Investigation of *Staphylococcus aureus,* prevailing in the environment of Khyber Teaching Hospital, Peshawar, Pakistan

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Abstract: The hospital environment plays an important role in the spread of microorganisms, including multi drug resistant (MDR) strains. Patients can acquire Methicillin-Resistant *Staphylococcus aureus* (MRSA) which can reside in the clinical setup that are not cleaned and can spread through air droplets, bed clothing, and healthcare workers. The purpose of this study was to investigate the prevalence of *S. aureus* in the Khyber Teaching Hospital (KTH). A total of 200 samples were collected from the floor, walls, air and inanimate objects in different wards of the KTH, during May 2012 to September 2012. These samples were screened for the recovery of *S. aureus.* Recovered organisms were subjected to susceptibility testing and investigated for the detection of various toxin and antibiotic resistance genes by Polymerase Chain Reaction (PCR). A total of 64 samples yielded *S. aureus*, out of which 37 (57.81%) were proved as MRSA. No isolate was found resistant to Vancomycin, however 81.25% of the isolates were found susceptible to Linezolid and Amikacin. The susceptibility to Fusidic acid, Chloramphenicol, Rifampicin, Doxycycline and Meropenem was observed as 79.69%, 76.56%, 75.00, 73.44% and 68.75% respectively. The frequency of *sea*, *seb* and *sec* genes were 56.25%, 43.75% and 12.5% in the recovered isolates. *Erm* C was more prevalent (28.12 %) than the *erm*A and *erm*B. The prevalence of *pvl* in MRSA was 21.62 % which is less than 33.33% in the MSSA isolates. *S. aureus* and especially MRSA are frequently prevalent in the KTH. Therefore, every immune-compromised patient is prone to infections caused by *S. aureus.* This will lead to high morbidity/mortality rate, prolong hospital stay and add extra cost to the health system.

Keywords: *Staphylococcus aureus*, multi drug resistant, antibiotic resistance genes by polymerase chain reaction.

INTRODUCTION

Environment plays a substantial role in the spread of microorganisms, including MDR strains. MRSA are known to survive in dry conditions and can persist in clinical areas that are not properly cleaned, therefore may persist in the hospital environment and hence can be transmitted to immune-compromised patients wherein responsible for enhanced morbidity and mortality (Kramer *et al*., 2006). Investigations of many epidemics have proved thisphenomena that MRSA can be easily spread by interaction, chiefly in hospital (Boyce *et al*., 1997, Solberg, 2000). Nasal carriage of MRSA could favor the spread of MRSA through air droplets (Williams, 1966, Lidwell *et al*., 1975, Mortimer *et al*., 1966, Bischoff *et al*., 2006). Bed making generates dust particles, which if harbor MRSA being shed from a previously MRSA positive patient could be transmitted to other patients (Shiomori *et al*., 2002). Thus, floating spread of MRSA from patients in respiratory secretions permits further studies, not only in terms of infection control for patients, but also for the use of personal protective strategies by healthcare workers.

Trauma, burn injuries and surgeries breach the physical barrier to opportunistic pathogens. Consequently a nutrients rich environment available to microbes favor growth/multiplication of these organisms acquired from healthcare personnel, patients and the hospital environment (Erol *et al*., 2004, Church *et al*., 2006). Infection caused by these MDR-MRSA strains makes them difficult and more costly to control and treat (Hacek *et al*., 1999, Rubin *et al*., 1999). The routes of transmission and causes of MRSA infections in burn units include patients susceptibility (Kaiser *et al*., 2011), surgical techniques (Olivo *et al*., 2009, Wibbenmeyer *et al*., 2010), health-care workers who are carriers for MRSA (Chambers, 2001), the capability of *S. aureus* to live on surfaces (Andrade *et al*., 2009) and air-borne propagation (Gehanno *et al*., 2009) therefore enhances chance of MRSA infection far more for these immune-compromised burn patients.

KTH is a tertiary care hospital in Peshawar that provides healthcare facilities to a large number of patients in the Khyber Pakhtunkhwa province of Pakistan. The purpose of this study is to determine the prevalence of *S. aureus* and particularly MRSA including their molecular characterization recovered from the environment of this tertiary care hospital.

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MATERIALS AND METHODS

A total of 200 samples from the KTH were collected during June 2012 to September 2012. These samples were taken from the floor, walls, tables, medical equipment, air and other inanimate objects in operation theater (OT), burn ward, surgical wards and obstetrics and gynecology wards. A sterile swab, wet in distilled water was used for the collection of samples. The swabs were streaked (fig.1a) on Mannitol salt agar (MSA), a selective medium for *S. aureus*, and blood agar. The yellow colonies on MSA were processed for catalase, coagulase and DNase tests to confirm *S. aureus.* Air samples were taken by settle plate method after exposing plates containing MSA for 2-4 hours in the operation theatre and other mentioned areas. The isolates were kept at -80^{o}C in cryo vials (Corning USA) containing Tryptone Soya Broth (TSB) with15% glycerol.

Confirmation of MRSA

The isolates were processed for the identification of MRSA on phenotypic and genotypic basis by the following methods.

Cefoxitin disc

On phenotypic basis the isolates were characterized as MRSA by 30µg Cefoxitin disc (Oxoid UK), applied in the culture sensitivity test as recommended by the Clinical Laboratory Standard Institute (CLSI) (Wayne, 2011). For positive control, *S. aureus* ATCC 43300 strain was used while ATCC 25923 strains was used as negative control.

Brilliance MRSA-2 agar

Brilliance MRSA-agar 2 (Oxoid, UK) is a selective media for the quick detection of MRSA. *S. aureus* isolates were refreshed in TSB and inoculated on Brilliance MRSA-2 agar. The media was incubated at 37°C for 16-20 hours to confirm MRSA. For positive control ATCC 43300 *S. aureus* strain was used while for negative control ATCC 25923 *S. aureus* strain was used. Blue denim color colonies (fig.1b) are positive MRSA strains (Sander, 2011).

Detection of nuc and mec a genes for the genetical confirmation of S. aureus and MRSA

Determinants of methicillin resistance i.e. *mec* A and *nuc* genes (which encode *S. aureus*-specific regions of the thermonuclease gene) were amplified using PCR. Sequences of both genes are given in the table 1.

