-Discussion
17. Discussion

Since the invention of antibiotics, every new drug has been followed by an emergence of a new strain of bacteria with resistance to that antibiotic. Interestingly, resistance has been reported to originate in North America and Europe which then slowly spread to other parts of the world including Asia. The proposed hypothesis for this was the global connection through air travel. It has been found true even in the case of MRSA. HA-MRSA was first reported from Europe in 1961. Now it is a global menace. It is only after a lapse of about two decades or more CA-MRSA originated in UK, but its undesirable impact was noticed only following the deaths of 4 US children who were devoid of any associated risk factors. This UK strain later acquired more virulence and emerged as an epidemic strain of MRSA (EMRSA), many variants of which viz. EMRSA-1 to EMRSA-17 have so far been reported. Now a more virulent strain of the same EMRSA-15 which made its appearance in India became one of the major causes of severe skin and soft tissue infections and meningitis.

17.1 Geography and population of Udupi

Udupi taluk is one among the three taluks in Udupi district of Karnataka state, India having a tropical climate. As per 2011 census report, the population at Udupi district is 117,7361. Male population constitutes 49% of the total population and females 51%. Population growth rate at Udupi district is 5.85% as per 2011 report. The average literacy rate is 83%, higher than the national average of 59.5%; male literacy is 86% and female literacy 81%. 

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17.2 Prevalence of nasal carriage of MRSA among school children of Udupi Taluk and associated risk factors

Complications associated with SA infections are severe and if the strain is methicillin resistant (MRSA), the consequences may be very complex and hard to manage. The famous proverb in English “Prevention is better than cure” is literally true with respect to the control MRSA infections. To plan and implement appropriate prevention strategies for any infectious diseases, one need to know the prevalence and distribution of that particular disease in the community and also should have a knowledge of the epidemiological type of the strain prevalent in that community. Nasal carriage is found to be the most important source of MRSA infections in the community as well as in the hospitals.\(^\text{104, 143, 144}\)

Prevalence of MRSA nasal colonization among children of Udupi taluk during the current study period was found to be 11 children per 1000 population with a 95% CI ranging between 6 and 17 cases per 1000 population. Prevalence of SA colonization during the same period was estimated as 29.3% \((95\% \text{ CI 27, 31.6})\). Stratified cluster sampling method adopted in this study, by way of its proportionate and methodical approach ensures a high degree of authenticity and acceptability. In the absence of adequate number of reports in this area, it may not be irrational to generalize and use the currently acquired data all over India, especially in the wake of not much difference being there in geography and population dynamics.

SA nasal carriage is more among children and the rates decreases with advancing age.\(^\text{13, 145, 146}\) Even though the exact reason is unknown, the increased frequency in contact with fellow children and being most of the time in a group make
children more susceptible to SA colonization. To the best of our information this is the first report of this kind from India which provides information about the prevalence of MRSA nasal carriage in children in the age group of 6 – 16 years. The limited data available is either collected from patients attending hospital outpatient departments or from family members accompanying the patients. Nasopharyngeal carriage of MRSA in children was reported from India by Chande et. al. According to them the prevalence of nasopharyngeal carriage of MRSA in children is very low (0.31%). Pathak et. al. reported the prevalence of SA colonization in children below 5 years as 16.3% and MRSA colonization as 6.3% in a hospital based study from Ujjain. Ramana KV et. al. reported 16% of SA colonization and 19% of MRSA colonization in school children of 5 to 15 years old from Narketpally, Andhra Pradesh. This 19% was among the SA isolates. When the prevalence was recalculated after keeping the subjects screened as denominator (12/392) the rate obtained was 3.1%. Chatterjee SS et. al. also reported a low prevalence of MRSA colonization (3.9%) among school children at Chandigarh. MRSA colonization rates inferred through all these three studies are more or less comparable with our findings (1.1%). While the risk factors associated with SA or MRSA colonization have not been addressed in the earlier studies, an earnest attempt has been made in this regard in the present study. It can be observed that the focus of sample size calculation was on the prevalence of SA colonization in the community rather than the prevalence of MRSA in the earlier studies. Moreover the exact mode of sampling is also lacking in the above reports. Sample size was noticeably low in Narketpally and Chandigarh studies. Sample size was satisfactory in the Ujjain study (n>1500) which was much similar to that of ours. The interesting finding in the present study is the high SA colonization rate (29.3%) and low MRSA colonization rate (1.1%) at Udupi when compared to the data from other parts of India. The true population value of MRSA colonization at Ujjain lies within a narrow (5.1, 7.5) range at 95% CI whereas at Udupi it is rather wider (0.6, 1.7). A recent
investigation by them at Ujjain itself in the same age group (< 6 years old) however, gives an alarmingly high colonization rate of MRSA (29%). It is interesting to note that the SA colonization rate of 35% reported by them does not deviate much from the national average.\textsuperscript{150}

