

Original Article

***Staphylococcus aureus* Nasal Carriage of ENT Patients in Ho Chi Minh City**

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Abstract *Staphylococcus aureus* nasal carriage is a significant risk factor for the epidemic staphylococcal infection in hospitals; however, few data are available for *Hospital in Ho Chi Minh City*. To measure the nasal carriage of *S. aureus* and methicillin-resistant *S. aureus* in ENT patients, two hundred seventy patients were screened for nasal swabs. The isolates were identified as *S. aureus* by routine microbiological methods. Susceptibility test was performed on *S. aureus* strains using disk diffusion method. MRSA strains were detected the presence of *mecA* gene by PCR. Sixty-four strains (23.7%) were isolated from 270 patients of ENT Hospital in Ho Chi Minh City. MRSA was isolated from 17 patients (6.3%) and MSSA was from 47 patients (17.4%). Of the 64 nasal carriers, 36 (56.25%) were women, and 28 (43.75%) were men, therefore no significant difference between the sexes with regard to rates of carriage was observed. No strain of *S. aureus* was found resistant to vancomycin. The susceptibility rates to the antibiotics were as follows: penicillin and erythromycin, 7.8%; ampicillin, 10.9%; cephalixin, 65.6%; chloramphenicol, 54.7%; doxycycline, 85.9%; gentamicin, 76.6%; cotrimoxazole, 68.8% and ciprofloxacin, 60.9%. All MRSA strains were multi-resistant to cephalixin and erythromycin. Of MRSA strains, 15/17 (88.23%) had corresponding *mecA* gene while 2 remaining strains were borderline-oxacillin resistant *S. aureus* phenotype. The determination of antibiotic sensitivity pattern of MRSA and the *mecA* gene among *S. aureus* nasal isolates will help the clinicians for strategy treatment in hospitals. ©All right reserved.

Keywords: *mecA* gene, MRSA, MSSA, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus has long been recognized as an important pathogen that causes human disease and nasal carriage of *S. aureus* is also an important risk factor for *S. aureus* infection and a reservoir for methicillin-resistant *S. aureus* (MRSA). Despite the use of antibiotic therapy, staphylococcal infection occurs regularly in hospitalized patients and has severe consequences. Therefore, the increased resistance of *S. aureus* to antimicrobials is a cause for concern. *S. aureus* infection is often difficult to treat, because many MRSA strains are also resistant to multiple other drugs and major causes of nosocomial infections worldwide.¹

Responsible gene of the resistance to methicillin is *mecA*, which encodes the low-affinity penicillin-binding protein PBP 2A. This gene is highly conserved among the methicillin-resistant species but is absent from susceptible strains, making it a useful molecular marker of beta-lactam resistance in all staphylococci with PCR used as the "gold standard" assay compared with several oxacillin screening medium.² Data on the rate of *mecA* gene among MRSA nasal carriage isolates was not available for many hospitals of developing countries including our city.

The ecological niche of *S. aureus* strains is the anterior nares. Nasal carrier rates among hospital personnel and patients (60-70%) are

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much higher as compared to those among community carriers (30-50%). For ENT patients, the rate can be observed from 41% to 54% of population.³ A vietnamese epidemiological study showed that the prevalence average of community-acquired of the organism was 26.2%.⁴ Most studies regarding the risks of acquiring *S. aureus* infections in the community concern skin and soft tissue infections. For the development of nosocomial infections, *S. aureus* nasal carriage has been identified as a risk factor in general hospital populations, surgical patients and patients admitted to intensive care units.⁵ Another vietnamese study demonstrated the rates of *S. aureus* carriage in the skin and the nose of pre-operative patients were 9.6% and 36.1%, respectively and the incidence of post-operative infection from nasal carriers was clearly higher than non-carriers (8.2% and 2.3%, respectively).⁶ Few data are available for Ho Chi Minh City.

This study aimed to estimate the rate of *S. aureus* carriage and to determine the antibiotic sensitivity pattern of MRSA and the *mecA* gene among *S. aureus* nasal isolates which will help the clinicians for prophylactic and treatment strategies to prevent staphylococcal disease and the spread of MRSA in our hospitals.

