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1. **Incidence and antibiotic susceptibility of methicillin resistant *Staphylococcus aureus* in pus samples of patients of Bastar region, Central India**

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2. **Emergence of linezolid resistant *Staphylococcus aureus* in Bastar tribal region, India**

   Journal of Microbiology and Infectious Diseases, Volume 2, Issue 3, Page 127-128

3. **Prevalence and drug resistance pattern of *Staphylococcus aureus* clinical isolates in Bastar region**

   Journal of Clinical and Analytical Medicine (Manuscript accepted and published online)
Incidence and antibiotic susceptibility of methicillin resistant *Staphylococcus aureus* in pus samples of patients of Bastar region, Central India

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**ABSTRACT**

Increasing prevalence of methicillin resistant *S. aureus* (MRSA) and resistance to multiple antibiotic classes is a global issue. Regional surveillance of antibiotic susceptibility of the organism is a necessary step to overcome the issues of antimicrobial resistance and treatment failure in MRSA infections. The study was conducted to find the pattern of antibiotic susceptibility in MRSA isolated from the pus samples of patients attending a tertiary care hospital in Bastar tribal region in Central India. The study was performed on 215 MRSA isolates cultured from pus samples of patients over a period of two years and five months. In the methicillin resistant organisms selected by oxacillin screen agar test and cefoxitin disk diffusion test, antibiotic susceptibility was determined by Kirby Baur disk diffusion test with CLSI guide lines. Of the total *S. aureus* isolates, the incidence of MRSA was 34.1% of which 82.8% were resistant to co-trimoxazole, 77.2% to tetracycline, 68.8% to gentamycin, 66% to erythromycin, 64.2% to ciprofloxacin, 1.4% to vancomycin, and 0.9% to linezolid. All these isolates were resistant to the β-lactam antibiotics tested. Emergence of linezolid resistance and relatively higher vancomycin resistance in the MRSA isolates is a worrisome finding of this study. The antibiotic prescribing must rely on both initial empirical therapy and microbiological antibiotic susceptibility result.

**1. Introduction**

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 (Gould, 2006; Klein et al., 2007; Köck et al., 2010). Resistance to all β-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. The hospitals in South and North India have reported the prevalence of MRSA as high as 55% (Khadri et al., 2010; Anupurba et al., 2003).

Vancomycin is described as the first-line intravenous drug for severe MRSA infections (Chambers et al., 2009). The first strain of *S. aureus* with reduced
susceptibility to vancomycin was isolated in 1996 from a Japanese patient (Hiramatsu et al., 1997). However, first clinical isolate of vancomycin resistant \( S. aureus \) (VRSA) was reported from United States in 2002 (CDC, 2002). Emergence of vancomycin-intermediate \( S. aureus \) (VISA) and vancomycin-resistant \( S. aureus \) (VRSA) has now become a global issue including India (Song et al., 2004; CDC, 2004; Howe et al., 1998; Assadullah et al., 2003; Tiwari et al., 2006; Menezes et al., 2008; Thati et al., 2011).

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). Though pyogenic wound and soft tissue infections are very common in the tribal patients attending the hospital, but work on MRSA prevalence and antimicrobial susceptibility has not been published earlier from this region. The pattern of antibiotic susceptibility of MRSA varies geographically, and there is an increasing need of antibiotic susceptibility surveillance (Khadri et al., 2010; Anupurba et al., 2003; Saikia et al., 2009; Murugan et al., 2008; Tyagi et al., 2008; Rajaduraipandi et al., 2006; Orrett et al., 2006; Shittu et al., 2006). The study is aimed to know the drug resistance pattern in the MRSA isolates with an intention to help start the appropriate empirical antibiotic treatment of patients even on the levels of primary healthcare centers.

