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ORIGINAL ARTICLE

Nasal screening and survey of pre-clinical medical students from Malaysia for nasal carriage of coagulase positive MRSA and rate of nasal colonization with *Staphylococcus* species

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ABSTRACT

Background: MRSA has long been implicated in the spread of nosocomial and community acquired infections which pose a threat for the emergence of carriers among the community and hospitals. This study was aimed at screening for methicillin resistant *Staphylococcus aureus* (MRSA) in students hailing from Malaysia, and characterizing the rate of carrier state along with nasal colonization with Staphylococcus species, among the different ethnicities of pre-clinical medical students before their entry into the clinical phase of their study.

Methods: 157 students were involved in the study. Samples were collected from the anterior nares of student volunteers. Biochemical tests were done to isolate *Staphylococcus aureus*. Species confirmation for *Staphylococcus aurues* was done using the tube coagulase test and the DNase test. Coagulase positive Staphylococci were subjected to oxacillin agar screen method to screen for MRSA.

Results: Out of 157 specimens, Staphylococcus species were isolated from 156 (99.3%) specimens, and one specimen showed no isolation of Staphylococcus species; 37 (23.7%) were Coagulase positive *Staphylococcus aureus* (CoPS), and 119 (76.2%) were Coagulase negative *Staphylococcus* species (CoNS). Of the total of 37 isolates of Coagulase positive Staphylococcus aureus, none were found to be resistant to methicillin (0%). All the 37 (100%) strains of CoPS isolated were methicillin susceptible *Staphylococcus aureus* (MSSA). The nasal carriage of CoPS among ethnic student communities were observed to be 22 (34.3%) in the Chinese; followed by Indians 12 (16.0%), and Malay 3 (17.6%).

Conclusions: The study revealed that out of the total specimens collected from student volunteers, none were carriers for MRSA. The highest percentage of nasal carriage for CoPS among the three main ethnicities of Malaysia was observed to be among the Chinese. All CoPS obtained were MSSA, while the highest rate of nasal colonisation with CoNS was observed in the Indian community. Screening should be made an essential protocol in order to assess and curb the in-flux of carrier transmitted drug resistant strains of Staphylococci from the community to the hospital setting.

Key words; MRSA, MSSA, CoPS, CoNS, Oxacillin agar screen, Nasal carriage, Antimicrobial drug resistance, methicillin susceptibility, Nasal carriage, Nasal colonisation Pre-clinical survey

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Introduction:

Staphylococcus aureus is one of the most common pathogen that has been known to cause a wide range of infections. *Staph aureus* colonization in the anterior nares is quite common, as its primary habitat is the moist squamous epithelium of the nares. Healthy individuals could become carriers of the organism and have a small risk of contracting an invasive infection due to *Staph aureus*.

The incidence of community-acquired and hospital-acquired *Staph aureus* infections has been on the rise, with the emergence of drug resistant strains called methicillin- resistant *Staph aureus* (MRSA). Prevalence of MRSA had been previously confined or limited to hospital settings, but as of late, incidences of MRSA infections in the community have also been reported in epidemiological surveys and studies. Bacterial, genetic and microbiological adaptive changes and properties gave rise to the emergence of antibiotic resistance of the organism. The drug of choice for Staphylococcal infections was penicillin, but indiscriminate use and genetic manipulations on the part of the organism slowly led to penicillin resistance.

Penicillin resistance was rampant during the antibiotic era, and in order to replace penicillin, methicillin was used. A year after the discovery of methicillin in 1959, Staphylococci started to show resistance even to methicillin, which led to the emergence of the 'super bug' MRSA (Methicillin resistant Staphylococcus aureus). Two years later, *Staphylococcus aureus* showed resistance to other beta-lactam antibiotics like oxacilllin, nafcillin, and the cephalosporins, that invariably contributed to a multiple drug resistance (MDR) pattern in this organism, [1] due to genetic mutations brought about by the *mec*A gene in the Staphylococcal chromosomal cassette. MRSA was first reported in 1961.[2] There were only sporadic outbreaks of MRSA, and this became a major problem only during the late 1970s and in the early 1980s. Many outbreaks were reported after that from different parts of the world [5]. Geographically, MRSA is distributed world wide.[11]

