Prevalence of community-acquired methicillin-resistant *Staphylococcus aureus* among tribal population of north-western Himalayas, India

**Abstract**

**Aim:** The objective of the study was to assess the percentage of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and their antibiotic resistant patterns among the native population of north-western Himalaya regions, India. The study was conducted on three tribal communities namely Bakarwal, Gujjar and Gaddi of Jammu and Kashmir (J&K) and Himachal Pradesh (HP) states of India.

**Methodology:** Identification and isolation of CA-MRSA was conducted by culturing it in the Mannitol Salt Agar and incubating at 37°C for 24 hrs. Single yellow colour colonies were selected and subjected to Gram staining as well other biochemical test such as, Coagulase, D-trehalose fermentation, maltose fermentation, DNAse and β haemolysis test were performed. The positive CA-MRSA strains were then subjected to antibiotic susceptibility testing and statistical analysis was done.

**Results:** In total, 1134 nasal swab samples were collected from healthy individuals for isolation of *S. aureus* and antibiogram was carried out for the screening of CA-MRSA; confirmed by oxacillin screen agar. Twenty four, CA-MRSA were isolated i.e., the frequency of nasal carriage CA-MRSA was 2.11% (24/1134). MRSA isolates were susceptible to gentamicin, doxycyclins hydrochloride, respectively.

**Interpretation:** CA-MRSA is highly prevalent in the young age groups of tribal population.
Introduction

South-eastern England sanatoriums in 1960 reported Methicillin Resistant *Staphylococcus aureus* (MRSA), and the first case was reported in the Health-care associated MRSA, in 1961 (Jevons, 1961). MRSA triggers a wide range of infections comprising of skin, soft tissue, endocarditis, bacteremia, infection of respiratory and central nervous system (Liu et al., 2011). In 1980, epidemic outbreak of Community-acquired MRSA from Detroit, Michigan, USA was reported among the intravenous drug users (Saravolatz et al., 1982) whereas, in 1993, the first case of CA-MRSA in non-intravenous drug users from New York, USA was reported (Berman and Kreishwirth, 1993). Both CA-MRSA and HA-MRSA are now distinguished by the molecular techniques. The SCCmec gene belonging to type I, II and III are most commonly found in HA-MRSA strains which provides resistance against non-β-lactam antibiotics of various classes. HA-MRSA strains are reported to occasionally carry the genes for the Panton-Valentine leukocidin (PVL) (David and Daum, 2010). In Australia, a hypothesis was stated that new clones of CA-MRSA ascend on numerous occasions in remote/native communities after establishment of SCCmec type IV, in previously prevailing and virulent MSSA (Methicillin sensitive *Staphylococcus aureus*) strains (Tong et al., 2008). In 1990, numerous cases of CA-MRSA were reported among the remote area people of Western Australia (Udo et al., 1993). In the late 1990s, an outbreak of CA-MRSA was reported in United States. Clones of CA-MRSA viz. USA-300 and USA-400 holds 60-75% of all *S. aureus* among American community (Moeluring, 2006). Similar increasing trend of CA-MRSA infections has been documented in Taiwan, South America and UK (Lu et al., 2005; Adedeji et al., 2007). In India, initially CA-MRSA was investigated in pyoderma and some other infections only (Nagaraju et al., 2004; Krishna et al., 2004), but later their prevalence among healthy adults and children belonging to rural and urban premises have also been documented (Pathak et al., 2010; Fomda et al., 2014).

There is only a single report from India representing the existence of CA-MRSA in ethnic populations (Nadig et al., 2010). Thus, there is a need to evaluate the prevalence of CA-MRSA in different part of the country over the distinct community. In the view of the above, the present study was conducted to assess the percentage of CA-MRSA and their antibiotic-susceptibility and resistant patterns in the native population of north-western Himalayas, India.

Materials and Methods

**Sampling site**: The study was executed over three different tribal communities namely Bakarwal, Gujjar and Gaddi belonging to Jammu and Kashmir geographically located between 33.45°N 76.24°E and Himachal Pradesh India, located between 31°6’12”N 77°10’20”E (Fig 1). Bakarwals are sheep and goat rearing transhumants, Gujjars are buffalo herding and both tribes oscillate between high to low altitudes in the hill tracts of Jammu and Kashmir State (Sofi, 2013; Sharma et al., 2014), whereas Gaddis are also sheep and goat herding nomadic tribe of Himachal Pradesh (Sharma et al., 2014).