DNA was extracted by taking 3-4 fresh colonies from blood agar and were suspended in 25µl of Lysostaphin (100µg/ml, Sigma Germany) solution (Kumari *et al*., 1997). This suspension was incubated for 10 minutes at 37ºC. In addition 25 µl of proteinase K (100 µg/ml) was added. Finally 75 µl of 0.1 M Tris HCl (pH 8.0) were also added. The mixture was then incubated for additional 10 minutes at 37˚C. This mixture was then boiled at 97°C for 5 min.

For each PCR reaction a total of 20µl of mixture was prepared. The PCR reaction mixture was composed of 2µl of 10x PCR reaction buffer (100 mMTris-HCl, 20 mM MgCl, 500 mMKCl, pH 8.3), 0.2µl *Taq* polymerase (Intron Biotechnology Inc., Korea), 2µl of dNTPs mixture (2.5mMof each dNTP), 2µl of template DNA, 1µl of forward and reverse primers (table 2.3) each with a concentration of 15 pmoles and 11.8µl of Nuclease-free water. The PCR products were run in 1.5% agarose gel processed in 1xTAE buffer. The bands stained by Ethidium bromide were snapped by a gel documentation system (Bio Rad Milan, Italy). Two strains i.e. ATCC 43300 and ATCC 25923 of *S. aureus* were processed as positive and negative control respectively in the detection of *mec*A gene. Similarly in the detection of *nuc* gene again ATCC 25923 *S. aureus* and *S. epidermidis* were processed as positive and negative control respectively.

Susceptibility test

In the culture and sensitivity test 21 antibiotics were processed (table 4) under the recommendation of CLSI (Wayne, 2011). Minimum inhibitory concentrations (MICs) of 5 antibiotics including Cephradine (CE), Cefoxitin (FOX), Vancomycin (VA), Ciprofloxacin (CIP) and Linezolid (LZD) were detected (table 5) by selecting the broth-micro dilution method (Wieg and *et al*., 2008). The MIC ranges of Fusidic Acid were recorded by another criterion recommended by (Jones *et al*., 2010). ATCC 43300 *S. aureus* strain was used for positive control in susceptibility tests. ATCC 25923 was used for negative controls in disc diffusion method while ATCC 29213 *S. aureus* strain was used for negative control in the MIC tests (Wayne, 2011).

Detection of toxin's genes

The isolates were screened for Panton Valentine Leucocidin (*pvl*), staphylococcal enterotoxins (*sea, seb, sec* and *sed*) and toxic shock syndrome toxin (*tsst*) genes by PCR. Cells lysed by lysostaphin (Sigma, Germany) enzyme and the DNA extracted was used as a template. 20µl of mixture was prepared for each PCR reaction composed of 2µl of 10xPCR reaction buffer (100 mMTris-HCl, 20mM MgCl, 500 mMKCl, pH 8.3), 0.2µl *Taq* polymerase (Intron Biotechnology Inc., Korea), 2µl of dNTPs mixture (2.5mMof each dNTP), 2µl of template DNA, 1µl of forward and reverse primers (table 2.3) each with a concentration of 15 pmoles primers (Oligonucleotides, Macrogen, Korea) and 11.8 µl of Nuclease-free water. A known *pvl* positive strain, *S. aureus* ATCC 49775 was used as positive control while for negative control a PCR mixture without template DNA was used. In case no positive standard were available then the amplicon obtained were sequenced (by Macrogen, Korea) and matched with the respective sequence of genes in the Gene bank database.

After each PCR run, the samples were run on the agarose gel (Invitrogen, UK). Generally 1.5% agarose gel prepared in 1xTAE buffer was used. The gel tank was filled with 1xTAE or 1xTBE 2-3mm above the gel slab. Before running on gel, the PCR products were mixed with 2µl loading buffer and 12µl from every tube was put in a single well in the gel already prepared. The gel was run in an electrophoresis gel tank for 45 minutes at 100V. A 1Kb DNA ladder (Fermentas, Lithania) as a size marker was run alongside the PCR products. After running the gel, it was stained with ethidium bromide for 15 minutes and then de-stained in water in plastic jar for 10 minutes. The bands stained with Ethidium bromide were photographed.

RESULTS

Out of 200 samples collected 64 *S. aureus* strains were isolated from the beds, walls, floor, equipment and air of OT and bed sheets etc of various wards of KTH. These samples were then subjected to susceptibility testing. In these 64 *S. aureus* strains 37 (57.81%) were found MRSA, while 27 (42.19%) were found as Methicillin-Susceptible *S. aureus* (MSSA). There was no isolate resistant to vancomycin. A total of 52 isolates (81.25%) were susceptible to Linezolid and Amikacin, 51 (79.69%) to Fusidic acid, 49 (76.56%) to Chloramphenicol, 48 (75.00) to Rifampicin, 47 (73.44%) to Doxycycline and 44 (68.75%) to Meropenem. The cephalosporins were not much effective (table 4).

Among 37 MRSA isolated from environment of KTH, 89.19% were susceptible to Vancomycin and 86.47% to linezolid while 10.81% were intermediately susceptible to each Vancomycin and Linezolid. Fusidic acid and Chloramphenicol also showed good activities (81.08% each). These were followed by Rifampicin, Doxycycline, Amikacin, Meropenem, Gentamicin, Ciprofloxacin, Clarithromycin showing 75.68, 67.57, 54.05, 40.54 and 35.14% respectively activities.

There were 27 MSSA isolates obtained from the environment of KTH. A total of 26 (96.3%) isolates were susceptible to Cefoxitin. Cephradine and Meropenem showed 88.89% activities each. Next were AMC, Ciprofloxacin and Vancomycin which inhibited 85.19% isolates each. Doxycycline inhibited 81.48% isolates, Clarithromycin and Fusidic acid inhibited 77.78% isolates, Cefaclor, Gentamicin, Linezolid and RD inhibited 74.07% isolates each. CPO and Chloramphenicol both showed 70.37% activities. Ceftazidime and CFM were completely inactive against the isolated MSSA showing 0% activities.