MRSA has been implicated in both community-acquired and hospital-acquired infections. They express heterogenous resistance to methicillin through the penicillin-binding protein 2a (PBP2a). This has been found to be the case in community acquired strains, as opposed to the hospital-acquired strains that show homogenous resistance patterns, though both contain the same gene [3], [4]. Some strains are called the epidemic strains, and can spread within or between hospitals, and can also spread between countries.[11] There is a greater risk now being posed, that these methicillin resistant strains could lead to heterogenous glycopeptide resistance which was first reported from Japan in 1997,[6] of intermediate resistance pattern to vancomycin of *Staphylococcus aureus* (VISA). It was thought that the resistant strains of *Staphylococcus aureus* had their origin in the hospital, but case studies throughout the world provided facts that were surprising to the scientific community in that, sporadic cases of MRSA isolations were being reported from the community reservoirs.

Since many clinical infections arise from spread from healthy carriers; it is important to assess and survey the population coming from other countries into India, as these become reservoirs of the organism. Clinical isolates from invasive infections can only focus on the severity of the disease, but does not give an estimate or prevalence of carriers among the healthy population. This formed the basis for our study and its importance of screening for healthy carriers of MRSA, and also to study the rate of colonisation of CoPs and CoNS among the student ethnicities from Malaysia.

There were no earlier studies conducted on the student carriage of the pathogen among Malaysian students studying in Manipal, India. The student volunteers were in their 1st and 2nd year pre-clinical phase of their medical degree. In this study, we investigated the probable carrier rate of the student community from Malaysia, and screened for carriers of MRSA as they could pose a potential risk factor for nosocomial transmission when the same carriers are exposed to the hospital setting during their clinical postings, which would start after the completion of the 2nd year of the medical course. This study was devised as a screening measure to isolate and characterize MRSA carriers in healthy student volunteers who could have acquired the organism from their home country or from India itself during their stay, and also to assess and compare colonization with CoPS and CoNS among the student population, especially from the three main ethnicities of Malaysia

which included the Malay, Chinese and the Indians; before their clinical exposure in the medical college hospital.

Materials and methods:

Study Design and sampling:

This study was approved by the institutional Kasturba hospital ethical clearance committee (KHEC) of Kasturba Medical College, Manipal, India, for the collection of samples from the student community which included 65 males and 92 females. This was a cohort study analyzing the carrier rate of MRSA in 157 students from Malaysia, whose ages were between 18-22 years. The 157 students were voluntary participants in this study, and samples were taken after written consent was obtained from the population under study.

Specimen Collection:

For the isolation of MRSA, samples were collected from the anterior nares of student volunteers using sterile cotton swabs soaked in sterile saline, and samples were directly inoculated onto sheep blood agar (FI-Chemechtron, Pvt. Ltd., Bangalore, India).

Processing of specimens:

Inoculated sheep blood agar plates were kept for 24 hours of incubation at 37^{o}C. Golden yellow to white, opaque, rounded, convex colonies were isolated for further analysis. These colonies were subjected to biochemical tests. Strains that were catalase positive and those that fermented mannitol, were identified as Staphylococcus species. Slide and tube coagulase tests were done for the confirmation of CoPS, followed by DNase test using DNase test agar with toludine blue. Control strains for the assays included, MRSA strains ATCC 33592 and ATCC 43300, and MSSA strain ATCC 29213.

Slide coagulase and tube coagulase test[7] : These tests were done in order to confirm coagulase positive Staphylococci.

DNase test[22]: Further confirmation of CoPS was done by detecting DNase activity by the use of DNase test agar w/ Toluidine Blue (M1041, HiMedia Laboratories Pvt. Ltd., Mumbai, India). The medium was prepared according to the manufacturer's instructions. Pinkish clearing around the colonies on the DNase test medium confirmed DNase activity.

Susceptibility screening:

Phenotypic detection of MRSA:

Oxacillin agar screen[8] :

Mueller-Hinton agar (MHA) No. 2 (M1084, HIMEDIA) with 4% NaCl (Universal Laboratories Pvt. Ltd., Mumbai, India) was used with 6µg/ml of Cloxacillin (500mg; Biochem Pharmaceutical Industries Pvt. Ltd., Mumbai, India) incorporated in MHA. CoPS were standardized to 0.5xMcFarland. The standardized suspensions were spot inoculated onto MHA.

