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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (9): 1282-1285, 2008
ISSN 1028-8880

Frequency of *mecA* Gene and Borderline Oxacillin Resistant *Staphylococcus aureus* in Nosocomial Acquired Methicillin Resistance *Staphylococcus aureus* Infections

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Abstract: The aim of the study was to determine the frequency and type of MRSA strains and antibiotic susceptibility in Al-Zahra Hospital, Isfahan, Iran. In an analytic descriptive survey in 2005 and early 2006, patients admitted to the hospital who contracted *S. aureus* nosocomial infections were enrolled in the study. All isolates were identified by the conventional laboratory tests. Minimal Inhibitory Concentration (MIC) of oxacillin on isolated bacteria was determined by E-Test method. According to Clinical and Laboratory Standard Institute (CLSI) criteria all strains with MIC of ≥4 μg for oxacillin were identified as MRSA. Intrinsic high level resistance (*mecA* positive) and borderline oxacillin resistant *Staphylococcus aureus* (BORSA) were detected by amoxicillin-clavulanate E-test strips. Strains with MIC of ≥4 μg for oxacillin and ≥8 μg for amoxicillin-clavulanate were identified as *mecA* positive MRSA. Other staphylococcus with MIC ≥4 μg for oxacillin and ≤4 for amoxicillin-clavulanate were identified as *mecA* negative MRSA (BORSA). MIC of vancomycin also was determined on isolated bacteria. Data were analyzed by SPSS version 13 and Who net version 5. Out of 134 *Staphylococcus aureus* samples which were isolated from nosocomial infections 90 (67.2%) were MRSA. Sixty seven out of 90 (74.5%) MRSA were *mecA* positive and 23 out of 90 (25.5%) were *mecA* negative (BORSA). Although most of the MRSA strains were isolated from surgical site infections, there were no statistically significant differences between types of *Staphylococcus aureus* growing from variant sites of infections. Only one (1.49) of the *mecA* positive MRSA had reduced susceptibility to vancomycin but all of the *mecA*-negative MRSA (BORSA) were sensitive to it. Because one fourth of our staphylococcus strains are *mecA* negative BORSA and there is no alternative for vancomycin against *mecA* positive MRSA and *Enterococcus* spp. in our hospital, vancomycin should be reserved only for life threatening infections due to these organisms. Thus MRSA typing should be done to choose appropriate antibiotic for optimal treatment of MRSA infections.

Key words: Methicillin resistant *Staphylococcus aureus*, nosocomial infections, *mecA*, BORSA, vancomycin

INTRODUCTION

Staphylococcus aureus plays key roles in causing various human infections. Severe nosocomial staphylococcal infections are encountered frequently and can result in significant morbidity and mortality (Kluitmans *et al*., 1997). The national nosocomial infection surveillance system identified *Staphylococcus aureus* as the most common cause of hospital acquired infections occurring between 1990 and 1996 (Rubin *et al*., 1999). MRSA is one of the most bacterial infections in many hospitals and is the major causes of nosocomial pneumonia, surgical site and blood steam infections (Boyce, 1994). Antibiotic efficacy studies have elucidated that the prevalence of MRSA has increased steadily around the world, reaching 25-50% of *S. aureus* isolates in 1997. In some countries that have taken active preventive measures such as patient isolation to avoid MRSA spread, the incidence of MRSA can remain very low (Jones, 2001). The national nosocomial infection surveillance system, reported that approximately 60% of all *S. aureus* nosocomial infections in intensive care units were methicillin resistant in 2003, denoting an 11% increase in resistance during preceding five years (NNIS, 2004). Compared with methicillin susceptible *S. aureus* (MSSA) strains, infections caused by MRSA strains are associated with longer hospital stay, higher mortality, more days of antibiotic administration and higher costs. (Abramson and Sexton, 1999; Engemann *et al*., 2003; Farr, 2004; Kopp *et al*., 2004; Blot *et al*., 2002; Cosgrove *et al*., 2003).

There are two types of methicillin resistance in staphylococci: intrinsic high level resistance and intermediate resistance (borderline resistance, borderline susceptibility, diminished susceptibility). This classification is obtained by methods detecting resistance

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patterns for example disk diffusion, kirby bauer; micro dilution and E-test (Andrew and Simor, 2001). Intrinsic high level resistance in MRSA is mediated by an abnormal penicillin binding protein called PBP2A or PBP2' encoded by chromosomal *mecA* gene (Hackbarth and Chambers, 1989). Penicillin binding proteins are bound to cell membrane and are the targets for all beta lactam antibiotics and have an important role in bacterial cell wall synthesis. PBP2a increases resistance to all beta lactam antibiotics including Penicillins, Cephalosporins, Cephamycins and Carbapenems by decreasing affinity for binding these antibiotics. Also *mecA* contains plasmids and transposons that make cross resistance to non beta lactam antibiotics such as erythromycin, clindamycin, garamycin, trimethoprim-sulfamethoxazole and ciprofloxacin (Andrew and Simor, 2001). Some strains of *S. aureus* produce large amounts of Penicillinase that hydrolyze the Penicillinase resistant Penicillins. Susceptibility tests to oxacillin or Methicillin in these strains may show reduction or borderline in susceptibility and they are named as BORSA. The mechanism of resistance of these *mecA* negative strains is production of modified PBPs 1 and 2 with reduced affinities for beta lactamase; production of a new beta lactamase; over production of PBP4 or increased beta lactamase production (Barber and Rozwadowska, 1948). The aim of the study was to determine the frequency and type of MRSA strains and antibiotic susceptibility in Al-Zahra Hospital, Isfahan, Iran.

