ORIGINAL ARTICLE

Staphylococcus Aureus Carriage in Selected Communities and their Antibiotic Susceptibility Patterns

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Summary

The carriage and antibiotic susceptibility patterns of *Staphylococcus aureus* in the community were determined. Nasal, throat and axillary swabs were taken from 100 healthy adults and 90 disabled nursing home inmates. Antibiotic disc susceptibility testing was conducted following the NCCLS method. *Staphylococcus aureus* carriage was noted in 29% of healthy adults and 47.7% of nursing home inmates. Out of 79 strains, resistance to antibiotics were as follows; penicillin (92.4%), gentamicin (2.5%), tetracycline (6.3%), fusidic acid (11.3%), erythromycin (3.8%), pefloxacin (5.1%), mupirocin (3.8%), amikacin (3.8%), ciprofloxacin (2.5%) and chloramphenicol (2.5%). Methicillin-resistant *Staphylococcus aureus* was not isolated. Multiple colonizations and multi-antibiotic resistant *Staphylococcus aureus* were shown to occur in healthy individuals without risk factors and not previously hospitalized.

Key Words: Staphylococcus aureus carriage, Community, Antibiotic susceptibility

Introduction

Staphylococcus aureus is by far the most important human pathogen among staphylococci. Although this organism is frequently a part of the human microflora, it can cause significant opportunistic infections under appropriate conditions[1]. *Staphylococcus aureus* carriage has been established as a major risk factor for the development of both community-acquired and nosocomial infections[2,3,4]. The increasing prevalence of *Staphylococcus aureus* resistant to multiple antibiotics in the hospital setting has led to concern for the emergence of such strains in the community. The prevalence of antibiotic resistance found among community-isolated pathogens including *Staphylococcus aureus* was similar to that reported in hospital-based studies, suggesting that resistance is as important an issue in the community as it is in hospitals[5]. The widespread use of broad-spectrum antibiotics and biocides in the community may also select for antibiotic resistance in *Staphylococcus aureus.* Besides limiting the therapeutic options available, there are also data reporting the increasing risk to

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patients infected with resistant strain as opposed to those infected with susceptible *Staphylococcus aureus* strains. Methicillin-resistant Staphylococcus aureus in nursing homes is also of concern because these institutions might serve as reservoirs of multiresistant *Staphylococcus aureus* and MRSA in the community. This study was conducted to determine the carriage and antibiotic susceptibility pattern of *Staphylococcus aureus* in a nursing home and a healthy population and also to determine whether MRSA is present in these communities.

Materials and Methods

Subjects

One hundred students from a local university were enrolled in this study. These students were chosen at random. These students are non-medical students and were not exposed to patients in hospitals. They were required to answer a questionnaire prior to the taking of samples. The questionnaire includes any history of antibiotic intake, hospital admission and contact with persons or close relatives admitted to hospital, all for the past six months. This group of students represented the healthy community.

Inmates of a long-term care facility in Rumah Sinar Harapan, Kuala Kubu Baru, represented the disabled community. This center cares for disabled persons who are mentally retarded, the majority being the consequence of cerebral palsy and were unable to attend to their own basic needs. Ninety subjects were chosen at random. Medical records of these inmates were referred to for history of antibiotic intake and hospital admissions for the past six months prior to study.

Sampling and culture

Nasal, throat and axillary swabs were taken from each of the students. Any skin infections observed during physical examination were also swabbed. From the disabled populations, only nasal and axillary swabs were taken. No throat swabs were taken because of difficulty in getting cooperation from these subjects. The swabs were immediately plated onto mannitol salt agar and labeled accordingly. The plates were then incubated at 37°C overnight. Colonies surrounded by yellow zones suggestive of *Staphylococcus aureus* were picked up and subcultured onto blood agar plates for further identification of *Staphylococcus aureus*. Strains that are gram-positive in clusters, tube coagulase test positive and deoxyribonuclease test positive are identified as *Staphylococcus aureus*.

Antibiotic disc susceptibility testing of community isolates of *Staphylococcus aureus*

Antibiotic disc susceptibility testing was carried out following the method outlined by the National Committee on Clinical Laboratory Standards (NCCLS)[6]. Eighteen antibiotics were tested, namely, penicillin (10 μg), oxacillin (1 μg), erythromycin (15 μg), gentamicin (10 μg), fusidic acid (10 μg), trimethoprim/sulfamethoxazole, tetracycline (30 μg), rifampicin (5 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), vancomycin (30 μg), amoxicillin-clavulanic acid, pefloxacin (5 μg), amikacin (30 μg), netilmicin (30 μg), ceftriaxone (30 μg), nitrofurantoin (300 μg) and mupirocin (5 μg).

