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Antibiotic Resistance and Antibiotic Resistance Genes in *Staphylococcus aureus* Isolates from Hospital Patients in Islamabad, Pakistan

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Key words
***Staphylococcus aureus*, Antibiotic resistance, MRSA, Resistance genes, Humans**

ABSTRACT

Two hundred and seventy-eight clinical samples from hospital patients including pus, blood, nasal swabs, ear swabs, urethral swabs, wounds and sputum were processed for isolation of *S. aureus*. From these samples 50 *S. aureus* isolates were recovered, which were subjected to sensitivity testing against 18 antibiotics. The isolates were also screened for antibiotic resistance genes through PCRs. In *S. aureus* isolates highest resistance rates were observed against co-trimoxazole (70%). The isolates also showed high resistance against erythromycin, sparofloxacin and ofloxacin to which 52%, 48% and 46% of the isolates were found to be resistant. Multiple drug resistance (MDR) was noted in 78% of the *S. aureus* isolates. Out of the 50 isolates, 28 (56%) were found to be methicillin resistant *S. aureus* (MRSA). In the MRSA isolates highest resistance rate was detected against co-trimoxazole (75.0%), followed by piperacillin-tazobactam (64.2%), ofloxacin (60.7%) and sparofloxacin (60.7%). All the 28 MRSA were found to possess the *mecA* gene and no *mecC* variant was detected. Thirty two percent of the *S. aureus* isolates possessed no antibiotic resistance gene. The resistance genes detected in the isolates were *aacA-D* (50%), *tetK* (38%), *ermC* (30%), *tetM* (8%) and *ermA* (6%), while *ermB*, *dfrA* and *cfr* were not found. Additional studies are needed to get a better picture of situation of antibiotic resistance and antibiotic resistance genes in the local *S. aureus* strains.

INTRODUCTION

Staphylococcus aureus occurs as a commensal organism on the nasal passages, skin and mucous membranes of humans and animals. At the same time, it is also the leading cause of bacteremia, infective endocarditis and osteoarticular, skin, soft tissue, pleuropulmonary, and device-related infections. *S. aureus* is also one of the major organisms responsible for nosocomial infections in humans and it can cause infections after surgery or from implanted medical devices (Balasubramanian *et al*., 2017). *S. aureus* has been recovered from a wide variety of animals (Monecke *et al*., 2016).

Resistance against several classes of antibiotics such as penicillins, glycopeptides, daptomycin, tetracyclines, aminoglycosides, linezolid, phenicols, lincosamides, pleuromutilins, macrolides, streptogramins, fusidic acid, mupirocin, fluoroquinolones, sulfonamides, trimethoprim and rifampicin has been observed in *S. aureus*. Resistance in *S. aureus* can occur by several mechanisms such as enzymatic modification or inactivation of antibiotic, alteration of antibiotic binding site, antibiotic efflux, acquisition of novel antibiotic-resistance target, change in the structure and composition of the bacterial cell wall and/or membrane to reduce the entry of the antibiotic into the bacterial cell (Foster, 2017).

In 1942, resistance to penicillin was reported in *S. aureus* that instigated the development of semi-synthetic penicillins such as methicillin and oxacillin. However, in the 1960s, the first isolates of MRSA were detected (Jevons, 1961). Methicillin resistance is due to *mecA* gene which codes for an altered Penicillin Binding Protein called PBP2a (Baba *et al*., 2002; Okuma *et al*., 2002). A novel *mecA* homologue called *mecC* was reported later. It was initially found in *S. aureus* isolates recovered from cattle and humans. The *mecC* gene has only 70% *nuc*leotide similarity with the *mecA* gene (Garcia-Alvarez *et al*., 2011). After its initial discovery in the UK in humans and

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bovine population, the *mecC* homologue of the *mecA* has been noted in 13 European countries and Australia and has been observed in MRSA from a variety of different animal species. It was also reported in MRSA from river water and urban waste-water in Spain (Aires-de-Sousa, 2017).

Many studies have been conducted on epidemiology *S. aureus* in Pakistan and high percentages of the *S. aureus* isolates have been found to be MRSA. In the cities of Rawalpindi and Peshawar 42% and 36.1% of the clinical *S. aureus* isolates were noted to be MRSA. High resistance rates against erythromycin (99.0%), moxifloxacin (85.1%), ciprofloxacin (80.2%) and gentamicin (56.4%) were observed in Pakistani MRSA isolates (Ali *et al*., 2007; Ullah *et al*., 2016).

The aim of the present study was to investigate the antibiotic susceptibility of *S. aureus* isolated from hospital patients in the city of Islamabad. This work also aimed to detect some selected antibiotic resistance genes in these isolates and to find the *mec* gene type (A or C) in the MRSA.

MATERIALS AND METHODS

Clinical samples and isolation of S. aureus

The study was carried out at Islamabad Diagnostic Center (IDC), which is a private diagnostic laboratory in Islamabad and at the Department of Zoology, Quaid-i-Azam University, Islamabad. A total of 278 clinical samples including pus (97), blood (126), nasal swabs (18), ear swabs (5), urethral swabs (3), wounds (3) and sputum (26) possibly containing *S. aureus* were received at IDC. The samples had been collected by the hospital staff from patients that had been admitted in various hospitals of Islamabad and were suffering from abscesses, wounds, pneumonia, throat infection, ear infection or urinary tract infection (community-acquired infections). Each sample was from a single patient. The samples were collected before any antibiotic was administered to the patients. Isolation of bacteria was done initially on Nutrient agar. Bacterial colonies were further sub-cultures on Blood agar and then on Mannitol Salt agar. To confirm the isolates as *S. aureus*, Grams' staining was performed, colonial morphology and hemolysis on blood agar were noted and coagulase, catalase, oxidase, DNase and other biochemical tests were performed following standard protocols (Versalovic *et al*., 2011). The isolates were also subjected to a *nuc* gene PCR (Brakstad *et al*., 1992) for molecular confirmation. Each *S. aureus* isolate was grown overnight in 5 ml of nutrient broth and the bacterial genomic DNA was isolated using DNAzol reagent (Thermofisher Scientific, Cat. No. 10503027) following manufacturer's protocol. A 25 µl PCR mix was prepared containing 5 µl template genomic DNA, IX PCR buffer (NH_4SO_4), 0.2 mM dNTPs, 1 µM of each of the two primers (given in Table I), 1.5 mM $MgCl_2$ and 2.5 U of *Taq* DNA polymerase (Thermofisher Scientific, Cat. No. EP0402). Cycling conditions were initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 30 sec, and extension at 72°C for 1 min. The final extension was at 72°C for 10 min.

