

## PREVALENCE OF NASAL CARRIAGE OF METHICILLIN RESISTANT STAPHYLOCOCCI IN HEALTHY POPULATION OF GANGTOK, EAST SIKKIM

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**Abstract:** Methicillin Resistant *Staphylococcus Aureus* (MRSA) strains emerged in the last decade as one of the most important nosocomial pathogens. MRSA may invade the blood and cause potentially serious complications such as bacteremia, septic shock, and metastatic infections. MRSA infections have recently been identified in the community. There are few studies regarding carriage state of MRSA in the community and hence, the Epidemiology of MRSA in the community is little understood. Objectives of the study were to (1) to determine the prevalence of staphylococcal nasal carriers among healthy adults of Gangtok, East Sikkim. (2) to determine the proportion of Methicillin Resistant *Staphylococcus* species isolated from nasal carriers in healthy adults of the community. One Nasal swab from each of a total of 280 apparently healthy individuals belonging to 5 different areas of Sikkim was collected using Simple Random Sampling. The collected data was tabulated in spreadsheets of Microsoft Excel version Office 2000 and analyzed by Epi Info version windows 2000. 247 of 280 swabs (88.2%) out of them were found positive for staphylococcus. Among 247 staphylococcus nasal carriers, 129(52.2%) individuals were positive for *S. aureus*. Staphylococcal nasal carriage among healthy adults not exposed to hospital environment was found to be high (88.2%). It was also found that 31(24.0%) nasal swabs were positive for MRSA among those positive for *S. aureus*. The prevalence of MRSA in community was thus estimated to be of 11.1%. Majority of MRSA nasal carriers in the community belonged to the age group of 20 and 40 years. Proportion of MRSA nasal carriers was lower in Ranipool (16.2%) but higher in Loomse (38.5%) than in other areas. High carriage rates in different areas located far away from tertiary care level hospitals reveal that living close to a hospital is not a risk factor for MRSA or MRCNS colonization. Epidemiological studies including genotyping are required to understand in detail, the dynamics of spread of MRSA and MRCNS in the community.

**Key words:** Prevalence, Methicillin Resistant, *Staphylococcus aureus*, Nasal Carriers, Community

### INTRODUCTION

*Staphylococcus aureus* (*S. aureus*), a Gram positive coccus, is frequently found as a part of the normal human microflora. The organism can be carried asymptotically for weeks or months on mucous membranes but only transiently on intact skin<sup>1</sup>. Nasal carriers of *S. aureus* are more prone to skin sepsis and postoperative staphylococcal infections than non-carriers<sup>2</sup>. Studies show that *S. aureus* teichoic acid, which is present in the surface of *S. aureus* and coagulase negative staphylococci, is the primary factor necessary for attachment to nasal vestibular mucosa<sup>3</sup>. *S. aureus* was the most common cause of nosocomial infections reported in USA during 1990-1996<sup>4</sup>. Methicillin Resistant *Staphylococcus Aureus* (MRSA) strains were initially described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens<sup>5</sup>. MRSA may invade the blood and cause potentially serious complications such as bacteremia, septic shock, and serious metastatic infections (endocarditis, pneumonia, osteomyelitis, and arthritis)<sup>6</sup>. Healthcare workers' hands, the environment, and airborne transmission (in the case of staphylococcal pneumonia) are the most common means of spreading MRSA. Infected and colonized patients provide the primary reservoir and transmission is mainly through hospital staff<sup>7</sup>. Common factors associated with acquiring MRSA in any acute care setting include prolonged hospital stay, use of broad spectrum antibiotics, greater number and longer duration of antibiotic

use, stay in an ICU or burn unit, surgical wounds, decubitus ulcers, poor functional status and proximity to another patient with MRSA<sup>8,9</sup>. MRSA is a strain of *S. aureus* that has developed resistance to methicillin and other beta $\beta$ -lactamase-resistant penicillins and cephalosporins<sup>10</sup>. However, MRSA infections have recently been identified in the community, which raised a question of whether these infections were transmitted from hospital, or they were caused by different resistant strains. The sharp increase in the prevalence of MRSA acquired infections in many communities had led to the consideration of outpatients as a source of infection in an institution<sup>11</sup>. However, there are few studies regarding carriage state of MRSA in the community. Majority of the studies, so far, had been conducted on the patients and staff members of the hospital<sup>6</sup>. Epidemiology of MRSA in the community is little understood or not studied at length. A few reports on MRSA in the healthy population of Nigeria, USA, Canada, Pakistan and Japan are available in the world literature. Till the beginning of study no report on the prevalence of MRSA in the community in India was available. Case reports of community acquired MRSA infections had been increasing since last 3 years in the tertiary care level hospitals in Gangtok of East Sikkim. Hence, there was an urgent need for evaluation of nasal carriage of staphylococci and Methicillin resistant staphylococci. With this background, a study was undertaken to determine the prevalence of MRSA among healthy subjects

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in the community in Gangtok of East Sikkim in India.

