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RESEARCH ARTICLE

Intervention points for community- acquired methicillin–resistant *Staphylococcus aureus* colonization and load in healthy population of lesser Himalayan Belt, South Asia, India

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OBJECTIVES: To trace the critical practicing, clinical and epidemiological risk factors in bacterial load and points of intervention in spread of community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) in healthy community.
STUDY DESIGN: 2872 individuals with no prominent clinical features were enrolled and administered a pre-tested questionnaire prepared on the basis of outcome of a prior pilot study in same region. Swab samples from skin, throat and nasal nares were tested for MRSA and molecular identification was done to track the strains moving from hospital to community.
METHODS: Swab samples from skin, throat and nasal nares were tested for MRSA culture followed by molecular characterization of isolates and antimicrobial resistance pattern. Bacterial load was estimated to better understand the burden in different categories. Statistical analysis was done using SPSS 16.0 version.
RESULTS: History of prior infection (OR 3.9, 95% CI 1.363 – 5.793), habit of self remedy (OR 3.2, 95% CI 0.991 – 1.473) and incomplete treatment (OR 0.26, 95% CI 0.08 – 0.80) ($P < 0.05$ for each) were the predominant factors that contributed to spread of CA-MRSA. Increased drug resistance in CA-MRSA was observed for 4 different clones: SCC*mec*$^+$ IVa/*PVL*$^+$, SCC*mec*$^+$ IVa/*PVL*$^-$ and SCC*mec*$^+$ IVc/*PVL*$^+$, SCC*mec*$^+$ IVc/*PVL*$^-$. Bacterial load was found significantly high in below poverty line dwellers and drug abusers ($P < 0.05$).
CONCLUSION: We identified habit of self remedy, drug abusing and incomplete treatment as practicing risk factors where interventions can be made to manage the dissemination of CA-MRSA in rural population.

Keywords community-acquired MRSA, risk factors, bacterial load, himalayan region, pantone-valentine leuckocidin

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) once known to be strictly a hospital acquired pathogen has now emerged as a persistent community pathogen (Berman et al., 1993; Shopsin et al., 2000). There have been several reports of incidences and rapid transmission of CA-MRSA around the globe. Centre for Disease Control and Prevention (CDC) has created a case definition for a CA-MRSA infection to distinguish it from strains from hospital settings (CDC, 2005; Morrison et al., 2006). The CA-MRSA is disseminated and colonized to the nares primarily by contaminated hands and transmission occurs mainly through person-to-person contact (Kluytmans et al., 1997) Spectrum of outbreak conditions caused by CA-MRSA spans from septicemia, wound infections, skin and soft tissue infections, toxic shock syndrome to life threatening conditions (Chatterjee et al., 2009; Honda et al., 2010) and it is now clear that CA-MRSA infections predominantly take the form of relatively minor skin and soft tissue infections (Naimi et al., 2003; Fridkin et al., 2005). Several lifestyle, demographic, epidemiological and clinical factors are known to be associated with the CA-MRSA infection and colonization (Naimi et al., 2001; Sattler et al., 2002).

The Garhwal Himalaya is the prominent part of North – Western mighty Indian Himalaya region (IHR). Although devoid of major facilities like tertiary healthcare centers and air bases, dwellers have been there for generations. Srinagar being the hotspot region in the entire route from lowlands to

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the foot of mountains, tourists in the pilgrimage seasons stays here for as long as 1-3 months. Establishments like H.N.B. Garhwal University with 2 campuses, polytechnic college, number of higher secondary schools and I.T.I campus further populates the region and 2 tertiary care hospitals (Base hospital & Combined state hospital) makes it more prone than other hilly regions in terms of rigorous interaction of individuals with each other. The humid season sustains for maximum part of the year and hence helps in propagation of CA-MRSA efficiently.

In this context, it has been observed that no comprehensive prevalence, associated risk factors based study with estimation of antibiotic resistance on CA-MRSA has been done from entire of Garhwal Himalayan regions. Hence, for the first time population based large study was conducted to understand the risk factors, potential points for intervention and epidemiology of CA-MRSA in this region.

Methods

Study design and setting

The site chosen for present study was Srinagar and adjacent areas. Srinagar is located at 30.22^0 N 78.78^0 E on the left bank of Alaknanda river. It has an average elevation of 560 m (1837 feet) and is the widest valley in Garhwal hills. The study was conducted on a large heterogeneous population dwelling in approximately 41.35 km^2 area of foothills of lesser Himalayan region comprising one university, three government institutions, six secondary schools, one teaching medical hospital (Base hospital) and one non – teaching hospital (Government combined state hospital). Besides of that, few primary health clinics were also in the area selected for the study.

