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Prevalence of Methicillin–Resistant *Staphylococcus aureus* (MRSA) in the Community of Al-Majmaah/Saudi Arabia and Possibility of Resistance to Vancomycin and other Antimicrobial Agents

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Abstract To determine the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) strains among clinical isolates collected from 2 tertiary hospitals in Al- Majmaah, Saudi Arabia and to test the possibility of resistance to vancomycin and other antimicrobial agents. A total of 106 *S. aureus* clinical isolates were collected during a period of 6 months. The sensitivity patterns of these isolates were determined using the Kirby-Bauer disc diffusion method. The prevalence of MRSA among *S. aureus* isolates was (43.4%) 46/106. Among 46 MRSA isolates, 82.61% showed multidrug resistance to Ciprofloxacin Tetracycline, Chloramphenicol, Kanamycin and Erythromycin. 82.6 % of MRSA were sensitive to gentamicin. No resistance to Vancomycin. The rate of MRSA resistance in this study was higher than what had been reported in other areas of Saudi Arabia and other countries and the majority shows multidrug resistance .

Keywords Methicillin, *Staphylococcus Aureus,* Resistance , Vancomycin, Antimicrobial Agents

1. Introduction

Staphylococcus aureus is a major pathogen associated with serious community and hospital-acquired diseases associated with high morbidity and mortality worldwide. The rapid evolution of antibiotic resistance in *S. aureus* is of considerable concern. Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *Staphylococcus aureus*. In 1961 the first *S. aureus* isolates that had acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) were reported from the United Kingdom[1] and were soon recovered from other European countries, and later from Japan, Australia, and the United States. MRSA is a major pathogen in hospitals worldwide and has become gradually more difficult to treat due to increasing resistance[2,3]. There is a wide range in the prevalence of MRSA strains between different countries and even between hospitals in the same country. The extent of the spread of these organisms from hospital to hospital also shows variation[4]**.** There are several mechanisms responsible for methicillin resistance. The most important is the production of the penicillin-binding protein PBP2a encoded by the *mecA* gene. In addition, hyperproduction of β –lactamase and modified drug affinities of the usual PBPs are considered as minor resistant mechanisms[4,10,11]**.**

Vancomycin and teicoplanin are glycopeptides with significant activity against gram positive bacterial pathogens [12] and over the past two decades, vancomycin has been considered the drug of choice for MRSA infections. Unfortunately, in 1996, the emergence of vancomycin-resistant *S. aureus* (VRSA) in Japan and thereafter from USA and other countries has caused additional concern[14,15]. Therefore, infections caused by these resistant strains are very serious and difficult to treat.

The aim of this study was to determine the prevalence of MRSA strains isolated from nosocomial infections and their antimicrobial resistant patterns including vancomycin in 2 tertiary hospitals in Majmaah, Saudi Arabia.

2. Materials and Methods

2.1. S. aureus Isolates

From October 2011 – April 2012, a total of 106 non replicate *S. aureus* clinical isolates from hospitalized patients were collected from 2 tertiary hospitals in the community of Almajmaah (Table 1). Infections were defined as hospital acquired when the patient had been hospitalized for more than 48 h[16]. The identity of these isolates was confirmed using colonial morphology on blood agar plates (Oxoid, UK), growth and fermentation of manitol in Manitol Salt agar

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(BBL ™), gram stain and positive catalase and coagulase tests (Murex Diagnostic Ltd., UK).

2.2. Antibiotic Susceptibility Testing

Susceptibility testing was performed using Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates (Oxoid) as recommended by the Clinical Laboratory

Standards Institute (CLSI), formerly NCCLS[17]. Briefly, the tests were performed by diluting colonies of *S. aureus* grown overnight on sheep blood agar (Oxoid) in normal saline equivalent in density to 0.5 McFarland barium sulfate standard unit. The entire surface of the Mueller-Hinton agar plate was covered with the required inoculum, and the plates were allowed to dry for 5 minutes before the antibiotic discs were placed on the surface and incubated for 18–24 h at 37°C. Following the incubation period, the sensitivity results were determined by comparing the diameter of the zones of inhibition with CLSI standards[17]. The antibiotics tested (Oxoid) were Pencillin 10µg, Oxacillin 1 µg, Ampicillin 10µg, Ciprofloxacin 5µg, Tetracycline 30µg, Chloramphenicol 30µg, Kanamycin 30µg, Erythromycin 15µg, Gentamycin 10µg, Amoxicillin/clavulanic acid 30µg and Vancomycin 30µg. *S. aureus* ATCC 25923 was used as a control strain.

2.3. MRSA Testing

Isolates were tested for methicillin resistance by the Kirby-Bauer disc diffusion method as described above using an oxacillin disc (1µg) on Mueller-Hinton agar supplemented with 4% NaCl and incubated at 35° C for 24 h. A zone of inhibition of ≥13 mm was considered as oxacillin sensitive[17]. Methicillin resistance results were confirmed by pencillin binding protein PBP2 –Latex agglutination test (Oxoid, UK) which is based on the agglutination of latex particles sensitized with monoclonal antibodies against PBP2a.

