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Original article

Molecular Epidemiology and Nasal Carriage of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* among Young Children Attending Day Care Centers and Kindergartens in Hong Kong

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Summary

Objectives: To investigate the prevalence and molecular epidemiology of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) nasal carriage in children.

Methods: We collected nasal and nasopharyngeal swabs from 2211 children aged 2-5 years attending 79 day care centers (DCCs) and 113 kindergartens (KGs) in all 18 geographical districts in Hong Kong.

Results: The overall carriage rates of *S. aureus* and MRSA were 27.6% (95% confidence interval [CI], 24.8%-28.5%) and 1.3% (95% CI, 0.8%-1.8%), respectively. Molecular typing (staphylococcal cassette chromosome *mec* [SCC*mec*], sequence type [ST], clonal cluster [CC]) showed that all the 28 MRSA isolates had SCC*mec* IV (n=13) or V (n=15) including 12 isolates with community-associated-MRSA genotypes (ST59-IV/V, ST30-IV, ST88-V), 10 isolates with healthcare-associated-MRSA genotypes (ST45-IV/V, CC5-IV and ST630-V) and six isolates with novel genotypes (ST10-V, CC1-IV). Spa typing indicated that there was some within and between DCCs/KGs transmission of certain MRSA and Methicillin-sensitive *S. aureus* strains but this was not extensive.

Conclusion: Our findings indicate the potential for DCCs to be a reservoir for emerging MRSA genotypes and highlight the need to enhance education and infection control measures to reduce their cross-transmission in this population.

(Word counts 186)

Introduction

Nasal carriage of *Staphylococcus aureus* is an important risk factor for staphylococcal infections.[1,2] Carriers of *S. aureus* have an increased risk of infection caused by the same strains they carry.[1] When *S. aureus* colonization is eradicated, the risk of infection is reduced.[1,2] Accordingly, nasal carriage of *S. aureus* has been widely used as an indicator to assess the antibiotic resistance of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) in different populations.[3-5] Young children and day care center (DCC) attendance are recognized to be important risk factors for community-associated (CA)-MRSA.[1,4] DCCs due to their crowded environment, inadequate hygienic conditions, frequent close contacts, and heavy exposures to antimicrobials among the attendees are a favorable environment for transmission of *S. aureus* and MRSA.

In Hong Kong, the 7-valent pneumococcal conjugate vaccine has been available in the market since October 2005.[6] Since September 2009, PCV7 was incorporated into the childhood immunization program with a three plus one schedule. The 10-valent and 13-valent pneumococcal conjugate vaccines were marketed in August 2009 and May 2010, respectively.[6] Previous studies suggested that use of the pneumococcal conjugate vaccines were associated with an increased risk for *S. aureus* otitis media[7] and that nasopharyngeal colonization with *S. pneumoniae*, especially the PCV7 types was inversely related to nasopharyngeal[8] and nasal[9,10] carriage of *S. aureus*. This has raised concerns that implementation of pneumococcal vaccination in children may facilitate the spread of *S. aureus* and CA-MRSA.[7,9]

In Hong Kong, about 30% of children aged 2 to 5 years attended DCC for more than 4 hours a day on a regular basis.[11] In this study, we investigate the prevalence of and risk factors associated with *S. aureus* and MRSA nasal carriage in a large sample of children

attending DCCs and kindergartens (KGs). The relationship between *Streptococcus pneumoniae* and *S. aureus* colonization were also studied.

Patients and methods

Study design and participants

Two specimens (one nasal and one nasopharyngeal swabs) were collected from children between 2 and 5 years of age who attended DCCs or KGs in Hong Kong during September 2009 to April 2010. In brief, Hong Kong is divided into 18 geographical districts, and the sample size of each district was calculated according to the number of DCC and KG places in each district.[11] 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 in 2007, 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.

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).

