Increase in the nasopharyngeal carriage of non-vaccine serogroup 15 *Streptococcus pneumoniae* after introduction of children pneumococcal conjugate vaccination in Hong Kong

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Abstract

This study assessed pneumococcal carriage in the early periods after routine use of 13-valent pneumococcal conjugate vaccine (PCV13) in Hong Kong. Nasopharyngeal swabs were obtained from 1110 children (<5 years) admitted with acute illness during September 2010-August 2013. Pneumococcal carriage rate was 13.5% in unvaccinated children, 14.1% in children who had ≥1 PCV dose and 15.3% in children who had ≥3 PCV doses. Nonv-PCV13 serotypes comprised 56.4% of all isolates. The most common serogroup/types were 15 (15A, 5.1%; 15B, 10.3%; 15C, 9.6%; 15F, 0.6%), 19F (17.9%), 6A (7.1%) and 6C (7.1%). Carriage of serogroup 15 was more common among vaccinated children (4.1% vs. 0.6%, *P*=0.033). Molecular typing revealed that expansion of several clones (clonal complex, CC63, CC199, CC1262, CC3397) was responsible for the increase in serogroup 15. Almost all CC63 and CC3397 isolates were nonsusceptible to both penicillin and erythromycin. The finding highlights the emergence of serogroup 15 following PCV13 use.

(150 words)

Introduction

Routine use of 7-valent pneumococcal conjugate vaccine (PCV-7) has been followed by reductions in vaccine serotypes invasive pneumococcal disease (IPD) and nasopharyngeal carriage among children targeted for vaccination (Ho et al., 2006; Ho et al., 2011b; Miller et al., 2011). The IPD rates have also reduced in older children and adults through herd immunity (Miller et al., 2011). However, these benefits have been counteracted by increased rates of IPD and carriage with non-PCV7 serotypes, notably multidrug-resistant 19A (Moore et al., 2008). However, the factors affecting serotype replacement are complex (Mehr and Wood, 2012; Dagan, 2009). Post-PCV7 surveillances have found variability in the pace of serotype replacement and in the predominating nonvaccine serotypes (Mehr and Wood, 2012). Broader coverage is provided by the 10-valent and 13-valent pneumococcal conjugate vaccines (PCV10 and PCV13) which were first approved by the European Medicines Agency in January 2009 and by the Food and Drug Administration in the United States in February 2010, respectively for use in children. 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. PCV13 has been approved in 128 countries and at least 83 of them have put PCV13 into routine use for children (Chiba et al., 2014). Concerns have been raised that increasing use of PCV13 would drive a shift towards non-PCV13 serotypes in both IPD and carriage (Mehr and Wood, 2012). Nonetheless, information on the changes in serotype

distribution and antimicrobial susceptibilities of pneumococcal isolates in the post-PCV13 era is limited (Chiba et al., 2014; Zuccotti et al., 2014).

In Hong Kong, PCV7 has been available in the market since October 2005 (Ho et al., 2011a). PCV10 and PCV13 were later marketed in August 2009 and May 2010, respectively. 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 (Ho et al., 2011a). From October 2010 onward, PCV7 was replaced by PCV10. This was mainly driven by vaccine cost consideration and the non-inferior safety and efficacy of PCV10 against IPD, when compared to PCV7. From December 2011 onward, PCV10 was replaced by PCV13 (Ho et al., 2011b). No catch-up vaccination was arranged at the time of switching to PCV10 and PCV13. For all formulations, the immunization schedule consists of a standard three-dose primary series (at 2, 4 and 6 months of age) and a booster dose at age 12-15 months. Before its routine use in our children, PCV7 was available as a self-financed item and its uptake in the vaccine target population had been low (Ho et al., 2011b). Here, we investigated the further changes in pneumococcal carriage and of the serotypes and antimicrobial resistance in children aged <5 years from 2010 to 2013.

