

MRSA: Prevalence and susceptibility pattern in health care setups of Karachi

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Abstract: This assessment aims to determine the prevalence of methicillin resistance and multidrug resistance (MDR) among the clinical isolates of *Staphylococcus aureus* and antimicrobial susceptibility profile of methicillin resistant *Staphylococcus aureus* (MRSA) to the frequently prescribed antibiotics in Karachi. Isolates of MRSA, recovered from various clinical samples were included in this prospective, cross-sectional study from Jan 2015 to June 2017. Agar diffusion method was employed according to the protocols of Clinical Laboratory Standards Institute. Out of total 346 *S.aureus* strains, the frequency rate of MRSA was 52 % (n = 180). MRSA infection was found higher among the age group 21-30 years i.e. 30% (n=54), followed by 20 % (n=36) in 31-40 years. Frequency of MRSA percentage in male and female was and 70 % and 30 % respectively. MRSA was more frequently observed in blood 20 % (n=36). MRSA showed high resistance (100 %) to Oxacillin and Cefoxitin while 25% Vancomycin resistant *S. aureus* (VRSA) isolates and 25% Teicoplanin resistance were also reported. MRSA exhibited 16% resistance to Minocycline. It was concluded that MRSA pose a challenging threat to public health in Karachi. In addition, MDR should be periodically checked to avoid treatment failure.

Keywords: Methicillin resistant *Staphylococcus aureus*, VISA, MRSA prevalence, susceptibility pattern, Kirby Bauer, Antibiogram, rationale use of antibiotics.

INTRODUCTION

Staphylococcus aureus is the most important pathogenic member of genus staphylococcus. It has overthrown most of the antibiotics that have been introduced against it in the recent years (Rajadurai et al., 2006). The development of β -lactamase resistant semi-synthetic penicillin in the 1960's contributed momentary alleviation which ended just after methicillin became commercially accessible for therapeutic utilization (Kaleem et al., 2010). Drug-resistant bacterial pathogens present a serious endangerment to the global health society (Levy et al., 2005).

Presently, most of MRSA isolates are showing multiresistant drug resistance (MDR) and are sensitive solely to glycopeptides like vancomycin. However, low level resistance to vancomycin has also been reported (Rajadurai et al., 2006). Multi-resistant bacteria (MDR) and extensively resistant bacteria (XDR) were well defined by European Centre for Disease Control (ECDC) and Centre for Disease Control & Prevention (CDC). According to their terminology MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or

fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) (Magiorakos et al., 2012).

The incidence of infectious diseases has escalated so much high that standards of public health in different parts of the world is comparable to pre-antibiotic era (Arias and Murray, 2009). In Pakistan, irrational prescribing practices of antibiotics lead to increased bacterial resistance, majority of nosocomial pathogens are resistant to multiple antibiotics and treatment fails to cure the infection. There is insufficient data available to assess the prevalence of MRSA strains in Pakistan. However, In Pakistan, the incidence of methicillin resistance in *S. aureus* has been reported with a varying range from 42% to 51% (Akinkunmi and Lamikanra, 2012). The current study is an attempt to contribute in demonstrating the same issue linked to MRSA and its susceptibility profiling.

MATERIALS AND METHODS

This prospective cross-sectional study was conducted in the Karachi from Jan 2015 to June 2017. The *S. aureus* samples were isolated from various clinical specimens and were identified by conventional methods (Winn, 2006). The clinical isolates were collected from outpatients and inpatients departments of a tertiary care

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teaching hospital of Karachi and were tested in the Research Laboratory of Department of Pharmaceutics, University of Karachi. Antibiotic susceptibility test of *S. aureus* was performed by Kirby Bauer agar diffusion method (Bauer *et al.*, 1966) as per Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI Guideline, 2006). Antibiotic disks used for Gram positive bacteria were oxacillin, cefoxitin, erythromycin, ciprofloxacin, clindamycin, tetracycline, teicoplanin vancomycin, nalidixic acid, minocycline and tetracyclin, all of Oxoid, UK. MDR and XDR strains were characterized by ECDC and CDC guidelines. Methicillin resistance in *S. aureus* strains was determined using cefoxitin (30µg) disk and oxacillin (6µg) disk on Mueller Hinton agar, Oxoid, UK. The plates were inoculated with *S.aureus* strains at 33-35°C for 18-24 hours after due validation of the system. As per CLSI guidelines, Cefoxitin disk with inhibition zone ≤ 21 mm was considered as *mecA* positive (CLSI Guidelines, 2006). It is used as a surrogate marker for oxacillin resistance.

