Study of the genetic traits associated with antibiotic resistance in *Staphylococcus aureus* isolated from skin wards of Khyber Pakhtunkhwa, Pakistan

Saeed Ullah Khattak¹, Nafees Bacha¹, Ghosia Lutfullah³, Jehan Bakhê², Sajid Ali³, Johar Ali⁴, Abid Ali Khan⁵*

¹Center of Biotechnology and Microbiology, University of Peshawar, 25120, KPK, Pakistan
²Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, 25120, KPK, Pakistan
³Department of Chemistry, Bacha Khan University, Charsadda, KPK, Pakistan
⁴Alvi-armani, 2680 Matheson Blvd. East, Suite 102, Mississauga, ON L4W 0A5, Canada
⁵Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad Campus, 22060, KPK, Pakistan

Objective: To investigate the prevalence of *Staphylococcus aureus* (*S. aureus*) isolated from skin wards of the hospitals of Khyber Pakhtunkhwa, its resistance against various commonly and commercially available antibiotics, as well as different genetic traits of resistance and their correlations with the phenotypic visible resistance.

Methods: In the present study a simple PCR technique were used to investigate the genetic traits of resistance in *S. aureus* isolated from skin wards of two major hospitals of Khyber Pakhtunkhwa, Pakistan. A total of 100 samples were collected from both the male and female, of which 50 were from patient’s site of infection and 50 from ward environment.

Results: These results demonstrated that the total prevalence of *S. aureus* both in ward as well as in patients was 48%. The *S. aureus* prevalence was the highest in female patients (50%) followed by ward environment (29%) and then male patients (21%). The antibiotic sensitivity tests revealed that the highest (91.6% isolates) sensitivity was shown to imipenem. However, the highest resistance was found to be against penicillin (100% isolates) followed by cefotaxime (75% isolates). In addition, only 29% of the isolates were found to be resistant to methicillin. PCR technique based on the previously designed primers targeting different genetic traits of resistance revealed that 13 out of the 14 isolates resistant to methicillin were positive for *mecA* gene. *blaZ* Genetic traits were found in all isolates resistant to penicillin. The multi-drug resistance traits, *vgaA* and *vgaB* each was detected only in 12.5% of *S. aureus* isolates. The phenotypic character of antibiotic resistance is highly correlated to different genetic traits of resistance.

Conclusions: Based on our findings, it is concluded that antibiotic resistance in *S. aureus* strains is increasing day by day due to self-medications and medication by non-registered medical practitioners. Therefore, for quick and fast detection, we propose next-generation sequencing be utilized to screen for antibiotic resistance.

1. Introduction

*Staphylococcus aureus* (*S. aureus*) is one of the most intimidating pathogens commonly found on skin and mucous membranes, e.g. in human nose. Almost 15%-40% of all healthy human beings are found to be carriers of this opportunistic pathogen[1]. *S. aureus* is a Gram positive cocci and a facultative anaerobe which can survive in high temperature (50 °C), salt concentrations and drying conditions[2]. Due to its formidable ability to survive in variable environmental conditions and remarkable ability to acquire resistance against antibiotics, it is considered as one of the most threatening microorganisms[3].

Different antibiotics have been used to treat some of the serious skin and other diseases including bacteremia, boils, bullous...
impetigo, cellulitis, endocarditis, folliculitis, food poisoning, lymphadenitis, lymphangitis, osteomyelitis, paronychia, scalded skin syndrome, septic arthritis, styes and toxic shock syndrome caused by *S. aureus*. In addition, *S. aureus* has the ability to become resistant to almost all the available antibiotics and bactericidal agents, either by acquiring the resistant genes from other strains or mutating its own genes. Thus through evolution, *S. aureus* has become resistant to several antibiotics, to which it was previously susceptible. Penicillin-G has been used against *S. aureus* since 1940, but soon it became resistant to this drug by acquiring beta-lactamase genes. Later it adapted ways to become resistant to penicillinase-resistant penicillins. Until recently this bacterium has been reported to be the best choice against *S. aureus*, however, the first methicillin resistant *S. aureus* (MRSA) was detected in 1961, which is now quite common in hospitals all over the world. After the emergence of MRSA, vancomycin were reported to be the best choice against *S. aureus*, unfortunately due to its genetic evaluation, *S. aureus* has evolved strains resistant to macrolides, lincosamides, tetracycline and gentamyacin. For some time methicillin were appropriate drug against *S. aureus*. However, the first methicillin resistant *S. aureus* (MRSA) was isolated in 2002 in USA.