Antibiotic susceptibility testing

All the isolates confirmed as *S. aureus* through staining, growth characteristics, biochemical and molecular tests were sub-cultured on Mueller-Hinton agar (Oxoid, UK) and subjected to antimicrobial susceptibility against 18 antibiotics using the disc diffusion method following standard procedures as described in the Clinical Laboratory Standards Institute Manual M100 (Wayne, 2017). The concentration of the antibiotics in the antibiotic impregnated discs (Oxoid, UK) were: linezolid (LNZ): 30µg, chloramphenicol (C): 30µg, clindamycin (DA): 2µg, erythromycin (E): 15 µg, cefoxitin (FOX): 30 µg, vancomycin (VA): 30µg, fusidic acid (FA): 10µg, tigecycline (TGC): 15µg, minocycline (MH): 30µg, amikacin (AK): 30µg, meropenem (MEM): 10µg, Co-trimoxazole (SXT): 25µg, sparofloxacin (SPX): 5µg, ciprofloxacin (CIP): 5 µg, levofloxacin (LEV): 5µg, piperacillin-tazobactam (PT): 110µg, ofloxacin (OFX): 5µg, gentamicin (CN): 10 µg. The results were interpreted as sensitive (S), intermediate (I) or resistant (R). Those isolates that were found to be resistant to cefoxitin were declared as MRSA as per guidelines (Wayne, 2017).

PCRs for mec *gene and other antibiotic resistance genes*

PCRs were performed to find the *mec* gene type (A or C) and to detect other antibiotic resistance genes in the confirmed as *S. aureus* using primers shown in Table I. A multiplex PCR (Stegger *et al*., 2012) to detect the *mec* gene type was done on DNA of isolates that were declared MRSA based on sensitivity to cefoxitin. A 25 µl PCR mix contained 5 µl template DNA, IX PCR buffer (NH_4SO_4), 0.2 mM dNTPs, 1 µM of each of the four primers, 1.5 mM $MgCl_2$ and 2.5 U of *Taq* DNA polymerase. Amplification conditions were 94°C for 5 min, followed by 30 cycles at 94°C for 30 sec, 59°C for 1 min and 72°C for 1 min. The final extension was at 72°C for 10 min.

PCRs were performed for the detection of the antibiotic resistance genes *aacA-D*, *tetK*, *tetM*, *ermA*, *ermB*, *ermC*, *cfr* and *dfrA*. For *aacA-D*, *tetK*, *tetM*, *ermA* and *ermC* genes, a multiplex PCR was performed (Kumar *et al*., 2010; Strommenger *et al*., 2003). The reaction mix (25 µl) contained 10 µl template DNA, IX PCR buffer (KCl), 0.2 mM dNTPs, 0.5 µM of each of the 10 primers,

Table I. Names of genes, sequences of the primers and expected sizes of PCR products.

Name of gene	Primer name	Sequence 5`→3`	Expected PCR product size (bp)
nuc	nuc-F	GCGATTGATGGT GATACGGTT	270
	nuc-R	AGCCAAGCCTTGACGAACTAAAGC	
mecA	mecA-F	TCCAGATTACAACTTCACCAGG	162
	mecA-R	CCACTTCATATCTTGTAACG	
mecC	mecC-F	GAAAAAAAGGCTTAGAACGCCTC	138
	mecC-R	GAAGATCTTTTCCGTTTTCAGC	
aacA-D	AacD-F	TAATCCAAGAGCAATAAGGGC	227
	AacD-R	GCCACACTATCATAACCACTA	
ermA	ErmA-F	AAGCGGTAAACCCCTCTGA	190
	ErmA-R	TTCGCAAATCCCTTCTCAAC	
ermB	ErmB-F	CTATCTGATTGTTGAAGAAGGATT	142
	ErmB-R	GTTTACTCTTGGTTTAGGATGAAA	
ermC	ErmC-F	AATCGTCAATTCCTGCATGT	299
	ErmC-R	TAATCGTGGAATACGGGTTTG	
tetK	TetK-F	GTAGCGACAATAGGTAATAGT	360
	TetK-R	GTAGTGACAATAAACCTCCTA	
tetM	TetM-F	AGTGGAGCGATTACAGAA	158
	TetM-R	CATATGTCCTGGCGTGTCTA	
cfr	cfr-F	TGAAGTATAAAGCAGGTTGGGAGTCA	746
	cfr-R	ACCATATAATTGACCACAAGCAGC	
dfrA	dfrA-F	CTCACGATAAACAAAGAGTCA	288
	dfrA-R	CAATCATTGCTTCGTATAACG	