MIC of KE isolates

MIC_{50} and MIC_{90} of cefoxitin and Cephradine remained high (table 5). The anti-staphylococcal agents showed good activity although the MIC_{90} of Linezolid was found high i.e. 32µg/ml. The MIC of MRSA strains were detected high in comparison to MSSA.

Distribution of various toxin and erythromycin resistance genes

Out of 64 *S. aureus* isolates, 37 (57.81%) were positive for *mec*A gene. *Pvl* gene was detected in 17 (26.56%) isolates. In MSSA it was detected in 9 (33.33%) and in MRSA it was detected in 8 (21.62%) strains. The *sea* gene was present in 36 (56.25%) *S. aureus* stains (fig. 2), 22 (59.45%) in MRSA and 14 (51.85%) in MSSA. *Seb* gene was detected in 28 (43.75%) *S. aureus* isolates, being 12 (44.44%) in MSSA and 16 (43.24%) in MRSA. fig.2; (2a: Agarose gel electrophoresis for *sea* gene, L: Ladder, lane 1: positive control, lane 2: negative control, lane 3: isolate 5 and from lane 4 to lane 15 are isolates 6 to 17. 2b: Agarose gel electrophoresis for *sec* gene, L: Ladder, lane 1: positive control, lane 2: negative control, lane 3: isolate 22 and from lane 4 to lane 15 are isolates 23 to 34)

Sec was found in only 8 (12.5%) isolates. *Sed* gene was not present in any environmental sample. Presence of *erm* C gene was detected in 18 (28.12%) *S. aureus* isolates (table 6).

mec A: Gene located on the staphylococcal cassette chromosome *mec* for the confirmation of MRSA; nuc: Thermonuclease gene in *S. aureus*, *pvl*: Panton Valentine Leukocidin; *erm*: erythromycin ribosomal methylase including *erm*A, *erm*B and *erm*C; Se: Staphylococcal enterotoxins including *sea*, *seb*, *sec*, *sed* and *tst*: Toxic shock syndrome toxin.

DISCUSSION

Prevalence of *S. aureus* is common in the hospital setup and makes it a prominent nosocomial pathogen. MRSA causes epidemics in the hospitals as well as in the community that is difficult to treat (Naimi *et al*., 2003, Ahmad *et al*., 2014). In the current study *S. aureus* was isolated from the bed sheets, beds, tables placed in the wards, OT equipment and even from the air in OT during surgeries. In these isolates 37 were MRSA. Other studies also suggest that MRSA can survive in dry environments and can reside in clinical places that are not properly cleaned (Kramer *et al*., 2006, Mehtar *et al*., 2008, Solberg, 2000).

All of the isolates recovered in this study were MDR as they exhibited resistance to more than three antibiotics. Ampicillin resistance was showed by all 64 *S. aureus* isolates. Although the MSSA strains were found more susceptible to the tested antibiotics but still some of these MSSA showed remarkable resistance. On the other hand most of the MRSA exhibited high level of resistance to the tested antibiotics. High levels of resistance in the clinical strains of MRSA were reported in an early study

from Peshawar, Pakistan which showed 100% resistance to oxacillin, ampicillin and penicillin while 92.4% resistance to cephalothin and 40 %resistance to meropenem (Naeem *et al*., 2013). Cefixime resistance was noted in 63 (98.44 %) while Ceftazdime resistance was also high i.e. 96.88 % in these isolates. Resistance to these commonly prescribed antibiotics was also reported in Pakistan in the previous studies by Yasmin Taj *et al*, who have found 100% resistance in *S. aureus* isolates to Cefixime (Taj *et al*., 2010).

In the current study, low level of resistance (7.81% and 32.81 %) was observed in *S. aureus* against Amikacin and Gentamicin. Yasmin Taj *et al.* (2010) have also reported 17.2%, a low level of resistance to Amikacin but high level of resistance to Gentamicin i.e. 96.3 % by *S. aureus* clinical isolates (Taj *et al*., 2010) Environmental isolates in this study have shown low level of resistance to Amikacin and Gentamycin which may be due to the environmental stress or due to the differences in prescription pattern in different areas of Pakistan.

Among the anti-staphylococcal agents tested by the broth micro-dilution method Vancomycin showed excellent activity (87.50 %) against these environmental *S. aureus* isolates followed by Linezolid (79.69 %) and Fusidic acid (78.13 %). Eight strains exhibited intermediate resistance to Vancomycin. 80 % of the strains were found susceptible to Fusidic acid reported from Agha Khan University Hospital in Karachi, Pakistan which correlates with the current study (Zafar *et al*., 2011). The observed rate of susceptibility (79.69 %) is similar to the rate of susceptibility (83%) reported by Tsiodras *et al.* (2001) in the clinical isolates of *S. aureus* (Tsiodras *et al*., 2001).

The *erm* genes are expressed constitutively ($cMLS_B$) or inducibly ($iMLS_B$) (Schreckenberger *et al*., 2004) which include (*erm*A, *erm*B and *erm*C). The main genetic factors in *S. aureus* are *Erm*A and *erm*C (Kim *et al*., 2004, Saribas*et al*., 2006, Otsuka *et al*., 2007). In the current study the prevalence of *erm*A and *erm* B was found the same (17.18%) in *S. aureus* isolates while *erm*C was found more prevalent (28.12 %) than the rest. The prevalence of *erm*A, *erm*B and *erm*C was found more in MRSA than MSSA.

Toxins

Staphylococcal-infections with more severity have been found to be associated with the ability of the cell attachment to biomaterial. In the current study the *sea* gene was found the most common among the classical-staphylococcal-enterotoxin genes detected in environmental *S. aureus* isolates 36 (56.25%). The MRSA isolated from environmental specimens screened for *sea* gene gave results of 22 (59.45%). Similarly the MSSA strains showed 14 (51.85%) prevalence of *sea*. Previously it was reported that 17.5% of MSSA and 80% of MRSA from the clinical strains of *S. aureus* harbor *sea* gene (Garbacz *et al*., 2007). Similarly in environmental MRSA isolates, the prevalence of *seb* gene is 43.24% while in MSSA isolates it is 44.44%. In MSSA *seb* gene was found more prevalent than in MRSA strains. *Sec* gene was found both in MRSA, 6 (16.22%) and MSSA 2 (7.40%) isolates collected in the current study but the prevalence rate was found low as compared to reports by others globally (Seguin *et al*., 1999).