*S. aureus* is becoming more and more prevalent in hospitals and other communities. Its extraordinary ability of becoming resistant to approximately all the available antibiotics makes *S. aureus* a formidable challenge for researchers all over the world. Therefore, the present study was conducted with the objectives to determine the prevalence of *S. aureus* isolated from skin wards of the hospitals of Khyber Pakhtunkhwa, and also its resistance against various commonly and commercially available antibiotics, i.e., cefixime, cefoperazone, cefotaxime, imipenem, methicillin, penicillin-G, streptomycin and tetracycline. The study was further focused to investigate different genetic traits of resistance and to determine their correlations with the phenotypic visible resistance.

2. Materials and methods

The present study was conducted at Microbiology Research Laboratory, Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar. A total of 100 samples were collected from skin wards of both male and female at the hospitals.

2.1. Sample collections

Commercially available sterile swabs dipped in sterile normal saline were used for the collection of samples. The samples were collected from skin ward of two local hospitals. Among these 50 samples were taken from the patient surroundings in the male and female ward while the other 50 samples were taken from patient’s infection site.

2.2. Preparation of selective media

These samples were directly streaked on staphylococcus selective media. The staphylococcus selective media was prepared by dissolving the ingredients in proper proportions in distilled water according to the manufacturer’s instructions. The media was autoclaved at 121 °C for 15 min at 15-20 psi. The media was then poured in the plates and waited till its solidification. Plates were kept in incubator for 24 h to check the sterility of the media.

2.3. Inoculation on selective media

The samples were directly streaked on the staphylococcus selective media plates and were kept for incubation of 24-48 h at 35-37 °C.

2.4. Identification of the isolates

The isolates were identified by microscopic examinations, Gram staining and several biochemical tests including catalase, coagulase, tryptophan and blood hemolysis.

2.5. Maintenance of bacterial isolates

Isolated bacterial strains were purified and preserved for further studies. For short term storage, the bacterial isolates were cultured on Muller Hinton agar slants and Petri dishes and maintained at 4 °C. These isolates were subcultured on monthly basis for routine use.

2.6. Preparation of 0.5 McFarland turbidity standard

For making 0.5 McFarland turbidity standard, 0.5 mL of 1.175% barium chloride were dissolved in 99.5 mL of 1% sulfuric acid. This solution was stored at room temperature in dark. Using standard McFarland turbidity enables us to compare the newly prepared bacterial suspension (1.5 × 10^8 CFU/mL).

2.7. Determination of antibiotic sensitivity by disc diffusion method

Disc diffusion method of Kirby and Bauer was used for determination of antibiotic resistance. Fresh broth culture which was incubated overnight at 35-37 °C was used. This culture was compared with 0.5 McFarland turbidity standard, by diluting the sample with sterile normal saline. Then the prepared broth culture was spread uniformly on the sterile Muller Hinton agar plates with the help of sterile cotton swabs. The antibiotic resistance capacities of all the bacterial isolates were determined against various commonly used antibiotics such as cefixime, cefoperazone, cefotaxime, imipenem, methicillin, penicillin-G, streptomycin and tetracycline. Using a sterilized forceps, antibiotic discs were placed carefully on inoculated Muller Hinton agar plates. All these plates were incubated at 35-37 °C overnight. Next day, the zones of inhibition were measured in millimeters, and the results were classified into resistant, intermediate and susceptible according to the NCCLS guidelines (Table 1).

