CHAPTER V

DISCUSSION AND CONCLUSION
Prevalence of *S. aureus* Infections

*S. aureus* has been reported in hospital acquired infections (HAIs) on top of the ten most common pathogens, namely coagulase-negative staphylococci, *Staphylococcus aureus*, *Enterococcus* species, *Candida* species, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* species, *Acinetobacter baumannii*, and *Klebsiella oxytoca*. (85) The prevalence of *S. aureus* infections in the present research was found 36.3% which was next to the Gram negative bacterial infections, but on the top of Gram positive bacterial infections. However, as the isolated Gram negative bacterial pathogens were not identified to their genera or species level, the *S. aureus* infections may be considered the top leading among all the infections in the observed Bastar population. Bastar is a tribal and Naxalites (Maoists) prone area of Chattisgarh state in central India located in the geographical coordinates between 19.07° North latitude and 82.03° East longitude, with least health awareness, low socioeconomic status and lack of healthcare facilities (Chopra et al., 2004). All the studied subjects were tribal and native of Bastar region only, and pyogenic and urogenital infections were found common in them. Unhygienic mode of living and least health awareness might be a cause of ease in acquiring infections. An earlier study by Chopra et al. (75) revealed the common health problems in the tribal community of Bastar District, and also gathered information that the majority of Bastar population prefers to avail PHC and government institutions for
their health issue and only 16 percent used services from private practitioners including traditional healing methods (*sirha* or *gunia*).

Many studies have revealed recovering highest number of *S. aureus* isolates from pus samples. Similarly, in this study also the number of *S. aureus* isolates remained high in pus samples constituting 68.9% proportion of the total sample size. The *S. aureus* infections were in the preponderance of male subjects with an approximate ratio of 1:3 for female to male subjects of the study (*p* < 0.001).

**MRSA Prevalence**

Methicillin resistant *Staphylococcus aureus* (MRSA), first described in 1961, is a leading cause of hospital acquired infections associated with significant increase in morbidity, mortality and hospital cost. Resistance to all ß-lactam antibiotics, and cephalosporins is an important feature of MRSA. The issue of increasing prevalence of MRSA and resistance to various classes of antimicrobial agents is a worldwide problem and challenging threat to the healthcare professionals.

MRSA is highly prevalent in hospitals worldwide. Although epidemiological data from separate studies are often not comparable owing to differences in study design and populations sampled, the highest rates (>50%) are reported in North and South America, Asia and Malta. Intermediate rates (25–50%) are reported in China, Australia, Africa and some European countries e.g. Portugal (49%), Greece (40%), Italy (37%) and Romania (34%). Other European countries have generally low prevalence rates e.g. Netherlands and Scandinavia. The prevalence of HA-MRSA has declined in recent years in some European countries, e.g. Austria, France, Ireland, the UK and Greece. In other European countries the prevalence has remained fairly stable. However, very high
rates of MRSA are reported in East Asia, especially in Sri Lanka (86.5%), South Korea (77.6%), Vietnam (74.1%), Taiwan (65.0%), Thailand (57.0%) and Hong Kong (56.8%).

The prevalence of MRSA varies between geographical regions and between tertiary care centers. Tertiary care centers in Central, South and East India has studied a very high MRSA prevalence of 60-68\%. \(^{110}\) High prevalence of 54-57\% has also been shown from hospitals in West, South, and North India. \(^{110, 112-114}\) Multicenter studies have revealed moderate prevalence of MRSA in India. \(^{110, 116, 125}\) A recent study by Joshi et al. has shown 41\% MRSA prevalence in India. In mono-center studies from Karnataka, and Assam, a moderate prevalence of 35\% has been observed. \(^{119, 124}\)

The present research has found 38.8\% MRSA prevalence which is moderate and similar to the findings of studies by Nishi et al. and Saikia et al.. The prevalence is also similar to that of a multicenter study published by Mehta et al. in 1996.