Study population

A total of 2872 healthy and potentially eligible individuals of different age group and profession participated in study. The Individuals who participated in the study completed and signed an informed consent form. Local language (Garhwali/ Hindi) was used by local translator to inform them of study and consent. An informed verbal and written consent was also taken by parents for children below 18 years (The study was approved and got clearance by Board of Studies of the University with the condition that the questionnaires will have to be explained to volunteers in local languages; the questionnaire hence was explained in local *Garhwali* language with the help of two local language translators). Eligible cases were screened on the basis of presence of certain risk factors identified from a pilot study conducted in the similar region 6 months prior to large scale investigation which included 454 individuals in all. Although, we adopted the standard statistical formula to estimate the sample size during pilot study i.e., $n = z^2 P (1 – P)/d^2$, keeping $z = 1.96$ (for 95% confidence), $P = 20\%$ (0.2) and $d = 0.05$ (for 5% precision), the sample size of 138 was not realistic to the area selected and hence a large random population screening method was chosen in a defined area.

Data collection and risk factor survey

A standardized pre-tested questionnaire was used to identify the risk factors for CA-MRSA carriage. All the subjects were interviewed by a trained team. Potentially eligible subjects were identified from the existing knowledge of pilot study and interviewed for demographic, epidemiological, practicing and clinical factors. The age, sex, profession and income economic status on the basis of Above Poverty Line and Below Poverty Line category classified according to the norms set by government of India (Tenth Five Year Plan 2002-2007), were considered as the socio-demographic factors in the study, whereas practicing factors included habit of self remedy or drug abuse and habit of incomplete treatment course for any disease and hand washing frequency per day. Clinical factors included history of infection at skin/ soft skin tissue, healthcare center visits in last 1 month prior to sampling and on antibiotics course during last 2 months.

Geospatial analysis and interpretation

Data was obtained from screening of 2872 subjects from an area of 41.35 km^2 and analyzed by geographic information system software (ArcGIS version 9.2). The prevalence of CA-MRSA in the different regions is shown in the ArcGIS map and classified on the basis of colors scaling from 0 to 5 in an increasing order.

Microbiological and antimicrobial susceptibility studies

Samples were procured from upper respiratory tract, nasal carriages and infected sites of human population using pre-sterilized swabs (Himedia). The swabs were immediately transported to microbiology laboratory for further processing.

Antimicrobial susceptibility testing was performed by Kirby-Bauer's disc diffusion method according to performance standards of Clinical & Laboratory Standards Institute (CLSI, 2010).

Bacterial load and genotyping of MRSA isolates

The bacterial load was estimated in individuals found positive for CA-MRSA. Samples were procured from any of the 3 anatomical sites (nasal nares, infected skin/cut and throat) which was found positive for CA-MRSA using swabs. Swabs were immediately inoculated into Tryptone soya broth containing 4μg/ml cefoxitin, 8 μg/mL aztreonam and 4mg/ mL methicillin/oxacillin and incubated at $37 \pm 1°C$ for 16-24h. The culture was serially diluted up to 10-fold in sterile

Tryptone soya broth and plated on to nutrient agar medium estimate the colony forming units. The turbidity of each dilution was measured at 590nm using UV visible spectrophotometer.

For genotyping, the recovered isolates were inoculated into 2 mL of lysogeny broth and grown for 16 h. Genomic DNA was isolated from MRSA isolates (Sambrook et al., 1989). Staphylococcal Cassette Chromosome mec (SCCmec) gene typing was done by multiplex PCR (Zhang et al., 2005). The primers used were:

SCC*mec* type I: F GCTTTAAAGAGTGTCGTTACAGG
R GTTCTCTCATAGTATGACGTCC
SCCmec type II: F CGTTGAAGATGATGAAGCG
R CGAAATCAATGGTTAATGGACC
SCCmec type III: F CCATATTGTGTACGATGCG
R CCTTAGTTGTCGTAACAGATCG
SCCmec type IVa: F GCCTTATTCGAAGAAACCG
R CTACCTTCTGAAAAGCGTCG
SCCmec typeV: F GAACATTGTTACTTAAATGAGCG
R TGAAAGTTGTACCCTTGACACC

SCC*mec* typing standards used were MRSA strains NCTC10442 for Type I, N315 for Type II, 85/2082 for Type III and JCSC 4744 for type IV. PCR reaction conditions were: initial denaturation step at 94°C for 5 min; 30 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min; Final extension at 72°C for 10 min. PCR products were visualized on 1.8% agarose gel stained with ethidium bromide under UV transillumination. For the presence of Panton – Valentine leukocidin (*pvl*) gene a polymerase chain reaction assay was applied as mentioned elsewhere (Lina et al., 1999).