Results

A total of 43 of 90 (47.7%) inmates were *Staphylococcus aureus* carriers compared to 29 of 100 (29%) of the healthy students. Using the Chi-square test, this is found to be significant with p value <0.005. From the 43 inmates with positive cultures, 35 have *Staphylococcus aureus* strains isolated from only one site, which is from the nose (26 subjects) or the axilla (9 subjects). Another seven had positive cultures from both nose and axilla and 1 had positive culture from nose and wound. Nasal carriage was observed in 34 out of 43 inmates (79.1%). Altogether, 51 positive cultures were obtained from these inmates.
From the 29 students with positive cultures, 22

students had *Staphylococcus aureus* isolated from only one site, which is from the nose (12), throat (6), axilla (4). Six students had isolates from both nose and throat and one had positive cultures from nose and axilla. Nasal carriage is therefore seen in 19 (65.5%) out of 29 students. A total of 36 *Staphylococcus aureus* isolates were obtained from these students irrespective of site of isolation. From both communities, a total of 87 *Staphylococcus aureus* strains were isolated (Table I).

Antibiotic susceptibility patterns

Antibiotic disc susceptibility testing was carried out on all the 87 *Staphylococcus aureus* isolates. The strains isolated from two sites from the same subject will be analysed as one strain per subject if the strains exhibited the same antibiotic susceptibility pattern. Strains that exhibited different susceptibility patterns even though isolated from the same subject will be analysed as separate strains (Table II).

Among the 7 subjects in the healthy community with isolates from two sites, 2 subjects have isolates showing the same antibiotic susceptibility patterns from both sites, while the isolates from two separate sites in another 5 subjects showed different antibiotic susceptibility patterns. Based on the criteria mentioned above, a total of 34 out of 36 strains from the healthy community were analysed.

Among the 8 inmates with isolates obtained from 2 sites, 6 inmates have their isolates showing the same antibiotic susceptibility patterns from both sites while 2 inmates had isolates showing different susceptibility patterns from the 2 sites. Therefore based on the citeria mentioned above a total of 45 out of 51 strains were analysed.

Out of the 34 strains isolated from the healthy community, only 20.6% were sensitive to penicillin. A low percentage of these strains were also resistant to erythromycin (8.8%), fusidic acid (11.8%), tetracycline (5.9%), ciprofloxacin (5.9%), chloramphenicol (2.9%), pefloxacin (11.8%) and amikacin (8.8%). All strains were sensitive to oxacillin, sulfamethoxazole/trimethoprim, gentamicin, rifampicin, amoxycillin-clavulanate, netilmicin, ceftriaxone, mupirocin and vancomycin. Resistance to 3 or more antibiotics was noted in 14.7% of the strains. One strain was multiply resistant to 6 of the antibiotics tested i.e penicillin, erythromycin, fusidic acid, tetracycline, pefloxacin and amikacin.

All the Staphylococcus aureus isolates from the disabled community were resistant to penicillin. Fusidic acid resistance of 11.1% was also observed in this community. A low percentage of the strains were also resistant to tetracycline (6.7%), gentamicin (4.4%), chloramphenicol (2.2%) and mupirocin (6.7%). There was no resistance to oxacillin, erythromycin, sulfamethoxazole/ trimethoprim, rifampicin, ciprofloxacin, amoxycillin-clavulanate, pefloxacin, amikacin, netilmicin, ceftriaxone, nitrofurantoin and vancomycin. A total of 6.7% of *Staphylococcus aureus* from this community was resistant to 3 or more antibiotics.

Past medical history

A total of 30 individuals (30%) of the healthy community had history of antibiotic intake within the past 6 months prior to sampling. Out of these only 7 students were *Staphylococcus aureus* carriers. Among the students with positive cultures only 5 of 29 (17%) had history of close contacts with relatives being admitted to the ward. History of hospital admission was noted in 3% of the healthy community but none of those admitted carry *Staphylococcus aureus.*

Only 10 (11.1%) of the disabled community was given antibiotics within the past six months prior to the study and out of these 6 were carriers of *Staphylococcus aureus.* History of hospital admission was recorded in 2 (2.2%) of the disabled community and out of them only 1 was positive for *Staphylococcus aureus* carriage.

