Isolation of methicillin-resistant *Staphylococcus aureus* from burn centers and evaluation of antimicrobial efficacy, in Faisalabad, Pakistan

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**ABSTRACT**

**Objectives:** The present study was conducted for the detection of MRSA infections in burn wards and for evaluation of different antibiotics efficacy.

**Methods:** A total of 100 samples were collected from burn ward of Allied hospital Faisalabad, Pakistan. Then all samples were cultured on Mannitol salt agar, S-110 agar and then selective biochemical tests were performed. Antibiotic sensitivity was checked by using different beta-lactam antibiotics including methicillin, and vancomycin, linezolid, amoxicillin along with clavulanic acid (ESBL inhibitor) and some others, according to criteria of Clinical Laboratory Standard Institute (CLSI), to find out better antimicrobial therapy for burn patients.

**Results:** A total of 28 (66.66%) isolates of MRSA were detected and remaining 14 (33.33%) were MSSA, out of 42 isolates of *S. aureus*. The results showed that highest risk of MRSA prevalence was associated with burn patients and had a percentage positivity of 66.66% among burn patients of Allied hospital, Faisalabad.

**Conclusion:** It was observed that maximum resistance was found against beta-lactam drugs especially oxacillin (98%), but maximum level of sensitivity was observed against linezolid (95%) and vancomycin (99.99%), means that, these are more effective drugs for the treatment therapy of burn patients.

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the normal flora to get the antimicrobial genes which can be transported to their pathogenic microbes. It causes different types of diseases from slight range to highly devastating due to some toxins and enzymes it produces and has very unique mechanisms of antimicrobial resistance that has made it very causative agent for the development of serious diseases both in hospitals and in community settings.

Among gram positive microbes, Methicillin-resistant *S. aureus* (MRSA) accounts for most common bacterial microbe in burn wound infections and it has significantly high ratio among burn patients. The patients with high burn scratches are susceptible to this infectious microbe. This microbe is considered highly predominant in nosocomial infections and causes threats in hospital acquired infections that remain a big barrier in controlling these infections. MRSA gets colonization in burn wounds within first 48 hours of infection.

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) crossed down all the options available to treat simple Staphylococcal infections. In healthy individuals MRSA can inhabit at 1-8% rate that is actually leading towards the *Staphylococcus* infections. MRSA has established a lot of resistance, not only to beta-lactam ringed anti-microbial, but also to few other antimicrobial classes. MRSA is a main pathogen in humans and a universal rise in the diseases rate caused by MRSA is alarming. Risk factors that enhance MRSA infections and establishment including unwise use of broad spectrum antibiotics, longer hospital stay, especially in intensive care (ICUs), intravascular catherization and patients with weak immune systems, which serve as a major reservoirs and its transmission mostly occur through the hands of health care staff that have polluted hands and poor hygienic conditions.

Multidrug resistance in *S. aureus*, especially methicillin resistance in *S. aureus*, makes it to cause remarkable clinical and epidemiological problems, because of their substitution amongst patients and staff. In sensitivity testing, resistance against commonly used beta-lactam antimicrobials including oxacillin was shown by MRSA strains. By the 1990s *S. aureus* were shown maximum resistance towards ampicillin that is most commonly used broad spectrum beta-lactam drug. During the course of the recent years, many beta-lactam antibiotics have originated and produced artificially, e.g. Monobactams, Carbapenems and Cephalosporins etc.

The mode of action of beta-lactam drugs against the cell wall production (peptidoglycan layer) is most common target in all drugs. Another modified group of drugs that affects and raptures the cell wall synthesis includes, glycopeptides of which vancomycin is the most significant serious infections caused by for *S. aureus* strains. But in recent years almost from 8 years, MRSA has gained resistance against vancomycin treatment by producing vancomycin resistant *S. aureus* strains (VRSA).

MRSA is a contributing agent of human toxicities due to numerous mechanisms of resistance including penicillinase enzymes, other virulence factors and low affinity of penicillin binding proteins (PBP) to beta lactam antibiotics that make the treatment options significantly lower. This all demands then administration of high efficacy antibiotics that are expensive as well as lethal to human health. This in turn results in increase of chances in spreading the organism to others and higher mortality rate.