2 mM $MgCl_2$ and 2.5 U of *Taq* DNA polymerase. The cycling conditions were 94°C for 5 min, followed by 30 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 1 min. This was followed by final extension at 72°C for 90 sec. The PCR mixture (25 µl) for the *ermB* gene incorporated 5 µl template DNA, IX PCR buffer (KCl), 0.2 mM dNTPs, 1 µM of each of the forward and reverse primers, 1.5 mM $MgCl_2$ and 2.5 U of *Taq* DNA polymerase. The amplification was performed at 95°C for 3 min, followed by 30 cycles at 95°C for 30 sec, 54°C for 30 sec, 72°C for 30 sec. The final extension was at 72°C for 4 min (Duran *et al*., 2012). In case of the *cfr* gene a 25 µl PCR mixture consisted of 5 µl template DNA, IX PCR buffer (KCl), 0.2 mM dNTPs, 1 µM of each of the forward and reverse primers, 1.5 mM $MgCl_2$ and 2.5 U of *Taq* DNA polymerase. The cycling conditions were 94°C for 1 min, followed by 34 cycles at 94°C for 1 min, 48°C for 30 sec, 72°C for 3 min. The final polymerization step was 72°C for 7 min (Kehrenberg and Schwarz, 2006; Osman *et al*., 2016). The PCR mix (25 µl) for the *dfrA* gene comprised of 5 µl template DNA, IX PCR buffer (KCl), 0.2 mM dNTPs, 1 µM of each of the forward and reverse primers, 1.5 mM $MgCl_2$ and 2.5 U of *Taq* DNA polymerase. The amplification protocol was 95°C for 2 min, followed by 30 cycles at 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec. The final extension was at 72°C for 4 min (Shittu *et al*., 2011).

RESULTS

S. aureus *isolates*

Fifty isolates of *S. aureus* were recovered from the 278 clinical samples that were processed for bacterial isolation, staining and biochemical tests. The distribution of *S. aureus* and MRSA isolates obtained from different clinical samples is shown in Table II. Highest number (28/97) of *S. aureus* isolates were from pus samples. The *nuc* gene PCR done for molecular confirmation of the 50 isolates produced an amplicon of expected size (270 bp).

Table II. Number of *S. aureus* and MRSA isolates recovered from various clinical samples.

	Pus	Blood	Nasal swab	Ear swab	Urethral swab	Wound	Sputum	Total
No. of samples	97	126	18	5	3	3	26	278
S. aureus positive	28	4	3	5	3	4	3	50
MRSA positive	18	2	1	3	2	1	1	28

Table III. Results of antibiotic sensitivity testing of the 50 *S. aureus* isolates.

Antibiotic	R No. (%)	I No. (%)	S No. (%)
E	26 (52%)	9 (18%)	15 (30%)
FOX	28 (56%)	7 (14%)	15 (30%)
C	4 (8%)	11 (22%)	35 (70%)
DA	7 (14%)	11 (22%)	32 (64%)
VA	0 (0%)	10 (20%)	40 (80%)
FA	6 (12%)	12 (24%)	32 (64%)
LNZ	2 (4%)	0 (0%)	48 (96%)
SXT	35 (70%)	7 (14%)	8 (16%)
CN	14 (28%)	9 (18%)	27 (54%)
AK	9 (18%)	9 (18%)	32 (64%)
LEV	22 (44%)	17 (34%)	11 (22%)
OFX	23 (46%)	14 (28%)	13 (26%)
CIP	20 (40%)	11 (22%)	19 (38%)
SPX	24 (48%)	13 (26%)	13 (26%)
PT	21 (42%)	13 (26%)	16 (32%)
TGC	8 (16%)	13 (26%)	29 (58%)
MH	4 (8%)	14 (28%)	32 (64%)
MEM	9 (18%)	13 (26%)	28 (56%)

LNZ, Linezolid; C, Chloramphenicol; DA, Clindamycin; E, Erythromycin; FOX, Cefoxitin; VA, Vancomycin; FA, Fusidic Acid; TGC, Tigecycline; MH, Minocycline; AK, Amikacin; MEM, Meropenem; SXT, Co-trimoxazole; SPX, Sparofloxacin; CIP, Ciprofloxacin; LEV, Levofloxacin; PT, Piperacillin-Tazobactam; OFX, Ofloxacin; CN, Gentamicin; R, Resistant; I, Intermediate; S, Sensitive.

Antibiotic susceptibility results

Results of antibiotic sensitivity testing are shown in Table III. Highest resistance rates were observed against co-trimoxazole (70%). This was followed by cefoxitin, erythromycin, sparofloxacin and ofloxacin to which 56%, 52%, 48% and 46% of the isolates, respectively were found to be resistant. Maximal sensitivity was observed to linezolid (96%), followed by vancomycin (80%) and chloramphenicol (70%). Twenty-eight (56%) isolates were found to be MRSA as determined by resistance to cefoxitin. The number of MRSA found in pus, blood, nasal swab, ear swabs, urethral swabs, wounds and sputum were 18, 2, 1, 3, 2, 1 and 1, respectively (Table II). The phenotypic antibiotic resistance combination patterns are shown in Table IV. A wide variation in antibiotic resistance phenotype was observed and 39 groups of antibiotic resistance patterns were seen. In general, the MRSA were resistant to larger number of antibiotics compared to the non-MRSA isolates. High levels of resistance were observed in the *S. aureus* isolates and multiple drug resistance (MDR) defined as resistance to ≥3 classes of antibiotics was noted in 39 (78%) isolates. Extensive drug resistance (XDR), determined as resistance to at least one antibiotic in all classes but susceptibility to at least two or fewer antimicrobial categories, was found in 2 (4%) isolates. Resistance to combinations of 4 or 5 antibiotics was the most common phenotype exhibited by 8 (16%) isolates each for both combinations. The next common phenotype was resistance to group of 7 or 9 antibiotics observed in 4 (8%) isolates each for both groups.

The antibiotic sensitivity of the 28 MRSA isolates is given in Table V. In MRSA highest resistance was against co-trimoxazole (75.0%). The resistance rates against piperacillin-tazobactam (64.2%), ofloxacin (60.7%) and sparofloxacin (60.7%) were also high. The antibiotics found to be most effective against MRSA were linezolid (92.8%), vancomycin (82.1%) and chloramphenicol (71.4%).

