Occurrence and Antimicrobial Susceptibility Pattern of Community and Hospital associated Methicillin resistant Staphylococcus aureus strains in Sikkim.

O. Kunsang Bhutia, T.S.K. Singh
Department of Microbiology, Sikkim Manipal Institute of Medical Sciences, East Sikkim, India

Abstract: The objective of this study was to determine the prevalence and antimicrobial susceptibility pattern of the community-associated (CA) and hospital-associated (HA) methicillin resistant Staphylococcus aureus (MRSA) strains in Sikkim. A total of 119 clinical strains of S. aureus were studied. Detection of MRSA and antimicrobial susceptibility testing were performed by multiplex PCR and Kirby-Bauer disc diffusion methods respectively. The interpretation of the results was done according to the CLSI guidelines 2008. Reference strains ATCC 29213 (MSSA) and 43300 (MRSA) were used as control strains. Out of 119 S. aureus strains, 46 (38.65%) were found to be mec-A positive (MRSA). Among 46 MRSA isolates, 38 (82.60%) and 8 (17.39%) were categorized as CA- and HA-MRSA respectively. All MRSA were found to be harboring pvl gene. The high and equivalent percentage of CA-and HA-MRSA isolates were found to be resistance to penicillin and co-trimoxazole, whereas resistant pattern to other antibiotics had varied. CA-MRSA strains were also found to be resistance to some of the newer drugs such as rifampicin (2.63%), fusidic acid (2.63%), and linezolid (10.52%), however, none of the HA-MRSA showed resistance to these antibiotics except linezolid (25%). Seven CA-MRSA and four HA-MRSA were found to be multidrug resistant (MDR). The study revealed that the prevalence of CA-MRSA infections is higher than HA-MRSA in Sikkim, which is in contrast to the findings of others elsewhere in India. Moreover, the high prevalence of MDR S. aureus strains in this small state of India with inadequate health facility is of major concern.

INTRODUCTION
Penicillin was the most effective antibiotic to treat S. aureus infections since 1940s, however the first case of penicillin resistant S. aureus strains was reported in 1944. Subsequently, penicillinase-stable penicillins such as methicillin and cephalosporins became available in the late 1950s. Soon after the introduction of these drugs, S. aureus strains resistance to methicillin was first reported in year 1961, termed as Methicillin-resistant S. aureus (MRSA)1. Based on the circumstances of acquiring disease, the organism has been often classified as community-associated MRSA (CA-MRSA) and hospital-associated MRSA (HA-MRSA) and current data suggest these are distinct strains of the bacterial species2. In the recent years, CA-MRSA has been increasingly reported as an important pathogen in India3, and wide spread in the world4. Although both CA- and HA-MRSA are resistant to commonly used staphyloccocal beta-lactam antibiotics, the former is usually susceptible to a wider spectrum of antimicrobial agents such as sulphonamides, trimethoprim, tetracycline and clindamycin. However, HA-MRSA is resistant to these drugs and susceptible only to vancomycin5. The present study was undertaken to determine the prevalence of MRSA in Sikkim, categorizing it depending upon the settings of acquiring infections and to determine the antimicrobial susceptibility patterns of these MRSA isolates. The result of the study will help us to understand the problem of MRSA in Sikkim and to formulate antibiotic policy in the hospitals and measures for the prevention of further spread of MRSA in the hospital as well as in the community.

MATERIALS AND METHODS

Design and Settings of the study
A point prevalence study conducted in 119 S. aureus strains isolated from the various clinical specimens during the period from August 2009 to “10” in teaching hospitals.

Case definition and source of data
Hospital-associated MRSA is defined as one cultured from a clinical specimen obtained ≤ 72 hrs after patient’s hospital admission or whose sources of isolation were associated with risk factors for HA-MRSA infection (e.g. recent hospitalization, recent surgery, residence in a long-term care facility, drug use)6,7 within one year of MRSA isolation date. Community- associated MRSA isolate is defined as one cultured < 72 hours of a patient’s hospital admission, or from patients whose sources of isolation were not associated with risk factors for HA-MRSA infection as mentioned above. MRSA isolates which are resistant to one of three non-beta lactam antibiotics were classified as multidrug-resistant MRSA (MDR-MRSA)8,9. The data of the patients were obtained from the laboratory investigation register and medical record file.

Identification of S. aureus isolates
All the isolates were identified as S. aureus using standard techniques, including slide and tube coagulase, DNase, Phosphatase and ModifiedHugh-Leifson tests. The S. aureus strains were inoculated into semi-solid nutrient agar in the screwed capped vials and stored at -20°C for further molecular analysis.

