COLONIZATION AND ANTIBIOTIC RESISTANCE PROFILING OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN PATIENTS FROM TERTIARY CARE HOSPITAL, LAHORE, PAKISTAN

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Abstract

The methicillin resistant *Staphylococcus aureus* (MRSA) is affecting the economy worldwide contributing to the increased morbidity and treatment costs. The present cross sectional study was conducted to determine the association of MRSA with kidney and liver patients from Tertiary Care Hospital in Lahore, Pakistan and its antibacterial resistance patterns. The study was conducted for the period of one year; from April 2019 to April 2020. A total of 1984 different samples were obtained and examined for bacterial cultures using standard procedures. From total samples, 51.05% were collected from male and the rest 48.95% were collected from females including different age groups. Samples were inoculated on different culture media for isolation of *S. aureus* and susceptibility testing. A total of 253/1984 clinical samples exhibited *S. aureus* characteristics while 39 of 253 *S. aureus* (15.41%) were identified as MRSA. MRSA strains were found highly resistant to Amikacin (AK), Cefoxitin (FOX), Clindamycin (DA), Fusidic acid (FD), Gentamicin (CN) and Tobramycin (TOB) while highest sensitivity was noted against Vancomycin (100%) and Linezolid (100%). The presence of MRSA and multidrug resistance pose serious therapeutic burden to critically ill patients. A systematic and concerted effort is essential to identify high-risk

patients rapidly and to reduce the burden of antimicrobial resistance.

Keywords: methicillin resistant *Staphylococcus aureus*; multidrug resistance; colonization; prevalence.

I. Introduction

Staphylococcus aureus (*S. aureus*) is a part of normal microflora of the nose, skin and respiratory tract in humans. About 20-30% of the total human population is a carrier of *S. aureus* where the nasal cavity remains the most common site for colonization (Banning. 2005; Sakr *et al.*, 2018). *S. aureus* can cause several infections ranging from superficial skin infections to invasive life-threatening diseases such as pneumonia, bacteremia, endocarditis, meningitis, sepsis and toxic shock syndrome (Tong *et al.*, 2015, Cadena *et al.*, 2016). It has high ability to develop resistance to frequently used antibiotics. The use of antibiotics promotes resistance and influences co-selection processes of antibiotic resistant bacteria leading to transmission of resistance genes in the bacterial community (Bengtsson *et al.*, 2018). *S. aureus* strains that are generally resistant to β -lactam antibiotics such as methicillin and oxacillin are referred to as MRSA. The resistance to β -lactams is mostly conferred by penicillin binding protein (PBP2a) encoded by *mecA* gene (Fuda *et al.*, 2004). The MRSA strains are associated with higher mortality rates than methicillin-sensitive *S. aureus* (MSSA) (Hassoun *et al.*, 2017).

The prevalence and resistance of *S. aureus* is associated with three types of infections i.e. hospital acquired (HA), healthcare-associated (HC) and community-acquired (CA) infection. Generally, HA-MRSA infections arise from persistent carriers those undergoing antibiotic therapy or from intermittent carriers. Both types of the carriers are at high risk of developing MRSA systemic infection such as bacteremia (Vigil *et al.*, 2015). The healthcare-associated community onset infections are usually associated with comorbidities, such as chronic renal disease, diabetes and ulcers. MRSA can colonize Kidney and Liver transplant patients. The factors which increase the risk of colonization of organisms include antimicrobial use, use of drains and catheters, length of surgery and duration of stay at hospital (Oliveira *et al.*, 2013). It has previously been reported that 10% to 25% of all liver transplant recipients are likely to acquire *S. aureus* infections. Moreover, the mortality rate may exceed 80% of all liver transplant recipients with MRSA pneumonia or MRSA intraabdominal infection (Singh *et al.*, 2000).

Keeping in view the significance of MRSA in liver and kidney patients the current study was designed to determine the prevalence of MRSA and vancomycin resistance infection in kidney and liver settings at a Tertiary Care Hospital in Lahore, Pakistan.