Statistical Analyses:

Chi-Square test was applied to test the association between organism type and ethnicities. A significant association was observed between the organism type and the three different ethnicities of Malay, Chinese, and Indian Malaysian student population screened.

Results:

Of the 157 samples screened, 156 isolates were Staphylococcus species. With the help of biochemical characterization; 37 were identified as CoPS, and 119 were CoNS [Table/Fig 1]. MRSA from the oxacillin agar screen obtained were none. One specimen did not show any growth of Staphylococci. 156 specimens showed nasal colonization with Staphylococcus species. Staphylococci were isolated from 17 (10.8%) specimens taken from Malay Malaysians, 64 (40.7%) from Chinese Malaysians, and 75 (47.7%) from Indian Malaysians. Of the 17

Table/Fig 1 Percentage isolations of Staphylococci from the anterior Nares of student volunteers from Malaysia (n=157)

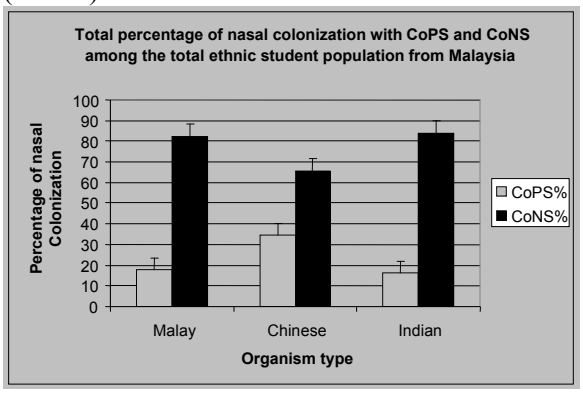
Organism type		Student Ethnicities		
		Malay (n=17)	Chinese (n=65)	Indian(n=75)
Total Staph. isolated		17	64	75
CoPS	Total	3	22	12
	CoPS %	17.6	34.3	16.0
	MRSA	0	0	0
	MSSA	3	22	12
CoNS	Total	14	42	63
	CoNS %	82.3	65.6	84.0

specimens from Malay Malaysians, 3 (17.6%) were CoPS; from a total of 65 Chinese specimens, 22 (33.8%) were CoPS, and from 75 Indian Malaysians, 11 (14.6%) were CoPS [Table/Fig 2].

Table/Fig 2: Nasal colonization with CoPS, CoNS, and MRSA in anterior nares of student ethnicities from Malaysia (n=157)

Organism type		Nos. Isolated	Total Percentage (%)
Staphylococci		156	99.4
COPS	MRSA	0	0
	MSSA	37	23.7

Although MRSA were not isolated, the highest rate of nasal carriage for CoPS according to this study was seen among the Chinese, followed by the Malay (even given their small sample size of 17), and the Indians in that order, while the highest rate of CoNS nasal colonisation was seen among the Indian students (84.9%) followed by the Malay (82.3%), and the Chinese (64.6%). 92 nasal swabs were collected



Table/Fig 3: *Total CoPS isolated from all three communities was 37 while CoNS was 119. The graph shows total percentage of nasal colonization for CoPS and CoNS among each community from total CoPS and CoNS isolated from all student ethnicities from Malaysia. The percentage ratio of CoPS:CoNS nasal colonization is higher in the Chinese while the percentage ratio of CoNS:CoPS is higher in the Indian community*

from females, and 65 were collected from male students. Out of 91 females (one sample from a Chinese Malaysian female yielded only Gram ne gative bacteria), 17 were carriers of CoPS (18.6%), while 74 were carriers of CONS (80.4%). Out of 65 specimens obtained from the males; 20 were COPS (30.7%), while 45 were CoNS (69.2%). From this study, from a sample size of 157, males showed a greater rate of nasal carriage of CoPS than the females.