| Disc (Potency) | Resistant | Intermediate | Susceptible |
|---------------|-----------|--------------|-------------|
| Imipenem (10 μg) | ≤ 13 mm | 14-15 mm | ≥ 16 mm |
| Ticarcillin (85 μg) | ≤ 14 mm | 15-18 mm | ≥ 19 mm |
| Cefoperazone (30 μg) | ≤ 14 mm | 15-22 mm | ≥ 23 mm |
| Cefotaxime (5 μg) | ≤ 15 mm | 16-18 mm | ≥ 19 mm |
| M ethicillin (5 μg) | ≤ 9 mm | 10-13 mm | ≥ 14 mm |
| Cefixime (75 μg) | ≤ 15 mm | 16-20 mm | ≥ 21 mm |
| Penicillin G (10 μg) | ≤ 20 mm | 21-28 mm | ≥ 29 mm |
| Streptomycin (10 μg) | ≤ 11 mm | 12-14 mm | ≥ 15 mm |
2.8. PCR screening of determinants of antibiotic resistance

All amplification reactions were prepared up to 25 µL containing 9.7 µL PCR grade water, 2.7 µL 10× PCR buffer, 2.5 µL MgCl₂, 4 µL dATP, dCTP, dGTP and dTTP, 1 µL oligonucleotide primers, 0.3 µL Taq polymerase and 5 µL template DNA. Antibiotic resistance genes, namely, blaZ (penicillin resistance); mecA (oxacillin resistance); tetK, tetM and tetL (tetracycline resistance); ermA, ermB, ermC (erythromycin resistance) and vgaA and vgaB (streptogramin-A and lincosamides group of antibiotic resistance) were examined in all bacterial strains by using PCR techniques. Oligonucleotide primers were used for the detection of antibiotic resistance-associated genes (Table 2)[10-12]. All the amplified DNA by PCR were confirmed by running on agarose gel in separate lane.

Table 2
PCR target genes and primers used in this work.

| Genetic trait | Primer pair                                      |
|---------------|--------------------------------------------------|
| mecA          | AACAGGTGAAATTAGCCTTGTAAAG                        |
|               | ATTCAGCTGAAATTAGGTGAA                            |
| blaZ          | ACTTCAACACCTGCTGCTTTC                            |
|               | TGACCACTTTTATACGAAACC                            |
| ermA          | TATCTATGTTGGAAGGATT                             |
|               | CTACACCTTTGCTAGATTGAAA                           |
| ermB          | CTATCTGATTTGGAAGGATT                             |
|               | GTTATCTTGGTTAGGTGAAA                             |
| ermC          | CTTTGTGATACCGATAATTCCC                          |
|               | ATCTTTTAGAACACCCTGTTAC                          |
| tetK          | CAGCAGATCCTACTCCTTT                             |
|               | TGGTACGGTCTGCTGATCTC                            |
| tetL          | GTACCACCAACTGAGGCG                               |
|               | GTGCAACAGGCGACACG                                |
| tetM          | AAGGGAGGCGGACACG                                 |
| vgaA          | CTCTGGGTCGCAATACG                                |
| vgaB          | TCTGCATAGAAGAGAC                                |

3. Results

3.1. Prevalence of S. aureus

The two largest hospitals in northwest region of Khyber Pakhtunkhwa, Pakistan were selected for studying the prevalence of S. aureus, which is a causal agent of various skin infections, abscesses, bacteremia, endocarditis, meningitis, mycobacteria, osteomyelitis and pneumonia at different sites. A total of 100 samples were collected, in which S. aureus was isolated from 48 samples. However, among the 48 isolates, 14 (29%) were identified from samples taken from male and female skin wards environment, and 34 (71%) were from samples taken from patient’s infection sites (Figure 1).

Figure 1. Infection sites of patients used for the collection of samples.

Out of the later 34 isolates, 10 were from samples taken from male patients while 24 isolates were from samples taken from female patients. These results demonstrate that the total prevalence of S. aureus both in ward as well as in patients was 48% (Figures 2 and 3).

Figure 2. Prevalence of S. aureus in different age groups.

Figure 3. Occurrence of S. aureus in skin wards and male and female patients.