The study has found higher rates of MRSA infections in adult population of 14-40 years of age with a preponderance of males over females (p < 0.001). The next common subjects of MRSA infections were the elderly population of >40 years age with comparatively higher incidence in male elders then females (p < 0.001). Incidence of MRSA was lowest in children with approximately equal percentage in both the genders (p > 0.001).

**Antibiotic Susceptibility of MRSA**

It was found that the previous studies did not use a complete array of antimicrobial drugs as recommended by CLSI for *S. aureus*, and there was a remarkable variation in antibiotic selection among the studies. However, the available resistance percentages of
antibiotics were arranged in the Table form for studying the pattern of resistance in the present research in comparison to the other studies.

High-level trimethoprim resistance is mediated by a single amino acid substitution in a DHFR gene, which is typically plasmid encoded. The trimethoprim-resistant DHFR seen in *S. aureus* and several species of coagulase-negative staphylococci, termed *dfrA*, probably evolved from *S. epidermidis*. *(189)* Lower level trimethoprim resistance may be mediated by a similar mutation in the *S. aureus* chromosomal DHFR gene. *(190)* Resistance to sulfonamides was earlier described as resulting from an overproduction of PABA. *(186)* Moreover, chromosomal mutations resulting in altered DHPS have been reported. *(187, 188)* The most common drug in the present research to which a high percentage of MRSA isolates were resistant was trimethoprim/sulfamethoxazole (cotrimoxazole). This finding is similar to most of the studies where cotrimoxazole has been shown most inactive antibiotic of the selected panel of antibacterial agents. *(112, 114, 119, 125-127)* In a multicenter study across India, Joshi *et al.* has recently observed ciprofloxacin as highly resistant antibiotic among the selected panel on MRSA isolates. *(110)* In other studies, clindamycin, azithromycin, and gentamycin have been studied occupying a top most position towards higher percentage of resistance among the selected antibiotics.

Tetracyclines inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit and blocking the association with aminoacyl-tRNA. *(129)* Two general mechanisms of resistance to this class of drugs have been described, and may be present in the same isolate. Active efflux is mediated by the inducible genes *tetK* and *tetL*, which are typically located on plasmids. *(129)* The *tetK* resistance gene may be found without other tetracycline resistance determinants, and these isolates are resistant to tetracycline but remain susceptible to minocycline. *(195)* The chromosomally encoded *tetM* gene is also
inducible, \(^{(196)}\) and confers resistance to all currently available tetracyclines by a proposed mechanism of ribosomal protection. \(^{(197, 198)}\) Unlike other studies, the resistance percentage of tetracycline was found at the second higher position of resistance next to cotrimoxazole. The second position of higher resistance in other studies has been commonly shown to be of gentamycin, \(^{(112, 114, 119, 125, 126)}\) while other researchers have observed ciprofloxacin \(^{(121, 127)}\) and macrolids. \(^{(110, 122)}\)

Resistance to aminoglycosides is primarily mediated by drug modification and inactivation. Enzymes encoded by resistance genes include phosphotransferases, acetyltransferases, and adenylyltransferases. \(^{(192, 193)}\) One protein with both acetyltransferase and phosphotransferase activities has been well characterized, and confers high-level resistance to all aminoglycosides. \(^{(194)}\) Aminoglycoside resistance genes are carried on plasmids and transposons, and may be integrated into the bacterial chromosome. The MRSA isolates in this research were found 61-68% resistant to gentamycin, ciprofloxacin, erythromycin, and clindamycin. The gentamycin resistance was found somewhat similar to the findings of studies by Murugan \textit{et al.} and Rajaduraipandi \textit{et al.}. A slightly lower gentamycin resistance percentage (59%) was shown by Joshi \textit{et al.} and Kumar \textit{et al.}. The MRSA isolates were shown 72-73% resistant to gentamycin in separate studies of tertiary care centres from Amritsar and Anantapur in year 2010. \(^{(112, 121)}\) Very high gentamycin resistance percentage of 86-90% has also been observed in some studies. \(^{(114, 119, 122)}\)

