DISCUSSION

Invention and the development of antibiotics was one of the greatest advances of modern medicine but the evolution of bacteria towards antibiotic resistance as well as multi-drug resistance, unfortunately is unavoidable because these conditions already exist in nature. Changes in the pattern of antimicrobial susceptibility of *Staphylococcus aureus* have been reported worldwide, especially in developing countries making antimicrobial agents increasingly less effective in treating bacterial infections.

The history of Methicillin Resistant *Staphylococcus aureus* starts with the development of antibiotics. Penicillin was discovered in 1928 by Alexander Fleming and was quickly put into production. The 1940s saw penicillin being commonly used to treat a number of different staphylococcal infections; virtually all staphylococcal species was susceptible to that drug. Over time and use, the *S. aureus* naturally developed a resistance to that drug, primarily due to the adaptive nature of the bacteria and overuse of the antibiotics in their early stages and by the 1950s; many of the antibiotics were already useless against treating staphylococcal infections.

Trends in the antibiotic susceptibility of MRSA are regularly investigated in many countries, but minimal countrywide data are available for India, particularly for in the study area. Changing pattern of resistance of *S. aureus* makes its periodic surveillance mandatory.

PCR method for detection of *mecA* gene is considered as the gold standard assay for the detection of methicillin resistance.\(^{190}\) PCR still remains a time consuming and expensive besides; it is not yet available in most of the routine clinical laboratories. Therefore phenotypic method still remains a method of choice in the laboratory settings with limited resources.

Currently available phenotypic methods for the detection of MRSA are problematic because of the heterogeneous resistance displayed by many clinical isolates.\(^ {191}\)

In present study, an attempt was made to detect the prevalence of MRSA, to know antibiotic sensitivity and resistant pattern of MRSA as well as MSSA isolates, to evaluate the performance of the four phenotypic methods that is oxacillin disc diffusion test, oxacillin MIC, oxacillin screen agar test and cefoxitin disc diffusion test for detecting MRSA isolates, using the PCR for *mecA* gene as the gold standard.
A total of 1000 consecutive non-duplicate isolates of *S. aureus*, identified using conventional biochemical procedures were included in the study.

The results obtained were compared with other studies and discussed as follows.

**Age and sex wise data** -

1000 isolates of *S. aureus* obtained from all age groups and both sexes were included. Male to female ratio was 1.68:1. The increase rate of staphylococcal infection in male patient could be due to active involvement of males in outdoor activities including agricultural work, more prone for injuries and infections acquired due to more exposure to contaminated environment and due to lack of knowledge, comparatively over use of antibiotics without prescriptions and its incomplete course. A similar observation has been made by Morgan M, who reported male to female ratio was 1.89:1.

Of 1000 isolates of *S. aureus*, 30% *S. aureus* and 34.7% MRSA isolates were found in the age group 51-60 years. This may be due to underlying hormonal abnormalities, decreased immunity as age advances and empirical therapy or indiscriminate use of antibiotics might be responsible for drug resistance which developed slowly and might have appeared in later part of life. Similar observation was made by Shakya et al. MRSA isolates were high among people more than 30 years age compared to less than 30 years age.

Majority of clinical cases had cellulitis and presence of abscess in this study. High rate of diabetic foot wound infection was found among male than female patients (1.7:1). Due to non-healing nature of diabetic wound it is prone to infections. This ratio correlates with study by Anandi et al, who observed male to female ratio 1.9:1 in diabetic foot wound.

**Ward wise data** -

When we observed ward wise distribution, orthopedic ward accounted for maximum isolation of *S. aureus* (35.50%), followed by Surgery (30%), Pediatric (12.50%), Medicine (10.90%), Skin (10.70%) while least from ICU (0.40%). Most of these cases were of post operative infections. Similarly, in a study by Loveena Oberoi, et al, the majority of the isolates were obtained from orthopedic ward (28.86%) followed by surgery (21.65%) and medicine (16.49%). The isolation of strains from orthopedic and surgery wards can be explained by the fact that these areas in hospitals
have been regarded as high risk areas as far as the rate of nosocomial infections is concerned.\[196,197]\n
Out of 265 MRSA isolates, 41.13% MRSA strains were isolated from surgery ward and 36.60% from orthopedic ward. Shilpa Arora et al found 54.8% MRSA strains from surgery unit and 27.8% from orthopedic ward.\[198]\nShrinivasan S et al also found 80% MRSA isolates in surgery ward and 28% from orthopedic ward.\[199]\n
The higher incidence of wound infection observed in department of surgery could be because of higher number of emergency procedures conducted in the department.

