PUBLICATIONS
Staphylococcus aureus is a major cause of both community acquired and health care associated infections and the treatment of suspected Staphylococcus aureus infections is becoming increasingly more complicated. The objective of the present study is to detect Staphylococcus aureus strains with vanA gene. Among 856 strains of Staphylococcus aureus along with ATCC 29213 quality control strain, all the Staphylococcal isolates have shown increased antibiotic resistance. All the isolated strains were resistant (100%) to penicillin, where as cloxacillin, oxacillin and methicillin have shown the resistance of 97.42%, 54.90%, and 50.93% respectively. 72.66%, 84.11%, 73.01% of the total isolates have showed resistance to tetracycline, erythromycin and rifampicin respectively. Where as ciprofloxacin, gentamycin, streptomycin and kanamycin resistance in Staphylococcus aureus was 67.75%, 86.5%, 95.75% and 94.97% respectively. Amoxicillin, cephraxone and clavulanic acid have also showed 86.68%, 85.51%, and 54.83% resistance respectively. In contrast 14 isolates (1.63%) of Staphylococcus aureus have shown increased resistivity to Vancomycin. The VRSA strain (MIC 32 µg/ml) has been isolated from the CSF of a 14 year old boy admitted in hospital having diagnosed for meningitis, 6 VRSA strains were isolated from pus of post operative wound infection and burn skin. Other 7 VISA (MIC 8µg/ml) strains were isolated from amputated wound and diabetic foot lesions. Primer for vanA (van A F'CA TGAA TAGAATAAAAGTTGCAA TA' 3') and (vanA R 5' CCCmAACGCTAATACGACGATCAA 3'). The PCR products were electrophorened, and the bands for van A gene was detected in only 4 strains out of 14 strains of Staphylococcus aureus. The remaining strains shown reduced susceptibility to vancomycin but van A gene was not detected, may be, the proposed mechanism resulting in reduced susceptibility to vancomycin is believed that thickening of the bacterial cell wall such that the vancomycin is trapped within the cell wall and is thus unable to reach its target site on the surface of the bacterial cytoplasmic membrane. The Electron Microscopic analysis to demonstrate the thickened cell wall is yet to be carried out.

It is believed that this is the first report from this part of the country. The emergence of Staphylococcus aureus with increased resistance to glycopeptides like vancomycin emphasizes the importance of the prudent use of antibiotics, the laboratory capacity to identify resistant strains, and the use of infection-control precautions to prevent transmission.

Keywords: Staphylococcus aureus, vancomycin, van A gene.

Introduction

Staphylococcus aureus is a major cause of hospital acquired infections, causing high morbidity and mortality throughout the world. Vancomycin has been the drug of choice since 30 years for the treatment of methicillin-resistant Staphylococcus aureus (MRSA). Over the last decade, methicillin resistant Staphylococcus aureus (MRSA) strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions. The emergence of high levels of penicillin resistance followed by development and spread of strains resistant to the semi synthetic penicillins (methicillin, nafcillin and oxacillin), macrolides, tetracyclins, and amino glycosides has made therapy of Staphylococcal infections in a number of regions. This had lead to increased reliance on vancomycin for treatment of documented MRSA infections. As a consequence, selective pressure was established that eventually lead to the emergence of strains of Staphylococcus aureus and other species of Staphylococci with decreased susceptibility to vancomycin and other glycopeptides. The first report of the Staphylococcus aureus with reduced susceptibility to vancomycin was from Japan1. This report was quickly followed by similar ones from other countries, including United States2, Belgium3, and India4. The extensive longitudinal study of current situation of vancomycin resistance and have reported the first incidence of VRSA emergence was reported from Northern part of India5. The first clinical infection with vancomycin resistant Staphylococcus aureus was reported from Michigan with second case in Pennsylvania. Further, the second confirmed VRSA from Pennsylvania was reported which represents the VRSA isolate from patient in United States. Emergence of decreased Vancomycin susceptibility in MRSA strain presents a significant clinical problem with few therapeutic options. The rapid evolution of antibiotic resistance is of considerable concern. Considering high prevalence of MRSA and increased use of vancomycin, the development of vancomycin resistance (VISA) in clinical strains seems likely to occur. In 1996, the documented infection caused by Staphylococcus aureus with reduced susceptibility to vancomycin (vancomycin-intermediate S. aureus (VISA)) was reported in Japan6. Thereafter about 20 cases7 of VISA infections have been reported in several countries, including Korea8. In addition to VISA and VRSA, another type of vancomycin resistance called hetero-VISA (hVISA), has been described9. This strain is susceptible to vancomycin but contains a sub population, at a frequency of 10^6 or higher with MIC of
vancomycin of more than 4 µg/ml. The potential importance of hVISA is that it may be associated with treatment failure8,10,11 and a precursor of VISA12,13. Although a number of studies have been undertaken to determine the prevalence of hVISA, reported frequencies have ranged from 0.209%14,15,9,16,17,18,19,20,21,22, depending on the definitions and methods employed for screening and confirmation. Four isolates of VRSA have been reported in the USA, with isolates found in June 2002 and February 2005 in Michigan23,24,25,26 in September 2002 in Pennsylvania26,17,28,29 and in March 2004 in New York. Common features of these four patients were a history of chronic underlying diseases (diabetes, morbid obesity, residence in chronic care facility, peripheral vascular insufficiency with skin ulceration) and isolation of VRSA from skin ulcers or urine. These isolates have been highly resistant to vancomycin (MICs ≥ 256 µg/ml), although resistance requires induction in some isolates and may be missed by some automated susceptibility test systems. The first two isolates have been shown to have plasmid-mediated vancomycin resistance due to vanA, which was probably acquired from VRE present in the lame lesion in the first Michigan case26,27. VRSA strains are resistant to glycopeptides, but are susceptible to lipopeptides such as daptomycin30. The first Michigan VRSA was highly resistant to vancomycin, with MICs of 1024 µg/ml by broth microdilution and >256 µg/ml by E-test. The main objective of the present study was to report the isolation of 14 VRSA strains including multi drug resistant, VISA and VRSA carrying vanA gene from community health care centers of Hubli-Dharwad, Karnataka, India.

