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Evaluation of Antibiotic Resistance for (Methicillin-Resistance) *Staphylococcus aureus*

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Abstract: Methicillin Resistant *Staphylococcus Aureus* (MRSA) bacteria is one of the famous widely distributed in the world and source of multiple diseases like cellulites and endocardities in all age groups. In the present study, fifty samples were taken as nasal swabs at time of admission to the hospital. About 44% were positive for *Staphylococcus aureus* (MRSA) and 0% were Methicillin-Sensitive *Staphylococcus aureus* (MSSA). MRSA colonization in males was 10% while in females were 12% which indicate that nasal colonization of MRSA in females is higher than males. MRSA nasal colonization among patients within 0-15 years old was 2%, patients within 16-30 years old was 6%, patients within 31-45 years old was 11%, patients within 46-60 years old was 2% and patients >70 years old was 1%. Sensitivity of the all *staphylococcus aureus* isolated samples to different antibiotics was studied using kanamycin, erythromycin, methicillin and vancomycin. It was found that all of the *S. aureus* strains were resistant to methicillin antibiotic (100%) and the rate of sensitivity to other drugs was72% for kanamycin, 9% for erythromycin and 22% for vancomycin. This study confirm that kanamycin is a drug of choice so far for treatment of MRSA although, there is slight resistance.

Key words: Antibiotic resistance, methicillin, *Staphylococcus aureus*, bacteria, Kingdom of Saudi Arabia

INTRODUCTION

Staphylococcus aureus is a ubiquitous human pathogen and a common cause of invasive and life threatening infections. It is the most common cause of community-associated cellulites and endocardites and is a common cause of bacteremia. *S. aureus* strains were once nearly uniformly susceptible to semi-synthetic penicillinase-resistant β-lactams (e.g., methicillin, oxacillin, dicloxacillin, nafcillin, amoxicillin), the most commonly used class of antibiotics for skin infection. These strains were termed Methicillin-Resistant *Staphylococcus aureus* or MRSA, a term that implied cross-resistance to all β-lactams including all penicillins and cephalosporins (Brian *et al*., 2010). Recently, the epidemiology of MRSA has changed with the emergence of Community-Acquired MRSA (CA-MRSA). Reports from different parts of the world indicate that CA-MRSA has emerged as a new pathogen. *S. aureus* is a transient or persistent part of the resident flora in the anterior nacres of the population. The anterior nacres are considered to be the primary colonization site and approximately 30% of healthy people carry this bacterium. Unrecognized carriers may spread MRSA and render infection control programs futile. Three main carriage patterns have been described when individuals are repeatedly sampled in the anterior nacres for *S. aureus* over longer periods of time. The so-called non-carriers who were never reported to carry the organism; the persistent carriers who repeatedly cultured positive and the occasional or intermittent carriers who from time to time yielded positive cultures. The persistence of single *S. aureus* clones in some of the carriers confirms previous reports on the exchange of *S. aureus* strains over time in nasal carriers (Hamdan-Partida *et al*., 2010).

The widespread availability of penicillin in the 1940s offered hope that modern medicine had defeated this human scourge. Yet by 1960, about half of *S. aureus* strains were resistant to the antibiotic. Fortunately, a new antibiotic, methicillin could treat most of these infections. Widespread resistance to methicillin eventually developed and by 1996, about one third of *S. aureus* strains were no longer susceptible to this drug. This led to the widespread use of the antibiotic vancomycin to treat *S. aureus* infections. In 2002, the first strains of *S. aureus* fully resistant to vancomycin were identified. Several new classes of antibiotics have been recently approved for use to treat multi drug resistant

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S. aureus. Based on past history, however it is likely to be only a matter of time before significant resistance to these drugs develops (Guilfoile *et al*., 2007). MRSA continues to cause outbreaks in many hospitals and nursing homes and is endemic in others. Some MRSA strains are resistant to all available agents except the glycopeptides antibiotics and isolates with mutations conferring low-level resistance to glycopeptides have already been reported (Greenwood, 2000).

Since, MRSA was first reported, it has become endemic in hospitals and communities around the world (Deresinski, 2005). The recent emergence of a highly virulent Community-Associated MRSA (CA-MRSA) and vancomycin-resistant, intermediate-resistant or heteroresistant *S. aureus* further heightens public health concerns (Barrett *et al*., 1968; Gillet *et al*., 2002).

