Abstract:
Burns wound infection accounts for nearly 75 percentage of deaths following burns. Staphylococcus aureus has been reported as a major cause of community and hospital acquired infections. Wide spread occurrence of Methicillin Resistant Staphylococcus aureus (MRSA) and the use of vancomycin to treat infections has resulted in low level resistance even to vancomycin. The objective of the study was to detect MRSA strains in the wound swabs of the burns patients. These MRSA strains were observed for low level vancomycin resistance. A total of 214 wound swabs was collected from the burns wound infection patients and processed according to standard laboratory techniques. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method using the antibiotic discs namely ampicillin (10 micro gm), erythromycin (15 micro gm), gentamycin (10micro gm), amikacin (30micro gm), ofloxacin (5micro gm), oxacillin (1micro gm), cefoxitin, (30micro gm), vancomycin (30 micro gm), linezolid (30 micro gm). The percentage of staphylococcus aureus isolated in wound swabs was 99 (46 percent). MRSA detection was done by Oxacillin disc diffusion method and Cefoxitin disc diffusion method. Percentage of MRSA detected by both methods was 48 (48.5 percent). VISA was detected by Disc diffusion method, Vancomycin screen agar method and Agar dilution method. In Agar dilution method two strains had MIC 8 micro gm per ml for vancomycin. None of the strains were VRSA with MIC more than 16 micro gm per ml. In Vancomycin screen agar the same two strains showed visible growth. But in Disc diffusion method all the strains were sensitive to vancomycin. Thus disc diffusion method may not be a reliable method for VISA detection. Vancomycin screen agar results coincides with agar dilution method. Thus vancomycin screen agar may be used as a preliminary screening test for VISA. In the present study both the VISA strains showed 100 percent susceptibility to linezolid. In conclusion,
though vancomycin intermediate resistant strains were susceptible to linezolid, a strict regulation has to be followed on the usage of glycopeptides like vancomycin to reduce the incidence of VISA and VRSA.

**Keyword:** Vancomycin intermediate resistant Staphylococcus aureus (VISA)

**INTRODUCTION**

Burns wound infection accounts for nearly 75% of deaths following burns (9). Staphylococcus aureus has been reported as a major cause of community and hospital acquired infections. The organism has differential ability to spread and cause outbreaks in hospitals. The emergence of high level of penicillin resistance followed by the development and spread of resistance to semisynthetic penicillins (Methicillin, nafcillin, oxacillin), macrolides tetracycline and aminoglycoside has made therapy of Staphylococcus aureus disease of great challenge. Wide spread occurrence of Methicillin Resistant Staphylococcus aureus (MRSA), and the use of vancomycin to treat infections has resulted in low level resistance even to vancomycin (1,4,5,12).

**OBJECTIVES**

1. To detect the occurrence of Staphylococcus aureus isolates from burns wound infection.
2. To study their susceptibility pattern.
3. To detect the Methicillin Resistant Staphylococcus aureus.
4. To detect the low level resistance to vancomycin in burns wound infection patients.

**MATERIALS & METHODS**

**Bacterial isolates**

A total of 214 wounds swabs were collected from burns wound infection patients and processed according to standard laboratory techniques (2).

**Antimicrobial susceptibility**

Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method using the antibiotic discs namely ampicillin (10 µg), erythromycin (15 µg), gentamycin (10 µg), amikacin (30 µg), ofloxacin (5 µg), oxacillin (1 µg), cefoxitin (30 µg), vancomycin (30 µg), linezolid (30 µg).

**MRSA detection methods**

1. Oxacillin disc diffusion method.
2. Cefoxitin disc diffusion method. All the Staphylococcus aureus that were resistant to oxacillin (zone diameter 10 mm) and cefoxitin disc (i.e. zone diameter of < 21 mm) were detected as MRSA positive strains. Cefoxitin zone diameter of ≥ 22 mm was considered methicillin sensitive staphylococcus aureus (MSSA) (14). Quality control was done using ATCC 29213 staphylococcus aureus.

