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CHARACTERISTICS OF NOSOCOMIAL MRSA IN ASSIR CENTRAL HOSPITAL, ABHA, KINGDOM OF SAUDI ARABIA

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The objective of this study is to determine the characteristics of nosocomial methicillin-resistant and sensitive *Staphylococcus aureus* (MRSA & MSSA) and their minimum inhibitory concentration (MIC) to vancomycin and oxacillin. Over a six-month period a study of *Staphylococcus aureus* isolates from clinical specimens of patients with nosocomial infections in Assir Central Hospital (ACH), Abha, Saudi Arabia, between September 2003 and February 2004, was carried out. Isolation and identification of *Staphylococcus aureus* was performed using standard microbiological methods. MIC to vancomycin and oxacillin was carried out using the E-test strips. Eighty-five *Staphylococcus aureus* isolates were identified. These were made up of 39 (45.9%) MRSA and 46 (54.1%) MSSA. The MIC to oxacillin showed that 37/39 (94.9%) MRSA had MIC >256 µg/ml and only 2/39 (5.1%) had MIC of 4 and 32 µg/ml. Thirty of forty six (65.2%) of the MSSA had MIC < 0.50 µg/ml and 16/46 (34.8%) had MIC of between 0.50 -2 µg/ml. All the 85 isolates were fully sensitive to vancomycin (MIC breakpoint < 4 µg/ml). There is even distribution of sensitivity pattern to vancomycin among MRSA and MSSA isolates. 31/39 (79.5%) of MRSA had MIC of 2 µg/ml while 34/46(74.0%) of MSSA had MIC of 2 µg/ml. The prevalence of MRSA in nosocomial infections in ACH is 45.9%. Thirty-seven out of thirty-nine (94.9%) of the MRSA strains show high resistance to oxacillin (MIC > 256 µg/ml). The use of oxacillin-related drugs to treat nosocomial Staphylococcal infections in ACH should be reviewed and infection control practices should be intensified so as to stem any future increase in MRSA prevalence in the hospital.

Key words: MRSA; Characteristics; MICs; Vancomycin; Oxacillin.

INTRODUCTION

Staphylococcus aureus is one of the most common causes of both hospital and community acquired infections worldwide [1]. Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant *Staphylococcus epidermidis* (MRSE) levels vary from 5-75% world-wide [2]. Strains of *Staphylococcus aureus* which are oxacillin and methicillin resistant (historically termed methicillin resistant *S. aureus* or MRSA), are traditionally resistant to all beta-lactam agents including cephalosporins and carbapenems as well as commonly used antimicrobial agents used as treatment options on patients infected with such strains [3]. Vancomycin has been used to treat many *Staphylococcus aureus* infections, particularly those caused by MRSA [4].

Accurate detection of methicillin and vancomycin resistance can be difficult using the routine disc-diffusion technique due to the presence of two sub-populations (one susceptible and the other resistant to oxacillin) that may coexist within a culture. Although, all cells in a culture may carry the genetic information for resistance, only a small number can express the resistance *in vitro*. This phenomenon is termed 'heteroresistance' and occurs in staphylococci resistant to penicillinase-stable penicillins, such as oxacillin. Heteroresistance frequently presents diagnostic problems to clinical laboratories, because cells expressing resistance may

grow more slowly than the susceptible population. One of the simplest and reliable methods of detecting heteroresistance in staphylococci is by using the Epsilon (E)-test (AB Biodisc, Solna, Sweden).

Conscious of the limitations of our routine disc-diffusion method to detect heteroresistance in *Staphylococcus aureus* isolates in our hospital, we decided to carry out E-test MIC determinations to oxacillin and vancomycin on *S. aureus* from nosocomial infections over a six-month period, September 2003 to February 2004. Our findings are presented and discussed in this communication.

MATERIALS AND METHOD

Staphylococcus aureus isolates were obtained from clinical specimens of patients with nosocomial infections in Assir Central Hospital (ACH), Abha, between September 2003 and February, 2004. The isolation and identification of *Staphylococcus aureus* were performed using standard microbiological methods [5]. Routine sensitivity testing to Gram positive antibiotics were carried out on Mueller Hinton agar according to NCCLS guidelines [6]. Strains with zone diameter ≤ 12 mm to 1μg oxacillin disc were regarded as methicillin resistant.

E-test method

E-test strips for oxacillin and vancomycin (0.016-256 μg/ml), from AB Biodisc, Solna, Sweden, were used for the study. Mueller Hinton agar plus 2% sodium chloride was used as the test medium. The *Staphylococcus aureus* isolates were sub cultured on 5% sheep Blood agar and incubated at 35°C in ambient air for 18-24 hours. Direct colony suspension was made in normal saline to 0.5 McFarland turbidity. The resulting suspension was inoculated on to Mueller Hinton agar containing 2% NaCl, using sterile cotton-tipped swabs. The E-test strips were placed on the inoculated plates aseptically. The plates were then incubated in ambient air at 35°C for 24 hours before reading. Quality control strains were included in every batch of tests.

Isolated colonies in the inhibition ellipse and fine hazes growing beyond the breakpoint are indicative of resistance. *Staphylococcus aureus* were considered oxacillin susceptible when the MIC is ≤ 2 μg/ml and resistant when oxacillin MIC is ≥ 4 μg/ml (Quality control *S. aureus* strain, ATCC 25923, MIC range 0.25-0.50 μg/ml) and vancomycin sensitive when vancomycin MIC is ≤ 4 μg/ml or resistant when MIC is ≥ 8 μg/ml (Quality control *S. aureus* strain, ATCC 25923, MIC range 1.5-3 μg/ml).

