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SCREENING OF MUPIROCIN RESISTANCE IN METHICILLIN RESISTANT S*TAPHYLOCOCCUS AUREUS* AMONG HEALTH CARE WORKERS AND DETECTION OF *MUPA* GENE BY PCR

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ABSTRACT

Increasing incidence of antibiotic resistance proves to be a major problem for health care industry. In particular methicillin-resistant *Staphylococcus aureus* (*MRSA*) seems to be increasingly a rapid emergence in nosocomial infections and colonization of the *MRSA* have resulted in development of further resistant to Mupirocin, antibiotic used in treatment of *MRSA* infections. This study investigated the occurrence of mupirocin resistance among 100 *MRSA* isolates from health care workers (HCWs) of Vinayaka Mission's Kirupananada Variyar (VMKV) Medical College and Hospital identified the prevalence of MuL and MuH resistance among the *MRSA* and isolated the *mupA* gene responsible for mupirocin resistant in MRSA. Investigation demonstrated that high incidence (63%) of *MRSA* isolation from an increased (79.9%) incidence of nasal carriage in HCWs. Prevalence of MuH resistance among *MRSA* isolates was significantly high in health care workers (75%), The study suggests periodical screening of HCWs for nasal carriage need to be carried out, and further steps to prevent transmission.

Keywords: *S. aureus*, MRSA, Mupirocin, MuH, MuL.

INTRODUCTION

Staphylococcus aureus, literally the 'golden cluster seed' is a facultative anaerobic, Gram positive coccus which appears as microscopic grape-like clusters and gold pigmentation of colonies that colonizes the nares, pharynx, axillae, vagina, or damaged skin surfaces (Nickerson *et al.,* 2009). Infections are initiated when a breach of the skin or mucosal barrier allows staphylococci access to adjoining tissues or the bloodstream. Hospitals are faced with the increasingly rapid emergence and dissemination of antibiotic resistant bacteria (*ARB*). *S. aureus* has been recognized as the main etiological agent in hospital-acquired infections (Askarian *et al*., 2009). Nasal colonization with *S. aureus* is common and it is an important step in the pathogenesis and spread of *S. aureus* infections,

these strains provide a reservoir for infection in other sites such as; surgical-site and bloodstream infections. Currently, the health problems associated with this microorganism have become more serious due to an increasing incidence of methicillin-resistant *S. aureus* (*MRSA*) (Sousa-Junior *et al.,* 2009). In certain subgroups, such as, frequently hospitalized people, senile and immune compromised patients, colonization with *S. aureus* occurs more frequently (Jones *et al*., 2007). *MRSA* colonization in health care workers (HCWs) presents as a significant reservoir for transmission of the bacteria to other HCWs and patients.

Mupirocin is a naturally occurring antibiotic produced by *Pseudomonas fluorescens* which acts by binding reversibly and specifically to bacterial isoleucyl-tRNA-synthetase (IRS, produced by *ile*S gene), thereby inhibiting bacterial protein synthesis. Mupirocin is used for *S. aureus* (methicillin-sensitive -*MSSA* and *MRSA*) carriers to prevent infection and for outbreak control. It was first introduced in the UK as one of the most effective topical antibiotic that is active against gram-positive pathogens, as well as some gram-negative bacteria and it is used for the eradication of *S. aureus* in the nasal carriage (Daskalaki *et al.,* 2009 and Liu *et al.,* 2010).

Nasal carriage therapy with mupirocin ointment appears to be effective in reducing the onset and severity of infections at surgical sites (Askarian *et al*., 2009). However, varying rates of resistance have been reported. Mupirocin was introduced into clinical practice in the UK in 1985, and the first reports of staphylococcal resistance developed 2 years later (Cooper *et al.,* 2004). Of the two mupirocin-resistant phenotypes, the low-level resistant (MuL) strain (MIC 8-256 μg/ml) is more common with a point mutation by the isoleucyl-tRNA synthetase gene (ileS-1) for the target enzyme, and a high-level of mupirocin (MuH) resistance (MIC ≥ 512 μg/ml), from the acquisition of a plasmid carrying a new gene, *ileS-2 or* mupA, it encodes an alternate isoleucyl-tRNA synthetase (Saderi *et al*., 2008 and Lim *et al*., 2010).

Studies suggest that *mupA* gene is transferred from *S. epidermidis* to *MRSA* during mupirocin prophylaxis (Hurdle *et al*., 2005). MuH resistant strains are important in the clinical field because they transfer resistance genes to other bacteria by plasmid conjugation, consequently protecting these bacteria from being eradicated with mupirocin (Park *et al*., 2012). Treatment with mupirocin is not likely to be effective in the presence of MuH resistance and there is some evidence to suggest that MuL may also predict treatment failure (Walker *et al.,* 2003).