Antibiotic resistance genes

The PCR for *mec* gene type applied on all the 28 MRSA isolates produced a 162 bp band showing that all the MRSA had the *mecA* gene and none had the *mecC* gene. The findings on presence of antibiotic resistance genes in the *S. aureus* isolates are shown in Table VI. The gene *aacA-D* had the highest occurrence (50%), followed by *tetK* (38%) and *ermC* (30%). *TetM* (8%) and *ermA* (6%) had low frequencies, while *ermB*, *dfrA* and *cfr* were not found. A representative gel picture of resistance genes PCR is shown in Figure 1. The diversity in occurrence of antibiotic resistance genes in the isolates is shown in Table VII. No antibiotic resistance gene was found in 32% of the isolates. *AacA-D* occurred as the sole antibiotic resistance gene in 22% of the isolates. *TetK* was also found to exist as a single antibiotic resistance gene in 6% of the isolates. Six various combinations of antibiotic resistance genes were observed in 2% to 8% of the isolates.

Table IV. Antibiotic resistance patterns in the 50 *S. aureus* isolates.

Antibiotic phenotype	No. of antibi-otics	No. of resistant isolates	Source
SXT	1	1	Pus
SXT, E	2	1	Sputum
SXT, E, DA	3	3	Pus, Nasal Swab
SXT, DA, FOX	3	1	Pus
SXT, FOX, MEM	3	1	Pus
SXT, OFX, CIP, SPX	4	3	Wound
OFX, CIP, SPX, LEV	4	1	Sputum, Pus, Urethral swab
FOX, MEM, CIP, VA	4	1	Wound
FOX, MEM, CN, AK	4	2	Pus
FOX, CN, AK, FA	4	1	Pus
SXT, OFX, CIP, SPX, LEV	5	1	Blood
SXT, E, DA, MEM, MH	5	3	Ear Swab
SXT, FOX, OFX, CIP, CN	5	2	Wound
SXT, OFX, CIP, SPX, PT	5	1	Pus
E, FOX, OFX, CIP, SPX	5	1	Nasal Swab
E, FOX, CIP, SPX, CN, PT	6	1	Pus
SXT, FOX, OFX, CIP, SPX, LEV	6	1	Pus
SXT, MEM, OFX, CIP, SPX, LEV	6	1	Pus
SXT, OFX, VA, CN, AK, FA, LNZ	7	1	Blood
SXT, FOX, OFX, CIP, SPX, LEV, FA	7	1	Urethral Swab
SXT, FOX, OFX, CIP, LEV, CN, FA	7	1	Ear swab
E, FOX, OFX, CIP, SPX, LEV, CN	7	1	Pus
FOX, OFX, CIP, SPX, LEV, CN, AK	7	1	Blood
SXT, E, DA, OFX, CIP, SPX, LEV, FA	8	1	Pus
SXT, FOX, OFX, CIP, SPX, LEV, CN, AK	8	1	Ear Swab
SXT, FOX, OFX, CIP, LEV, CN, AK, FA	8	1	Blood
SXT, E, DA, FOX, OFX, CIP, SPX, LEV, FA	9	1	Pus
SXT, E, DA, FOX, VA, CN, AK, FA, LNZ	9	1	Sputum
SXT, E, FOX, OFX, CIP, SPX, LEV, CN, AK	9	1	Ear Swab, Urethral Swab
SXT, FOX, OFX, CIP, SPX, LEV, AK, TGC, MH	9	1	Pus
SXT, E, FOX, OFX, CIP, SPX, LEV, CN, AK, PT	10	1	Pus
SXT, E, FOX, OFX, CIP, SPX, LEV, CN, AK, TGC, C	11	1	Pus
SXT, E, FOX, OFX, CIP, SPX, LEV, CN, AK, PT, MH	11	1	Pus
SXT, E, FOX, MEM, OFX, CIP, SPX, LEV, CN, AK, C, MH	12	1	Pus
SXT, FOX, MEM, OFX, CIP, SPX, LEV, CN, AK, FA, PT, MH	12	1	Pus
SXT, DA, FOX, MEM, OFX, CIP, SPX, LEV, CN, AK, FA, PT, MH	13	1	Pus
SXT, E, FOX, MEM, OFX, CIP, SPX, LEV, CN, FA, LNZ, PT, TGC, MH	14	1	Pus
SXT, E, DA, FOX, OFX, CIP, SPX, LEV, VA, CN, AK, FA, LNZ, PT, TGC, C	16	1	Wound
SXT, E, DA, FOX, MEM, OFX, CIP, SPX, LEV, CN, AK, FA, LNZ, PT, TGC, C, MH	17	1	Pus

Table V. Antibiotic sensitivity of the 28 MRSA isolates.

Antibiotic	R No. (%)	I No. (%)	S No. (%)
E	15 (53.5%)	5 (17.8 %)	8 (28.5%)
C	2 (7.1%)	6 (21.4%)	20 (71.4%)
DA	3 (10.7%)	6 (21.4%)	19 (67.8%)
VA	0 (0%)	5 (17.8%)	23 (82.1%)
FA	4 (14.2%)	7 (25.0%)	17 (60.7%)
LNZ	2 (7.1%)	0 (0%)	26 (92.8%)
SXT	21 (75.0%)	4 (14.2%)	3 (10.7%)
CN	14 (50.0%)	7 (25.0%)	7 (25.0%)
AK	7 (25.0%)	5 (17.8%)	16 (57.1%)
LEV	10 (35.7%)	9 (32.1%)	9 (32.1%)
OFX	17 (60.7%)	8 (28.5%)	3 (10.7%)
CIP	14 (50.0%)	7 (25.0%)	7 (25.0%)
SPX	17 (60.7%)	7 (25.0%)	4 (14.2%)
PT	18 (64.2%)	5 (17.8%)	5 (17.8%)
TGC	8 (28.5%)	6 (21.4%)	14 (50.0%)
MH	2 (7.1%)	8 (28.5%)	18 (64.2%)
MEM	6 (21.4%)	7 (25.0%)	15 (53.5%)

For abbreviations of antibiotics, see Table III.