Convenience sampling procedures were used for sample collection. Total 1134 nasal swab samples were collected from the healthy individual belonging to defined area (Fig. 1). A verbal consent was obtained from the local authority before sampling as per Helsinki declaration and Indian Council of Medical Research guidelines. Sterile cotton swab sticks pre-dipped in saline were swirled inside the anterior-nares of individuals five times and put back into the Amies transport medium with charcoal.

**Microbiological examination**: The primary cultures were streaked on Mannitol Salt Agar (Hi Media) and then incubated at 37°C in bacteriological incubator for 24 hrs. From each MSA plate, a single colony with a yellow colour origin was picked and Gram staining was performed so as to identify the colony morphology. Gram-positive colony from each isolate was further identified using biochemical tests such as tube coagulase using rabbit plasma, D- trehalose fermentation, maltose fermentation (Cheesbrough, 2006; Cunha et al., 2004; Hazek, 1976), DNase test and β haemolysis on sheep blood agar were done accordingly to the guidelines by the manufacturer (Hi Media).

**Antibiotic susceptibility testing**: The drug susceptibility test was done by employing the Standard Bauer method (Bauer et al., 1966). The various antibiotic discs such as penicillin (10 units), oxacillin (1 µg), gentamicin (10 µg), cefoxitin (30 µg), erythromycin (15 µg), doxycycline hydrochloride (30 µg), co-trimoxazole (1.25/23.75 µg), methicillin (5 µg) and ciprofloxacin (5 µg) were used. The discs were placed over the Muller Hinton plates previously seeded with 18 hrs old broth of the positive cultures. *S. aureus* (CLSI, 2012), MTCC-740 were used as reference strains and the petri plates were incubated at 37°C in bacteriological incubator for 24 hrs and the inhibition zones were measured according to the guidelines of CLSI (CLSI, 2012).

**Statistical analysis**: Means of gender were compared using T-test and age groups were compared by one-way ANOVA at significance level of 5%. To analyze the drug susceptibility patterns of MRSA isolates against different drugs among the different groups (expressed in mean± standard deviation), the samples were subjected to one-way ANOVA, means were compared using post Hoc Tukey’s at significance level of p<0.05 with SPSS v16.

**Results and Discussion**

In total, seventy one *S. aureus* isolates were isolated from the nasal swabs of 1134 healthy individuals. The rate of *S. aureus* occurrence in nasal carriage was found to be 6.26%. The number of *S. aureus* in nasal carriage was 36 (50.70%) in males and 35 (49.29%) in females. The mean difference between nasal carriage in female and male was not found to be statistically
CA-MRSA among tribal population

Significant with \( p > 0.05 \). Maximum nasal carriage, 20 (28.16\%) of S. aureus was recorded in the age group of 10-19 yrs followed by 18 (25.35\%), 12 (16.90\%), 6 (8.45\%), 5 (7.04\%), 4 (5.63\%), 3 (4.22\%), 2 (2.80\%), 1 (1.40\%) in the age groups of 0-9, 20-29, 30-39, 40-49, 50-59, 60-69, 80-89 and 70-79 years respectively. There was no significant variation among the means of age groups with \( p > 0.05 \). Out of 71 S. aureus isolates, 24 (33.80\%) were MRSA by cefoxitin disc diffusion method, which was further confirmed by Oxacillin screen agar (Hi Media) (CLSI, 2012). The prevalence of nasal carriage was 2.11\% (24/1134). In male 11, (45.83\%) and 13 (54.16\%) nasal carriage were recorded. Highest nasal carriage, 9 (37.5\%) MRSA was documented from the age group of 0-9 yrs followed by 4 (16.66\%), 3 (12.5\%), 2 (8.33\%), 1 (4.16\%) in the age group of 10-19, 20-29 and 30-39, 40-49, 50-89 and 50-59 years respectively. The mean difference between nasal carriage MRSA in female and male in age groups were not statistically significant \( p > 0.05 \).

MRSA isolates showed susceptibility to (10µg) gentamicin, (30 µg) doxycycline hydrochloride (Table 1). Most of the results of erythromycin (15µg) fell in the intermediate category and only few isolates showed resistance against it. Similarly for cotrimoxazole (1.25/23.75 µg), maximum isolates showed resistance and few showed intermediate results against the drug. Results of both the drugs were statistically significant, as shown in Table 1, having \( p > 0.05 \). Most frequently MDR pattern for MRSA was evaluated by using the combination of penicillin, cotrimoxazole, ciprofloxacin (n=7, 29.16\%) followed by methicillin, penicillin, ciprofloxacin (n=5, 20.83\%), methicillin, cotrimoxazole, penicillin (n=2, 8.33\%), methicillin, cotrimoxazole, penicillin and ciprofloxacin (n=2, 8.33\%), respectively.

