African Journal of Microbiology Research Vol. 5(21), pp. 3446-3451, 9 October, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.600
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

The prevalence, antibiogram and characterisationof methicillin resistant *Staphylococcus aureus* among the patients from the Doon Valley hospitals

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Accepted 25 August, 2011

In 1880, *Staphylococcus aureus* was first discovered by a surgeon named Sir Clifton Smithin pus from surgical abscesses in Aberdeen, Scotland. Methicillin, as the first beta-lactamase resistant penicillin, was used to treat *S. aureus* infection in 1961. The first methicillin-resistant *Staphylococcus aureus* (MRSA) was identified in the United Kingdom in the same year. It appeared in the United States in 1981 among intravenous drug users. MRSA is an important agent of hospital-acquired infection. Two hundred patients who were admitted in the Doon valley hospitals were screened for nasal colonization of MRSA. Morphological and biochemical identification was also done. Out of 200 nasal samples, 97 *S. aureus* were recovered. Crome agar was used in order to detect MRSA, only 23 *S. aureus* were recovered out of total 97 isolates. Antibiotic susceptibility was tested by using the disk diffusion technique on Mueller-Hinton agar. A total of 12 antibiotics were used. Our study reveal the presence of MRSA in the Doon valley hospitals this might also be prevalent in other parts of India as antibiotic misuse is equally common there. This will help in treating this problem in referral hospitals.

Key words: Methicillin-resistant *Staphylococcus aureus* (MRSA), Doon valley, prevalence, antibiotics.

INTRODUCTION

MRSA is an important cause of hospital-acquired infections leading to high morbidity and mortality. MRSA infection places an addition burden on the patient care budget due to prolonged hospital stays. Further, it adds an inestimable human suffering, which could be avoided by taking the proper infection control precautions (Duckworth, 2003). According to a European Antimicrobial Resistance Surveillance System (EARSS) report, MRSA was responsible for 0.5 to 44% of cases of staphylococcal bacteraemia in Europe, with the highest incidence of 44% in Greece and lowest of 0.5% in Iceland (Tiemersma et al., 2004). According to National Nosocomial Infection Surveillance System (NNIS) report, 50% of hospital acquired infections in ICUs in the USA are due to MRSA (Salgado et al., 2003). Apart from high transmissibility in the hospital, MRSA also carries certain virulence factors that are associated with toxic shock syndrome, necrotizing pneumonia and skin infections (Dufour, 2002). MRSA is of greater concern in females due to its higher prevalence rate in comparison to males (Mongkolrattanothai et al., 2003; Melish et al., 2004). Nasal colonization with MRSA is a significant risk factor for hospital acquired infections. Nasal colonization of MRSA among 1 to 3% of the out patients population in the USA has been reported (Kenner et al., 2003; Jernigan et al., 2003; Leman et al., 2004), whereas nasal colonization of MRSA was absent among the out patient population in Turkey (Erdenizmenli et al., 2004). A high prevalence of nasal colonization (18.1%) among healthy community individuals has been reported in India (Saxena et al., 2003). The prevalence of MRSA nasal colonization in the community needs to be determined before instituting measures to prevent the transmission of MRSA. There is no information on the prevalence of MRSA nasal colonization among patients at the time of admission in Saudi Arabia. We determined the prevalence of MRSA and MSSA nasal colonization among patients at the time of admission to the hospital and examined possible risk factors involved in the nasal

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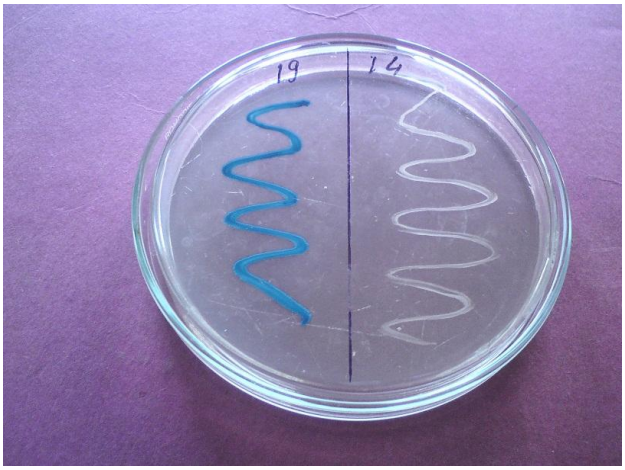


Figure 1. MRSA positive (on left) and negative (on right) on Hi-Crome MeReSa agar medium.

