Research Journal of Biological Sciences 5 (2): 150-154, 2010
ISSN: 1815-8846

Nasal Carriage of Methicillin-Resistant *Staphylococcus aureus* Isolates from Intensive Care Unit Patients

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Abstract: Nasal carriage of the patients hospitalized in the ICU is at high risk for acquiring MRSA infections. Hence, the researchers conduct the study to evaluate the nasal carriage of MRSA isolates from ICU and to investigate resistance patterns against various antimicrobial agents. Among 327 *S. aureus* strains, 255 MRSA (77.9%) was detected. The high incidence of MRSA was seen in cardiac ICU (92.4%) followed by trauma ICU (71.9%), renal ICU (58.6%) and medical ICU (30.7%). Penicillin resistance was found to be high (92.9%). None of the isolate was resistant to glycopeptides. The *mec*A gene has been identified by PCR amplification. Monitoring and stopping the spread of MRSA in hospital environment can control the nasal carriage of MRSA.

Key words: *S. aureus*, MRSA, nasal carriage, ICU, nosocomial infection, India

INTRODUCTION

Staphylococcus aureus is one of the most important human pathogens causing skin and tissue infections, deep abscesses formation, pneumonia, endocarditis, osteomyelitis, toxic shock syndrome and bacteremia (Tenover and Gaynes, 2000). The progressive emergence and rapid dissemination of antibiotic resistance in *S. aureus* and its association with the use and consumption of antibiotics constitute a major health concern and have been considered a global crisis (Chambers, 1997; Hashimoto *et al*., 1994; Martinez and Baquero, 2000). MRSA has gradually disseminated and began causing serious nosocomial infections worldwide in the 1970 (Locksley *et al*., 1982). Methicillin resistance is due to the presence of mecA genes coding for Penicillin Binding Protein (PBP2A) with a low affinity for β-lactum antibiotics (Ito *et al*., 2001).

By the mid-1990s, MRSA had become a major problem because the strains generally exhibited multiple resistance to tetracyclines, aminoglycosides, macrolides, lincosamides and some other antimicrobial drugs and has continued to spread through new communities (Mandell *et al*., 1995; Voss and Doebbeling, 1995; Ayliffe, 1997). MRSA prevalence varies markedly between different countries and between different regions and hospitals within countries (McGowan and Tenover, 2004). Within hospitals, the ICU characteristically has higher rates of infections and increased transmission rates, high antibiotic use and large numbers of vulnerable patients (Safdar and Maki, 2002). In Indian hospitals, MRSA is one of the common causes of hospital-acquired infections and different hospitals have reported anywhere from 30-80% Methicillin-resistance based on antibiotic sensitivity tests (Anupurba *et al*., 2003). Detection of MRSA carriage is essential for the prompt implementation of barrier isolation o f colonized patients (Muto *et al*., 2003; Nijssen *et al*., 2005; Verbrugh, 2005). The aim of this study was to evaluate the nasal carriage of MRSA isolates from ICUs and to investigate resistance patterns against various antimicrobial agents.

MATERIALS AND METHODS

Study subject: The study conducted on 550 inpatients of ICU from Durgabai Deshmukh Hospital and Research Center and Osmania Hospital, Hyderabad, South India. *S. aureus* strains were isolated from nasal specimens collected from these patients after 48 h of admission to Intensive care units such as medical ICU, renal ICU, cardiac ICU and trauma ICU.

Nasal specimen collection: A swab from both anterior nares was obtained from patients. Swab was carefully inserted into each nostril so that the tip is entirely at the nasal osteum level (about 2.5 cm from the edge of the nare) and rubbing the swab 4 times around the inside of nostril by applying an even pressure and rotating the

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swab without interruption. The swabs were immediately placed in peptone water (Hi-Media, India) and kept at 4°C until inoculation.

Media and culture conditions: All clinical samples were first inoculated into blood agar and brain heart infusion agar plates (Hi-media, India) and incubated at 37°C for 24 h. The identification of isolates was done according to standard methods. Mannitol fermentation was observed by inoculating the isolates onto mannitol salt agar (Hi-media, India) and plates were incubated at 37°C for 24-48 h (Baird, 1996).

Coagulase test: Slide coagulase tests were performed by emulsifying few pure colonies of Staphylococci from blood agar on diluted plasma. Tube coagulase test were performed by diluting the plasma in freshly prepared normal saline (1:6). Three to four pure colonies were emulsified in 1 mL of diluted plasma and the tubes were incubated at 37°C. Readings were taken at 1, 2, 3 and 4 h and further incubated overnight at room temperature if no clot formation was observed (Baird, 1996).

