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ORIGINAL ARTICLE

Difference in epidemiology and antibiotic susceptibility of methicillin resistant and methicillin susceptible *Staphylococcus aureus* isolates

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ABSTRACT

Background: Antimicrobial resistance of *Staphylococcus aureus* especially methicillin resistant *S. aureus* (MRSA) continues to be a problem for clinicians worldwide. Although difference in epidemiology and antibiotic susceptibility of methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA) isolates has been proposed by investigators, few data have been reported to now.

Patients and methods: Disk diffusion method was used for determination of in vitro susceptibility of *S. aureus* isolates to 15 antibiotics. Susceptibility pattern of 192 non-duplicates *S. aureus* isolated from clinical specimens in four university hospitals in Tehran, from November 2007 to August 2008, were compared. In addition, distribution of MRSA and MSSA isolates were investigated in different specimens, on various wards, and in different age groups.

Results: MRSA isolates were constituted 49% of all isolates. While only 1.7% of the MSSA isolates were multidrug resistant, all of MRSA were shown resistance to at least five antibiotics. Majority of isolates from patients ≥65 years old were MRSA and prevalence of methicillin resistance was highest among *S. aureus* isolated from respiratory specimens. Also, MRSA appeared to be more prevalent in intensive care units and operation wards than in other departments.

Conclusion: This study has shown a relationship between methicillin resistance and resistance to other antibiotics in *S. aureus* isolates. Also, considerable differences were observed in epidemiology of MRSA and MSSA isolates.

Keywords: *Antimicrobial susceptibility, Iran, Staphylococcus aureus, MRSA, MSSA.*
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INTRODUCTION

Methicillin resistance in *Staphylococcus aureus* is now common in many areas of the world and the frequencies of infections and outbreaks due to methicillin resistant *S. aureus* (MRSA) have continued to increase. It is noteworthy that the prevalence of MRSA varies from one geographic region to another and between different institutions in a given area (1-3). Also, relationship between methicillin resistance and resistance to other antibiotics in *S. aureus* isolates was proposed by some investigators, there are scanty reports.

The aim of the present study was to compare the epidemiology and susceptibilities of methicillin resistant *S. aureus* and methicillin susceptible *S. aureus* (MSSA) isolates.

PATIENTS and METHODS

A total 192 *S. aureus* were collected for the period of 10 months from November 2007 to

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August 2008. The strains were isolated from various clinical specimens including pus, urine, wound swabs, catheters, blood, sputum and CSF of patients in different inpatient and outpatient departments of four university hospitals (A to D) in Tehran. The identification of isolates as *S. aureus* was done according to colonial morphology, gram-staining, growth and fermentation on mannitol salt agar and catalase, coagulase and DNAase tests (4).

The susceptibility testing of *S. aureus* isolates to 15 antibiotics was carried out by the disk diffusion method according to the Clinical Laboratory Standards Institute guidelines (5), using commercial disks (MAST Diagnostics, Merseyside, UK). The antibiotics used and their disk potencies were as follows: penicillin G (10U), ampicillin-sulbactam (20µg), oxacillin (1µg), gentamicin (10µg), ciprofloxacin (5µg), chloramphenicol (30µg), erythromycin (15µg), tetracycline (30µg), co-trimoxazole (25µg), vancomycin (30µg), clindamycin (2µg), rifampicin (5µg), mupirocin (5µg), linezolid (30µg) and quinupristin-dalfopristin (15µg). The test medium was Mueller-Hinton agar (Merck, Darmstadt, Germany) and were overlaid with the inoculum (turbidity equivalent to that of a 0.5 McFarland Standard) of the *S. aureus* strains. *S. aureus* ATCC25923 was used as reference strain. Zone diameters were measured at 24 h following CLSI criteria (5). The interpretative criteria for mupirocin disk diffusion susceptibility which are not stated in the CLSI guidelines were considered as: ≥14mm, susceptible and ≤13mm, resistant (6). Strains showing intermediate zone sizes were recorded as resistant.

The resistance rate to each antibiotic was calculated as the number of resistant isolates divided by the total number of isolates. The epidemiology of methicillin-susceptible *S. aureus* (MSSA) and MRSA isolates were studied by determining their prevalence in different specimens, on various wards, and in different age groups. Isolates were considered to be multidrug resistant when they displayed resistance to at least 5 examined antibiotics, and the percentages of multidrug resistant isolates were determined for MSSA and MRSA isolates separately.