2. Material and Methods

The study was conducted in the microbiology laboratory of School of Life Sciences in MATS University, Raipur, Chattisgarh, India. Permission was sought to collect the pus swab samples from patients of Maharani Hospital, Jagdalpur, Bastar. The institutional ethical committee approved the study protocol. Informal consent was obtained from patient/relatives to sample the pus for microbiological investigations. The study included 215 MRSA isolates cultured from various pus samples during January 2010 to May 2012.

2.1. Culture and identification of the organisms

Pus samples were inoculated onto nutrient agar, blood agar and MacConkey agar media. The isolates of \( S. aureus \) were identified based on standard tests (Gram staining, catalase, and coagulase). The identification was further confirmed by culturing the organism on mannitol salt agar and by Dry Spot Staphytec Plus\(^*\) (Oxoid); latex agglutination assay kit for \( S. aureus \). The study excluded Gram negative bacilli, \( Streptococci \), MSSA and coagulase negative \( Staphylococci \) (CoNS) cultured from the pus samples.

2.2. MRSA screening

2.2.1. Oxacillin screen agar

Mueller-Hinton agar (MHA) plates containing 4% NaCl and 6 μg/ml of oxacillin were prepared. Plates were inoculated with 10 μL of 0.5 Mc Farland suspension of the isolate by streaking in one quadrant and incubated at 35°C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant (Swenson et al., 2001).

2.2.2. Cefoxitin disk diffusion test

All the isolates were subjected to cefoxitin disk diffusion test using a 30 μg disk. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture was done on MHA plate. Plates were incubated at 37°C for 18 h and zone diameters were measured. An inhibition zone diameter of ≤ 19 mm was reported as oxacillin resistant and ≥ 20 mm was considered as oxacillin sensitive (Anand et al., 2009).

2.3. Antibiotic susceptibility testing

The antibiotic susceptibility pattern of all MRSA isolates was determined by Kirby Bauer disk diffusion method against the following antibiotics (HiMedia Laboratories Pvt. Ltd, India): penicillin-G (10 units), ampicillin (10 μg), ampicillin/sulbactam (10/10 μg), erythromycin (15 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), gentamycin (10 μg), ciprofloxacin (5 μg), tetracycline (30 μg), linezolid (30 μg), and vancomycin (30 μg). All the tests were performed on MHA, and were interpreted after incubation for 24 h at 37°C. Following CLSI criteria, the susceptibility was noted as per the zone diameter measured around each disk (CLSI, 2010).

3. Results

Of the 2074 pus samples cultured, growths were obtained from 1343 samples of which 55.8% (749), 4.7% (63), and 39.5% (531) were Gram positive staphylococci, Gram positive streptococci, and Gram negative bacilli respectively. All the staphylococci were catalase positive. Of the catalase differentiated staphylococci, 8.8% (118) were coagulase
negative staphylococci (CoNS). The incidence of S. aureus in the culture positive pus samples was found to be 47% (631) (Figure 1).

Out of 631 S. aureus isolate, 215 (34.1%) were mecA-mediated MRSA; all resistant to penicillin-G, ampicillin, and ampicillin-sulbactam. Resistance in the MRSA to erythromycin, cotrimoxazole, gentamicin, ciprofloxacin, tetracycline, linezolid, and vancomycin was 66%, 82.8%, 68.8%, 64.2%, 77.2%, 0.9%, and 1.4% respectively (Table-1).

![](image)