Table 1. The prevalence of MRSA from nasal samples of Doon Valley hospitals.

Number of samples	*Staphylococcus aureus*	MRSA
200	97	23

carriage of *Staphylococcus aureus*.

MATERIALS AND METHODS

Collection and enrichment of samples

Patients admitted in the hospitals were examined for MRSA infection. Nasal samples were taken from each patient. A Performa including age, sex, health status and relevant data were also collected from each patient. A total of 200 patients were monitored for the proposed study of nasal carrier state of Methicillin Resistant *S. aureus.* Autoclaved cotton swabs (dipped in normal saline 0.9%) were used for nasal swabbing of the anterior nares of the patients. The swabs were rubbed very well by rotating 5 times over the inner wall of the ala and nasal septum and immediately processed for culture and isolation.The nasal swabs collected were cultured on Mannitol Salt agar (selective medium for *S. aureus*) within one hour after collection by spreading as per the conventional technique. The culture plates were incubated at 37°C for 24-48 h in the incubator.

Identification of *Staphylococcus aureus*

The suspected *S. aureus* yellow colored colonies showing Mannitol fermentation were selected and subjected to Gram staining and sub-cultured into nutrient agar slopes. The isolates showing gram-positive cocci in clusters were subjected to Catalase test, DNase, Coagulase test by slide and test tube technique using undiluted and 1:5 diluted human plasma, respectively.

Identification of MRSA by "Crome agar" plate method

For the identification of the MRSA among the isolates of *S. aureus*, the Hi-Media (India) made HIMEDIAHiCrome MeReSa Agar Base (M1674) was used. The media was prepared by mixing 41.65 gms of the media into 500 ml of the distilled water. The medium is cooled to around 50-55°C and MeReSa Selective Supplement (FD229) (reconstituted with 5 ml sterile distilled water into each Methicillin vials having 2.0 mg of Methicillin as per the direction of the supplier (Hi Media - India) was added and mixed very thoroughly. Soon after that the medium was poured into Petri plates and cooled. After checking the plates for sterility by keeping at 37°C overnight the *S. aureus* strains were streaked onto the Hi Crome Me Re Sa agar and incubated at 35°C for 24 h. The MRSA only grew on this Hi Crome Me Re Sa agar, while the MSSA was inhibited on the same agar plate. All cultures showing bright blue colored growth were taken as MRSA positive strains, while all others are recorded as MSSA strains.

Antibiotic susceptibility testing

All nasal isolates of *S. aureus* were subjected to *in vitro* anti-microbial testing method on Muller-Hinton agar containing 2-3% NaCl, using 2-hour-old nutrient broth culture and HIMEDIA make antibiotic discs as per the method described by Kirby and Bauer (1966). The zone of inhibition around the discs were measured and interpreted as sensitive, moderately sensitive and resistant using the interpretation chart supplied by the antibiotic disc manufacturers (HIMEDIA, Mumbai).

Detection of β-lactamase production

The production of β-lactamase enzyme in MRSA was detected by performing double-disc synergy test (DDST) that employs a combination of piperacillin and piperacillin-tazobactam. A total of 23 clinical isolates of MRSA were selected for the study. DDST detected β-lactamase production in 19/23 of the isolates.

RESULTS

Out of 200 nasal samples, 97 *S. aureus* were recovered and were further subjected to biochemical testing. The detection of MRSA among the isolates of the *S. aureus* was carried out using the Hi-Crome MeReSa agar medium.