RESULTS

Of three hundred and forty six *S.aureus* specimens, one hundred and eighty samples (52%) were found MRSA positive. Out of 180 positive samples for MRSA, 126 (70%) belonged to male subjects while 54 (30%) were from females. Methicillin-resistant *S. aureus* infections gender an age wise distributions are shown in table 1 and fig. 1 while specimen wise distribution, in fig. 2 respectively. The drug resistance profile of MRSA isolated from non-duplicated clinical specimens was observed to be highly variable (fig. 3).

Table 1: Gender wise distribution of MRSA Isolates (N=180)

Males	Females
126	54

DISCUSSION

Methicillin resistant *S. aureus* also called super bug or bad bug has created a serious concern for health care planners as limited option available to treat MRSA (Akhter *et al.*, 2009). Problems with MRSA are not only confined to industrialized countries but an alarming high rate of MRSA infections were also observed in Pakistan in the past decade. The early MRSA incidence was reported in Pakistan in 1989 and then its prevalence has been observed to be increasing (Ashiq and Tareen, 1989). Earlier studies reports prevalence of MRSA that varied among various cities of Pakistan such as 57% in Karachi, 61% in Lahore, 54 % in Peshawar and 46% in Rawalpindi (Ahmed *et al.*, 2000, Hafiz *et al.*, 2002, Qureshi *et al.*, 2004, Shafiq *et al.*, 2011). The present study has reported 52% prevalence rate of MRSA strains among *S. aureus*. Similar finding was also reported form Faisalabad that

observed the same percentage of MRSA (Chaudary and Qureshi, 2017). A study from Lahore revealed 50% MRSA out of all *S. aureus* isolates (Perwaiz *et al.*, 2007). This is in agreement with the study conducted in Peshawar which documented 48.1% MRSA strains (Vandenbroucke-Grauls, 1994) another study done at Karachi in 2005 showed 43% MRSA prevalence (Ullah *et al.*, 2016). There is continuous increase in the global prevalence of MRSA, A European study documented 65% MRSA samples from ICUs (Ullah *et al.*, 2016).

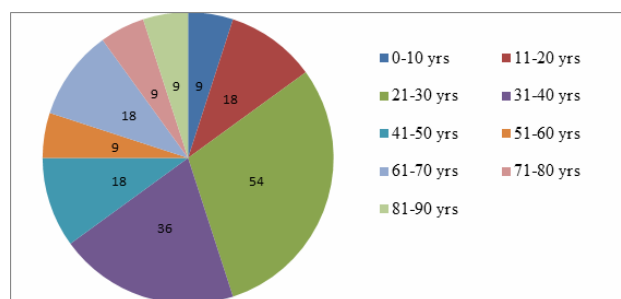


Fig. 1: Patient agegroup wise distribution of MRSA isolates

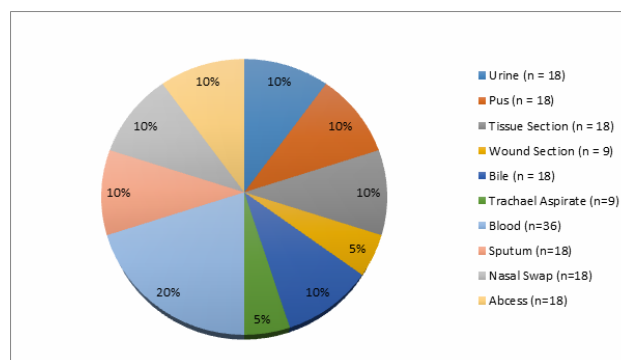


Fig. 2: Distribution of MRSA with respect to source specimen N = 180

MRSA resistance to rifampicin was found 28%, while other studies reported low resistance of MRSA isolates towards rifampicin such as 18.8 % and 14 % respectively (Idrees *et al.*, 2009, Yameen *et al.*, 2010). Another finding demonstrated relatively higher resistance of MRSA (50%) towards rifampin in comparison to the present study (Akhter *et al.*, 2009). The current study reports 65% MRSA resistance against erythromycin that is in line with the 70% and 69.10%, of MRSA resistance as described by earlier studies conducted in Pakistan (Aghazadeh *et al.*, 2009, Akhter *et al.*, 2009). However, the prevalence of resistance found in this study was more than what was reported from Iran 27.7% (Baker and Frenck Jr, 2004). A higher resistance rate of MRSA to clindamycin has existed (Butt *et al.*, 2004), however, that was lower than the previous studies from Karachi and Rawalpindi having 79% and 70% respectively (Akhter *et al.*, 2009, Hussain *et al.*, 2013). The present study has reported 47% MRSA resistance against Ciprofloxacin which is in line with other study that reported 44% resistance (Kaleem *et al.*,