Discussion:

The data obtained from this study revealed that there were no reservoirs or carriers of MRSA in the student population from Malaysia that were screened. Out of the 157 samples collected from student volunteers; 156 yielded Staphylococcus species, while one of the plates interestingly showed no growth of Staphylococcal colonies, but showed growth of Gram negative bacteria from the nasal swab collected from the anterior nares of the Chinese subject (female). Retrospective clinical history of this volunteer only showed that she had had a recent attack of diarrhoea and vomiting. The subject had also been administered with typhoid vaccine. Other than that, there were no other complaints related to, or that which could lead to nasal isolation of Gram negative rods. There have been interesting publications regarding the association of Staphylococcal nasal carriage with glucocorticoid receptor gene polymorphisms,[10] which states that genotype-dependent variation leading to glucocortocoid insensitivity may cause immune enhancement precipitating autoimmune disorders, while protecting from *S.aureus* colonization. This is just descriptive, which warrants for further investigation into the field and the organism that was isolated in this case was a gram negative bacterium that was not subjected for further analysis, as it did not fall within the scope of the project. The factors that distinguish between a carrier and a non carrier are still unknown.[17] Enhanced adhesion of *Staphylococcus* aureus[18], to cell associated and cell free secretions, along with induction of reduced mucociliary activity[19], could well explain the nasal colonization by *Staphylococcus aureus*.

Further, 23.56% of students screened showed nasal carriage of CoPS, while 75.79% had nasal colonization with CoNS. This goes to prove that one of the ecological niche for the colonization of Staphylococci is the anterior nares, as all the nasal specimens yielded Staphylococcal growth on culture, except for one specimen.[9] The greater amount of bacteremia cases recorded, have been due to *Staphylococcus aureus* of endogenous origin, since they originate from colonies of nasal mucosa. Most invasive infections are assumed to originate from nasal

carriage[13]. Hence, it is imperative that nasal carriage due to *S.aureus* strains should be prevented in order to stem the rate of infection, and in preventing the transmission of resistant strains of the organism.

Although nasal carriage of *S. aureus* is harmless in healthy individuals[14], they can become carriers[15] who could pose the risk of spreading infections to the community at large, and since the section of individuals under this study were medical students; their interaction and exposure to hospital environs could cause major risks in transmitting to hospital patients and spreading nosocomial infections. In one of the studies conducted in Australian pre-clinical and clinical students; hospital exposure to the students gave rise to Staphylococci, that showed a decreased percentage of isolates that showed sensitivity to three or more antibiotics, while there was an increase in the rate of carriage of *Staphylococci* that showed increased resistance to three or more antibiotics.[16] Studies on antimicrobial peptide analysis of nasal fluid revealed greater markers of inflammation in healthy nasal carriers.[20],[21].

The ratio of carriage of CoPS in the males and females in our study showed that the rate of carriage of CoPS was higher in males than in the females, although the sample size collected was much larger in females than in the males. There are no significant data available to correlate this finding. Two major ethnic communities in this study comprised of the Indians (75 in number) and Chinese (65 in number), apart from the Malays who were a minimum (17 in number). Taking the mean of the samples of these two ethnicities (Indian and Chinese Malaysians) into consideration; the ratio-proportion of nasal carriage of CoPS in Chinese to the Indians was 2:1. It is very interesting to see that the Chinese seem to have a greater rate of nasal carriage of CoPS. If we take the hypothesis of inflammatory markers into question, the most probable explanation to this could mean that the healthy Chinese population could well be having immunological tolerance towards the nasal colonization of CoPS, or could be harbouring genotype-dependent glucocorticoid insensitivity.[20], [21]. The explanations are purely hypothetical, and warrant for further study at the molecular and genetic level for analysis of *S.aureus* predilection in certain ethnicities.

It would be further interesting to study the same student volunteers after they are exposed to the hospital setting in their clinical exposure, during the second phase of their medical term; in order to get a comparative data to analyze the rate of carriage of Staphylococci and isolation of MRSA during their pre and post exposure to clinical hospital settings. Screening for resistant strains of Staphylococci in healthy students should be adopted as a protocol in medical colleges, in order to curb the spread of drug resistant Staphylococci from the community to the hospital. This will also help in monitoring the student population who might pose a risk to patients and hospital personnel; and the community at large.

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Conflicts of interest: None to declare

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