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NASAL CARRIAGE RATE OF POTENTIAL PATHOGENS AMONG HEALTHY POPULATION

Microbiology

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ABSTRACT

Introduction: Nasal cavity can act as filter of unwanted particles entering the body & also has potential to cause endogenous infection by harbouring potential pathogens. Many studies have been done in hospital set up assessing nasal carriage, but only few studies are available on healthy adults in community . This study aims to know the prevalence of nasal carriers with potential pathogens in the community. **Material & Methods:** 100 Nasal swabs were taken aseptically from the anterior nares of, healthy people from the community & processed in the lab for potential pathogens.

Results: All 100 participants have shown some growth. 86% of the samples grew Coagulase Negative Staphylococcus (CONS) , predominantly *S.epidermidis. Total of* 54.0% were colonized with at least one of the known potential bacterial pathogens assessed. The overall prevalence of nasal *S. aureus* carriage was 30.0% ; amongst them 11 (36.6%) were MRSA. 34 % of the participants were nasally colonized with Enterobacteriaceae (*Escherichia coli* (9.0 %), *Proteus mirabilis* (4.0%), *Citrobacter koseri* (8.0 %), *Enterobacter aerogenes* (7.0 %), *Klebsiella pneumoniae* (8.0 %), Morganella morganii (1 %)) and 3 % with non-fermenters *Acinetobacter baumannii* (1.0%), *Pseudomonas sps* (2 %).

Conclusion: Significant variation in nasal microbiota and differences in their composition between *S. aureus* carriers and noncarriers was observed. Cocolonisation rates with other organisms were less in MRSA carriers when compared to *S.aureus* carriers. MRSA carrier rate was higher in this study, as our study population was from male gender which is correlating with other studies. Epidemiological studies with genotyping are required to understand this in detail.

KEYWORDS

Nasal carriers, Healthy population, Nasal swabs, MRSA, Nasal colonization of GNB.

INTRODUCTION:

Microorganisms colonizing the human body will help in preventing the establishment of potentially harmful pathogens and thus assist in improving the immune system. Sometimes microbiota may promote the development of allergic diseases and can act as a major reservoir for endogenous infections. [1] Nasal cavity is one such rich area which has potential to cause endogenous infection.

Main function of nasal cavity is to avoid entry of foreign or unwanted particles from entering the lungs through the nasal cavity. Large particulate matter is removed from inhaled air in the anterior naris, a relatively dry environment lined by squamous epithelial cells, sebaceous glands and hair. Smaller particulate matter like bacteria, are trapped in a mucus blanket flowing deeper in the nose towards the oropharynx by the steady beating of cilia. Antimicrobial compounds such as lysozyme, lactoferrin, and secretory IgA are present in mucus and, along with mucosal immune cells, contribute to the innate immune system. Anterior nares are colonized by microbiota that is distinct from other regions of the integument which may play a crucial role in determining the reaction patterns of the mucosal and systemic immune system. The presence of microbes in this sticky milieu is an important component of immune priming[2].

The bacterial flora of the nasal cavity has potential bacterial pathogens such as *Staphylococcus aureus, Enterobacteriaceae, Pseudomonas's,* non-fermenters etc. Enterobacteriaceae and *S. aureus* which were increasingly found as colonizers of the nares and the intestinal tract are associated with travel activities, contact with or ingestion of contaminated food items or contact with livestock husbandries [1]. However, little is known about the normal microbiota of the nasal cavity in healthy adults.

Staphylococcus aureus is an important cause of infections both in the community and hospital. Most of the colonizers remain asymptomatic, but it can become invasive in susceptible hosts. Carriage of *S. aureus* is a significant risk factor for nosocomial invasive infections by *S. aureus*. Approximately 20% of humans are persistently colonized by this pathogen [3]. Bacterial colonization can progress into primary infection or secondary superinfection after viral infection in its host. They act as asymptomatic carriers & are also recognized source of community-acquired Respiratory tract infections [2].

Methicillin resistant *Staphylococci aureus* (MRSA) is a strain of *S. aureus* that has developed resistance to methicillin and other beta ß-lactamase resistant penicillins and cephalosporins. However, MRSA infections have frequently 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 [4]. MRSA in community can spread by contact with an infected wound or by sharing personal items, such as towels or razors, that have touched infected skin. Risk factors associated are crowding, skin-to-skin contact, and shared equipment or supplies. Athletes, refugees, day-care children and school students, military personnel who stay in barracks and those who recently received inpatient medical care are at higher risk. According to CDC update in 2016, studies show about 1 in 3 people carry *Staphylococcus sps* in their nose, usually without any illness. Two in 100 people carry MRSA (CDC). There are only 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. Epidemiology of MRSA in the community is little understood or not studied at length.