The *sed* gene was not found in the *S. aureus* strains. A study conducted in 1995, in France, reported the association between the skin infections and *pvl* production, especially in the cases of furuncles (Prevost *et al.*, 1995). In the current study the prevalence of PVL in MRSA was found in 8 (21.62%) isolates. In the MSSA isolates it was found in 9 (33.33%) strains.

This suggests that the rate of occurrence of *S. aureus* strains positive for *pvl* in Pakistan is high in the MSSA as compared to MRSA. Same is also reported by Lina *et al.*, from France (Lina *et al*., 1999). Low occurrence of *pvl*-positive MRSA detected in the current study proposes that the ability of these strains to cause diseases could be significantly due to some other genes or features other than the *pvl* gene. The current results are much similar with Lina *et al.*, (1999) who have found that there was 85% definite association between *pvl* gene and the detection of community-acquired-pneumonia (Lina *et al*., 1999).

The *tst* gene was detected in 3 (11.11%) MSSA isolates. Prevalence of the *tst* gene is reportedly low (7.5%) among *S. aureus* clinical isolates and 4.5% among stable *S. aureus* nasal carriers (Nashev *et al*., 2004, El-Ghodban *et al*., 2006). The incidence of *Staphylococcal* enterotoxin, *tst* and *pvl* genes in clinical isolates of *S. aureus* from Pakistan has not been frequently published. The consequence is the deficiency of data regarding these genes in clinical strains from Pakistan and adjacent countries.

CONCLUSION

S. aureus isolates were present on the inanimate objects including bed sheets, walls, floor, devices in OT and air in the OT while the routine work was in progress. Most importantly many of the isolates were MRSA and were found resistant to many commonly prescribed antibiotics. This data show that adequate cleaning procedures (Rafla and Tredget, 2011) must be adopted otherwise these prevailing *S. aureus* isolates will cause infections in the immune-compromise patients and will increase hospital stay and cost of treatment.

Table 1: PCR primer sequences, amplicon sizes and PCR conditions

Gene	Primer sequence (5′-3′)	Product Size	PCR conditions		References
			Annealing temp.	Cycles	
mecA	F-CTCAGGTACTGCTATCCACC	449	55^0C	30	(Bignardi *et al*., 1996)
	R-CACTTGGTATATCTTCACC				
nuc	F-GCGATTGATGGTGATACGGTT	280	55^0C	30	(Brakstad *et al*., 992)
	R-AGCCAAGCCTTGACGAACTAAAGC				

Table 2: Oligonucleotide primers used for amplification of various toxin genes

Target Gene	Primer	PCR primer sequence 5'-3'	PCR conditions	Amplicon Size (bp)	Reference
Pvl	PVL-1 PVL-2	ATCATTAGGTAAAATGTCTGGACATGATCC GCATCAACTGTATTGGATAGCAAAAGC	2	433	(Lina et al., 1999)
Tst	TSST-1 TSST-2	ATGGCAGCATCAGCTTGATA TTTCCAATAACCACCCGTTT	3	350	(Nashev *et al*., 2004)
Sea	SEA-1 SEA-2	AAAGTCCCGATCAATTTATGGCTA GTAATTAACCGAAGGTTCTGTAGA	3	216	(Nashev *et al*., 2004)
Seb	SEB-1 SEB-2	TCGCATCAAACTGACAAACG GCAGGTACTCTATAAGTGCC	3	278	(Nashev *et al*., 2004)
Sec	SEC-1 SEC-2	GACATAAAAGCTAGGAATTT AAATCGGCTTAACATTATCC	3	257	(Nashev *et al*., 2004)
Sed	SED-1 SED-2	CTAGTTTGGTAATATCTCCT TAATGCTATATCTTATAGGG	3	317	(Nashev *et al*., 2004)

1: 94 ^{o}C 5min then 50 x (94 ^{o}C 30 sec, 55.5 ^{o}C 30 sec, 72 ^{o}C 30 sec), 2: 35 x (94 ^{o}C 30 sec, 64 ^{o}C 30 sec, 72 ^{o}C 60 sec), 3: 30 x (94^{o}C 120 sec, 55^{o}C 120 sec, 72^{o}C 60 sec)

