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Original Research Article

A Study on Methicillin Resistant Staphylococcus AureusCarriage in Medical Students before and after Exposureto Hospital Environment

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Abstract:

Background and Objectives: Methicillin resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that has developed resistance to methicillin and other beta lactam antibiotics as well as cephalosporins and monobactams. It harbors a gene, *mecA*, coding for altered penicillin binding protein (PBP 2a). Exposure to hospital environments is known for MRSA carriage. Health care workers (HCW) have often been implicated in outbreaks of MRSA associated hospital acquired infections due to MRSA carriage in their anterior nares, hands, axillae etc. To evaluate the prevalence of MRSA carriage in among the medical students. To assess the impact of hospital environment on the colonization by MRSA instudents with no previous exposure to the organism.

Materials and Methods: A total of 86 students were involved in the study. Nasal swabs were taken before exposure to the hospital, 6 months and 1 year after the exposure to the hospital environment. Swabs were cultured onto mannitol salt agar and blood agar. Standard microbiological methods were followed to identify *S. aureus* and methicillin resistance was checked for, using cefoxitin discs.

Results: No MRSA carriage was seen before hospital exposure. At the end of 6 months 2 students (2.3%) were colonized with MRSA and 3 students (3.48%) were colonized at theend of 1 year. The male: female ratio at the end of 1 year was 2:1.

Conclusion: Increasing rate of colonization with MRSA was seen with increase in duration of exposure to the hospital environment. Since MRSA is one of the major causes of Hospitalacquired infections (HAI) and HCW are considered the source of the infection, periodic screening and treatment of the carriers will go a long way in controlling HAIs caused by MRSA.

Keywords: *S. aureus,* Methicillin, Cephalosporins And Monobactams.

Introduction

Staphylococcus aureus (S.aureus) has been recognized as an important cause of disease around the world. It has become a major pathogen causing significant morbidity and mortality in both the community and hospital settings [1]. Methicillin-resistant Staphylococcus aureus (MRSA) has become widespread in hospitals worldwide and is responsible for causing skin and soft tissue infection, bacteraemia, septicemia, pneumonia, surgical site infections, and other nosocomial infections. The impact of Staphylococcus aureus infection on human health has dramatically increased as a result of its remarkable ability to become resistant to antimicrobials [2]. The difference between methicillin-resistant Staphylococcus aureus and methicillin susceptible Staphylococcus aureus is resistance to β-lactam antibiotics; this is often associated with resistance to multiple other antibiotics, which limits the therapeutic options [3,4]. Methicillin-resistant Staphylococcus aureus by definition harbors a gene, mecA, for methicillin resistance. The mecA gene codes for altered penicillin binding protein (PBP 2a) which is different from the indigenous PBPs of S. aureus. PBP 2a allows MRSA to continually synthesize its cell wall even in the presenceof β-lactam antibiotics. [5,6] MRSA strains were initially described in1961 and emerged in last decade as one of the most important nosocomial pathogens [7]. Exposure to the hospital environment of a prolonged duration have been established as a cause for increase in carriage of MRSA among the personnel [8,9]. This has raised our concern on MRSA carriage among theclinical medical students, who have been exposed to the hospital environment. Because of its primary habitat is moist squamous epithelium of the anterior nares, the source of most invasive S.aureus infections are assumed to arise from nasal carriage [3,8]. The reports regarding this issue have been scanty. Therefore, we conducted this study to figure outthe prevalence and epidemiology of nasal carriage of MRSA among

the medical students.

Objectives

- To evaluate the prevalence of MRSA carriage in among the medical students.
- To assess the impact of hospital environment on the colonization by MRSA instudents with no previous exposure to the organism.

Materials and Methods

The present study was undertaken at the department of Microbiology, at Patna Medical College and Hospital Patna, Bihar. Atotal of 86 medical students were included in the study of which 47 were males and 39 were females.

Inclusion Criteria

All students of PMCH Patna, entering 2nd year MBBS.

Exclusion Criteria: Students having active infections and a history of antibiotic usage in 15 days before the start of study.