Statistical analysis

Statistical analysis was performed by using SPSS version 19.0 (SPSS Inc. Chicago, IL, USA). Student's t – test was applied to continuous variables whereas for categorical variables differences between groups were measured with Pearson Chi – square test. Logistic regression analysis was done for bivariate or multivariate analysis by calculating odds ratios (OR) with 95% confidence intervals (CI). All variables with $P <$ or equal to 0.10 in the bivariate analysis were taken for multivariate logistic regression analysis. Bacterial load was taken as dependent variable to compute linear regression analysis and ANOVA. All variables were considered significant at the $P = 0.05$ level.

Results

A total of 2872 individuals of different age groups, profession, economic status and habits were enrolled in the study from April 2011 to October 2013. MRSA and methicillin resistant *Staphylococcus aureus* (MSSA) carriage was observed in 622 and 812 individuals respectively (Table 1). In all 2872 screened individuals, 622 cases (21.65% prevalence) were found to have CA-MRSA colonized.

Risk factors associated with CA-MRSA nasal carriage

By the multivariate logistic regression analysis, factors that were found to be significantly associated with CA-MRSA nasal carriage includes the profession (farmers and shopkeepers), practice of self remedy, habit of incomplete treatment, and prior history of infection ($P < 0.05$) (Table 2).

Risk factors associated with overall occurrence of CA-MRSA

Bivariate regression analysis demonstrated that independent factors that significantly ($P < 0.05$) contributed to the overall occurrence of CA-MRSA comprised of the age group (31 to 40 years and 40 above), gender, profession (daily wagers, farmers and shopkeepers). Practicing and clinical factors that were associated with occurrence of CA-MRSA were frequency of hand washing, self remedy habit, habit of going through incomplete treatment, prior history of infection, frequent visits to healthcare centers and receipt of antibiotics 2 months prior to study (Table 1).

Molecular analysis and antibiotic susceptibility pattern of MRSA isolates

All the 812 confirmed MRSA isolates recovered were typed for the SCC*mec* and presence of *PVL* gene. A total of 5 different types of SCC*mec* were observed. Strains were assigned with community or hospital acquired on the basis of presence of IVa, IVc and I, II, III SCC*mec* type respectively. SCC*mec* IVa and IVc predominated significantly (Table 3). However, variation in presence of *PVL* gene was observed in CA-MRSA with 465 isolates possessing *PVL* gene. Antibiotic susceptibility pattern shows that highest sensitivity for CA-MRSA isolates was recorded against ciprofloxacin, vancomycin, amikacin, clindamycin, kanamycin and chloramphenicol (100% each). Eight different patterns were observed for drug resistance in the recovered 622 CA-MRSA isolates (Fig. 1). Maximum of the isolates were resistant for Methicillin + Oxacillin pattern (168), followed by Methicillin + Oxacillin + Erythromycin type (147) with highest resistivity for oxacillin and erythromycin after methicillin (Fig. 2). With E test, all the isolates were found to be resistant up to 30 μg/mL of methicillin and showed prominent growth, whereas for other antibiotics, number of isolates that showed growth varied with the concentration.

Impact of risk factors on MRSA bacterial load

Analysis of variance demonstrated the impact of risk factors on MRSA load. Age, economic status, practicing drug abuse

Table 1 Independent factors associated with overall occurrence of CA-MRSA by bivariate regression analysis

Factors		Subjects				
	$n = 2872$	MRSA carrier	MSSA carrier	OR (95% CI)	*P*	Non carriers
Age group(years)						
1 to 10	281	42	53	Reference		186
11 to 20	448	158	209	0.954 (0.606 – 1.503)	0.839	81
21 to 30	1187	324	276	1.956 (1.259 – 3.039)	0.103	587
31 to 40	554	78	134	0.357 (0.221 – 0.574)	< 0.001	342
40 above	402	20	140	0.188 (0.101 – 0.350)	< 0.001	242
Total		622	812			
Gender						
Male	1514	354	479	Reference		681
Female	1358	268	333	2.590 (2.025 – 3.314)	< 0.001	757
Family size						
1 to 3	1327	202	393	Reference		732
4 to 7	1027	152	209	0.888 (0.687 – 1.147)	0.364	666
7 to 10	518	268	210	2.495 (1.947 – 3.197)	0.051	40
Profession						
Student	1132	254	301	Reference		577
Daily wager	201	125	44	0.705 (0.535 – 0.931)	0.013	32
Farmer	714	77	82	2.074 (1.381 – 3.113)	< 0.000	555
Shopkeepers	307	180	97	2.601 (1.908 – 3.547)	< 0.001	30
Others	518	165	238	2.016 (1.491 – 2.725)	0.811	115
Economic status						
APL	1861	411	567	Reference		883
BPL	1011	211	245	1.223 (0.977 – 1.531)	0.079	555
No. of toilets	1.64±1.2	1.7±1.3	1.5±1.1	0.676 (0.088 – 1.423)	0.879	
Practicing factors						
Hand washing frequency	4.9±3.1	4.6±3.1	5.4±3.8	0.57 (0.071 – 2.021)	0.032	
Self remedy	943	112	131	2.435 (1.813 – 3.269)	0.001	700
Drug abuse	402	74	129	1.403 (1.032 – 1.907)	0.301	199
Incomplete treatment	354	69	186	2.163 (1.605 – 2.914)	< 0.000	99
Clinical factors						
History of infection	222	114	8	9.8 (4.44-21.87)	< 0.001	100
Frequent visits to healthcare center	814	414	280	0.697 (0.574 – 0.845)	0.014	120
On receipt of antibiotics 2 months prior to study	464	74	131	0.541 (0.391 – 0.748)	< 0.001	259