Microbiological methods

Nasopharyngeal cultures were obtained with a alginate-tipped swab on a flexible alumin wire (TRANSWAB per nasal, Medical Wire and Equipment Co. Ltd, Corsham, Wilts, England) and nasal cultures of both nares were obtained with sterile rayon swabs (TRANSWAB; Medical Wire and Equipment Co. Ltd). All specimens were obtained by three 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. Nasal swab from each subject was inoculated into a mannitol-salt broth medium (Oxoid) and incubated aerobically at 35 ^{o}C for 24 hours. Turbid broths were subcultured onto two agar plates, ChromID MRSA (bioMerieux, France) and mannitol salt agar. 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% CO2 for 16 to 24 hours. Bacterial colony morphology, Gram stain, and the following tests were used to identify an organism as *S. aureus*: latex agglutination (Slidex Staph Plus, bioMerieux, France), tube coagulase, mannitol, ornithine and DNase. For *S. pneumoniae*, the 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). Antimicrobial susceptibility testing was performed by the disc diffusion method in accordance with the Clinical and Laboratory Standard Institute (CLSI) recommendations. Cefoxitin discs were used for phenotypic detection of methicillin resistance. The D-test was used to detect inducible resistance to clindamycin. Quality control strains were included on each day of testing. The serotypes of the *S. pneumoniae* isolates were determined by multiplex PCR and the quellung reaction.[6] Findings for the serotype distribution have been reported elsewhere.[12]

Molecular studies

The primers SpaF1 and SpaR1 were used to amplify the polymorphic X region of protein A.[13,14] Repeat regions and spa types were analyzed according to RIDOM. MLST was performed as described elsewhere.[15] The SCC*mec* types and presence of PVL genes were determined as previously described.[16-18] SCC*mec* types were designated according to the ccr type and *mec* class combinations.[14,19] MRSA clones were named according to their MLST and SCC*mec* types.[20] The following MRSA strains were used as a positive control for SCC*mec* types (conventional/ alternative nomenclature): COL (type I/1A), PER34 (type IA/1A), BK2464 (type II/2A), ANS46 (type III/3A), HU25 (type IIIA/3A), HDE288 (type VI/4B) and HK1A2 (type V/5C).[14,16,17] Multiplex PCR assays were used for detection of genes encoding resistance to macrolides-linocosamindes-streptogramin B antibiotics (*ermA*, *ermB*, *ermC* and *mef*) and aminoglycosides (*aacA-aphD*).[21,22]

Statistic analysis

The StatMate (GraphPad Software, CA, USA) was used to determine the power for the sample size in this study. We expected the *S. aureus* and *S. pneumoniae* carriage rates to be 30% and 15%, respectively.[8,11] To detect a difference of at least 6% in *S. aureus* carriage rates among *S. pneumoniae* carriers and noncarriers with alpha = 0.05 (two-tail significance) and 80% power, a sample size of 1000 children in each group was needed. The chi-square test or the Fisher exact test was used for categorical variable. Continuous variables were tested by the Student *t* test. The statistical package SPSS, version 14.0 (SPSS, Hong Kong), was used for all analyses. A *P* value of less than 0.05 was considered to be statistically significant.

Results

Characteristics of the study population

From September 2009 to April 2010, a total of 2211 children were enrolled from 79 DCCs and 113 KGs in the study. The mean age (± standard deviation [SD]) for the children was 3.9 ± 0.9 years. About one-half of the children were male (52.0%). The mean ± SD household size was 4.4 ± 1.2. Twenty-eight percent (614/2211) of surveyed children had siblings five years old or below. One hundred and five (4.7%) of the 2211 children lived in an overcrowed environment. In this cohort of children, 9.7% (214/2211) reported acute upper respiratory symptoms at the time of sampling, 62.7% (1386/2211) reported prior physician visit(s), 30.6% (677/2211) reported prior antibiotic use, and 28.1% (622/2211) had received at least one dose of the 7-valent pneumococcal conjugate vaccine (PCV7). Among children with pneumococcal vaccination, 80.7% (502/622) were considered age appropriately vaccinated for PCV7.

S. aureus carriage and association with _S. pneumoniae_ carriage

Overall, 610 (27.6%) children were found to carry *S. aureus*, including 582 children with MSSA alone and 21 children with MRSA alone and 7 children with both MSSA and MRSA. The nasal carriage rates of MRSA and MSSA were 1.3% (28/2211, 95% confidence interval [CI] 0.8%-1.8%), and 26.6% (589/2211, 95% CI, 24.8%-28.5%), respectively. The age-stratified *S. aureus* carriage rate was 19.8% (34/172) for children aged 2 years, 24.2% (133/549) for children aged 3 years, 30.3% (230/759) for children aged 4 years and 29.1% (213/731) for children aged 5 years ($P = 0.007$). Those for MRSA carriage were 2.3% (4/172) for those aged 2 years, 1.5% (8/549) for those aged 3 years, 0.9% (7/759) for those aged 4 years and 1.2% (9/731) for those aged 5 years. The 28 MRSA isolates were recovered from children attending 10 DCCs and 15 KGs. Two isolates each was found in three DCCs/KGs. The remaining 22 DCCs/KGs had one isolate each. The 25 DCCs/KGs with MRSA carriers had distribution in 14 of the 18 geographical districts in Hong Kong. The four geographical

districts without MRSA carriers were K4, NT2, NT4 and NT7. The MRSA carriage rate in the other 14 districts ranged from 0.7% (NT8) to 3.4% (K5).