Table I: Number of positive isolations from various sites in the community studied

Isolation sites	Healthy community (No. of subjects)	Disabled community (No. of subjects)	Total isolates (No. of subjects)
Nose only	12 (12)	26 (26)	38 (38)
Axilla only	4 (4)	9 (9)	13 (13)
Throat only	6 (6)	ND	6 (6)
Nose and Axilla	2 (1)	14 (7)	16 (8)
Nose and Throat	12 (6)	-	12 (6)
Nose and Wound	0	2 (1)	2 (1)
Total	36 (29)	51 (43)	87 (72)

ND = not done.

Table II: Percentage susceptibility of *Staphylococcus aureus* to tested antibiotics

Antibiotics	Healthy Community				Disabled Community			
	S (No.)	R (No.)	% S	% R	S (No.)	R (No.)	% S	% R
Penicillin	7	27	20.6	79.4	0	45	0	100
Oxacillin	34	0	100	0	45	0	100	0
Erythromycin	31	3	91.2	8.8	45	0	100	0
Fusidic acid	30	4	88.2	11.8	40	5	88.9	11.1
Co-trimoxazole	34	0	100	0	45	0	100	0
Tetracycline	32	2	94.1	5.9	42	3	93.3	6.7
Gentamicin	34	0	100	0	43	2	95.6	4.4
Rifampicin	34	0	100	0	45	0	100	0
Ciprofloxacin	32	2	94.1	5.9	45	0	100	0
Chloramphenicol	33	1	97.1	2.9	44	1	97.8	2.2
Vancomycin	34	0	100	0	45	0	100	0
Amoxy/clav	34	0	100	0	45	0	100	0
Pefloxacin	30	4	88.2	11.8	45	0	100	0
Amikacin	31	3	91.2	8.8	45	0	100	0
Netilmicin	34	0	100	0	45	0	100	0
Ceftriaxone	34	0	100	0	45	0	100	0
Nitrofurantoin	34	0	100	0	45	0	100	0
Mupirocin	34	0	100	0	42	3	93.3	6.7

S = sensitive; R = resistant

Discussion

Staphylococcus aureus carriage appears to play a key role in the epidemiology and pathogenesis of infections because carriage often precedes infection[7]. *Staphylococcus aureus* carriage has been extensively studied amongst in-patients and healthy individuals in other countries but such studies in Malaysia are lacking. *Staphylococcus aureus* carriage differs from one individual to another. In this study 29% of the healthy population carry *Staphylococcus aureus* while nearly 50% of the inmates of a long term nursing facility were carriers. The carriage rate in this healthy population is in concordance with other studies in healthy communities. Carriage rates in the disabled population in this study were higher than in the healthy population. Several assumptions can be made as to why the rate is higher among these inmates. It could be due to the personal hygiene of these inmates, as they could not take care of themselves. The close proximity to other inmates could possibly lead to easy transmission of the strains among themselves and also spread of the bacteria from one inmate to another by the health care workers in the facility.

Studies have shown that the nares are the most consistent area from which this organism can be isolated[8]. The other consistent carriage site is the perineal area[7]. Most of the positive cultures in this study were from nasal swabs. The subjects who had *Staphylococcus aureus* isolated from other sites also had positive isolation from the nose. Colonisation of the nares frequently lead to hand carriage and spread to other parts of the body. However in some cases nasal colonization with *Staphylococcus aureus* may be secondary to chronic ischemic skin lesions. Skin damage caused by minor lesions, eczema, psoriasis or the insertion of foreign bodies increases the risk of nasal carriage[9]. In this study an inmate with positive culture from her skin lesion was also a nasal carrier. However the isolate from the skin lesion showed a different antibiotic susceptibility pattern from the nasal isolate, in which the skin isolate was fusidic acid resistant but the nasal isolate was sensitive. This suggests that she may be colonized by multiple *Staphylococcus aureus* strains. In this study, colonization with multiple strains was mainly observed more frequently in the healthy community as compared to the disabled community. Five out of 7 subjects in the healthy community have multiple isolates from separate sites with different susceptibility patterns while only 2 out of 8 subjects from the disabled community had isolates that have different susceptibility patterns. Multiple isolates with different susceptibility pattern suggest that the multiple strains can be isolated from the same subject. However, even though multiple strains isolated from the same patient may show the same susceptibility pattern, it may not imply that they were of the same strains. Differentiation of these strains can be done using DNA fingerprinting technique such as pulsed-field gel electrophoresis. In hospital settings, where heterogeneous strains are endemic, it is usual to find multiple strains of *Staphylococcus aureus* colonizing the same patient particularly when hospitalized for a long period or if immunocompromised. In this study, multiple strain colonization was observed mainly in the healthy community but not seen in the disabled community staying in a long-term care facility, which is quite similar to the hospital environment. This showed that multiple colonizations could occur in a healthy population outside the hospital environment.