2. Materials and method

2.1 Study design and data collection

Nasopharyngeal swabs were collected from children aged <5 years hospitalized in a regional hospital from September 2010 to August 2013. A standardized questionnaire was used to obtain the following participant information from parents of participating children: demographic characteristics, number of siblings (and their ages), day care attendance, prior antibiotic treatment (in the last 3 months), current antibiotic treatment, smoking habit of family members, the physician's diagnosis on the screening day, medical and immunization history (PCV7, PCV10 and PCV13). Young children aged <5 years who were hospitalized for acute illness (e.g. respiratory illness, diarrhea) were enrolled. Children with major underlying condition (e.g. immunosuppression, malignancy, chronic heart disease, chronic lung disease, chronic renal disease, diabetes mellitus) were excluded. The protocol is approved by the Institutional Review Board at the Hong Kong West Cluster/University of Hong Kong.

2.2 Microbiological methods

Nasopharyngeal cultures were obtained with alginate-tipped swabs (Ho et al., 2011b). All specimens were obtained by trained nurses. The swabs were transferred to the microbiology laboratory at the University of Hong Kong within 6 hours of collection in Amies transport media (TRANSWAB per nasal; Medical Wire and Equipment, Wilts, United Kingdom). For selective isolation of *S. pneumoniae*, nasopharyngeal swabs were inoculated onto 5% horse blood agar supplemented with gentamicin (2 μg/ml) and incubated in 5% CO_2

for 16 to 24 hours. The isolates were identified by colony morphology, Gram stain, optochin susceptibility, bile solubility and a slide co-agglutination test (Phadebact Pneumococcus Test, Remel). Susceptibility of the isolates was determined by Etest (penicillin, cefotaxime) or disc diffusion method (erythromycin) (Clincal and Laboratory Standard Institute, 2014). The criteria for penicillin-nonsusceptible and cefotaxime-nonsusceptible were ≥0.12μg/ml (oral penicillin breakpoint) and ≥2μg/ml (nonmeningitis breakpoint).

2.3 Molecular studies

The serotypes of the *S. pneumoniae* isolates were determined by multiplex PCR and the Quellung reaction (Ho et al., 2011a; Ho et al., 2011b). The isolates were initially tested by a sequential multiplex PCR approach (Pai et al., 2006). Strains that could not be serotyped and those that required further testing to the serotype level were then tested by the Quellung method with group, type and factor antisera from the Statens Seruminstitut (Copenhagen, Demark). Serotype 6C and 6D were identified by PCR assays (Ho et al., 2010). Multilocus sequence typing (MLST) was performed as previously described (http://pubmlst.org/spneumoniae/) and results were analyzed by eBURST v3 (Enright and Spratt, 1998).

2.4 Statistical analysis

The serotype coverage for the 7-valent and 13-valent PCVs for carriage isolates was calculated as the proportion of all isolates included in the vaccine formulations, without

taking into account the potential serogroup cross-protection. Potential risk factors for pneumococcal carriage were studied by univariate analysis using Chi square or Student's *t* test. Variables that were significant in the univariate analysis and those that could increase the risk of carriage from a clinical point of view were further tested by logistic regression using the forward-conditional models. The following parameters were entered for analysis in the logistic regression models: age, gender, having sibling aged <5 years, having smoker in household, day care center (DCC) attendance, recent antibiotic use and any use of PCV. To further understand how pneumococcal carriage might have changed before and at different time periods after implementation of PCVs, selected data from this study was tabulated with those from two previous studies conducted by us (Chiu et al., 2001; Ho et al., 2011b). A *P* value of <0.05 was considered to indicate statistical significance. All statistical analysis was performed by the SPSS statistic package (version 20.0, SPSS Hong Kong Ltd., Hong Kong).

3. Results

3.1 Demographics

A total of 1110 children were enrolled in the study. The mean age (± standard deviation [SD]) of the children was 1.7 ± 1.2 years. The mean ± SD household size was 3.6 ± 1.3. Fifty-eight percent of the children were male. In this cohort of children, 29.6% had household siblings aged ≤5 years; 53.4% had recent antibiotic use; 27.6% had exposure to household