Overall, the *S. pneumoniae* carriage rate was 15.7% (347/2211). *S. aureus* carriage among *S. pneumoniae* carriers was 29.1% (101/347) vs. 27.3% (509/1864) in *S. pneumoniae* noncarriers ($P = 0.1$) (Table 1). *S. pneumoniae* carriage among *S. aureus* carriers was 16.6% (101/610) vs. 15.4% (246/1601) in *S. aureus* noncarriers. If carriage of the two organisms were independent, the expected dual carriage would be 4.3% (i.e. *S. aureus* 27.6% × *S. pneumoniae* 15.7%). Dual colonization with *S. aureus* and *S. pneumoniae* was found in 101 (4.6%, 95% confidence interval 3.7%-5.5%) children. There was no association between *S. aureus* carriage and PCV7-type or non-PCV7 type *S. pneumoniae* carriage ($P = 0.3$). Similar lack of association with *S. pneumoniae* carriage was found following further stratification by MRSA and MSSA. The findings indicated that *S. aureus* colonization was not affected by *S. pneumoniae* or vaccine-type *S. pneumoniae* co-colonization.

Potential risk factors for *S. aureus* carriage

Table 2 summarized findings from the univariate analyses. *S. aureus* carriage was significantly and negatively associated with younger age but not the other variables. Due to the small number of MRSA carriers, none of the variables were found to have statistically significant association with MRSA carriage.

A history of PCV7 vaccination did not interfere with *S. aureus* carriage. *S. aureus* and MRSA carriage was 29.7% (95% CI, 25.9%-33.8%) and 1.6% (0.8%-3.1%) among fully vaccinated children, 25.0% (18.1%-33.4%) and 1.7% (0.5%-5.9%) among partially vaccinated children and 27.1% (95% CI, 24.9%-29.4%) and 1.1% (95% CI, 0.7%-1.8%) among non-vaccinated children, respectively. To further evaluate whether PCV7 vaccination and *S. pneumoniae* carriage modified the risk of *S. aureus* colonization, a multivariate

analysis was conducted to adjust for confounding from the other child and household factors. The finding showed that only young age (OR 0.6, 95% CI 0.4-0.9, P = 0.02) was a negative predictor for *S. aureus* carriage. No significant association was found for at least one dose of PCV7 vaccination (P=0.3), fully vaccinated for PCV7 (P=0.2), colonization with *S. pneumoniae* (P=0.5) and colonization with PCV7-type *S. pneumoniae* (P=0.4).

Antimicrobial susceptibility

Drugs active against >90% of both MSSA and MRSA isolates include cotrimoxazole, minocycline, fusidic acid and rifampicin. Overall, the erythromycin resistance rate was 41.8% (258/617) including 40.3% (241/598) for MSSA and 60.7% (17/28) for MRSA. Among erythromycin-resistant isolates, 17.4% (45/258) and 75.6% (195/258) had constitutive (cMLS phenotype) and inducible (iMLS phenotype) resistance to clindamycin, respectively. The remaining 18 erythromycin-resistant isolates (all MSSA) had the M phenotype (i.e. resistant to erythromycin only). Another six isolates (all MSSA) were resistant to clindamycin alone (L phenotype). The iMLS phenotype predominated among MSSA (78.4%, 189/241), while the cMLS phenotype was prevalent among MRSA (64.7%, 11/17). MRSA isolates were significantly more likely than MSSA to have multidrug resistance (i.e. co-resistance to three or more non-β-lactam drugs). The antimicrobial agents that were less likely to be susceptible among MRSA isolates than MSSA isolates included erythromycin (MRSA, 39.3% sensitive vs. MSSA, 59.1% sensitive, P = 0.04), clindamycin (39.3% vs. 61.1%, P = 0.02), gentamicin (82.1% vs. 97.6%, P < 0.001), tetracycline (67.9% vs. 86.4%, P = 0.006), chloramphenicol (85.7% vs. 97.5%, P < 0.001) and ciprofloxacin (75.0% vs. 96.1%, P < 0.001).