DETECTION OF MRSA

1. DNA isolation: The test inoculum was prepared by inoculating two to three isolated colonies of S. aureus into 3 to 4 ml of BHI broth (Hi-Media) and incubated overnight at ambient temperature of 35-37°C. The DNA was extracted by using the HiPura TM Bacterial and Yeast Genomic DNA MiniPrep Purification Spin kit (Hi-Media).

2. Multiplex PCR for the detection of mec-A and pvl gene: The primers for the amplification of mec-A (Gen Bank accession no:Y00688) and pvl gene (Gen Bank accession no: -X72700) were MECAP4 (5′- TCCAGATTACAACTTCACCAGG-3′), and MECAP7 (5′- CCACCTTCATCTTGTGAA-3′) as described by Oliveira et al10, and luk-PV-1 (5′- ATCATAGGTAAAGTTCTGTGAATGATCC-3′) and luk-PV-2 (5′- GCATCAAGTGTTATGTGATAGGCAAAACG-3′) as described by McFarr et al11 respectively. PCR was performed by using Qiagen Multiplex PCR kit with slight modification. A 25-µl final reaction volume consisting of 12.5 µl mastermix, 2.5 µl primer mix (0.2µM of each primer) and 3µl of DNA template and 7µl of RNase free water was prepared. DNA samples were subjected to thermocycling conditions with initial inactivation step (95°C,15min) with three step cycling condition of denaturation (94°C,30 sec), annealing (60°C,90sec) and extension (72°C,90sec) for 35 cycles with final extension (72°C,10min) and soak at 4°C. Then 5µl of amplified products were mixed with 2µl of ethidium bromide and loaded on 2% agarose gel (Amresco) along with GeneRuler TM 100bp Plus DNA Ladder (Fermentas) and electrophoresis at 100 volt for 50-60min and visualized under UV transilluminator (Bio-Doc analyzer, Biometra). Reference strains ATCC 29213 (MSSA) and 43300(MRSA) were used as control strains.

Antibiotic susceptibility testing of MRSA isolates
Kirby-Bauer disc diffusion method was performed with following antibiotics discs
(Hi-Media); penicillin-G (10 units), co-trimoxazole (25µg), erythromycin (15µg), ofloxacin (5µg), gentamicin (10µg), linezolid (30µg/ml), rifampacin (5µg), chloramphenicol (30µg), fusidic acid (30µg). Five discs in one and four in another agar plate were tested. The testing conditions and interpretation of the test was done as per CLSI criteria.46

RESULTS

A total of 119 S. aureus isolates tested, 46 (38.65%) were found to be mec-A positive (Fig 1). Thirty-eight (82.60%) met the definition of CA-MRSA, and 8(17.39%) of HA-MRSA and all MRSA isolates were found to be harboring pvl gene (Fig 1). All MRSA isolates were from skin and soft-tissue infections, except one (HA-MRSA) which was isolated from blood culture. The majority of CA (24 or 63.15%) and HA-MRSA (5 or 62.5%) were isolated from male patients in comparison to females. All HA-MRSA strains were isolated from the clinical specimens obtained 72 hours of patient’s hospital admission.

The results of in-vitro susceptibility testing of MRSA are given in (Table 1). The very high percentage of CA-MRSA (92.10%) and all isolates of HA-MRSA were resistance to penicillin-G and co-trimoxazole. The resistant pattern of CA- and HA-MRSA strains to other antibiotics were not so significant; ofloxacin (23.68%), followed by erythromycin (21.05%), gentamicin (13.15%), linezolid (10.52%) and least (2.3%) with rifampacin, fusidic acid and chloramphenicol. HA-MRSA strains showed 37.5% resistance to erythromycin and 25% to gentamicin, ofloxacin and linezolid, and least with chloramphenicol (12.5%). However, none of the HA-MRSA strains was found to be resistant to rifampicin and fusidic acid. Among CA- and HA-MRSA, 18.42% (7/38) and 50% (4/8) were found to be multi-drug resistant (MDR) respectively. MDR-CAMRSA isolates were grouped into three antibiotics: RP-I (resistance to P, Co, Lz, E), RP-II (resistance to P, Co, G, Of) and RP-III (resistance to P, Co, G, Of, E, C). Two (28.57%), four (57.14%) and one (14.28%) isolates were fell under RP-I, II and III respectively. Whereas, all four MDR-HAMRSA isolates had different RPs, RP-I (P,Co,Fc,Lz,R,C), II (P,Co,G,E) III (P,Co,GO,LE,C) and IV (P,Co,OIFL-Z).

