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Research Article

Antimicrobial Sensitivity Pattern of Methicillin Resistant *Staphylococcus aureus* Isolated from Hospitals of Kohat District, Pakistan

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ABSTRACT

The study was conducted to determine prevalence and analyze antibiotic susceptibility patterns of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates in two hospitals of District Kohat, Khyber Pakhtunkhwa province, Pakistan. Staphylococcus strains (n = 434) comprising of 191 (44 %) MRSA and 243 (56 %) coagulase negative were isolated from different clinical samples and identified using conventional microbiological procedures. Majority of the isolates (15.55%) were identified from surgical ward followed by outdoor patient department (OPD, 14.70%). The percentage of MRSA in wound samples was highest (24.08 %) followed by pus samples (21.46 %) and vaginal swab (3.14 %). MRSA strains (n = 191) isolated from two hospitals of Kohat were resistant to amoxycillin (100%), followed by cefotaxime (76.43 %), ofloxacin (74 %), levofloxacin (70.15 %), erythromycin (69.10 %) and chloramphenicol (34.03 %). Highest sensitivity was found against vancomycin (99.55 %). A potentially alarming prevalence and multi drug resistance pattern found in MRSA isolates indicates major health concerns in remote district Kohat.

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INTRODUCTION

Hospital associated methicillin resistant *Staphylococcus aureus* (HA-MRSA) is emergence of MRSA from the patients having current or recent hospitalization, receives dialysis, or resides in a long-term care facility (Milyani and Ashy, 2012). MRSA has become a leading cause of nosocomial infections worldwide since the first European isolate of MRSA detected in 1960s (Lowy, 1998; Tristan *et al*., 2007). During the period 1970 to 2010, outbreaks of hospital acquired infections of MRSA were observed around the world including Saudi Arabia (Madani *et al*., 2001), Austria (Krziwanek *et al*., 2009), Argentina (Reyes *et al*., 2009), South Africa (Shittu *et al*., 2009), Italy (Soavi *et al*., 2010). These outbreaks were mostly associated with neonatal, intensive care and burns units (Liu *et al*., 2011).

MRSA shows resistance to multiple antibiotics such as gentamicin, norfloxacin, fucidic acid, erythromycin and clindamycin (Shai *et al*., 2004). Infections caused by resistant MRSA are prominent threat to animal and human health. Infection outbreaks have been reported from burn wards, nurseries, intensive care units as well as in clinical and surgical patients and due to misuse of antibiotics, lack of hand washing, irresponsible nursing care and presence of carriers among the hospital staff (Zermina *et al*., 2012). There are two types of *Staphylococcus aureus* found in nosocomial environments: permanent and transitory. The former can be found on healthcare workers and in the hospital environment (Yao *et al*., 2010; Persoons *et al*., 2009, Shai *et al*., 2004). These infection has also been reported in different animal species such as sheep, goat, cows, dogs (Bassim and El-Maghraby, 2005) and hospitalized horses (Hartmann *et al*., 1997)

In developing countries like Pakistan, antibiotic resistant bacteria are persistent challenge and difficult to control because of irrational use of antibiotics. There exists a paucity of data regarding MRSA in Khyber Pakhtunkhwa, province of Pakistan. The present study, therefore, has been conducted for the first time to investigate the antibiotic susceptibility profiles of MRSA isolated from human population in district Kohat, Pakistan.

MATERIALS AND METHODS

Sampling

From January to December 2012, a total of 552 samples were collected from different wards (OPD, Ear Nose and Throat (ENT), Medical, Burns, Orthopedic, Surgical, Children, Gynaecological ward and Main Operation Theater) of Liaqat Memorial hospital and DHQ hospital KDA district Kohat (Khyber Pakhtunkhwa) Pakistan. These samples were collected from patients admitted in different wards of above said hospitals with history of different clinical problems. Samples included whole blood, pus, wound swabs, throat swabs, sputum, high vaginal swab (HVS) and urine samples. Samples were labeled accordingly and were subjected to screening of *Staphylococcus aureus* and subsequent antimicrobial susceptibility.