RESULTS

In this study, all the isolates were sensitive to vancomycin. The proportion of isolates susceptible to chloramphenicol, mupirocin, quinupristin-dalfopristin and linezolid were higher than 90%. Susceptibility to other antibiotics was as follows: co-trimoxazole 76%, rifampicin 65.9%, ampicillin-sulbactam 64.3%, gentamicin 55.2%, ciprofloxacin 55%, oxacillin 51%, clindamycin 47.9%, erythromycin 47.4% and tetracycline 38.5%. Penicillin had the least antibacterial activity on *S. aureus* (2.6%).

According to results of oxacillin disk, 49% of the *S. aureus* isolates were considered MRSA. The prevalence of MRSA was confirmed to vary considerably among different hospitals; 73, 46, 38 and 7 percent for hospital A, B, C and D, respectively. The prevalence of methicillin resistance was highest among *S. aureus* isolated from respiratory specimens (79%) and was lowest among isolates associated with skin infections (31.7%). The prevalence of methicillin resistance was 43.8% and 37.5% among blood and urinary tract infection isolates, respectively. Considerable differences were observed when the distributions of MRSA isolates in different wards were compared. Almost 66.7% of the *S. aureus* isolates from intensive care units (ICUs) and 71.4% of the isolates from operation wards were MRSA, whereas only 37.5% of the isolates from emergency rooms were MRSA. The distributions of both MSSA and MRSA were similar in children and adults (nearly 35%). However, the prevalence of *S. aureus* infections had rise after 65 years of age (83.9%).

The comparative in vitro activities of 15 antimicrobial agents against MSSA and MRSA isolates are shown in figure 1. All MRSA isolates

were resistant to penicillin, while 94.9% of the MSSA isolates were resistant. The percentage of MSSA isolates which were susceptible to erythromycin was more than sixty fold higher than the percentage of MRSA isolates which were susceptible to erythromycin (5.3%). While 85.7% of the MSSA isolates were susceptible to clindamycin, only 8.5% of the MRSA isolates exhibited susceptibility. The percentage of MRSA isolates showing susceptibility to gentamicin (10.6%) was more than 9-fold lower than that of MSSA isolates. While MSSA isolates were all susceptible to ampicillin-sulbactam, only 33.3% of MRSA were susceptible. Susceptibility to tetracycline fell from 66.7% among MSSA isolates to 10% among MRSA isolates. Totally, 99% of all MSSA isolates were susceptible to ciprofloxacin, whereas less than 10% of all MRSA isolates tested were susceptible.

The percentage of MSSA isolates which were susceptible to rifampicin (96.7%) and co-trimoxazole (98%) were almost 2-fold higher than the percentage of MRSA isolates which were susceptible to rifampicin and co-trimoxazole. While 96.6% and 100% of the MSSA isolates were susceptible to quinupristin-dalfopristin and mupirocin, respectively, this rates were slightly decreased to 88.4% and 92.6% for the MRSA isolates. Susceptibility rate of MRSA and MSSA to chloramphenicol and linezolid were almost same. Vancomycin was the only compound tested to which reduced susceptibility was not recognized for any of the *S. aureus* isolates tested.

The results of multidrug resistance are shown in table 1. While only 1.7% of the MSSA isolates were shown resistance to at least five antibiotics, all of MRSA were multidrug resistant.