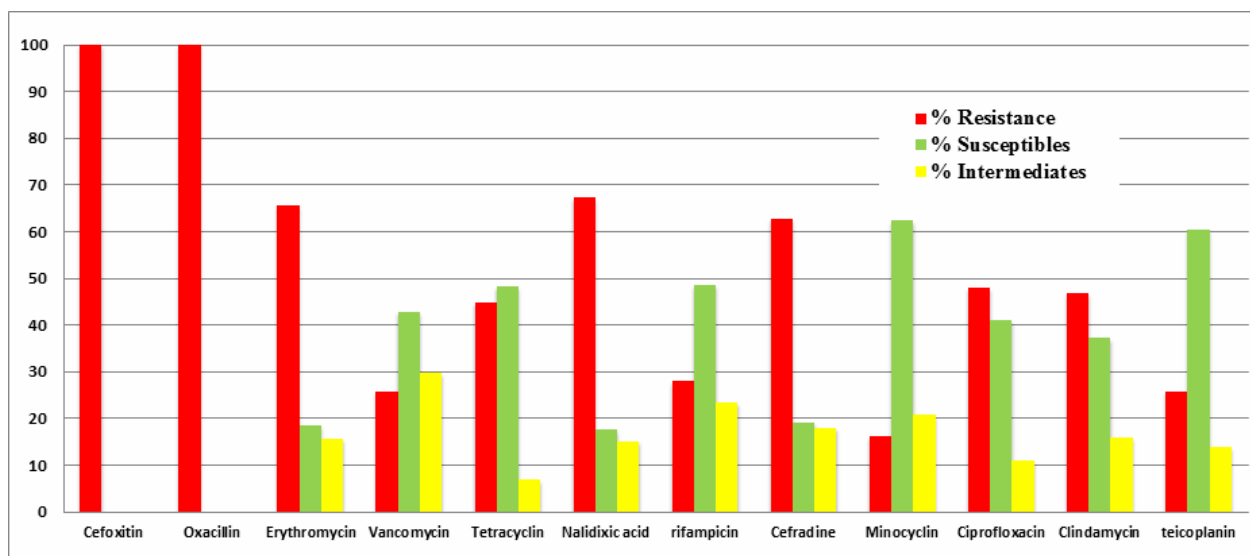


Fig. 3: Antibiogram of selected antibiotics against MRSA isolates (N=180)

2010). MRSA resistance to tetracycline was also found 42% in the present study, this correlates with earlier findings where it has been reported that MRSA isolates were 51% however, another finding claimed 82% resistance against tetracycline (Akhter *et al.*, 2009, Hussain *et al.*, 2013).

The present study found that all MRSA strains tested were 16 % resistant to minocycline. The study found slightly higher resistance than an earlier research performed at Military Hospital Rawalpindi in 2009 which indicated that only 9% of MRSA isolates were resistant to minocycline (Okonko *et al.*, 2009). The present study documented 67% resistance of MRSA isolates against Nalidixic acid that is in agreement with the study conducted in Nigeria in 2009 which reported 63.6% MRSA resistance to nalidixic acid (Taj *et al.*, 2016). The current study reported 62% of MRSA strains were resistant to cephadrine, which varied from previous reports (Liaqat *et al.*, 2015, Perveen *et al.*, 2013).

This study indicates the higher rate of VRSA occurrence 25% in different clinical settings in Karachi. An earlier work in Pakistan reported the presence of 43.1% VISA and 9.8% VRSA strains (Shahriar *et al.*, 2012). This is the first time when this high Vancomycin resistance has been reported from Pakistan. Results in the present study show unique and distinctive percentage in contrast with literature existed. A study conducted in Bangladesh revealed an alarming result that *S. aureus* strains showed very low sensitivity (6.56%) towards vancomycin in-contrast 93.44% were found to be vancomycin intermediate strains (VISA) (Styers *et al.*, 2006).

The present study reported 68% multi-drug resistant *S.aureus* isolates which is comparable to the data from USA that have documented 67.8% multi-drug resistant

(MDR) strains for inpatients and 65% for outpatients MRSA isolates (Onanuga *et al.*, 2005). A total of 43 *S. aureus*, out of 60, were observed to be multidrug resistant in Nigerian women (Tiwari *et al.*, 2008). Another study from India also revealed a high frequency of multidrug-resistant MRSA i.e. 72.1% (Archer, 1991).

CONCLUSION

The current report identified a high occurrence of VRSA and MDR strains in isolated MRSA that is a significant threat to the public health at Karachi as this bad bug can disseminate in community setup resulting in various clinical conditions. Minocycline was the most effective drug against MRSA. This high prevalence of MDR MRSA highlights the need for periodic assessment to understand and characterize MRSA infections. In addition, the maintenance of proper hygiene by hospitalized patients and staff and avoidance to irrational use of broad-spectrum antibiotics and provision of antibiotic stewardship and establishment an adherence to antibiotic policies could effectively reduce the rate and dissemination of multi drug resistant strains Thus, it was found that all isolates of MRSA resistant to multiple antibiotics tested.

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