SA and MRSA colonization rates vary from community to community and it is of low occurrence in resource poor countries compared to those reported from developed countries. A convincing explanation was offered by Sivraman et. al. that a higher prevalence of SA nasal colonization (26% to 35%) in industrialized countries is attributable to better personal hygiene and advanced living conditions.\textsuperscript{151} The proposed reason for this could be the lack of protective antibodies produced in them because of lower exposure to SA. Udupi is one among the fastest developing region in India where people enjoy better health care and educational facilities. This could be a good reason for the higher rate of SA colonization in our population, as well. Among the parents of our study subjects 100% of the male and 70% of the female counter parts are gainfully employed which bestows better hygienic practices. A reasonably good percentage (60%) of the parents have attained at least a primary level education which also can be a factor favorable for keeping better hygiene and thus the resultant lesser exposure to SA. The fact that a higher exposure to SA, for example, a job in a hospital can be a risk factor for MRSA colonization has been brought about by a study conducted by Nakamura et. al. in 2001.\textsuperscript{152} A good number of reports from Indian population are much warranted to afford a consistent and acceptable data pertaining to SA/MRSA distribution rate. It will be worthwhile looking at the colonization rates procured from other countries to get a better picture in this regard (Table 23).
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year of publication</th>
<th>Country</th>
<th>Age group</th>
<th>Study setting</th>
<th>Sample size</th>
<th>SA nasal carriage rate</th>
<th>MRSA carriage rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dey S et. al.</td>
<td>2013</td>
<td>India</td>
<td>1-6 years</td>
<td>Day care centers</td>
<td>1002</td>
<td>35%</td>
<td>29%</td>
</tr>
<tr>
<td>Ho PL</td>
<td>2012</td>
<td>Hong Kong</td>
<td>2-5 years</td>
<td>Day care centers</td>
<td>2211</td>
<td>27.6%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Juan RP</td>
<td>2012</td>
<td>Colombia</td>
<td>2-6 years</td>
<td>Pre school</td>
<td>104</td>
<td>38.5%</td>
<td>4.8%</td>
</tr>
<tr>
<td>Ashish P et. al.</td>
<td>2010</td>
<td>India</td>
<td>1 month to 59 months</td>
<td>Hospital out patients</td>
<td>1562</td>
<td>16.3%</td>
<td>6.3%</td>
</tr>
<tr>
<td>Ramana KV et. al.</td>
<td>2009</td>
<td>India</td>
<td>5-15 year</td>
<td>Schools</td>
<td>392</td>
<td>16%</td>
<td>3.1%</td>
</tr>
<tr>
<td>Lamaro-Cardosa J et. al.</td>
<td>2009</td>
<td>Brazil</td>
<td>2 months to 5 years</td>
<td>Day care centers</td>
<td>1192</td>
<td>31.1%</td>
<td>1.2%</td>
</tr>
<tr>
<td>Regev YG et. al.</td>
<td>2009</td>
<td>Israel</td>
<td>0-40 months</td>
<td>Primary care center</td>
<td>4648</td>
<td>7.6%</td>
<td>Low (3 children)</td>
</tr>
<tr>
<td>Ko KS et. al.</td>
<td>2008</td>
<td>Korea</td>
<td>1-11 years</td>
<td>Hospital out patients</td>
<td>296</td>
<td>32.1%</td>
<td>6.1%</td>
</tr>
<tr>
<td>Ciftci H et. al</td>
<td>2007</td>
<td>Turkey</td>
<td>4-6 year</td>
<td>Schools</td>
<td>1134</td>
<td>28.4%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Lo WT et. al.</td>
<td>2007</td>
<td>Taiwan</td>
<td>Less than 7 years</td>
<td>Day care centers</td>
<td>68</td>
<td>25%</td>
<td>13.2%</td>
</tr>
</tbody>
</table>
Discussion