S. aureus is one of the utmost pathogen, which is linked to community-acquired and nosocomial infections. Nasal colonisation by S. aureus is considered to be the major risk factor responsible for infection which effect the host life (Mason et al., 2003). The prevalence of CA-MRSA in village populations was published from the northern parts of India (Fomda et al., 2014). The only few reports on the geographical distribution of CA-MRSA among the tribal communities in various parts of the world have come up from Western Australia, Gabonese Republic of Central Africa and South India (O’Brien et al., 2009; Schaumburg et al., 2011; Nadig et al., 2010). This is the second study of Indian origin focussing on the CA-MRSA prevalence in the tribal communities belonging to the northern part of India.

CA-MRSA Colonisation rates in the tribal community are known to range from null to 9.2\% (Chatterjee et al., 2009). In the present study, the prevalence of CA-MRSA nasal colonisation was 2.11\%. This rate was slightly higher than a previous study carried out in the village population in north India (Fomda et al., 2014). On the other hand, the percentage of CA-MRSA was much lesser in the present study and the study carried out by Fomda group in the village populations, as compared to the two previous researches, reported 18.1\% MRSA among the urban populations of India (Fomda et al., 2014; Saxena, 2002; Saxena et al., 2003). Previous studies have reported the prevalence of CA-MRSA (0.2\%) among the healthy population of the USA in the year of 2000, and 2.8\% in urban population in 2002, whereas other two reports published in 2006 provide information regarding the CA-MRSA prevalence, which was 0.8\% to 0.84\% in the USA (Shopsin et al., 2000; Charlebois et al., 2002; Kuehnert et al., 2006; Mainous et al., 2006). Amongst other countries, the rate of CA-MRSA was 3.8\% in Taiwan, 0.23\% New Zealand and 0.9\% in Brazil (Wang et al., 2009; Best et al., 2011; Piers et al., 2014). Colonisation rates of CA-MRSA in the tribal communities of Western Australia, South India were 2.28\% and 0.8\% respectively, whereas no CA-MRSA were detected from the tribal community in Gabonese Republic of Central Africa (O’Brien et al., 2009; Nadig et al., 2010; Schaumburg et al., 2011). In the present study, the colonisation rates of CA-MRSA was found to be 0.7\% and 0.35\% in the age group of 0-9 yrs and 10-19 yrs, respectively, and the overall rate in age group of 0-19yrs was 1.14\%. In India, a study conducted by Chatterjee et al. (2009) reported about the 3.89\% incidence of CA-MRSA belonging in the age group of 5-15yrs. In 2010 (Pathak et al., 2010), found 1.02\% occurrence of CA-MRSA in the age group of 1-month to 5 yrs. Bhash et al.

### Table 1: Mean susceptibility patterns of MRSA in three tribal communities

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Bakarwal</th>
<th>Gujar</th>
<th>Gaddi</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin</td>
<td>9.44±5.1</td>
<td>11.09±4.1</td>
<td>13±3.7</td>
<td>0.421</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>22±1.6</td>
<td>21.91±4.0</td>
<td>13±2.9</td>
<td>0.000*</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>18±2.1</td>
<td>18.55±2.8</td>
<td>18±0.8</td>
<td>0.856</td>
</tr>
<tr>
<td>Doxycycline Hydrochloride</td>
<td>19.22±2.6</td>
<td>22.82±4.1</td>
<td>22.25±1.8</td>
<td>0.332</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>7.11±2.6</td>
<td>8±1.6</td>
<td>7.8±0.8</td>
<td>0.598</td>
</tr>
<tr>
<td>Co- Trimoxazole</td>
<td>10.33±1.3</td>
<td>10±2.6</td>
<td>14.25±4.9</td>
<td>0.042*</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>19.67±5.0</td>
<td>21±1.6</td>
<td>19.75±2.0</td>
<td>0.651</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10.89±1.9</td>
<td>12.82±1.2</td>
<td>13.25±3.3</td>
<td>0.062</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>14.78±5.1</td>
<td>14.91±3.9</td>
<td>16±5.4</td>
<td>0.903</td>
</tr>
</tbody>
</table>