Prevention of *S. aureus* infection and reduction of the spread of virulent and resistant strains are therefore of great importance. On the other hand, *S. aureus* is a member of the commensally microflora. The anterior nacres of the nose are the primary reservoirs of *S. aureus* colonization in humans and many *S. aureus* infections occur in persons with prior nasal bacterial carriage (Wertheim *et al*., 2005; Brooks *et al*., 2010). Nasal colonization is an important step in the pathogenesis of *S. aureus* infection and is a risk factor for acquiring nosocomial infection. It has been shown that 80% of nosocomial *S. aureus* bacteremia episodes in carriers of this bacterium were attributed to an endogenous source. Nosocomial *S. aureus* bacteremia was three times more frequent in *S. aureus* carriers than in non-carriers (Qu *et al*., 2010). Numerous studies of *S. aureus* nasal carriage have been carried out in various geographic regions in the United States and the Netherlands cross-section surveys of nasal carriage prevalence and transmission mechanisms in special healthy populations are beneficial in assessing risk factors associated with *S. aureus* infections (Abdelgadir *et al*., 1998; Ji, 2007).

Nosocomial infections cause significant patient morbidity and mortality. The mode of entry of MRSA in the hospital might be on admission of patients of MRSA infection or nasal colonization. Prevention of *S. aureus* infection and reduction of the spread of virulent and resistant strains are of vital significance. The present study is therefore suggested to: Investigate resistance of *Staphylococcus aureus* mainly MRSA among the patients at health care and nursing houses, isolate and identify Staphylococcus bacteria from the anterior nacres, skin and mouth of patients at health care units and nursing houses, collect data on age and sex and correlate antibiotic susceptibility patterns of the isolated bacteria to a number of antibiotics.

MATERIALS AND METHODS

Study population and data collection: The study was conducted at private clinic during the period of April to December 2012. Nasal swabs were collected within 48 h from 50 patients at admission and data on age and sex were also preformed. All the procedures of laboratory work were carried out in Khartoum and Shaqra University Laboratories.

Isolation, identification and preservation of bacteria: Culture samples were obtained from anterior nacres, skin and mouth using swabs; the swabs were pre-moistened by normal saline and then inserted into a single nacre (approximately 1 cm) and rotated also in the skin and mouth (Cheesbrough, 2000). Isolation attempts were made on all samples on the same day of collection. Each tube was labeled and taken to the laboratory within 2 h of arrival. At the laboratory each culture sample was direct-plated onto Manitol salt agar. Swabs were placed on the plates and incubated at 37°C for 24 h. *S. aureus* was identified by gram stain, biochemical, i.e., catalase, slide coagulase test and DNAse tests (Rollins *et al*., 2003).

Application of sensitivity discs: Antibiotics susceptibility was tested by the disc diffusion technique according to criteria of the national committee for clinical laboratory standard (NCCLS, 2004) using Muller Hinton agar medium. The tested antibiotics were kanamycin, erythromycin, methicillin and vancomycin and the examination for zones of inhibition of bacterial growth around the respective discs was determined (Table 1, 2 and Fig. 1).

Interpretation of inhibition zone sizes: Using the interpretative chart, interpretation of the zones sizes of each antibiotic against each organism was done as resistant, intermediate/moderately sensitive and sensitive (susceptible) (Table 3 and Fig. 1).

Table 1: Biochemical reactions of Gram-positive isolated species

Bacterial species	No. of samples	Coagulase test	Catalase test	Manitol fermentation test	DNAse test
Staphylococcus aureus	22	+	+	+	+
Staphylococcus epidermidis	19	-	+	-	-
Gram +ve rods	9	-	+	-	-

Table 2: Sensitivity tests (measurement of zone inhibition (mm) for *S. aureus* aganist different certain antibiotics

	Antibiotics			
Samples	Kanamycin	Erythromycin	Methicillin	Vancomycin
1	12.0	0.0	0.0	1.5
2	21.5	27.5	0.0	18.0
3	17.0	23.5	9.0	17.5
4	15.0	10.5	4.5	10.5
5	12.5	10.5	0.0	21.0
6	0.0	0.0	0.0	22.5
7	21.5	6.5	0.5	9.0
8	0.5	8.5	0.5	8.5
9	22.5	9.5	0.0	7.5
10	24.0	8.5	0.0	9.5
11	23.5	8.5	0.0	6.5
12	21.5	8.0	0.5	0.0
13	23.5	11.0	0.0	9.0
14	25.0	10.5	0.5	10.5
15	21.5	9.5	0.0	14.5
16	22.5	9.5	0.0	0.5
17	20.5	11.5	1.5	0.5
18	21.0	9.5	1.5	2.0
19	21.5	11.5	0.5	13.0
20	21.5	11.5	0.0	11.0
21	19.5	12.0	1.5	9.0
22	15.5	10.0	2.0	7.0