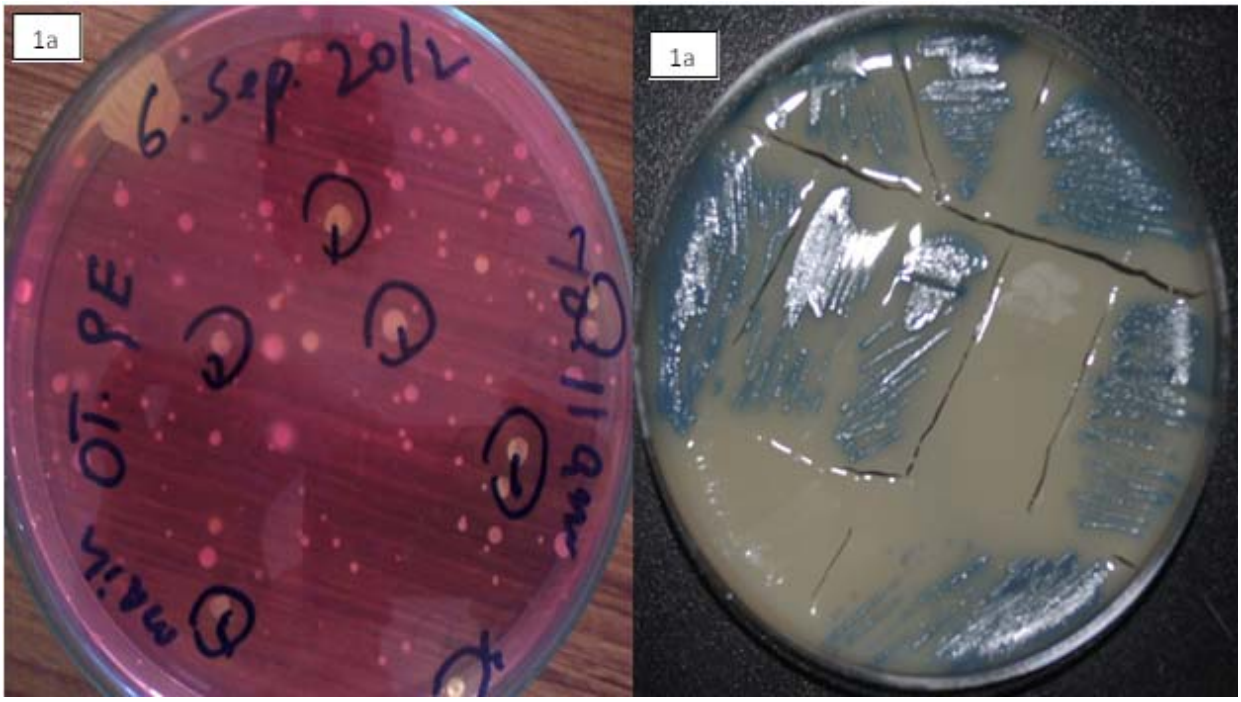

Fig. 1: (1a: Growth of *S. aureus* on MSA with yellow colonies) (1b: Growth of MRSA on Brilliance MRSA agar2)

Table 3: Primer used with the PCR conditions to detect erythromycin resistance genes in *S. aureus*

Resistance gene	PCR primer sequence 5'-3'	PCR reaction conditions	Amplicon Size (bp)	Reference
*erm*A	F-GTTCAAGAACAATCAATACAGAG R-GGATCAGGAAAAGGACATTTTAC	94 °C for 5min then 30 cycles (30s at 94°C; 30s at 52°C; 1 min. at 72°C)	421	(Leclercq *et al*., 1989)
*erm*B	F-CCGTTTACGAAATTGGAACAGGTAAAGGGC R-GAATCGAGACTTGAGTGTGC	As *erm* (A)	359	(Trieu-Cuot *et al*., 1990)
*erm*C	F-GCTAATATTGTTTAAATCGTCAATTCC R-GGATCAGGAAAAGGACATTTTAC	As *erm* (A)	572	(Leclercq *et al*., 1989)

Table 4: Susceptibility of *S. aureus*, MRSA and MSSA isolated from the hospital set-up

Antibiotic Symbol	*S. aureus* (n=64)			MRSA (n=37)			MSSA (n=27)		
	Resistance n (%)	Susceptible n (%)	Intermediate n (%)	Resistance n (%)	Susceptible n (%)	Intermediate n (%)	Resistance n (%)	Susceptible n (%)	Intermediate n (%)
FOX	37 (57.81)	27 (42.19)	0 (0)	37 (100)	0 (0.00)	0 (0.00)	0 (0.00)	27 (100)	0 (0.00)
AMC	35 (54.69)	27 (42.19)	2 (3.13)	31 (83.78)	4 (10.81)	2 (5.41)	4 (14.81)	23 (85.19)	0 (0.00)
AMP	64 (100)	0 (0.00)	0 (0)	37 (100)	0 (0.00)	0 (0.00)	27 (100)	0 (0.00)	0 (0.00)
CE	30 (46.88)	33 (51.56)	1 (1.56)	28 (75.68)	9 (24.32)	0 (0.00)	2 (7.41)	24 (88.89)	1 (3.70)
CEC	29 (45.31)	29 (45.31)	6 (9.38)	23 (62.16)	9 (24.32)	5 (13.51)	6 (22.22)	20 (74.07)	1 (3.70)
CAZ	62 (96.88)	0 (0.00)	2 (3.13)	35 (94.59)	0 (0.00)	2 (5.41)	27 (100)	0 (0.00)	0 (0.00)
CFM	63 (98.44)	0 (0.00)	1 (1.56)	36 (97.30)	0 (0.00)	1 (2.70)	27 (100)	0 (0.00)	0 (0.00)
FEP	32 (50.00)	26 (40.63)	6 (9.38)	24 (64.86)	9 (24.32)	4 (10.81)	8 (29.63)	17 (62.96)	2 (7.41)
CPO	31 (48.44)	28 (43.75)	5 (7.81)	24 (64.86)	9 (24.32)	4 (10.81)	7 (25.93)	19 (70.37)	1 (3.70)
CIP	22 (34.38)	38 (59.38)	4 (6.25)	18 (48.65)	15 (40.54)	4 (10.81)	4 (14.81)	23 (85.19)	0 (0.00)
CLR	20 (31.25)	34 (53.13)	10 (15.63)	14 (37.84)	13 (35.14)	10 (27.03)	6 (22.22)	21 (77.78)	0 (0.00)
MEM	15 (23.44)	44 (68.75)	5 (7.81)	13 (35.14)	20 (54.05)	4 (10.81)	2 (7.41)	24 (88.89)	1 (3.70)
CN	21 (32.81)	40 (62.50)	3 (4.69)	15 (40.54)	20 (54.05)	2 (5.41)	6 (22.22)	20 (74.07)	1 (3.70)
AK	5 (7.81)	52 (81.25)	7 (10.94)	5 (13.51)	25 (67.57)	7 (18.92)	0 (0.00)	27 (100)	0 (0.00)
DO	2 (3.13)	47 (73.44)	15 (23.44)	2 (5.41)	25 (67.57)	10 (27.03)	0 (0.00)	22 (81.48)	5 (18.52)
SXT	35 (54.69)	23 (35.94)	6 (9.38)	23 (62.16)	10 (27.03)	4 (10.81)	12 (44.44)	13 (48.15)	2 (7.41)
VA	0 (0.00)	56 (87.50)	8 (12.5)	0 (0.00)	34 (91.89)	3 (8.11)	0 (0.00)	22 (81.48)	5 (18.52)
LZD	12 (18.75)	52 (81.25)	0 (0.00)	5 (13.51)	32 (86.49)	0 (0.00)	7 (25.93)	20 (74.07)	0 (0.00)
FD	13 (20.31)	51 (79.69)	0 (0.00)	7 (18.92)	30 (81.08)	0 (0.00)	6 (22.22)	21 (77.78)	0 (0.00)
RD	15 (23.44)	48 (75.00)	1 (1.56)	9 (24.32)	28 (75.68)	0 (0.00)	6 (22.22)	20 (74.07)	1(3.70)
C	11 (17.19)	49 (76.56)	4 (6.25)	4 (10.81)	30 (81.08)	3 (8.11)	7 (25.93)	19 (70.37)	1 (3.70)