Catalase test: The catalase test was done by transferring a small portion of the culture with a clean rod onto a side with 3% (V/V) Hydrogen peroxide (H_2O_2) which is kept under cover of a Petri plate to avoid aerosols. If the bacteria produce catalase, they will split hydrogen peroxide and oxygen will be evolved. The evolution of gas causes bubbles to form and is indicative of a positive test.

Antimicrobial susceptibility test: Susceptibility was measured by disc agar diffusion method, using the following antibiotic discs; Amikacin (Ak) 10 μg, Ampicillin (A) 10 μg, Amoxicillin (Am) 25 μg, Cephalexin (Cp) 30 μg, Cephotaxime (Cx) 30 μg, Ceftazidime (Ca) 30 μg, Chloramphenicol (C) 30 μg, Clindamycin (Cd) 10 μg, Erythromycin (E) 15 μg, Gentamycin (G) 50 μg, Methicillin (M) 30 μg, Nalidixic acid (Na) 30 μg, Norfloxacin (Nx) 10 μg, Oxacillin (Ox) 1 μg, Penicillin G (P) 10 U, Rifampin (R) 15 μg, Streptomycin (S) 25 μg, Tetracycline (T) 30 μg, Vancomycin (Va) 30 μg.

Mueller-Hinton agar plates were overlaid with the inoculum (turbidity equivalent to that of a 0.5 McFarland standard) of the *S. aureus* clinical strains. Zone diameters were measured at 24 and 48 h as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

Amplification of mecA gene: The presence of mecA gene was identified by means of PCR. The oligonucleotide primers for amplification of *mec* A_1 (5' AAAATCGATGGTAAAGGT TGGC-3') and mec A_2 (5' AGTTCTGCAGTACCGGATTTGC-3'), yielding a PCR product of 533 bp were used (Murakami *et al*., 1991). The reaction conditions were 30 cycles of denaturation at 94°C for 40 sec, primer annealing at 52°C for 45 sec and extension at 92°C for 30 sec.

RESULTS AND DISCUSSION

In this study, from the 550 patients, 327 *S. aureus* strains were identified using convectional method. All were catalase and coagulase positive. Among these 255 MRSA (77.9%) was detected. The high incidence of staphylococci and MRSA was obtained in cardiac ICU (83.8 and 92.4%) as shown in Table 1.

The most effective antimicrobial agent against *S. aureus* and MRSA is glycocpeptides and out of 327 *S. aureus* strains, no resistance was found against vancomycin. A part from glycopeptides, most effective antibiotics are chloramphenicol (9.8%), clindamycin (10%) and ciprofloxacin (19.3%). The resistance pattern of *S. aureus* isolates to different antibiotics is as shown in Fig.1.

The genotypic characterization of MRSA was done by PCR for 24 MRSA strains. The results of the amplification study and agarose gel analysis shows a PCR product of 533 bp mecA gene.

Nosocomial infections affected about 30% of patients in ICUs and are associated with substantial morbidity and mortality. Staphylococci are pathogens of special concern in ICUs (Vincent, 2003). Nasal carriage of MRSA varies in different geographical areas (Madani *et al*., 2001; Abudu *et al*., 2001; Alghaithi *et al*., 2000; Araja and Kanj, 2000; Sa-Leao *et al*., 2001). While, prevalence of carriage of Methicillin resistance is high, it is increasing in hospital environments (Alghaithi *et al*., 2000).

The development of antibiotics for treatment of bacterial infection has lead to improvement in health and elongation of life (Voss and Doebbeling, 1995). However, improper use of antibiotics creates problems such as the emergence of bacterial resistance to antibiotics (Chambers, 1997; Saroglou *et al*., 1980). Patients hospitalized in ICUs are 5-10 times more likely to acquire nosocomial infection than other hospitalized patients.