Table 3: Percentage of resistant and sensitive *Staphylococcus aureus* isolates to different antibiotics

	Disk	Resistant		Intermediate		Sensitive	
Antibiotic	potency (mcg)	No.	Percent	No.	Percent	No.	Percent
Kanamycin (K)	30	4	18.0	3	13.0	16	72
Erythromycin (E)	15	20	90.9	0	0.0	2	9
Methicillin (M)	5	22	100.0	0	0.0	0	0
Vancomycin (VA)	30	11	50.0	6	27.2	5	22

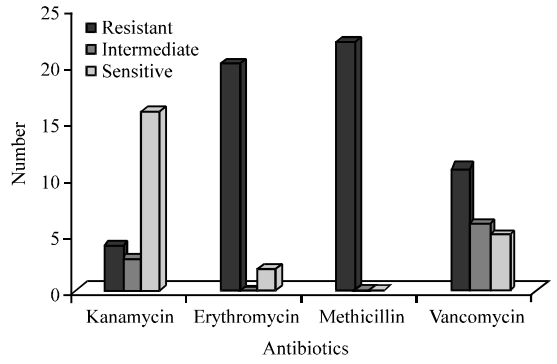

Fig. 1: *Staphylococcus aureus* antibiotic resistance for certain antibiotics

RESULTS

Bacteriological findings: In this study, isolation and identification of bacteria were done on samples on the same day of collection under strict hygienic condition to ensure the prevention of entrance of contaminants from surrounding environment. The total samples collected were 60 samples. The total number of Gram-positive bacteria isolated was 50 (10 were excluded because it left open for long time swabs).

Table 4: Methicllin-Resistance (MRSA) and Methicllin-Sensitive *Staphylococcus aureus* (MSSA) nasal colonization at the time of admission to the hospital by age

Age groups (years old)	MRSA	MSSA	Total
0-15	2	0	2
16-30	6	0	6
31-45	11	0	11
46-60	2	0	2
≥70	1	0	1

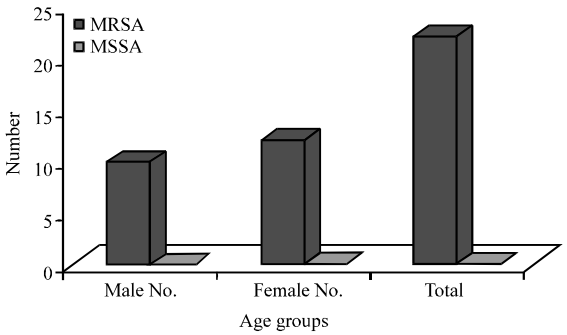

Fig. 2: MRSA and MSSA nasal colonization in males and females at time of hospital admission

The organisms isolated were Gram-positive, they were made of the genus *Staphylococcus* and they were classified as *Staphylococcus aureus* (22), *Staphylococcus epidermidis* (19), Gram +ve rods (9) (Table 1).

Biochemical reactions of the isolates: The biochemical reactions of the different organisms were similar to those described by Cheesbrough (2000) (Table 1).

Catalase test: The 22 of Gram-positive isolates were positive to the test.

Coagulase test: *Staphylococcus aureus* isolates were positive in slide test and other *Staphylococcus* species were negative to the test.

DNAse test: All *Staphylococcus aureus* isolates were positive to the test.

Prevalence of nasal MRSA colonization: *Staphylococcus aureus* isolated from the nasal swabs were 22 out of 50 patients (26%) at the time of admission. Of the 50 isolates, 22 (44%) were positive for MRSA (0%) were positive for MSSA, *Staphylococcus aureus* nasal colonization in males was 10 and in females was 12. Nasal colonization of MRSA in females is higher than males which were 12 and in male's were 10 (Table 4 and Fig. 2).

MRSA nasal colonization among patients (0-15 years old) was 2, patients (16-30 years old) was 6 and patients

Table 5: Methicllin-Resistance (MRSA) and Methicillin-Sensitive *Staphylococcus aureus* (MSSA) nasal colonization at the time of admission to the clinic by gender

Staphylococcus groups	Male	Female	Total
MRSA	10	12	22
MSSA	0	0	0

Table 6: Statistical analysis (Appendix) for antibiotics inhibition zones of antibiotics

Groups	Variance	Count	Sum	Average
Kanamycin	22	403.5	18.340910	47.009200
Erythromycin	22	228.0	10.363640	34.385280
Methicillin	22	22.50	1.022727	4.297078
Vancomycin	22	209.0	9.500000	41.547620