II. Methods

Collection of samples

The samples were collected from liver and kidney patients from the Tertiary Care Hospital in Lahore. A total number of 1984 samples were obtained from these patients. The various samples included in study were Urine (704), Folly's Tip (101), cystoscopy urine (32), nephrostomy urine (54), pus (114), blood (654), wound swabs (89) and sputum (236).

Processing of samples

After the collection of samples, all were transported to the laboratory of University of Central Punjab, Lahore for further processing. The isolation and identification of pathogenic bacteria was done using various culture media. The Cystine Electrolyte Deficient (CLED) agar was used for the processing of urine samples. Blood agar and MacConkey agar were used to inoculate samples other than urine. After inoculation, the samples were incubated for 18-24 h at 37°C.

***S. aureus* identification**

Biochemical profiling was done by various tests including catalase, coagulase and DNase test using Bergey's manual identification scheme (Bergey *et al.*, 1939).

Antibiotic susceptibility testing

The Kirby Bauer disk diffusion method was used for antibiotic susceptibility testing. Various common antibiotics used for all sample types were Amikacin (30µg), Cefoxitin (30µg), Ciprofloxacin (5µg), Co-trimoxazole (1.25/23.75µg), Gentamicin (10µg), Linezolid (30µg), Erythromycin (15µg), Clindamycin (2µg), Penicillin (10units), Tetracycline (30µg), Teicoplanin (30µg), and Tobramycin (10µg). The nitrofurantoin (300 µg) was additionally used for urine samples while chloramphenicol (30µg) and Fusidic acid (10µg) were used for samples other than urine. The results were interpreted in accordance with CLSI Guidelines (Clinical and Laboratory Standards Institute, 2015). All *S. aureus* isolates were classified into two groups according to presence of oxacillin resistance as MRSA and methicillin sensitive *S. aureus* (MSSA). The strain was considered MDR when it was found resistant to three or more of the tested antibiotics.

E-Strip test

The MRSA isolates were further tested for resistance against vancomycin (VRSA) by using E-strip (Biomeriux) determining minimum inhibitory concentration (MIC). The strains with MIC $\geq 16\mu\text{g/ml}$ were considered as vancomycin resistant.

Statistical analysis

The percentage sensitivity data of antibiotic resistance was analyzed using SPSS version 20.0.

III. Results

Demographic characteristics

The highest positive isolates were obtained from females 17.40% (169/971). The male population is presented with almost half the number of *S. aureus* isolates than female population. The age group 40-49 showed strongest association with *S. aureus* positive isolates while this association was less common below 30 years of the age (Table 1).

Table 1: Demographic characteristics of *S. aureus* isolates.

	Variables	Total Samples	Positive for <i>S. aureus</i>
Gender	Male	1013	84 (8.29%)
	Female	971	169 (17.40%)
Age (Yrs)	0-9	29	01 (4.3%)
	10-19	112	02 (1.7%)
	20-29	246	04 (1.6%)
	30-39	237	14 (5.9%)
	40-49	556	106 (19%)
	>50	804	126 (15.6%)

***S. aureus* isolation and identification**

A total of 253 out of 1984 samples were confirmed as *S. aureus* by macroscopic and microscopic characteristics followed by biochemical testing including coagulase, catalase and DNase tests. The maximum number of *S. aureus* isolates was obtained from cystoscopy urine comprising of (42.8%), folly's tip (24%), nephrostomy urine (22.2%) followed by pus (18.4%), wound swabs (15.7%), urine (11.7%), blood (11.6%) and sputum (3.3%) (Table 2).

Table 2: Prevalence of MRSA strains in different clinical samples.