Specimens were collected from the anterior nares by gently rotating the pre- moistened sterile cotton swabs for 5 times. In those who did not harbor MRSA in the 1stculture, nasal swabs were collected again at the end of 6 months and 1year respectively and examined for MRSA colonization. In those who showed positive for MRSA were treated with mupirocin and if showed negative for MRSA in 3 consecutive samplings were again included in the study.

Specimens were transported to the laboratory without delay.

Blood Agar: Medium sized (1-2µm diameter), circular, smooth, shiny, convex, easily emulsifiable colonies with or without beta-hemolysis were seen. **Chocolate Agar**: colonies similar to that of blood agar seen that is Medium sized (1-2µm diameter), circular, smooth, shiny, convex, easily emulsifiable colonies with or without beta-hemolysis were seen. **Mannitol Salt Agar:** similar colonies with golden yellow pigmentation seen.

1) Crystal violet was poured on the slide and kept for 1 minute.
2) After washing with water. Gram's iodine was poured and kept for 1 minute.
3) After washing with water, decolorization was done with acetone for 1-2 seconds.
4) Slide was then washed with water and dilute carbon fuchsin was poured on theslide and kept for 30 seconds.
5) Slide was washed with water, blotted and air dried. Slide was observed under oilimmersion.

Gram staining showed Gram Positive Cocci arranged in clusters, short chains and singles.

Positive: Any degree of visible clot formation.

Negative: When plasma remains wholly liquid or showed only a flocculent or ropyprecipitate.

DNase Test: Most of the strains of S.aureus hydrolyze DNA and give positive results. This test helps in identifying S.aureus strains that have given doubtful tube coagulase test. DNase agar with toludine blue indicator is used to detect deoxyribonuclease activity. DNase polymerizes the DNA resulting in the formation of a pink halo around the microbial growth

Method: inoculate the test organism onto DNase agar and incubated at 35˚C for 18-24hours

Interpretation: Positive- bright pink colour around the test organismsNegative- medium remains blue

All the isolates were tested for the methicillin resistance according to CLSI 2012 criteria: by Kirby-Bauer's disc diffusion method.

Procedure: Growth from an 18-24 hour culture was inoculated into peptone water using a sterile inoculating needle. The broth was incubated at 37^0C for approximately four hours. The turbidity of the broth was matched with the turbidity of the 0.5 McFarland turbidity standards (i.e. 1.5 x 10^8 CFU/ml).

A swab was immersed into the broth, rolled and squeezed against the sides of the tube to remove excess broth. The swab was then used to inoculate the plate of Mueller- Hinton agar, in 3 different directions to ensure an even and complete distribution of the inoculum over the entire plate. The plates were allowed to dry for 3-5 minutes. With the help of a sterile forceps, the cefoxitin discs were applied within 15 minutes of inoculationof plate and the plate was incubated in inverted position overnight at 37°C.

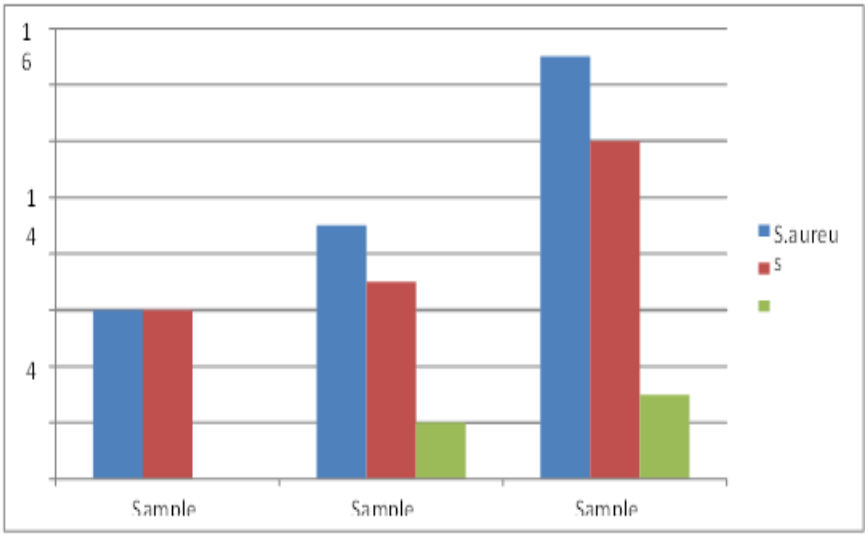
Commercially obtained Hi media discs were used. The strength of discs used and their interpretative zone size were read according to guidelines by CLSI.