**Specimen wise data –**

Out of the 1000 *S. aureus* isolates, maximum *S. aureus* were isolated from pus and wound swab. Similar observation was made by Mehta, who in his study on control of MRSA in a tertiary care center, had reported an isolation rate of *S. aureus* highest from pus and wound swab.\[200]\nHowever, Qureshi from Pakistan reported a high rate of *S. aureus* isolates (83%) from wound swab and pus.\[201]\n
Out of 265 MRSA isolates, frequency of isolating MRSA was maximum from wound swab (51.82%) and pus (26.66%) specimens which might be due to more chances of MRSA infection in deep seated lesions compared to superficially infected wounds,\[202]\nfollowed by blood, fluid and urine specimens. Our findings correlates with the previous studies conducted in India by Loveena oberoi, Chandrashekhar DK and Bandaru Narasinga Rao, they have reported maximum number of MRSA isolates from wound swab and pus.\[196, 197,203,]

**Results of phenotypic methods for detection of MRSA -**

During the last several years, the CLSI-AST has attempted to improve the accuracy of detecting mecA positive strains of *S. aureus*. Detection of mecA gene or its product, penicillin binding proteins (PBP2a), is considered the gold standard for MRSA confirmation.\[190]\n
From a clinical perspective, it is important to differentiate isolates that have mecA positive resistance from the infrequently encountered isolates that have borderline resistance because it may affect therapy. Strains that possess mecA classic
resistance are either heterogenous or homogenous in their phenotypic expression of resistance. It is the testing of heteroresistant isolates which may appear as susceptible.

Many laboratories use multiple tests and often get a conflicting oxacillin and cefoxitin susceptibility results which are most likely to occur for isolates with reduced susceptibility to oxacillin by a non mecA mediated mechanism or are mecA positive but are very heteroresistant. [204]

Conventional methods are still widely used. Identification and determination of the susceptibility to antibiotics by conventional methods require a minimum of two day period, whereas the detection of antibiotic resistance genes by PCR assay can be done within a few hours. The PCR based tests are rapid and reliable methods for identification of MRSA strains and to detect antibiotic susceptibility. In our study we emphasized on this problem and for evaluation of four phenotypic methods as well as for confirmation of cryptically MRSA strains, we used PCR method for detection of mecA gene as gold standard test. We have selected four phenotypic methods for evaluation which are widely used for detection of MRSA.

i) Oxacillin Disc Diffusion Method (OXDD) -

The oxacillin disc diffusion method is most widely used method for confirming methicillin resistance in clinical microbiology laboratories. The conventional MRSA detection assays are simple and relatively cheap methods for detecting methicillin resistance.

We found 301 (30.1%) MRSA by oxacillin disc diffusion method. This result correlates with Vidya Pai et al who reported 29.1% MRSA by this method.

When we compared oxacillin disc diffusion method with gold standard PCR for mecA gene, we found that mecA gene was present among 265 (26.5%) isolates and it is absent amongst 36 isolates out of 301 MRSA isolates detected by oxacillin disc diffusion method. These same 265 (26.5%) mecA positive isolates were accounted for MRSA by cefoxitin disc diffusion test, oxacillin MIC and oxacillin screen agar tests. These 36 strains were falsely characterized as MRSA by oxacillin disc diffusion, the method formerly advised by NCCLS. It is suggested that 36 strains were given false positive by oxacillin disc diffusion method. Our report correlates with Anand and Pramodhini. [155,179] The Oxacillin disc diffusion method was found least reliable by
Prasad et al also. Among these 12 and 5 strains were falsely characterized as MRSA by oxacillin MIC and oxacillin screen agar methods respectively. The false positivity of oxacillin disc diffusion method in this study could be due to hyper production of β-lactamase which may lead to phenotypic expression of oxacillin resistance. Anand and Swenson 2001. [155,205]