MATERIALS AND METHODS

ENT Patients

Patients (18 years or older) who visited the ENT center during March to June 2007 primarily because of nose-specific complaints as signs of rhino-sinusitis, nasal trauma or wound and snoring were screened for nasal carriage of *S. aureus*. A nasal examination was performed by an ENT specialist and nasal swabs from both anterior nares were obtained. Patients were excluded if they were currently receiving antibiotic therapy.

Bacterial Isolates

The isolates were identified as *S. aureus* by routine microbiological methods. Only one isolate per patient was included. Nasal swabs were inoculated directly onto Columbia agar with 5% of sheep blood (Difco). Plates were

incubated at 37°C for 48 hours. Typical colonies were subcultured onto Mannitol salt agar (Difco) overnight at 37°C. Presumptive identification was performed on the basis of colony morphology, catalase reaction and Gram stain; isolates were confirmed to be *S. aureus* using a rabbit plasma lyophilized.

Susceptibility Testing

All isolates were screened for susceptibility to 11 antibiotics (Table 1) by disk diffusion, according to National Committee for Clinical Laboratory Standards guidelines. Reference strain used for quality control was *S. aureus* ATCC 25923. Isolates were considered multiresistant when they displayed a decreased susceptibility to at least three of antimicrobial agents tested.

Detection and Amplification of mecA Gene by PCR

In the oxacillin-resistant isolate, the presence of the *mecA* gene was confirmed by PCR reaction as described previously, using primers MecA-F (5'-ACT GCT ATC CAG CCT CAA AC -3') and MecA-R (5'-CTG GTG AAG TTG TAA TCT GG -3').⁷ A few colonies were picked from blood agar; DNA extraction was carried out with an Instagene™ Matrix kit (Bio-Rad Laboratories, Hercules, Calif.), according to manufacturer's instruction. The thermocycling protocol consisted of 5 minutes of preliminary denaturation at 94°C, followed by 35 cycles of 30-second denaturation at 94°C, 45 seconds of primer binding at 47°C and 1 minute of polymerisation at 72°C and then a final 10-minute polymerisation at 72°C using a thermocycler (ATC401 model, Apollo-CLP). Reference strain used for quality control was *S. aureus* ATCC 43300.

RESULTS AND DISCUSSION

During a 4-month period (March-June 2007), a total of 270 nasal swabs were taken from 270 subjects. Sixty-four subjects (23.7%) were identified as colonized with *S. aureus*; 36 (56.3%) were women and 28 (43.7%) were men, no significant difference between the sexes with regard to rates of carriage. Among 64 *S. aureus* isolates, MRSA was

isolated from 17 patients (26.6% of isolates or 6.3% of population) and MSSA from 47 patients (73.4% of isolates or 17.4% of population). The antimicrobial resistance rates ranged from 26.6% (17 isolates) for methicillin (MRSA) to 92.2% for penicillin and erythromycin with a 90.6% (58/64 isolates) profile of multi-drug resistance (3-10 antibiotics at the same time); no vancomycin resistant isolates were found. All MRSA isolates were also multi-resistant, the greatest rate of resistance was seen for erythromycin (100%) followed by chloramphenicol (52.9%), gentamicin and cotrimoxazole (35.3%), ciprofloxacin (23.5%) and doxycycline (11.8%). The most frequent MRSA multi-resistant pattern recorded was the association of erythromycin, gentamicin and cotrimoxazole (Table 1).

Table 1. Antimicrobial susceptibility of *S. aureus* (n = 64) and MRSA isolates (n = 17)

Antibiotics	Resistance (%)	
	<i>S. aureus</i>	MRSA
Penicillin	92.2	100
Ampicillin	89.1	100
Oxacillin	26.6	100
Cephalexin	34.4	100
Erythromycin	92.2	100
Chloramphenicol	45.3	52.9
Doxycycline	14.1	11.8
Gentamicin	23.4	35.3
Cotrimoxazole	31.3	35.3
Ciprofloxacin	39.1	23.5
Vancomycin	0	0

According to the result of the antibiogram, all 17 MRSA isolates were detected the presence of *mecA* gene by PCR; 15 strains (88.2%) were positive (Figure 1); 2 negative remaining strains had oxacillin disc diffusion zone diameter of less than 12 mm which were borderline-oxacillin resistant *S. aureus* phenotype (BORSA)⁸ (Figure 2).