Results

Nasal swabs were collected from 86 medical students who were involved in the study. Of these 47 were male and 39 were female. Swabs were collected before exposure to the hospital environment and thereafter at the end of 6 months and one year during clinical postings.

In the first set of nasal swabs collected before exposure to hospital environment 6 students were found to be colonized with S.aureus all of which were methicillin sensitive (MSSA). The male: female MSSA colonization was in the ratio of 4:2.

Sample II, which was collected 6 months later showed 9 isolates of S.aureus of which 2 were MRSA and 7 MSSA. Both MRSA isolates were from the male students. The male: female MSSA colonization was in the ratio of 4:3. Sample III, the last set of nasal swabs taken at the end of one year showed higher colonization with S.aureus. Totally 15 samples showed S.aureus of which 3 were MRSA and 12 were MSSA. The male: female ratio of MRSA was 2:1 whereas 6 MSSA were isolated from both males and females respectively.



Graph-1: Showing MRSA carriage in all the 3 sets of nasal swabs:

Table-1: Showing MRSA and MSSA carriage based on sex distribution:

	SAMPLE I (n=6)		SAMPLE II (n=9)		SAMPLE III (n=15)	
S.aureus	M	F	M	F	M	F
MRSA	Nil	Nil	2(2.3%)	Nil	2(2.3%)	1(1.2%)
MSSA	4(4.7%)	2(2.3%)	4(4.7%)	3(3.5%)	6(7%)	6(7%)

Discussion

In this study 3.48% of the medical students were colonized with MRSA at the endof one year of hospital exposure, whereas MSSA colonization was seen in 18% of the students. At the beginning of the study, that is before exposure to hospital environment (pre clinical) no candidates were found colonized with MRSA. The same set of students when exposed to the hospital environment during their clinical postings showed increasing rates of colonization with MRSA. Colonization at theend of 6 months was 2.4% and at the end of 1 year was 3.48%. The results of our study atthe end of 1 year (3.48%) are comparable with previous studies from United States by Charles B et al; which showed 3.4% and Brazil by Karina A P et al. which showed 3.6%[9,12]. Karina A P et al. screened 250 students, 93 males and 157 females, including first and second-year undergraduate students in pharmacy, nursing, dentistry and medicine ata Brazilian university for MRSA. They found the nasal carriage was 2.4% for MRSA [9]. The time duration of this study is not mentioned. However their rate of colonization is similar to the rates in our study at the end of 6 months. Out of the 150 HCW screened by R Goyal et al, in Delhi; Staphylococcus aureus was detected in 37.3% and MRSA was detected in 6.6% [10]. Few studies showed higher results compared to our study. In a study done by OduNN and Okonko IO in Nigeria, involving 100 medical students, showed very high nasal carriage for S.aureus (32%) as well as MRSA (12%). Also there was significant difference in MRSA nasal carriage between male and female students with male preponderance [11].

The lower rate of MRSA carriage in our candidates in comparison with above mentioned studies could be a result of strict hand hygiene policy and very effective infection control program of our hospital. In our study we found a progressive increase incolonization (as in the Graph-1) over the one year period of the study. However the time duration of their study is not mentioned. So the higher carriage rate in the studies by Odu NN and Okonko IO in Nigeria and R Goyal et al in Delhi may be due to longer duration of the study or may be due to lack of adherence to hand hygiene policies. Economically backward countries like Nigeria have the highest colonization (12%). This high colonization may be attributed to low standards of hygiene, resulting from economic backwardness. This may be the reason behind higher Nasal carriage of MRSA in different geographical areas [9]. In our study we also found

that male students had little higher colonization with MRSA nasal carriage throughout the study as shown in Table -1 compared to that of female students. Male: female ratio for MRSA nasal colonization at the end of one yearwas 2:1. Similar results have been found in a study done by Odu NN and Okonko IO in Nigeria, where the male gender had a significantly higher colonized with MRSA than female[11]. During our study we also found progressively increasing rate of MSSA colonization as shown in Graph-1. This has also been corroborated by the other studies done by Charles B et al which showed 3.4% and by Karina A P et al. which showed 3.6%. [9,12]