Out of these 36 strains, 12 isolates which were resistant to oxacillin had oxacillin MICs of 4-8 µg/ml. These strains probably are BORSA (Borderline oxacillin resistant S. aureus) or MODSA (Moderately resistant S. aureus) that hyper produce β-lactamase or with alterations to the existing PBPs respectively and while they appear oxacillin resistant, do not possess the usual genetic mechanism for such resistance. Remaining 24 isolates which were resistant to oxacillin had oxacillin MICs of 0.25-2 µg/ml. The clinical importance of MODSA and BORSA strains had been doubtful, confirmation is important as BORSA infection can be treated with β lactam and cephalosporin. [155]

Oxacillin disc diffusion method is easy to perform and cheap. The accurate determination of methicillin resistance in staphylococci by the oxacillin disc diffusion method may be affected by various components of medium, temperature, and the duration of incubation. This method requires special media Muller-Hinton agar with 2-4% NaCl supplement and optimum temperature required for incubation also differs slightly 350C than others 370C. Further the results may get affected by variables like concentration of NaCl, pH of medium and incubation temperature. So this method requires an extra efforts and investment of material. Hence, other phenotypic methods like the agar screen method and the cefoxitin disc diffusion method have been evaluated. [206]

ii) Oxacillin Screen Agar Method (OSA) -

We found 270 (27%) MRSA out of 1000 S. aureus by using the oxacillin screen agar method. Among these 270 strains, mecA gene was absent in the 5 strains of S. aureus. It is suggested that these 5 strains were given false positive by oxacillin screen agar method when compared with gold standard method (PCR for mecA gene).

All these 5 isolates were sensitive to cefoxitin but resistant to oxacillin by disc diffusion method. These same 5 strains were found to be heterogenous resistant at the end of 48 hrs of incubation. Among these, 3 strains had MICs of 2µg/ml and 2 strains
had MICs of 4µg/ml. The 2 strains which had MICs of 4µg/ml probably are Borderline Oxacillin Resistant *S.aureus* or Modified oxacillin resistant *S.aureus*. The presence of growth of *S.aureus* on oxacillin screen agar generally suggests that strains have *meca* gene. Occasionally, however, heteroresistant strains which have *meca* gene, is not detected due to low expression of resistance. Our results correlate with results of Swenson et al. He suggested that the oxacillin screen agar test generally does not detect BORSA in which resistance is heterogenous. [205]

Oxacillin screen agar method is easy to read and cheap but is cumbersome to perform. This method also requires oxacillin powder which is not available easily and the method takes more time to show result as compared to other phenotypic methods.

It was also observed that oxacillin screen agar is as efficient as oxacillin MIC, provided the media was freshly prepared and exact concentration of oxacillin is included in this media.

Sensitivity of the oxacillin screen agar test for the detection of resistant strains was 100% and specificity 99.31%. It was excellent. However, two reports by Cavassini and Resende noted that when heteroresistant strains were tested, sensitivity decreased.[207,208] Cells expressing heteroresistance grow more slowly than the oxacillin-susceptible population and may be missed at temperatures above 35°C, so this method also required restricted temperature (35°C) according to CLSI guideline.

### iii) Minimum Inhibitory Concentration (MIC) -

Oxacillin MIC for isolates was tested by agar dilution method. Out of 1000 *S. aureus*, we found 277 strains of *S. aureus* had MICs of ≥ 4 µg/ml detected as MRSA and 723 strains of *S. aureus* ≤ 2µg/ml detected as MSSA. Among these 277 MRSA stains, *meca* gene was absent in 12 strains. It is suggested that these 12 strains were given false positive by oxacillin MIC method when compared with PCR for *meca* gene. Strains possessing *meca* gene are either heterogenous or homogenous in their expression. Heteroresistant strains may show lower expression resulting in MICs that appear susceptible.

Out of these 12 strains, 8 strains had MICs of 8µg/ml and 4 strains had MICs of 4µg/ml

These 12 strains of *S. aureus* were sensitive to cefoxitin but resistant to oxacillin and *meca* gene was absent, probably they are BORSA or MODSA that
hyper produce β-lactamase and while they appear oxacillin resistant phenotypically but do not possess the usual genetic mechanism for such resistance. MICs of these isolates were higher than that of those that were sensitive to both cefoxitin and oxacillin.\cite{178} As per criteria for BORSA by Louie et al., (oxacillin MIC when 2-8 μg/ml and meca negative) 12 strains were characterized as BORSA strains. Confirmation is important as BORSA infections can be treated with β-lactams and cephalosporins.