MRSA, Methicillin resistant *Staphylococcus aureus;* MSSA, Methicillin Sensitive *Staphylococcus aureus;* OR, odds ratio; CI, confidence interval.

and gender were the risk factors ($P < 0.05$) associated with MRSA bacterial load (Table 4).

Linear regression model

To show the behavior of one variable to another on MRSA load the regression approach was adopted where bacterial load was considered dependent variable and age, drug abuse and incomplete treatment habits were taken as the independent variables.

With adjusted $R^2 = 0.76$ and standard error = 0.67 the three predictors collectively best fitted the model. The Durbin – Watson value for co linearity was 0.747 (Data not shown). The model showed that the 75.8% variations on 'Bacterial load' were due to independent variables Age, Drug abuse and Incomplete treatment. Fitted regression equation was:

Table 2 Risk factors for methicillin-resistant *Staphylococcus aureus* nasal carriage: Multivariate logistic regression analysis

Factors	OR (95% confidence interval)	*P*
Farmers in field	0.21 (0.091 – 0.142)	0.002
Shopkeepers near THCC[a]	1.6 (0.381 – 2.241)	0.012
Self remedy	3.2 (0.991 – 1.473)	0.003
Incomplete treatment	0.26 (0.08 – 0.80)	0.022
History of infection	3.9 (1.363 – 5.793)	0.001
Have taken antibiotics in the prior 2 months	1.1 (0.065 – 0.699)	0.81

[a], Tertiary health care center (Base hospital and Government hospital); OR, odds ratio.

Table 3 Distribution of MRSA strains

SCC*mec* type	No. of isolates	Virulence factor (*pvl*)	
		+	–
IVa	414	384	30
I	41	–	
II	121	–	
IVc	208	181	27
III	28	–	

SCC*mec*, Staphylococcal Cassette Chromosome mec; *pvl*, Pantone valentine leukocidin.

$$\text{Bacterial load} = 0.221_{\text{age}} + 0.132_{\text{drug abuse}} + 0.199_{\text{Incomplete treatment}}$$

$$Z_{\text{Bacterial load}} = 0.511_{\text{age}} + 0.202_{\text{drug abuse}} + 0.196_{\text{Incomplete treatment}}$$

ANOVA showed the $P < 0.000$ for the model which rejects the null hypothesis. $F = 540.481$ (0.00).

Discussion

Owing to its diverse and adverse geographical structure, earlier studies have been confined to the lowlands where accessibility to subjects was much easier in a hospital setting. We investigated the problem to trace the critical points where intervention can be applied in order to manage the CA-MRSA transmission and infections. Several demographic, clinical and practicing factors were included in the study as the prior pilot study in the same region indicated that the habits of dwellers were the prominent drivers for CA-MRSA prevalence.

Our results regarding prevalence (21.65%) are in line with a smaller study performed earlier in 2013, where 17.59% prevalence of CA-MRSA was reported (Kainthola and Bhatt, 2013). However, keeping in view the adversity and remoteness of villages, a 4% rise in prevalence is substantial. The plausible reason for this rise in prevalence within a short duration may be the heavy incumbent of visitors to famous Hindu's and Sikh's pilgrimage site in this region. Also, our investigation suffered a dramatic fluctuation in prevalence of CA-MRSA in those areas which were affected by Kedarnath flood of large magnitude in June 2013 which covered major part of the area of study. CA-MRSA and methicillin sensitive *Staphylococcus aureus* (MSSA) were recovered approx. in the unit ratio (622 and 812 respectively). A school of thought states that CA-MRSA phenotype replaces CA-MRSA in the community, however there are certain evidences that MSSA gradually emerged alongside without any reduction in its infection or colonization (Ala'Aldeen, 2002; Hota et al., 2008). Health care centers were considered as the large reservoir of Hospital Acquired MRSA (HA-MRSA) for long. In recent time, it is now been reported that resistance to methicillin has raised in the community strains as well. This rise in methicillin resistance is attributed to the transmission