Isolation of S. aureus from clinical samples

Staphylococcus aureus was isolated from each sample as per protocol produced. Each sample was inoculated onto Mannitol Salt Agar (MSA) (Oxoid, UK) followed by identification of *S.*

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aureus on the basis of Gram staining reaction and biochemical tests such as catalase, coagulase and DNase tests as discussed previously by Perveen *et al*., (2013).The positive results of these tests were considered to be the positive isolates of *S. aureus*.

MRSA screening

Methicillin was used to screen *S. aureus* isolates for presence of methicillin resistance. Briefly, each isolate was streaked on surface of Muller-Hinton agar (Oxoid UK) containing 6 µg/ml of methacillin and incubated at 37 °C for 24 hours as performed previously (Ma *et al*., 2007). Isolates showing growth on this media were preserved and labeled as MRSA for further studies.

Anti-biogram Assay

The Kirby-Bauer disc diffusion method was used to evaluate the antimicrobial susceptibility pattern of the MRSA isolates against different commonly used antibiotics.

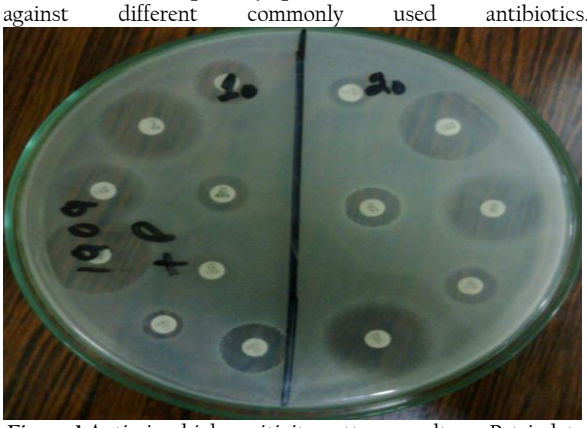

Figure 1 Antimicrobial sensitivity pattern results on Petri plate

Amoxicillin (60 µg), cefotaxime (20 µg), cephradin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), doxycycline (1µg), gentamicin (10 µg), levofloxacin (5µg), ofloxacin (10 µg), sparfloxacin (20 µg), norfloxacin (5 µg) and vancomycin (30 µg) (Oxoid UK), were used in the present study.

and the results were interpreted by measuring zone of inhibitions according to standard guidelines as described by Perveen et al. (2013).

RESULTS

Out of 552 samples tested, we isolated 434 *Staphylococcus* strains containing 191 (44 %) MRSA and 243 coagulase negative *Staphylococcus* strains, while rest of the samples (109) were negative for *Staphylococcus*. Out Of these *Staphylococcus* positive samples, 51.79% were from male patients while remaining 48.21% were from female patients. Statistically there was no difference (P › 0.05) in prevalence of MRSA on sex and age basis (data not shown). Isolates were mostly taken from surgical ward (15.55%) followed by OPD (14.70 %). Highest percentage of MRSA was isolated from wound swabs (24.08 %) followed by pus samples (21.46 %) while the lowest percentage of MRSA was from HSV (3.14 %). Isolation of MRSA from clinical samples is detailed in Table 1.

Table 1: MRSA isolated from different clinical samples of hospitals of Kohat

Clinical Samples	Number of MRSA isolates		Total (%)
	KDA Hospital	Liaqat Hospital	
Blood	19	8	27 (14.13)
Urine	15	12	27 (14.13)
Pus	23	18	41 (21.46)
HVS	6	0	6 (3.14)
Throat	13	13	26 (13.61)
Wound	26	20	46 (24.08)
Sputum	11	7	18 (9.42)
Total	113	78	191 (100)

Table 2: Antibiotic sensitivity pattern of Methicillin Resistant *Staphylococcus aureus*

Antibiotic	DHQ, Hospital KDA (n = 113)			Liaqat Memorial Hospital (n = 78)			(n = (191)
	**S	*R	Percent Resistant (%)	**S	*R	Percent Resistant (%)	Total Resistant (%)
Amoxicillin	0	113	100	0	78	100	100
Cefotaxime	25	88	78	20	58	74.35	76.43
Cephradine	37	76	67.25	23	55	70.51	68.58
Erythromycin	32	81	71.68	27	51	65.38	69.10
Chloramphenicol	69	44	39	57	21	27	34.03
Ciprofloxacin	47	66	58.40	31	47	41.59	59.16
Doxycyclin	45	68	60.17	25	53	68	63.35
Gentamicin	37	76	67.25	26	52	66.66	67.01
Levofloxacin	41	72	64	16	62	79.48	70.15
Norfloxacin	49	64	56.63	20	58	74.35	64
Ofloxacin	36	77	68.14	14	64	82.05	74
Sparfloxacin	55	58	51.32	23	55	70.51	59.16
Vancomycin	112	01	0.88	78	0	0	0.46
Methicillin	0	13	100	0	78	100	100