All the isolates resistant to both cefoxitin and oxacillin had an MIC ≥4 μg/ml. 20 strains had MICs of 256 μg/ml and 3 strains had MICs of 512 μg/ml. MIC values of these isolates were higher than that of those strains that were sensitive to oxacillin as well as cefoxitin disc diffusion method. These values are higher than the results obtained in previous studies done by Vidhani and Gupta et al., studied on methicillin resistant S. aureus isolates from high risk patients and reported 64 μg/ml MIC as highest value.\cite{133,209}

Oxacillin MIC method is useful to identify BORSA or MODSA strains of S. aureus but requires oxacillin powder, which is costly and not available easily. The temperature has to be maintained to 35°C for incubation, requires skilled laboratory staff and is time consuming. It is difficult to be implemented in routine laboratories. Determination of oxacillin MIC values of isolates that show such discrepancy will give additional information.

iv) Cefoxitin Disc Diffusion Method (CXDD) -

Out of the 1000 isolates of S. aureus, 265 and 735 strains were detected as MRSA and MSSA respectively by cefoxitin disc diffusion method. All these 265 strains showed presence of meca gene by PCR. Study done by Anand et al. reported cefoxitin disc diffusion method for detection of MRSA was in concordance with the PCR for meca gene.\cite{135}

All the 265 MRSA isolates detected by cefoxitin disc diffusion method were also MRSA by other three phenotypic methods.

Cefoxitin disc diffusion test is easy to perform, cost effective. It does not require special technique of media preparation and addition of NaCl in medium. It is easier to interpret than those of oxacillin due to the frequent hazy oxacillin zones, which are commonly misinterpreted as evidence of oxacillin susceptibility. Oxacillin...
must also be read using transmitted light, unlike most other antimicrobials, including cefoxitin, to ensure correct interpretation.\textsuperscript{[210]}

As compared to other three phenotypic methods, cefoxitin disc can be placed on the routine media. Plate can be incubated at $37^0\text{C}$ for 18 hours. Hence it can be used along with other antibiotic discs.\textsuperscript{[173]}

Cefoxitin is a better inducer of \textit{mecA} expression; this could explain why heterogeneous MRSA populations variably expressing the \textit{mecA} are better detected by disc diffusion with cefoxitin than with oxacillin, which is a weak inducer of PBP2a production. The main advantage of using cefoxitin is that the test conditions are similar to those used for other antibiotics.\textsuperscript{[211,212]}

**Sensitivity and specificity of phenotypic methods for detection of MRSA**

Sensitivity and specificity value of phenotypic methods were used for identification of MRSA. Several studies have showed that detection of \textit{mecA} gene is a gold standard method for detection of MRSA in clinical microbiology laboratories.\textsuperscript{[17]} However, most laboratories especially in developing countries are not in position to perform molecular methods.

Sensitivity and specificity value of various phenotypic methods vary depending on the media used for inoculum size, medium pH, salt concentration of medium, the incubation time, temperature and the experience of personnel’s which carry out the tests.\textsuperscript{[213]} However, difficulties occur when organisms have their MICs near the break-point. It is in such instances that detection of \textit{mecA} gene is useful by molecular technique. The strains that posses \textit{mecA} gene which is responsible for MRSA are called classical resistant.\textsuperscript{[155]} These classical resistant strains are either homogenous or heteroresistance in their expression of resistance.

In present study, sensitivity of all four phenotypic methods is 100% but specificity is 95.10%, 100%, 98.36% and 99.31% of oxacillin disc diffusion, cefoxitin disc diffusion, oxacillin MIC and oxacillin screen agar test respectively.