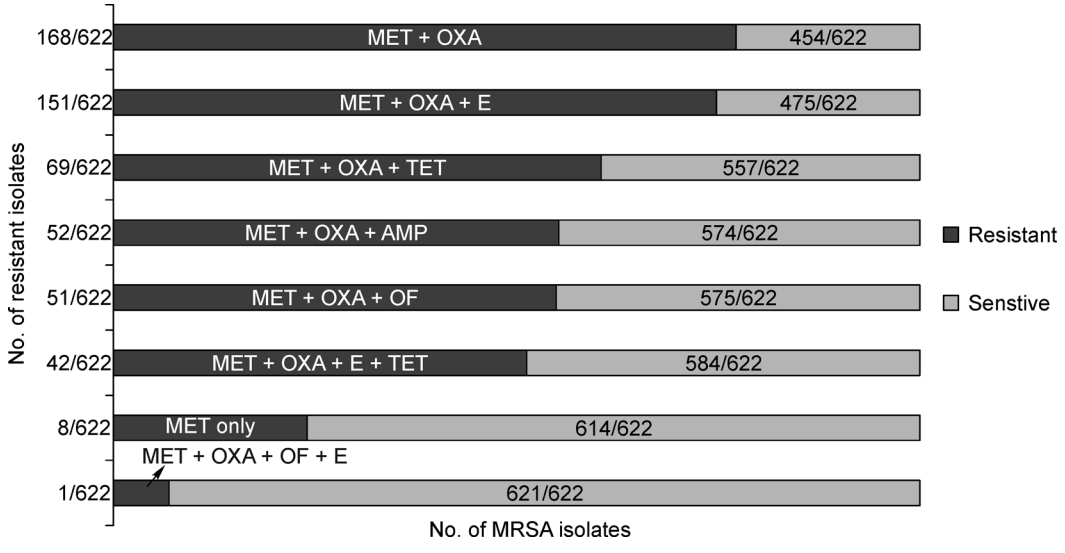

Figure 1 Patterns of antimicrobial susceptibility for 622 community acquired Methicillin-resistant *Staphylococcus aureus* isolates. MET, methicillin; OXA, oxacillin; E, erythromycin; AMP, ampicillin; TET, tetracycline; OF, ofloxacin.

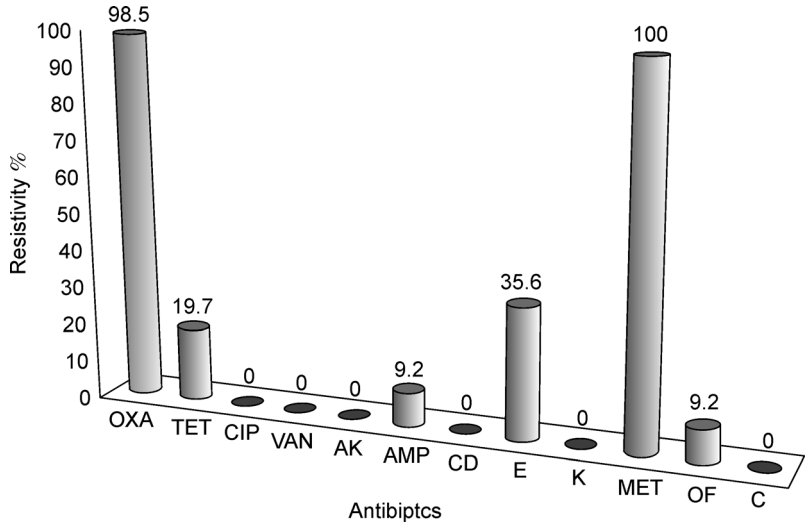

Figure 2 Resistivity (%) pattern of 622 recovered CA-MRSA isolates. OXA, oxacillin; TET, tetracycline; CIP, ciprofloxacin; VAN, vancomycin; AK, amikacin; AMP, ampicillin; CD, clindamycin; E, erythromycin; K, kanamycin; MET, methicillin; OF, ofloxacin; C, chloramphenicol.

of resistance from hospital strain to the community strain. The study area and population is more or less a closed community with frequent interaction. An increased sample size was expected to show a slight increase in the CA-MRSA prevalence. Hence, an increased prevalence (approximately of 4%) has been reported in this study compared to the pilot study.

