ORIGINAL ARTICLE

Staphylococcus aureus carriage in selected kindergartens in Klang Valley

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ABSTRACT

Introduction: Nasal colonisation of *S. aureus* in healthy children was 18% to 30%. One to three percent of them were colonised by Methicillin-resistant *Staphlycoccus aureus* (MRSA). Although MRSA infection has become increasingly reported, population-based *S. aureus* and MRSA colonisation estimates are lacking. The main objective of this study was to determine the prevalence of *S. aureus* carriage among children.

Methods: Nasal samples for *S. aureus* culture were obtained from 250 children from three kindergartens in the Klang Valley, after consent was obtained from the children and their parents. Swabs were transported in Stuart medium, and inoculated on mannitol-salt agar within four hours of collection. Identification and disk diffusion test were done according to guidelines. Polymerase chain reaction was done on MRSA isolates for the presence of *mecA* and lukS/F-PV genes.

Results: Overall prevalence of *S. aureus* and MRSA carriage were 19.2% (48/250) and 1.6% (4/250) respectively. *mecA* gene was present in all isolates, 50% isolates carried Panton-Valentine leucocidin (PVL) gene. *Sccmec* type I was found in 2 isolates and the remaining isolates has Sccmec type V.

Conclusion: The prevalence of *S. aureus* and MRSA carriage were similar to other studies. However, risk of contracting severe infection might be higher due to presence of PVL gene in half of the MRSA isolates.

KEY WORDS:
Nasal colonisation; Staphylococcus aureus; children

INTRODUCTION

Staphylococcus aureus is a Gram positive bacterium that usually colonises skin and anterior nares. The bacterium gets into the skin through abrasion or small openings in the skin, particularly around hair follicles. S. aureus usually causes skin and soft tissue infections such as a boil, pimple, abscess and cellulitis with purulent drainage. Methicillin-resistant *Staphlycoccus aureus* (MRSA) refers to isolates that are resistant to all currently available β-lactam antibiotics (except for the fifth generation cephalosporin).

Carriage of *S. aureus* in the nose appears to play important role in pathogenesis and epidemiology of infection. Nasopharyngeal carriage of *S. aureus* is present in one-third of population, and is more prevalent among children.[1] Most individuals who develop *S. aureus* infections are infected with their own colonising strains.

Children cared for at day-care centres exhibit two to three time greater risk of acquiring infections, which impact both the individual health and on the dissemination of diseases through to the community.[2] The objective of this study were to determine the prevalence of nasal colonisation of *Staphylococcus aureus* and its antibiotic susceptibility patterns, among healthy children in kindergartens.

MATERIALS AND METHODS

This random sampling, cross-sectional study involved pre-schoolers aged five- and six-year-old studying in afternoon session at preschools under *Majlis Agama Islam Wilayah Persekutuan* (MAIWP) in Malaysia. There were eight preschools running afternoon session with a total of 694 pre-schoolers. The calculated sample size was 149. Only 3 pre-schools (total number of students 352) were randomly selected using "Stat Trek Random Number Generator" Software after considering the inclusion and exclusion criteria.

Written approval from related agency (*Majlis Agama Islam Wilayah Persekutuan*) was obtained prior to commencement of the study. Consent forms and self-explanation hand-outs with regards to this study were distributed to the parents a month ahead. Verbal consent from the children was also sought. Those without consent were excluded. Kindergarten A, was located in an army camp, and almost all of the parents were army personnel. Kindergarten B was located in one of elite areas in the Klang Valley, while Kindergarten C was situated in the middle of high-rise low-cost residences.

Nasal swab was taken using sterile cotton swabs with sterile 0.9% saline solution, rotated two or three times in the vestibule of both anterior nares and immediately placed in Stuart transport medium and processed in the microbiology laboratory within four hours of collection. Swab was inoculated onto mannitol salt agar (MSA) and incubated at 37°C for 24 hours. Colonies with mannitol-salt fermentation and morphology suggestive of *Staphylococcus* were sub-

This article was accepted: 9 March 2016
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Table I: Distribution of S. aureus Carriers according to gender

Kindergarten		A (n=18)	B (n=13)	C (n=17)
Gender	Male	9	5	12
	Female	9	7	5

Table II: MRSA Antibiotic Susceptibility Pattern

	Antibiotic									
No	Pen	Ox	Ery	Clin	Dox	Rif	Fus	Cip	Gen	Van (MIC µg/L
1	R	R	R	S	R	S	R	R	S	S (2.0)
2	R	R	R	I	R	S	S	I	S	S (2.0)
3	R	R	R	R	S	S	S	I	S	S (2.0)
4	R	R	R	I	S	S	S	I	S	S (1.5)