**Figure 1: Incidence of S. aureus in culture positive pus samples; n = 1343.**

<table>
<thead>
<tr>
<th>Disk</th>
<th>R</th>
<th>I</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>100.0 (215)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>100.0 (215)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A/S</td>
<td>100.0 (215)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>66.0 (142)</td>
<td>4.2 (9)</td>
<td>29.8 (64)</td>
</tr>
<tr>
<td>Sxt</td>
<td>82.8 (178)</td>
<td>2.8 (6)</td>
<td>14.4 (31)</td>
</tr>
<tr>
<td>G</td>
<td>68.8 (148)</td>
<td>0</td>
<td>31.2 (67)</td>
</tr>
<tr>
<td>Cp</td>
<td>64.2 (138)</td>
<td>1.9 (4)</td>
<td>33.9 (73)</td>
</tr>
<tr>
<td>T</td>
<td>77.2 (166)</td>
<td>0</td>
<td>22.8 (49)</td>
</tr>
<tr>
<td>Lz</td>
<td>0.9 (2)</td>
<td>0</td>
<td>99.1 (213)</td>
</tr>
<tr>
<td>V</td>
<td>1.4 (3)</td>
<td>0</td>
<td>98.6 (212)</td>
</tr>
</tbody>
</table>

**Table 1: Antibiotic susceptibility of MRSA (n = 215) isolated from pus samples.**


4. Discussion and Conclusion

The present study found a higher incidence of S. aureus in pus samples (Tyagi et al., 2008). The percentage of MRSA in the isolates was observed not exceeding those shown earlier in other parts of India (Khadri et al., 2010; Anupurba et al., 2003; Saikia et al., 2009; Murugan et al., 2008; Tyagi et al., 2008; Rajadurai et al., 2006). Linezolid resistance and a higher percentage of vancomycin resistance is, though, an important finding of our study, but a relatively lower percentage of resistance in MRSA to other antibiotics of different classes is surprising (Anupurba et al., 2003; Saikia et al., 2009; Rajadurai et al., 2006). Like most of the studies in India, cotrimoxazole in our study was found to be highly inactive (only 14.4% sensitive) drug for the MRSA isolates. The second common drug for that MRSA had higher resistance was tetracycline (77.2%) instead of gentamycin as in other regional studies from India (Khadri et al., 2010; Saikia et al., 2009; Murugan et al., 2008). Resistance to ciprofloxacin, erythromycin, and gentamycin in the isolates ranged from 64% to 69% which is rather lower than what is shown in eastern Uttar Pradesh, Assam and Tamilnadu (Anupurba et al., 2003; Saikia et al., 2009; Tyagi et al., 2008).

Drug of choice in severe MRSA infections is vancomycin, whereas for treatment of VISA and VRSA infections linezolid is one of the newer drugs (Chambers et al., 2009; Tsiodras et al., 2001). Our study found 1.4% VRSA, comparatively higher than those shown from southern and northern parts of India. However, Thati et al. (2011) have recently revealed 2.46% VRSA in MRSA isolates from intensive care units of tertiary care hospitals in Hyderabad. Resistance to linezolid in MRSA has not yet been reported from any part of India, but we found 0.9% linezolid resistance in our isolates. The probable reason for the significant variation in the anti-
biotic 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.

The study concluded a relatively lower prevalence of MRSA in pus and found a significantly higher percentage of the isolates susceptible to the CLSI recommended panel of antibiotics. However, emergence of linezolid resistance and higher percentage of vancomycin resistance in MRSA from this tribal part of central India is an alarming threat. Although, our study highlights linezolid and vancomycin as the most sensitive agents of the entire selected panel of antibiotics, we emphasize the antibiotic prescribing must rely on both initial empirical therapy and streamlining and adjustment of therapy once microbiological antibiotic susceptibility result becomes available. Also, we suggest the respective government health authorities to pay attention to this tribal region in providing sufficient facility for microbiological diagnostics and culture-sensitivity.

Acknowledgement

Present work is a part of Ph.D. research of Mr. Mohammad Fareed Khan. The authors are thankful to the respected Registrar, and Vice-Chancellor of MATS University, Raipur for providing the research facilities, and to the Hospital Superintendent of Maharani Hospital, Jagdalpur for permitting the collection of clinical samples.

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4928.