*Statistically significant
Fig. 1: (A) Map of India showing Jammu & Kashmir (J&K) and Himachal Pradesh (HP), (B) Map of Jammu & Kashmir showing the district: (1) Anantang, (2) Pulwama, (3) Srinagar, (4) Budgam, (5) Baramullah, (6) Kupwara and (7) Rajouri and (C) Map of Himachal Pradesh showing the district: (8) Obri Chamba and (9) Sultanpur.
(2014) reported 10.17% prevalence of CA-MRSA in 1-6 yrs age group. In 2014, reported the rate of CA-MRSA was 7.39% in the age group of 5-10 yrs. Study carried out in Turkey in 2008 by Oguzkaya and group found 1% of CA-MRSA among the 0-6yrs of age group (Oguzkaya-Artan et al., 2007). The present study illustrated that the overall rate of nasal carriage CA-MRSA was 0.97% in the aged ≥ 20 yrs. Previous report published in Queensland, Australia had found the CA-MRSA rate 0.7% in the aged ≥ 18 yrs (Munkchhof et al., 2009). Present study illustrated that 0.26 proportion of CA-MRSA were in the age group of 20-29 yrs, previous finding in the Brazilin adults having the age group 18-27 claimed the percentage of CA-MRSA was 2.4 (Prates et al., 2010).

Excluding resistance against penicillin, the overall susceptibility results showed that ciprofloxacin was the least effective drug against CA-MRSA with resistance rate 79.2%. This results are consisted with the previous studies conducted by Saxena et al. (2003) reporting resistant 71%, Alvarez-Uria and Reddy et al. (2012) claiming 84.4% resistant rate and Sharma et al. (2014) showing 70% resistant rate (Saxena et al., 2003; Alvarez-Uria and Reddy, 2012; Sharma et al., 2014). But this finding contradicts several other studies illustrating low rate of resistance to ciprofloxacin for nasal carriage CA-MRSA (Fomda et al., 2014; Chatterjee et al., 2009). The results of high ciprofloxacin resistant rate in CA-MRSA is matched up with those of healthcare-associated MRSA (HA-MRSA) having resistance 66%, 79.3% and 89.0% (Askanian et al., 2009; Joshi et al., 2013; Sapri et al., 2013). In present study co-trimoxazol was found to be second least effective drug with resistant rate 58.3% but is in an agreement with the previous studies who claimed a high resistance 88%, 88.23% and 75% against the same (Saxena et al., 2003; Bharathi et al., 2014; Saxena, 2002). Studies conducted in USA showed that CA-MRSA was completely susceptible against co-trimoxazol (Charlebois et al., 2002; Kuehnert et al., 2006). Current in the present study, gentamicin and doxycyline hydrochloride was considered as drugs of choice against CA-MRSA and this claim is also supported by the previous findings in India (Krishna et al., 2004; Fomda et al., 2014; Chatterjee et al., 2009; Alvarez-Uria and Reddy, 2012). CA-MRSA showed complete susceptibility against gentamicin in USA also (Charlebois et al., 2002; Kuehnert et al., 2006).

Research in developed countries put forward that factors associated with CA-MRSA mostly include poor socio-economic conditions and overcrowding (Rihn et al., 2005; CDC, 2006). But how did CA-MRSA enter into such kind of remote populations is a matter of debate. If we follow the conventional statements which claim these community-acquired strains has progressed from the hospital strains which underwent certain genetic amendments or it is due to the transfer of mec gene from the formerly susceptible strains (Tong et al., 2008; Chambers, 2001). However, the resistance patterns of CA-MRSA in the study generate another possibility that they might be wild descendants of the hospital isolates, as our antibiogram patterns showed different result as compared to which were isolated from fish in Cochin and Mumbai India (Visnuvinayagam et al., 2015).

There were three limitations in the present investigation. First study on the risk factors associated with nasal carriage CA-MRSA, like family size, zoonotic reservoir and environment was not done. Second, the nasal samples study did not provide the data related to colonisation and its prevalence (Hamdan-Partida et al., 2010). Third, the small sample size because there were 18 tribes excluding sampled population in both the states. The high proportion of nasal carriage CA-MRSA cases and occurrence of antibiotic resistance against commercial available drug is an alarming event. Thus, there is a need of antibiotic stewardship programmes which endorse cautious exploitation of the antibiotic with addition to regulated approaches for ceasing the widespread of resistant bacteria, especially amongst far-fetched area.

Acknowledgments

Authors are indebted to all the tribe’s head and the participants for their cooperation and hospitality.

References