*R: Resistant, **S: Sensitive

The antibiotic sensitivity patterns of MRSA isolated from clinical specimens were found to be highly variable. All the 191 MRSA strains isolated from two hospitals of Kohat were resistant to methicillin (used for screening) and penicillin

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(100%), followed by cefotaxime (76.43 %),ofloxacin (74 %), levofloxacin (70.15 %), erythromycin (69.10 %) and chloramphenicol (34.03 %). Highest sensitivity was found against vancomycin (99.55 %). The antimicrobial susceptibility pattern of MRSA isolates against different classes of antibiotics is shown in Table 2.

DISCUSSION

The results of present study showed that the prevalence MRSA in clinical samples from two government hospitals was 44 %. Previous reports have showed variable prevalence of MRSA strains among various cities of Pakistan including 61% in Lahore, 57% in Karachi, 46% in Rawalpindi and 54 % in Peshawar (Ahmad et al. 2000, Hafiz et al. 2002, Qureshi et al. 2004, Shafiq et al. 2011). This raised isolation of MRSA with the passage of time may be attributed to the transfer of resistance genes between bacterial cell and persistence of bacteria in hospital environment due to antibiotic resistance (Saima et al. 2007). Absence of control program for antibiotics usage pattern is another factor which facilitates MRSA to increase in concentration (Hacek *et al*., 1999). In present study statistically no significant difference was observed in prevalence of MRSA on sex and gender basis that is in accordance with previous studies conducted by Khatoon *et al*., (2002).

The results of present study showed multiple drug resistance of MRSA strains isolated from two hospitals of Kohat as shown in figure 2. We have shown that all 191 MRSA strains were 100% resistant to methicillin and amoxycillin. The resistance pattern of methicillin and amoxycilline observed against MRSA was similar to what has been reported from other part of the world. Zermina et al. 2012 conducted study in Rawalpindi and found 92% of MRSA were resistant to Ampicillin. Amoxycillin, a derivative of penicillin, has been in use for last two decades, so development of high resistance is obvious. In present study 68.58 % and 76 % of MRSA strains showed resistance against cephradine and cefotaxime respectively. James and Reeves (1996) described that MRSA strains are resistant to first, second, third and fourth generation of cephalosporins. In a study conducted by Perveen *et al*. (2013), 83% of the MRSA showed resistance against cephradine whereas Mahmood et al. (2001) reported 29% resistance of generation cephalosporins. In this study, we have examined that67.01% MRSA showed resistance against gentamicin that is lower than the 76.35% of MRSA resistance towards gentamicin as described by Perveen et al. (2013). Gentamicin is an aminoglycoside and is most often prescribed because of its low cost and synergistic activity with ß-lactum antibiotics (Hafiz *et al*., 2002). In this study 69.10 % of MRSA were resistant to erythromycin, which isin accordance with previous reports conducted by Perveen *et al*. (2013). Among quinolones the percentage resistance found in MRSA in present study was quite higher (ciprofloxacin (59.16), ofloxacin (74 %), levofloxacin (70.15 %). Previously reported resistance of ciprofloxacin and other quinolones also showed higher pattern as reported by Auckenthaler et al. (2000), Zermina et al. 2012. The development of *S. aureus* resistance to ciprofloxacin might be due to previous antimicrobial chemotherapy of patient before hospitalization. When quinolones are used to treat infections caused by other bacterial pathogens, *S. aureus* colonized on these patients (e.g., on their skin or mucosal surfaces) are likely to be exposed to sub therapeutic antibiotic doses and are therefore at risk of developing resistance. These resident, resistant strains then become the reservoir for future infections (Mandell, 2005). We observed that all MRSA isolates gave 99.55 % sensitivity to vancomycin. There was only one isolate that was found to be resistant to vancomycin. These results coincide with the findings of multi drug resistance in the study conducted by Perveen et al. (2013). It is well known that emergence of bacterial resistance is promoted by excessive use of antibiotics. The presence of antibiotics residues in livestock products like milk and meat could be another responsible for maintaining resistant strains in environment (Persoon *et al*., 2009). The healthcare workers inserted in this epidemic chain have great importance in the increasing resistance of contaminants, serving as a source of transmission and information for empirical prescription of antibiotics.

