Nasopharyngeal colonisation and antimicrobial resistance of *Streptococcus pneumoniae* in Hong Kong children younger than 2 years

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KEY MESSAGES

1. Serotype replacement by non-vaccine serotypes in circulating pneumococci among healthy young children in Hong Kong was evident after introduction of pneumococcal conjugate vaccine into the childhood immunisation programme.
2. The predominant carriage serotypes were serogroup/type 15 and 6C.
3. Further monitoring and evaluation of these and other emerging serotypes among invasive disease and carriage isolates are warranted.

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Introduction

Introduction of the heptavalent pneumococcal conjugate vaccine (PCV7) substantially reduced invasive pneumococcal disease (IPD) in children.[1] However, serotype replacement has been observed, with increasing proportions of IPD caused by non-vaccine serotypes.[1,2] The nasopharynx of children is a natural reservoir where pneumococcal colonisation can give rise to IPD. Therefore, surveillance of nasopharyngeal pneumococcal carriage is important in the monitoring of PCV impact.[3] In Hong Kong, PCV7 was first incorporated into the universal childhood immunisation programme in September 2009, replaced by PCV10 in October 2010 and by 13-valent PCV (PCV13) in December 2011. With the use of PCV13, it was predicted that the prevalence of both carriage and IPD would be further reduced.[4] However, it may also pose an additional selective pressure that may lead to a non-PCV13 serotype shift. A community-based epidemiological study is needed to investigate the nasopharyngeal carriage serotype distribution and antimicrobial susceptibilities of pneumococcal isolates in children. This study aimed to assess nasopharyngeal pneumococcal carriage rates, serotypes, and antimicrobial resistance patterns in children younger than 2 years.

Methods

Healthy children aged 2 months, 12 months, and 18 months were recruited by convenience sampling from June 2013 to June 2014 when they attended Maternal and Child Health Centres for routine vaccination. Informed consent was obtained from parents or primary caregivers of participants. Parents or caregivers of participants were asked to complete a questionnaire about demographics and possible predictors of pneumococcal carriage.

Deep nasopharyngeal samples were taken transnasally by a trained nurse according to World Health Organization standard procedures.[5] The nasopharyngeal swab processing and identification of pneumococcal serotypes were based on the Centers for Disease Control and Prevention study protocol.[6] PCR-based serotyping of the broth-enriched culture was performed in parallel with the isolation-based study, using the primers and conditions based on the latest updates.[6] In brief, sequential multiplex PCRs would be able to detect a total of 40 serotypes. Other untypable strains by conventional multiplex-PCRs were further resolved by a combination of traditional Quellung reaction-based testing method, as described previously and by additional primers as described.[7] Antimicrobial susceptibilities were performed by microbroth dilution using cation-adjusted Mueller-Hinton broth with lysed horse blood (5% v/v), according to Clinical and Laboratory Standards Institute 2014.[8] Interpretation of results was based on the published breakpoints.[8] Isolates identified as intermediate or resistant were grouped together as non-susceptible. Non-susceptible breakpoints for penicillin, cefotaxime, and erythromycin were minimum inhibitory concentration (MIC) of ≥4 μg/mL, ≥2 μg/mL, ≥0.5 μg/mL, respectively.

Pneumococcal carriage rate, proportion of non-vaccine serotypes carriage, and proportion of carriage that was antibiotic non-susceptible were calculated. Potential risk factors for pneumococcal carriage were determined using univariate analysis by Chi-squared test for categorical variables and Student's *t* test or Mann-Whitney *U* test for continuous variables. Variables that were significant in the univariate analysis and those that increase

the risk of carriage were further tested by logistic regression using the forward-conditional models. A P value of <0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics (Windows version 21.0; IBM Corp., Armonk [NY], US).

Results

A total of 2435 eligible children were approached and 1541 of them (782 were male) with a mean age of 0.93 (standard deviation, 0.53) years were included after consent was obtained. All included children had completed the recommended vaccination schedule for their age. The overall pneumococcal carriage rate was 5.5% (84/1541). Children of older age-groups had higher colonisation rate (P<0.001) and prevalence of recent respiratory symptoms and recent antibiotic use (P<0.001) [Table 1].

In multivariate logistic regression, age-groups of 12 months and 18 months, presence of siblings younger than 6 years, and recent respiratory symptoms were significantly associated with pneumococcal carriage (Table 2).

Of 84 pneumococcal isolates obtained from 84 children, 16 different serotypes were identified. The most common serotypes were 15B/C (16.67%), 6C (15.5%), 23A (13.1%), and 15A/F (9.5%) [Table 3].