S= sensitive, R=resistance, I=intermediate
Pen: penicillin, Oxa: oxacillin, Ery: erythromycin, Clin: clindamycin, Dox: doxycycline, Rif: rifampicin, Fus: fusidic acid, Cip: ciprofloxacin, Gen: genatmicin, Van: vancomycin
MIC: minimum inhibitory concentration

Table III: MRSA Genotypic Profiles

No	lukS/F-PV gene	mecA gene	SCCmec type
1	Yes	Yes	I
2	No	Yes	V
3	No	Yes	V
4	Yes	Yes	I

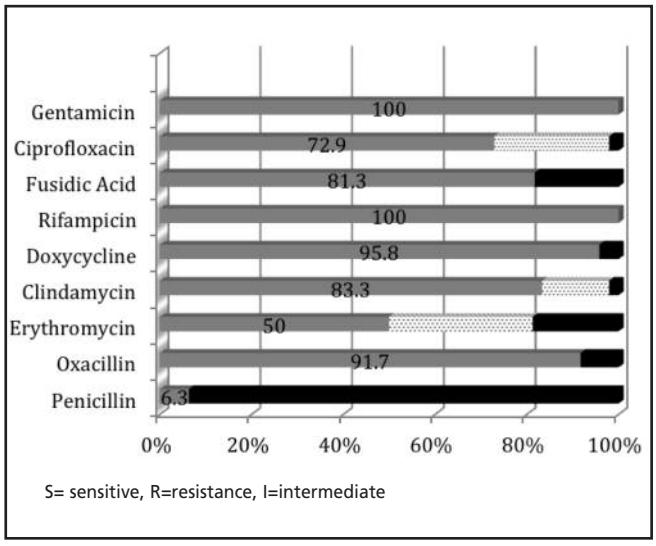

Fig. 1: Aureus antibiogram.

cultured onto blood agar plates. Gram staining, catalase and coagulase tests were performed for identification.

Antibiotic susceptibility test against cefoxitin (30µg), rifampicin (5µg), gentamicin (10µg), clindamycin (2µg), doxycycline (30µg), erythromycin (15µg) and penicillin (10µg) was done on Mueller Hinton agar based on Kirby Bauer method. The results were interpreted according to the CLSI guideline, 2014. Isolates that were resistant to cefoxitin (MRSA) were also tested with vancomycin E-test® strip. Polymerase chain reaction was done on all four MRSA isolates to check for the presence of mecA and lukS/F-PV genes. mec gene complex and ccr were also done to determine *Sccmec* type.

RESULTS

A total of 250 Malay children aged five to six years from three kindergartens participated in this study: 120 from kindergarten A, 71 from kindergarten B and 59 from kindergarten C. The prevalence of S. aureus carriage in kindergarten A, B and C were 15% (18/120), 18.4% (13/71) and 28.8% (17/59) respectively. Overall prevalence was 19.2% (48/250). Table I shows the gender distribution of *S. aureus* carriers.

Out of 48 *S. aureus* isolates, 91.7% (44/48) was susceptible to cefoxitin. This isolates were also known as Methicillin Sensitive *S. aureus* (MSSA). All isolates were susceptible to gentamicin and rifampicin. Susceptibility to other antibiotics ranged from 6.3% (penicillin) to 95.8% (doxycycline). Table II shows the result of antibiotic susceptibility test.

Four out of 48 *S. aureus* isolates were resistant to oxacillin. This is known as Methicillin Resistant *S. aureus* (MRSA). All MRSA were isolated from kindergarten C. In other words, 23.5% (4/17) of carriers in Kindergarten C carries resistant organism i.e., MRSA. No MRSA was isolated from kindergartens A and B. The overall prevalence of MRSA was 1.6% (4/250). All MRSA were sensitive to vancomycin. Table III shows the MRSA profiles.

Table IV shows the result of further molecular tests on MRSA isolates. *mecA* gene was detected in all isolates. Genes *lukS/F-PV* that encodes for leucocidin was found in 50% (n=4) of isolates. Isolates No.1 and No. 4 revealed SCCmec type I, whereas isolates No.2 and No.3 revealed *SCCmec* type V.