Colonization with enterobacteria and non-fermenters is associated

with several factors like hospitalisation, diabetes mellitus, intravenous drug abuse, dialysis treatment, topical antimycotic therapy, age of more than 70 years and oral antibiotic therapy, as well as certain characteristics of the place of residence i.e. contact with livestock husbandries. According to some studies, people colonized with Enterobacteriaceae show significantly less frequent S. aureus carriage, smoking and haemato-oncologic diseases. This may be due to previous antibiotic consumption, the bactericidal effects of smoking and bacterial competition in the nasal habitat [1]. Males were more likely to be MRSA carriers than females[1] [5][6] .

Many studies have reported nasal carriage of *S. aureus*, but little is known about other bacterial pathogens like GNB in Indian healthy adults. Limited data is available on occurrence and persistence of Enterobacteriaceae & other GNB in the nares, which may be transmitted to the environment and to other persons. Data on studies investigating nasal carriage with GNB are rare because most reports focus on colonization of the intestinal tract.

Therefore, the objective of this prospective study was to assess the prevalence of nasal colonization of important facultative pathogenic bacteria including *S. aureus*, Enterobacteriaceae and non-fermentative bacteria in samples from healthy population at Pune.

MATERIAL & METHODS:

Design and specimen collection :- Nasal samples were taken from the anterior nares of healthy people presenting for routine medical examination . Collection was performed using a moist Dacron/ cotton swab with a rotating technique in both anterior nasal vestibules aseptically.

Exclusion criteria:-. Those with symptoms of URTI, fever, who are on antibiotics in the preceding 1 month & individuals who did not give consent were excluded from the study.

Isolation and identification of bacteria

Nasal swab samples from nose of 100 healthy people on day 1 were enriched in BHI broth for 4 hrs. Then they are plated to 5% sheep blood agar, 7.5% mannitol salt agar (MSA), MacConkey agar and Chocolate agar then incubated in the incubator of 37°C.

Phenotypic methods like colony morphology on culture plates, Gram staining, biochemical reactions, etc were used to presumptively identify the type of microorganisms.

The plates were read and the staphylococcal colonies were identified from colony morphology . They were subjected to gram staining and catalase test. Gram positive coccus (GPC) in clusters giving positive catalase test were considered as *Staphylococci sps*. These were further processed by coagulase test to identify *S.aureus*. Isolates giving a positive slide & tube coagulase test were taken as *S.aureus*. Those who were negative for slide and tube coagulase were labelled as CoNS . *S. aureus* ATCC 25923 was used as a control strain. The 30 µg cefoxitin disk diffusion test was used for detection of MRSA strains according to CLSI guidelines.

GNB were phenotypically identified by automated culture system (Vitek 2 bioMe´rieux).

RESULTS:

A total of 100 nasal swabs were collected from healthy volunteers . All of them were Males with mean age of 30 ± 5 years. All showed growth with various organisms. Of these 86 sample grew CoNS . In addition, 54 (54.0%) of them were found to be colonized with at least one of the known potential bacterial pathogens. The overall prevalence of nasal *S. aureus* carriage was 30.0% (n = 30); among them 11 were MRSA. . 34 % of the participants were nasally colonized with Enterobacteriaceae (n = 34) and 3 % with non-fermenters (n =3). At the species level *Escherichia coli* (9.0 %), *Klebsiella pneumoniae* (8.0 %), *Proteus mirabilis* (4.0%), *Citrobacter koseri* (8.0 %), *Enterobacter aerogenes* (7.0 %), *Acinetobacter baumannii* (1.0%), *Pseudomonas aeruginosa* (1.0%), *Pseudomonas luteola* (1 %) & Morganella morganii (1 %) were detected.

Among those participants carrying Enterobacteriaceae (n =34), 30 were colonized with single enterobacterial species, 04 with two different species . Of the 03 participants colonized by non-fermenters, 2 carried one non-fermentative species & 1 *Acinetobacter sps* was with *Klebsiella pneumoniae*. Significant differences in the co-colonization patterns were mainly found for MRSA carriers (n=2) than *S. aureus* carriers (n = 9), who were less frequently colonized with various Enterobacteriaceae members.

DISCUSSION:

Various studies are done on nasal carriage of pathogens in various study groups worldwide mostly in children. Most researchers have studied *S. aureus* & MRSA carriage among health care workers. Very few Indian studies are available on prevalence of potential pathogens in healthy adults from community.