3.2. Susceptibility pattern

The results showed that several S. aureus strains have developed resistant to antibiotics, especially methicillin, whereas, they were previously susceptible. Therefore, we have reported the antibiotic resistance of all the 48 isolates against various commonly used antibiotics such as cefixime, cefoperazone, cefotaxime, imipenem, methicillin, penicillin-G, streptomycin and ticarcillin. The results have been shown in Figure 4, while the resistance profiles in terms of zone of inhibition of all the isolates are given in Table 3.

Figure 4. Inhibition zones formed by antibiotic discs.

We have further observed that all the clinical isolates were resistant to penicillin-G; however, all the isolates were susceptible to imipenem except the isolate No. 38 and 40. A total of 14 isolates were found to be resistant to methicillin, 6 isolates have shown an intermediate response and 28 were susceptible. Then a total of 36 isolates were resistant to cefotaxime while 12 isolates were with intermediate range of inhibition (15-22 mm). A total of 40 isolates...
showed resistance to streptomycin, 6 isolates showed intermediate activity and only 2 isolates were found susceptible. Further a total of 46 isolates showed complete resistance while only 2 isolates were found to have intermediate activities and the rest of 46 were resistant. Staphylococcus isolates were mostly found resistant to ticarcillin. The resistant samples constituted of 79.2% of the total samples, 20.8% were found in the intermediate zone of inhibition, whereas no sample was found susceptible to ticarcillin.

3.3. Molecular screening of antibiotic resistance

Related to the respective phenotypic pattern of resistance, subsequent PCR was performed to analyze the genetic basis of resistance. These results demonstrated that 13 out of 14 isolates resistant to methicillin were positive for meca gene (Table 4). One resistant isolate and 6 intermediate resistant isolates showed no sign of amplification for this gene. In addition to the phenotypic based genetic traits, we have further investigated the presence of erythromycin resistance methylase traits, i.e., ermA, ermB and ermC, tetracycline resistance traits, i.e., tetK, tetL and tetM and

Table 3

Zone of inhibition (in mm) of different antibiotics.