Material and Methods

Staphylococcal Cultures: The bacterial strains were collected from various clinical specimens like urine, infected blood, pus, wound swabs, catheters and cerebrospinal fluid (CSF) from different inpatients of Karnataka Institute of Medical sciences (KIMS), Civil Hospital, some local hospitals and Nursing Homes of Hubli-Dharwad. The clinical samples were collected in sterile screw cap bottles containing sterile peptone water as a transport media and immediately transported to the laboratory. A total of 856 Staphylococcus aureus were collected and investigated for the period of January 2006 to October 2008.

Media and Culture Conditions: All the samples were first inoculated onto blood agar and brain heart infusion agar plates (Hi-Media). The plates were incubated at 37°C for 24 hrs. The identification of isolates was done according to the standard methods like colony morphology and biochemical characteristics.

Catalase Test: The catalase test was done by transferring a small portion of the culture with a clean glass rod onto a slide with 3% (v/v) hydrogen peroxide (H2O2) which is kept under cover of a Petri plate to avoid aerosols. If the bacteria produce, they will split hydrogen peroxide and oxygen will be evolved. The evolution of gas causes bubbles to form and is indicative of a positive test.

Oxidation Fermentation Test: Bacteria were inoculated into two oxidation fermentation tubes, one tube sterile liquid paraffin was added to provide anaerobic condition, and the tubes were incubated at 37°C. The tubes were observed for change indicating oxidative and fermentative breakdown of the sugars and the observations were recorded.

Mannitol Fermentation: To perform this test Mannitol salt agar (Hi-Media) was used which contains D-Mannitol and phenol red as pH-indicator. The MSA plates were inoculated with test organism and incubated at 37°C for 24hrs. The colony characters and colour change was recorded.

Coagulase Test: A single colony of overnight cultures on Trypticase soya agar (TSA) was used for a coagulase test following the conventional protocol except that the pre-incubation times in brain heart infusion (BHI) prior to the enzyme assay was 24 h. The BHI broth was used for the coagulase. The formation of a clot was examined at 2, 4, 6, and 24 h.

Antibacterial Susceptibility Testing: Antibiotic susceptibility screening was done as per the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). Kirby- Bauer's disc diffusion technique was adapted for antibiogram31. The antibiotic discs and Mueller-Hinton Agar purchased from Hi-Media, Mumbai. The plates were prepared as per the manufacturer's instructions and checked for sterility by incubating the plates overnight at 37°C. The antibiotics used in this study shown in Table-1. The antibiotics discs were kept at room temperature for 1 hour. The agar plates were incubated at 37°C for 24hrs. The colonies and colour change was recorded.

Determination of Minimum Inhibitory Concentration (MIC): MIC of oxacillin and Vancomycin (Hi-Media, India) were determined by agar dilution method32. Brieﬂy, gradient plates of Mueller-Hinton Agar (Hi-Media, India) were prepared with oxacillin (0.25–256µg/ml) (with 2% NaCl), Vancomycin (0.5–128µg/ml) and teicoplanin (0.5–128µg/ml). By direct colony suspension method 0.5 McFarland equivalent inoculum were prepared in normal saline from 18–24 h agar plate culture. The suspension was further diluted to achieve desired inoculum concentration of 10 CFU/ml. All strains were spotted onto gradient plates. Plates were incubated overnight at 35°C for any visible growth. Readings were taken according to NCCLS guidelines. Staphylococcus aureus ATCC 29213 strain was used as control.