MATERIALS AND METHODS

This analytic descriptive survey was conducted from 2005 to early 2006 in Al-Zahra Hospital; a state runs educational hospital in Isfahan, Iran. Patients who contracted nosocomial infection after hospital admission (absence of fever, signs and symptoms of infections before hospitalization and at least 48 h after hospital residence) were enrolled in the study. Specimens taken from surgical wound, lower respiratory tract and blood steam were cultured. A wound infection was identified by the presence of purulent discharge from the incision with erythematous cellulitis, induration or pain and demonstrable fluid collection noted on ultrasound after surgery. Aspirates were obtained by preparing the wound area with alcohol, inserting a sterile needle through the healing incision and aspirating fluid into a sterile syringe. For the patients with nosocomial pneumonia (fever, increase sputum production and infiltration in chest radiography), specimens from lower respiratory tract were obtained with Broncho Alveolar Lavage (BAL). For all of the patients suspected with nosocomial infections bloodstream cultured were performed too. All of the specimens were cultured in blood agar media and incubated at 35° for 18-24 h. After incubation, plates were examined for the presence of moderately sized smooth mauve colored colonies. All white colonies with or without beta hemolysis were processed to rule out *S. aureus*. *S. aureus* was identified by gram stain, catalase, slide coagulase test and growing in DNase, manitol salt agar media. MIC of oxacillin on isolated bacteria was determined by gradient concentration method (E-test; AB BIODISK Co. Sweden). Quality control was tested by staphylococcus ATCC29213. Minimum Inhibitory Concentrations (MICs) were determined by Mueller Hinton plates containing 2% NaCl which were inoculated with a direct colony suspension equivalent to 0.5 Mac Farland standards in accordance with the National Committee for Clinical Laboratory Standards. The breakpoints mentioned in the last edition of CLSI tables M_7A_6 were used to determine susceptibility and resistance. The plates were incubated at 35°C for 24 h. All strains with MIC of $\geq$4 μg for oxacillin were identified as MRSA.

mecA and BORSA were detected by amoxicillin-clavulanate strips (E-test; AB BIODISK Co. Sweden), a beta lactam plus a beta lactamase inhibitor. Strains with MIC of $\geq$4 μg for oxacillin and $\geq$8 μg for amoxicillin-clavulanate were identified as *mecA* positive MRSA. Other staphylococcus with MIC $\geq$4 μg for oxacillin and $\leq$4 for amoxicillin-clavulanate were identified as *mecA* negative MRSA (BORSA).

Sensitivity of all isolates for vancomycin also was tested by E-test (AB BIODISK Co. Sweden). Orgamisms with MIC of $\geq$32 μg for vancomycin were known as VRSA (Vancomycin Resistant *Staphylococcus aureus*).

The data were analyzed by the Statistical Package for the Social Sciences (SPSS) version 13 and Who net version 5. Comparisons were made by using Student's t-test and comparisons of the categorical data by χ^2 statistics or Fisher's test. A p-value < 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

One hundred and thirty four cultures of nosocomial infections grew *Staphylococcus aureus*. 44 (32.8%) of these cultures were positive for MSSA (MIC <4) and 90 (67.2%) for MRSA (MIC of oxacillin $\geq$4). Table 1 shows frequency of MSSA and MRSA in different nosocomial infections. Although most of the MRSA strains were obtained from surgical site infections but there were no statistically significant differences between types of *Staphylococcus aureus* growing from various sites of infection. Sixty seven out of 90 (74.5%) MRSA were *mecA* positive. Forty six out of 67 (68.7%) were from

Table 1: Frequency of MSSA and MRSA infections in different sites

	Surgical site	Abscess	Blood steam	Bal	Total
MSSA*	29 (66.0%)	6 (13.6%)	8 (18.2%)	1 (2.2%)	44
MRSA**	63 (70.0%)	9 (10.0%)	14 (15.5%)	4 (4.5%)	90
Total	92 (68.7%)	15 (11.2%)	22 (16.4%)	5 (3.7%)	134

*: MIC oxacillin <4, **: MIC oxacillin ≥4

Table 2: Frequency of *mecA* and BORSA infections in different sites

	Surgical site	Abscess	Blood steam	Bal	Total
*mec-A**	46 (68.7%)	6 (8.9%)	11 (16.5%)	4(5.9%)	67
BORSA**	17 (74.0%)	3 (13.0%)	3 (13.0%)	-	23