N: number, FOX; Cefoxitin, AMC; Amoxicillin + Clauvalinic acid, AMP; Ampicillin, CE; Cephradine, CEC; Cefaclor, CAZ; Ceftazidime, CFM; Cefixime, FEP; Cefepime, CPO; Cefpirome, CIP; Ciprofloxacin, CLR; Clarithromycin, MEM; Meropenem, CN; Gentamicin, AK; Amikacin, DO; Doxycycline, SXT; Trimethoprim+Sulphamethoxazole, VA; Vancommycin, LZD; Linezolid, FD; Fusidic acid, RD; Rifampicin, C; Chloamphenicol

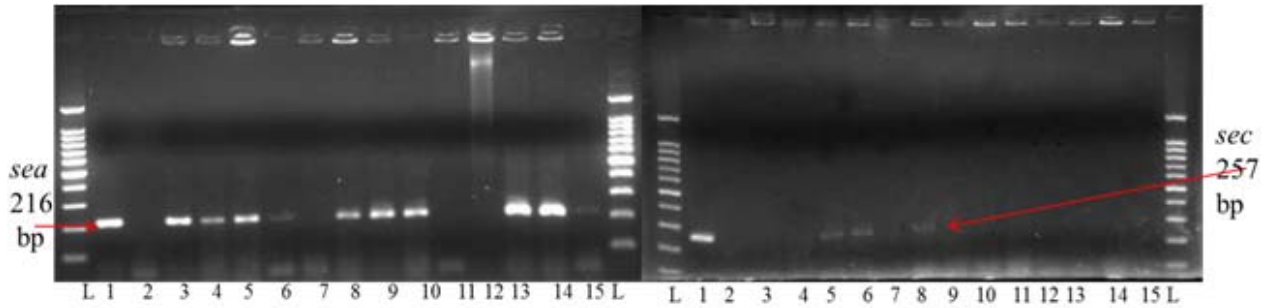

Fig. 2: (2a: Agarose gel electrophoresis for *sea* gene, L: Ladder, lane 1: positive control, lane 2: negative control, lane 3: isolate 5 and from lane 4 to lane 15 are isolates 6 to 17. 2b: Agarose gel electrophoresis for *sec* gene, L: Ladder, lane 1: positive control, lane 2: negative control, lane 3: isolate 22 and from lane 4 to lane 15 are isolates 23 to 34)

Table 5: Minimum inhibitory concentration (MIC) of different antibiotics against *S. aureus*, MRSA and MSSA isolates.

Symbol	MICs of *S. aureus* in µg/ml													MIC 50%	MIC 90%	Total samples
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256			
FOX	0	0	3	14	10	0	0	0	2	7	15	12	1	64	256	64
Ce	0	0	1	7	9	7	3	0	0	11	16	10	0	64	256	64
FD	6	10	12	22	2	2	3	4	3	0	0	0	0	1	8	64
VA	1	3	8	17	27	6	2	0	0	0	0	0	0	2	4	64
LZD	0	0	0	15	21	16	1	2	6	3	0	0	0	2	32	64
CIP	0	4	12	19	0	5	2	2	6	8	6	0	0	1	64	64
Symbol	MICs of MRSA in µg/ml													MIC 50%	MIC 90%	Total samples
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256			
FOX	0	0	0	0	0	0	0	0	2	7	15	12	1	128	256	37
Ce	0	0	0	0	0	0	0	0	0	11	16	10	0	128	256	37
FD	3	7	8	12	0	0	3	3	1	0	0	0	0	1	8	37
VA	1	1	5	9	18	3	0	0	0	0	0	0	0	2	2	37
LZD	0	0	0	11	13	8	0	0	4	1	0	0	0	2	32	37
CIP	1	1	4	7	0	4	1	2	5	7	5	0	0	16	128	37
Symbol	MICs of MSSA in µg/ml													MIC 50%	MIC 90%	Total samples
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256			
FOX	0	0	3	14	10	0	0	0	0	0	0	0	0	1	2	27
Ce	0	0	1	7	9	7	3	0	0	0	0	0	0	2	4	27
FD	3	3	4	10	2	2	0	1	2	0	0	0	0	1	4	27
VA	0	2	3	8	9	3	2	0	0	0	0	0	0	2	4	27
LZD	0	0	0	4	8	8	1	2	2	2	0	0	0	4	32	27
CIP	0	2	8	12	0	1	1	0	1	1	1	0	0	1	8	27

FOX; Cefoxitin, CE; Cephradrine, VA; Vancomycin, LZD; Linezolid, FD; Fusidic acid, CIP; Ciprofloxacin.

Table 6: Distribution of various toxin and erythromycin resistance genes in *S. aureus*, MRSA and MSSA isolates.