The factor significantly associated with the overall occurrence of CA-MRSA was the age group 31 to 40 years and 40 above (OR 0.357, 95% CI 0.221 – 0.574 and OR 0.188, CI% 0.101 – 0.350 respectively) evidently due to their frequent interaction with tertiary care hospital and unhygienic living conditions. However, maximum numbers of MRSA and MSSA were recovered from youngsters of age group 21 to 30 years followed by 11 to 20 years. Published studies have reported earlier the independence of CA-MRSA infections in youngsters from hospital settings (O'Brien et al., 1999; Carleton et al., 2004; Paintsil, 2007) and hence community may itself act as an independent variable for transmission of CA-MRSA. Results are in agreement of the earlier investigation (Chatterjee et al., 2009). Unlike previous studies, surprisingly, females were tested more positive in acquiring CA-MRSA (OR 2.590, 95% CI 2.025 – 3.314, $P < 0.001$). Professionals like daily wagers, farmers and shopkeepers (OR 0.705, 95% CI 0.535 – 0.931, OR 2.074, 95% CI 1.381 – 3.113, OR 2.601, 95% CI 1.908 – 3.547 respectively) had greater susceptibility for CA-MRSA probably due to the compromised hygienic environment they work in.

Practicing factors identified by bivariate regression analysis which significantly contributed to the occurrence of CA-MRSA in individuals included frequency of hand washing per day (OR 0.57, 95% CI 0.071 – 2.021, $P < 0.032$), self remedy and habit of incomplete treatment. Unavailability of healthcare centers/ private hospitals in the interior foot hills of Himalayan region has instilled the habit of self remedy in local population. Taking antipyretics and antibiotics by individuals without medico prescription was a general practice which has increased the drug pressure in this region.

Table 4 ANOVA: Impact of independent factors on occurrence and load of MRSA

Factors	Occurrence of MRSA		Load	
	F	*P*	*F*	*P*
Age	6.501	0.000	40.688	< 0.000
Economic status	6.521	0.003	40.932	< 0.000
Prior incomplete treatment	2.078	0.150	0.329	0.566
Have taken antibiotics	4.399	0.036	2.125	0.096
Drug abuse	2.272	0.079	20.938	0.001
Profession	9.351	0.004	1.808	0.165
History of prior infection	6.491	0.002	0.322	0.725
Gender	3.028	0.029	11.329	< 0.001
Frequent visits to healthcare center	3.555	0.029	2.613	0.074

In addition to the dissemination of determinants of methicillin resistance across the community, practicing factors further developed a favorable environment where resistant CA-MRSA could thrive and propagate. The adverse geographical conditions, below par transport facilities and remoteness further increased the drug resistance in MRSA as dwellers are compelled to leave the antibiotic course in between.

We further identified three major clinical factors responsible of MRSA acquisition which include history of skin infections/burns/wounds, frequent visits to the healthcare centers and taking antibiotics 2 months prior to study ($P < 0.05$). Individuals who had infection history did act as MRSA and MSSA reservoirs as MRSA colonization can persist from months to years (Thompson et al., 1982; Sanford et al., 1994). These reservoirs either untreated or discharged from hospital play key role in spreading the CA-MRSA in community whereas population which was residing close enough to the two major healthcare centers namely Veer Chandra Singh Garhwali (VCSG) medical college and Government combined hospital were at much higher risk of CA-MRSA acquisition (Fig. 3). Figure 3 clearly depicts that the transmission of CA-MRSA was higher in the vicinity of the two healthcare centers. Prevalence map shows that the CA-MRSA was originating and disseminating through these two healthcare centers. ANOVA depicted that among demographic factors, age ($F = 40.688$, $P = 0.000$), economic status ($F = 40.932$, $P = 0.000$) and gender ($F = 11.329$, $P = 0.001$) (Table 4) had significant rolein having heavy bacterial load. More than 50% of the individuals enrolled in the study falls between the age of 1 to 30 years and hence comprise an active group which is supposed to have a rigorous interaction with each other in the community. Arguably, our results demonstrate that females had greater MRSA burden while in reality men are the active members of the family in this region. Drug abusing was the only clinical factor that was statistically significant for MRSA load which is clearly an outcome of sharing of needles and objects of general usage like door handles, towel etc.