Sensitivity as well as specificity of cefoxitin disc diffusion test was 100%. As compared to high specificity of cefoxitin disc diffusion method, oxacillin disc diffusion method had only 95.10% specificity. In a number of studies, sensitivity and specificity of disc diffusion method have been reported between 61.3 - 100 % and 50 - 99.1 % respectively.\textsuperscript{[215,218,219]} Some other studies showed better specificity of oxacillin than cefoxitin disc.\textsuperscript{[220]}
In present study specificity of oxacillin disc diffusion was 95.10%. Our result correlates with Fawzi et al. (2007), where the oxacillin disc diffusion test showed 100% sensitivity and 95.6% specificity.\textsuperscript{[214]} In contrast to our result sensitivity of oxacillin disc diffusion 96.9% and specificity was 100% by Zeeshan M. also dissimilar to the sensitivity of 61.3% and specificity of 96.7% reported by Cavassini et al.\textsuperscript{[207]}

This study has shown that the oxacillin disc diffusion method had higher sensitivity but lower specificity when compared with the cefoxitin disc diffusion and OSA methods. Krishnan et al. reported that the specificity of routine laboratory tests for MRSA detection was variable and it was difficult to perform PCR in routine diagnostic laboratories.\textsuperscript{[215]}

The good sensitivity (100%) but relatively poor specificity (99.31%) for the oxacillin screen agar test was seen in our study, this result is similar with study by Pramodini S, she reported 100% sensitivity of the oxacillin screen agar.\textsuperscript{[179]} Most of the studies reported the sensitivity of OSA is more than 97\%.\textsuperscript{[205,216]} This difference may be due to the inclusion in our study of more isolates and the greater probably of heteroresistance. Presence of heteroresistant strains leads to decreased sensitivity.\textsuperscript{[207,208]} 100% sensitivity and 98.36% specificity by oxacillin MIC method reported in this study. Wallet et al, compared the MIC method with PCR and the sensitivity was 96\%, which was lower than results of present study.\textsuperscript{[217]}

The higher sensitivity to cefoxitin can be explained by the increased expression of the \textit{meca}-encoded protein PBP2a, cefoxitin being an inducer of the \textit{meca} gene.\textsuperscript{[173]} This is considered to be the underlying mechanism for the higher sensitivity of cefoxitin than oxacillin. Anand et al and Priya Datta\textsuperscript{[37]} reported the sensitivity and specificity of the cefoxitin disc method to be 100\%.\textsuperscript{[221]}

Several studies including the current one have reported that disc diffusion testing using cefoxitin is far superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion, oxacillin screen agar testing and oxacillin MIC and correlate better with the presence of \textit{meca} than oxacillin based phenotypic tests.\textsuperscript{[212,219, 222]}

Study done by Qi W, Boeotia and Rao Venkatakrishna et al., similarly reported cefoxitin disc to be superior and easier to interpret than oxacillin disc and correctly identified all low-level resistant MRSAs and provide evidence that results of cefoxitin disc diffusion test is in concurrence with the PCR for \textit{meca} gene. Thus the
Cefoxitin disc diffusion method is very suitable for detection of MRSA and it can be an alternative to PCR. [24,225]

When we compared specificity of cefoxitin disc diffusion test and oxacillin screen agar, there is slight difference amongst both but cefoxitin disc diffusion is easy to perform, does not require special equipment, particular temperature, can perform with routine antibiotic test as compared with oxacillin screen agar test. Cefoxitin disc diffusion method also demonstrated high sensitivity (100%) and specificity (100%) for MRSA detection. These findings are consistent with those of Anand et al, where the sensitivity and specificity of the cefoxitin disc method were reported as 100%. [155]

Also, cefoxitin is a surrogate marker and detects all staphylococci that are mecA positive. The discs are stable and give accurate results allowing the discrimination between MRSA and Borderline resistant S. aureus. Felton et al compared cefoxitin with oxacillin disc diffusion test. [222,245]

Recently, CLSI outperformed oxacillin with cefoxitin in obtaining more appropriate results. Broekeme NV et al compared oxacillin disc diffusion method with cefoxitin disc diffusion method for detection of MRSA, by using mecA PCR as the reference method. [226]

They found the cefoxitin disc diffusion method (specificity 100%, sensitivity 96.5%) superior to the oxacillin disc diffusion methods (specificity 99%, sensitivity 90.4%). They concluded that combining the results of tests with both cefoxitin and oxacillin would give a sensitivity of 100% and a specificity of 99.1%.