Detection of vanA gene by PCR: Staphylococcal DNA was isolated as described above33. Oligonucleotide primers for vanA (vanA F 5’CATGATAGAATTAAAAGTTGCAATA 3’and vanA R 5’CCCGCTTACGGATTACGATCAAA 3’) gene and reaction condition previously reported were used34. A Bio-rad DNA thermocycler was programmed with the initial denaturation, 10 min at 94°C; 30 cycles with a 30s denaturation step at 94°C, a 45s annealing step at 50°C and a 30s extension step at 72°C and 10 min extension step at 72°C and a holding step at 4°C until the sample was analyzed. The PCR products were
Results and Discussion

All the strains of *Staphylococcus aureus* have been identified and confirmed using conventional methods. The results for disc diffusion test using 14 antibiotics for 856 isolates of *Staphylococcus* are shown in Table No.1. Disc diffusion test revealed that all the isolated strains were resistant (100%) to penicillin, where as cloxacillin, oxacillin and methicillin have shown the resistance of 97.42%, 54.90%, and 50.93% respectively. The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semi synthetic penicillins (methicillin oxacillin and nafcillin), macrolides, tetracyclines and aminoglycosides has made the therapy of Staphylococcal disease a global challenge. 72.66%, 84.11%, 73.01% of the total isolates have showed resistance to tetracycline, erythromycin and rifampicin respectively. Where as ciprofloxacin, gentamycin, streptomycin and kanamycin resistance in *Staphylococcus aureus* was 67.73%, 56.5%, 95.79% and 94.97% respectively. Ampicillin, ceftriaxone and clavulanic acid have also showed 86.68%, 85.51%, and 94.85% resistance respectively. In contrast 14 isolates (1.63%) of *Staphylococcus aureus* have shown increased resistivity to Vancomycin (Table 2.). Earlier reports have showed that almost all (> 95%) methicillin-resistant *staphylococci* produce penicillinase in large amounts (60), but lose this property at high frequency in vitro. Penicillinase production also tends to be lost from methicillin sensitive *staphylococci* both in vitro and in vivo, the extensive use of some penicillins (e.g., ampicillin) probably accounts for the large number of methicillin-sensitive *staphylococci* that produce penicillinase.

The reports reveal that the glycopeptide antibiotic vancomycin was introduced clinically in 1958 for the treatment of gram-positive bacteria. Use of this agent has increased dramatically in the last 20 years, in large part because of the increasing prevalence of methicillin resistance in both coagulase-negative *staphylococci* and *Staphylococcus aureus* [21]. Data from the December 2000 report of the National Nosocomial Infection Surveillance (NNIS) System indicated that about 75% of coagulase-negative *staphylococci* and 47% of S. aureus isolates from intensive care units were resistant to methicillin (www.cdc.gov/ncidod/hsp/NNIS/DEC2000sar.FDF). Vancomycin remains the drug of choice for these infections. Vancomycin resistance among *staphylococci* was reported in laboratories even before the drug was in use clinically. However, this resistance was so difficult to induce that many felt it would be unlikely to occur in a clinical setting. That no vancomycin-resistant *staphylococci* were reported in the first 20 years the drug was used only strengthened this assumption. Unfortunately, this confidence was shattered by the first reports of vancomycin resistance in coagulase-negative *staphylococci* in 1979 and 1983. In present study the VRSA strain (MIC 32 µg/ml) has been isolated from the CSF of a 14 year old boy admitted in hospital having diagnosed for meningitis, 6 VRSA strains were isolated from pus of post operative wound infection and burnt skin. Other 7 VISA (MIC 8µg/ml) strains were isolated from amputated wound and diabetic foot lesions. Since the first report of VRSA being reported earlier, the threat of vancomycin resistance in *Staphylococcus aureus* has been the topic of intensive research and discussion. Though the vancomycin resistance in *Staphylococcus aureus* remains extremely rare, there is widespread concern that VRSA poses the greatest risk to patients.

Similar results have been reported that out of 783 *S. aureus* two *S. aureus* strains were found to be vancomycin and teicoplanin resistant (one strain with MIC 32 µg/ml and the other strain with MIC 64 µg/ml); six strains of *S.aureus* have shown to be vancomycin intermediate (two strains with MIC 16 µg/ml and four strains with MIC 8 µg/ml); and two strains with teicoplanin intermediate (MIC 16 µg/ml). One CoNS strain was resistant to vancomycin and teicoplanin (MIC 32 µg/ml), and two CoNS strains were intermediate to vancomycin and teicoplanin (MIC 16 µg/ml). All VRSA, VISA and vancomycin resistant CoNS had shown growth on BHI vancomycin screen agar (vancomycin 6 µg/ml) and were mecA PCR positive. None of these isolates have demonstrated vanA/vanB gene by PCR. Another report reveals on the isolation of four vancomycin-resistant *staphylococcal* strains from healthy carriers inside and outside the hospital environment. These carriers did not receive treatment with any antibiotic. All coagulase-negative *staphylococcal* strains showed variable levels of resistance to several antimicrobial agents, including oxacillin, and unstable resistance to vancomycin, with decreased vancomycin MICs (<4 µg/liter) after 10 days of passage in a nonselective medium. However, exposure of these revertants to vancomycin selected *staphylococcal* strains resistant to vancomycin at very high frequencies. The vancomycin resistance in these *staphylococcal* strains was not mediated by the van gene. The cell wall of the *staphylococcal* strains studied became thickest after culture in medium containing vancomycin. In present study the strains with primers for the van A gene revealed that 4 VRSA in this study were vancomycin-resistant because of the presence of the vanA gene, and no others could be detected in remaining 3 VRSA isolates. The attempt has not been made to detect vanB and vanC genes. Although the precise genetic mechanism for vancomycin resistance in these *Staphylococcus aureus* strains awaits elucidations. The remaining strains shown reduced susceptibility to vancomycin but van A gene is not detected, may be, the proposed mechanism, resulting in reduced susceptibility to vancomycin is believed that thickening of the bacterial cell wall such that vancomycin is trapped within the cell wall and is thus unable to reach its target site on the surface of the bacterial cytoplasmic membrane. Hence, the detection of van B, van C and thickening of the cell wall is yet to be carried out.
Conclusion

