



Prevalence and Antibiotic Susceptibility Profile of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated from Different Clinical Samples in District Peshawar

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ABSTRACT

Purpose: *Staphylococcus aureus* is a flexible pathogen causing variety of infections in humans and other animals. The current investigation was aimed to study the prevalence rate and sensitivity profile of MRSA strains isolated from clinical samples to various antimicrobial agents.

Methods: Isolates from three different clinical specimens; pus, body fluids and blood were obtained from patients, belonging to various parts of District Peshawar of Khyber Pakhtunkhwa. Each sample was processed by conventional method using blood, MacConkey and CLED agar. *Staphylococcus aureus* was identified and confirmed by Gram staining, catalase test and coagulase test. Antibiotic susceptibility pattern of MRSA to different antibiotics was confirmed by standard Kirby-Bauer disc diffusion method.

Results: A total of 855 clinical samples were studied, out of which 45 (5.26%) were found positive for MRSA, among which 32 (71%) were from pus samples, 8 (18%) were from fluids samples and 5 (11%) were from blood samples. The frequency of MRSA strains was more in males (69%) than in females (31%). All MRSA isolates showed complete resistance to oxacillin, ampicillin and penicillin and complete sensitivity to linezolid, teicoplanin, and vancomycin. These MRSA strains also showed various degrees of resistance to other antimicrobials such as ceftriaxone (77.77%), cefoxitin (64.44%), erythromycin (82.22%), clindamycin (71.11%), co-amoxiclav (75.55%), fusidic acid (66.66%), and gentamycin (73.3%).

Conclusion: Our results show lower MRSA prevalence in Peshawar than the previous reports in Peshawar and other areas of Pakistan. However; since MRSA is multidrug resistant, proper culture sensitivity test should be carried out for proper choice of antibiotic/s to treat MRSA infections.

KEYWORDS: *Staphylococcus aureus*; MRSA; Prevalence; Antibiotic susceptibility; Peshawar

INTRODUCTION

Staphylococcus aureus is a versatile human pathogen that causes diseases ranging from relatively mild infections of skin and soft tissue to life-threatening sepsis in humans [1], and increases hospital and health system costs [2-4]. *Staphylococcus aureus* causes both the hospital-associated infections [5] and community-associated infections [6, 7]. MRSA was first reported in United Kingdom in 1961 [8], since then it is increasingly prevailing and poses a high therapeutic challenge [9]. Presently, more than 50% of the infections caused by *S. aureus* are due to methicillin-resistant strains of *S. aureus* [10]. It has been associated with many infection sites including bones and joints, lungs, and the urinary tract [11]. It also causes bacteremia which possibly leads to endocarditis and osteomyelitis [11]. Majority of MRSA affects skin and soft tissues of the body. MRSA is genetically adapted to such an extent that it poses considerable resistance to a greater variety of antibiotics like β -lactams [12].

MRSA infections have recently become the focus of intense media attention. In 2005, the United States press described MRSA as the "superbug", because it killed more people than the AIDS did [13]. MRSA was reported to have become totally resistant to gentamycin, erythromycin, penicillin, ceftriaxone and ampicillin in Trinidad and Tobago [14]. Similar results have been reported for oxacillin and penicillin among MRSA strains

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in some Latin countries of America such as Mexico (penicillin and oxacillin), Brazil (penicillin), Chile (oxacillin and penicillin) [15]. The prevalence rate of MRSA is rapidly increasing worldwide. In 11 metropolitan cities of United States, community-associated MRSA caused more than 50% of all the suppurative skin infections [16]. MRSA prevalence rate was 29% in 1997 which augmented to 74% in 2002 [9] in San Francisco.