130 smokers and 86.0% had at least one dose of any PCVs (79.7% in 2010/2011, 86.3% in
131 2011/2012 and 95.2% in 2012/2013). Among vaccinated children, 44.1% had ≥1 dose PCV7,
132 39.1% had ≥1 dose PCV10, 29.9% had ≥1 dose PCV13 and 67.7% had ≥3 doses of any PCVs.
133 Combination of >1 PCV formulations was received by 24.9% of the vaccinated children.
134 *3.2 Pneumococcal serotype distribution and antimicrobial susceptibilities*
135 Pneumococcal carriage was detected in 14.1% (156/1110) of the children (16.7% in
136 2010/11, 12.5% in 2011/12, and 12.5% in 2012/13). Table 1 showed distribution of the
137 serotypes and antimicrobial resistance. Overall, PCV13 serotypes accounted for 43.6%
138 (68/156) and non-vaccine serotypes accounted for 56.4% (88/156). Four PCV13 serotypes
139 including 1, 7F, 9V and 18C were not found. The common serotypes were 19F (17.9%), 15B
140 (10.3%), 15C (9.6%), 6A (7.1%) and 6C (7.1%). Serogroup 15 accounted for 45.5% (40/88)
141 of all non-vaccine isolates (including 15A, 9.1% [8/88]; 15B, 18.2% [16/88]; 15C, 17.0%
142 [15/88] and 15F, 1.1% [1/88]). MIC_{50}/MIC_{90} (range) of penicillin and cefotaxime for all the
143 isolates were 0.125/4 μg/ml (0.002–4 μg/ml) and 0.125/4 μg/ml (0.008–16 μg/ml),
144 respectively. Isolates with penicillin (MIC ≥0.12μg/ml), cefotaxime (MIC ≥2μg/ml),
145 erythromycin and dual penicillin/erythromycin nonsusceptibility were found among a wide
146 range of different serotypes (Table 1). Eighteen isolates had penicillin MIC ≥4 μg/ml. They
147 belong to serotypes 19F (n=11), 19A (n=2), 15C (n=2), 15B (n=1), 6C (n=1) and 34 (n=1).
148

149 *3.3 Changes in serotypes of carried pneumococci over time*

150 The data from this study was tabulated with those from two previous studies (Table 2).
151 Compared to the previous two groups of children, there was a small decline in overall
152 pneumococcal carriage and antibiotic-resistant pneumococcal carriage rates. Prevalence of
153 PCV7 serotypes progressively declined. In contrast, pneumococcal isolates of non-PCV13
154 serotypes, especially serogroup 15 have increased in prevalence over time (Table 2). Within
155 the three years in period 3, no significant trends in serotype replacement were observed. The
156 prevalence of carriage of serogroup 15 was 3.6% in 2010/2011, 3.3% in 2011/2012 and 4.0%
157 in 2012/2013 (P=0.883). Serogroup 15 accounted for 21.7% of all pneumococcal isolates in
158 2010/2011, 26.4% in 2011/2012 and 32.4% in 2012/2013 (P=0.504).

159

160 *3.4 Pneumococcal carriage and PCV use*

161 In the current cohort (period 3), total pneumococcal carriage was not associated with
162 pneumococcal immunization status. Carriage rate was 13.5% in unvaccinated children, 14.1%
163 in children who had ≥1 PCV dose (P = 0.845) and 15.3% in children who had ≥3 PCV doses
164 (P = 0.437). The effect of PCVs on pneumococcal carriage was further investigated by
165 multivariate analysis. A history of PCV use was independently associated with lower carriage
166 of PCV7 serotypes (P=0.042; odds ratio [OR], 0.5; 95% confidence interval [CI], 0.2 to 0.9)
167 and higher carriage of non-PCV13 serotypes (P=0.025; OR, 2.9; 95% CI, 1.1 to 7.2).

Carriage of serogroup 15 was significantly more common among children with a history of PCV use (4.1% [39/955] vs. 0.6% [1/155], P=0.033). However, the association failed to reach statistical significance in the multivariate analysis (P=0.064, OR, 6.6; 95% CI, 0.9 to 48.1).