Sample	Total samples (n= 1984)	<i>S. aureus</i> Positive (n=253)	MRSA (n=39)
Urine	704 (35.5%)	83 (11.7%)	14 (16.86%)
Folly's Tip	101 (5.1%)	24 (24.0%)	0
Cystoscopy urine	32 (1.6%)	15 (42.8%)	1 (6.66%)
Nephrostomy urine	54 (2.7%)	12 (22.2%)	1 (8.33%)
Pus	114 (5.7%)	21 (18.4%)	4 (19.04%)
Blood	654 (33.0%)	76 (11.6%)	9 (11.84%)
Wound Swabs	89 (4.5%)	14 (15.7%)	2 (14.28%)
Sputum	236 (11.9%)	08 (3.3%)	8 (100%)

Antibiotic susceptibility testing

All the biochemically confirmed *S. aureus* isolates were analyzed for MRSA by resistance to oxacillin disc. Out of 253 *S. aureus* strains, 39 (15.41%) were resistant to oxacillin recognized as MRSA while the remaining 214 were identified as MSSA. The prevalence of MRSA strains of *S. aureus* isolated from different clinical samples is illustrated in table 2. All 8 strains isolated from sputum samples were MRSA while no MRSA strain was isolated from Folly's tip. Moreover, these MRSA strains were also tested for MDR using antibiotic discs. Figure 1 shows the representative plate of antibiotic susceptibility pattern to different antibiotics against *Staphylococcus aureus*.

Figure 1: *Staphylococcus aureus* representative plate of antibiotic susceptibility pattern to different antibiotics.

Table 3 manifested the resistance pattern of MRSA and MSSA strains to various antibiotics. MRSA strains were found totally resistant to Amikacin (AK), Cefoxitin (FOX), Clindamycin (DA), Fusidic acid (FD), Gentamicin (CN) and Tobramycin (TOB). MSSA strains were observed with relatively high-level resistance to these drugs. A high antibiotic resistance of MRSA was also observed against Chloramphenicol (C) (36.5%), Co-trimoxazole (SXT) (32%), Erythromycin (E) (35%) and Ciprofloxacin (CIP) (16%) and Tetracycline (TE) (11.2%). Two drugs Linezolid and Vancomycin were observed as highly sensitive (100%) among our isolates and remains as the drug of choice for treating these patients (Table 3).

Table 3: Percentage of *S. aureus* resistance pattern to various antimicrobial agents.

Antibiotics	Sensitive	Resistance	
		MRSA (%)	MSSA (%)
Amikacin (AK)	237	16 (100)	0 (0)
Chloramphenicol (C) *	201	19 (36.5)	33 (63.5)
Cefoxitin (FOX)	214	39 (100)	0 (0)
Ciprofloxacin (CIP)	156	19 (16)	98 (84)
Co-trimoxazole (SXT)	191	20 (32)	42 (68)
Clindamycin (DA)	236	17 (100)	0 (0)
Erythromycin (E)*	171	29 (35)	53 (65)
Fusidic acid (FD) *	232	21 (100)	0
Gentamicin (CN)	229	24 (100)	0
Linezolid (LZD) **	39	0	0
Penicillin (P)	36	N/A	178 (100)
Tetracycline (TE)	128	14 (11.2)	111 (88.8)
Teicoplanin (TEC)	253	0	0
Tobramycin (TOB)	237	16 (100)	0
Nitrofurantoin (F)	69	N/A (0)	145 (100)
Vancomycin (VA) **	39	0	0

*Not reported in urinary isolates **Reported in MRSA only

Vancomycin resistance screening

MRSA strains were tested for vancomycin resistance to detect the VRSA emergence among MRSA strains. No VRSA strain was isolated as all isolates were sensitive for vancomycin.