| No. | Sample | Penicillin | Imipenem | Cefotaxime | Streptomycin | Cefixime | Cefoperazone | Ticarcillin | Methicillin |
|-----|---------|------------|----------|------------|--------------|----------|--------------|-------------|-------------|
| 2   | 5       | 26         | 14       | 11         | 10           | 10       | 5            | 14          |
| 4   | 11      | 23         | -        | 10         | 12           | 11       | 8            |
| 8   | 9       | 34         | 4        | 10         | -            | 7        | 15           |
| 11  | 15      | 28         | 13       | 16         | 6            | 14       | 11           |
| 13  | 11      | 22         | 19       | 9          | 12           | 12       | 7            |
| 20  | 13      | 24         | 18       | 12         | 11           | 12       | 12           |
| 21  | 18      | 23         | 18       | 10         | 9            | 5        | 14           |
| 26  | 8       | 17         | 12       | 11         | 4            | 13       | 9            |
| 27  | 18      | 22         | 17       | 9          | -            | 14       | 16           |
| 28  | 19      | 24         | 5        | 11         | -            | 15       | 15           |
| 29  | 14      | 19         | -        | 10         | -            | 12       | 15           |
| 30  | 6       | 22         | 12       | 10         | 4            | 11       | 5            |
| 31  | 7       | 24         | 12       | 10         | 7            | 12       | 6            |
| 32  | 19      | 21         | 4        | 9          | -            | 14       | 16           |
| 33  | 8       | 27         | 12       | 11         | -            | 11       | 7            |
| 34  | 9       | 18         | 12       | -          | -            | 12       | 8            |
| 37  | 8       | 17         | 7        | 11         | -            | 13       | 7            |
| 38  | 8       | 14         | 7        | -          | -            | 10       | 5            |
| 39  | 7       | 25         | 11       | 11         | 4            | 12       | 6            |
| 40  | 10      | 15         | 17       | 13         | 16           | 19       | 9            |
| 41  | 13      | 26         | 13       | 10         | 4            | 13       | 12           |
| 42  | 8       | 23         | 5        | 10         | -            | 12       | 8            |
| 43  | 11      | 20         | 14       | 9          | -            | 14       | 10           |
| 44  | 17      | 23         | 16       | 13         | -            | 13       | 15           |
| 47  | 12      | 22         | 14       | 10         | -            | 13       | 5            |
| 51  | 15      | 27         | 6        | 8          | -            | 9        | 15           |
| 52  | 16      | 29         | 3        | 6          | -            | 11       | 11           |
| 53  | 11      | 20         | 2        | 3          | -            | -        | 6            |
| 56  | 6       | 18         | 6        | 7          | 2            | -        | 5            |
| 59  | 12      | 20         | 12       | 12         | -            | 10       | 12           |
| 62  | 14      | 19         | 10       | 6          | 4            | 9        | 9            |
| 66  | 10      | 32         | 15       | 13         | 7            | 6        | 15           |
| 71  | 17      | 18         | 10       | 10         | -            | 12       | 11           |
| 74  | 13      | 17         | 6        | 10         | -            | 10       | 6            |
| 77  | 19      | 34         | 18       | 16         | 16           | 17       | 16           |
| 78  | 4       | 17         | 7        | 9          | 4            | 6        | 8            |
| 79  | 8       | 20         | 10       | 11         | 5            | 11       | 9            |
| 80  | 11      | 20         | 12       | 13         | 8            | 11       | 7            |
| 82  | 13      | 24         | 4        | 2          | -            | 4        | 5            |
| 84  | 16      | 28         | 16       | 4          | 2            | 8        | 10           |
| 85  | 18      | 30         | 15       | 6          | 8            | 10       | 12           |
| 87  | 9       | 22         | 11       | 4          | 10           | 12       | 7            |
| 89  | 5       | 18         | 9        | 8          | 2            | 8        | 6            |
| 90  | 17      | 26         | 17       | 10         | 2            | 8        | 15           |
| 91  | 12      | 23         | 15       | 11         | 7            | 12       | 15           |
| 92  | 10      | 19         | 7        | 5          | -            | 12       | 10           |
| 95  | 14      | 28         | 11       | 9          | -            | 13       | 11           |
| 98  | 8       | 17         | 4        | 4          | -            | 11       | 8            |
multidrug resistance plasmid, i.e., vgaA and vgaB in all 48 S. aureus isolates. A bout 79.2% of the isolates were positive for ermA gene, 10.4% for ermB gene and 25% for ermC gene respectively. However, 14.6% of the isolates were positive for tetK, and 6.25% of isolates were positive each for tetL and tetM traits respectively. The multi-drug resistance traits, vgaA and vgaB each were detected only in 12.5% of S. aureus isolates.

4. Discussion

4.1. Prevalence of S. aureus

Various studies have been conducted to determine the resistance of different strains of S. aureus where the nosocomial infections cause significant patient morbidity and mortality. It has been reported in literature that prevalence of S. aureus varies among different populations, and is influenced by age, underlying illness, race, certain behaviors, and the environment in which the person lives or works[13]. In our study we have reported that the prevalence of S. aureus was 59% in patients with age of above 40 years, 21% in patients with age between 20 to 40 and 20% in patients below 20. Among these patients 70.5% were female and 29.5% were male. These results indicate that the occurrence of S. aureus is high in female and patients aged above 40 years.

Another study about the prevalence of S. aureus reported that the rate of nosocomial infections can be reduced by maintaining good hygiene in the hospital[14]. Their specific program to prevent S. aureus transmission had led to a significant decrease of this prevalence rate by 32%. In our study, the prevalence was determined to be 29% in wards environment, which depicts the poor hygienic conditions of the skin wards.