Table VI. Prevalence of antibiotic resistance genes in the 50 *S. aureus* isolates.

Name of gene	Isolates found positive No. (%age)
aacA-D	25 (50%)
cfr	0 (0%)
dfrA	0 (0%)
ermA	3 (6%)
ermB	0 (0%)
ermC	15 (30%)
tetK	19 (38%)
tetM	4 (8%)

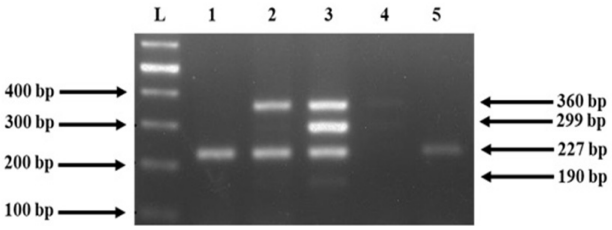

Fig. 1. A representative gel showing results of PCR for detection of antibiotic resistance genes. M: molecular size marker. PCR product bands in the figure represent *tetK* (360 bp), *ermC* (299bp), *aacA-D* (227 bp) and *ermA* (190 bp).

Table VII. Diversity of antibiotic resistance genes in the 50 *S. aureus* isolates.

Antibiotic resistance genes combinations	No. of isolates (% age)
No gene	16 (32%)
tetK (alone)	3 (6%)
aacA-D (alone)	11 (22%)
tetK, ermC	4 (8%)
tetK, aacA-D	2 (4%)
tetK, ermC, aacA-D	3 (6%)
ermC, aacA-D, tetM	1 (2%)
tetK, ermC, aacA-D, ermA	3 (6%)
tetK, ermC, aacA-D, tetM	2 (4%)

DISCUSSION

S. aureus is one of the most common causes of both community and hospital acquired infections. Although *S. aureus* causes a wide variety of clinical infections, the most common are those that affect skin and soft tissues and often lead to the formation of abscesses (Kobayashi *et al*., 2015). In our study also most isolations of *S. aureus* were from pus samples. We found a high percentage of MRSA (56%) in our study and this is in line with previous international reports. In general, there has been an increase in the prevalence of MRSA in the Asia-pacific and in various other regions of the world with few exceptions (Loewen *et al*., 2017; Wong *et al*., 2018). We tested our MRSA isolates for the presence of *mecA* or *mecC* alleles by a multiplex PCR. However, we found no isolate harboring the *mecC* allele. The occurrence of the *mecC* has generally been found to be low in humans (Paterson *et al*., 2014).

In the present study the highest resistance rates were observed against co-trimoxazole. High resistance against co-trimoxazole has been reported in previous studies also. In a study in Nigeria 72.1% of *S. aureus* clinical isolates were found to be resistant to co-trimoxazole (Shittu *et al*., 2011). Ninety six percent of Staphylococci isolated from meat samples have been found to be resistant to co-trimoxazole (Osman *et al*., 2017). Other antibiotics against which high rates of resistance were observed in *S. aureus* and MRSA in this study were the quinolones sparofloxacin and ofloxacin and the macrolide erythromycin. A total of 107 out of 122 (87.7%) of milk *S. aureus* isolates from various locations in South Africa were found to be resistant to erythromycin (Akindolire *et al*., 2015). In Iran 89.1% of MRSA were found to be resistant to erythromycin (Dehkordi *et al*., 2017). Another study in Egypt found clinical MRSA isolates to be highly insusceptible to quinolones. In this, study 58% of the isolates were

observed to be resistant to ofloxacin (Hashem *et al*., 2013).

In the present study highly diverse patterns of antibiotic resistance were detected against the 18 antibiotics tested and 39 antibiotic resistance phenotype groups were observed. Wide variation in antibiotic resistance patterns in *S. aureus* have been noted in previous studies. Ninety-seven *S. aureus* clinical isolates tested for susceptibility to 14 antibiotics showed high divergence in resistance phenotype and 36 antibiotic resistance groups were observed (Yilmaz and Aslantas, 2017). Sixteen resistance patterns were observed against 9 antibiotics in 33 *S. aureus* isolates recovered from ear discharges (Deyno *et al*., 2017). Thirty *S. aureus* isolates were collected from seawater and sand from beaches and were tested against 15 antibiotics. The isolates showed 23 antibiotic resistance phenotypic patterns (Akanbi *et al*., 2017). In our study a high percentage (78%) of *S. aureus* isolates were found to carry MDR. High rates of MDR in *S. aureus* are being increasingly reported. Very high rates of MDR *S. aureus* were reported from China (100%) and Ireland (84.3%) (Earls *et al*., 2017; Wong *et al*., 2018).

In the present study the drugs found to be most effective against *S. aureus* were linezolid (96% for *S. aureus* and 92.8% for MRSA), vancomycin (80% for *S. aureus* and 82.1% for MRSA) and chloramphenicol (70% for *S. aureus* and 71.4% for MRSA). In previous studies both linezolid and vancomycin have shown excellent results against *S. aureus*. In a study 1116 *S. aureus* isolates collected over six years time (2009-2014) were tested for antibiotic sensitivity. None of the isolate was found to be resistant to linezolid or vancomycin (Ragbetli *et al*., 2016). In a study in India, out of 250 *S. aureus* isolates, none was found to be resistant to either linezolid or vancomycin (Gade and Qazi, 2013). Chloramphenicol has also been found to be highly effective against *S. aureus* in previous investigations. In a previous study in Pakistan 132 out of 174 (75.86%) of the MRSA isolates were found to be sensitive to chloramphenicol and this drug was suggested to be a good substitute to the latest expensive antibiotics in resource constrained-countries (Fayyaz *et al*., 2013). Five out of 30 (83.3%) *S. aureus* isolates from seawater and beaches sand were found to be susceptible to chloramphenicol (Akanbi *et al*., 2017). None of the 221 *S. aureus* isolates recovered from various animal species were found to be resistant to chloramphenicol (Rubin *et al*., 2011).