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Emergence of linezolid resistant *Staphylococcus aureus* in Bastar tribal region, India

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Dear Editor,

Methicillin resistant *Staphylococcus aureus* (MRSA) is a well-known threat to the healthcare systems for its increasing global prevalence, intrinsic ability of resistance to β-lactam and cephalosporin, and for acquiring resistance to multiple classes of other antibiotics, causing difficult-to-treat infections with significant increase in morbidity, mortality and treatment cost. Although for severe MRSA infections vancomycin is described as the first-line intravenous drug, vancomycin-resistant and intermediate isolates of *S. aureus* (VRSA & VISA) have been increasingly reported throughout the world. The therapeutic and life-saving option for VRSA and VISA infections remain linezolid, first antimicrobial of oxazolidinone group available since 2000. The first case of linezolid-resistant staphylococci appeared within 1 year after linezolid was approved for therapeutic use.⁴ Although linezolid resistance in *S. aureus* is uncommon, emergence has been shown from some parts of the world.² From India, first case report of linezolid resistance was published in 2011 from Kashmir.³ This is the first report from the Chattisgarh state in Central India where we found two linezolid-resistant *Staphylococcus aureus* isolates which were cultured in March 2011 from pus samples collected from the male surgical ward of Maharani Hospital, Jagdalpur, Bastar.

Linezolid acts by inhibiting bacterial protein synthesis through binding to the peptidyltransferase center (PTC) of the 50S ribosomal subunit.⁴ 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 RNA gene,⁵ (ii) acquisition of the plasmid-mediated ribosomal methyltransferase *cfr* gene,⁶ and (iii) deletions or mutations in the ribosomal protein L3 of the PTC.⁷ 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 linezolid-resistant *S. aureus* strains.⁷

The study was conducted in the microbiology laboratory of School of Life Sciences in MATS University, Raipur, Chattisgarh, India. Permission was sought to collect the pus samples from patients of Maharani Hospital, Jagdalpur, Bastar. The institutional ethical committee approved the study protocol. The study included 631 *S. aureus* isolates cultured from various pus samples during January 2010 to May 2012.

Pus samples were inoculated onto nutrient agar, blood agar and MacConkey agar media. The isolates of *S. aureus* were identified based on standard tests (Gram staining, catalase, and coagulase). The identification was further confirmed by culturing the organism on mannitol salt agar and by Dry Spot Staphytec Plus® (Oxoid); latex agglutination assay kit for *S. aureus*. MRSA screening was done by oxacillin screen agar (Swenson et al., 2001) as well as by cefoxitin disc diffusion test.⁸

Antibiotic sensitivity was done by Kirby-Bauer disk diffusion method using disks of erythromycin (15 μg), clindamycin (2 μg), cotrimoxazole (1.25/23.75 μg), gentamycin (10 μg), ciprofloxa-
cin (5 μg), tetracycline (30 μg), and vancomycin (30 μg) by HiMedia Lab Pvt. Ltd, India, and minimum inhibitory concentration (MIC) of linezolid was determined using Etest (AB Biodisk, Solna, Sweden) as per manufacturer’s instructions. Following CLSI, 2010 breakpoints, the susceptibility was noted for the disc diffusion and MIC. Isolates with an MIC ≤ 4.0 mg/L are considered susceptible to linezolid, and isolates with an MIC ≥ 8.0 mg/L are resistant.

All the methicillin sensitive S. aureus (MSSA) were sensitive to linezolid, whereas two (0.9%) MRSA were linizolid resistant, both with MIC 8 mg/L. The isolates were resistant to erythromycin, clindamycin, and gentamycin, whereas sensitive to the rest of the antibiotics used including vancomycin (Table 1).

| Table 1. Susceptibility of the two linezolid resistant (MIC 8 mg/L) MRSA. |
|-----------------|---|---|---|---|---|---|---|
| Strain         | E  | Cd | Sxt | G  | Cp | T  | V  |
| LZR-1          | R  | R  | S   | R  | S  | S  | S  |
| LZR-2          | R  | R  | S   | R  | S  | S  | S  |

E=Erythromycin, Cd=Clindamycin, Sxt=Cotrimoxazole, G=Gentamycin, Cp=Ciprofloxacin, T=Tetracyclin, V=Vancomycin, R=Resistant, S=Sensitive