In contrast to our result, some investigator has recently been reported the oxacillin disc diffusion test better as compared to cefoxitin disc diffusion and Cefoxitin disc diffusion testing is now an accepted method for the detection of MRSA by many reference groups including CLSI. [212, 223]

**Cryptically MRSA**

Out of 1000 S. aureus strains, 319 were subjected to PCR for detection of mecA gene. Among these, 301 were MRSA by various phenotypic methods and 18 MSSA strains detected by all phenotypic methods were selected randomly for confirmation of cryptically methicillin resistant strains. [131]

Study by Mohansoundaram et al revealed that one of 117 (0.85%) MSSA isolates when subjected to PCR revealed the presence of mecA gene, remaining were found to be negative. This isolate namely mecA positive but probably non-PBP-2a
producing has been referred as ‘cryptically methicillin resistant. We do not found meca gene (cryptically methicillin resistant strains) in all 18 MSSA strains. [131] Kunsung Bhutia et al and Nikbakht et al. they reported 7.84% and 3.75% percentage of cryptically methicillin resistant strains respectively in their studies. [180,227]

In the present study, in a PCR reaction, out of 301 strains were confirmed by all four phenotypic methods. 265 meca-positive strains and 36 meca-negative strains were identified. However, 36 meca-negative strains were confirmed to be MRSA by oxacillin disc diffusion test. This finding may be due to point mutation or deletion in the meca gene, or other mechanisms of oxacillin resistance such as hyperproduction of β-lactamase, which was not assessed in our study, or modified PBPs. Our results were in accordance with that of Warren et al. who found three meca-negative isolates phenotypically resistant when grown on oxacillin screen agar, suggesting that methicillin resistance in these isolates was mediated by methods other than PBP2a, such as hyperproduction of β-lactamase or modified PBPs. [228]

**Prevalence of MRSA –**

Sensitivity and specificity value of phenotypic methods were used for identification of MRSA. We found prevalence of MRSA to be 26.50%. The epidemiology of MRSA appears to be variable and fluctuating since its emergence was reported. Studies show that the epidemiology of MRSA over different parts of India is not uniform. Rate of prevalence of MRSA vary from place to place and from time to time. Our result correlates with Indian studies by Choudhary et al who reported 26.80% [229] and Vidya Pai et al who reported 29.1%.[230] Kumari N et al from Nepal also reported prevalence of MRSA to be 26.14% in their study,[231] but there are many other studies which show prevalence of MRSA less or markedly higher than above mentioned studies. [8, 232,233,234,235]

The above discrepancy may not only be because of difference in the study design, population involved in study, variation in antibiotic usage, infection control practices in different hospitals but might also be due to differential clonal expansion and drug pressure in community.

The prevalence of MRSA isolates from various clinical specimens reported by different investigators varies over a wide range in India.
Following table showed prevalence of MRSA from different places in India.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Author</th>
<th>Place of study</th>
<th>Year</th>
<th>MRSA%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vidhani et al [133]</td>
<td>New Delhi</td>
<td>2001</td>
<td>51.60%</td>
</tr>
<tr>
<td>2</td>
<td>Majumdar et al [236]</td>
<td>Assam</td>
<td>2001</td>
<td>52.9%</td>
</tr>
<tr>
<td>3</td>
<td>Hanumantappa et al [171]</td>
<td>Davangere</td>
<td>2002</td>
<td>43%</td>
</tr>
<tr>
<td>4</td>
<td>Kakru et al [235]</td>
<td>Kashmir</td>
<td>2003</td>
<td>33%</td>
</tr>
<tr>
<td>5</td>
<td>Anupurba et al [234]</td>
<td>Varanasi</td>
<td>2003</td>
<td>54.80%</td>
</tr>
<tr>
<td>6</td>
<td>Mohanty et al [237]</td>
<td>New Delhi</td>
<td>2004</td>
<td>38.56%</td>
</tr>
<tr>
<td>7</td>
<td>Chawla K et al [233]</td>
<td>Manipal</td>
<td>2008</td>
<td>10.74%</td>
</tr>
<tr>
<td>8</td>
<td>Vidya Pai et al [230]</td>
<td>Mangalor</td>
<td>2008</td>
<td>29.10%</td>
</tr>
<tr>
<td>9</td>
<td>Lahari Saikia et al [238]</td>
<td>Assam</td>
<td>2009</td>
<td>34.78%</td>
</tr>
<tr>
<td>10</td>
<td>INSAR [239]</td>
<td>Hyderabad</td>
<td>2009</td>
<td>24%</td>
</tr>
<tr>
<td>11</td>
<td>Kunsang et al [180]</td>
<td>New Delhi</td>
<td>2011</td>
<td>43.58%</td>
</tr>
<tr>
<td>12</td>
<td>Loveena et al [196]</td>
<td>Amritsar</td>
<td>2011</td>
<td>45.36%</td>
</tr>
<tr>
<td>13</td>
<td>Bandaru et al [203]</td>
<td>Andra pradesh</td>
<td>2012</td>
<td>52%</td>
</tr>
<tr>
<td>14</td>
<td>Present study</td>
<td>Sangli</td>
<td>2013</td>
<td>26.50%</td>
</tr>
</tbody>
</table>

