Global Journal of Pharmacology 5 (2): 106-111, 2011
ISSN 1992-0075

Antibiotic Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* in Three Hospitals at Hodeidah City, Yemen

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Abstract: In this study, sixty *Staphylococcus aureus* isolates were collected from nosocomial carriers in three hospitals (Al-Aqsa, Al-Olafy and Al-Thowra hospitals) at Hodeidah city, Yemen. Nasal swabs were collected from physicians and nurses working in surgery section, intensive care unit and burn section. The prevalence ratio of *Staphylococcus aureus* isolates vs. other staphylococci isolates was 2.5. The isolates were screened for methicillin–resistance and 29 isolates of methicillin–resistant *S. aureus* (MRSA) were selected depending on the standard methods. The susceptibility and drug resistance patterns of *S. aureus* isolated from nosocomial swabs was found to be highly variable.The pattern of antibiotic resistance of *S. aureus* was determined by using the modified Kirby Bauer disc diffusion method against 8 antibiotics (chloramphenicol, clindamycin, erythromycin, lincomycin, oxacillin, penicillin, co-trimoxazole and vancomycin). The results showed that the tested isolates of *S. aureus* had 89.7% resistance to penicillin, 86.2% resistance to oxacillin, 75.7% resistance to lincomycin, 51.7% resistance to co-trimoxazole, 31.0% resistance to erythromycin, 13.8% resistance to clindamycin and 10.3% resistance to chloramphenicol. All tested isolates of MRSA were sensitive to vancomycin.

Key words: *S. aureus* · Antibiotics · Multidrug resistance · Nosocomial-MRSA

INTRODUCTION

Staphylococcus aureus is important cause of community- and hospital-acquired infections. Infections caused by methicillin- or oxacillin-resistant *S. aureus* (MRSA) are mainly nosocomial and are increasingly reported from many countries worldwide [1]. As MRSA strains are frequently resistant to many different classes of antimicrobial drugs, second- and third-line antimicrobial resistance is a growing concern [2]. Methicillin resistance in staphylococci is mediated by the *mecA* gene, which encodes for the penicillin-binding protein 2A (PBP2A) resulting in reduced affinity for the beta-lactam antibiotics including the penicillinase-resistant penicillin. MRSA has become a major hospital pathogen in human medicine [3]. Since the emergence of MRSA after one year of launching methicillin, there have been many reports of MRSA causing various infections throughout the world. *S. aureus* especially methicillin resistance *S. aureus* is relatively ubiquitous and is the cause of many community, endemic and epidemic nosocomial colonization and infections. MRSA is of concern not only because of its resistance to methicillin but also because it is generally resistant to many other chemotherapeutic agents [4-7].

At present, MRSA has become an endemic pathogen worldwide [8] and multidrug resistant [9], with most isolates exhibiting resistance to both quinolones and aminoglycosides [10]. However, vancomycin resistant *S. aureus* is not widely seen even though a low level resistance to vancomycin is being reported [11, 12].

The rate of multidrug resistance was found to be 69% for tested MRSA strains. Antibiotic resistance pattern of these isolates was high against penicillin 100%, erythromycin 83%, co-trimoxazole 82%, respectively. Among the isolates, 83% of MRSA were beta-lactamase positive. However all strains of *Staphylococcus* were sensitive to vancomycin [12]. Thus the knowledge of the prevalence of MRSA and their antibiotic susceptibility pattern becomes fundamental in the selection of appropriate empirical treatment especially in a hospital setting.

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In the present study, the *in vitro* antimicrobial susceptibility pattern of MRSA strains isolated from nosocomial carriers was analyzed in three hospitals at Hodeidah city, Yemen.

MATERIALS AND METHODS

Isolation and Identification of Clinical Specimens: In this study, sixty staphylococcal isolates were collected from nosocomial carriers in three hospitals (Al-Aqsa, Al-Olafy and Al-Thowra hospitals) at Hodeidah city, Yemen. Nasal swabs were collected from physicians and nurses working in surgery section, intensive care unit and burn section. The isolates were screened for methicillin-resistance and 29 strains of methicillin-resistant *S. aureus* (MRSA) were selected depending on the standard methods.

