ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AT SRI RAMACHANDRA MEDICAL CENTRE

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ABSTRACT:

BACKGROUND: Methicillin resistant *Staphylococcus aureus (*MRSA) is an important nosocomial pathogen causing significant mortality and morbidity . It is associated with a wide spectrum of infections ranging from mild skin and soft tissue infections to life threatening sepsis. Infected and colonised patients are the main reservoirs of infection and hand carriage by health care workers is the predominant mode of transmission .

AIM: This study was undertaken to determine the prevalence of MRSA and their susceptibility pattern at Sri Ramachandra Medical Centre.

METHODS: Forty isolates of *Staphylococcus aureus* were obtained from different clinical specimens from patients hospitalised for > 48 hours. They were screened for methicillin resistance by standard laboratory procedures. Susceptibility to beta lactams, aminoglycosides, macrolides, fluoroquinolones , glycopeptides and Oxazolidinones were determined by disc diffusion method. RESULTS AND DISCUSSION: Among the 40 *Staphylococcus aureus* isolates studied, 18(45%) were MRSA. The MRSA isolates were associated with a high degree of co-resistance to other groups of antimicrobial agents. Active screening and compliance with recommended infection control practices play an important role in the control of MRSA

Key words: MRSA, Antimicrobial tests, infection control;

INTRODUCTION:

Staphylococcus aureus is one of the most common pathogens causing a variety of infections ranging from relatively benign skin infections to life threatening systemic illness such as pneumonia, endocarditis, septic arthritis, subcutaneous or visceral abscesses[1].

Before the introducion of penicillin in the late 1940s, Staphylococcal septicemia was associated with an extremely high mortality rate. Penicillin dramatically improved the prognosis of this infection[2]. However , penicillin resistant strains were discovered shortly and penicillin became ineffective both in the hospital and community settings[3,4]. The development of beta-lactamase resistant penicillins such as methicillin and oxacillin in the early 1960s once again revolutionized the treatment of Staphylococcal infections. Within a year of the use of methicillin, methicillin resistant *Staphylococcus aureus*(MRSA) strains were reported worldwide and over the next few decades, MRSA has reached epidemic proportions[5,6].

MRSA is a resistant variant of *Staphylococcus aureus* which has evolved an ability to survive treatment with beta lactam antibiotics which includes penicillin, methicilllin and cephalosporins and to various other groups of antimicrobial agents. They are often referred to as super bugs. Most isolates remain susceptible to Glycopeptides (Vancomycin, Teicoplanin), Oxazolidinones (linezolid), Streptograminins (quinupristin-dalfopristin), and polycyclic compounds (tetracycline, tigecycline)[7,8].

MRSA is well recognised now as a major cause of nosocomial infections worldwide and these infections impose a high burden on health care resources[9].A significant concern now is the spreading of MRSA in the community, possibly because of antibiotic pressure outside the hospital and transfer from hospital settings .Community acquired MRSA (CA-MRSA) strains differ from health care associated MRSA (HA-MRSA) in that they are more frequently recovered from skin and soft tissue infecions and also cause severe pneumonia in otherwise healthy patients[10,11].

Accurate and rapid identification of MRSA and their antimicrobial susceptibility profile is therefore necessary for the selection of appropriate therapy[12]. This study was carried out to determine the prevalence of MRSA and their susceptibility pattern to various antimicrobial agents.

MATERIALS AND METHODS

STUDY DESIGN: *Staphylococcus aureus* strains isolated from cultures of specimens from patients who have been hospitalised for > 48 hours were included in the study.

Staphylococcus aureus were characterised by their morphology on Gram staining , growth characteristics and coagulase production.

PERIOD OF STUDY: July to August 2007

SOURCE OF ISOLATES: The source of the isolates were exudative specimens (pus, wound swabs, ear swabs and body fluids), blood, respiratory secretions and urine obtained from cultures of specimens from patients who had been hospitalised for > 48 hours.

SAMPLE EVALUATION: A total of 40 consecutive ,clinically significant, nonrepetitive *Staphylococcus aureus* strains were included in the study.

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METHODS: The *Staphylococcus aureus* isolates were subjected to susceptibility testing by disc diffusion technique according to the Clinical Laboratory Standards International (CLSI) guidelines with quality controls (*Staphylococcus aureus* ATCC 29213) [13].

The antimicrobials tested included ampicillin (10μg), cephalexin(30μg), cefotaxime(30μg) , cloxacillin (30μg), gentamycin(10μg), amikacin(30μg), netilmycin(30μg), erythromycin (30μg), clindamycin(30μg), ciprofloxacin (5μg), chloramphenicol(30μg), vancomycin (30μg), teicoplanin (30μg) and linezolid(30μg).

Screening for MRSA

Methicillin resistance was screened by disc diffusion method using 30μg cefoxitin disk (Becton Dickinson) .The diameter of the zone of inhibition was measured and interpretation was done in accordance with the CLSI guidelines. An isolate was considered to be a MRSA strain if cefoxitin inhibition zone diameter was < 21 mm[13].