*: MIC oxacillin ≥4 and MIC amoxicillin/clavulanic acid ≥8, **: MIC oxacillin ≥4 and MIC amoxicillin/clavulanic acid ≤4

surgical site infections, 6 (8.9%) from abscess aspiration, 11 (16.5%) from blood stream infections and 4 (5.9%) from bronchial lavage (Table 2). Twenty three (25.5%) of MRSA isolates were *mecA* negative BORSA. Seventeen (74%) of them were from surgical site infections, 3 (13%) from abscess aspiration and 3 (13%) from blood steam infections (Table 2). There was no statistically significant difference regarding isolation of *mecA* positive and *mecA* negative BORSA from various sites of infection. Only one (1.49) of the *mecA* positive MRSA had reduced susceptibility to vancomycin but all of the *mecA* negative MRSA (BORSA) were sensitive to it.

MRSA may become a very serious infection and makes potentially dangerous complications such as bacteremia, septic shock and serious metastatic infections for example pneumonia, endocarditis, osteomyelitis and arthritis (Kasper *et al*., 2005). The National Nosocomial Infections Surveillance system (NNIS) reports an increasing incidence of MRSA. A 40% increase in resistance in 1999 was noted compared to 1994-1998 data (NNIS, 2000). The epidemiologic factors may play an important role in MRSA prevalence different regions. For example, the percentage of MRSA increased from 2.1% in 1975 to 35% in 1991 in US hospitals (Panlilio *et al*., 1992) and amounted to 36% in 1999 reports (Andrew and Simor, 2001). Scandinavian and Northern European countries report low rates of nosocomial MRSA (<2%), but many other European countries report rates as high as 30 to 60% (Voss *et al*., 1994). In this assay, the prevalence of MRSA was approximately 67.2% of *S. aureus* nosocomial infections. This variability may be due to geographic variation of MRSA strains with different virulence or colonization properties, or it may reflect differences in antimicrobial utilization and hospital infection control practices. Common factors affecting MRSA prevalence in critical hospital situations include prolonged hospital stay, use of broad spectrum antibiotics, greater number and longer duration of antibiotic use, staying in ICU or burn unit, surgical wounds, decubitus ulcers, poor functional status and proximity to another patient with MRSA, healthcare workers' hands, the environment and airborne transmission (Boyce, 1998).

Fong *et al*. (2000) reported 53.7% of staphylococcal isolates were designated as *mecA* positive that was higher than our study (50% of staphylococcal aureus and 74.5% of MRSA strains). In Levi *et al*. (2003) study 14 of 109 (12.8%) MRSA isolates were identified by conventional culture as borderline oxacillin-resistant *S. aureus* (BORSA), Gebhardt (2003) reported 15.8% frequency but in present study this frequency was 25.5% and was higher their reports.

Vancomycin should be used for treatment of *mecA* positive staphylococcus infections in high risk patients. As vancomycin resistant strains are being encountered more often, great search is in progress for finding alternative therapies, for example the newly discovered drug, linezolid which is a member of a new class of antibiotics (Oxazolidinones) is being used (Murray *et al*., 1995). Daptomycin which is a newer semi synthetic glycopeptides antibiotic, could be used for the treatment of gram positive orgamisms and VRSA (Vancomycin Resistant *Staphylococcus aureus*) (Andrew and Simor, 2001). But infections with *mecA*-negative BORSA strains can be treated with Penicillinase-resistant Penicillins for example the Methicillin group or first and second generation cephalosporins (Barber and Rozwadowska, 1948). Unfortunately resistance of staphylococci to glycopeptides is rising. In the past few years, there have been reports from the United States, Japan and several European countries indicating reduced susceptibility (intermediate resistance) of *S. aureus* strains to vancomycin and other glycopeptides (Smith *et al*., 1999). Clinical infections with these strains lead to significant morbidity and prolonged antimicrobial therapy. When vancomycin is used for a long time, bacterial cell wall proteins may be modified which is probably responsible for the emergence of Glycopeptides resistance in these isolates (Andrew and Simor, 2001). We found only one of MRSA specimens was resistant to vancomycin. Vancomycin use increases as MRSA infections become more prevalent in our hospital and all around the worlds. This results in increased selective pressure for the emergence of Vancomycin resistant orgamisms such as Vancomycin-resistant Enterococcus and Vancomycin-resistant *S. aureus*.

Because one forth of our staphylococcus strains are *mecA* negative BORSA and there is no alternative for vancomycin in treatment of *mecA* positive MRSA and *Enterococcus* spp. in our hospital, Vancomycin should be reserved only for life threatening infections due to *mecA* positive MRSA. So we recommend the use of vancomycin as empiric antibiotic therapy in suspected *Staphylococcus aureus* nosocomial infections until susceptibility results became available.

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