The isolates collected from nasal swabs submitted at the microbiology laboratory were processed and all *Staphylococcus* isolates were identified by conventional techniques [13]. Isolates were screened by preliminary Gram's staining and were inoculated on 10% sheep blood agar, mannitol salt agar, McConkey agar and subcultured on nutrient agar. Slants were made and tested further for the production of DNase, catalase and coagulase. Isolates found to be positive by these tests were identified as *S. aureus*.

Antibiotics Susceptibility Tests: The pattern of antibiotic resistant of *S. aureus* was determined by using the modified Kirby Bauer disc diffusion method against 8 antibiotics; chloramphenicol (30 μg), clindamycin (2 μg), erythromycin (15 μg), lincomycin (2 μg), oxacillin (1 μg), penicillin (60 μg), co-trimoxazole (trimethoprim-sulfamethoxazole; 1.25 μg + 23.75 μg) and vancomycin (30 μg). All tests were performed on Muller-Hinton agar and were interpreted after incubation for 24 hrs at 37°C. The zone diameters (mm) measured around each disk were interpreted on the basis of guidelines published by the Clinical and Laboratory Standards Institute [14]. According to the breakpoints for susceptibility testing of oxacillin, *S. aureus* isolate is considered as sensitive (S) at ≥13 mm, intermediate (I) at 11- 12mm and resistant (R) at ≤10 mm. Most guidelines agree that *S. aureus* isolates should be considered nonsusceptible (R) to oxacillin if the MIC is ≥ 4 mg/L.

Screening test for MRSA: Screening test was performed in accordance to CLSI guidelines [14] using oxacillin agar. Briefly, a bacterial suspension of 10^6 cfu/ml was prepared from each isolate. Then a swab was dipped and streaked on the surface of a Mueller-Hinton agar supplemented with 6 μg/ml oxacillin and 4% NaCl. After incubation for 24 hrs at 35°C [14], if any growth was detected, the isolate was considered oxacillin- or methicillin-resistant (MRSA).

RESULTS

Isolation and Identification of Staphylococci: Based on our identification methods, we isolated a total of 60 staphylococcal isolates from different nosocomial swabs collected from nosocomial carriers in three hospitals (Al-Aqsa, Al-Olafy and Al-Thowra hospitals) at Hodeidah city, Yemen. The yellow colonies grew on mannitol salt agar and the golden yellow colonies appeared on nutrient agar showing G+ve grape-like coccoid clusters and producing DNase and coagulase were identified as *S. aureus*.

Prevalence of *S. aureus* in Nosocomial Carriers: Table 1 shows prevalence of *S. aureus* in nosocomial carriers according to the personal characteristics (gender, age and job). The data generally revealed that prevalence ratio of *Staphylococcus aureus* isolates vs. other staphylococci isolates was 2.5. According to the gender, the prevalence of *S. aureus* did not significantly differ between both sexes. Persons younger than 30 years of age seemed to be *S. aureus*-carriers more than those older than 30 years (prevalence rate = 1.15). Taking the job in consideration, the prevalence ratio of *S. aureus* in nurses to physicians was 1.9.

Multidrug Resistance of *S. aureus*: The drug resistance patterns of *S. aureus* isolated from nosocomial swabs was found to be highly variable. Table 2 shows the antibiotic susceptibility of two *S. aureus* isolates.

S. aureus 1 appeared to be sensitive to all tested antibiotics except oxacillin which showed intermediate effect. Whereas, *S. aureus* 2 was recorded as a multidrug resistant MRSA sense it was resistance to oxacillin, erythromycin, lincomycin and penicillin. On the other hand, it was sensitive to chloramphenicol, clindamycin, co-trimoxazole and vancomycin.

Antibiotics Susceptibility Pattern of MRSA: Out of sixty, 29 isolates were found to be MRSA. The antimicrobial susceptibility pattern of MRSA isolates against antibiotics of different classes is summarized in Table 3.