There are many reports where the incidence of this microbe is at increasing rate consistently along with other contributing factors like long hospital stay, costly treatment etc. However, the outbreak of MRSA infections in community after the hospital infections appeals for achieving priorities for prevention and control of this pathogenic microbe.

Vancomycin and Clindamycin are highly effective against MRSA but other than that innovations in fluid management, better ventilation, proper isolation protocols and discovery and administration of new and effective antibiotics can result in significant decline in morbidity and mortality ratio. MRSA is highly resistant to all available beta lactam antibiotics including semi synthetic penicillinase resistant antibiotics (methicillin and vancomycin) due to co-transfer of beta lactamase enzymes and genetic DNA transfer between bacterial species make treatment strategy dwindling.

This significant increase in MRSA infections highlights the diminished efforts in developing new therapeutic drugs. So this consistently exposure in resistant strains such as MRSA demands for improving the antibiotics using schedule and production of new antibiotics.

However the emergence of antibiotics resistance have been described for some antibiotic groups: aminoglycosides, Lincosamides, Tetracyclines, chloramphenicol, macrolides. Since 2000, new group Oxazolidinones including linezolid, which is accessible in recent years. But some sort of direct resistance against linezolid has been reported.

**MATERIALS AND METHODS**

**Ethical Statement:**
From the association moral review group for human clinical samples, a moral explanation was accomplished. For performance of tests, composed consent from medical superintendent of clinic was taken.

**Study Setting:**
This study was approved by chairman of burn ward, Allied hospital, Faisalabad. This study was organized in compliance with the Institutional Biosafety Committee, University of Agriculture Faisalabad.

**Study Duration:**
Almost 100 burn wounds (swab) specimens were collected during January 2018 to March 2018 by using appropriate sampling after getting vocal and written agreement and were analyzed.

**Study Sample:**
Specimens were collected by using sterile techniques from all patients. All swab specimens were transported to microbiology lab for culturing and sensitivity tests.
The whole procedure was performed according to microbiological diagnosis of WHO standard for *S. aureus*. The history data of patients (epidemiological parameters) was also collected to correlate the findings.

**Media preparation and sample inoculation:**
Culture media sterilization was done by using autoclave, having suitable temperature of 121°C for sterilization and it was maintained for 15-20 min/151lb.

For the inoculation of collected samples from burn patients, nutrient broth was used. Bacterial growth was transferred on nutrient agar, after overnight incubation. It is used to maintain growth of all microbes mostly. For confirmation of *S. aureus* from the collected samples, colonies grown on nutrient agar were further streaked on mannitol salt agar (MSA) that acts as differential and selective media for *S. aureus* from other bacteria due to higher concentration (7.5%) of NaCl that inhibits growth of other microbes and further more Staph-110 media was also used to get pure growth of *S. aureus* from the MS agar growth, as it acts as selective and differential media maintaining only growth of pathogenic *S. aureus*. Furthermore blood agar was also used for confirmation of *Staphylococcus aureus* growth from mannitol salt media and Staph-110 media through observation of haemolysis pattern. Gram staining was done for differentiation of gram positive bacteria from gram negative bacteria.

**Biochemical Identification:**
Further confirmation and identification of this bacterial species was done after performance of the following biochemical tests\(^29\). In which catalase test, coagulase test, sugar fermentation test, Voges-Proskauer test, urease and oxidase test were performed.

**Antimicrobial Susceptibility Test for *S. aureus* Isolates:**
After overnight incubation of inoculum, antimicrobial susceptibility of different antibiotics was checked out by using Muller Hinton agar and different antibiotics discs were used and their zone of inhibition were measured as resistant, intermediate and sensitive. Appropriate zone diameters were checked out according to the criteria of the national committee for clinical laboratory standard institute\(^26\). Antibiotic susceptibility was tested by using Kirby Bauer susceptibility methods for antibiotic, according to CLSI guidelines\(^18\). These some antimicrobial agents are oxacillin, levofloxacin, amoxicillin, ampicillin and amoxicillin +clavulanic acid, gentamycin, meropenem, linezolid, Ciprofloxacin, Levofloxacin, Doxycycline, Vancomycin, Trimethoprim-sulfamethoxazole, Ceftriaxone, Penicillin G, Penicillin, Cefotaxime, and Ceftazidime