TABLE 1. Characteristics of participants

Characteristic	No. (%) of participants				P value
	Age 2 months (n=477)	Age 12 months (n=522)	Age 18 months (n=542)	Total (n=1541)	
Male sex	241 (50.5)	267 (51.1)	274 (50.6)	782 (50.7)	0.975
Vaginal delivery	311 (65.2)	352 (67.4)	367 (67.7)	1030 (66.8)	0.65
Ever breastfed	386 (80.9)	417 (79.9)	417 (76.9)	1220 (79.2)	0.26
Child-care attendance	2 (0.4)	57 (10.9)	138 (25.5)	197 (12.8)	<0.001
Presence of young siblings aged <6 years	168 (35.2)	171 (32.8)	159 (29.3)	498 (32.3)	0.13
Household tobacco exposure	130 (27.3)	172 (33.0)	183 (33.8)	485 (31.5)	0.06
Overcrowding (a living space of <5.5 m^2/person)	36 (7.5)	27 (5.2)	33 (6.1)	96 (6.2)	0.296
Household income ≤HK$20 000 per month	115 (24.1)	157 (30.1)	175 (32.3)	447 (29.0)	0.01
Recent respiratory symptoms (3 days)	44 (9.2)	110 (21.1)	122 (22.5)	276 (17.9)	<0.001
Recent respiratory symptoms (1 month)	50 (10.5)	231 (44.3)	234 (43.2)	515 (33.4)	<0.001
Recent use of antibiotics (3 months)	44 (9.2)	117 (22.4)	137 (25.3)	298 (19.3)	<0.001
Pneumococcal carriage	11 (2.3)	41 (7.9)	32 (5.9)	84 (5.5)	<0.001

TABLE 2. Univariate and multivariate analyses of risk factors for pneumococcal carriage

Variable	No. (%) of participants		Univariate analysis		Multivariate analysis	
	Total (n=1541)	Pneumococcal carriage (n=84)	Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value
Age-group						
2 months	477 (31.0)	11 (13.1)	1		1	
12 months	522 (33.9)	41 (48.8)	3.61 (1.83-7.11)	<0.001	2.88 (1.41-5.87)	0.004
18 months	542 (35.2)	32 (38.1)	2.67 (1.32-5.33)	0.01	2.19 (1.05-4.57)	0.04
Ever breastfed	1220 (79.2)	71 (46.1)	1.46 (0.80-2.68)	0.22	-	-
Child-care attendance	197 (12.8)	13 (15.5)	1.27 (0.69-2.33)	0.45	-	-
Young siblings aged <6 years	498 (32.3)	53 (63.1)	3.88 (2.46-6.14)	<0.001	3.90 (2.44-6.23)	<0.001
Respiratory symptoms in recent 3 days	276 (17.9)	29 (34.5)	2.58 (1.61-4.13)	<0.001	2.13 (1.31-3.47)	0.002
Respiratory symptoms in recent 1 month	515 (33.4)	44 (52.4)	2.30 (1.48-3.58)	<0.001	1.71 (1.07-2.73)	0.03
Doctor visit in recent 3 months	950 (61.6)	64 (76.2)	2.06 (1.24-3.45)	0.01	1.17 (0.65-2.10)	0.60
Hospitalisation for all causes in recent 3 months	94 (6.10)	7 (8.3)	1.43 (0.64-3.20)	0.38	-	-
Use of antibiotics in recent 3 months	298 (19.3)	21 (25)	1.42 (0.85-2.37)	0.18	-	-
Respiratory symptoms in household members in recent 1 month	806 (52.3)	54 (64.3)	1.69 (1.07-2.67)	0.03	1.00 (0.60-1.65)	0.98
Antibiotic use by household members in recent 3 months	352 (22.8)	24 (28.6)	1.38 (0.84-2.25)	0.20	-	-

Overall, 2.4% of the isolates were PCV7 serotypes, 10.7% were PCV13 serotypes, and 89.3% were non-PCV13 serotypes. Multiple serotypes were detected in two samples by PCR-based serotyping of the broth-enriched culture, which were co-colonisation of 22F/A and 10A in one sample, and 15B/C and 10F/C/33C in another one. Discrepancy was noted in one sample, in which PCR-based serotyping of the broth-enriched culture was 23A but the isolation-based PCR serotyping was 15A/F.

Antibiotic susceptibility patterns for 82 out of 84 isolates were available. The proportions of penicillin (MIC ≥4μg/mL), cefotaxime (MIC ≥2μg/mL), and erythromycin (MIC ≥0.5μg/mL) non-susceptible isolates were 7.3%, 13.4%, and 79.3%, respectively. Non-PCV13 serotypes accounted for 33.3%, 45.5%, and 86.2% of the penicillin, cefotaxime, and erythromycin non-susceptible isolates, respectively (Table 4).