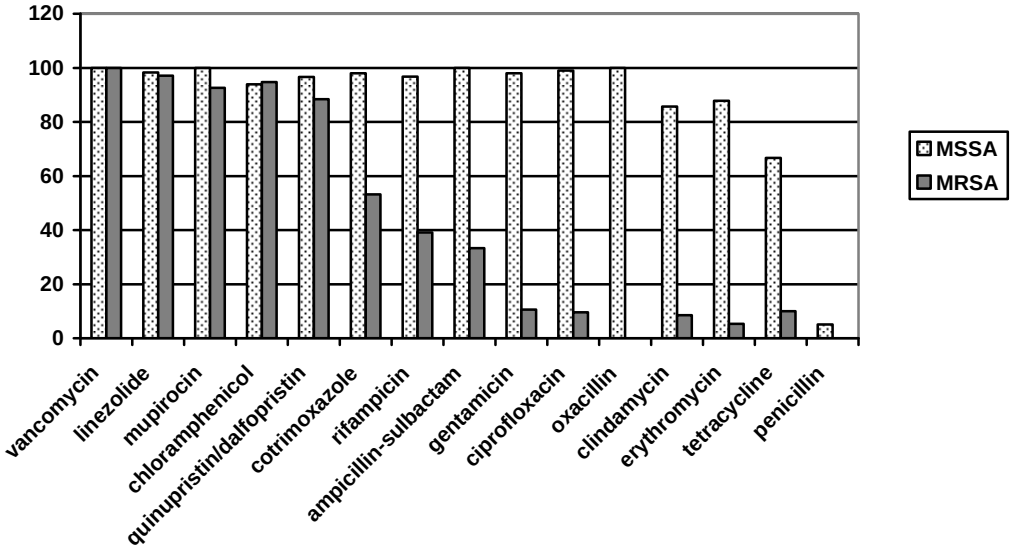

Figure 1. Comparison of percentage of susceptibility to 15 antibiotics in methicillin resistant *Staphylococcus aureus* MRSA (n=94) and methicillin susceptible *S. aureus* MSSA (n=98) isolates

Table 1. Number of antibiotics to which methicillin resistant *Staphylococcus aureus* MRSA and methicillin susceptible *S. aureus* MSSA isolates were resistant

Isolates	Number of antibiotics to which isolates were resistant												
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
MRSA	0	0	0	0	0	0	0	11.6	69.6	7.2	5.8	4.3	1.5
MSSA	6.8	57.6	23.7	8.5	1.7	0	0	1.7	0	0	0	0	0

DISCUSSION

The rates of resistance to *S. aureus* in this study are comparable to other studies in various parts of world, including the study of Mamishi and co-workers in Tehran (7). However, overall resistance rates of *S. aureus* to most antibiotics in this study were higher than what mentioned in Mamishi study, except for resistance to chloramphenicol, co-trimoxazole and oxacillin.

The prevalence of MRSA in this study is comparable to that found in United States, Canada, Europe and Iran (1,3,7,8), but the percentage of MRSA isolates is less than half of the percentage reported from Japan (9). The prevalence of MRSA was confirmed to vary considerably among different hospitals. The reason for the low prevalence in some hospitals may be related to the rapid detection and strict policies of isolation of patients with MRSA colonization or infection, combined with the restricted use of antibiotics (8). Differences in prevalence of methicillin resistance among *S. aureus* isolated from various specimens might be due to prolonged antibiotic treatment of severely sick patients, which generally have longer hospital stays, resulting in enhanced selection pressure. Distributions of MRSA isolates were varied in different wards which partly reflects the fact that some patients, e.g., critically ill patients in ICUs, have a greater chance of becoming colonized or infected (8). Our results concerning the high prevalence of MRSA in ICUs are largely in accordance with other studies (2,10); as CDC reported that more than 50% of *S. aureus* isolates from ICUs are now resistant to methicillin and, in other hospital units, this rate is close behind at 40% (2). The low prevalence of MRSA in emergency rooms suggests that the level of MRSA in the community is still lower than that in hospitals. Also, in this study it had been shown an obvious relationship between oxacillin resistance and resistance to other antibiotics as noted in other investigations (8,11-13).

The glycopeptide agent vancomycin is still the drug of choice for the treatment of life-threatening infections caused by multidrug resistant MRSA strains although recently isolation of vancomycin resistant *S. aureus* from many countries has confirmed that emergence of these strains is a global issue (14). In this study, neither of *S. aureus* isolates had shown resistance to vancomycin in disk diffusion agar method, although it is reported that disk diffusion agar test did not accurately identify resistance to vancomycin in *S. aureus* and broth or agar dilution methods or E-test are needed (14,15). Nevertheless, it is important to carefully monitor the prevalence of vancomycin resistant *S. aureus*, especially in MRSA populations because of the almost invariable multidrug resistant nature of MRSA.

In Conclusion, this study has provided baseline information in assisting physicians, clinical microbiologists and public health officials on critical issues regarding empirical and pathogen specific therapy. The wide dissemination of multidrug resistant MRSA in various hospitals in Tehran, Iran, indicate that adequate steps in limiting spread are urgently needed. Continuous surveillance on resistance patterns of *S. aureus* in understanding new and emerging trends is of utmost importance.

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