**Antibiotic Sensitivity pattern of MRSA:**

Susceptibility test profiles revealed a higher level of resistance to commonly prescribed antimicrobial agents among MRSA. The resistance of MRSA to a wide range of antibiotics is well documented. Hence MRSA strains should be routinely tested using the cefoxitin disc diffusion test as per the CLSI guidelines for prompt patient treatment and also for controlling the transmission of MRSA strains in any health-care setup.

In a study from north India, the prevalence of MRSA was 46 per cent and MRSA isolates were found to be more resistant to various antibiotics than MSSA. [198]

As expected, all the MRSA strains (100%) were resistant to penicillin. 93.87% MSSA strains were resistant to Penicillin. NCCL recommends not reporting penicillin sensitivity in MRSA. [22]

Among MRSA, high degree of resistance was encountered for Ampicillin (93.20%) and Gentamycin (94.33%), CO- trimaxazole (80%), Ciprofloxacin (79.62%). Other studies have also reported quite high resistance to these antibiotics e.g.
resistance to Ampicillin (77.27%) Ciprofloxacin (75%), Gentamicin (75%) reported by Loveena Oberoi[196], 70% resistance to Ampicillin noted in a study by Shobha KL et al. [240]

Our study also reported high level resistance to fluoroquinolones. High level of ciprofloxacin resistance has emerged very rapidly after its introduction in to general use. We have 79.62% of the MRSA strains resistant to ciprofloxacin correlates with study of Anupurba et al 84.1%. Our report is higher than Majumder et al (22.8%) [236] and Uma choudhary (5.7%) [229] and less than study done by K Rajaduraipandi, on spectrum of antimicrobial resistance among MRSA, ciprofloxacin resistance was as high as 90% [20] and by Qureshi et al where ciprofloxacin resistance as high as 98.9% was reported. [201] The rapid emergence of ciprofloxacin is probably due to the indiscriminate and empirical use of these drugs.

Glycopeptides remain the mainstay of the treatment of MRSA. Gentamicin resistance is increased since 1996. An increase of gentamicin resistance ranging from 0% before 1996 to 80% after 1996 has been reported. In present study we observed 94.33% resistance to gentamicin in MRSA. However, contrary to the result of our study, Pulimood had observed only 8% resistance of MRSA to gentamicin [241] and Saxena et al reported 76% MRSA strains were resistant to gentamycin as against 94.33% in our study. [241] Qureshi had reported a gentamicin resistance of 97.8%,[2] which is higher compared to our study. [201]

Recently more than 90% isolates from South Maharashtra have been found resistant to ampicillin, tobramycin, penicillin, erythromycin, kanamycin and gentamicin, whereas only 39.1% of strains are resistant to methicillin . [243]

The results of our study are supporting the other studies conducted nationally and internationally in India, Pakistan, Colombia and Romania etc. [243,244]

Awad SS et al in review of MRSA susceptibility over the seven year period showed a constant susceptibility rate to vancomycin at 100%, co-trimoxazole 98%.[17] Only 15% MRSA were susceptible to erythromycin in 2000, which have still decreased to 4% in year 2006. Resistance to co-trimoxazole (97%) by Mohansundaram is higher than present study 80%. [131]

According to Archer and Scott 1991 et al, > 50% of MRSA are also resistant to macrolides, lincosamides, fluoroquinolones and aminoglycosides. [245]

High level of sensitivity was observed to Erytromycin (53.96%) and Clindamycin (38.49%) in MRSA strains in the present study. In MSSA, moderate
level of resistance was seen to Ciprofloxacin (78.63%), Gentamicin (70.06%) Clindamycin (84.62%) and Erythromycin (93.74%). Low level resistance was observed to Penicillin (6.12%) and Ampicillin (14.69%).

