2y	0.0	2.9	-	0.33
3y	7.6	5.5	-	0.23
4y	5.0	4.7	-	0.85
5y	5.1	4.2	-	0.36
All ages	5.3	4.6	-	0.5

y, year

Table 4. Serotype distribution of nasopharyngeal isolates and their antimicrobial susceptibility according to serotype and time periods, Hong Kong.

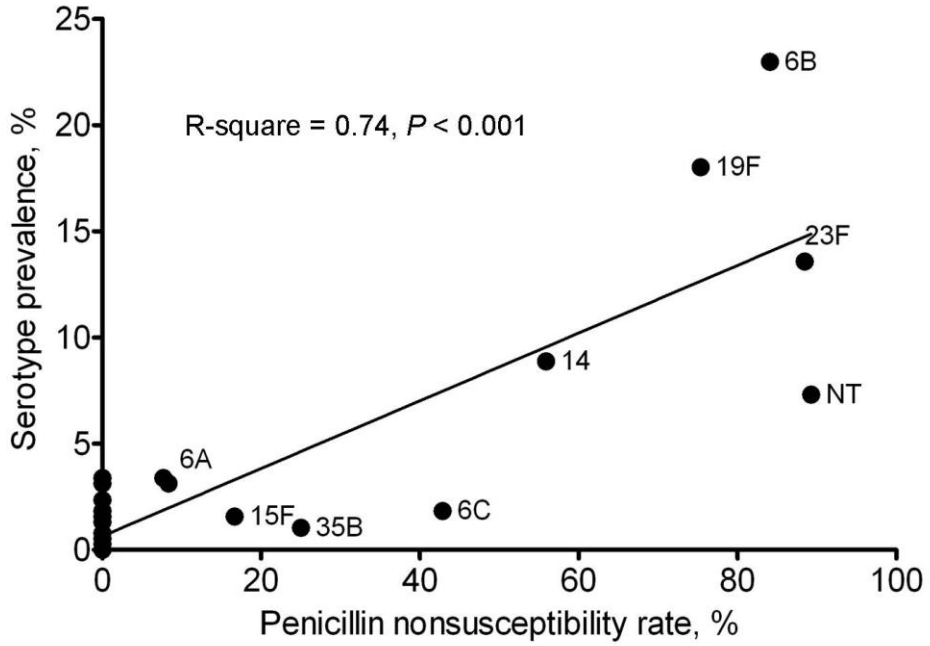
	% Pen-NS[a, b]			% Ery-NS			% Pen/Ery-NS		
Serotype	Period 1	Period 2	*P*	Period 1	Period 2	*P*	Period 1	Period 2	*P*
All	58.2	70.6	<0.001	77.0	86.7	<0.001	52.5	70.3	<0.001
PCV7									
4	-	-	-	-	-	-	-	-	-
6B	84.1	77.3	0.3	95.5	90.7	0.4	81.8	77.3	0.5
19F	75.4	100	<0.001	95.7	100	0.2	75.4	100	<0.001
23F	88.5	78.0	0.2	92.3	82.9	0.2	86.5	78.0	0.3
14	55.9	100	0.01	97.1	100	1.0	55.9	100	0.01
18C	0	0	-	11.1	50	0.3	0	0	-
9V	0	0	-	100	100	-	0	0	-
PCV13-nonPCV7									
3	8.3	0	1.0	25.0	0	0.5	0	0	-
19A	-	75.0	-	-	87.5	-	-	75.0	-
6A	7.7	87.8	<0.001	76.9	95.1	0.08	7.7	87.8	<0.001
Non-PCV13									
6C	42.9	59.3	0.7	85.7	96.3	0.4	42.9	59.3	0.7
23A	0	78.9	0.001	100	100	-	0	78.9	0.001
15B	0	22.2	1.0	0	55.6	1.0	0	22.2	1.0
others	29.7	19.1	-	40.7	63.8	-	9.9	17.0	-

[a] The percentages referred to the proportion of penicillin-nonsusceptible (Pen-NS, MIC >0.06 μg/ml), erythromycin-nonsusceptible (Ery-NS) or dual penicillin/erythromycin-nonsusceptible (Pen/Ery-NS) isolates within each serotype group.

[b] The penicillin MIC_{50} and MIC_{90} for all the isolates were 0.5 μg/ml and 2 μg/ml, respectively. Forty-nine isolates had penicillin MIC 4 μg/ml. They belonged to 19F (n=35), 23F (n=9), 6B (n=2), 14 (n=1), 15C (n=1) and 19A (n=1). Two isolates had penicillin MIC 8 μg/ml (both 19F).

Figure 1. Relationship between serotype-specific prevalence and penicillin-nonsusceptibility rates for (A) period 1 and (B) period 2. The line showed regression result according to the linear model.

(A)



(B)