Since *Staphylococcus aureus* can be a coloniser [6,7], special emphasis was laid on the clinical significance of all the isolates.This was done by correlating with Gram stained smear examination and ascertaining significance with the clinical history.

RESULTS

A significant proportion of the *Staphylococcus aureus* isolates were obtained from the exudative specimens such as pus(20) ,wound swabs(7), ear swab(3), Pericardial fluid(1) and drain fluid(1). Five isolates were from blood, two from urine and one from bronchoalveolar lavage(BAL).

Among the 40 *Staphylococcus aureus* isolates 82.5% showed resistance to ampicillin and cephalexin (Beta lactamase producers), 65% were resistant to gentamycin, 62.5 % to erythromycin, 60% to ciprofloxacin. Suceptibility to clindamycin, amikacin and netilmycin were 65%, 77.5% and 75% respectively.
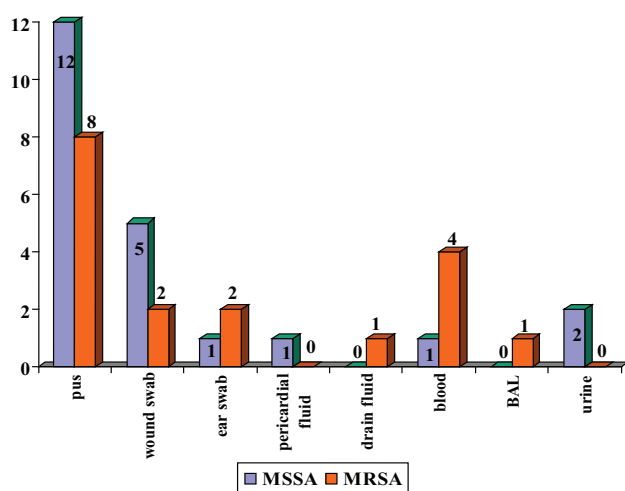

Figure-1
Isolation of *Staphylococcus aureus* from different clinical specimens

Of the 40 *Staphylococcus aureus* isolates 18 (45%) exhibited < 21 mm zone of inhibition to cefoxitin (30μg). They were considered as MRSA. All the MRSA were susceptible to vancomycin, teicoplanin, linezolid and chloramphenicol. Of the 18 MRSA isolates 66% (n = 12)were susceptible to clindamycin and 55.5% (n = 10) to amikacin and netilmycin.

The source of the *Staphylococcus aureus* and MRSA from different clinical specimens is shown in figure 1.

The susceptibility pattern of *Staphylococcus aureus* is shown in Figure 2.
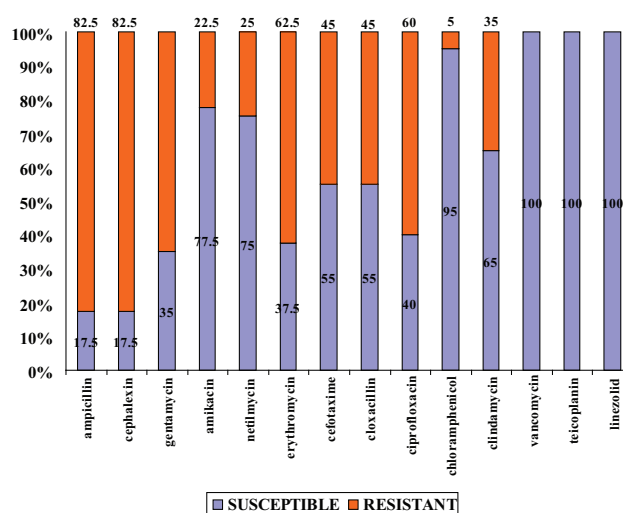

Figure -2
Susceptibility Pattern of *Staphylococcus aureus*

DISCUSSION

MRSA is a major cause of nosocomial infections worldwide.Serious endemic and epidemic MRSA infections occur globally as infected and colonised patients in the health care settings are the reservoirs. Transient hand carriage by the health care workers is the predominat mode for patient -to -patient transmission[7,14].

The risk factors for infection with MRSA that are unique to the hospital environment are well established. The emergence of MRSA as a cause of infection in the community (CA-MRSA) in patients who have never been hospitalised and who have no other risk factors for MRSA infection is a significant concern. CA-MRSA strains carry the gene for Panton -Valentine leucocidin (PVL) which has been associated with hightened virulence[11,15].

The prevalence of MRSA in this study was 45.5%.The prevalence from several centres in India as reported ranges from 20-80% [12,16,17,18]. In a surveillance study conducted simultaneously at three centres across India ,using the same methodology, the MRSA isolation rates were 27%, 42.5% and 47 % in each of the centres and the over all rate was 32%[19].