3.5 Changes in antimicrobial resistance and clonal structure of serogroup 15 isolates

A total of 82 serogroup 15 isolates (serotype 15A, 17 isolates; 15B, 26 isolates; 15C, 35 isolates; and 15F, 4 isolates) were obtained during the three time periods and all were investigated by MLST. The relationship between MLST and serogroup 15 subtypes was summarized in Table 3. An eBURST analysis of our isolates with all other serogroup 15 isolates in the MLST database was shown in Figure S1 (supplementary file). In the three time period, either 15C or 15B was the most prevalent subtype. The isolates belonged to 24 different sequence types (STs) of which 68 isolates belonged to five clonal complexes (CCs, with 6 to 27 isolates each) and 14 isolates were singletons (12 different STs). The prevalence of four clones had increased over time (Figure 1): CC199-serotype 15B/C clone, 1.3%, 2.6% and 8.3% (P < 0.001); CC1262-serotype 15B/C clone, 0%, 0.9% and 6.4% (P<0.001); CC63-serotype 15A/C/F clone, 0.3%, 1.4% and 5.8% (P < 0.001); and CC3397-serotype 15B/C clone, 0%, 0.6% and 2.6% (P = 0.004) in period 1, period 2 and period 3, respectively. The nonsusceptibility rates among serogroup 15 isolates from period 1, period 2 and period 3

were 7.1%, 32.1% and 37.5% for penicillin (P = 0.102), 50.0%, 57.1% and 47.5% for erythromycin (P = 0.732), and 7.1%, 32.1% and 30.0% (P = 0.185) for dual penicillin/erythromycin nonsusceptibility, respectively. The penicillin- and erythromycin-nonsusceptibility rates by clonal groups were as follows: CC199-serotype 15B/C, 11.1%/18.5%; CC63-serotype 15A/C/F, 80%/100%; CC1262-serotype 15B/C, 7.7%/15.4%; CC156-serotype 15A/C/F, 14.3%/57.1%; CC3397-serotype 15B/C, 100%/100% and singletons, 14.3%/71.4%. High level penicillin resistance (MIC ≥4 μg/ml) was observed in four isolates, three from period 3 and one from period 2. The isolates belonged to ST3397-serotype 15C (n=2), ST199-serotype 15B (n=1) and ST1262-serotype 15C (n=1).

4. Discussion

This study extends our previous observations on the changes in pneumococcal carriage, antimicrobial resistance rates and serotype distribution of nasopharyngeal isolates before and since the routine use of PCVs in our children (Ho et al., 2011b). The findings showed that overall carriage declined and was due mainly to a loss of PCV7 serotypes. At the same time, there was replacement with non-vaccine serotypes. We previously noted an increase in several PCV13-nonPCV7 (6A and 19A) and nonPCV13 serogroup/serotypes (6C, 23A and

15) among children from period 2 (Ho et al., 2011b). Here, we found that the incidences of 6A, 19A and 6C have declined or stabilized following a switch to PCV13 for routine use.

Of note, serogroup 15 isolates continued to rise and it is now the predominant colonizing serogroup/serotype. When all the subtypes of serogroup 15 are considered together, their prevalence had increased by 6.9 folds from 3.7% in 1999-2000 to 8.1% in 2009-2010 and 25.6% in 2010-2013. In serially sampled Massachusetts communities, 15B/C had increased from 6% in 2001 to 11% in 2008-2009 following the widespread use of PCV7 (Wroe et al., 2012). Among Pittsburgh-area's children presenting with acute otitis media (Martin et al., 2014), an increase in the carriage of serogroup 15 pneumococci was also noted. In 2012-2013 (Martin et al., 2014), serogroup 15 (all subtypes) was the most frequently occurring serogroup/serotype comprising 23% of all colonizing pneumococcal isolates. At present, the clinical significance of the emergence of serogroup 15 in NP carriage is unclear. Previous studies found that serotype 1, 3, 5, 7F and 19A (total 7.7% in this study) have a high propensity to cause invasive disease while the ability of serogroup 15 to cause invasive disease is low (Del et al., 2014; Yildirim et al., 2010). In the post-PCV13 era, only small numbers of invasive pneumococcal disease were caused by serogroup 15 (Levy et al., 2014). In Japan where PCV7 vaccination in children began in 2010, an increase in the rate of pediatric invasive pneumococcal disease caused by 15A, 15B and 15C during 2010-2012 was noted (Chiba et al., 2014). In our locality, ongoing surveillance has not detected any increase

224 in the frequency of occurrence of serogroup 15 among children with invasive pneumococcal
225 disease (Ho et al., 2011a).