Table 1: Percentage of *S. aureus* and MRSA isolated from ICUs

Types of ICU	No. of patients (n = 550)	*S. aureus* (%) (n = 327)	MRSA (%) (n = 255)
Medical	153	30.7 (47)	44.6 (21)
Renal	140	58.6 (82)	65.8 (54)
Cardiac	111	83.8 (93)	92.4 (86)
Trauma	146	71.9 (105)	89.5 (94)

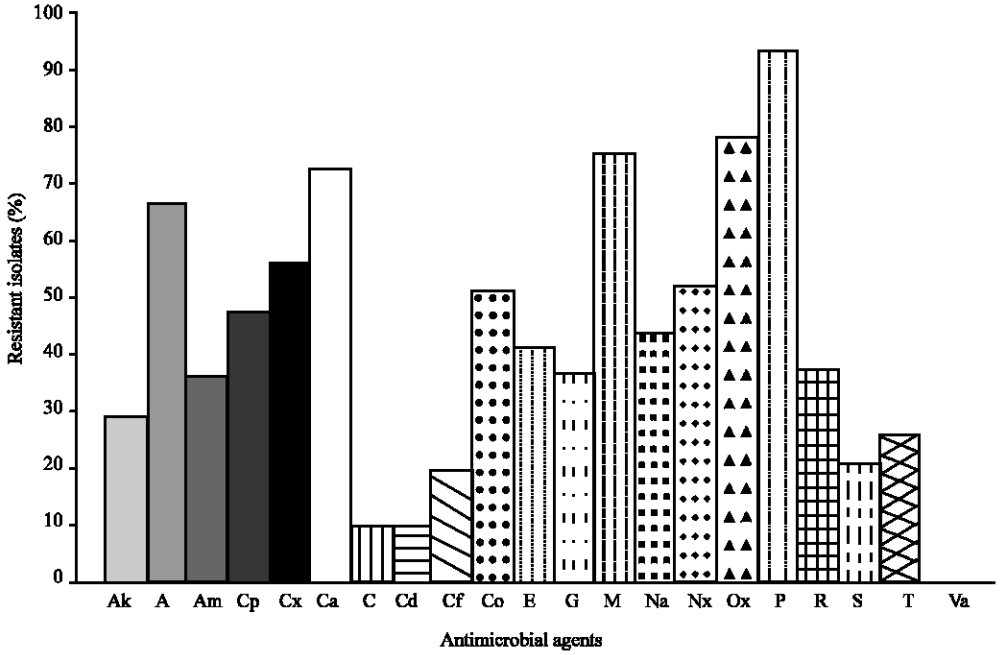

Fig.1: Resistance pattern of *S. Aureus* isolates to antimicrobial agents

Infections by MRSA require rapid and accurate diagnosis for elimination at an early stage, because these strains can be of severe damage to infected sites and may be widespread in the environment (Lee *et al*., 2004). Knowledge of the antibiotic susceptibility of the organisms isolated in the ICU helps to formulate an antibiotic policy for the ICU. This also avoids unnecessary use of broad-spectrum empirical antibiotics and prevents emergence of drug resistant bacterial strains (Weber *et al*., 1999).

The results from several studies on MRSA in intensive care units have also revealed that MRSA colonization predisposed to MRSA infection during the same hospitalization period (Garrouste-Org *et al*., 2001). The present study yields a high rate (77.9%) of MRSA nasal carriage in ICU patients. Low colonization and nasal carriage rates were reported in other studies from Turkey (56.7%) (Duran *et al*., 2006) and Saudi Arabia (38%) (Saxena and Panhotra, 2003). In this study the highest *S. aureus* isolation (83.89%) was obtained in cardiac ICU which is followed by trauma ICU (71.9%), renal ICU (58.6%) and medical ICU (30.7%). Distribution of MRSA strains in different ICU was different Methicillin-resistance rate was 92.4% in cardiac ICU. Also it was 89.5% in trauma ICU, 68.8% in renal ICU and 44.6% in medical ICU. No vancomycin resistance was encountered. Penicillin resistance was found to be 92.9%. The higher resistance was observed for oxacillin (77.9%), methicillin (75.2%), ceftazidime (72.1%). Lowest rate of resistance was seen in chloramphenicol (9.78%), clindamycin (10%), ciprofloxacin (19.3%) and tetracycline (25.9%).

The mec A gene encoding Methicillin resistance is widely disseminated among various staphylococcus species (Archer and Niemeyer, 1994). In this study, mecA gene is identified by PCR amplification and the result shows 533 bp mec A gene after analyzing of PCR product on agarose gel.

CONCLUSION

In ICUs, patients nasal carriage of MRSA must be regularly screened and give an early warning of the presence of antimicrobial-resistant pathogens. Measures should be taken to control the spread of MRSA infection including laboratory-based survivallence, isolation of the colonized and infected patients, use of barrier precautions etc. Eradicating MRSA nasal colonization among affected patients and health care personal has also been an effective control measure, with variable success.

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