Bastar is a tribal area of Chattisgarh state in Central India with least health awareness, low socioeconomic status and lack of sufficient health facilities. Preferential therapeutic use of the drug of choice as a substitute for bacterial identification and sensitivity testing in absence of sufficient microbiology laboratory facility at this tribal region might be one reason for the emergence of antibacterial resistance. The linezolid resistance may have been acquired following the prior linezolid exposure of the patients. Because Maharani Hospital is tertiary care center, so it is most likely that patients may have taken the drug previously before coming here. We emphasize the antibiotic prescribing must rely on both initial empirical therapy and streamlining and adjustment of therapy once microbiological antibiotic susceptibility result becomes available. The antibiotic policy at the primary health care and hospital level need to be revised and the drugs of choice should be kept reserved as the final lifesaving option. Also, we suggest the respective government health authorities to pay attention to this tribal region in providing sufficient facility for microbiological diagnostics and culture-sensitivity.

Acknowledgement

The authors are thankful to the respected Registrar, and Vice-Chancellor of MATS University, Raipur for providing the research facilities, and to the Hospital Superintendent of Maharani Hospital, Jagdalpur for permitting the collection of clinical samples.

REFERENCES

Prevalence and Drug Resistance Pattern of Staphylococcus aureus Clinical Isolates in Bastar Region

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Abstract

Aim: The aim of this research was to study the prevalence and antibiotic resistance of S. aureus in the Bastar region. Material and Method: From the clinical samples cultured from Jan 2010 to May 2012, 916 S. aureus isolates were identified by the standard tests. Screening of MRSA was done by oxacillin screen agar and cefoxitin disk diffusion tests. Antibiotic susceptibility was examined by Kirby-Bauer disk diffusion test. For MIC of vancomycin and linezolid, Etest was performed. Result: Of the isolates, 34.8% were MRSA. In the MRSA isolates, 63% (165) were found resistant to erythromycin, 39.3% (103) to azithromycin, 61.8% (162) to clindamycin, 81.5% (260) to cotrimoxazole, 0.6% (2) to linezolid, 0.9% (3) to vancomycin, 76.5% (244) to tetracycline, 67.7% (216) to gentamycin, 63.3% (202) to ciprofloxacin, 8.5% (27) to gatifloxacin, 16.4% (43) to chloramphenicol, 68.4% (39) to norfloxacin, 12.3% (7) to nitrofurantoin, 80.7% (46) to sulfisoxazole, and 80.7% (46) to trimethoprim antibiotics. Discussion: The study was conducted first time from this region. The prevalence and drug resistance percentage is compared with other studies. Emergence of linezolid resistance and relatively higher vancomycin resistance in the MRSA isolates is a worrisome finding of this study. Cotrimoxazole and/or gentamycin may be considered as initial empiric treatment, but must be replaced immediately with the correct antibiotic according to the antibiogram.

Keywords

Bastar; MRSA; Prevalence; Drug Resistance
Introduction

Staphylococcus aureus is a well known nosocomial pathogen and the methicillin resistance in the organism has been more than a half century old issue [1–4]. Methicillin resistant S. aureus (MRSA) isolates not only possess intrinsic ability to resist the standard concentrations of β-lactam antibiotics and cephalosporins, but also show multiple drug resistance (MDR) to various other antimicrobial classes. MDR leaves a limited choice for antimicrobial therapy and the infections become difficult-to-treat that in turn have a significant impact in increasing morbidity, mortality, and hospital cost [5–7].

Increasing MRSA prevalence is a global problem, and regional periodic survey for antimicrobial susceptibility is a necessary step for understanding the correct empiric therapy and to develop antimicrobial stewardship to help stop the further emergence of drug resistance. A recent multicenter study including various national regions found 41% MRSA prevalence in India with the maximum prevalence being 60-68% from tertiary care centers in Central, South, and East India [8]. A very low prevalence of 2.4% was reported in 1996 from Vellore [9]. The prevalence has also been shown to be 54-57% reported from the hospitals in West, South, and North India [8, 10–12].