226 Genotypic analysis of the serogroup 15 strains by MLST revealed that increasing
227 prevalence of serogroup 15 was mainly caused by expansion of several preexisting clones
228 (CC63-serotype 15A/C/F, CC199-serotype 15B/C, CC1262-serotype 15B/C and
229 CC3397-serotype 15B/C). Two of them, CC63-serotype 15A/C/F and CC199-serotype 15B/C
230 are internationally recognized clones designated by the Pneumococcal Molecular
231 Epidemiology Network (http://web1.sph.emory.edu/PMEN/pmen_table1.html) as
232 Sweden^{15A}-25/ST63 and Netherlands^{15B}-37/ST199, respectively. In the MLST database (last
233 accessed 25 August 2014) CC/ST63 and CC/ST199 are the commonest genotypes associated
234 with serotype 15A and 15B/C, respectively. Besides serogroup 15 subtypes, ST63 was also
235 commonly associated with serotype 14, 19A and 19F, and ST199 associated with serotypes 3,
236 6, 19A, 19F, 23A and 23F. Prior to the introduction of PCV13, ST199 is one of the major
237 STs involved in the massive expansion of serotype 19A (Scott et al., 2012). ST1262 and
238 ST3397 are minor genotypes among serogroup 15 strains in the MLST database. All ST3397
239 strains deposited in the database were of serogroup 15 and all were recovered from patients in
240 China (Li et al., 2011). As suggested previously (Scott et al., 2012), some STs may be
241 preferentially favored in serotype replacement. This could occur as existing clones switch

their capsules or by expansion of preexisting STs with nonvaccine serotypes (Croucher et al., 2013).

This study has some potential limitations. First, we studied a convenient sample of children hospitalized to a regional hospital. The fact that many children had history of recent antibiotic use may influence the results of this study. Therefore, caution is required in interpreting the findings involving comparisons with results from our previous territory-wide carriage studies (Chiu et al., 2001; Ho et al., 2011b). Second, the number of children with carriage was relatively small. Therefore, it was not possible to stratify the findings by age groups. Third, the methodology that we adopted for culture is not capable of detecting carriage of multiple serotypes. The strengths of this study include molecular typing of the serogroup 15 isolates, and analysis of the culture result against the vaccination history and other epidemiological data.

In conclusion, this study showed that nasopharyngeal carriage by previously prevalent pneumococcal vaccine serotypes have largely been replaced by non-PCV13 types, especially serogroup 15. The results demonstrate that expansion of preexisting clones was mainly responsible for the recent increase in serogroup 15. Future pneumococcal vaccines may need to be planned according to increasing serogroup 15.

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Table 1

Distribution of antimicrobial non-susceptible nasopharyngeal *Streptococcus pneumoniae* isolates according to serotypes, Hong Kong 2010-2013

	Total		%. with resistance phenotype[d]			
	%	n	Pen-NS	Ctx-NS	Ery-NS	Pen/Ery-NS
PCV13 types[a]						
19F	17.9	28	78.6	53.6	92.9	71.4
6A	7.1	11	63.6	0	100	63.6
19A	5.1	8	87.5	37.5	87.5	87.5
14	4.5	7	100.0	0	85.7	85.7
23F	3.8	6	50.0	0	66.7	50.0
6B	1.9	3	66.7	0	100	66.7
3	1.9	3	0	0	33.3	0
4	0.6	1	0	0	100.0	0
5	0.6	1	0	0	0	0
Non-PCV13 types						
15B	10.3	16	18.8	6.3	25.0	6.3
15C	9.6	15	46.7	13.3	40.0	40.0
6C	7.1	11	81.8	18.2	81.8	72.7
15A	5.1	8	50.0	12.5	100.0	50.0
23A	3.2	5	60.0	0	100.0	60.0
34	2.6	4	75.0	25.0	25.0	25.0
35B	2.6	4	50.0	0	0	0
11D	1.3	2	0	0	50.0	0
28A	1.3	2	0	0	100.0	0
35A/C/42	1.3	2	0	0	100.0	0
35F	1.3	2	0	0	50.0	0
15F	0.6%	1	100.0	0.0	100.0	100.0
Others[b]	5.1	8	0	0	50.0	0
NT	5.1	8	37.5	0	62.5	37.5
Total	100.0	156	53.2	16.0	69.2	46.2

[a] PCV7 serotypes were underlined. Four PCV13 serotypes including 1, 7F, 9V and 18C were not found.