The present study demonstrates for the first time emergence of VRSA from this part of the country and indicates the prevalence of the antibiotic resistance. Vancomycin resistance in Staphylococcal species is beginning to emerge as a clinical threat, yet the attention it has received and serves to underscore the seriousness of the problem. A better understanding of these issues will be a key to helping the prevention and treatment of these infections in the future. The heightened awareness of the issues and strict adherence to current guidelines for vancomycin use and infection control practices may help limit the impact these organisms.

Acknowledgement

The authors are grateful to the Post Graduate Department of Studies in Microbiology and Biotechnology, Karnatak University Dharwad for providing the necessary facilities.

Table No. 1: Resistant Pattern of Various Antimicrobial Agents Determined by Disc Diffusion Method

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No. of Resistant Strains (%)</th>
<th>No. of Intermediate Strains (%)</th>
<th>No. of Sensitive Strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>97.42</td>
<td>2.58</td>
<td>-</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>54.90</td>
<td>30.1</td>
<td>15</td>
</tr>
<tr>
<td>Methicillin</td>
<td>50.93</td>
<td>36</td>
<td>13.07</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>72.66</td>
<td>20.34</td>
<td>7</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>84.11</td>
<td>14</td>
<td>1.99</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>73.01</td>
<td>26.99</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>67.75</td>
<td>20</td>
<td>12.25</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>86.5</td>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>95.75</td>
<td>4.25</td>
<td>-</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>94.97</td>
<td>5.03</td>
<td>-</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>86.68</td>
<td>10</td>
<td>3.32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>85.51</td>
<td>10.49</td>
<td>4</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>94.85</td>
<td>5.15</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Detailed Description of Vancomycin Resistant Staphylococcal Strains.

<table>
<thead>
<tr>
<th>Strains</th>
<th>No. of isolates</th>
<th>MIC µg/ml</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus *</td>
<td>6</td>
<td>32 µg/ml</td>
<td>Pus</td>
</tr>
<tr>
<td>Staphylococcus aureus *</td>
<td>1</td>
<td>74 µg/ml</td>
<td>CSF</td>
</tr>
<tr>
<td>Staphylococcus aureus **</td>
<td>3</td>
<td>8 µg/ml</td>
<td>Pus</td>
</tr>
<tr>
<td>Staphylococcus aureus **</td>
<td>4</td>
<td>8 µg/ml</td>
<td>Diabetic foot lesion</td>
</tr>
</tbody>
</table>

References

5. Hare Krishna Tiwari and Malay Ranjan Sen Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infectious Diseases, 6, 156 (2006)


48. Hare Krishna Tiwari and Malay Ranjan Sen. Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infectious Diseases, 6, 156 (2006)

* * *
To

Mr. C G Unakal
And Prof. B.D. Kaliwai
Dept. of Studies in Biotechnology and Microbiology
Karnatak University
Dharwad.

Sub: Acceptance of paper for publication.

Sir,

With reference to the above subject, your paper entitled
"PHENOTYPIC CHARACTERIZATION AND RISK FACTORS OF
NOSOCOMIAL Staphylococcus aureus FROM HEALTH CARE
CENTRES" has been accepted for publication in the referred proceedings of
the conference "Role of Life Sciences in Global Warming".

Thanking you,

Yours faithfully,

[Signature]
Organizing Secretary and Co-Ordinator
National Symposium on
Role of Life Sciences in Climate Change & Global Warming,
K.R. Road, Bangalore - 560 004.
ABSTRACT

Multidrug resistant *Staphylococcus aureus* (MDRS) is a serious threat to hospitalized patients globally and now represents a challenge for public health, as community-acquired infections appear to be on the increase in both adults and children. S. aureus colonization has been shown to be a risk factor for community-acquired and nosocomial infections. A total of 130 subjects from the community and 100 subjects from health care-related facilities were evaluated for the prevalence of *Staphylococcus aureus* colonization and to identify risk factors associated with methicillin-resistant *S. aureus* (MRSA) and Vancomycin resistant *S. aureus* (VRSA) colonization. Among the community subjects, 35.38% had MRSA and 1.53% VRSA colonization. Subjects from health care-related facilities had a lower MRSA colonization rate (17%) than community subjects and the colonization VRSA has not been found. Age was a risk factor for S. aureus colonization, with subjects under age 20 years or between 60 and 80 years showing higher rates of colonization. In conclusion, a high prevalence of MRSA colonization was observed among people with relationship to the hospital setting. The high level of multiple-drug resistance among community MRSA strains in association with the previously reported excessive use of antibiotics highlights the importance of the problem of antibiotic selective pressure. Our results indicate that the spread of both MRSA and VRSA and the transmission of hospital isolates contribute to the high MRSA/VRSA burden in the community.