All the 622 recovered CA-MRSA isolates were phenotypically methicilin resistant. Of these, 168 were also having resistance for oxacillin, another beta lactam antibiotic which is much higher than previous study (Pathak et al., 2010). Although clindamycin is a good choice of antibiotics for many types of CA-MRSA infections, the possibility of clindamycin resistance must be recognized. A study of CA-MRSA in Australia (Stevens et al., 2006) suggested that erythromycin and clindamycin cannot be relied upon empirically as non beta lactam alternatives due to the emergence of 57% non multidrug resistant CA-MRSA. Our study is in agreement with the former by reporting 35.6% resistivity to erythromycin in CA-MRSA isolates (Fig. 2). The notable finding was pattern of antibiotic resistance which

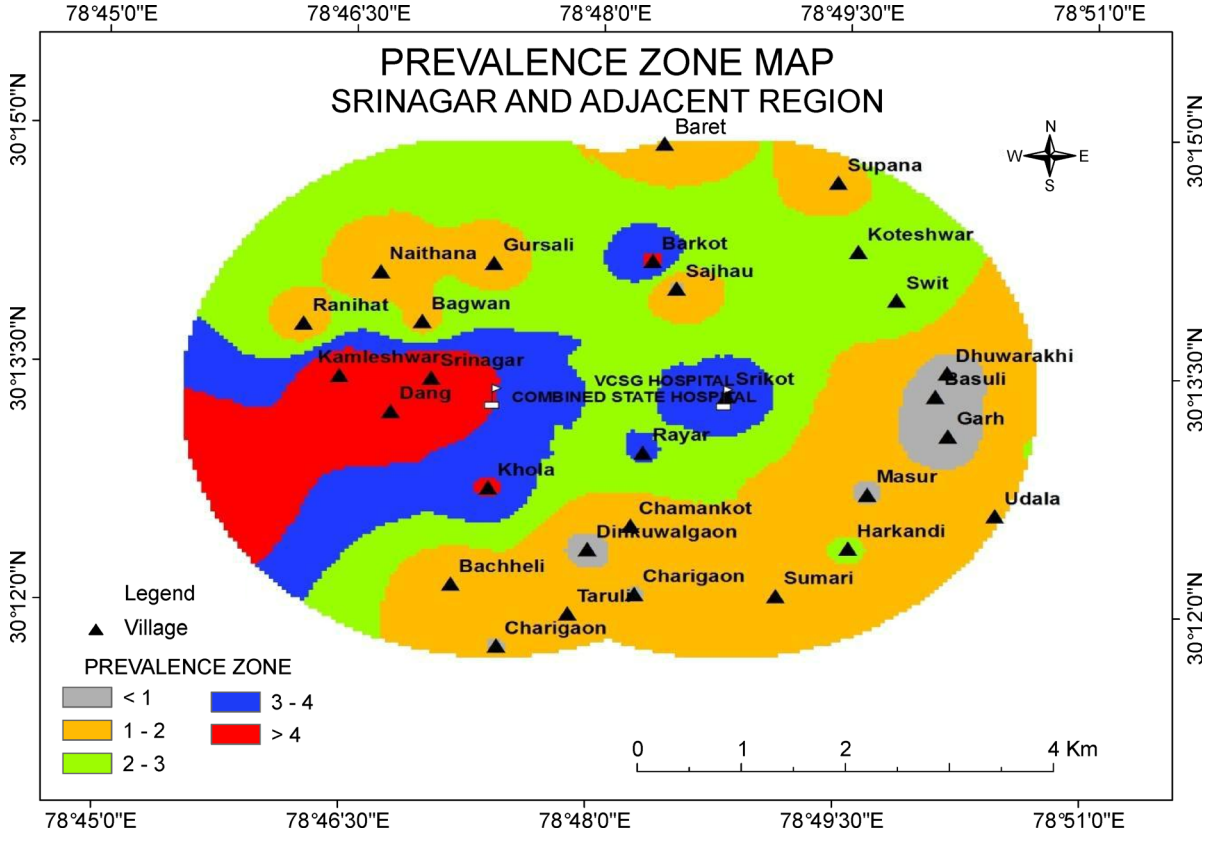

Figure 3 Prevalence of CA-MRSA in the study site (41.35 km^2).

was suggestive of 'tending to' nature of CA-MRSA to HA-MRSA as far as drug resistance is concerned. Five major MRSA clones were obtained in this study with majority of the isolates belonging to the SCC*mec* IVa type (414,Fig.4), followed by the SCC*mec* IVc type (208) (Table 3). The presence of *pvl* gene varied among the CA-MRSA clones characterized as SCC*mec*$^{+}$ IVa/*PVL*$^{+}$ (384), SCC*mec*$^{+}$ IVa/*PVL*$^{-}$ (30) and SCC*mec*$^{+}$ IVc/*PVL*$^{+}$ (181), SCC*mec*$^{+}$ IVc/*PVL*$^{-}$ (27). SCC*mec*$^{+}$ IVa/*PVL*$^{+}$ were the most prevalent clone observed in the study which clarifies the emergence of CA-MRSA isolates with increased drug resistance. However, recovery of HA-MRSA (190) from very interiors of the Himalayan villages is of serious concern. This region is almost separated from the local community by substantial margin. Occurrence of HA MRSA in this region suggests that people traveling from this farthest region to the active community are acting as carriers. Notably, negligible numbers of CA-MRSA were recovered from here which indicates that dwellers may have visited hospitals. However with the recovery of HA-MRSA, emergence of CA-MRSA in this very region is expected now. A prospective study may further reveal the dyanamics of resistance transmission in this area. Three clones of HA-MRSA, SCC*mec*$^{+}$ I/*PVL*$^{-}$, SCC*mec*$^{+}$ II/*PVL*$^{-}$ and SCC*mec*$^{+}$ III/*PVL*$^{-}$ were observed which suggest that in a way or other the HA-MRSA clones have triggered the dissemination of drug resistance in the CA-MRSA strains. Previous studies have demonstrated the higher prevalence of other clones of MRSA (Maier et al., 2005) may be because of selection of homogenous well defined study population and different geographical regions.