A statistically significant difference (p < 0.05) in colonization rates of SA and MRSA among boys and girls were noticed in Udupi taluk during the study period, with the boys bearing a risk of 1.8 times more than that of the girls. As far as the MRSA colonization is concerned it is even more striking with a 4.67 fold increase in risk for the boys. Other demographic factors had negligible influence on MRSA colonization. However, when it comes to the SA colonization demographic factors like category of schools and age group had some influence (p<0.05) evident from univariate analysis. Multivariate analysis data negated the association between SA colonization and categories of school (Government, private aided and private unaided) but not that of the different age groups. This is discernible from the observation that the children of upper primary age group (10-12 years) were at a 33% less risk compared to lower primary age group (5-9 years). This tendency turns out to be little more evident with a 67% less risk for high school children (13-16 years). A plausible hypothesis could be a more frequent interaction among smaller children in their own ways at school coupled with deficient immunity. Many other reports from India corroborate this view.143, 144, 148

The influence of seasonal variation on SA colonization was also studied. It was noticed that the risk associated with both summer and winter seasons was found to be 1.5 times that found in the monsoon season. Since prevalence of MRSA colonization was extremely low in our study population, logistic regression model could not be developed to study the strength of association between MRSA colonization and various risk factors. However, when the different risk factors were subjected to univariate analysis, only the factor ‘gender’ was found to have statistically significant
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association. This could be ascribed to deficiency in the number of cases (17/1503) in the population. Recurrent sinusitis and diabetes mellitus afflicted population gave a strong indication for SA colonization in univariate analysis. Though this preponderance could be sustained in the multivariate analysis also, the logic involved was, however, poor in the sense that it depended on the ‘don’t know’ response. It may not be out of place here to recognize the findings of Kutlu SS et. al. that MRSA colonization among diabetic patients reached as high a level as 9.9%. The increased risk was assessed to be 1.6 fold by another two groups of workers.

17.3 Characterization of MRSA

Characterization of MRSA isolates were carried out by determining antibiotic susceptibility pattern, urease production, presence of *pvl* gene, SCC mec types and PFGE patterns. Antibiotic susceptibility pattern gives an initial clue whether a particular strain of MRSA is hospital associated or community associated. Unlike multi-drug resistant HA-MRSA, CA-MRSA in general shows resistance only to beta lactam antibiotics such as penicillin and cephalosporin groups. This is attributed to the low number of drug resistance gene harbored by the short SCC mec (21kb to 25 kb) element of CA-MRSA compared to the large cassettes (34 kb to 67 kb) of HA-MRSA. This is evident in the present work as well. None of the SA isolates were multi-drug resistant. However resistance to ampicillin was more pronounced among SA and MRSA isolates (88%). Resistance to ciprofloxacin, erythromycin and amoxicillin – clavulanic acid combination was also noticed in a few isolates (38%, 30% & 20% respectively). All the isolates were susceptible to vancomycin and linezolid. This resistance pattern is characteristic of CA-MRSA type.
Emergence of CA-MRSA as a nosocomial pathogen is a challenging issue. This was even pointed out by Seybold et. al. in one of their reports.\(^\text{157}\) Boucher et. al. in their review article exemplified a case of multi-drug resistant CA-MRSA infection.\(^\text{104}\) Major consequence of such infections is the treatment failure. A soothing fact to be noted here is the absence of such multi-drug resistant MRSA in our communities. This indicates that neither HA-MRSA has invaded into our community settings nor CA-MRSA has acquired multi-drug resistance genes in this area.

Among all the clinical isolates of MRSA that we studied, resistance pattern was almost the same indicating that they are all purely of CA-MRSA type. It is thus assumable that majority of the strains causing infection in our community and the ones which colonize children belong to the same epidemiological type.

Another major concern for the clinicians while choosing the appropriate antibiotic regimen has been the emergence of inducible clindamycin resistance among MRSA isolates. Routine investigations of MLSBi status would, therefore, be of great significance in the choice of antibiotics. The same holds good in the case of the colonizing strains as well. It needs to be emphasized here that, in the population investigated, a high incidence of inducible clindamycin resistance (71\%) was noticed among colonizing strains of MRSA. When the inducible clindamycin resistance crops up, a roughly 25 fold increase is registered in MRSA when compared with MSSA.