	S. aureus n=64		MRSA n=37		MSSA n=27	
Genes	Positive n (%)	Negative n (%)	Positiven (%)	Negative n (%)	Positive n (%)	Negative n (%)
*mec*A	37 (57.81)	27 (42.19)	37 (100.00)	0 (0.00)	0 (0.00)	27 (100.00)
nuc	64 (100.00)	0 (0.00)	37 (100.00)	0 (0.00)	27 (100.00)	0 (0.00)
Pvl	17 (26.56)	47 (73.44)	8 (21.62)	29 (78.38)	9 (33.33)	18 (66.67)
*erm*A	11 (17.19)	53 (82.82)	7 (18.92)	30 (81.08)	4 (14.81)	23 (85.18)
*erm*B	11 (17.19)	53 (82.81)	8 (21.62)	29 (78.38)	3 (11.11)	24 (88.89)
*erm*C	18 (28.12)	46 (71.87)	10 (27.03)	27 (72.97)	8 (29.63)	19 (70.37)
Sea	36 (56.25)	28 (43.75)	22 (59.46)	15 (40.54)	14 (51.85)	13 (48.15)
Seb	28 (43.75)	36 (56.25)	16 (43.24)	21 (56.76)	12 (44.44)	15 (55.55)
Sec	8 (12.50)	56 (87.50)	6 (16.22)	31 (83.78)	2 (7.40)	25 (92.59)
sed	0 (0.00)	64 (100.00)	0 (0.00)	37 (100.00)	0 (0.00)	27 (100.00)
Tst	3 (4.6875)	61 (95.31)	0 (0.00)	37 (100.00)	3 (11.11)	24 (88.89)

*mec*A: Gene located on the staphylococcal cassette chromosome *mec* for the confirmation of MRSA; nuc: Thermonuclease gene in *S. aureus*, *pvl*: Panton Valentine Leukocidin; *erm*: erythromycin ribosomal methylase including *erm*A, *erm* B and *erm* C; Se: Staphylococcal enterotoxins including *sea*, *seb*, *sec*, *sed* and *tst*: Toxic shock syndrome toxin.

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REFERENCES

Ahmad B, Khan F, Ahmed J, Cha SB, Shin M, Bashir S and Yoo HS (2014). Antibiotic resistance pattern and molecular epidemiology of Methicillin-Resistant *Staphylococcus aureus* colonization in burns unit of a tertiary care hospital in Peshawar, Pakistan. *Trop. J. of Pharma. Res.*, **13**: 2091-2099.

Andrade C, Champagne S, Caruso D, Foster K and Reynolds K (2009). Methicillin-resistant *Staphylococcus aureus*: An assessment of environmental contamination in a burn center. *American J. of Infec. Cont.,* **37**: 515-517.

Bignardi GE, Woodford N, Chapman A, Johnson AP and Speller DCE (1996). Detection of the *mec-A* gene and phenotypic detection of resistance in *Staphylococcus aureus* isolates with borderline or low-level methicillin resistance. *J. of Antimic. Chemother.*, **37**: 53-63.

Bischoff WE, Wallis ML, Tucker BK, Reboussin BA, Pfaller MA, Hayden FG and Sherertz RJ (2006). Gesundheit Sneezing, common colds, allergies and *Staphylococcus aureus* dispersion. *J. Infect. Dis.,* **194** (8): 1119-1126.

Boyce JM, Potter BG, Chenevert C and King T (1997). Environmental contamination due to methicillin-resistant *Staphylococcus aureus*: Possible infection control implications. *Infect. Cont. Hosp. Epid.,* **18**(9): 622-627.

Brakstad OG, Aasbakk K and Maeland JA (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J. Clin. Microbial.,* **30** (7): 1654-1660.

Chambers HF (2001). The changing epidemiology of *Staphylococcus aureus*? *Emer. Infect. Dis.,* **7**(2): 178-182.

Church D, Elsayed S, Reid O, Winston B and Lindsay R (2006). Burn wound infections. *Clin. Microbial. Rev.,* **19**: 403-434.

El-Ghodban A, Ghenghesh KS, Marialigeti K, Esahli H and Tawil A (2006). PCR detection of toxic shock syndrome toxin of *Staphylococcus aureus* from Tripoli, Libya. *J. Med. Microbiol.,* **55**: 179-82.

Erol S, Altoparlak U, Akcay MN, Celebi F and Parlak M (2004). Changes of microbial flora and wound colonization in burned patients. *Burns,* **30**: 357-361.

Garbacz K, Dajnowska-Stanczewa A and Piechowicz L (2007). Occurrence of selected enterotoxins and toxic shock syndrome toxin genes among sensitive and resistant to methicillin *Staphylococcus aureus* isolated from clinical samples. *Med. Dosw. Mikrobiol.,* **59**: 201-6.

Gehanno JF, Louvel A, Nouvellon M, Caillard JF and Pestel CM (2009). Aerial dispersal of meticillin-resistant *Staphylococcus aureus* in hospital rooms by infected or colonised patients. *J. of Hosp. Infect.,* **71**: 256-262.

Hacek DM, Suriano T, Noskin GA, Kruszynski J, Reisberg B and Peterson LR (1999). Medical and economic benefit of a comprehensive infection control program that includes routine determination of microbial clonality. *Am. J. Clin. Pathol.,* **111**: 647-654.

Jones RN, Castanheira M, Rhomberg PR, Woosley LN and Pfaller MA (2010). Performance of fusidic acid (cem-102) susceptibility testing reagents: Broth micro dilution, disk diffusion and test methods as applied to *Staphylococcus aureus*. *J. Clin. Microbiol.,* **48**: 972-976.

Kaiser ML, Thompson DJ, Malinoski D, Lane C and Cinat ME (2011). Epidemiology and risk factors for hospital-acquired methicillin-resistant *Staphylococcus aureus* among burn patients. *J. Burn Care Res.,* **32**: 429-434.

Kim HB, Lee B, Jang HC, Kim SH, Kang CI, Choi YJ, Park SW, Kim BS, Kim EC, Oh MD and Choe KW (2004). A high frequency of macrolide-lincosamide-streptogramin resistance determinants in *Staphylococcus aureus* isolated in South Korea. *Microb. Drug Resist.,* **10**: 248-254.

Kramer A, Schwebke I and Kampf GN (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis.,* **6**: 130.

Kumari DN, Keer V, Hawkey PM, Parnell P, Joseph N, Richardson JF and Cookson B (1997). comparison and application of ribosome spacer DN Aamplicon polymorphisms and pulsed-field gel electrophoresis for differentiation of methicillin-resistant *Staphylococcus aureus* strains. *J. Clin. Microbiol.,* **35**: 881-885.

Leclercq R, Bauduret F and Soussy CJ (1989). Selection of constitutive mutants of gram-positive cocci inducible resistant to macrolides, lincosamides and streptogramins (MLS): Comparison of the selective effects of the MLS. *Pathol. Biol. (Paris).,* **37**: 568-572.