The tribal area Bastar is located in the central part of India as a district of Chhattisgarh state. The region is a beautiful dense forest area and a well known red zone for Naxalite (Maoist) activity. Bastar natives are mostly tribes living in a primitive style under below poverty line [13, 14]. Though pyogenic wound and soft tissue infections are very common in the tribal patients attending the hospital, but there is a lack of published literature on the prevalence of pathogens and their pattern of drug resistance from this region. The aim of this research was to study the prevalence of S. aureus infections in the Bastar tribes and the drug resistance pattern in the isolates with an intention to help start the appropriate empirical antibiotic treatment of patients even on the levels of primary healthcare centers.

Material and Method

Study population

The study was conducted in the microbiology laboratory of School of Life Sciences at MATS University, Raipur, Chhattisgarh, India. The study included the samples of patients of Bastar region attending Maharani Hospital, Jagdalpur, Bastar. The patients were mostly the tribes and belonging to the in and around area of Jagdalpur under Bastar district only. Permission for sample collection was taken from the Superintendent of the hospital. Informed consent was taken from the patients or their relatives to take samples for microbiological investigations. The institutional ethical committee approved the study protocol. Those samples which were drawn for the microbiological investigations under hospital were also included.

From January 2010 to May 2012, a total 916 S. aureus isolates were cultured from the clinical samples of 573 male, and 343 female patients. According to their ages, the subjects in the studied population were observed into 3 groups: (1) up to 13 yrs age, (2) 14 to 40 yrs age, and (3) >40 yrs age. The studied male population was comprised of 71 subjects of up to 13 yrs age, 347 subjects of 14-40 yrs age, and 155 subjects with >40 yrs age. In the studied female population, there were 72 subjects of up to 13 yrs age, 218 subjects with 14-40 yrs age, and 53 subjects of >40 yrs age (Table 1).

Table 1. Studied population satisfying the total study load of S. aureus isolates (n = 916).

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Study population</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 13 yrs</td>
<td>143</td>
<td>71</td>
<td>72</td>
</tr>
<tr>
<td>14-40 yrs</td>
<td>565</td>
<td>347</td>
<td>218</td>
</tr>
<tr>
<td>&gt;40 yrs</td>
<td>208</td>
<td>155</td>
<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>916</td>
<td>573</td>
<td>343</td>
</tr>
</tbody>
</table>

Culture and identification of the organisms

All the dehydrated culture media were purchased from HiMedia Lab, and prepared under instructions of the manufacturer. The pus and wound samples were taken with sterile swab sticks (HiMedia Lab). The swabbed samples were inoculated onto sterile nutrient agar, blood agar and MacConkey agar media in Petri dishes. For throat swab, two swabs were taken from the same areas; one to prepare a smear for Gram staining, while the other to inoculate onto blood agar, chocolate agar and MacConkey agar plates. Freshly voided midstream urine samples were obtained in sterile wide-mouth screw capped universal containers. The urine specimens were well mixed and inoculated onto blood agar, MacConkey agar and cystine lactose electrolyte deficient (CLED) agar plates. The plates were incubated aerobically at 37°C for 24 hours.

For blood culture, 3 ml blood sample was aseptically transferred to 50 ml brain-heart infusion (BHI) broth in bottles. Covering the bottle-caps with aluminium foil, the bottles were incubated at 37°C. The bottles were routinely inspected twice a day (at least for the first 3 days) for signs of microbial growth for the maximum of 7 days. The aspirated broth was examined microscopically by Gram-staining, and sub-cultured aseptically onto sterile blood agar, MacConkey agar, and mannitol salt agar plates. The blood cultures were considered negative, where there were no growth occurred after 7 days.