Genotypic characteristics

Table 3 summarized the genotypic and epidemiologic features for the 28 MRSA isolates. Molecular typing showed that the isolates had either SCC*mec* type IV (*n*=13) or type V (*n*=15). Most (26/28) the isolates were found to belong to six lineages (clonal complex [CC]-SCC*mec* type): CC1-IV, CC5-IV, CC10-V, CC30-IV, CC45-IV/V and CC59-IV/V. The remaining two sporadic isolates belonged to ST630-V and ST88-V. The PVL gene was detected in seven isolates of the ST30 (*n*=2), ST59 (*n*=4) and ST88 (*n*=1) lineages. A total of 15 different spa types were found. The spa types with >1 isolate included t437 (*n*=6), t1081 (*n*=6), t1244 (*n*=3) and t019 (*n*=2). The remaining spa types were detected one isolate each: t002, t062, t114, t1861, t3485, t3590, t441, t5554, t6294, t6383 and t7316.

Analysis of spa type distribution in the DCCs and KGs showed that there was evidence of intra- and inter-DCCs/KGs transmission. The genotypic features for the three MRSA clusters in the three DCCs/KGs were: HKS02 (two isolates, both t1081, ST45-V and PVL negative), ST02 (two isolates, both t437, ST59-V and PVL positive) and YTM02 (two isolates, both PVL negative, one had t6383, ST1774-V and one had t1244, ST10-IV). Of the spa types with >1 isolates, t437 was found in five DCCs/KGs, t1081 in five DCCs/KGs, t1244 in three DCCs/KGs and t019 in two DCCs/KGs.

Three MRSA clones, including CC5, CC45 and CC59 were found to exhibit multidrug resistance phenotypes involving chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin and/or tetracycline. In contrast, CC1, CC10 and ST88 had resistance limited to erythromycin and/or clindamycin. CC30 and ST630 isolates were sensitive to all the non-β-lactam antibiotics. The 17 erythromycin-resistant isolates were found to possess the *ermB* (*n*=8, all had cMLS phenotype) or *ermC* gene (*n*=9, three had cMLS and six had iMLS phenotype).

A subset of randomly chosen 101 MSSA isolates was further analyzed by spa typing. This subset of isolates had the following MLS phenotype: cMLS (*n*=7), iMLS (*n*=29), M

(*n*=2), L (*n*=1) and none (*n*=62). The isolates belonged to 51 spa types. Of these, 61 isolates had 11 spa types with two or more isolates each: t338 (*n*=13), t189 (*n*=12), t091 (*n*=11), t084 (*n*=5), t701 (*n*=4), t012 (*n*=3), t164 (*n*=3), t363 (*n*=3), t5864 (*n*=3), t5864 (*n*=3), t002 (*n*=2) and t122 (*n*=2). The remaining 40 spa types had one isolate each: t008, t019, t021, t034, t091, t094, t1081, t114, t1156, t118, t127, t148, t1950, t2019, t2091, t2883, t304, t3272, t3697, t4445, t458, t4615, t5078, t5622, t571, t584, t7254, t7315, t7316, t7318, t7319, t7320, t7321, t7322, t7325, t7397, t7402, t7407, t935 and t937. These results indicated that there were only limited MSSA transmissions among the children. Six (6.0%) of the 101 MSSA isolates shared spa types found in the MRSA isolates. These include two isolates with t002 and one isolates each with t019, t114, t1081, and t7316. The 36 MSSA isolates with iMLS and cMLS phenotypes were found to belong to 18 different spa types. Twelve (92.3%) of the 13 spa t338 strains had the iMLS phenotype. The 12 strains were obtained from 12 different DCCs/KGs. All the remaining 17 spa types associated with MLS resistance had one to three isolates each.

Discussion

The prevalence of *S. aureus* carriage among children who attended DCCs (28.1%) was similar to those who attend KGs (27.4%). In comparison, the *S. aureus* carriage rate among DCC attendees was 18.1% for 1163 children in the United States in 2007-2009 [5] and 31.1% for 1192 children in Brazil in 2005.[4] According to Census data, there were 181,000 children aged 2-5 years in Hong Kong in 2009. The 1.3% MRSA prevalence translates into 2292 (95% CI, 1593-3312) children colonized with MRSA in the community. The MRSA burden is six times the number of CA-MRSA notified to the Department of Health in 2009.[14] The results are similar to the 1.3% and 1.2% MRSA carriage rates found among DCC attendees in the two studies conducted in the United States and Brazil, respectively.[4,5] but was

lower than the MRSA colonization rates among Taiwanese (6.2% to 9.2% in 2005-2008)[9] and Korean (9.3% in 2008) children.[23] In Hong Kong, large scale public health campaigns on hand hygiene have been implemented in DCCs and KGs during 2003-2009.[24] Such interventions could have reduced within centers transmission of MRSA and explained why our MRSA prevalence was substantially lower than those reported for other Asian countries.[9,23,25]