A total of 97 *S. aureus* strains were tested on this medium. The strains were inoculated on this special medium and incubated at 35°C for 24 h. The cultures showing bright blue color were taken as MRSA positive and the color less growth were recorded as MSSA strains (Figure 1). A total of 23 *S. aureus* were found to be MRSA.The prevalence of MRSA from nasal samples of Doon Valley hospitals was 23.71%. The details of the results are given in Table 1.

Maximum MRSA positive strains were found among the females than the males (60.86 and 39.13 %, respectively). The details of the results are given in Table 2 and Figure 2.

Antibiogram

We have tested 14 different types of antibiotics for the susceptibility pattern of MRSA isolates on Mueller-Hinton agar (MHA) plates (Bauer, Kirby, Sherris and Turck,

Table 2. Prevalence of the MRSA in different sexes.

Gender	MRSA	Percentage (%)
Male	9	39.13
Female	14	60.86

Table 3. Antibiogram of MRSA.

Antimicrobial agent	Disc potency (µg)	Zone diameter (mm)		
		Resistant (%)	Intermediate (%)	Susceptible (%)
Ciprofloxacin	10	78.26	21.73	0.01
Amoxicillin	10	34.78	4.34	60.86
Cephalexin	30	60.86	4.34	34.78
Cloxacillin	5	73.91	17.39	8.69
Methicillin	5	100	-	-
Cefotaxime	10	100	-	-
Oxacillin	**5**	86.95	-	13.05
Gentamicin	50	34.78	39.13	26.09
Kanamycin	5	86.95	13.04	0.01
Tetracycline	10	34.78	52.17	13.04
Chloramphenicol	10	52.17	43.47	4.36
Amikacin	10	91.30	4.34	4.36
Vancomycin	30	-	4.34	95.65
Erythromycin	15	56.52	43.47	-

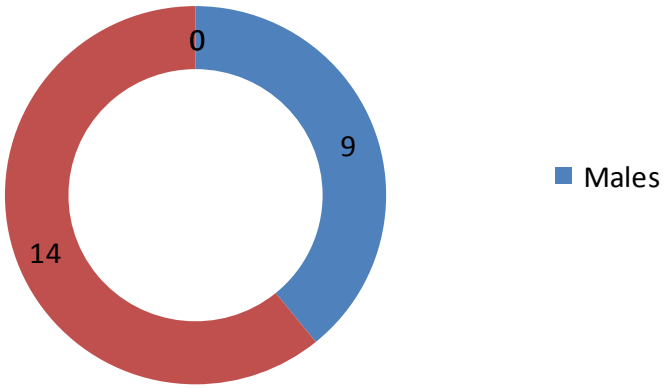


Figure 2. Prevalence of the MRSA in different sexes.

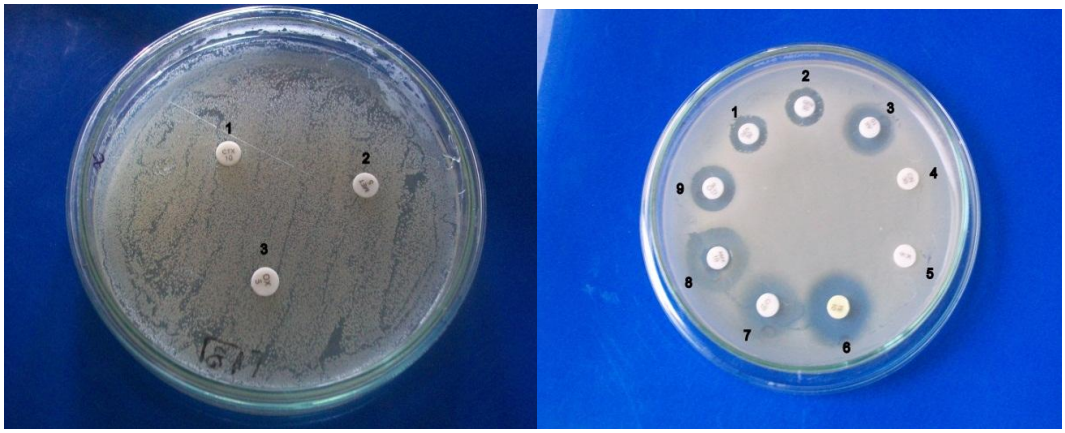
1966). The drug resistance patterns of MRSA isolated from clinical specimens and carrier screening samples were found to be highly variable. Almost all the MRSA strains (91.3%) screened from nasal samples were resistant to Amikacin, 86.95% to kanamycin and Cloxacillin, 78.26% to ciprofloxacin, 56.52% to erythromycin, 52.17% to chloramphenicol, and 34.78% to both tetracycline and gentamycin. In general, all MRSA provided were multidrug resistant (Table 3).