Table 1: Prevalence of staphylococci in nosocomial carriers according to the personal characteristics (n= 60)

	Staphylococcus aureus		Other staphylococci		
Characteristics	No.	%	No.	%	Prevalence ratio*
Gender					
Male	21	35.0	5	8.3	4.2
Female	22	36.7	12	20.0	1.8
Total	43	71.7	17	28.3	2.5
Age					
< 30 y	23	38.4	11	18.3	2.1
30-40 y	14	23.3	5	8.3	2.8
> 40 y	6	10.0	1	1.7	6.0
Total	43	71.7	17	28.3	2.5
Job					
Physicians	13	21.7	4	6.6	3.3
Nurses	25	41.7	13	21.7	1.9
Others	5	8.3	-	-	-
Total	43	71.7	17	28.3	2.5

*Prevalence ratio for comparison of *Staphylococcus aureus* isolates vs. other staphylococci isolates

Table 2: Antibiotic susceptibility profiles of two *Staphylococcus aureus* isolates

Antibiotics	*S. aureus* 1	*S. aureus* 2
Chloramphenicol	S	S
Clindamycin	S	S
Erythromycin	S	R
Lincomycin	S	R
Oxacillin	I	R
Penicillin	S	R
Co-trimoxazole	S	S
Vancomycin	S	S

S: Sensitive, I: Intermediate, R: Resistant

Table 3: Antibiotics susceptibility patterns of Methicillin resistant- *Staphylococcus aureus* (MRSA)

	Sensitive		Intermediate		Resistant	
Antibiotics	No.	%	No.	%	No.	%
Chloramphenicol	25	86.2	1	3.5	3	10.3
Clindamycin	23	79.3	2	7.0	4	13.8
Erythromycin	6	20.7	14	48.3	9	31.0
Lincomycin	4	13.8	3	10.3	22	75.9
Oxacillin	1	3.5	3	10.3	25	86.2
Penicillin	2	6.9	1	3.5	26	89.7
Co-trimoxazole	14	48.3	0	0.0	15	51.7
Vancomycin	29	100.0	0	0.0	0	0.0

Total number of *S. aureus* strains = 29 MRSA

The drug resistance patterns of MRSA isolated from nosocomial swabs was found to be highly variable. Out of the 29 MRSA strains, the resistance to penicillin was 89.7%, followed by oxacillin (86.2%), lincomycin (75.9%), co-trimoxazole (51.7%), erythromycin (31.0%), clindamycin (13.8%) and chloramphenicol (10.3%). On the other hand, all MRSA strains tested were recorded as sensitive to vancomycin (100%).

DISCUSSION

Methicillin resistant *Staphylococcus aureus* (MRSA) are prevalent worldwide and are an important cause of nosocomial infection, resulting in increased morbidity and mortality in the hospital settings worldwide [15-17]. Methicillin was first introduced in human medicine in the 1960s for the treatment of infections caused by penicillin's resistant *S. aureus*, but within a few years, methicillin-resistant *S. aureus* (MRSA) emerged [18]. Methicillin and its derivatives were indicated for treatment of staphylococcal infection due to penicillinase production. However, these bacteria have become a major concern with the extraordinary ability to adapt to antibiotics stress. MRSA were gradually reported [5, 7, 12, 19].

In our study, the prevalence of MRSA was found to be 48.3% of the investigated *S. aureus* isolates. In India, a higher prevalence rate (54.9%) of MRSA was recorded [12]. In comparison with our results, higher prevalence rates of MRSA were recorded in other previous studies [6, 20]. According to the gender, our data revealed that the prevalence of *S. aureus* did not significantly differ between both sexes. Persons younger than 30 years of age seemed to be *S. aureus*-carriers more than those older than 30 years (prevalence rate = 1.15). These findings agreed with those obtained by other investigators who reported that MRSA groups did not significantly differ between the sexes and persons in the community-acquired MRSA USA 300/USA 400 group were more likely to be younger than 50 years of age [21].

In this investigation, the resistant rate to different antibiotics among MRSA strains was higher than those sensitive to methicillin and this phenomenon was reported elsewhere [22]. In addition, high multidrug resistance rates were observed in our MRSA isolates. In this concern, multidrug resistance rates of MRSA isolates reached 55 % were recorded [12]. Other published reports have indicated a closely similar or higher percentage of resistant [11, 23]. Overall, the sensitivity of MRSA isolates were significantly more resistant to different classes of antibiotic compared to the methicillin sensitive staphylococci.

CA-MRSA isolates were reported to be susceptible to numerous antimicrobial drugs. The bacteria were sensitive to 13 tested antibiotics. Among them were chloramphenicol, lincomycin and vancomycin [24].

Most of the microbial resistance which is now making difficult to treat some infectious diseases is of genetic origin and transferable between species and genera of bacteria, due to extensive use of antimicrobial drugs [25]. Thirty percent (30%) of nosocomial isolates of *S. aureus* are resistant to methicillin and 17% of enterococcal blood isolates are resistant to vancomycin.