When it comes to the question of molecular characterization of MRSA using PVL assay, a large proportion (59\%) has been found to be producing PVL toxin. It is a matter of immediate concern that needs to be addressed effectively, lest it should become a threat to the control of infectious diseases. A regular screening of children...
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for MRSA colonization at the time of hospitalization would go a long way in controlling the spread of such infections. PVL production though not a unique feature of MRSA, the virulence associated with it is sufficient to cause severe skin and soft tissue infections such as necrotizing fasciitis or necrotizing pneumonia.\textsuperscript{53, 158} Now that CA-MRSA has evolved from PVL positive MSSA\textsuperscript{159–161} and that the occurrence is of a significant proportion, utmost care and caution need to be exercised in dealing with the MRSA carriers. A much welcome approach would be to include PVL detection also as an integral part of the routine diagnostic panel of MRSA infections.

When the clinical isolates sampled in this study were considered with respect to PVL production, it was found to be of a sizeable percentage (78.7%). Permitting the spread of such strains among the patients or in the community would meet with dire consequences. Thus it becomes a compelling need to subject all the MRSA isolates for PVL assay in order to initiate effective precautionary measures.

SCC mec typing has become more popular and accepted molecular typing technique among microbiologists and epidemiologists as a method to discriminate HA and CA – MRSA types. Employing this technique we could detect 41% MRSA strains from the anterior nares of school children classified as SCC mec IV with a total absence of type V. While clinical isolates were taken up, 64% of these were confirmed to be of CA-MRSA type, out of which 34% could be categorized as type IV and the rest 30% as type V. The remaining 36% of strains could not be typed with the methods employed. The fact that a major 90% of CA-MRSA were assigned PVL positive in the current study, corroborates the generally accepted hypothesis concerning PVL production in CA-MRSA. A statistically significant association
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(p<0.05) was noticed between clinical CA-MRSA isolates and PVL production which lends further support to the aforementioned findings.

DNA finger printing by PFGE is considered as the ‘gold standard’ test for epidemiological typing of MRSA which normally is adopted in outbreak investigations of infectious diseases, thanks to its high discriminating power, reproducibility and reliability.\textsuperscript{162} Only a few random selected strains were subjected to the above mentioned method. Four isolates collected from anterior nares of school children and 2 isolates from a case of necrotizing fasciitis (first isolate and the repeated isolate) were included in this DNA finger printing method. The classical EMRSA-15, NCTC 13142, NCTC 8325 and an Indian variant EMRSA-15 isolated at Bangalore\textsuperscript{84} were used as reference strains. The rationale for selecting EMRSA-15 as one of the references was the recent reports on the emergence EMRSA-15 infections in South East Asian countries.\textsuperscript{84, 85, 122, 163} Almost all the CA-MRSA isolates (6/7) collected from the school children belonged to the urease non-producing category, an observation which has been well recognized as a feature of EMRSA-15. None of the six clinical/colonizing strains studied were identical to NCTC 8325 strain (HA-MRSA) in their PFGE pattern. However, the strains can be assigned as the Indian variant EMRSA-15 in spite of there being a variation in only 3-4 bands of the classical EMRSA-15 (NCTC 13142).

Further detailed molecular characterization of the clinical isolates (Ro 1087 & Ro 370) selected for PFGE were carried out to get a deeper insight into the presence of other more virulent genes such as accessory gene regulator (agr), Enterotoxin gene cluster (egc) and staphylococcal protein A (spa). Detection of egc came out to be
positive for all the tested strains. Both the isolates belonged to \textit{agr} type I, ST 22 and \textit{spa} type t 852 (Published as a case report by Govindan et. al.).

The findings obtained so far bear ample testimony to the fact that all MRSA strains endemic in this region (Udupi taluk) originally belonged to a single clone identical to the Indian variant of EMRSA-15 irrespective of the source (colonizer or clinical). The epidemiological significance of the latter gets enhanced through the acquisition of \textit{pvl} and \textit{egc} genes. Perhaps, the minor difference in the banding pattern of current study isolates in comparison with those of classical EMRSA-15 in PFGE, accounts for the probable genetic changes that the strain would have inherited during the evolution process.

Emergence of the virulent Indian variant EMRSA-15 in our community is a worrisome fact to be reckoned with, notwithstanding the low prevalence of nasal colonization of MRSA. Highly organized precautionary measures on the part of health care professionals are warranted for an effective control of the spread of these deadly strains, in the light of the findings evolved from this epidemiological study.