[b] Including one isolate each of the following eight serotypes: 11A, 13, 17F, 18A, 21, 22F, 29 and 33F.

[d] Penicillin-nonsusceptible (Pen-NS, MIC ≥0.12μg/ml), cefotaxime-nonsusceptible (Ctx-NS, MIC ≥2μg/ml); erythromycin-nonsusceptible (Ery-NS); dual penicillin/erythromycin -nonsusceptible (Pen/Ery-NS).

Table 2

Comparison of selected characteristics of children and nasopharyngeal *Streptococcus pneumoniae* carriage, Hong Kong

	Sampling time[a]			*P* value[b]
	Period 1	Period 2	Period 3	
No. of children tested	1978	2221	1110	-
Recent antibiotic use, %	50.1	30.6	53.4	<0.001
Children with at least one dose of				
Any PCVs, %	0.0	28.1	86.0	<0.001
PCV13, %	0.0	0.0	25.8	<0.001
Children with pneumococcal carriage, %				
Any serotypes	19.4	15.6	14.1	<0.001
PCV7 serotypes	12.8	8.6	4.1	<0.001
PCV13-nonPCV7 serotypes	1.3	2.5	2.1	0.019
Non-PCV13 serotypes	5.3	4.6	7.9	<0.001
Serotype 6C	0.4	1.2	1.0	<0.001
Serogroup 15	0.7	1.3	3.6	<0.001
Antibiotic- resistant pneumococcal carriage, %				
Pen-NS	11.3	11.1	7.5	0.428
Ery-NS	14.9	13.6	9.7	0.266
Total no. of pneumococcal isolates	383	347	156	-
Proportion of pneumococci, % all isolates				
PCV7 serotypes	66.1	54.8	28.8	<0.001
PCV13-nonPCV7 serotypes	6.5	15.9	14.7	<0.001
Non-PCV13 serotypes	27.4	29.4	56.4	<0.001
Serotype 6C	1.8	7.8	7.1	0.001
Serogroup 15	3.7	8.1	25.6	<0.001

[a] Data for period 1 and period 2 were from two previous carriage studies involving healthy children aged ≤5 years attending kindergartens or day care centers (Ho et al., 2011b; Chiu et al., 2001) while period 3 involved children hospitalized in a regional hospital. The sampling times were as follows: period 1, December 1999-June 2000; period 2, September 2009-April 2010; and period 3, September 2010-August 2013.

[b] Chi Square for comparison of data in the three time periods.

Table 3

Subtypes of serogroup 15 by pneumococcal clones and collection periods, Hong Kong

Variable	n	Serotype, % by row			
		15A	15B	15C	15F
Collection time					
Period 1	14	21.4	7.1	50.0	21.4
Period 2	28	21.4	32.1	46.4	0.0
Period 3	40	20.0	40.0	37.5	2.5
Clones					
CC199[a]	27	0.0	48.1	51.9	0.0
CC63	15	80.0	0.0	6.7	13.3
CC1262	13	0.0	46.2	53.8	0.0
CC156	7	57.1	0.0	14.3	28.6
CC3397	6	0.0	16.7	83.3	0.0
Singletons[b]	14	7.1	42.9	50.0	0.0
Total	82	20.7	31.7	42.7	4.9

CC, clonal complex; ST, sequence type

[a] The following STs were found in the CCs.: CC199 (ST199, 22 isolates; ST200, 3 isolates; ST1200, 1 isolate; ST9763, 1 isolate), CC63 (ST63, 15 isolates); CC1262 (ST1262, 13 isolates); CC156 (ST2647, 2 isolates; ST5453, 2 isolates; ST6011, 1 isolate; ST1078, 1 isolate; ST3280, 1 isolate); CC3397 (ST3397, 6 isolates).

[b] Two STs (ST8914 and ST9765) has two isolates each. Ten STs had one isolate each: ST83, ST193, ST1835, ST2758, ST8496, ST9762, ST9764, ST9766, ST9767 and ST9768.

New STs found for the first time in this study were underlined.