The detection of beta-lactamases was carried out with the MRSA isolate using agar double disc diffusion method (Figure 3). The results obtained are given in Table 4 and Figure 4.

DISCUSSION

Methicillin resistant *S. aureus* has become an enormous problem for health care providers because it is hard to treat and is sometimes called super bug. Multiple studies have been carried out on growing concern over multidrug resistance including India. MRSA is becoming a problem in paediatric population including hospital setting. The previous inclination of MRSA is in high intensity in the surgical and intensive care services, where antibiotic usage is the greatest. According to our study, there is high occurrence of MRSA in surgical wound infection, due to overcrowding, workload, and understaffing of wards. The MRSA could be prevented by identifying and screening MRSA carriers inside high-risk wards.

A study from Eritrea revealed low MRSA (9%) prevalence (Teclu and Nazik, 2009) which is less than the prevalence observed in our study. The rate of prevalence of MRSA isolates have increased over the years as reported by a study where they found 85.9% MRSA in 2003, decreasing to 57.8% in 2005 and again increasing to 90.8% in 2006 (CLSI). In our study the frequency of MRSA is 23% which is less than that reported from



A B

Figure 3. Antibiogram of MRSA.

Table 4. β- Lactamase production.

Total samples	β-lactamase production	Percentage
23	19	82.60%

Table 5. MRSA prevalence in different age groups.

Age group	MRSA	Percentage (%)
0-35	4	18
35-55	7	30
55 and above	12	52

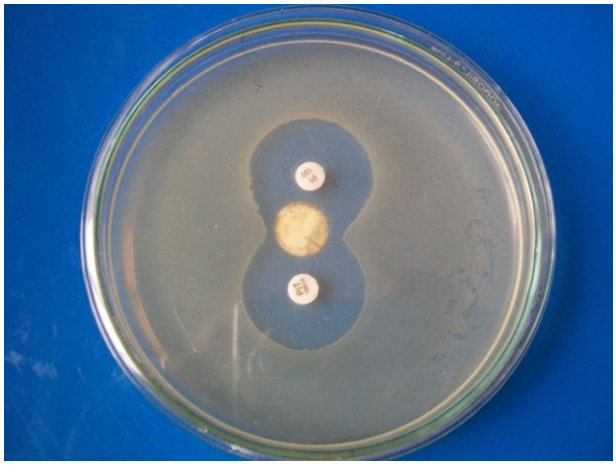


Figure 4. β- lactamase production shown by MRSA stains.

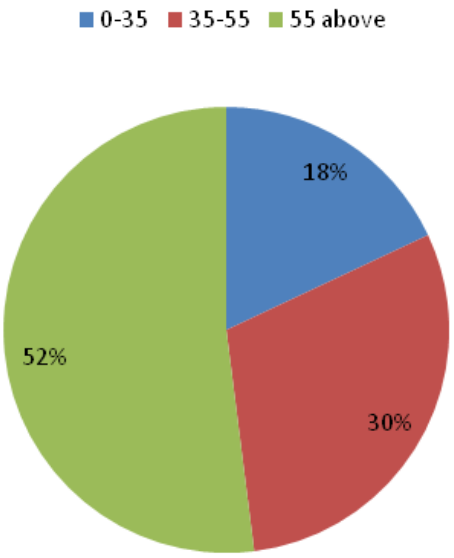


Figure 5. Prevalence of the MRSA in different age groups.