A prospective study was performed by Hafiz *et al.*, in 2002, which reported percent prevalence of MRSA infection in some of the major areas of Pakistan like Karachi (57%), Lahore (61%), Rawalpindi-Islamabad (46%), Peshawar District (36%), Azad Jammu and Kashmir (32%), Quetta District (26%) and Sukkur (2%) [17]. Some later studies reported a significant decrease in the prevalence rate of MRSA in some of these regions including in Karachi (43% in 2007 and 38.6% in 2010) [18, 19] and Lahore (27.77% and 34.7%) [20, 21]. In this study the prevalence rate of MRSA was determined in District Peshawar and antimicrobial susceptibility of these MRSA strains isolated from blood, pus and fluids was checked. Our results show lower prevalence rate of MRSA in Peshawar as compared to previous reports.

MATERIALS AND METHODS

Sample Collection and Identification of Bacterial Isolates

Different clinical samples (including pus, body fluid and blood) were collected during August 2012 to March 2013 and brought to microbiology section of united Laboratory, Dabgari garden hospital Peshawar, Pakistan. A total of 855 samples, collected from patients throughout Peshawar District, were processed for isolation of MRSA. While collecting blood samples, a proforma was filled to collect medical/clinical information from all the individuals and obtain their written consent. No duplicate samples were included from a single subject. Blood agar, MacConkey agar and Cysteine Lactose Electrolyte Deficient (CLED) agar were used as growth media for culturing of samples. Each sample was inoculated on blood, MacConkey and CLED agar with the help of wire loop. The plates were then incubated at 37 °C for 24 hours to get the colonies growth. Positive samples were then processed further for identification using standard operating procedures. Gram staining was conducted to differentiate and identify gram-positive and gram-negative bacteria. For the confirmation of *Staphylococcus aureus*, biochemical tests including catalase test and coagulase test were performed [22].

Detection of Methicillin Resistance:

MRSA identification was carried out using oxacillin screen plates following the guidelines of NCCLS. Briefly, a suspension equivalent to 0.5 McFarland standards, prepared from each strain, was inoculated homogeneously on the entire surface of the Mueller-Hinton agar plate (Oxoid-UK) containing 4% NaCl and 6 µg/mL oxacillin, with the help of sterile swabs. All the plates were incubated at 35 °C for 24 hours. Indication of growth (>1 colony) identified the isolates as oxacillin/methicillin-resistant [23].

Antibiotic Susceptibility Testing:

Antibiotic susceptibility pattern of MRSA to different antibiotics was confirmed by standard Kirby-Bauer disc diffusion method [24]. Muller-Hinton agar was prepared and sterilized by autoclaving at 121 °C for 15 minutes. 25 mL of media was poured in 90 mm sterile petri dishes and incubated at 37 °C overnight to check sterility. All the clinical isolates of MRSA were tested for their sensitivity against antimicrobials including ampicillin, penicillin, ceftriaxone, cefoxitin, erythromycin, clindamycin, oxacillin, co-amoxiclav, fusidic acid, gentamycin, linezolid, teicoplanin, and vancomycin of standard strengths. The plates were incubated at 37 °C for 18 hours and after incubation, plates were examined for zones of inhibition and reported the organism sensitive, intermediate, resistant according to national committee for control laboratory standards.

RESULTS

Prevalence of MRSA in District Peshawar

Eight hundred and fifty five different clinical samples were processed for isolation of MRSA during August 2012 to March 2013. Among these samples, forty five samples (5.26%) were found positive for MRSA (Figure 1). Out of forty five positive samples for MRSA, thirty one (69%) belonged to male subjects while fourteen (31%) were from females. Of these forty five MRSA positive samples, thirty two (71%) were

from pus samples, eight (18%) from fluids samples and five (11%) were from blood samples.

Table 1: Distribution of MRSA isolates

Total Samples (+ve)	MRSA	Male	Female	Pus samples	Fluid samples	Blood samples
45		31 (69%)	14 (31%)	32 (71%)	8 (18%)	5 (11%)

Antibiotic Susceptibility Profile of MRSA

All MRSA isolates showed complete resistance to oxacillin, ampicillin and penicillin while complete sensitivity to linezolid, teicoplanin and vancomycin. These MRSA strains also showed various degree of resistance to other antimicrobials such as ceftriaxone (77.77%), cefoxitin (64.44%), erythromycin (82.22%), clindamycin (71.11%), co-amoxiclav (75.55%), fusidic acid (66.66%), and gentamycin (73.3%).