All *S. aureus* isolates, irrespective of their methicillin status, were sensitive to Vancomycin. This is in accordance with other studies. However, Vancomycin intermediate and Vancomycin resistant *S aureus* strains have been reported recently from various part of country. \[246,247\]

In present study we found marked difference between sensitivity patterns of MRSA and MSSA isolates which correlates with report of Arora et al\[198\] and Mounir M et al\[248\] who also found marked difference between sensitivity pattern of MRSA and MSSA isolates. The degree of resistance or sensitivity of MRSA towards commonly used antibiotics is found to be variable from region to region.

Moreover, antibiotics are prescribed without doing drug sensitivity testing due to lack of laboratory facilities in most of the health care centers of this region. Even where the facility is available, medical practitioners do not routinely recommend the test because of negligence or patients’ poor economic status. All these factors might have contributed to the data showing very high prevalence reported by this study. \[249\]

**Multidrug resistance in MRSA -**

Multiple drug resistance of *S. aureus* is due to several drug resistant genes in a single plasmid, each with its own resistance markers. A bacterial cell may carry more than one plasmid with resistance markers. The resistance development in *S. aureus* dates back to 1940s. Most common reason for multi drug resistant MRSA is indiscriminate use of antibiotics without drug sensitivity testing which may be due to lack of advanced laboratory facilities or negligence on the part of medical practitioners or patients poor economic status. There is a difference between antibiogram of MRSA and MSSA isolates and routine testing of methicillin resistance should be done using cefoxitin disc which at present is the most sensitive method.

Most of the MRSA in this study were actually resistant to many classes of antimicrobials at the same time and thus qualify as multiply drug resistant *S. aureus* (MDR-MRSA). \[131\] In the various reports from other parts of India, rate of MDR strains among MRSA range from 23.2% to 73%. \[20,198\]

In the present study MRSA strains were found more multidrug resistant as compared to MSSA strains. 78.86% *S. aureus* strains were resistant to more than
three non-beta lactam antibiotics i.e. multidrug resistant. Correlates with study of Arunava Kali et al who recorded 79% multi drug resistant MRSA and less than study by Chandrashekhar DK et al, who observed 100% MRSA were MDR-MRSA in his study. \cite{250,197} E Marais, recorded (81.5%) multidrug resistant MRSA in his study. \cite{251} Multi-drug resistant MRSA accounted for 83% and 72.1% of MRSA isolates in study by Mohansundaram and Hare Krishna Tiwari respectively. \cite{131,249} and less MDR-MRSA recorded by Bandaru (32.9%). High rate of MDR-MRSA leads to possibility of exploitation of Vancomycin by clinicians. \cite{203}

Various factors like use of antibiotics without doctor’s prescription, inadequate dose, use of poor quality antibiotics and using antibiotics without doing drug sensitivity testing might have contributed to the data showing high degree of resistance to MRSA as reported in this study.

As regards the antibiotic susceptibility to 8 antibiotics (other than oxacillin and cefoxitin), all MRSA isolates were sensitive to vancomycin and resistant to penicillin.

A major problem is the fact that infections caused by MRSA have a significantly higher mortality rate than those caused by sensitive staphylococcus. Moreover, healthcare-associated infections caused by MRSA significantly increase the costs of hospital treatment. \cite{252}

Antibiotics other than vancomycin can be used as anti-MRSA agents after a sensitivity test so as to prevent the emergence of resistance to it and that current problems for treatment to MRSA infecting patients will rise unless indiscriminate and irrational usage of antibiotics is checked. VRSA was first reported in India by Tiwari and Sen in 2006. \cite{95} Glycopeptides and linezolid which is the first oxazolidinone antibiotic approved for treatment of Gram-positive infections continue to remain the mainstay for treatment for MRSA infections. \cite{253}