

(Hi-Media); penicillin-G (10 units), co-trimoxazole (25µg), erythromycin (15µg), ofloxacin (5µg), gentamicin (10µg), linezolid (30µg/ml), rifampicin (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 criteria¹⁴.

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 e⁷² hours of patient's hospital admission.

The results of *in-vitro* susceptibility testing of MRSA are given in (Table1). 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-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 rifampicin, 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 resistance profiles (RPs) according to the resistant pattern to the panel of nine 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,G,Of,E,C) and IV (P,Co,Of,Lz).

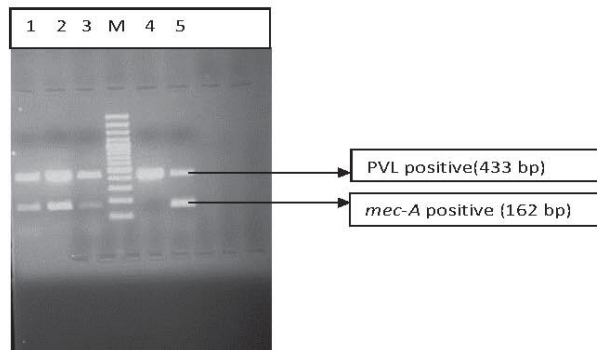


Figure 1: Multiplex PCR (*mec-A* and *pvl* gene). Lane 1,2,3,5 = Positive *mec-A* (162bp) and *PVL* (433bp), M= Marker (100bp DNA ladder), Lane 4 =Negative *mec-A* (162bp) and positive *PVL* (433 bp).

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

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

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 %)¹⁵, 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 years^{21, 22}. Several reports from Asia including India have highlighted the prevalence of MRSA in the community and community-acquired pyoderma^{23, 5}.²⁴ 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)²⁵. In contrast high prevalence of HA-MRSA was reported from Maharashtra, India (77%)²⁶, Korea (94.7%)²⁷, and USA (85%)⁷. 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 only²⁷, as CA-MRSA is mainly associated with skin and soft-tissue infections²⁵. However, over the years since mid 1990s the prevalence of CA-MRSA has been on the rise⁹.

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*²⁸ and Gorwitz *et al*²⁹ 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 UP²¹ 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 antibiotics²⁸. 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 isolates¹¹. Similarly, Fey *et al*³⁰ 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 acquired the MRSA infections in the hospitals and returned to community without complete cure or asymptomatic carriers and complete treatment and cure of MRSA infected