IV. Discussion

MRSA is involved in multiple infections starting from common infections like skin infections to life threatening infections such as meningitis, kidney failure, lung abscess and endocarditis. It is one of the most common infections encountered in health care settings and results in huge economic burden. Patients with chronic kidney obstruction and liver diseases are immunocompromised and the propensity of them acquiring HAI MRSA is even higher. Thus, it becomes very imperative to analyze the prevalence of MRSA in such patients to introduce early and appropriate antimicrobial therapy. The current study was designed to investigate the prevalence of methicillin resistance pattern in kidney and liver patients. Our results showed an overall 12.7% prevalence of *S. aureus* in different hospital samples, in which the highest percentage (42.8%) was recovered from cystoscopy urine samples as compared to pus and blood samples (Table 1). This suggests that although *S. aureus* may not be the typical etiological agent involved in urinary tract infections, there are high chances of their presence in patients that are catheterized or go through cystoscopy procedures (Stokes *et al.*, 2019).

The highest incidence of *S. aureus* among middle age (40-49 yr; 19%) and aged people (>50: 15.6%) as compared to the children (0-12 yr; 1.5%) teenagers (13-19 yr; 1.7%) and young adults (20-39 yr; 5.9%) indicate the increased vulnerability of *S. aureus* infection with age. The elderly people develop more chronic diseases and bacteremia as a result of comorbid conditions such as smoking, organ transplant, dysfunctional kidneys and livers, catheterization or prior hospitalization. Our study is in good agreement with other studies which have reported higher prevalence of 35.7% *S. aureus* mediated infections among elderly (>65 yr) as compared to the young adults. The higher percentage (17.4%) of female patients infected with *S. aureus* as compared to male counterparts (8.29%) is also in close alignment with this study, where they have reported higher percentage (45.3%) of females infected with *S. aureus* as compared to males (39.7%) (Kang *et al.*, 2011).

The present study reports highest resistance against penicillin, (70%), nitrofurantoin (57%), tetracycline (49.4%), ciprofloxacin (46%), co-trimoxazole (42%), and erythromycin (32.4%). Our results show an increased resistance as compared to another study reported from Pakistan in 2014 (Khan, Rasheed and Zahra. 2014). The study by Khan *et al.* (2014) reported 12.2% and 28.9% resistance against tetracycline and erythromycin respectively, which is plausibly lower than our study, indicating high resistance among *S. aureus* isolates with passing time. MRSA is of great concern in kidney and liver patients because of their immunocompromised state. MRSA in such settings often lead to life threatening infections.

Our study reports, over-all 15.4% MRSA, which is in agreement with the surveillance study conducted in year 2018 and reports 26.3%–100% MRSA in liver recipient patients. The study also reports 1-23.5% in liver cirrhosis patients which is in close association with our results (Liu, Zhang and Wan. 2018). Unfortunately, there are no studies in Pakistan from liver and kidney settings to support our data. However, another study reports 33.9% MRSA isolated from pus, blood, sputum, tracheal aspirate and urine (Ahmed *et al.*, 2014). In another study pus and wound samples collected from patients with diagnosed

malignancies had 38/68 *S. aureus* isolates that were recognized as MRSA (Duwadi *et al.*, 2020). When tested against different drugs, MRSA were found significantly more resistant against amikacin (100%), ceftiofur (100%), clindamycin (100%), gentamicin (100%), fusidic acid (100%) and tobramycin (100%) as compared to other drugs such as chloramphenicol (36.5%), ciprofloxacin (16%) and tetracycline (11.2%) among others.

In this study the highest sensitivity of MRSA was noted against vancomycin (100%) and linezolid (100%) suggesting that these two drugs seem the choice of drugs under prevailing MRSA conditions. However, efforts need to be combated against the possible arise of VRSA which has been reported in other studies from Pakistan.

V. Conclusion

In conclusion, this study suggested the frequent colonization of MRSA in liver and kidney patients. These critically ill individuals are at high risk of developing severe infection with the resistant strains. Only limited data is available in Pakistan for the association of MRSA with liver and kidney patients. Therefore, further studies are required for effective monitoring of these patients to combat MRSA emergence and to control its spread and transmission in the community.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MK, WK, and ZR performed experiments. MAS and NA analyzed the data. MK and SK wrote the manuscript. AR helped in manuscript editing. All authors approved the final manuscript.

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