Table 1: Antibiotic resistance pattern of CA-MRSA and HAMRSA isolates.

<table>
<thead>
<tr>
<th>Antibiotic (µg/ml)</th>
<th>CA-MRSA(n=38)</th>
<th>HA-MRSA(n=8)</th>
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<tbody>
<tr>
<td>Penicillin (10)</td>
<td>35 (92.10%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Co-trimoxazole(25)</td>
<td>35(92.10%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Erythromycin (15)</td>
<td>8 (21.05%)</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>Ofloxacin (30)</td>
<td>9 (23.68%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Gentamicin (10)</td>
<td>5 (13.15%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Linezolid (30)</td>
<td>4 (10.52%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Chloramphenicol (30)</td>
<td>1 (2.63%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Fusidic acid (30)</td>
<td>1 (2.63%)</td>
<td>0</td>
</tr>
<tr>
<td>Rifampicin (5)</td>
<td>1 (2.63%)</td>
<td>0</td>
</tr>
</tbody>
</table>

DISCUSSION

The prevalence rate of MRSA in Sikkim was found to be 38.65 %, which is comparable to the prevalence rate reported from different parts of India; Tamil Nadu (35 %)4, New Delhi (38.56 %)16, Maharashtra (39.1%)17 except Assam (23.6%)18. Similarly, MRSA prevalence rate of 26.14% and 35% were reported from Nepal19 and China20 respectively. On the contrary, an alarmingly high prevalence of MRSA infections (54.85 %) was reported from Uttar-Pradesh21. MRSA once regarded as almost exclusively a hospital-associated pathogen has been increasingly identified as a cause of community-associated infections in the recent years22,23. Several reports from Asia including India have highlighted the prevalence of MRSA in the community and community-acquired pyodermas24,25.

The present study has revealed that the prevalence of MRSA in the CA-infections (82.60%) was much higher than the HA-infections (17.39%) in Sikkim. The majority of CA-MRSA strains were isolated from patients with skin and soft-tissue infections, which is in agreement with the finding of Fergie et al (2001)26. In contrast high prevalence of HA-MRSA was reported from Maharashtra, India (77%)27, Korea (94.7%28, and USA (85%)29. The low prevalence of MRSA in the community-associated infections in the studies (stated above) might be due to differences in case definitions of CA and HA-MRSA with ours26 and inclusion of patients with bloodstream infections only30, as CA-MRSA is mainly associated with skin and soft-tissue infections31. However, over the years since mid 1990s the prevalence of CA-MRSA has been on the rise32. Antibiogram analysis has been a good epidemiological marker for MRSA. Most contrasting finding in the present study, was very high percentage (92.10% of CA-MRSA isolates were resistant to co-trimoxazole. On the contrary, Benoit et al 33 and Gorwitz et al 34 reported that CA-MRSA strains were susceptible to multiple antimicrobial agents, most importantly co-trimoxazole. On the basis of their findings, co-trimoxazole may be a viable, cost-effective treatment option for many CA-MRSA infections. A study from eastern UP35 also reported high percentage of MRSA isolates were resistant to co-trimoxazole. The increased incidence of co-trimoxazole resistant MRSA in India may be due to the misuse of the drug as it is cheap and easily available drug alternative to penicillin, similarly in Sikkim co-trimoxazole is most frequently used antibiotics for the treatment of Staphylococcal infection, in alternative to penicillin suggest possible abuse of this drug in our region. In our study too the number of HA-MRSA isolates resistance to different antibiotics comparatively higher than CA-MRSA, however, very little insignificant number of isolates of CA-MRSA was also found to be resistance to newer drugs like fusidic acid and rifampicin.

Besides time based criteria, in microbiology MRSA has been often categorized based on susceptibility pattern to various antibiotics36. Based on this definition, CA-MRSA has wider spectrum of susceptibility to antibiotics compare to HA-MRSA. In support of this a study has reported 33% of MRSA isolates as MDR-MRSA, where CA-MRSA isolates were less likely to be resistance to antibiotics than HA-MRSA isolates31. Similarly, Fey et al 38 in their study, reported 87.5% of HA-MRSA was MDR, whereas no MDR was found among the CA-MRSA isolates. Our study in agreement to them, that occurrence of MDR-MRSA strains is more prevalent in HA-MRSA (50%) than CA-MRSA (18.42%), but contrasting at the same time indicating that HA-MRSA strains may be the important reservoirs of MDR strains, but now it is being slowly acquired by CA-MRSA strains. Therefore, our finding proposed that microbiological definition would be unreliable in the near future for the proper categorization of MRSA isolates due to emergence of MDR resistance strains among CA-MRSA as well. Antibiogram pattern of MRSA varies in different geographical areas. Therefore, the choice of antibiotic for the treatment of infections caused by CA-MRSA and HA-MRSA should be guided by the antibiotic susceptibility test of the isolate and or current antibiotic policy whenever possible not based on the type of MRSA infections. The data on the antibiotic susceptible pattern of common bacterial pathogens should be made available to the clinicians.