RESULTS

Out of the 85 *Staphylococcus aureus* isolates, 39 (45.9%) were MRSA while 46 (54.1%) were methicillin sensitive *Staphylococcus aureus* (MSSA). The MSSA strains had very low MIC to oxacillin, 30 (65.2%) had MIC less than 0.50 μg/ml. In contrast, the MRSA were highly resistant to oxacillin; 37 (94.8%) had MIC > 256 μg/ml (Table 1). The MIC to vancomycin was more evenly distributed between the MRSA and MSSA strains; 31/39 (79.5%) and 34/46 (73.9%) respectively, had MIC of 2 μg/ml (Table 1).

Table 1 also shows the antibiogram to some commonly used antibacterial drugs for treating Gram-positive cocci infections. Apart from penicillin G, these relatively cheap drugs are still effective against MSSA strains. On the contrary, only vancomycin was effective against the MRSA strains.

Table 1: E-Test MIC and Antibiogram of MRSA and MSSA isolates to some routine antibiotics

	E-TEST MIC						ANTIBIOGRAM								
	Vancomycin MIC (µg/ml)			Oxacillin MIC (µg/ml)			VA n (%)	OX n (%)	PG n (%)	KF n (%)	CD n (%)	E n (%)	TS n (%)	OT n (%)	GM n (%)
	2	3	≥4	≤2	4-32	>256									
MRSA n =39	32 (82.1)	7 (17.9)	0 (0.0	0 (0.0	2 (5.1)	37 (94.9)	39 (100	0 (0.0)	0 (0.0)	0 (0.0)	4 (10.3)	0 (0.0)	1 (2.6)	6 (15.4)	6 (15.4)
MSSA n =46	39 (84.8)	7 (15.2)	0 (0.0)	46 (100	0 (0.0)	0 (0.0)	46 (100	0 (0.0)	0 (0.0)	43 (93.5)	41 (89.1)	36 (78.3	42 (91.3)	37 (80.4)	45 (97.8)

Percentages susceptible are shown in brackets ( )
Abbreviations: VA=Vancomycin; OX=Oxacillin; PG=Penicillin G; KF= Cephalothin; CD= Clindamycin; E=Erythromycin; TS = Co-trimoxazole; OT = Oxytetracycline; GM=Gentamicin

DISCUSSION

Methicillin resistant *Staphylococcus aureus* (MRSA) has become a global concern. They are common causes of both hospital and community-acquired infections [7, 8]. Both forms of infections have been reported from the Kingdom of Saudi Arabia [9, 10]. Detection of MRSA has been problematic when using different antimicrobial susceptibility testing methods [11]. Consequently, NCCLS recommended incubating isolates being tested against oxacillin at 35°C for a full 24hours before reading [6]. Similar problem is encountered when testing *S. aureus* for vancomycin susceptibility. One way of getting round this problem is by using the E-test method (AB Biodisc, Solna, Sweden).

The *S. aureus* vancomycin MIC breakpoint is 4µg/ml. Most susceptible strains have MIC between 0.5-2 µg/ml. In contrast, *S. aureus* isolates for which vancomycin MICs are 8-16 µg/ml are classified as vancomycin-intermediate and isolates for which vancomycin MICs are >32 µg/ml are classified as vancomycin resistant. Strains of staphylococci for which vancomycin MICs are 4-8 µg/ml are not reliably detected using the disc-diffusion method even when these tests are incubated a full 24 hours.

If disc diffusion is the primary method used in a laboratory for testing vancomycin, laboratories should also use a supplemental testing method such as vancomycin screen plate or MIC method. In our study, 76.5% of all our isolates (79.5% of MRSA and 74% of MSSA), have vancomycin MIC of 2 µg/ml while 16.5% have MIC of 3 µg/ml. This is a welcome development from the therapeutic perspective, confirming the absence of vancomycin resistant *S. aureus* in our hospital. It however calls for caution and constant vigilance especially when vancomycin-resistant enterococci have been reported from this hospital [12].

The results of the oxacillin MIC however are not as cheering as those for vancomycin. The MIC susceptibility breakpoint for *S. aureus* is 2µg/ml for oxacillin. The majority of the MSSA (65.2%) have MIC <0.5 µg/ml, while about 95% of the MRSA have MIC > 256 µg/ml. An earlier report from this hospital [13] reported 61% MRSA colonization in hospital personnel (not nosocomial infections). Although at that time MIC determinations were not performed, no isolate was resistant to vancomycin by the disc diffusion method.

The results of the antibiogram of our isolates show that MRSA are completely

resistant to all antibiotics except vancomycin. The MSSA on the other hand, show appreciable susceptibility to all the antibiotics tested except penicillin G. The greatest danger posed by MRSA lies in their multiple antibiotic resistance and their ability to enter the blood stream causing bacteraemia, thus, clinicians are left with few treatment options for infections caused by these organisms and the use of relatively toxic agents. While infection control efforts are being made to identify and control those factors that facilitate the acquisition and spread of MRSA at hospital and community levels, there is an urgent need to source for alternative effective drugs besides the glycopeptides, which currently are over-used in many hospitals, in view of the looming threat of the advent of vancomycin resistant *S. aureus*.

Fortunately, some new agents such as linezolid, quinu/dalfo (Synercid), daptomycin and oritavancin are coming into clinical practice, but these are still in the investigational stage. Since the prevalence rate of MRSA is almost 50%, it is only reasonable to suggest that clinicians should use oxacillin and related drugs to treat infections caused by *S. aureus* in our hospital, only after the susceptibility to oxacillin has been determined.

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