In \textit{S. aureus}, fluoroquinolone resistance is conferred by point mutations occurring primarily in the subunit ParC (also designed GrlA) of topoisomerase IV, and secondarily in the subunit GyrB of DNA gyrase. \(^{(154)}\) Most of the resistant MRSA have at least two mutations, one in ParC and one in GyrA. \(^{(143)}\) In some strains, overexpression of an efflux pump termed NorA contributes to the resistance phenotype. \(^{(155)}\) Multiple

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mutations and combination of resistance mechanisms also confer cross-resistance to newer fluoroquinolones, including those with increased activity against Gram-positive bacteria.\(^\text{153}\) In the present study, the percentage of ciprofloxacin resistance in the MRSA isolates was found 63.3% which is nearly similar to the finding in a study at GMC, Amritsar.\(^\text{121}\) Studies have reported lower rates of 40–48%,\(^\text{112, 125, 126}\) as well as high rates of 79–88% for ciprofloxacin.\(^\text{110, 114, 119, 122, 127}\) Gatifloxacin is a fourth generation fluoroquinolone. Rao\ et\ al.\ revealed 71.1% MRSA resistant to gatifloxacin. This study has found a very low gatifloxacin resistance percentage of 8.5% in the MRSA isolates.

Three related erythromycin ribosomal methylase genes, \(ermA\), \(ermB\), and \(ermC\), alter the ribosomal target site and confer MLS\(_B\) resistance in \(S.\ aureus\).\(^\text{167, 168}\) The \(erm\) genes are carried on plasmids and transposable elements, and may be produced in an inducible or constitutive manner.\(^\text{169-171}\) When the methylase is constitutively produced, strains will demonstrate \textit{in vitro} resistance to macrolides, clindamycin, and streptogramin B antibiotics. Tertiary care centres from North-west and South India have reported 60–62% erythromycin resistance in their MRSA isolates which is quite similar to the present research.\(^\text{121, 125, 126}\) Hospitals in other parts of our nation has increasingly reported not only 71%,\(^\text{110, 112}\) but also 80–83% erythromycin resistance in the methicillin resistant isolates of \(S.\ aureus\).\(^\text{112, 114, 119, 127}\) A very high erythromycin resistance percentage of 95.6% has been quoted by Rao\ et\ al..\ Though there is a lack of investigation of susceptibility of azithromycin in the published research articles, but Rao\ et\ al.\ has also quoted an amazingly very high azithromycin percentage of 95.6%. The present research has found a low percentage (39.3%) of MRSA resistant to azithromycin. A recent multicentre study across India found 46.6% clindamycin resistance in MRSA. Other studies reported 22% clindamycin resistance in 2012 from Pondicherry, 56.2% in 2009
from Assam. This doctoral research has observed rather higher percentage (61.8%) of clindamycin resistance in MRSA.

There are three mechanisms of resistance to chloramphenicol: reduced membrane permeability, mutation of the 50S ribosomal subunit and elaboration of chloramphenicol acetyltransferase. High-level resistance is conferred by the cat-gene; this gene codes for an enzyme called chloramphenicol acetyltransferase, which inactivates chloramphenicol by covalently linking one or two acetyl groups, derived from acetyl-S-coenzyme A, to the hydroxyl groups on the chloramphenicol molecule. The acetylation prevents chloramphenicol from binding to the ribosome. Earlier research studies were not found investigating the susceptibility of chloramphenicol. The probable reason for this might be a therapeutic withdrawal of chloramphenicol from clinical practice. However, the MRSA isolates in the present study were observed to have 16.4% resistance to chloramphenicol. The resistance might be due to constitutive efflux of chloramphenicol.