**VISA detection methods**

All the MRSA isolates were subjected to MIC for vancomycin by Agar dilution method as described by CLSI methods (2,8,12). Various gradient plates of Mueller Hinton Agar were prepared with vancomycin dilution of 0.5, 1, 2, 4, 8, 16, 32 µg/ml. By direct colony suspension method 0.5 McFarland equivalent inoculums were prepared in normal saline. All strains were spotted onto gradient plates. Plates were incubated overnight at 35°C for any visible growth. All the strains that had MIC 4-8 µg/ml were determined as VISA strains and those strains that had MIC > 16 µg/ml were to be determined as VRSA strain as per CLSI guidelines (jan 2006).
Vancomycin Screen Agar (5,12,13) Muller Hinton agar screen plates containing 6 µg/ml vancomycin were prepared. Inoculums suspension was prepared by selecting colonies from overnight growth on nutrient agar plates. The colonies were transferred to normal saline to produce a suspension that matches the turbidity of 0.5 McFarland std. The suspension was inoculated onto MHA screen agar plates and incubated at 35°C for 24 hrs. Any visible growth indicated VISA & VRSA.

RESULTS
Total No. of pus samples -214
Total No. of culture positive -195
Percentage of staphylococcus aureus isolates Collected from wound samples -99 (46%)
Percentage of GNB isolated -84 (39%)
Others (Cons, Enterococci) -12 (6%)
Percentage of No Growth -19 (9%)
Out of 99(46%) staphylococcus aureus Percentage of MRSA isolates - 48 (48.5%) Out of 48(48.5%) MRSA strains Percentage of VISA isolates with MIC 8 µg/ml -2 (4%)
### Antibiotic susceptibility for MRSA strains (BY DISC DIFFUSION METHOD)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MRSA strains sensitivity %</th>
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<tbody>
<tr>
<td>Ampicillin</td>
<td>0 (0%)</td>
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<tr>
<td>Erythromycin</td>
<td>0 (0%)</td>
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<tr>
<td>Gentamycin</td>
<td>25 (52%)</td>
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<tr>
<td>Vancomycin</td>
<td>48 (100%)</td>
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<tr>
<td>Amikacin</td>
<td>24 (50%)</td>
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<tr>
<td>Ofloxacin</td>
<td>27 (56%)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>48 (100%)</td>
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### Antibiotic susceptibility of VISA strains (by disc diffusion method)

<table>
<thead>
<tr>
<th>VISA strains 1 MIC 8 μg/ml</th>
<th>Ampicillin</th>
<th>Erythromycin</th>
<th>Gentamycin</th>
<th>Vancomycin</th>
<th>Amikacin</th>
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<tr>
<th>VISA strains 2 MIC 8 μg/ml</th>
<th>Ampicillin</th>
<th>Erythromycin</th>
<th>Gentamycin</th>
<th>Vancomycin</th>
<th>Amikacin</th>
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DISCUSSION
In the present study the total number of wound swabs from burns wound infection patients was 214. The percentage of staphylococcus aureus in burn patients was 99 (46%), GNB 84(39%) and others (Cons,Enterococci) 12(6%). This was contrast to studies conducted by Rastegar Lari A.R. et al, Kahiinde A.O. et al where GNB (Pseudomonas aeroginosa) percentage was higher than staphylococcus aureus(10). According to professor Keichii Hiramatsu (6), the reduction in consumption of broad spectrum cephalosporins (which are ineffective against MRSA ) would reduce the number of MRSA in the Hospitals. Recently, successful reduction of MRSA was achieved in a Japanese hospital by cutting the total use of broad-spectrum cephalosporins by half for surgical prophylaxis. In our study according to antibiotic susceptibility by Kirby Bauer method all the MRSA strains and two VISA strains were found sensitive to Linezolid (100%) . None of the isolates were sensitive to Ampicillin and Erythromycin. Early detection and treatment with specific antibiotic will reduce the incidence of MRSA which will ultimately reduce emergence of VISA and VRSA(6). 

CONCLUSION:
In our study from 99 (46%) staphylococcus aureus isolates 48(48.5%) were MRSA strains. From 48(48.5%) MRSA, VISA with MIC 8µg/ml was 2(4%). By disc diffusion method both the VISA strains with MIC 8µg/ml was detected to be sensitive. By Agar dilution method 2 strains of VISA was detected So in our study Agar dilution method was better method compared to disc diffusion method for VISA detection as per CLSI guideline( jan 2006) Thus disc diffusion method may not be a reliable method for VISA detection (6). Vancomycin screen agar results coincides with agar dilution method, (i.e) both VISA strains showed visible growth on it. 

Thus vancomycin screen agar can be used as a preliminary screening test for VISA and VRSA detection. (1,8) In the present study according to VISA antibiotic susceptibility pattern, both the VISA strains were susceptible to linezolid. In conclusion, though vancomycin intermediate resistant strains were susceptible to linezolid a strict regulation has to be followed on the usage of glycopeptides like vancomycin to reduce the incidence of VISA and VRSA.

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