MRSA has been detected in nursing and long-term care facilities in other countries[10, 11]. In this study, no MRSA was detected among the inmates of a long-term care facility and also in the healthy community. Multi-antibiotic resistance was not an uncommon occurrence among the *Staphylococcus aureus* strains isolated. High penicillin resistance was observed in both communities. This is expected because emergence of penicillin resistance had occurred since the 1950's[12]. All the isolates from the disabled community were resistant to penicillin and nearly 80% of the isolates from the healthy community were resistant to penicillin. However, the sample sizes were small and may not be representive of the whole country.

In a study by Cheong et al.[13] on antimicrobial resistance pattern of bacteria isolated from patients seen by private practitioners in Klang Valley, *Staphylococcus aureus* was a common pathogen isolated. None of the *Staphylococcus aureus* was methicillin resistant, 90% were penicillin resistant and resistance to tetracycline and erythromycin was 23% and 13% respectively. In this study, resistance to tetracycline in both communities is still less than 10%. Resistance to erythromycin was seen only among the healthy population while all the strains in the disabled community remain susceptible to erythromycin. In this study interestingly it is noted that more resistant strains were observed among the isolates obtained from the healthy community rather than in the disabled community. Again, this observation could be the result of the small number of samples involved. Strains that showed low rates of resistance to erythromycin (8.8%), ciprofloxacin (5.9%), pefloxacin (11.8%) and amikacin (8.8%) were observed among the healthy community but not in the disabled group. Gentamicin and mupirocin resistance of 4.4% and 6.7% respectively was observed among the disabled group but not among the strains from the healthy carriers.

Resistance to quinolones such as ciprofloxacin and pefloxacin has not been documented in any community-based studies in Malaysia but was observed in this study. None of the healthy community with quinolone resistance had a history of hospitalization. Fusidic acid resistance usually reported in hospitals has also emerged in our community. The rates of resistance in the communities studied were slightly more than 10%. Similar rate of resistance was noticed in hospital isolates of methicillin resistant *Staphylococcus aureus* but in this study fusidic acid resistance occurred in methicillin-sensitive Staphylococcus aureus (MSSA). The subjects in whom fusidic acid resistant strains were isolated also had no history of previous hospitalization, a small percentage of them had a history of antibiotic intake within the past 6 months but none of the subjects were treated with fusidic acid. Mupirocin resistance was observed in the isolate from inmates who had a history of skin-lesions. There is a possibility that the inmates were treated before with mupirocin, however, no mupirocin applications were recorded in the medical records.

Interestingly this study found that there is a higher rate of multi-resistant Staphylococcus aureus among the healthy community (14.7%) as compared to the disabled community (6.7%) even though those exposed to antibiotics were mainly among the disabled groups. A strain that was multiresistant to 6 antibiotics was also isolated from the healthy population, who was not involved in any way with health care. However since the number studied is small it is difficult to make a general assumption based on this study.

In recent years there has been a trend towards the use of biocides in the home environment. These products are marketed for the decontamination of food preparation area, floors and toilets. The widespread use of these biocides has led to the concern that it may select for antibiotic resistance. Biocide resistance was first reported 70 years ago and cross-resistance between biocides and antibiotics have been observed in Mycobacterium species[14]. Furthermore biocide resistance can also be plasmid mediated and thus can be easily transferred from one bacterial species to another[15, 16].

In conclusion, *Staphylococcus aureus* colonization varies from one individual to another. Multiple colonizations can occur in healthy individuals outside a hospital environment, without risk factors. *Staphylococcus aureus* in the community is already resistant to various classes of antibiotics. The emergence of multiantibiotic resistant strains in the community were mainly from individuals who had not been hospitalized. This may imply that the emergence of resistance is independent from the influence of antibiotic resistance in the hospitals and antibiotic resistances are no more confined to the hospital. The widespread use of broad-spectrum antibiotics and biocides in the community may also play a part in the selection for antibiotic resistance in *Staphylococcus aureus.*

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