The prevalence of MRSA infections as reported by the National Nosocomial Infection Surveillance System (NNIS)

in The United States has been steadily increasing from 2.4% in 1974, 5% in1981, 29% in 1991 to 43% in 1997[2]. In intensive care units (ICU) the proportion of MRSA isolates is between 59.5%-64.4%. Furthermore, the percentage of hospitals treating patients with MRSA infections is also increasing. In a survey by the Society for Health care Epidemiology of America in 1990, 97%, reported having managed patients with MRSA in their hospitals.An understanding of the magnitude of the problem requires accurate National estimates of incidence[3].

In this study majority of the MRSA isolates were from exudative specimens.There were four isolates from blood and one from bronchoalveolar lavage(BAL). Many investigators have reported an increase in the incidence of MRSA originating from wounds (pus)[14,16,17]. Blood stream infections caused by MRSA is also frequently reported and a cause of concern especially in patients with intravascular catheterisations[14,16]. In one study bacteremia occured in 27% of patients with microbiologically documented primary sites of MRSA infection [6].In the same study the body sites that were affected by overt MRSA infections were surgical sites(31.1%), pneumonia(27%), and endovascular catheter infections(20.3%).Approximately 25% of patients with MRSA infections had bacteremia but only 6.5% had overt septic shock[6].

Carriage of *Staphylococcus aureus* in the anterior nares plays a key role in the epidemiology and pathogenesis of infection.Patients with Diabetes mellitus, those on hemodialysis, IV drug abusers, patients with skin and soft tissue infections and those with HIV infection are at increased risk for carriage of *Staphylococcus aureus* in their anterior nares [20]). Upto 80% of cases of *Staphylococcus aureus* bacteremia are due to the strain isolated from the patients anterior nares[21]. A significant reduction in the rate of infection is achieved after nasal decolonisation in surgical and dialysis patients.Therefore it is necessary to screen high risk patients for *Staphylococcus aureus* carriage because they have a greater probability of infection[7,20].

Measures to control the spread of MRSA include swab sampling of the anterior nares, isolating colonised and infected patients until complete decolonisation and implementing hygenic precautions such as handwashing and antisepsis, the efficacy of which has been well established [7,14]. Application of mupirocin (2%) in the anterior nares twice daily for 5 days is highly efficacious in eliminating *Staphylococcus aureus* in both healthy carriers and carriers belonging to high risk groups[7].

Protective measures for health care workers against MRSA include contact isolation of the patient,using protective gown, gloves, mask and googles and most importantly cleaning hands with alcoholic solution at glove removal and between patients .These measures are also of paramount importance to prevent the transmission of MRSA from patient-to patient[7,20].

Methicillin resistance in *Staphylococcus aureus* is associated with the production of an altered low affinity penicillin binding protein PBP 2a encoded by the chromosomal *mec* gene complex. Because of its low beta lactam affinity, PBP 2a can take over the cell wall assembly when the normal Staphylococcal penicillin binding protein are blocked by the beta lactam compounds[7,8]. Expression of the methicillin resistance in the laboratory setting is subject to environmental conditions (ie) temperature, pH, incubation time and salt concentration in the medium. Conditional expression of PBP 2a gene may cause ambiguity in susceptibility testing .To complicate this issue further methicillin resistance is often expressed heterogenously, masking the genetic information for resistance that the bacteria carry. Cells expressing heteroresistance grow more slowly than the oxacillin suscepibility population and may be missed at temperatures > 35°C. The microbiology laboratory has to take particular care in the identification of MRSA[7,8,12].

The conventional methods to detect MRSA in the Microbiology laboratory include oxacillin agar screen,disk diffusion using 1μg oxacillin disc or by minimun inhibitory concentration(MIC) testing[12,19].

Cefoxitin ,a cephamycin is a more potent inducer of the PBP2a and several groups of investigators have reported that the results of cefoxitin disk diffusion tests correlate better with the presence of *mec* gene responsible for methicillin resistance[5,22,23,24].

Errors in the detection of methicillin resistance can have adverse clinical consequences . False susceptibility results may result in treatment failure and the spread of MRSA if appropriate infection control measures are not applied.Conversely, false resistance results in increasing health care costs following unnecessary isolation precautions and overusage of glycopeptides. Hence accurate detection of MRSA is necessary[22].

Emergence of vancomycin and linezolid resistance among *Staphylococcus aureus* is an alarming threat. The prevalence of vancomycin intermediate *Staphylococcus aureus* (VISA) strains in India is reported to be 6.3%[25]. This has potential for increasing incidence and rapid spread, further complicating the treatment of Gram positive infections.

The limitations of this study are that ,the isolates obtained after 48 hours of admission were included in the study. This could possibly include some strains the patients had acquired before admission. Clinical history was obtained for all the isolates to ascertain its clinical significance.

However risk factor and other contributing factors for acquisition was not obtained or analysed for selection of the isolates in the laboratory.

CONCLUSION

The prevalence of MRSA is 45.5% among clinical isolates of *Staphylococcus aureus*. Active screening and compliance with recommended infection control practices play an important role in the control of MRSA. Attention

should be paid to halt the transmission of MRSA by health care workers by meticulous handwashing.

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