Infections involving methicillin-resistance *S.aureus* (MRSA) or vancomycin-resistance entercocci (VRE) for example; are associated not only with increased risk of mortality but also longer hospitals stays and more costly life saving interventions. The most significant problems of drug resistance in Gram+ve bacteria are oxacillin resistance, vancomycin resistance, penicilin resistance in *S. pneumoniae* and fluoroquinolone resistance in *S. pneumoniae*. Oxacillin, like penicilin, methicillin and nafcillin, is a member of the β-lactam group of antimicrobial agents. It works by binding to transpeptidases that catalyze the crosslinking of subunits comprising the peptidoglycans cell wall, so the bacterial cell is unable to protect against differences in osmotic pressure between inside and outside of the cell, which result in cell lysis. With the exception of penicilin, other antimicrobials are resistant to bacteria encoded β-lactamase enzymes that clear a β-lactam ring thereby inactivating the agent [26].

An earlier investigation concerned with the prevalence of MRSA and MRCoNS (methicillin-resistant and coagulase-negative staphylococci) and their rate of resistance to different antistaphylococcal antibiotics used broadly for treatment. Out of 235 isolates, the frequency of MRSA by oxacillin screen agar method, 54.2% strains were MRSA. The rate of multidrug resistance observed was 69% for MRSA. Antibiotic resistance pattern of these isolates was high against penicillin 100%, erythromycin 83%, co-trimoxazole 82% for MRSA strains. In order to test beta-lactamase production, 83 % of MRSA isolates were beta-lactamase positive [12]. Other investigators recorded similar rates of β-lactamase production by MRSA [7, 27].

Vancomycin remains the drug of choice in the treatment and prophylaxis of infection caused by organism resistance to β-lactam antibiotics. This antimicrobial inhibits peptidoglycans biosynthesis binding to the D-alanyl-D-alanine peptide subunit and is, unaffected by bacterial β–lactamases [26].

However, the emergence of methicillin-resistant *S. aureus* (MRSA) mutants with reduced susceptibility to vancomycin (MIC ≥ 8 μg/ml) was reported. The first reported heterogeneous resistance to vancomycin in Thailand gave an early warning for the possible emergence of vancomycin resistance in *S. aureus* in

Southeast Asia [28]. The Eurpean Antimicrobial Surveillance system (EARSS) database did not show vancomycin resistance; a few vancomycin intermediate *S. aureus* (VISA) isolates were reported from France only [29]. It was reported that no USA 300 isolate was resistant to trimethoprim-sulfamethoxazole (co-trimoxazole) or vancomycin [21]. Our study revealed that 51.7% of MRSA strains were resistant to co-trimoxazole, whereas all tested MRSA strains recorded 100% sensitivity to vancomycin. Thus, the herein obtained results are in agreement with a previous study which reported that all tested strains of *Staphylococcus* were sensitive to vancomycin [12].

Findings presented in this study indicated a high level of resistance to widely used therapeutic agents. An appropriate knowledge on the current antibiotic susceptibility pattern of MRSA is essential for appropriate therapeutic scenario.

CONCLUSION

The emergence of drug resistance in MRSA is worrisome in the present therapeutic scenario. A regular surveillance of hospital associated infection including monitoring antibiotic sensitivity pattern of MRSA and is mandatory to controlling the spread in the hospital and strict drug policy are of importance or else the threat will increase. According to this study, vancomycin seems to be the only antimicrobial agent which shows 100% sensitivity even with multi-drug resistance. Vancomycin remains the first choice of treatment for MRSA and to preserve its value, vancomycin use should be limited to those cases where there are clearly needed. However, due to increasing of vancomycin MICs for MRSA, regular monitoring of vancomycin sensitivity and routine testing of other new glycopeptides should be carried out further.

ACKNOWLEDGMENTS

Authors would like to thank the Directors and staffs in the three Hospitals (Al-Aqsa, Al-Olafy and Al-Thowra hospitals) at Hodeidah city, Yemen for excellent cooperation and providing swabs. Members of Microbiology Lab., Faculty of Medical Sciences, Hodeidah University are also gratefully acknowledged for technical assistance. Deep thanks are due to Mr. Omar A. Sinan for helping in the preparation of the manuscript.

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