The isolates of S. aureus were identified based on standard tests (Gram staining, catalase, and coagulase). The identification was further confirmed by culturing the organism on mannitol salt agar and by Dry Spot Staphytect Plus® (Oxoid); latex agglutination assay kit for S. aureus. The study excluded Gram negative bacilli, streptococci, and coagulase negative staphylococci (CoNS) cultured from the clinical samples.

MRSA screening

Mueller-Hinton agar (MHA) plates containing 4% NaCl and 6 μg/ml of oxacillin were prepared. Plates were inoculated with 10 μL of 0.5 Mc Farland suspension of the isolate by streaking in one quadrant and incubated at 35°C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant [15, 16].

For further confirmation, all the isolates were subject to cefoxitin disk diffusion test using a 30 μg disk. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture was done on MHA plate. Plates were incubated at 35°C for 18 h and zone diameters were measured. An inhibition zone diameter of ≤ 21 mm was reported as oxacillin resistant and ≥ 22 mm was considered as oxacillin sensitive [15, 17–19].
Kirby-Bauer disk diffusion method for antibiotic susceptibility

The antibiotic susceptibility pattern of all isolates was determined by Kirby Bauer disk diffusion method against the following antibiotics (HiMedia Laboratories Pvt. Ltd, India): penicillin (10 μg), ampicillin (10 μg), ampicillin/sulbactam (10/10 μg), erythromycin (15 μg), azithromycin (15 μg), clindamycin (2 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), linezolid (30 μg), vancomycin (30 μg), tetracycline (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), gatifloxacin (5 μg), and chloramphenicol (30 μg). For the isolates cultured from urine samples, norfloxacin (10 μg), nitrofurantoin (300 μg), sulfisoxazole (300 μg) and trimethoprim (5 μg) were tested in addition to the above mentioned antibiotics except erythromycin, azithromycin, clindamycin and chloramphenicol. All the tests were performed on Mueller Hinton agar, and were interpreted after incubation for 24 h at 37°C. Following CLSI criteria, the susceptibility was noted as per the zone diameter measured around each disk [15].

Etest method for MIC

Minimum inhibitory concentration (MIC) of vancomycin and linezolid was determined using Etest (AB Biodisk, Solna, Sweden) as per manufacturer’s instructions [20, 21]. Following CLSI, 2010 breakpoints [15], the susceptibility was noted.

Statistical analysis

The values were represented in the relative frequencies. The data were entered in the Microsoft Excel 2007 and analyzed statistically using chi-squared test and student t test to see the significance at 0.001 level.

Results

A total number of 3591 clinical samples were processed which included 2074 pus samples, 1130 urine samples, 260 blood samples, 116 wound swabs, and 11 throat swabs. Of the entire number of clinical samples processed, only 2520 samples were found positive for bacterial growth. The prevalence of Staphylococcus aureus in the culture positive samples was found 36.3% (916). The other organisms cultured were: 6.2% (155) coagulase negative staphylococci, 6.7% (169) streptococci, and 50.8% (1280) Gram negative bacilli. The incidence of S. aureus in the types of clinical samples is shown in Table 2.

The prevalence of MRSA and MSSA in the clinical samples has been shown in Table 3. The prevalence of MRSA was found to be 34.4% (916) in the entire studied population. In the male region only, and pyogenic and urogenital infections were found leading among all the infections in the observed Bastar population. The incidence of MRSA in pus samples (631), urine samples (144), blood samples (65), wound swabs (71), and throat swabs (5) were found to be 34.1% (215), 39.6% (57), 27.7% (18), 40.8% (29), and 0% (0) respectively (Table 3).