Lidwell OM, Brock B, Shooter RA, Cooke EM and Thomas GE (1975). Airborne infection in a fully air-conditioned hospital: IV. Airborne dispersal of *Staphylococcus aureus* and its nasal acquisition by patients. *J. of Hyg.,* **75**: 445-474.

Lina G, Piemont Y, Godail-gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F and Etienne J (1999). Involvement of Panton-Valentine-Leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin. Infect. Dis.,* **29**: 1128-1132.

Mehtar S, Wiid I and Todorov SD (2008). The antimicrobial activity of copper and copper alloys against nosocomial pathogens and *Mycobacterium tuberculosis* isolated from healthcare facilities in the Western Cape: an in-vitro study. *J. Hos. Infect.,* **68**: 45-51.

Mortimer EA, Wolinsky E, Gonzaga AJ and Rammelkamp CH (1966). Role of airborne transmission in staphylococcal infections. *BMJ.,* **1**: 319-322.

Naeem M, Adil M, Naz SM, Abbas SH, Khan A and Khan MU (2013). Resistance and sensitivity pattern of *Staphylococcus aureus*; A study in Lady Reading hospital Peshawar. *J. Post. Med. Insti. (Peshawar-Pakistan),* **27**: 42-47.

Naimi TS, Ledell KH, Como SK, Borchardt SM, Boxrud DJ, Etienne J, Johnson SK, Vandenesch F, Fridkin S and O'boyle C (2003). Comparison of community-and health care associated methicillin-resistant

Staphylococcus aureus infection. *JAMA,* **290**: 2976-2984.

Nashev D, Toshkova K, Salasia SI, Hassan AA, Lammler C and Zschock M (2004). Distribution of virulence genes of *Staphylococcus aureus* isolated from stable nasal carriers. *FEMS. Microbiol. Lett.,* **233**: 45-52.

Olivo TET, Demelo EC, Rocha C and Fortaleza CM (2009). Risk factors for acquisition of methicillin-resistant *Staphylococcus aureus* among patients from a burn unit in Brazil. *Burns.,* **35**: 1104-1111.

Otsuka T, Zaraket H, Takano T, Saito K, Dohmae S, Higuchi W and Yamamoto T (2007). Macrolide-lincosamide-streptogramin B resistance phenotypes and genotypes among *Staphylococcus aureus* clinical isolates in Japan. *Clin. Microbiol. Infect.,* **13**: 325-327.

Prevost G, Couppie P, Prevost P, Gayet S, Petiau P, Cribier B, Monteil H and Piemont Y (1995). Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. *J. Med. Microbiol.,* **42**: 237-245.

Rafla K and Tredget EE (2011). Infection control in the burn unit. *Burns,* **37**: 5-15.

Rubin RJ, Harrington CA, Poon A, Dietrich K, Greene JA and Moiduddin A (1999). The economic impact of *Staphylococcus aureus* infection in New York City hospitals. *Emerg. Infect. Dis.,* **5**: 9-17.

Rutala WA, Kat EB, Sherertz RJ and Sarubbi FA (1983).Environmental study of a methicillin-resistant S*taphylococcus aureus* epidemic in a burn unit. *J. Clin. Microbiol.,* **18**: 683-688.

Sander C, Kalka-Moll W and Scopes E (2011). Methicillin-resistant staphylococcus screening using Brilliance MRSA 2 agar. C*onference proceedings, 21st ECCMID/27th ICC, Milan, Italy)* p.1035.

Saribas Z, Tunckanat F and Pinar A (2006). Prevalence of *erm* genes encoding macrolide-lincosamide-streptogramin resistance among clinical isolates of *Staphylococcus aureus* in a Turkish university hospital. *Clin. Microbial. Infect,* **12**: 797-799.

Schreckenberger PC, Ilendo E and Ristow KL (2004). Incidence of constitutive and inducible clindamycin resistance in S*taphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. *J. Clin. Microbial.,* **42**: 2777-2779.

Seguin JC, Walker RD, Caron JP, Kloos WE, George CG, Hollis RJ, Jones RN and P faller MA (1999). Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: Potential human-to-animal transmission. *J. Clin. Microbiol.,* **37**: 1459-1463.

Shiomori T, Miyamoto H, Makishima K, Yoshida M, Fujiyoshi T, Udaka T, Inaba T and Hhiraki N (2002). Evaluation of bed making-related airborne and surface methicillin-resistant *Staphylococcus aureus* contamination. *J. Hos. Infect.,* **50**: 30-35.

Solberg CO (2000). Spread of *Staphylococcus aureus* in hospitals: Causes and prevention. *Scand. J. Infect. Dis.,* **32**: 587-595.

Taj Y, Abdullah FE and Kazmi SU (2010). Current pattern of antibiotic resistance in *staphylococcus aureus* clinical isolates and the emergence of vancomycin resistance. *JCPSP.,* **20**: 728-732.

Trieu-Cuot P, Poyart-Salmeron C, Carlier C and Courvalin P (1990). Nucleotide sequence of the erythromycin resistance gene of the conjugative transposon tn 1545. *Nuc. acids Res.,* **18**: 3660.

Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, Moellering, RC and Ferraro MJ (2001). Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. *Lancet,* **358**: 207.

Wayne P (2011). Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing: Twenty-first informational supplement M100-s21.

Wibbenmeyer L, Williams I, Ward M, Xiao X, Light T, Latenser B, Lewis R, Kealey GP and Herwaldt L (2010). Risk factors for acquiring vancomycin-resistant enterococcus and methicillin-resistant *Staphylococcus aureus* on a burn surgery step-down unit. *J. Burn care Res.,* **31**: 269-279.

Wiegand I, Hilpert K and Hancock REW (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols.,* **3**: 163-175.

Williams RE (1966). Epidemiology of airborne staphylococcal infection. *Bact. Rev.,* **30**: 660.

Zafar A, Stone M, Ibrahim S, Parveen Z, Hasan Z, Khan E, Hasan R, Wain J and Bamford K (2011). Prevalent genotypes of Methicillin Resistant *Staphylococcus aureus*: Report from *Pak. J. Med. Microbiol.,* **60**: 56-62.