4.2. Susceptibility pattern

All kind of bacterium especially S. aureus has emerged as a major clinical and epidemiological problem in hospitals. It has been previously reported that most of the methicillin resistant MRSA were not only resistant to all type of β-lactam antibiotics but also resistant to many other classes of antibiotics, e.g., imipenem[15,16]. Our results clearly demonstrate that all the strains resistant to methicillin were also resistant to penicillin, i.e., β-lactam, but in contrast to the literature, S. aureus remained susceptible to imipenem. A previous survey about the antibiotic susceptibility pattern of S. aureus strains from clinical and skin isolates grown at 37 and 44 °C respectively were carried out in Irrua Nigeria[17]. The isolates were susceptible to streptomycin (30.0%) and were resistant to penicillin. According to our study, out of 100 different specimens, 48% S. aureus isolates were found, in which only 4% isolates were susceptible to streptomycin, which is very much different from that of the above survey. But the results for penicillin are same in both cases. It is further concluded that the genetic diversity may result in the generation of different strains of S. aureus. The antibiotic resistance results indicate that S. aureus isolates showed greatest resistance to penicillin-G and cefixime and minimum resistance to imipenem.

4.3. Molecular screening of antibiotic resistance

For molecular screening, the pair of primers utilized for the

| Isolates No. | Gene Characteristics |
|-------------|----------------------|
| 2           | - + + - - + + + + + |
| 4           | + + + - - + + + + + |
| 8           | - + + - - - - - - - |
| 11          | - + - - - - - - - - |
| 13          | - + - - - - - - - - |
| 20          | - + + - - - - - - - |
| 21          | - + + - - - - - - - |
| 26          | + + + - - - - - - - |
| 27          | + + + - - - - - - - |
| 28          | + + + - - - - - - - |
| 29          | + + + - - - - - - - |
| 30          | + + + - - - - - - - |
| 31          | + + + - - - - - - - |
| 32          | + + + - - - - - - - |
| 33          | - + + - - + + + + + |
| 34          | + + + - - - - - - - |
| 37          | + + + - - - - - - - |
| 38          | + + + - - - - - - - |
| 39          | + + + - - - - - - - |
| 40          | + + + - - - - - - - |
| 41          | + + + - - - - - - - |
| 42          | - + + - - + + + + + |
| 43          | - + + - - - - - - - |
| 44          | - - - - - - - - - - |
| 47          | - - - - - - - - - - |
| 51          | - - - - - - - - - - |
| 52          | - - - - - - - - - - |
| 53          | - + + + + + - - - + |
| 56          | - + + + + + - - - + |
| 59          | - + + + + + - - - + |
| 62          | - + + + + + - - - + |
| 66          | - + + + + + - - - + |
| 71          | - + + + + + - - - + |
| 74          | + + + + + + + + + + |
| 77          | + + + + + + + + + + |
| 78          | + + + + + + + + + + |
| 79          | + + + + + + + + + + |
| 80          | + + + + + + + + + + |
| 82          | + + + + + + + + + + |
| 84          | + + + + + + + + + + |
| 85          | + + + + + + + + + + |
| 87          | + + + + + + + + + + |
| 89          | + + + + + + + + + + |
| 90          | + + + + + + + + + + |
| 91          | + + + + + + + + + + |
| 92          | + + + + + + + + + + |
| 95          | + + + + + + + + + + |
| 98          | + + + + + + + + + + |
detection of mecA gene was already well known primers for the detection of methicillin resistant plasmid in *S. aureus*. It is clear from the literature that majority of the MRSA strains analyzed were mecA positive. While blaZ genetic traits were present in all *S. aureus* isolates resistant to penicillin. The blaZ were found to be the characteristic genetic traits responsible for resistance to β-lactam group of antibiotics [10-12]. Our results further confirm the observations of correlation between penicillin and blaZ genetic traits. Previously it has been demonstrated that ermA trait is the most prevalent trait in MRSA strains [18,19], which strongly support our findings that only one strain resistant to methicillin was ermA negative, i.e. isolate No. 13. As we have mentioned previously that the same isolate No. 13 was phenotypically resistant to methicillin but was negative for mecA trait.

Based on our findings, it is concluded that antibiotic resistance in *S. aureus* strains is increasing day by day due to medication by non-registered medical practitioners and self-medication as well, because 100% and 29% resistance were observed against penicillin and methicillin respectively, which is a really very challenging situation. We have further concluded that the phenotypic resistance of 48 different *S. aureus* isolates is well correlated to the genetic traits of resistance as well as with the hygienic conditions. Therefore, the hygienic conditions of the hospital need improvement, in order to decrease rate of nosocomial infections caused by *S. aureus* in hospitalized patients. Further, for quick and fast detection, we propose next-generation sequencing be utilized to screen for antibiotic resistance.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgements**

The authors are thankful to the Centre of Biotechnology and Microbiology, University of Peshawar for financial support of this research work. The work was also supported by HEC under PIN No. 074-1053-Bm4-207.