Discussion

S. aureus is a leading pathogen in hospital acquired infections (HAI’s). The prevalence of S. aureus infections 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. 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.
The prevalence of MRSA varies between geographical regions and between tertiary care centers. Tertiary care centers in Central, South and East India have studied a very high MRSA prevalence of 60-68% [8]. High prevalence of 54-57% has also been shown from hospitals in West, South, and North India [8, 10–12]. Multicenter studies have revealed moderate prevalence of MRSA in India [8, 22, 23]. A recent study by Joshi et al. [8] has shown 41% MRSA prevalence in India. In mono-center studies from Karnataka, and Assam, a moderate prevalence of 35% has been observed [24, 25]. The present research has found a moderate prevalence of MRSA similar to the findings of studies by Nishi et al. [25] and Saikia et al. [24]. The prevalence is also similar to that of a multicenter study published by Mehta et al. [22].

The study recovered large number of MRSA from pyogenic infections. 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 earlier studies [10, 12, 23–27]. The MRSA isolates were found 61-68% resistant to gentamicin, ciprofloxacin, erythromycin, and clindamycin. The gentamicin resistance was found somewhat similar to the findings of studies by Murugan et al. [26] and Rajaduraiapandi et al. [23]. A slightly lower gentamicin resistance percentage (59%) was shown by Joshi et al. [8] and Kumar et al. [27]. The MRSA isolates were shown 72-73% resistant to gentamicin in separate studies of tertiary care centres from Amritsar and Anantapur in year 2010 [10, 28]. Very high gentamicin resistance percentage of 86-90% has also been observed in some studies [12, 24, 28].

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 monocentric study in Amritsar [28]. Studies have reported lower rates of 40-48% [10, 23, 26], as well as high rates of 79-88% for ciprofloxacin [8, 12, 24, 27, 29]. Gatifloxacin is a fourth generation fluoroquinolone. Rao et al. [29] revealed 71.1% MRSA resistant to gatifloxacin. A remarkably low percentage of gatifloxacin resistance (8.5%) was found in the studied MRSA isolates.

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 [23, 26, 28]. Hospitals in other parts of our nation has increasingly reported not only 71% [8, 10], but also 80-83% erythromycin resistance in the methicillin resistant isolates of S. aureus [10, 12, 24, 27]. A very high erythromycin resistance percentage of 95.6% has been quoted by Rao et al. [29]. Though there is a lack of investigation of susceptibility of azithromycin in the published research articles, but Rao et al. [29] has also quoted an amazingly high erythromycin 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 [8]. Other studies reported 22% clindamycin resistance in 2012 from Pondicherry [27], 56.2% in 2009 from Assam [24]. We have observed rather higher percentage (61.8%) of clindamycin resistance in MRSA.

Vancomycin is the potential glycopeptides drug that can reliably treat MRSA infections [30]. However, the massive use of vancomycin for treating MRSA has caused the emergence of vancomycin resistant S. aureus (VRSA) and vancomycin intermediate S. aureus (VISA) cases [31]. The therapeutic and life-saving option for VRSA and VISA infections remains linezolid, first antimiicrobial of oxazolidinone group available since 2000 [30]. The first case of linezolid-resistant staphylococci appeared within 1 year after linezolid was approved for therapeutic use [32]. Although linezolid resistance in S. aureus is uncommon, emergence has been shown from some parts of the world [33]. From India, first case report of linezolid resistance was published in 2011 from Kashmir [34]. Present research has found 0.9% (3) VRSA in the MRSA, comparatively higher than that shown from north part of India [35]. However, Thati et al. [36] have recently revealed 2.46% VRSA in MRSA isolates from intensive care units of tertiary care hospitals in Hyderabad. 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 study is very important as no such work has been conducted earlier from this tribal region. We found a moderate prevalence of MRSA in Bastar, and significantly higher percentage of the isolates susceptible to the CLSI recommended panel of antibiotics. However, emergence of linezolid resistance and higher percentage of vancomycin resistance in MRSA is alarming. 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. We observed the higher generation of macrolides and fluoroquinolone more promising than the lower one. Cotrimoxazole and/or gentamicin 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.

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Competing Interests
The authors do not have any competing interest in this manuscript.

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