## MATERIALS & METHODS

**Background Information:** Gangtok with Geographic-locations of (Lat/Lon Bounding Box: North=27.333332 South=27.333332 East=88.61667 West=88.61667 and altitude of 1547 m) has a population of 550,000. There are two tertiary care level hospitals, one government and the other private, at a distance of 5 miles apart in East Sikkim.

**Study Period:** Two months (March 2005 - April 2005).

**Sample Size:** One Nasal swab from each of a total of 280 apparently healthy individuals was collected for the study.

**Sampling Technique:** Simple Random Sampling by using the probability proportionate to sample size (PPS) method was used.

**Selection Method:** At the beginning, a spot-map of dwellings of different areas of East Sikkim was prepared. Then each village and nodal areas were identified in it and numbered serially. From these numbers, five spots (areas) in the map were randomly selected through lottery method. Thus, the areas selected for survey were - Deorali-Daragaon, Metro-point, Sarswati temple area, Loomse & Ranipool in East Sikkim. Households in the selected survey areas were numbered serially and specific number of households in each area (as calculated by power analysis from software package of epi-info version windows 2000) was chosen according to PPS method. Individual households were selected by using the random number table. Only one individual from each household was selected for the study through lottery method.

**Study Area:** Five areas that include Deorali-Daragaon, Metro-point, Sarswati temple area, Loomse & Ranipool.

**Inclusion Criteria:** Only one individual was included in the study from each household. Selection was done through lottery method after arranging all household members in ascending order of age.

**Exclusion Criteria:** Since, *Staphylococcus aureus* (*S. aureus*) was frequently found as a part of the normal human micro flora, children below 13 years of age were excluded from the study. Persons who had been admitted in a hospital in the preceding 12 months or had used any antibiotic during that period or worked in a health care center were also not included in this study.

**Materials used for the survey:** Sterile cotton-swabs, sterile test tubes, nutrient agar (HiMedia Laboratories Private Ltd.), Mueller-Hinton agar (HiMedia Laboratories Private Ltd.) supplemented with 4% NaCl, Oxacillin disk-1 $\mu$ g (HiMedia Laboratories Private Ltd.), Control strain NCTC 6571 (ICMR, Dibrugarh), Other reagents for catalase, oxidase, Coagulase, phosphates, DNase and sugar fermentation tests.

**Data collection procedure:** Nasal swabs were collected by sterile, dry cotton swabs from anterior nares of each nostril of a subject, inserting the swab and then gently rotating the swab three times<sup>12</sup>. The swabs were immediately placed in test tubes for further processing in the laboratory. All the isolates were

tested for coagulase production following standard procedures. *Staphylococci spp* isolated were tested for Methicillin resistance by using modified Stokes same plate comparative disc diffusion method<sup>23</sup> using 1 $\mu$ g Oxacillin disk. Mueller-Hinton agar with 4% NaCl medium was used to detect Oxacillin resistance, incubated at 35°C for 24 hours<sup>13</sup>. Zone diameter of the test strain was measured in millimeter with a scale. Strains were classified as resistant or sensitive following standard procedure.

**Data Analysis:** The collected data was tabulated in spreadsheets of Microsoft Excel version Office 2000 and analyzed by Epi Info version windows 2000.

## RESULTS

Nasal swabs from 280 healthy adult subjects were examined and among them 247(88.2%) were found to be positive for staphylococcus. Of 247 *Staphylococcal* nasal carriers, 129(52.2%) were positive for *Staphylococcus aureus* (nasal carriers of *S. aureus*) and the remaining coagulase negative staphylococci. Out of 129 *S. aureus* isolates, 31(24%) isolates were Oxacillin resistant and these are referred as MRSA. The prevalence of MRSA in community was thus estimated to be of 11.1%.

Among 247 staphylococcus nasal carriers, 171(69.2%) were males and 76(30.8%) were females. *S. aureus*, isolated from 19(21.6%) out of 88 male, and 12(29.3%) from the 41 female carriers were Methicillin resistant. However, difference in carriage rates of MRSA among male and female subjects was statistically not significant [ $\chi^2=1.79$ ,  $p=0.181$ (Yates corrected)].

MRSA nasal carriers in the community were high (26.6%) in age group of (20-40) years of age and less (10%) in age groups below 20 years and above 40years. MRSA nasal carriage was lower in Ranipool (16.2%) and higher in Loomse (38.5%) than in other areas. But these differences are statistically not significant [ $\chi^2=1.72$ ,  $p=0.156$  (Fisher exact 2-tailed)].