**RESULTS**
As 100 samples were taken from Allied hospital, 42 samples out of 100 samples (n=100) were found positive for *Staphylococcus aureus*, based on morphology and biochemical tests and rest 58 samples were of *Pseudomonas aeruginosa*, *E. coli* and other bacteria as shown in Table 1. From 42 *S. aureus* samples, 28 (66.66%) samples were positive for MRSA (methicillin resistant *Staphylococcus aureus*) and remaining 14 (33.33%) were MSSA (methicillin sensitive *Staphylococcus aureus*). All isolates of MRSA were detected to be resistant to Oxacillin, Penicillin and Ampicillin and sensitive against Linezolid and vancomycin. From the study conducted during Feb-April 2018, in burn center of Allied hospital Faisalabad, MRSA was 66.66% prevalent in burn patients admitted during that period as shown in Figure 3, which indicates a significant ratio of this microbe in burn wounds.

<table>
<thead>
<tr>
<th>Bacterial groups detected</th>
<th>Numbers</th>
<th>Percentage (%)</th>
</tr>
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<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td><em>E. coli</em> + other bacteria</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
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Figure 1: The column chart is illustrating the information regarding to the number of bacterial isolates from burn ward of Allied Hospital, Faisalabad, Pakistan.

Figure 2: The pie graph is showing the prevalence of various isolates in the form of %age.
In the second part of study, 22 antimicrobials, Oxacillin, Amoxicillin, and Ampicillin, Amoxicillin + clavulanic acid combination, Levofloxacin, gentamycin, Vancomycin, Amikacin and Linezolid, Cefotaxime and others were used to check the antimicrobial efficacy of these antibiotics against MRSA as shown in Figures 4 and 5. Maximum resistance was observed against oxacillin (n=27/28), ampicillin (n=28/28), chloramphenicol (n=26/28), Ceftazidime (n=25/28), levofloxacin (n=23/28), ciprofloxacin (n=24/28), ofloxacin (n=26/28), penicillin (n=28/28), penicillin G (n=27/28) and intermediate resistance was observed against Amikacin (n=20/28), gentamicin (n=21/28), doxycycline (n=21/28), azithromycin (n=15/28) and amoxicillin+ clavulanic acid combination (n=21/28) (Fig. 4, 5, 6.1). However maximum level of sensitivity was detected against clindamycin (n=6/28), ceftriaxone (n=5/28), linezolid (n=3/28), piperacillin-Tazobactam (n=6/28) and trimethoprim-sulfamethoxazole (n=5/28), with almost zero percent resistance and 99.99% sensitivity was shown against vancomycin (n=0/28) drug (Fig 5, 6.2), which is showing that vancomycin is most effective drug to heal burn wound infections in patients admitted in burn ward of Allied hospital and also helpful to lessen over and misuse of antibiotics that only enhance antibiotic resistance capacity of microbes.

**Discussion**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is contributing continuously in spreading infections both in hospitals and in communities around the world\(^2\).
MRSA is a persistent issue in hospitals especially in less developed countries. Present review also indicates the pervasiveness of MRSA in Allied hospital in Faisalabad, Pakistan.

MRSA related infections are more serious and persistent one because they lead to longer hospital stays and economic burden as compare to methicillin sensitive strain of S. aureus (MSSA). With the passage of time MRSA infections are also present in community settings other than hospital acquired infections. Moreover, transmission of MRSA strains can occur via zoonotic transmission between humans and animals because of sharing of their common ancestry. The present review was focused for investigating MRSA prevalence in burn center of Allied hospital Faisalabad, Pakistan, only a few reviews on prevalence of MRSA in Pakistani health care facilities and in hospitals have been reported.

The patients with high burn abrasions are vulnerable to this infectious microbe and it causes out breaks in hospital acquired infections that are creating a big hurdle in handling these infections as many strains are showing resistance to a lot of available remedial drugs and other therapeutic strategies.