We screened our isolates for presence of 8 selected antibiotic resistance genes. The highest occurrence was that of *aacA-D* and 50% of the isolates were positive for it. The *aacA-D* gene has been detected at high levels in *S. aureus* in many previous studies. In Palestine 74.5% of MRSA isolates were found to harbor *aacA-D* gene (Adwan *et al*., 2014). Hospital MRSA isolates recovered in Poland contained *aacA-D* at a rate of 72.3% (Szymanek-Majchrzak *et al*., 2018). The other genes detected at considerable levels in our *S. aureus* isolates were *tetK* (38%) and *ermC* (30%). Both these genes have been detected at high levels in *S. aureus* in some of the earlier studies. *TetK* and *ermC* were detected in *S. aureus* clinical isolates at rates of 43.7% and 91.9%, respectively (Yilmaz and Aslantas, 2017). MRSA isolates contained *tetK* and *ermC* at rates of 76.4% and 74.5%, respectively (Adwan *et al*., 2014). A high percentage (85.5%) of MDR *S. aureus* clinical isolates in China were found to contain *ermC* gene (Yang *et al*., 2017). The genes found in low percentages in our study were *tetM* (8%) and *ermA* (6%). *TetM* has been observed at low levels in *S. aureus* in former studies in Palestine (16.4%) and Iran (27%) (Adwan *et al*., 2014; Dehkordi *et al*., 2017). *ErmA* was noted in 19.4% of clinical *S. aureus* isolates in Turkey (Yilmaz and Aslantas, 2017), while no *ermA* was found in *S. aureus* collected from seawater and sand from beaches (Akanbi *et al*., 2017). *Cfr*, *dfrA* and *ermB* genes were not detected in the present study. No *cfr* gene was detected in 23 *Staphylococcus* isolates collected from meat samples (Osman *et al*., 2017). The occurrence rate of *ermB* in *S. aureus* clinical isolates in Turkey was 6.5% (Yilmaz and Aslantas, 2017). No *dfrA* gene was detected in any of the 49 co-trimoxazole resistant Nigerian *S. aureus* isolates (Shittu *et al*., 2011).

Regarding the diversity of antibiotic resistance gene occurrence pattern, 32% of our *S. aureus* isolates contained no resistance gene, while *aacA-D* occurred as sole antibiotic resistance gene in 22% of the isolates. Wide variation in occurrence of combinations of resistance gene was observed. Such patterns have been observed in earlier studies also (Egyir *et al*., 2015; Szymanek-Majchrzak *et al*., 2018).

The main factors that have been attributed to the development of antimicrobial resistance in Pakistan are irrational use of antibiotics and their extensive use in animal farming (Saleem *et al*., 2018). There is a dire need to contain rising antibiotic resistance in the country. A plan called the "National Action Plan for the Control of Antimicrobial Resistance" in the country was devised by the Government of Pakistan. In this regular surveillance of antibiotic resistance, raising public awareness on the issue, enforcement of regulations of antimicrobial use in human and veterinary practice, research on antibiotic resistance and vaccines and improved hygiene etc. were suggested as antimicrobial resistance control strategies (Anonymus, 2011).

CONCLUSION

This study is one of the very few studies that have

addressed the situation of antibiotic resistance and antibiotic resistance genes in Pakistani *S. aureus* strains. Detailed analysis of the findings of this study have been presented in the results part. More than half (56%) of the isolates were found to be MRSA. High resistance rates against co-trimoxazole, erythromycin, ofloxacin, sparofloxacin and piperacillin-tazobactam were observed. MDR was detected in a high proportion (78%) of the isolates. All the MRSA isolates carried the *mecA* gene and the *mecC* variant was not detected. The antibiotic resistance gene *aacA-D* had the highest occurrence and *tetK* and *ermC* were also detected in a significant number of isolates. Further studies are required to know better the prevalence of antibiotic resistance and antibiotic resistance genes in Pakistani *S. aureus* strains.

DECLARATIONS

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IRB approval

This study was approved by the Advanced Studies and Research Board of Quaid-i-Azam University, Islamabad, Pakistan.

Ethics statement

This study was approved by the Bioethics/Biosafety Committee of Quaid-i-Azam University, Islamabad, Pakistan.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Adwan, G., Adwan, K., Jarrar, N. and Amleh, A., 2014. Molecular detection of nine antibiotic resistance genes in methicillin resistant *Staphylococcus aureus* isolates. *Roum. Arch. Microbiol. Immunol.,* **73**: 9-17.

Aires-de-Sousa, M., 2017. Methicillin-resistant *Staphylococcus aureus* among animals: Current overview. *Cl. Microbiol. Infect.,* **23**: 373-380. https://doi.org/10.1016/j.cmi.2016.11.002

Akanbi, O.E., Njom, H.A., Fri, J., Otigbu, A.C. and Clarke, A.M., 2017. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from recreational waters and beach sand in Eastern Cape Province of South Africa. *Int. J. environ. Res. Publ. Hlth.,* **14**: 1001. https://doi.org/10.3390/ijerph14091001

Akindolire, M.A., Babalola, O.O. and Ateba, C.N., 2015. Detection of antibiotic resistant *Staphylococcus aureus* from milk: A public health implication. *Int. J. environ. Res. Publ. Hlth.,* **12**: 10254-10275. https://doi.org/10.3390/ijerph120910254

Ali, A.M., Abbasi, S.A., Arif, S. and Mirza, I.A., 2007. Nosocomial infections due to methicillin resistant *Staphylococcus aureus* in hospitalized patients. *Pak. J. med. Sci*., **23**: 593.

Anonymus, 2011. Ministry of national health services, regulations and coordination Government of Pakistan National Institute of Health, Islamabad, Pakistan. *J. Glob. Infect. Dis.,* **3**: 285.