There are certain strengths to this study. To best of our knowledge, this is the first large and population based prospective study in the Indian lesser Himalayan belt. Different topological areas which structure the community into open and closed community system were included in this study. Our study certainly puts forward the fact that clones of CA-MRSA and MSSA have reached in this part of world at worrisome level. Further, we have come up with the critical points where interventions like regular workshops to enhance their knowledge about infections and outcomes of unhygienic living conditions may be arranged. Also, the active age group may be targeted (which comprised 80% of college students) to upgrade their knowledge about cleanliness and protective interactions with each other by local Non Government Organizations (NGO's) and government bodies. A time based regular surveillance and such epidemiological studies are of utmost importance here at this region to ensure the effectiveness of interventions made to check and manage the prevalence of CA-MRSA and MSSA in healthy people. Also, our study has provided a platform for medicos to rationalize their choice of antibiotics to treat infections and finally our study paves the way for future investigations in this remote region.

Compliance with ethics guidelines

All authors declare that they have no conflicts of interest. The study was approved by Board of Studies of research i.e. HNB Garhwal Central University, Srinagar, Uttarakhand, India. The study was supported financially by University Grant Commission, New Delhi vide Grant No-F-65-3/2012 (CU); dated 07 August 2012.

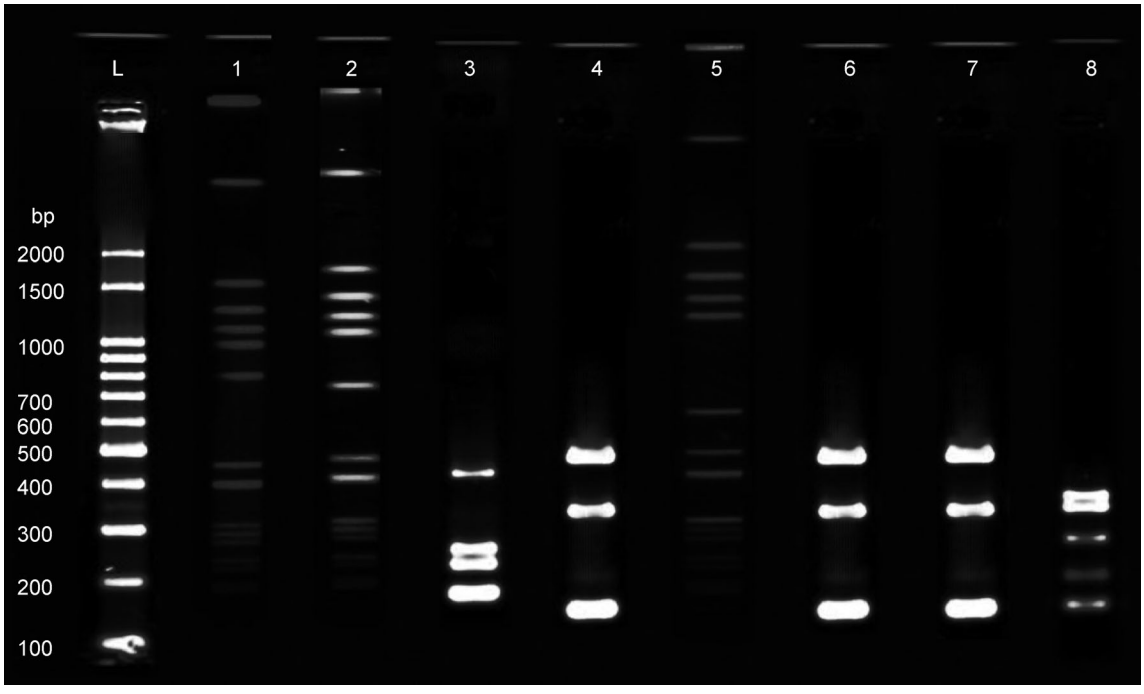

Figure 4 Multiplex PCR of samples run to type the *SCCmec* gene.

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