DISCUSSION

Child day-care centre or nursery is a place where pre-school children are supervised while the parents are at work. In the day-care centres, infections may spread by contact with infected skin or shared items, such as towels, toys, or stationeries. Child day-care centres represent special risks for transmission of infectious agents because young children lack natural immunity to many community-acquired viruses and bacteria; they lack developmental understanding of the importance of good hygiene; their natural intimacy with the susceptible children and they frequently receive antibiotics (appropriately and inappropriately).[3]

In this study setting, the overall prevalence of *S.aureus* and MRSA carriage in children were 19.2% and 1.6% respectively. These findings were similar to the prevalence in the other part of the world. Earlier reports from other countries showed that *S. aureus* carriage among healthy paediatric population ranged from 7.6% to 53.8% and the frequency of MRSA was between 0.3% to 13.2%.[4,5] A meta-analysis study revealed that MRSA nasal colonisation prevalence in children ranged from 2.3% to 5.4%: highest in hospitalised children and lowest in healthy chidlren.[6]

Skin-to skin contact, primarily via the hands of carriers, is thought to be the primary route of transmission of MRSA. MRSA carriers repeatedly contaminate their hands by touching colonised body parts, e.g. the nose, especially in cases of unknown asymptomatic carriage, and the capacity of *S. aureus* to survive for months in a dry, often relatively hostile, environment contributes to environmental reservoirs of MRSA, e.g. door handles and beds.[7]

From our observation, kindergarten C has the highest prevalence of *S. aureus* carriage and it was the only place with MRSA carriers. This kindergarten is located in a lower socioeconomic neighbourhood as compared to the other two kindergartens. Chen *et al.* reported that crowded environment such as living with greater number of children and attending day care was a significant factor for MRSA colonisation.[8] An earlier study from Colombia, also reported that higher frequencies of colonisation was associated with low socioeconomic neighbourhoods.[9]

We found that majority of isolates were susceptible to oxacillin, the mainstay antimicrobial for *S. aureus* infection. As expected, the rate of penicillin resistance was very high (94.7%), thus the use of penicillin is not recommended for empirical coverage of *Staphylococcus aureus*. On the other hand, empirical treatment with penicillin and oxacillin combination might be useful in certain cases such as cellulitis where both *Staphylococcus aureus* and *Streptococcus pyogenes* are the most common causative agents.[10] However, children with beta lactam allergies might not get better with erythromycin due to its susceptibility rate of only 50%. Even though doxycycline and ciprofloxacin showed good susceptibility rate of 95.8% and 72.9% respectively, their use in the paediatric population is generally contraindicated.

We did not find any isolates with reduced susceptibility to vancomycin, it has been reported that, the incidence of vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) in this part of the world was reported to be very low.[11] For hospitalised patient without bacteraemia or intravascular infections, therapy with clindamycin was recommended, provided that the resistance rate was low (<10%).[12] With the resistance rate of 16.7% from our study, treatment with clindamycin should be monitored closely. Moreover, larger study on MRSA should be done to understand the actual behaviour of isolates.

Although only 1.6% MRSA was isolated in this study, 50% of the isolates carried CA-MRSA genotype i.e. SCC*mec* type V and the remaining isolates were regarded as HA-MRSA as they carried SCCmec type I. PVL is encoded by *lukF-PV* and *lukS-PV* genes, and its presence in *Staphylococcus aureus* isolates is associated with tissue necrosis and leukocyte destruction by forming pores in the cell membrane. Earlier reports have shown that PVL has always been associated with CA-MRSA [16]. In contrast, our PVL genes were found in HA-MRSA isolates but not found in CA-MRSA isolates. In China, a study on HA-MRSA showed that 28.6% isolates were PVL positive and most of them were associated with skin and soft tissue infections (SSTIs) but not with pneumonia or bacteremia.[13] This finding highlighted the impending risk of the appearance of HA-MRSA strains with increased virulence in the community.

This study had several limitations. Children with cultures positive for MSSA and MRSA were not followed up. Thus, further data on the risk of acquiring MSSA and MRSA were not established. Since all MRSA were isolated from the same kindergarten, clonal typing should have been done to confirm their relatedness. This study might also underestimate the actual prevalence of MRSA carriage as colonisation can also be found in other sites such as axilla and groin.

In conclusion, the prevalence of *S. aureus* and MRSA carriage in this study setting were similar to other studies. However, risk of contracting severe infection might be higher due to presence of PVL gene in half of the MRSA isolates. Preventive strategies are therefore needed to interrupt the chain of transmission. A structured personal hygiene particularly hand hygiene program should be conducted in the kindergarten. If it is successful, such program will also help to minimize transmission of other infectious diseases.

ACKNOWLEDGEMENT

This study was funded by Universiti Sains Islam Malaysia (USIM) Short Term Grant (PPP/USG-0213/FPSK/30/11713) and the molecular work was funded by Universiti Kebangsaan Malaysia Grant (UKM-GUP-TKP-08-19-067). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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