Baba, T., Takeuchi, F., Kuroda, M., Yuzawa, H., Aoki, K., Oguchi, A., Nagai, Y., Iwama, N., Asano, K., Naimi, T., Kuroda, H., Cui, L., Yamamoto, K. and Hiramatsu, K., 2002. Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet*, **359**: 1819-1827. https://doi.org/10.1016/S0140-6736(02)08713-5

Balasubramanian, D., Harper, L., Shopsin, B. and Torres, V.J., 2017. Staphylococcus aureus pathogenesis in diverse host environments. *Pathog. Dis*., **75**. https://doi.org/10.1093/femspd/ftx005

Brakstad, O.G., Aasbakk, K. and Maeland, J.A., 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *J. clin. Microbiol.,* **30**: 1654-1660. https://doi.org/10.1128/jcm.30.7.1654-1660.1992

Dehkordi, F.S., Gandomi, H., Basti, A.A., Misaghi, A. and Rahimi, E., 2017. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant *Staphylococcus aureus* isolated from hospital food. *Antimicrob. Resist. Infect. Contr.*, **6**: 104. https://doi.org/10.1186/s13756-017-0257-1

Deyno, S., Toma, A., Worku, M., Bekele, M., 2017. Antimicrobial resistance profile of *Staphylococcus aureus* isolates isolated from ear discharges of patients at University of Hawassa comprehensive specialized hospital. *BMC Pharmacol. Toxicol.,* **18**: 35. https://doi.org/10.1186/s40360-017-0141-x

Duran, N., Ozer, B., Duran, G.G., Onlen, Y. and Demir, C., 2012. Antibiotic resistance genes and susceptibility patterns in staphylococci. *Indian J. med. Res.,* **135**: 389-396.

Earls, M.R., Kinnevey, P.M., Brennan, G.I., Lazaris, A., Skally, M., O'Connell, B., Humphreys, H.,

Shore, A.C., Coleman, D.C., 2017. The recent emergence in hospitals of multidrug-resistant community-associated sequence type 1 and spa type t127 methicillin-resistant *Staphylococcus aureus* investigated by whole-genome sequencing: Implications for screening. *PLoS One,* **12**: e0175542. https://doi.org/10.1371/journal.pone.0175542

Egyir, B., Guardabassi, L., Monecke, S., Addo, K.K., Newman, M.J. and Larsen, A.R., 2015. Methicillin-resistant *Staphylococcus aureus* strains from Ghana include USA300. *J. Glob. Antimicrob. Resist.,* **3**: 26-30. https://doi.org/10.1016/j.jgar.2014.11.006

Fayyaz, M., Mirza, I.A., Ahmed, Z., Abbasi, S.A., Hussain, A. and Ali, S., 2013. *In vitro* susceptibility of chloramphenicol against methicillin-resistant *Staphylococcus aureus. J. Coll. Phys. Surg. Pak.,* **23**: 637-640.

Foster, T.J., 2017. Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. *FEMS Microbiol. Rev*., **41**: 430-449. https://doi.org/10.1093/femsre/fux007

Gade, N.D. and Qazi, M.S., 2013. Fluoroquinolone therapy in *Staphylococcus aureus* infections: Where do we stand? *J. Lab. Phys.,* **5**: 109-112. https://doi.org/10.4103/0974-2727.119862

Garcia-Alvarez, L., Holden, M.T., Lindsay, H., Webb, C.R., Brown, D.F., Curran, M.D., Walpole, E., Brooks, K., Pickard, D.J., Teale, C., Parkhill, J., Bentley, S.D., Edwards, G.F., Girvan, E.K., Kearns, A.M., Pichon, B., Hill, R.L., Larsen, A.R., Skov, R.L., Peacock, S.J., Maskell, D.J. and Holmes, M.A., 2011. Meticillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: A descriptive study. *Lancet Infect. Dis*., **11**: 595-603. https://doi.org/10.1016/S1473-3099(11)70126-8

Hashem, R.A., Yassin, A.S., Zedan, H.H. and Amin, M.A., 2013. Fluoroquinolone resistant mechanisms in methicillin-resistant *Staphylococcus aureus* clinical isolates in Cairo, Egypt. *J. Infect. Dev. Countr.,* **7**: 796-803. https://doi.org/10.3855/jidc.3105

Jevons, M.P., 1961. Celbenin-resistant staphylococci. *Br. med. J.,* **1**: 124. https://doi.org/10.1136/bmj.1.5219.124-a

Kehrenberg, C. and Schwarz, S., 2006. Distribution of florfenicol resistance genes fexA and cfr among chloramphenicol-resistant *Staphylococcus* isolates. *Antimicrob. Agents Chemother*., **50**: 1156-1163. https://doi.org/10.1128/AAC.50.4.1156-1163.2006

Kobayashi, S.D., Malachowa, N. and DeLeo, F.R., 2015. Pathogenesis of *Staphylococcus aureus* abscesses. *Am. J. Pathol*., **185**: 1518-1527. https://doi.org/10.1016/j.ajpath.2014.11.030

Kumar, R., Yadav, B.R. and Singh, R.S., 2010. Genetic determinants of antibiotic resistance in *Staphylococcus aureus* isolates from milk of mastitic crossbred cattle. *Curr. Microbiol.,* **60**: 379-386. https://doi.org/10.1007/s00284-009-9553-1

Loewen, K., Schreiber, Y., Kirlew, M., Bocking, N. and Kelly, L., 2017. Community-associated methicillin-resistant *Staphylococcus aureus* infection: Literature review and clinical update. *Can. Fam. Phys.,* **63**: 512-520.