**Key words:** *Staphylococcus aureus*, Nosocomial, MRSA, VRSA
INTRODUCTION

In the past few decades, methicillin-resistant Staphylococcus aureus (MRSA) has been recognized as an important nosocomial pathogen worldwide.\textsuperscript{1,2} The emergence and rapid spread of this organism has created important new challenges for infection prevention and control services in hospitals and other health care facilities. Interestingly, there appears to be significant variability in the epidemiology and prevalence of MRSA in different parts of the world and even in different regions of a country.\textsuperscript{3}

Patients in the intensive care units (ICU) are at a higher risk of acquiring nosocomial infections compared with patients in general wards.\textsuperscript{4,5} This is partly because of the severity of the underlying illnesses and partly because of iatrogenic factors related to the high frequency of invasive procedures required for monitoring and treatment.\textsuperscript{6} Bacteremia continues to be a major cause of morbidity and mortality in hospitals.\textsuperscript{7} The overall or crude rate of death does not distinguish between the contribution of the patients' underlying diseases and the contribution of bloodstream infections.\textsuperscript{8} The prognosis of postbacteraemia infection (true bacteremia or fungaemia) is very variable. In recent years, many studies have analyzed the mortality rates in relation to pathogens, source of infection, patient age, and underlying diseases.

In the preantibiotic era S. aureus bacteremia resulted in 80% mortality\textsuperscript{9} however, with the advent of antibiotics, the organism was reported to be susceptible to the earliest antimicrobial agents, the sulfonamides and penicillin. The widespread use of these antibiotics in the 1950s induced the predominance of b-lactamase-producing resistant strains. To solve the problem, the b-lactamase-resistant penicillins were developed, but reports of resistance to this new group started to appear in the 1960s in Europe\textsuperscript{10} and in the 1970s in the United States.\textsuperscript{11} The emergence of antibiotic-resistant strains of S. aureus is now considered to be a major problem in most hospitals. Virtually, all nosocomial strains produce a b-lactamase and thus are resistant to penicillins. Moreover, data from the Centers for Disease Control and Prevention indicate that throughout the United States there has been an increase in the frequency of methicillin-resistant S. aureus (MRSA) strains resistant to multiple antibiotics in both large and small hospitals.\textsuperscript{12} Thus far, all strains of MRSA have been susceptible to vancomycin, although certain strains have exhibited tolerance. It is possible, however, that vancomycin resistant grampositive cocci such as Enterococcus spp.
and *Staphylococcus haemolyticus* may transmit the gene(s) responsible for this phenotype to *S. aureus*, leaving few if any options for antimicrobial chemotherapy of infections caused by the organism.

Recent studies suggest that the infection due to MRSA is not only hospital-acquired but community acquired as well. MRSA now represent a global problem. Some large outbreaks have been reported from different parts of the world, where it had caused severe infections including septicemia, endocarditis and meningitis. A study by Dickinson in England and Wales has concluded an increase in the trend of death due to MRSA infection. Infections caused by MRSA can be expensive in terms of antibiotic therapy, isolation facilities and materials and length of hospital stay. According to a World Health Organization literature, the global financial burden because of MRSA infection has been worked out to be $20,000 to $114,000 for outbreaks and from $28,000 to $1600,000 for endemic infections per year. The common sources of these infections are human patients and carriers. The risk factors that contribute to MRSA are antibiotics abuse, prolonged hospitalization, intravascular instrumentation and hospitalization in an intensive care unit. There is considerable variation in numbers of clinical infections among units, hospitals and countries.

The analyses of the data showed a higher prevalence of *S. aureus* in nursing staff and attendants compared to the doctors. Age, sex, health status could not be correlated with the rate of infection, however, it could be due to the nature of job and place of work of the individuals. The prevalence of *S. aureus* was found higher in surgical wards than the general wards. Our study shows that the risk of infection is higher in individuals occupationally exposed to such microbes. MRSA has been reported earlier from hospitals in various parts of the world. There is a need to screen individuals in hospitals for risk exposures and infections, to avoid outbreak and cross infections.