Drug of choice in severe MRSA infections is vancomycin. The intermediate resistance in VISA has been associated to the presence of a thickened cell wall. (130) According to the accepted vancomycin susceptibility breakpoints, the vancomycin MIC for VISA has been shown to be 4–8 μg/ml. (138) VRSA are usually high-level vancomycin resistant (MIC ≥ 16 μg/ml). While the mechanism of resistance in VISA is quite elusive, in VRSA it is well understood. VRSA strains have acquired the complete genetic apparatus for glycopeptide resistance from vancomycin-resistant enterococci (VRE). To date, six VRSA strains have been described, and all have acquired the vanA operon that confers high level resistance to both glycopeptides, vancomycin and teicoplanin. (148-151) The vanA operon contains an assembly of genes that encode the synthesis of modified peptidoglycan precursors containing a terminal D-Ala-D-Lac instead of D-Ala-D-Ala (vanH and vanA genes) and the elimination of the susceptible wild-type targets (vanX
and the accessory genes). Present research has found 0.9% (3) VRSA in the MRSA, comparatively higher than that shown from north part of India. (45) However, Thati et al. (47) have recently revealed 2.46% VRSA in MRSA isolates from intensive care units of tertiary care hospitals in Hyderabad. For treatment of VISA and VRSA infections linezolid is one of the newer oxazolidinones. (37, 48) Linezolid acts by inhibiting bacterial protein synthesis through binding to the peptidyltransferase center (PTC) of the 50S ribosomal subunit. (159) To date, the following mechanisms responsible for linezolid resistance have been reported in clinical isolates of S. aureus: (i) mutations in the domain V region of one or more of the five or six copies of the 23S rRNA gene, (160) (ii) acquisition of the plasmid-mediated ribosomal methyltransferase cfr gene, (161) and (iii) deletions or mutations in the ribosomal protein L3 of the PTC. (162) Additional mutations in domain V of the 23S rRNA genes and substitutions in ribosomal protein L4 of the PTC are also reported in laboratory-derived lenizolid-resistant S. aureus strains. (162) Resistance to linezolid in MRSA has not yet been reported from any part of India, but we found 0.6% (2) linezolid resistance in our isolates. The probable reason for the significant variation in the antibiotic susceptibility of the isolates in our study might be due to the preferential therapeutic use of vancomycin and linezolid, the drugs of choice, as a substitute for bacterial identification and sensitivity testing in the absence of sufficient microbiology laboratory facility at this tribal region.

**Antibiotic Susceptibility of MSSA**

To the β-lactam antibiotics; penicillin and ampicillin, 81.2% MSSA isolates of present study were resistant, which was quite similar to study by Arora et al. (121) and slightly lower than that in another study by Saikia et al.. However, a low percentage of MSSA
isolates resistant to β-lactam antibiotics, i.e. 23.6% and 58.5% has been shown in studies by Joshi et al. and Kumar et al. respectively. (110, 127)

Except β-lactam antibiotics, the resistance in MSSA to rest of the antibiotics was not more than 12% which is very lower than as shown in studies from other parts of India. However, the resistance frequency to clindamycin in this research is somewhat similar to study by Saikia et al. (119) and higher than that which is reported by Kumar et al. (127) Studies published in year 2009 to onwards have reported 25-49% resistance in erythromycin, whereas the present study has found only 12% erythromycin resistance. Although studies were not found selecting azithromycin antibiotic in the antimicrobial susceptibility testing, the MSSA isolates in the present research were found 0.6% resistant to azithromycin.

The present doctoral study found a moderate prevalence of MRSA in Bastar, and significantly higher percentage of the isolates susceptible to the CLSI recommended panel of antibiotics (P < 0.001). However, emergence of linezolid resistance and higher percentage of vancomycin resistance in MRSA is alarming. We observed the higher generation of macrolides and fluoroquinolone more promising than the lower one. Cotrimoxazole and/or gentamycin may be considered as initial empiric treatment, but must be replaced immediately with the correct antibiotics according to the antibiogram. Although, the study highlights linezolid and vancomycin as the most sensitive agents of the entire selected panel of antibiotics, these classes must not be used commonly in therapy if the other sensitive antibiotics are available in the microbiological report. Also, it is suggestive to the respective government health authorities to pay attention to this tribal region in providing sufficient facility for microbiological diagnostics and culture sensitivity.