Monecke, S., Gavier-Widen, D., Hotzel, H., Peters, M., Guenther, S., Lazaris, A., Loncaric, I., Muller, E., Reissig, A., Ruppelt-Lorz, A., Shore, A.C., Walter, B., Coleman, D.C. and Ehricht, R., 2016. Diversity of *Staphylococcus aureus* isolates in European Wildlife. *PLoS One,* **11**: e0168433. https://doi.org/10.1371/journal.pone.0168433

Okuma, K., Iwakawa, K., Turnidge, J.D., Grubb, W.B., Bell, J.M., O'Brien, F.G., Coombs, G.W., Pearman, J.W., Tenover, F.C., Kapi, M., Tiensasitorn, C., Ito, T. and Hiramatsu, K., 2002. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J.clin. Microbiol*., **40**: 4289-4294. https://doi.org/10.1128/JCM.40.11.4289-4294.2002

Osman, K., Alvarez-Ordonez, A., Ruiz, L., Badr, J., ElHofy, F., Al-Maary, K.S., Moussa, I.M.I., Hessain, A.M., Orabi, A., Saad, A. and Elhadidy, M., 2017. Antimicrobial resistance and virulence characterization of *Staphylococcus aureus* and coagulase-negative staphylococci from imported beef meat. *Annls clin. Microbiol. Antimicrob*., **16**: 35. https://doi.org/10.1186/s12941-017-0210-4

Osman, K., Badr, J., Al-Maary, K.S., Moussa, I.M., Hessain, A.M., Girah, Z.M., Abo-Shama, U.H., Orabi, A. and Saad, A., 2016. Prevalence of the antibiotic resistance genes in coagulase positive and negative *Staphylococcus* in chicken meat retailed to consumers. *Front. Microbiol*., **7**: 1846. https://doi.org/10.3389/fmicb.2016.01846

Paterson, G.K., Harrison, E.M. and Holmes, M.A., 2014. The emergence of mecC methicillin-resistant *Staphylococcus aureus*. *Trends Microbiol.,* **22**: 42-47. https://doi.org/10.1016/j.tim.2013.11.003

Ragbetli, C., Parlak, M., Bayram, Y., Guducuoglu, H. and Ceylan, N., 2016. Evaluation of antimicrobial resistance in *Staphylococcus aureus* isolates by years. *Interdiscip. Perspect. Infect. Dis.*, **2016**: 9171395. https://doi.org/10.1155/2016/9171395

Rubin, J.E., Ball, K.R. and Chirino-Trejo, M., 2011. Antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from various animals. *Can. Vet. J.,* **52**: 153-157.

Saleem, Z., Hassali, M.A. and Hashmi, F.K., 2018. Pakistan's national action plan for antimicrobial resistance: Translating ideas into reality. *Lancet Infect. Dis.,* **18**: 1066-1067. https://doi.org/10.1016/S1473-3099(18)30516-4

Shittu, A.O., Okon, K., Adesida, S., Oyedara, O., Witte, W., Strommenger, B., Layer, F. and Nubel, U., 2011. Antibiotic resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. *BMC Microbiol.,* **11**: 92. https://doi.org/10.1186/1471-2180-11-92

Stegger, M., Andersen, P.S., Kearns, A., Pichon, B., Holmes, M.A., Edwards, G., Laurent, F., Teale, C., Skov, R. and Larsen, A.R., 2012. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either mecA or the new mecA homologue mecA(LGA251). *Clin. Microbiol. Infect.,* **18**: 395-400. https://doi.org/10.1111/j.1469-0691.2011.03715.x

Strommenger, B., Kettlitz, C., Werner, G. and Witte, W., 2003. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *J. clin. Microbiol.,* **41**: 4089-4094. https://doi.org/10.1128/JCM.41.9.4089-4094.2003

Szymanek-Majchrzak, K., Mlynarczyk, A. and Mlynarczyk, G., 2018. Characteristics of glycopeptide-resistant *Staphylococcus aureus* strains isolated from inpatients of three teaching hospitals in Warsaw, Poland. *Antimicrob. Resist. Infect. Contr.,* **7**: 105. https://doi.org/10.1186/s13756-018-0397-y

Ullah, A., Qasim, M., Rahman, H., Khan, J., Haroon, M., Muhammad, N., Khan, A. and Muhammad, N., 2016. High frequency of methicillin-resistant *Staphylococcus aureus* in Peshawar Region of Pakistan. *Springerplus,* **5**: 600. https://doi.org/10.1186/s40064-016-2277-3

Versalovic, J., Carroll, K., Funke, G., Jorgensen, J., Landry, M. and Warnock, D., 2011. *Manual of clinical microbiology*. ASM, Washington DC. https://doi.org/10.1128/9781555816728

Wayne, P., 2017. *Clinical and laboratory standards institute: Performance standards for antimicrobial susceptibility testing: 27th informational supplement*. CLSI document M100-S27. 2017.

Wong, J.W., Ip, M., Tang, A., Wei, V.W., Wong, S.Y., Riley, S., Read, J.M. and Kwok, K.O., 2018. Prevalence and risk factors of community-associated methicillin-resistant *Staphylococcus aureus* carriage in Asia-Pacific region from 2000 to 2016: A systematic review and meta-analysis. *Clin. Epidemiol.,* **10**: 1489-1501. https://doi.org/10.2147/CLEP.S160595

Yang, Y., Hu, Z., Shang, W., Hu, Q., Zhu, J., Yang, J., Peng, H., Zhang, X., Liu, H., Cong, Y., Li, S., Hu, X., Zhou, R. and Rao, X., 2017. Molecular and phenotypic characterization revealed high prevalence of multidrug-resistant methicillin-susceptible *Staphylococcus aureus* in Chongqing, Southwestern China. *Microb. Drug Resist.,* **23**: 241-246. https://doi.org/10.1089/mdr.2016.0078

Yilmaz, E.S. and Aslantas, O., 2017. Antimicrobial resistance and underlying mechanisms in *Staphylococcus aureus* isolates. *Asian Pac. J. trop. Med.,* **10**: 1059-1064. https://doi.org/10.1016/j.apjtm.2017.10.003