Other factors including prolonged hospitalization, multiple antibiotic therapy sessions, and intravenous catheterization also increase the risk of nosocomial infections in burn patients. MRSA is an important causative agent of nosocomial infections in India. According to an Indian study, the prevalence of infections caused by MRSA has increased from 12 percent in 1992 to 80.03 percent in 1999. Many of these MRSA isolates are becoming multidrug-resistant, and are susceptible only to glycopeptides.
In the 1980s, due to the widespread occurrence of MRSA, empiric therapy for staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health-care institutions. Vancomycin use in many countries also increased during this period because of the growing numbers of infections with Clostridium difficile and coagulase-negative staphylococci in health-care facilities. Thus, the early 1990s saw a discernible increase in vancomycin use. As a consequence, selective pressure was established that eventually led to the emergence of strains of Staphylococcus aureus and other species of staphylococci with decreased susceptibility to vancomycin, but in 1997 the first clinical isolate of Staphylococcus aureus with reduced susceptibility to vancomycin was reported from Japan. Data from the December 2000 report of the National Nosocomial Infection Surveillance (NNIS) System indicated that about 75% of coagulase-negative staphylococci and 47% of S. aureus isolates from intensive care units were resistant to methicillin (www.cdc.gov/ncidod/hip/NNIS/DEC2000sar.PDF). Vancomycin remains the drug of choice for these infections. Vancomycin resistance among staphylococci was developed in laboratories even before the drug was in use clinically. However, this resistance was so difficult to induce that many felt it would be unlikely to occur in a clinical setting. That no vancomycin-resistant staphylococci were reported in the first 20 years the drug was used only strengthened this assumption. Unfortunately, this confidence was shattered by the first reports of vancomycin resistance in coagulase-negative staphylococci in 1979 and 1983. In our previous study it has been reported that the VRSA strain (MIC 32 µg/ml) has been isolated from the CSF of a 14 year old boy admitted in hospital having diagnosed for meningitis, 6 VRSA strains were isolated from pus of post operative wound infection and burnt skin. Other 7 VISA (MIC 8µg/ml) strains were isolated from amputated wound and diabetic foot lesions. The present study was carried out to determine the risk factors for nosocomial acquisition of MRSA/VRSA, phenotypic characterization of the Staphylococcus aureus and mortality in health care centers.

MATERIALS AND METHODS

Study duration and population.

This study was carried out at the Department of Microbiology and Biotechnology, Karnataka University, Dharwad from April 2007 to November 2008. The samples were obtained from the persons served as subjects of various Nursing Homes, Hospitals and other Health Care Centers of Hubli-Dharwad. Nasal swabs from both anterior nares were
obtained from healthy subjects working in health care centers of Hubli-Dharwad. Samples were also taken from healthy volunteers including adults of both sexes residing the hospital premises. Any person with a history of Hospitalization, undergoing surgery or any kind of treatment and intake of antibiotics in the past three months was ruled out of the study.

**Microbiological study.**

All study participants underwent swabbing of the anterior 1.5 cm of the nasal vestibule of both nares with a sterile swab. The swab specimen was streaked onto two mannitol salt agar plates (Hi-Media, Mumbai) one of which was supplemented with oxacillin (6µg/ml). These inoculated plates were incubated at 37°C for 48 h, after which morphological and Gram stain examinations were conducted. Colonies of interest were selected for further inoculation onto Brain Heart Infusion agar plates (Hi-Media, Mumbai.) at 37°C overnight. The coagulase test was used to identify S. aureus. Methicillin-susceptible S. aureus (MSSA) was preliminarily detected by its characteristic growth on mannitol salt agar and the absence of growth in the presence of oxacillin, while growth on both agar plates was presumed to indicate the presence of MRSA. All isolates were inoculated onto Mueller-Hinton agar (Hi-Media, Mumbai) containing 6µg of oxacillin per ml and 4% NaCl to confirm methicillin resistance.

**Catalase Test.**

The catalase test was done by transferring a small portion of the culture with a clean glass rod onto a slide with 3% (v/v) hydrogen peroxide (H₂O₂) which is kept under cover of a Petri plate to avoid aerosols. If the bacteria produce, they will split hydrogen peroxide and oxygen will be evolved. The evolution of gas causes bubbles to form and is indicative of a positive test.

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Bacteria were inoculated into two oxidation fermentation tubes, in one tube sterile liquid paraffin was added to provide anaerobic condition, and the tubes were incubated at 37°C. The tubes were observed for colour change indicating oxidative and fermentative breakdown of the sugars and the observations recorded

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A single colony of overnight cultures on Trypticase soya agar (TSA) was used for a coagulase test following the conventional protocol except that the pre-incubation times in brain heart infusion (BHI) prior to the enzyme assay was 24 h. The BHI broth was used for the coagulase. The formation of a clot was examined at 2, 4, 6, and 24 h.

Antibacterial Susceptibility Testing

Antibiotic susceptibility screening was done as per the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). Kirby- Bauer’s disc diffusion technique was adapted for antibiogram. The antibiotic discs and Mueller- Hinton Agar purchased from Hi-Media, Mumbai. The plates were prepared as per the manufacturer’s instructions and checked for sterility by incubating the plates overnight at 37°C. The antibiotics used in this study were obtained from Hi-Media, Mumbai. The antibiotics discs were kept at room temperature for 1 hour. The agar plates were overlaid with inoculums of Staphylococcus aureus showing the turbidity equivalent to that of a 0.5 McFarland standard.