In the present review, total 100 samples were taken, for this purpose, 100 swabs were collected from burnt patients from Allied hospital Faisalabad and were selected for MRSA. In the second part of study, Twenty two antimicrobials Oxacillin, Amoxicillin, and Ampicillin, Amoxicillin +clavulanic acid combination, Levofloxacin, gentamycin, Vancomycin, Amikacin and Linezolid, Cefotaxime etc. were used to check the antimicrobial efficacy of these antibiotics against MRSA. Total MRSA percentage from burnt patients was 42%. All isolates of MRSA were detected to be resistant to Oxacillin, Penicillin and Ampicillin and sensitive against Linezolid and vancomycin. Out of 100 samples, 42 samples of S. aureus were detected based on morphology and biochemical tests. From 42 S. aureus samples, 28 samples were positive for MRSA and remaining were MSSA (methicillin sensitive Staphylococcus aureus).

In this review, 100 burn samples from Allied hospital were observed to check out MRSA contamination but each of them was contaminated with more than microbe, similar results were conducted by Rehman in 2011, where it was observed that along with other microbial contamination, MRSA infections are going to rise in Peshawar every year, especially in burn patients. In 2009, there were 207 cases, in 2010, there were 284 cases reported and in 2011, there were 438 cases detected.

However in this study among Gram +ve bacteria S. aureus showed maximum prevalence specially MRSA strains of S. aureus is present at higher numbers in burn patients due to immuno-compromised situation of their body, that is unable to protect them against microbes as burn wound is an ideal place for microbe residing and proliferation because the underlying vasculature of skin has been damaged so other immune cells of body cannot reach there to provide protection against infection.

In 1961, when first strain of MRSA reported in England, with but passage of time this bacterium acquired resistance against most commonly used antibiotics. However methicillin resistance in S. aureus was found because of presence of meca gene (an exogenous gene) present on mobile genetic element called Staphylococcal cassette chromosome mec (SCCmec). This gene encodes a modified form of penicillin-binding protein called PBP2a that makes it less vulnerable to commonly used beta-lactam antibiotics.

The MRSA issue has remained not only a hospital related microbe but also emerging rapidly in community acquired infections. CA-MRSA infections are causing a critical threat to health care systems due to their attack on normal individuals by causing different soft tissue and skin infections. MRSA is of prime importance from the category of Gram positive microbes. Now it has been recognized about the fact that microbial colonization and other diseases in hospitalized patients are a key source to spread to other wards and in other patients staying in those hospitals. After gaining of resistance, MRSA strains become more aggressive and spread effectively on other contaminated objects that make their removal less effective.

Another review focused by National Nosocomial Infections Surveillance System recognized that about 60% cases due MRSA were related to hospitals called them HA-MRSA (NNIS, 2004). The total incidence of MRSA cases are raising and a big surveillance program conducted in U.S discovered that during 1995-2001 an increase in MRSA rates occurred from (22-57%).

Another review acknowledged that during 1997-1999, the total frequency of MRSA isolates in hospitals was high; such as 67% in Japan, 35% in Latin America, 32% in USA, and 23% in Australia, 40% in South America, 26% in Europe.

In developing countries the problems of antimicrobials resistance are occurring more frequently rather than the developed countries because of higher rates of infections due to unhygienic conditions and financial burden also acts as big hurdle to combat this enemy completely and hospital acquired infections are chief morbidity and mortality sources.

There are many reports given that resistance mechanisms of hospitals acquired MRSA (HA-MRSA) infections against many antimicrobial agents such as aminoglycosides, fluoroquinolone and Tetracyclines, and these antimicrobials groups which are showing minimum level of affectivity. Another antibiotic vancomycin remains a front-line treatment linezolid resistant MRSA strains but some reports have been published now against vancomycin resistance. Some strains are emergent in USA that are showing intermediate resistant to Vancomycin (VISA) and also full resistant to Vancomycin (VRSA), that in turn is very frightening condition to combat this deadly pathogen with full potential.
Conclusions
In this study antimicrobial resistance pattern was observed through disc diffusion mechanism using Muller Hinton agar that showed maximum resistance was shown by MRSA against methicillin and other common beta-lactam drugs means that these drugs are ineffective in treating the burn infections that only leads to increase the antimicrobial resistance among burn patients but it showed maximum sensitivity against Linezolid and Vancomycin, as out of 100%, almost more than 95% sensitivity was observed against these two drugs, means that, these are more effective drugs for the treatment therapy of burn patients.

REFERENCES