Table 2. Sensitivity profile of MRSA against selected antibiotics

S/No	Antibiotics	Sensitive (%)	Resistant (%)	Intermediate (%)
1	Ampicillin	00 (00)	45 (100)	0 (00)
2	Penicillin	00 (00)	45 (100)	0 (00)
3	Ceftriaxone	03 (06)	35 (77)	7 (15.6)
4	Cefoxitin	04 (08)	29 (64.4)	12 (26.7)
5	Erythromycin	05 (11)	37 (82.2)	3 (6.7)
6	Clindamycin	08 (18)	32 (71.11)	5 (11.11)
7	Oxacillin	00 (00)	45 (100)	0 (00)
8	Co-amoxiclav	06 (13)	34 (75.5)	5 (11.11)
9	Fusidic acid	09 (02)	30 (66.7)	6 (13.33)
10	Gentamycin	05 (11)	33 (73.3)	7 (15.6)
11	Linezolid	45 (100)	00 (00)	0 (00)
12	Teicoplanin	45 (100)	00 (00)	0 (00)
13	Vancomycin	45 (100)	00 (00)	0 (00)

DISCUSSION

The MRSA frequency observed in the current investigation is very small as compared to the previous reports about the MRSA pinnacle prevalence in Pakistan and throughout the world as well. There is a significant variation in the prevalence rate of MRSA in Pakistan, ranging from 2% to 61% [17][11] and throughout the world [25-27]. However MRSA offer considerable resistance to various antibiotics here in Peshawar which is comparable to the other reports about the chemotherapeutic activity of various antimicrobials against MRSA. The observed frequency of MRSA in Peshawar is less than the previous reports in Rawalpindi (60.40%) [28], in Johannesburg and Cape Town, the major cities of South Africa (33–43%) [29], in Trinidad and Tobago (12.8%) [14], in eastern Uttar Pradesh, India (54.85%) [30], in California (86.3%) [4], 69.1% in western Nepal [31], (32% in Kuwait [32], 71.4% in Sudan [33], 13% in Sri Lanka [34], 42% in Iran [35], and 33.5% in Russia [36]. The prevalence rate of MRSA in various European countries is reported as; Austria (8.8%), Belgium (23.6%), Bulgaria (33.9%), Croatia (36.7%), Czech Republic (5.9%), Denmark (0.6%), Estonia(0.9%), Finland (1%), France (33.1%), Germany (13.8%), Greece (44.4%), Hungary (7.1%), Iceland (0.5%), Ireland (41.2%), Israel (38.4%), Italy (40.9%), Luxemburg (19.2%), Malta (43.8%), Netherlands (0.6%), Poland (17.7%), Portugal (34.7%), Slovakia (10.5%), Slovenia (18.4%), Spain (24.8%), Sweden (0.8%), and united kingdom (41.5%) with 20% overall prevalence rate of MRSA in Europe [26]. MRSA prevalence rate was 29% in the session 2001-2002 that rose to 64% in the session 2003-2004 in the county jail system of Los Angeles, USA [37].

Vancomycin, Teicoplanin and Linezolid showed excellent therapeutic activity against MRSA and no resistant MRSA strain was detected against these antibiotics in this study. Similar reports have been obtained in other areas of Pakistan for Vancomycin [14, 18, 28], for Teicoplanin [18, 38] and for Linezolid [19]. MRSA has also been found as completely sensitive to vancomycin [25, 39-43], teicoplanin [25, 39, 43] and linezolid [36, 39, 42]. All MRSA strains were resistant to Ampicillin, Oxacillin and Penicillin and showed no therapeutic activity against MRSA infections. Similar results have been obtained in some of the major areas of Pakistan. MRSA is totally resistant to oxacillin and penicillin [18, 28]. MRSA has also been reported resistant

to Oxacillin and Penicillin in some of the Latin countries of America like Mexico (penicillin and Oxacillin), Brazil (penicillin) and Chile (Oxacillin and penicillin; [15]. High degree of resistance has been reported for Ampicillin in India and Nepal [44].