RESULTS

A total of 230 samples were collected from various health care centers including males and females of the age 18 to 80 years. Of the total the samples were collected from 149 male and 81 female individuals (Table No.1). Locations for the sample collection were both the anterior nares, forearm and dorsum of palm. A total of 200 (87%) Staphylococcus isolates were obtained (142 males, 58 females) (Table No. 2) and 130 (57%) Staphylococcus aureus were confirmed based on staining character, growth on mannitol salt agar, coagulase test and other biochemical parameters (Table No. 3). The results for disc diffusion test using 14 antibiotics for 130 isolates of Staphylococcus aureus are shown in Table No.4. Disc diffusion test revealed that all the isolated strains were resistant (100%) to penicillin, where as cloxacillin, oxacillin and methicillin were shown the resistance 97.42%, 35.90%, and 35.38% respectively. 72.66%, 84.11%, 73.01% of the total isolates have showed resistance.
to tetracycline, erythromycin and rifampicin respectively. Where as ciprofloxacin, gentamycin, streptomycin and kannamycin resistance in *Staphylococcus aureus* was 67.75%, 86.5%, 95.75% and 94.97% respectively. Ampicillin, ceftriaxone and vancomycin have also showed 86.68%, 85.51%, and 1.53% resistance respectively. Many of the MRSA strains were resistant to all the tested antibiotics.

**DISCUSSION**

*S. aureus*, antibiotic-resistant Gram-negative bacteria, and *Candida* spp. are among the pathogens responsible for bloodstream infections, which are usually associated with the poorest outcomes\(^3\), \(^4\). In recent years, the *Staphylococcus aureus* causing nosocomial infections has increased because of their ability to express certain resistance phenotypes\(^5\) and hence they continue to be one of the major sources of morbidity and mortality\(^6\), \(^7\). While in these studies the most frequently isolated pathogens that caused NB infections were *Klebsiella* spp., *Candida* spp., *Enterococcus* spp. and *P. aeruginosa*, in our study we concentrated on, methicillin-resistant *S. aureus*, as a source of secondary bacteraemia, has been reported to be more frequent among patients \(^3\). However, primary bloodstream infection, in which no source could be determined, was also reported to be the most frequent source of NB\(^9\). Broad-spectrum antibiotic use was found to be the most important risk factor associated with the occurrence of this undesirable medical practice. Nosocomial infection, nosocomial pneumonia, older age, mechanical ventilation, enteral nutrition, tracheostomy and use of steroids or chemotherapy were found to be the most important risk factors for mortality in ICU \(^4\), \(^4\), \(^4\) similar to our study and those of others\(^4\), \(^4\), \(^4\), \(^4\). Although, the clinical significance of Methicillin-resistance has been questioned in the past there is now a widespread acknowledgement of the pathogenicity of MRSA. It has emerged as a significant cause of both nosocomial and community acquired infections. Furthermore, during the past decade there has been a steady increase in the incidence of infections caused by this bacterium\(^4\).

Many investigators have reported an increase in the incidence of MRSA during recent years, most of which originated from wounds (pus)\(^4\), \(^4\). We also found a high rate of MRSA isolates i.e. 35.38% from the clinical specimens also showed multiple drug resistance. In our study (64.62%) MSSA (Methicillin sensitive *Staphylococcus aureus*) isolates were resistant most of the antibiotics tested. Prevention of MRSA infections merit discussion as once introduced in a hospital MRSA is very difficult to eradicate\(^5\). After
introduction within hospital, MRSA spreads rapidly by hands of medical personnel. Colonized employees of the hospital such as asymptomatic nasal carriers and infected patients acting as reservoirs are important sources in the spread of this organism. Multiple, prolonged use of antibiotics and prolonged hospitalisation are other important factors which make hospitals an ideal place for transmission and perpetuation of MRSA.

Resistant strains are not only a major obstacle in treatment, but once established, infections are also likely to ratchet up the possibility of further transmission. In 1982, an American hospital outbreak reported an incidence of 30% MRSA. Other studies conducted in Japan reported an incidence of about 41.5% by 1992. There are many reports of increasing resistance of S. aureus from our country. In order to reduce the problem of antibiotic resistance, it is mandatory to survey and screen all clinical isolates for resistance. Our efforts should be concentrated not only on antibiotic use, but also on other confounding factors that contribute to resistance such as infection control practices. Contact isolation and strict asepsis should be enforced. This area warrants further studies on a larger number of isolates from various parts of the country in order to develop and apply evidence based guidelines on countering resistance.

CONCLUSION

Therefore, regular surveillance of hospital-associated infections including monitoring of antimicrobial (especially Vancomycin and other newer glycopeptides) susceptibility pattern of MRSA and formulation of a definite antimicrobial policy may be helpful for reducing the incidence of these infections. Infected or colonized patients may be isolated in a single room or isolation unit to prevent the spread of MRSA. Knowledge about MRSA and carrier status needs to be raised among the health staff of the hospital and control measures need to be implemented consistently in order to reduce the burden of MRSA infection in the hospital environment. A further study of MRSA may be done for the epidemiological mapping of these infections.

Acknowledgements:
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RESULTS

Table No. 1: Number of individuals screened for Staphylococcus isolates.