Molecular analysis of the colonizing MRSA strains revealed that they were genetically diverse and no single clone predominated. These include CA-MRSA clones that are associated with infections in the community (ST30-IV and ST59-V)[18] as well as a major MRSA clone that is endemic in our hospitals (ST45-IV/V).[14,19] Although the ST239-III and ST5-II clones are also endemic in our hospitals and had been reported among patients without risk factors for healthcare-associated (HA)-MRSA infections in other Asian countries,[14,26] these HA-MRSA clones were not found in the present study. The relatively smaller sizes of the SCC*mec* types in ST45-IV/V might give this clone an advantage for its spread from hospitals to the community. Since the children with ST45-IV/V colonization had no history of prior hospitalization, they might have acquired the strains from other people with healthcare risk factors. Two MRSA genotypes including ST10-V and ST1-IV were detected for the first time in Hong Kong. ST1-IV is one of the indigenous CA-MRSA clone in West Australia and have been found in United States (known as USA400), Europe and Asia.[27] Unlike USA400, ST1-IV in Western Australia is usually PVL-negative. Similar PVL-negative ST1-IV variants have also been found to colonized DCC attendee in South Korea.[23] ST10-V is a novel MRSA genotype and has not been reported previously. In the MLST database, there were only three ST10 *S. aureus* isolates and all three were MSSA. These new MRSA strains could have arisen *de novo* within Hong Kong or be imported from elsewhere.

Spa typing analysis showed that the colonizing MSSA isolates were genetically more diverse than the colonizing MRSA isolates. Although a few of the MSSA isolates shared common spa types with the MRSA isolates, the leading spa types among MSSA isolates were different from those of the MRSA isolates suggesting that the MRSA isolates evolved independently.

Previous studies indicated that the difference in *S. aureus* rates among *S. pneumoniae* carriers and noncarriers ranged 6% to 22.7%.[8-10,28,29] The completed results indicated that the present study was powered to detect a difference of at least 7.4% which is within the expected range. However, our investigations did not find an association between nasal carriage of *S. aureus*, MRSA and nasopharyngeal carriage of *S. pneumoniae*. It is important to point out that the inverse relationship between *S. aureus* colonization and *S. pneumoniae* carriage was not observed in all the studies. One study involving 1968 healthy children aged 3 month to 7 years (80% power to detect 5% difference) and one study involving 1783 young children aged 6-24 months with acute otitis media (80% power to detect 4% difference) did not find any difference in colonization with *S. aureus* among children who were and were not colonized with *S. pneumoniae.*[30,31] The carriage of *S. aureus* is also influenced by interactions from other co-colonizing organisms such as *Haemophilus influenzae*.[28] In addition, we found no association between PCV7 use and *S. aureus* carriage. As reported recently, PCV7 vaccination may only temporary induce an increase in *S. aureus* carriage in children around 12 months of age.[32] Our study was cross-sectional in nature and the vaccinated children received PCV7 over a wide range of different times. This may explain the discordant observations. Finally, comparison of findings from the published studies are cofounded by variations in specimen collection techniques (one nasopharyngeal swab for both organisms[8,31] versus two separate nasal and nasopharyngeal swabs[9,30] for *S. aureus* and *S. pneumoniae*) and

laboratory methodologies (culture of *S. aureus* by direct plating[30,31] versus a broth enrichment[8] before plating).

The main strength of this study is that it is a comprehensive study involving children who were enrolled according to the distribution of DCCs and KGs places in the whole territory. Nonetheless, it has several limitations. Firstly, it is a cross-sectional study. It would not be possible to assess the dynamic changes in *S. aureus* carriage. Secondly, the small number of MRSA-colonized children limited our ability to statistically demonstrate risk factors associated with MRSA colonization. The variables that was more common among the MRSA colonized children in the present study, including young age, DCC attendance, and overcrowding have previously been shown to be associated with MRSA colonization.[4,9]

In summary, this study showed that 1.3% of children aged 2-5 years attending DCCs and KGs were colonized with MRSA of diverse genotypes. In addition, erythromycin-resistant MSSA was highly prevalent in this population. The results indicate the potential for DCCs and KGs to act as reservoirs for different types of antimicrobial-resistant *S. aureus*. Thus, continued efforts to enhance hygiene in the DCCs and KGs are necessary to slow down the transmission of these pathogens in the community.[24,33]