TABLE 3. Serotype distribution of the pneumococcal isolates

***Streptococcus pneumoniae* serotype**	**No. (%)**
Pneumococcal conjugate vaccine 7/10/13	
19F	2 (2.38)
Pneumococcal conjugate vaccine 13	7 (8.33)
6A	2 (2.38)
3	1 (1.19)
19A	4 (4.76)
Non-vaccine serotypes	75 (89.29)
15B/C	14 (16.67)
6C	13 (15.48)
23A	11 (13.10)
15A/F	8 (9.52)
10A	1 (1.19)
13	1 (1.19)
16F	1 (1.19)
35A/35C/42	1 (1.19)
35B	1 (1.19)
35F/47	1 (1.19)
34	1 (1.19)
CpsA negative/ LytA negative	14 (16.67)
CpsA negative/ LytA positive	5 (5.95)
Non serotypable	3 (3.57)
Total	**84 (100)**

Discussion

The overall pneumococcal carriage in children younger than 2 years was 5.5%; 89.3% of the isolates were non-PCV13 serotypes. Pneumococcal carriage was associated with older age-groups, presence of young siblings, and presence of recent respiratory symptoms. All of these have been reported to be risk factors.[3,9] The acquisition rate of *Streptococcus pneumoniae* from the nasopharynx is higher among children with respiratory tract infection. This may be due to the increase in secretions and a higher bacterial load within the nasopharynx during a respiratory tract infection.[10,11] In cohorts in Milan and Massachusetts, the incidence of recent antibiotic use has been reported to be 6.5%[3] and 15.5%,[9] respectively. In our cohort, it was 19.3%, which was relatively high. Nonetheless, we did not find an inverse association between recent antibiotic use and pneumococcal carriage, as has been reported in other studies.[3,9] Our cohort had a younger age and lower child-care attendance rate (12.8%) than other studies of older children (37.3%-54.4%).[3,9] There was no significant association between child-care attendance and pneumococcal carriage in our study.

The significant reduction in vaccine serotypes in nasopharyngeal colonisation reflected the effectiveness of the immunisation. This was consistent with the results from other post-PCV surveillance

TABLE 4. Antibiotic susceptibility patterns

Antibiotic	**Minimum inhibitory concentration (μg mL-1)**			**% Non-susceptible**	**Clinical and Laboratory Standards Institute breakpoint (μg mL-1)**		
	Range	**50%**	**90%**		**Susceptible**	**Intermediate**	**Resistant**
Ciprofloxacin	0.5 to 4	1	2	-	-	-	-
Levofloxacin	0.5 to 2	1	1	0	≤2	4	≥8
Lincomycin	≤0.25 to >32	2	>32	-	-	-	-
Vancomycin	≤0.03 to 1	0.25	0.5	0	≤1	-	-
Cefotaxime	≤0.25 to 32	0.25	2	13.41	≤1	2	≥4
Penicillin	0.015 to 16	0.25	2	7.32	≤2	4	≥8
Chloramphenicol	≤1 to 16	2	4	2.44	≤4	-	≥8
Erythromycin	≤0.015 to >64	4	>64	79.27	≤0.25	0.5	≥1
Tetracycline	0.12 to >32	32	>32	71.17	≤1	2	≥4
Linezolid	≤0.12 to 2	1	1	0	≤2	-	-

studies.[3,9,12-14] Our surveillance was conducted more than 1 year after the introduction of PCV13 into the childhood immunisation programme. The decline in vaccine serotypes in pneumococcal carriage and the emergence of non-vaccine serotypes were more evident than in previous local surveillance studies in hospitalised children.[12-14]

The strength of our study was that the surveillance was conducted in healthy children attending Maternal and Child Health Centres for routine vaccination, whereas previous local studies included hospitalised children with fever or respiratory illnesses.[12-14] Our study might better reveal the circulating serotypes among young children in our community, as there is possible change of the nasopharyngeal bacterial colonisation in young children with the presence of a respiratory tract infection at the time of swabbing.[9] Limitations of our study include the number of children with carriage, which was too small to perform subgroup analysis of the carriage serotypes. In addition, the response rate was suboptimal. Many parents declined to consent for nasopharyngeal swab because they considered the procedure invasive and unnecessary. This might explain why local data about the pneumococcal carriage rate in young children are lacking. Despite these limitations, our cohort is considered representative, owing to the community-based nature and territory-wide recruitment.

Conclusion

Since the introduction of PCV into the childhood immunisation programme, most circulating pneumococci in healthy young children in Hong Kong have been non-vaccine serotypes. The predominant carriage serotypes in the present study were serogroup/type 15 and 6C. Further monitoring and evaluation of these emerging serotypes is warranted.

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