Abstract— Nosocomial infections are infections that develop as a result of a stay in hospital or are produced by microorganisms and viruses acquired during hospitalization. Nasal carriage of *S. aureus* is very common and may be due to hand to nose transmission. The objective of the present study was to screen individuals from various sectors as carriers of MRSA. The study group included hospital staff such as nurses, doctors and visitors. Nasal swabs were taken from the volunteers and cultured on media selective for *Staphylococcus aureus*. Pathogenecity was confirmed using PCR for the coagulase gene. Coagulase positive strains were subjected to PCR for the mec gene. A comparison was done with a student population to serve as a group non exposed to MRSA.

Keywords— MRSA, coagulase gene, mec gene, nosocomial infections, PCR based detection

I. INTRODUCTION

Nosocomial infections are infections that develop as a result of a stay in hospital or are produced by microorganisms and viruses acquired during hospitalization. They may be endogenous, arising from an infectious agent present within a patient's body, or exogenous, transmitted from another source within the hospital. In addition to patient-to-patient spread, others may be involved, including staff, students, visitors and voluntary workers[1]. *Staphylococci* and *Enterococci* are major causes of nosocomial infections. They cause superficial skin lesions such as boils, styes and more serious infections such as osteomyelitis and endocarditis. Methicillin-resistant *S. aureus* (MRSA) is a strain of *S. aureus* which by definition is resistant to the semi- synthetic penicillins (i.e. methicillin, nafcillin, and oxacillin). As such, it is resistant to all other beta-lactam antibiotics (including other penicillins, cephalosporins and cephamycins). Additionally, MRSA is often resistant to other classes of antibiotics including aminoglycosides, macrolides and quinolones. Thus, MRSA is not only methicillin-resistant but also multiply-resistant as well[2]. MRSA colonization and infection in acute and non-acute care facilities have increased dramatically over the past two decades, evidenced by the increasing number of reported outbreaks in the medical literature. Because of its resistance to antibiotics, management of MRSA infections requires more complicated, toxic and expensive treatment. It is important for the health care professional to understand the difference between colonization and infection. Colonization indicates the presence of the organism without symptoms of illness. *S. aureus* permanently colonizes the anterior nares of about 20% to 30% of the general population. Hospital workers are more likely to be colonized than persons in the general population, presumably because of increased exposure [3]. The resistance to antibiotics is due to a gene called mecA which is part of the staphylococcal cassette chromosome. It codes for a Pencicillin Binding Protein (PBP2a) that prevents the action of beta lactamococcal cassette chromosome. This study is hence focused on rapid detection of the MRSA using gene specific primers designed to detect the mecA gene. Recently, coagulase gene typing is being used as an important tool to characterize pathogenic *Staphylococci*. Its discriminatory power relies on the heterogeneity of the region containing the 81-bp tandem repeats at the 3' ends of the coagulase gene. PCR amplification of this particular region produces DNA fragments of different sizes which can then be further discriminated by Alul digestion [5]. In this study I have used coagulase gene PCR and RFLP to type the MRSA strains from various populations.

II. EXPERIMENTAL PROCEDURE

Samples were taken from hospital workers such as nurses and technicians. The sample group consisted of volunteers from various clinics and hospitals. A total of 50 samples was used for the study. All volunteers signed a consent form for ethical approval of the study. A control group of students not exposed to hospitals was also used for the study. Swabs were taken from both the anterior nares using sterile swabs moistened in saline. The swabs were used to spread on Media specific for *Staphylococcus aureus* (Fig 1). Cultures were then subjected through three rounds of purification and subculturing to get single colonies.
Colonies were boiled in lysis buffer containing 1% Triton X 100 at 95°C for 15 mins. The suspension was centrifuged and 10 ul of the supernatant was used as template for PCR. Primers specific for the mecA gene were used to amplify the methicillin gene. mecA positive strains were then screened for presence of the coagulase gene using primers specific for the coagulase gene. Primers used and PCR conditions are shown in table 1[5-8].

### III. RESULTS AND DISCUSSION

PCR based screening for carriers of MRSA showed that most of the health care workers such as staff nurses and doctors were MRSA positive, but asymptomatic. Some of the hospital visitors also were positive for MRSA. On comparison with the student population consisting of undergraduate students, it was observed that the student population was free from nasal carriage of MRSA, indicating non exposure to the pathogen (Fig 2 and 3).

**Fig 1.** Nasal swabs cultured on media specific for Staphylococcus aureus

**Fig 2.** Mec A gene PCR products from health care workers

**Fig 3.** Mec A gene PCR from student population.

PCR products were visualized by electrophoresis using 1% agarose gel. Mec A positive strains were subjected to PCR for coagulase gene. The different size coagulase gene products were subjected to restriction digestion using AluI restriction enzyme for 1 hour at 37°C. ATCC 700699 strain was used as MRSA positive control.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers used</th>
<th>PCR conditions</th>
<th>No.of cycles</th>
<th>PCR product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mec A</td>
<td>F′: 5′ AATAACGTAGCTAAAGTGTCG3′  R′: 5′ AGTTCGAGTAACCGATCG3′</td>
<td>94°C – 30 secs</td>
<td>40</td>
<td>533 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55°C – 30 secs</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>72°C – 1 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>72°C – 5 min</td>
<td></td>
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<td></td>
<td>final extension</td>
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</tbody>
</table>

**Table 1.** Primers and PCR conditions used for detection of mecA and coagulase genes.

Lane 1 - 100 bp DNA ladder  
Lanes 2-12 – PCR products from health care workers  
Lane 13 – mecA positive control ATCC 700699 strain

Lanes 4-11 – PCR negative from student population

Mec A positive strains were subjected to PCR for the coagulase gene. Four different strain types were found based on polymorphisms in the size of the coagulase gene such as 350 bp, 430 bp, 570 bp and 630 bp (Fig 4). The PCR products also yielded different restriction patterns on digestion with the enzyme AluI (Fig 5), indicating that amongst the strains isolated from this study there were four different types.
Fig 4. Coagulase PCR product of various sizes from the MRSA isolates

Lane 1: 100 bp DNA ladder
Lane 2: Negative control
Lane 3: coagulase product of size 350 bp
Lane 4: coagulase product of size 430 bp
Lane 5: coagulase product of size 570 bp
Lane 6: coagulase product of size 630 bp

Fig 5. Restriction analysis of coagulase PCR products with Alu

Lane 1 - 100 bp DNA ladder
Lanes 2-7 – coagulase PCR products showing different restriction patterns

IV. CONCLUSION

MRSA colonization and infection in acute and non-acute care facilities have increased dramatically over the past two decades, evidenced by the increasing number of reported outbreaks in the medical literature. Because of its resistance to antibiotics, management of MRSA infections requires more complicated, toxic, and expensive treatment. It is important for the health care professional to understand the difference between colonization and infection. Colonization indicates the presence of the organism without symptoms of illness. *S. aureus* permanently colonizes the anterior nares of about 20% to 30% of the general population. Hospital workers are more likely to be colonized than persons in the general population, presumably because of increased exposure. In most cases culture methods are used to identify MRSA, but the results take at least 2 days\(^1\). The need of the hour is developing faster and precise methods for identifying MRSA. This study shows that rapid identification of MRSA can be done using the PCR specific for the mecA gene. The technique can be used as a regular screening program in hospitals to avoid spread of MRSA and also to educate health care professionals on the importance of frequent and proper hand sanitization methods to prevent the MRSA infections from spreading.

REFERENCES

[8] Graham, J.C., Murphy, O.M., Stewart, D., kearns, A.M., Galloway, A and Freeman, R.