The largest populations of *S. aureus* were found in the regions of the skin and mucous membranes surrounding openings in the body surface, including the anterior nares. Although nasal carriage of *S. aureus* has been suggested as the source of infections, previous studies have been found in defined

patient groups and health care workers.^{1,5} The rate of nasal carriage was 23.7% which was much higher as compared to those of our recently study among healthy student carriers (15.3%) but was lower as compared to those of a vietnamese study among healthcare workers and patients in general hospital (36.1-38%).^{4,6} The rate of nasal carriage of *S. aureus* in other countries or other specialities was also different. For example, a study of patients with rhino-sinusitis showed a carriage rate of 35%.⁹ In general, for ENT patients, the rate could be observed from 41% to 54% of population, and numerous studies demonstrated that it was a risk factor of acquiring infections, and was necessary to measure for the eradication of this organism in nasal carriers.³

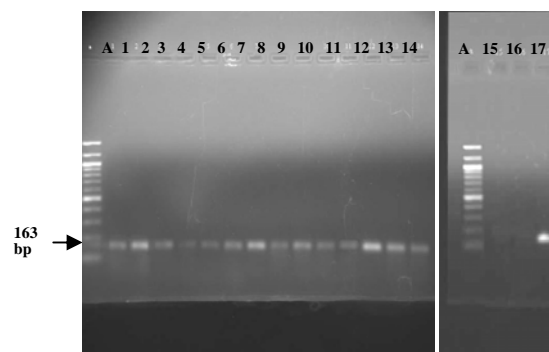


Figure 1. Detection of *mecA* gene by PCR. Lane A: ladder marker 100 bp; Lane 1-17: PCR product of MRSA isolates.

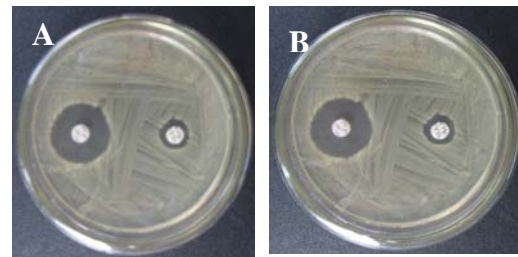


Figure 2. BORSA phenotype isolates. A: isolate 15, B: isolate 16, left disc: clavulanic acid/ amoxicillin, right disc: amoxicillin.

The nasal carriage rate of MRSA in the present study was 26.6% or 6.3% of population. This rate was also variable according to various hospital studies which

has been reported from 2.7%-16%. A reason for the difference in the prevalence of MRSA could be the different techniques used for the detection.^{1,10} *S. aureus* isolates were highly resistant to commonly used antibiotics in our ENT center. The data presented in this study appear to validate that the widespread use of antibiotics concerns regarding the possibility of accelerated development of resistance.

Indeed, susceptibility testing of methicillin resistance in *S. aureus* may be problematic because of the heterogeneous resistance displayed by many clinical isolates. Although methods of susceptibility testing are standardized, few MRSA isolates that have been found to contain *mecA* yet that appear to be phenotypically susceptible have the potential to become highly resistant if exposed to antistaphylococcal penicillins.¹¹ In contrast, BORSA phenotype isolates (11.8% of MRSA in this study) were still sensitive when antistaphylococcal penicillin was associated with a stable beta-lactamase inhibitor as clavulanic acid. The PCR result for the presence of *mecA* gene is a detection for early initiation of appropriate antimicrobial therapy and to limit the inappropriate use of glycopeptide agents,⁸ but it is applied even less for hospitals of developing countries including our city. Furthermore, standard susceptibility testing requires an additional 24-hour incubation period compared to the time required for molecular techniques.

In conclusion, the determination of antibiotic sensitivity pattern of MRSA and the *mecA* gene among *S. aureus* nasal isolates will help the clinicians for prophylactic and treatment strategies to prevent staphylococcal disease and the spread of MRSA in our hospitals.

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