CONCLUSION

The CA-MRSA has indeed emerged in Sikkim as an important cause of skin and soft tissue infections. The high prevalence of MDR strains among CA-MRSA suggests the significant change in the microbial characteristics and epidemiology of MRSA in the community and hospitals. The possible factors that contribute the increased prevalence of CA-MRSA infections are patients who have aquired the MRSA infections in the hospitals and returned to community without complete cure or asymptomatic carriers and complete treatment and cure of MRSA infected
patients before discharging from the hospitals and proper awareness in use of anti-biotics may significantly reduce the further spread of MRSA in the community and as well as in the hospitals.

FUTURE WORK PROPOSED

Future molecular typing (SCC-mec and MLST) of such discrepant isolates, will be helpful to know the genetric basis of MRSA and also to establish the proposed finding that MRSA circulating in our health-care settings are mainly of community origin. It will be helpful in taking appropriate measures in control and prevention of further spread of MRSA.

REFERENCES


Tolvaptan

Mechanism of action: Tolvaptan is a selective vasopressin V2-receptor antagonist with an affinity for the human V2-receptor 1.8 times that of native vasopressin. Tolvaptan antagonize the effect of vasopressin V2 and cause an increase in urine water excretion that results in an increase in free water clearance (aquareasis), a decrease in urine osmolality, and a resulting increase in serum sodium. Urinary excretion of sodium and potassium, and plasma potassium concentrations are not significantly changed, serum potassium levels should be monitored in situations of serum potassium >5 mEq/L or hyperkalemia. Pharmacodynamics/pharmacokinetics: In healthy subjects receiving a single dose of tolvaptan 60 mg, the onset of the aquareas and sodium-increasing effects occurs within 2 to 4 hours post-dose. The peak effect of about a 6mEq increase in serum sodium and about a 9mL/min increase in the urine excretion rate occurs 4 to 8 hours after the dose; the effects of tolvaptan in the recommended dose range of 15 to 60 mg once daily appear to be limited to aquareasis and the resulting increase in serum sodium concentration. At least 40 % of the dose is absorbed as tolvaptan or metabolites. Peak concentrations of tolvaptan are observed between 2 and 4 hours post –dose. Tolvaptan is eliminated entirely by nonrenal routes and mainly, if not exclusively, metabolized by CYP 3A. Moderate or severe hepatic impairment or congestive heart failure decrease the clearance and increase the volume of distribution of tolvaptan. Indication and Important Limitations; (Tolvaptan) is indicated for the treatment of clinically significant hypervolemic and euvoelastic hyponatremia (serum sodium <125mEq/L or less marked hypernatremia that is symptomatic and has resisted correction with fluid restriction), including patients with heart failure, cirrhosis, and syndrome of Inappropriate Antidiuretic Hormone (SIADH). Drug Interaction: Coomitant use of tolvaptan is contra indicated with strong inhibitors of CYP3A. Such as : Ketoconazole, Clarithromycin, Itaconazole, Telithromycin, Saquinavir, Nelfinavir, Nefazodone, Erythromycin, Fluconazole, Aprepitant, Diltiazem, Verapamil, Rifampin, Phenytoin, Carbamazepine. Co-administration with medications known to raise potassium: Treatment with tolvaptan is associated with an acute reduction of extracellular fluid volume which could result in increased serum potassium. Although specific interaction studies were not performed, in clinical studies when used concomitantly with beta-blockers, angiotensin receptor blockers (ARBs), angiotensin-converting enzyme inhibitors (ACEIs), and potassium – sparing diuretics. Adverse reactions of hyperkalemia were approximately 1% to 2 % higher tolvaptan was administered with ARBs, ACEIs, and potassium sparing diuretics compared with administered with placebo.Electrocardiogram monitoring should begin immediately and continue until ECG parameters are within normal ranges. Dialysis may not be effective in removing tolvaptan because of its high binding affinity for human plasma protein (99%). Close medical supervision and monitoring should continue until the patient recovers.