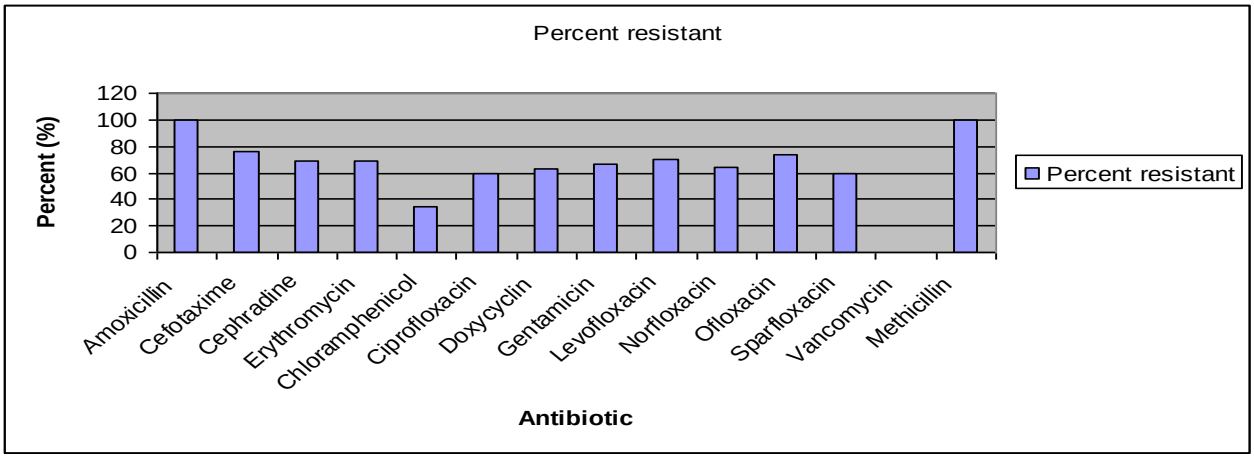

Figure 2: Antimicrobial sensitivity pattern shown by multi drug resistant MRSA

The results of present study clearly showed high prevalence of MRSA in hospitals of district Kohat. The antibiotics used in this study are frequently prescribed by clinicians without carrying out anti-biogram assays on samples of their patients. Moreover, common people have easy access to these antibiotics available to them at local pharmacies without any prescriptions from doctors. Therefore, the rise in resistance is clearly due to irrational use of antibiotics and non existence of protocols and guidelines for safe practice in terms of treatment of MRSA.

CONCLUSION

MRSA were isolated in high concentration from clinical samples in Kohat and showed a high risk to the patients and staff working in the hospital. The overcrowding in hospital, lack of facilities and lack of knowledge about HA-MRSA result

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in the presence of nosocomial pathogen in high concentration. MRSA strains isolated showed resistance to multiple antibiotics. We suggest an implementation of preventive measure in order to minimize the bacterial resistance to antibiotics. An electronic record of antibiotic usage should be made to prevent unnecessary usage of broad spectrum antibiotics and increase recommended usage of antibiotics. All health care personnel should strictly follow the preventive guideline for patient as well as for their own safety. Proper antibiotics susceptibility test should be made for all suspected infection caused by MRSA. Vancomycin showed good results against MRSA in the present study. But the prescription of this antibiotic should be limited and subjected to presentation of proper antimicrobial sensitivity assay reports.

ETHICAL CONSIDERATION

The samples were obtained from patients with prior informed consent and explaining to them the importance of this study. The study was finally approved by Research and ethical committee, Department of Microbiology, Kohat University of Science and Technology, Pakistan.

CONFLICT OF INTEREST

The authors declare no conflict of interest of any type.

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