Karnataka (77.9%), Delhi (44%) and Uttar Pradesh (38.44%) (Naseer et al., 2010, Tyagi et al., 2008 and Tiwari et al., 2008). Frequency of MRSA in our study is comparable with another study done in Kashmir, where MRSA prevalence was 23.9% (Ahmed, 2009).

Regarding the carriage rate in relation to the age group, the prevalence of the MRSA carriage in our study was different than reported by other workers (Chaudhary et al., 2007). A much higher prevalence rate was seen in (52%) old persons, that is, more than 55 years than (30%) those aged 35-55 years (Table 5 and Figure 5).

A total of 23 patients who developed MRSA infection/colonization were evaluated in our study. Out of which 9 were males (39.13%) and 14 were females (68.9%). A higher prevalence rate was seen infemales than in the males which are similar to studies conducted by other workers (Chaudhary et al., 2007).

A majority of the MRSA isolates showed multiple drug resistance and were fully sensitive to vancomycin only. A majority of the strains were resistant to Oxacillin,

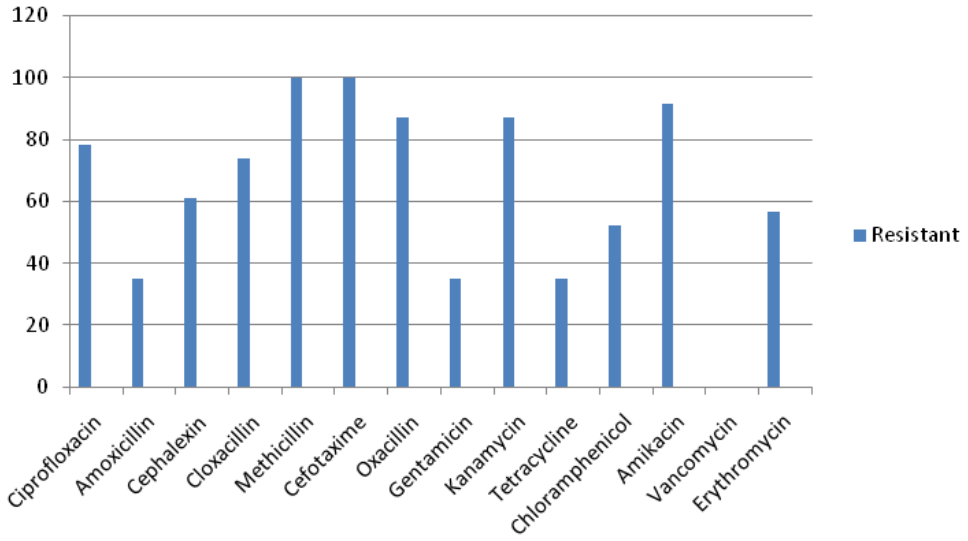


Figure 6. Bar graph showing different Antibiotic resistant pattern of MRSA.

Ciprofloxacin, Kanamycin and Amikacin (Figure 6).

Similarly, the β-lactamase production rate observed was 83% in MRSA which have been shown in other studies (Paradisi et al., 2001).

Conclusion

MRSA is a persistent and ever growing problem for healthcare institutions, and HA-MRSA adds another degree of complexity. Minimizing the emergence of this organism and its spread remain to be the challenges that need to be addressed. In conclusion, a high rate of the carriage of *S. aureus*in this hospital, with a large proportion of strains being resistant to penicillin and the isolation of MRSA strains from these carriers calls for the periodic surveillance of nosocomial infections due to *S. aureus* and other important bacterial pathogens. The usual hygienic methods such as hand disinfection after each contact with patients and the use of masks when appropriate must be performed by all workers in hospitals to protect the patients from nosocomial infections. Alcohol hand rub must be placed at every bedside in hospitals and promotional materials must be used to remind health workers and visitors to use the hand rub.

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