Out of 247 staphylococcal isolates, 118 (48%) were Coagulase Negative Staphylococci (nasal carriers of CNS) and among them, 26(22%) CNS was found to be resistant to oxacillin, referred to as Methicillin Resistant Coagulase Negative Staphylococci (MRCNS). Sex-wise break up of nasal carriers of CNS and MRCNS did not show any significant difference [ $\chi^2=0.04$ ,  $p=0.835$  (Yates corrected)] in the rates of nasal carriage among male and female carriers. MRCNS prevalence was high near the Saraswati temple area (28.6%) of lower Tadong and Loomse (28.6%). MRCNS nasal carriage rate was also higher in the age group below 40 years of age (24.75%).

## DISCUSSION

*S. aureus* nasal carriage rates in various populations have been investigated in the developed countries with temperate climate<sup>14</sup> but no such study among healthy population had been reported from India so far. Researchers reported that nasal carriage of *S. aureus* varied in different communities. The

results of the present study showed that nasal carriage of staphylococci was as high as 88.2% and in 52.2% cases, *S. aureus* were isolated. The prevalence of MRSA in the apparently healthy community of East Sikkim was estimated to be of 11.1%. A total of 129(46.1%) among 280 healthy individuals screened were nasal carriers of *Saureus*. Similar findings were reported by Anwar *et al* in their study in Lahore, Pakistan who screened 1024 and 636 apparently healthy persons from urban and rural area respectively for nasal carriage of *Staphylococcus aureus* and MRSA and reported that in urban areas prevalence of nasal carriers of *S. aureus* was estimated to be 16.99%, but in rural areas, it was 11.32%. In urban areas prevalence of nasal carriers of MRSA was found to be 22.98% as against 11.11% in rural areas<sup>11</sup>. In a study by Lamikanra *et al* it was observed that 56.4% of healthy Nigerian students were nasal carriers of *S. aureus*<sup>14</sup>. Tanaka *et al*, while studying *S. aureus* in healthy individuals in Japan reported 24.3% of them to be of nasal carriers<sup>15</sup>. In a study conducted at University of Texas, F. Moreno *et al* reported that 99 (58%) of 170 isolates of *S. aureus* were from community cases; the community to nosocomial case ratio was 2:1; no significant risk factors differentiated patients with community MRSA from community MSSA<sup>16</sup>.

There was no statistically significant difference in the prevalence of *S. aureus* nasal carriage between male and female subjects in the present study. This finding was contrary to that observed in the study done in Nigerian population where females harbored *S. aureus* significantly more often than males<sup>14</sup>.

An area-wise analysis of methicillin sensitive vs. resistant strains of CNS infections showed that statistically there was no significant difference in nasal carriage rate of MRCNS in areas near a tertiary care level hospital and away from the hospital. The reason for much higher rate of MRSA nasal carriage in Gangtok needs to be further investigated. A surveillance centre is necessary to be established for monitoring the problem of MRSA and MRCNS among the general population in India since Methicillin Resistant strains are increasing in other parts of the world. It is clear that epidemiological studies including genotyping are required to understand in detail, the dynamics of spread of MRSA and MRCNS in the community. Moreover, acute and recurrent infections with *S. aureus* and MARSAs are a possibility of developing drug resistant staphylococcal strains in the community. As the threat of acquiring multi-drug resistant staphylococcal infection increases with increasing prevalence of MRSA in general population, it is recommended that more studies be carried out in a larger scale in the general population along with the hospitals in order to keep updated information on nasal carriage of MRSA.

## CONCLUSION

Case reports of community acquired MRSA infections had been increasing since last 3 years in the tertiary care level

hospitals in Gangtok of East Sikkim. Hence, there was an urgent need for evaluation of nasal carriage of staphylococci and Methicillin resistant staphylococci. The prevalence of MRSA in the apparently healthy community of East Sikkim was estimated to be of 11.1%. Majority of MRSA nasal carriers in the community belonged to the age group of 20 and 40 years. Proportion of MRSA nasal carriers was lower in Ranipool (16.2%) but higher in Loomse (38.5%) than in other areas. Staphylococcal nasal carriage among healthy adults, not exposed to hospital environment, was found to be high (88.2%). It was found in this study that there was no age / gender / location specific difference in nasal carriage rate of MRSA or MRCNS around the tertiary care level hospitals. High rate of MRSA nasal carriage among healthy adults in general population needed further investigation. High carriage rates in different areas located far away from tertiary care level hospitals reveal that living close to a hospital is not a risk factor for MRSA or MRCNS colonization. Epidemiological studies including genotyping are required to understand in detail, the dynamics of spread of MRSA and MRCNS in the community.

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