**References**

[1] Stanway A, Rademaker M, Newman P. Healing of severe ulcerative necrobiosis lipoidica with cyclosporin. Australas J Dermatol 2004; 45: 119-22.

[2] Grundmann H, Anensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW, et al. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med 2010; 7(1): e1000215.

[3] McCallum N, Berger-Bächi B, Senn MM. Regulation of antibiotic resistance in *Staphylococcus aureus*. Int J Med Microbiol 2010; 300: 118-29.

[4] Abbott Y, Leonard FC, Markey BK. Detection of three distinct genetic lineages in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from animals and veterinary personnel. Epidemiol Infect 2010; 138: 764-71.

[5] Wunderink RG, Niederman MS, Kollef MH, Shorr AF, Kunkel MJ, Baruch A, et al. Linezolid in methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia: a randomized, controlled study. Clin Infect Dis 2012; 54(5): 621-9.

[6] Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leukocidin. Lab Invest 2007; 87: 3-9.

[7] Drew WL, Barry AL, O’toole R, Sherris JC. Reliability of the Kirby-Bauer disc diffusion method for detecting methicillin-resistant strains of *Staphylococcus aureus*. Appl Microbiol 1972; 24(2): 240-7.

[8] Paterson DL, Ko WC, von Gottberg A, Casellas JM, Mulazamoglu L, Klugman KP, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. J Clin Microbiol 2001; 39(6): 2206-12.

[9] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility testing. Seventeenth informational supplement. Wayne PA: Clinical and Laboratory Standards Institute; 2007. [Online] Available from: http://www.microlablab-bg.com/CLSI.pdf [Accessed on 18th September, 2014]

[10] Martineau F, Picard FJ, Lansac N, M enard C, Roy PH, Ouellette M, et al. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob Agents Chemother 2000; 44: 231-8.

[11] Ng LK, Martin I, Alfa M, Mulvey M. Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes 2001; 15: 209-15.

[12] Werner G, Cuny C, Schmitz FJ, Witte W. Methicillin resistant, quinupristin-dalfopristin-resistant *Staphylococcus aureus* with reduced sensitivity to glycopeptides. J Clin Microbiol 2001; 39: 3586-90.

[13] Schijffelen MJ, Boel CH, van Strijp JA, Fluit AC. Whole genome analysis of a livestock-associated methicillin-resistant *Staphylococcus aureus* ST398 isolate from a case of human endocarditis. BMC Genomics 2010; 11: 376.

[14] Shore AC, Deasy EC, Slickers P, Brennan G, O’Connell B, Monncke S, et al. Detection of staphylococcal cassette chromosome mec type X1 carrying highly divergent mecA, mecr, mecR1, blaZ, and ccr genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2011; 55(8): 3765-73.

[15] Gould IM. The clinical significance of methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 2005; 61: 277-82.

[16] Zhang Y, Agidi S, Lejune JT. Diversity of staphylococcal cassette chromosome in coagulase-negative staphylococci from animal sources. J Appl Microbiol 2009; 107(4): 1375-83.

[17] Obiazi HAK, Nmorsi OPG, Ekundayo AO, Ukwandu NCD. Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* from clinical isolates grown at 37 and 44 °C from Irrua, Nigeria. Afr J Microbiol Res 2007; 1: 57-60.

[18] Petrelli D, Repetto A, D’Erocole S, Ripa S, Prenna M, Vitali LA. Analysis of methicillin susceptible and resistant biofilm forming *Staphylococcus aureus* from catheter infections isolated in a large Italian hospital. J Med Microbiol 2008; 57: 364-72.

[19] MCarthy AJ, Lindsay JA. Genetic variation in *Staphylococcus aureus* surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol 2010; 10: 173.