Figure 1

Changes in the prevalence of *Streptococcus pneumoniae* serogroup 15 isolates according to clonal complexes and sampling periods, Hong Kong. The sampling times were as follows: period 1, December 1999-June 2000; period 2, September 2009-April 2010; and period 3, September 2010-August 2013. The serotypes of the clonal complexes (CCs) were labeled.

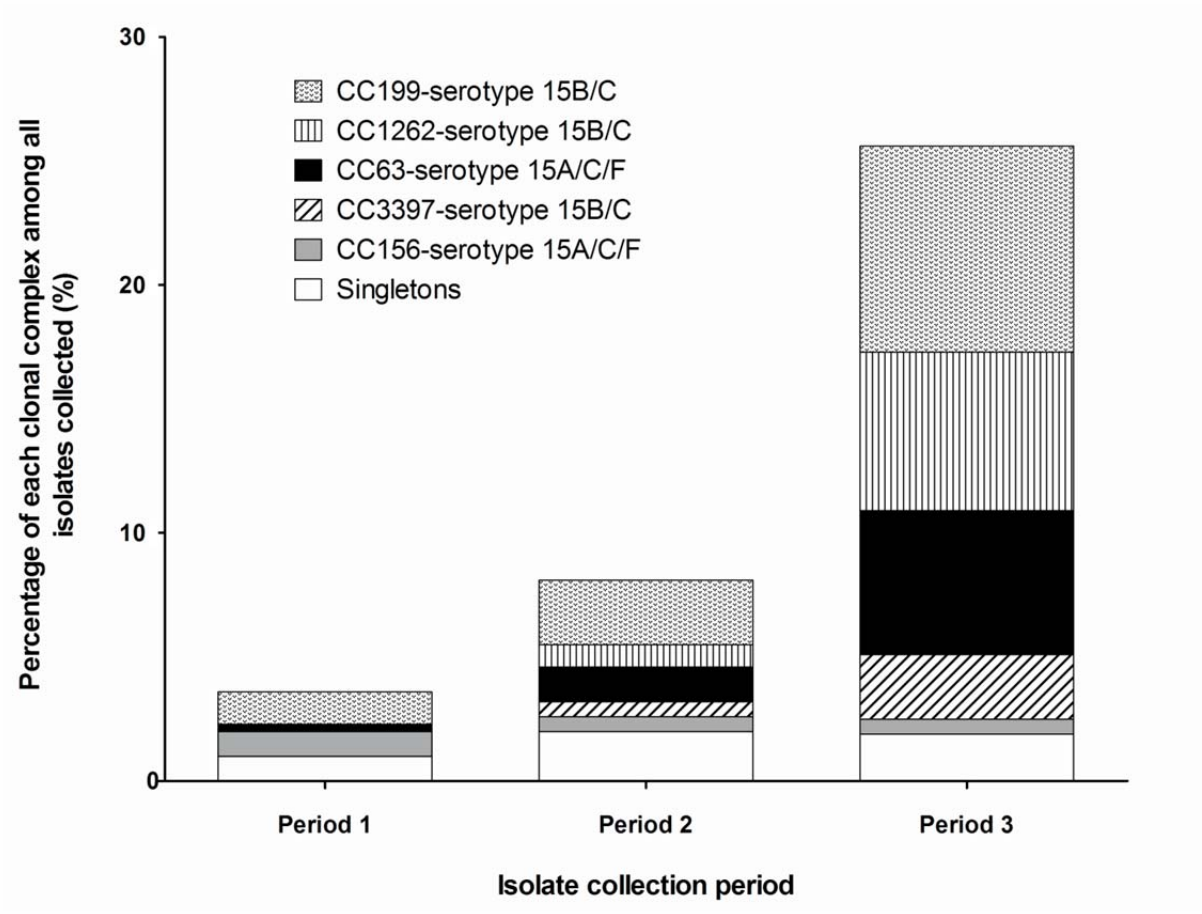

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Figure S1. eBURST analysis showing the ST distribution of serogroup 15 isolates. A total of 849 serogroup 15 isolates including 767 isolates from the MLST database (accessed on 25 August 2014) and 82 isolates form the current study were included. The four major clonal clusters (CC) found in this study were indicated with arrows.