Conclusion

MRSA is a pathogen of high concern because of its ability to cause a variety of life threatening infections and its capacity to adapt to different environmental conditions. It is one of the leading causes of hospital acquired infection. It has often been suggested that certain strains of S.aureus have a special ability tocolonize health care workers, doctors, staff and that certain MRSA strains are among these. Many outbreaks of MRSA infections in hospitals have been traced to hospital personnel. Study of nasal carriage of MRSA is important to the community since it plays a key role in epidemiology and pathogenesis of the disease. Elucidating the ambiguous determinants of this phenomenon is of major public health interest. We have assessed the prevalence of colonization of MRSA among medical students and thus the possibility of its spread in hospital. As a first step towards understanding the microbial ecology of S.aureus carriage, the nasal carriage among medical students was assessed.

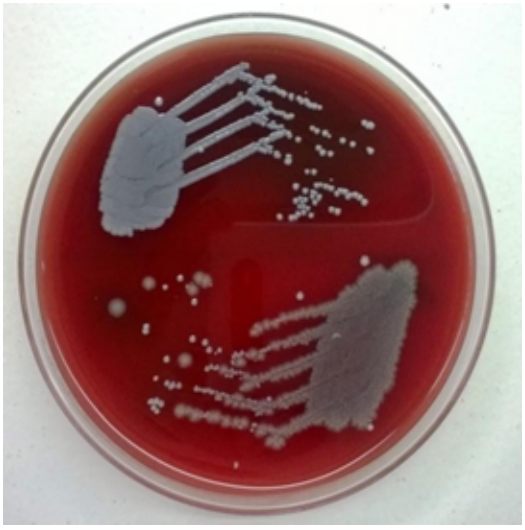
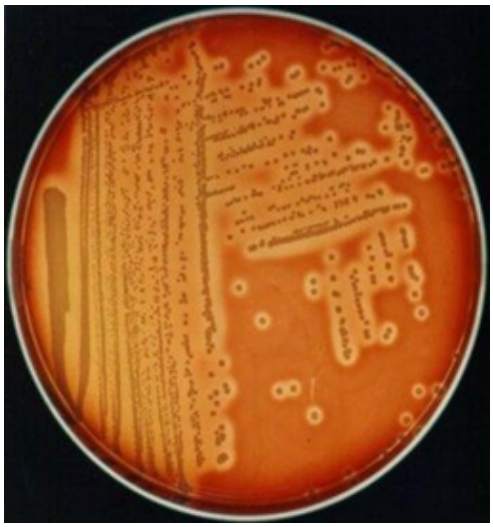


Photo 1: *S.aureus* colonies on blood Agar photo



2: *S.aureus* showing beta haemolysis on ba

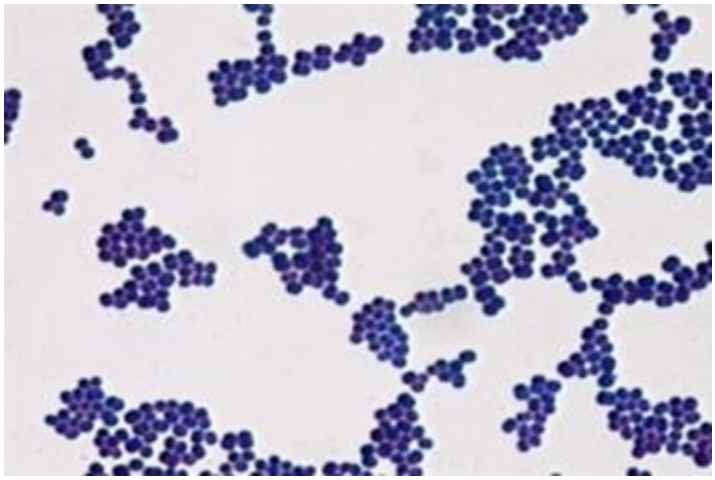


Figure 3: Grams staining -- gram positive cocci in clusters

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