<table>
<thead>
<tr>
<th>Location</th>
<th>Population Group</th>
<th>Age Group</th>
<th>Total No. of Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursing Home</td>
<td>Nurses, Staff</td>
<td>18-30 Yrs</td>
<td>54 26 80</td>
</tr>
<tr>
<td>Hospital</td>
<td>Nurses, Staff</td>
<td>18-50 Yrs</td>
<td>32 28 60</td>
</tr>
<tr>
<td>Private Clinic</td>
<td>Staff</td>
<td>18-60 Yrs</td>
<td>25 15 40</td>
</tr>
<tr>
<td>Medical College</td>
<td>Duty Doctors Staff</td>
<td>20-80 Yrs</td>
<td>38 12 50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>149 81 230</strong></td>
</tr>
</tbody>
</table>

Table No. 2: Location wise Distribution of Staphylococcus isolates

<table>
<thead>
<tr>
<th>Location</th>
<th>Isolate</th>
<th>Anterior Nares</th>
<th>Forearm</th>
<th>Dorsum of palm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursing Home</td>
<td>Staphylococci</td>
<td>14 04</td>
<td>20 08</td>
<td>10 04</td>
<td>44</td>
</tr>
<tr>
<td>Hospital</td>
<td>Staphylococci</td>
<td>16 08</td>
<td>10 10</td>
<td>06 -</td>
<td>32</td>
</tr>
<tr>
<td>Private Clinic</td>
<td>Staphylococci</td>
<td>11 02</td>
<td>06 02</td>
<td>03 01</td>
<td>20</td>
</tr>
<tr>
<td>Medical College</td>
<td>Staphylococci</td>
<td>10 09</td>
<td>22 08</td>
<td>14 02</td>
<td>46</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>142 58 200</td>
</tr>
</tbody>
</table>

Table No. 3: Proportion of Staphylococcus aureus among the total staphylococci isolated from different location

<table>
<thead>
<tr>
<th>Location</th>
<th>Isolate</th>
<th>Anterior Nares</th>
<th>Forearm</th>
<th>Dorsum of palm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursing Home</td>
<td>Staphylococcus aureus (%)</td>
<td>40 6.7</td>
<td>33.3 10</td>
<td>6.7 3.3</td>
<td>80 20</td>
</tr>
<tr>
<td>Hospital</td>
<td>Staphylococcus aureus (%)</td>
<td>25 20</td>
<td>20 25</td>
<td>10 -</td>
<td>55 45</td>
</tr>
<tr>
<td>Private Clinic</td>
<td>Staphylococcus aureus (%)</td>
<td>30 10</td>
<td>30 10</td>
<td>15 5</td>
<td>75 25</td>
</tr>
<tr>
<td>Medical College</td>
<td>Staphylococcus aureus (%)</td>
<td>25 10</td>
<td>25 7.5</td>
<td>27.5 5</td>
<td>77.5 22.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>70.77 29.23</strong></td>
</tr>
</tbody>
</table>
Table No. 5: Resistant pattern of various antimicrobial agents determined by disc diffusion method

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No. of Resistant strains (%)</th>
<th>No. of Intermediate strains (%)</th>
<th>No. of Sensitive strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>97.42</td>
<td>2.58</td>
<td>-</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>54.90</td>
<td>30.1</td>
<td>15</td>
</tr>
<tr>
<td>Methicillin</td>
<td>35.38</td>
<td>-</td>
<td>64.62</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>72.66</td>
<td>20.34</td>
<td>7</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>84.11</td>
<td>14</td>
<td>1.99</td>
</tr>
<tr>
<td>Refampicin</td>
<td>73.01</td>
<td>26.99</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>67.75</td>
<td>20</td>
<td>12.25</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>86.5</td>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>95.75</td>
<td>4.25</td>
<td>-</td>
</tr>
<tr>
<td>Kannamycin</td>
<td>94.97</td>
<td>5.03</td>
<td>-</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>86.68</td>
<td>10</td>
<td>3.32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>85.51</td>
<td>10.49</td>
<td>4</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>94.85</td>
<td>5.15</td>
<td>-</td>
</tr>
</tbody>
</table>

Table No. 5: Prevalence of MRSA/MSSA and VRSA/VISA from Staphylococcus aureus isolates

<table>
<thead>
<tr>
<th>S. aureus Isolated (n=130)</th>
<th>No. of isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>46</td>
<td>35.38</td>
</tr>
<tr>
<td>MSSA</td>
<td>84</td>
<td>64.62</td>
</tr>
<tr>
<td>VRSA</td>
<td>02</td>
<td>1.53</td>
</tr>
<tr>
<td>VISA</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

MRSA- Methicillin Resistant S. aureus, MSSA- Methicillin Susceptible S. aureus
VRSA- Vancomycin Resistant S. aureus, VISA- Vancomycin Intermediate susceptible S. aureus
REFERENCES


Dis, 1983; 15, 347–360

31. C. G. Unakal and B. B. Kaliwal. Vancomycin-Resistant *Staphylococcus aureus* containing van *A* Gene isolated from Clinical Samples of Community Health Care Centers of North Karnataka, India