Gentamycin, an amino glycoside, showed poor activity against MRSA showing 73.3% resistance. Variable degree of gentamycin resistant MRSA has been reported in other areas of Pakistan as; in Karachi (96.55%) [19], in PIMS hospital Islamabad (100%) (Shagufta Hussain 2005), 67% in Kohat [45] and 76% MRSA prevails in Rawalpindi (Perveen, Majid et al. 2013. In India, a lower resistance (60%) has been reported [44].

In the current investigation, ceftriaxone, a third generation cephalosporin antibiotic, shows a high degree of resistance (77.77%), which is greater than that of Karachi where it is 45% [19], however, lower than Trinidad and Tobago where MRSA is completely resistant to ceftriaxone [14]. Cefoxitin, a second generation cephalosporin antibiotic, showed a high therapeutic value than ceftriaxone. 82.22% of MRSA strains are found resistant to erythromycin, comparable to the previous reports in Rawalpindi [28] [30] and Islamabad (Shagufta Hussain 2005), 95% resistance to erythromycin has been reported in Karachi [18] and 68.5% of MRSA have been reported resistant to erythromycin [45]. MRSA poses 93% resistance to erythromycin in Sacramento, California [4], and in rural southwestern Alaska, MRSA pose 80% resistance to erythromycin [46].

MRSA shows considerable resistance to Fusidic acid (66.66%), which is higher than 2% in Karachi [18] and (20%) in Islamabad (Shagufta Hussain 2005), however lower than the previous reports (95.7%) in Riyadh Saudi Arabia [47]. However, Fusidic acid is a drug of choice in most of the countries [36, 39, 48]. MRSA shows 71% resistance to clindamycin, a lincosamide antibiotic, a higher resistance (90%) has been reported in Karachi [18]. However, a lower resistance has been reported in UK (18%) [48], in Russia (27%) [36]

The present study shows that the prevalence rate of MRSA is higher in males 31/45 (69%) than in females 14/45 (31%). In 2009 Rahman et al, have also reported the greater percentage of MRSA in males (58%) than in females (42%) in Peshawar [49]. In Riyadh, Saudi Arabia, Baddour et al have also reported the greater frequency of MRSA in males (64.4%) than in females (35.6%) [47]. Tiemersma et al have also reported the higher rate of MRSA prevalence in males than female [26], however in India S. Sharma and A. Mall have reported the greater rate of prevalence of MRSA in females 14/25 (60.86%) than males 9/25 (39.13%) [50]. Maximum frequency of MRSA strains was found in pus samples (71%) and least number of MRSA strains was collected from blood samples (11%). Similar results have been obtained in India [51][39], in European countries [26] and in Pakistan [28].

The implementation of preventive measures is recommended for the control and prevention of MRSA infections. "Cleanliness is next to Godliness". Environment should be kept clean and hands should be regularly washed with soap. Awareness should be produced among the mob and self-medication should be discouraged. Proper antibiotic sensitivity tests should be carried out for MRSA suspected infections. Colonized/infected patients should be properly isolated/ treated. Vancomycin, teicoplanin and linezolid showed best chemotherapeutic activity against MRSA infections in this study, but their prescription should be kept limited, followed by antibiotic susceptibility tests.

CONCLUSION

The observed prevalence rate of MRSA in the people of District Peshawar is less than that of its previous reports. Vancomycin, teicoplanin and linezolid showed best chemotherapeutic activity against MRSA infections. Proper preventive measures should be introduced to avoid resistance of MRSA to these drugs and spread of MRSA in District Peshawar.

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