Source of variation	SS	df	MS	F	p-value	F_{crit}
Between groups	3308.693	3	1102.898	34.67164	1.11E-14	2.713227
Within groups	2672.023	84	31.80979	-	-	-
Total	5980.716	87	-	-	-	-

ANOVA: Single Factor (summary)

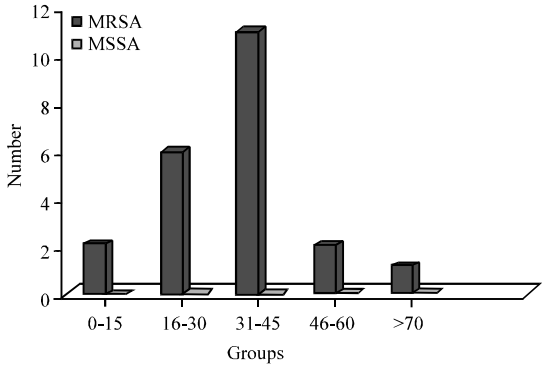

Fig. 3: Age groups of MRSA and MSSA nasal colonization

(31-45 years old) was 1, patients (46-60 years old) was 2 and patients (<70 years old) was 1 (Table 5, 6 and Fig. 3).

DISCUSSION

Nasal carriage of *S. aureus* is a complex phenomenon that affects the health care capabilities considerably, both in terms of social and economic fallouts. While the reasons behind the phenomenon are unclear, it is unambiguous that steps should be taken to control and if possible prevents the spread of the pathogen in a community as well as hospital setting (Sivaraman *et al*., 2009). With the emergence of worldwide dissemination of *S. aureus* and evidence of cross-transmission between humans, surveillance of this pathogen has become crucial. In general, *S. aureus* is present in the noses of 20-30% of healthy people (Wertheim *et al*., 2005) and in higher frequencies in crowded populations (Ellis *et al*., 2004). The *S. aureus* nasal carriage rate varies depending on the specialized populations studied. In this study, the total *Staphylococcus aureus* isolated from the nasal swabs were 22 of 50 patients (44%) at the time of admission. Hidron *et al*. (2005) in their study in Saudi Arabia explained that *S. aureus* was isolated from the nasal swabs of 20.2% patients at the time of admission which is similar to rates found in general community-based nasal carriage studies in the United States, Turkey, Australia and Malaysia (Munckhof *et al*., 2009) indicating that *S. aureus* nasal colonization is common in healthy communities all over the world. In contrast, MRSA colonization had significantly geographic distribution variations. MRSA nasal carriage rates are higher in developed countries (Qu *et al*., 2010). In the study of the 50 isolates, 22 (44%) were positive for MRSA, 0 (0%) were positive for MSSA. This is different than the results obtained by Hidron *et al*. (2005) who revealed that 1.1% had MRSA and 19.1% MSSA colonization at admission. Also, in the same study they found that a total of 53 (7.3%) of 726 patients had a nacres culture positive for MRSA and 119 (16.4%) had a nacres culture that was positive for methicillin-susceptible *S. aureus* (Hidron *et al*., 2005) (Table 5 and 6).

Resistance patterns, in addition to varying by geographic region also vary by age. In the present study, MRSA nasal colonization among patients (0-15 years old) was 2, patients (16-30 years old) was 6, patients (31-45 years old) was 11, patients (46-60 years old) was 2 and patient (>70 years old) was 1. Which is different than the results obtained by Hidron *et al*. (2005) in their study in Saudi Arabia who found that the prevalence of MRSA is high among 10-40 years age group.

In this study, nasal colonization of MRSA in females is higher than males which was 12 and in males' was 10. Also, Hidron *et al*. (2005) in their study found that more female patients were nasal colonizers of MRSA than males. Sensitivity of the all staphylococci isolated to about 5 antibiotics (other than methicillin) was studied. All of the *S. aureus* strains were resistant to methicillin (100%) and the rate of sensitivity to other antibiotics was kanamycin (72%), erythromycin (9%) and vancomycin (22%). This study confirms that kanamycin is a drug of choice for treatment of MRSA although, there is slight resistance (18%).

CONCLUSION

This study revealed that the prevalence of nasal carriage of *S. aureus* strains among patients at admission to hospital pattern is similar to that found in other studies in Saudi Arabia, Sudan and other countries. The within hand study represent a valuable source of information for researchers to study prevalence of nasal carriage of

Methicillin-Resistant Staphylococcus Aureus (MRSA) at admission to hospitals and among healthcare workers and also the local antibiotic resistance pattern which can increase the knowledge of antibiotic resistance profile.

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