The nasal cavity is a physical transition zone between a space that is in constant contact with the external environment & a protected and highly regulated internal space. Anterior nares are major reservoir for S. aureus. Humidity and moisture may provide favourable environment for Staphylococcus species and other pathogens. An altered host immune state could contribute to susceptibility to *S. aureus* colonization as well as the selection of a distinct bacterial community structure[2].

Some studies show that interactions between microorganisms themselves may also influence which of the species are able to persist. Converse associations have been suggested between *S. aureus* and *Str. pneumoniae*. In our study also we didn't grow any *Str .pneumoniae* in swabs showing *S. aureus*. [3]

In our study among the 100 nasal swabs collected, 86 grew CONS predominant being *Staphylococcus epidermidis* , which is almost correlating with a study by Human Microbiome Project Consortium (HMPC)which was showing nasal carriage rates, 93% of *Staphylococcus epidermidis*. 54.0% of samples were also colonized with at least one of the bacterial pathogens assessed. Of positive nares cultures, 85 % had more than 100 CFU of each organism. 37 % were showing mixed growth of organism with GPC & GNBs. 19 swabs were showing pure growth of GPCs (10 MSSA & 9 MRSA).

Staphylococcal nasal carriage among healthy adults, was found to be about 30 % ,including MRSA (36.6%) which is similar to Devjyothi et al, Arucha et al & HMPC study where nasal carriage of 29% *S. aureus* including MRSA infections was reported . In their study 26.6 % of the MRSA nasal carriers were in age group of 20-40 years. In our study participants are 25 – 40 years of age.

Of the total *S.aureus* isolated, 36.6 % of them were MRSA which is higher than other studies. It may be because, our study group contains men only. As described by the other authors MRSA carriers are predominantly men[1][5][6] . Kock et al reported *S. aureus* colonization is more among males, and found that smokers were less frequently carriers of S. aureus, putatively due to the bactericidal activity of the smoke & occupational livestock contact had a 31 times higher risk for MRSA carriage compared to the rest of the cohort [1] . Anwar et al reported 14.82% of nasal swabs were positive for S. aureus & 19.51 % were MRSA. Nasal carriage was higher in males (15.47%) as compared to females (13.26%) .

Among 34 Enterobacteriaceae members co-colonisation with *S.aureus* were observed in 11 (32.35%) cases (9 MSSA & 2 MRSA), which is in contrary to Kock et al. 24 swabs have shown co-existence of CONS with Enterobacteriaceae.

In our study 34 % of the participants were nasally colonized with Enterobacteriaceae (n = 34) and 3 % with non-fermenters (n =3). Similarly Kock et al reported that 33.4 % of all participants from community were colonized with Enterobacteriaceae (with *K. oxytoca, E. coli, Citrobacter spp. and Pantoea spp*.) and 3.7% by non-fermenters. They also found that nasal colonization with Enterobacteriaceae also prevented *S. aureus* carriage. Arucha et al. reported *Klebsiella pneumoniae* 7.0% , *Citrobacter koseri* 3.9% , *Enterobacter spp* 3.1% , *Klebsiella oxytoca* 0.8% and *Proteus spp0*.8% were isolated among medical students [7] None of the studies reported *Pseudomonas aeruginosa*. Whereas Weil et al recovered only 12 % of GNBs (*Proteus mirabilis, Enterobacter aerogenes, K. pneumoniae, Escherichia coli*) from nares [9].

This suggest that the carriage pattern of some species in the human microbiome may be analogous to genetic traits, where recessive alleles of modest risk are maintained in a population. In the case of the human microbiome, high-risk pathogens remain absent, whereas species that pose a modest degree of risk also seem to be stably maintained in this

ecological niche[8].

CONCLUSION:

This prospective study provided insight into nasal colonization patterns of healthy adults with known potential pathogens like *S. aureus,* Enterobacteriaceae and non-fermenters. MRSA carrier rate was 11% in this study, which is correlating with other studies. Co colonisation rates with other organisms like GNBs were less in *S. aureus* carriers (32.35 %) when compared to S. aureus non carriers (67.65%). There is also significant difference in co colonisation of GNBs with MRSA carriers (2%) when compared to *S. aureus* carriers (9%). This shows significant variation in nasal microbial communities and differences in community composition between *S. aureus* carriers and noncarriers. High-risk pathogens like *Pseudomonas sps, Acinetobacter sps* were found less frequently when compared to species that pose a modest degree of risk (*E.coli, Citrobacter sps* etc) . Thus immune system is maintaining the niche of human microbiota in balance but chances are there for these moderate risk pathogens in causing opportunistic infections , in conditions associated with suppressed immune status of these healthy adults carrying them . It will be helpful to screen them before undergoing any major surgery especially for MRSA & treatment should be given for its eradication.

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