2.4. Vancomycin Resistance Testing

MRSA were tested for vancomycin resistance by Kirby-Bauer disc diffusion method as described above using vancomycin disc (30µg). A zone of inhibition of ≤14 mm was considered as vancomycin resistant[17]. Multidrug resistance was defined as resistance to penicillin and oxacillin plus 3 or more of the following agents: Erythromycin, Ciprofloxacin, Kanamycin and Tetracycline [18].

3. Results

Of the 106 *S. aureus* strains tested, 46 (43.4%) contained PBP2a and were methicillin resistant according to the disc diffusion test. Similar results were found with the latex agglutination test which further confirmed MRSA results. Rates of resistance of methicillin-sensitive *S. aureus*(MSSA) and MRSA to the other antibiotics tested are shown in (Table 3). Of 46 MRSA isolates, 82.61% were multidrug resistant.

Infections with MRSA were more common among males (60.87%) and older (≥ 50 years) than females and younger patients. Among the 46 MRSA strains, 28 (60.87%) were isolated from wounds, 8 (17.4%) from nose, 6 (13.04%) from skin abscess, 2 (4.35%) from urine and 2 (4.35%) from eye. While the 60 MSSA strains, Wound = 16 (26.67%), Skin = 12 (20%), Ear = 12 (20%), Blood = 8 (13.33%), Nose = 6 (10%), Eye = 4(6.67%), Urine = 2 (3.33%). (Table 2).

Table 1. Distribution of clinical specimens from which *S. aureus* was isolated

Sample source	*S. aureus* Isolates
Wound	44
Skin abscess	18
Nose	14
Ear	12
Blood	8
Eye	6
Urine	4
Total	106

Table 2. Distribution of MRSA based on clinical samples, sex and age

106 *S. aureus*		Samples	Sex	Age
MRSA	46 (43.4%)	Wound = 28. Nose = 8. Skin = 6. Urine = 2. Eye = 2.	Male = 28. (60.87%).	≥ 50 years = 26. (56.52%)
			Female= 18. (39.13%).	
MSSA	60 (56.6%)	Wound = 16. Skin = 12. Ear = 12. Blood = 8. Nose = 6. Eye = 4. Urine = 2.	Male = 16 (26.66%)	≥ 50 years = 7. (11.67%)
			Female = 44. (73.34%)	

Table 3. Antibiotics sensitivity results of 11 antimicrobial agents against 106 *S. aureus* clinical isolates (**MRSA** and **MSSA**)

No	Antibiotics	Resistance %		
		Diameter of zone of inhibition/ mm	MRSA n=46	MSSA n=60
1	Pencillin 10µg	R≤ 28	100	96.66
2	Oxacillin 1 µg	R≤ 13	100	0
3	Ampicillin 10µg	R≤ 28	100	96.66
4	Ciprofloxacin 5µg	R≤ 20	86.96	70
5	Tetracycline 30µg	R≤ 18	91.3	60
6	Chloramphenicol 30µg	R≤ 17	69.57	53.33
7	Kanamycin 30µg	R≤ 17	69.57	11.67
8	Erythromycin 15µg	R≤ 23	82.61	70
9	Gentamycin 10µg	R≤ 14	17.4	3.33
10	Amoxicillin/ clavulanic acid 30µg	R≤ 19	100	53.33
11	Vancomycin 30µg	R≤ 14	0	0

4. Discussion

In the United States and in some European countries, MRSA accounts for 10 to 40% of all *S. aureus* isolates. In Spain, the prevalence of MRSA has increased from 1.5% in 1986 to 31.2% in 2002[5,6]. In Algeria, the rate increased from 10% in 1997 to 14% in 2001[7]. In Saudi Arabia hospitals ranged from 12% to 49.4% in Riyadh and 38.9% in Makkah hospitals without any report of resistance to vancomycin[8,9]. In this study, the rate of resistance (43.4%) of *S. aureus* is much higher than what has been reported by researchers in other regions of Saudi Arabia as well as in some other Studies[8,9,19].

Microbes have genetic plasticity, which means that they have the capacity to evolve in response to their environment. The major impetus for developing resistance is selective pressure resulting from antibiotic use. The bacteria that survive are those that develop mechanisms to avoid being killed by antibiotics. Although new antibiotics can effectively treat some resistant pathogens, bacteria will eventually develop resistance to any antibiotic with time. The misuse and overuse of antibiotics drive the emergence and spread of resistance.

In Japan, the first strain of *S. aureus* with reduced susceptibility to vancomycin (VISA/VRSA) was isolated in 1996[14] and as of 2002, infections with such strains in was confirmed patients in USA[15]. Later on, the emergence of such cases has been confirmed in India[13]. hVISA isolated in some Asian countries like South Korea, Japan, Philippines, Singapore and Thailand[24,26]. All MRSA isolates in this study were sensitive to vancomycin and the majority sensitive to gentamicin, but the majority of our isolates (82.61%) showed multidrug resistance to Erythromycin, Ciprofloxacin, Kanamycin and Tetracycline.

5. Conclusions and Recommendations

The rate of MRSA resistance in this study is much higher than what has been reported in other areas of Saudi Arabia and many other international countries. Keeping in mind the increasing prevalence rate of MRSA, it is extremely important to implement a revised strategy for MRSA isolates in hospitals and in community because any delay or wrong choice of antibiotics is avoidable and to improve treatment and control.

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