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Table

Table 1. Tabulation of *S. aureus*, MRSA, MSSA and *S. pneumoniae* carriage among children aged 2-5years in Hong Kong, 2009-2010

Category	*S. aureus* carriage[a]			
	All serotypes (A)	Non-PCV7 serotypes (A1)	PCV7 serotypes (A2)	*S. pneumonie* noncarrier (B)
Any *S. aureus*	29.1 (101/347)	27.4 (43/157)	30.5 (58/190)	27.3 (509/1864)
MRSA	1.7 (6/347)	1.9 (3/157)	1.6 (3/190)	1.2 (22/1864)
MSSA	27.4 (95/347)	25.5 (40/157)	28.9 (55/190)	26.5 (494/1864)

[a] Percentage (Number/subtotal) of children with *S. aureus*, MRSA or MSSA carriage among those children who were or were not colonized with *S. pneumoniae*. The *S, aureus*, MRSA and MSSA carriage rates in the groups with different *S. pneumoniae* colonization status were compared (A vs. B, A1 vs. B and A2 vs. B). No significant difference was found for all pairwise comparisons (Chi square or Fisher exact test, >0.05).

Table 2. Demographic and clinical characteristics of *S. aureus* and MRSA carriage among children, Hong Kong

	S. aureus carriage (%)		*P*
	No (*n*=1601)	Yes (*n*=610)	
Child factors			
Male sex	51.8	51.3	0.8
Young age	8.6	5.6	0.02
DCC attendance	32.4	33.1	0.7
PCV7 vaccination			
At least one dose	27.7	29.3	0.4
Completely vaccinated	22.0	24.4	0.2
Current URTI	9.9	9.2	0.6
Prior antibiotic use	29.9	32.5	0.2
Prior hospitalization	7.9	6.2	0.1
Recent physician visit	62.5	63.3	0.9
Household factors			
No. of household members	4.4 ± 1.2	4.4 ± 1.2	0.8
Overcrowding	4.4	6.0	0.1
PCV7 vaccination in siblings	11.1	10.8	0.8
Having young siblings	27.4	28.9	0.5

DCC, day care center; PCV7, 7-valent pneumococcal vaccine

Table 3. Genotypic and phenotypic characteristics of 28 MRSA isolates carried by children in Hong Kong, 2009-2010

MRSA clones	n	MLST[a]	spa type	Resistance pattern[b]	Resistance determinants[c]
ST59-IV/V	9	ST59 (9)	t437 (6), t441 (1), t3485 (1), t3590 (1)	CHL (4), ERY (8), TET (6)	ermB (8)
ST45-IV/V	7	ST45 (7)	t1081 (6), t1861 (1)	CIP (6), ERY (2), GEN (4), TET (3)	ermC (2), aacA-aphD (4)
ST10-V	4	ST10 (4)	t7316 (1), t1244 (3)	ERY (4)	ermC (10)
CC1-IV	2	ST1 (1), ST1774 (1)	t114 (1), t6383 (1)	ERY (1)	ermC (1)
ST30-IV	2	ST30 (2)	t019 (2)	None	
CC5-IV	2	ST5 (1), ST1773(1)	t002 (1), t062 (1)	CIP (1), ERY (1), GEN (1)	ermC (1), aacA-aphD (1)
ST630-V	1	ST630 (1)	t5554 (1)	None	
ST88-V	1	ST88 (1)	t6292 (1)	ERY	ermC (1)

[a]Parentheses indicate the number of isolates. CC, clonal cluster; ST, sequence type. ST630 (12-3-1-1-4-4-3) is single locus variant (SLV) of ST8 (3-3-1-1-4-4-3). ST1774 (10-1-1-8-1-1-2) differ from ST1 (1-1-1-1-1-1-1) in three loci. ST1773 (1-255-1-4-119-1-10) is a double loci variant of ST5 (1-4-1-4-12-1-10).

[b]For the following eight drugs: chloramphenicol (CHL), ciprofloxacin (CIP), cotrimoxazole), erythromycin (ERY), fusidic acid, gentamicin (GEN), rifampicin and tetracycline (TET).

[c] *aacA-aphD*, aminoglycoside resistance gene encoding the bifunctional enzyme, 6'-aminoglycoside N-acetyltransferase/2"-aminoglycoside phosphotransferase; *ermB and ermC*, genes encoding macrolide-lincosamide-streptogramin B resistance.