The objectives of this study were to determine the prevalence of mupirocin resistance among *MRSA* isolates from health care workers of Vinayaka Mission's Kirupananada Variyar (VMKV) Medical College and Hospital with genotypic studying, to identify the prevalence of MuL and MuH resistance among the *MRSA* by disc diffusion method and to detect *mupA* gene by Polymerase Chain Reaction (PCR).

MATERIALS AND METHODS

Sample collection

Clinical specimens of swabs from anterior nares, palms and web spaces of HCWs were collected in sterile containers from the subjects reporting at the Department of Microbiology, VKMV Medical College and Hospital, Salem. The samples were collected from November 2012 to April 2014. All the collected samples were analysed immediately.

Sample analysis, isolation and identification of *S. aureus*

All the samples were screened for 100 consecutive *Staphylococcus aureus* individually among health care workers. The samples were inoculated on sterile Nutrient agar, MacConkey agar, Mannitol Salt agar and Blood agar plates and incubated at 37^{o}C for 24 hrs. After incubation, the plates were examined for colony characteristics such as size, shape, consistency, haemolytic properties and pigmentation. Primary identification was done on the basis of colony and cell morphology by Gram staining (Cadilla *et al.,* 2011). Representative colonies that appeared on NA plates were checked for purity through microscopy and pure isolates were streaked on NA slants and stored at 4˚C for further secondary identification. Secondary identification was carried out by performing a series of biochemical tests. Both primary and secondary identifications were carried out on the physical characterization and the biochemical tests outlined in Bergy's manual of Determinative Bacteriology (Holt, 1994). *Staphylococcus aureus* ATCC 25923, was used as a quality control strain.

Biochemical tests

Tube coagulase

Hundred microliter of overnight BHI broth culture was added to 0.5 ml of undiluted plasma and incubated at 37°C in a water bath for 4 hrs the tubes were examined at 1, 2 and 4 hrs for clot formation by tilting the tube 90˚C. A positive test was shown by a clot formation indicating the production of the enzyme coagulase by the organism (Yanagisawa *et al.*, 1994).

Mannitol Fermentation

A pure inoculum was transferred aseptically to a sterile tube containing Mannitol broth. The inoculated tube was incubated at 37˚C for 24 hrs and the results were determined. A positive test was determined by the color change from red to yellow, indicating the pH change to acidic (Cadilla *et al*., 2011).

Cefoxitin Disc Diffusion Test

All the isolates were subjected to Cefoxitin (30µg) disc diffusion test. Well isolated colonies of *Staphylococcus aureus* c were inoculated into peptone water and incubated at 37^{o}C for 2 hrs and turbidity compared with 0.5 McFarland's. Disc diffusion test performed based on Kirby – Bauer technique. Plates were incubated at 35^{o}C for 24 hrs and the zone of inhibition was measured as per CLSI guidelines. An inhibition zone of ≤ 21 mm was reported as Cefoxitin resistant and ≥ 22 mm was considered as Cefoxitin sensitive.

Detection of the mupirocin resistance

In the disk diffusion method, mupirocin disks of 5µg (SD748, Himedia Labs, India) and 200µg (CT0523B, Oxoid, India) concentration were used. Zone diameter of > 14 mm for both discs was taken as susceptible for mupirocin. Whereas, isolates that showed zone diameters < 14 mm in the 5 µg disk but > to 14 mm in the 200 µg disk were considered to be low-level mupirocin resistant strains. All isolates with zone diameters < 14 mm for both 5µg and 200µg disks were considered to be high-level mupirocin resistant strains (Singh *et al*., 2013).

Detection of the *mupA* gene by PCR

The genomic DNA was isolated from *S. aureus* by method described by Jegadeesh *et al*. (2012). The extracted DNA was dissolved in 20µl of Tris EDTA (TE) buffer (pH 8.0), vortexed gently for 30sec and the DNA solution was stored at 4°C for further experiments.

PCR assay was performed according to Braoios *et al.* (2009) procedure with slight modification. The primers was obtained from Sigma, India and used in the PCR comprised Primer 1 *mup*A (5'-

TAT ATT ATG CGA TGG AAG GTT GG-3') *mup*B (5'- AAT AAA ATC AGC TGG AAA GTG TTG-3') for amplification of a 456bp fragment. Each PCR reaction mixture (20µl) contained 1µl of genomic DNA (Genomic DNA), 2µl of 10 × PCR buffer, 0.5µl of 2.0mM of each primers, 1µl of 25mM of each deoxynucleotide triphosphate and 0.5µl of Taq DNA polymerase (5U/µl) and 15.5µl of molecular grade water. The amplification reaction involved an initial denaturation phase at 94^{o}C for 5 minutes, followed by 30 amplification cycles (denaturation at 94^{o}C for 30s, annealing at 64^{o}C for 30s and elongation at 72^{o}C fro 45s) and a final elongation phase at 72^{o}C for 10 min. After the reaction, 20µl of the final product was resolved into amplified fragments by electrophoresis in 2% agarose gel at 100V (45mA) for 1 hour. The gel was visualized by UV illumination and photographed.

RESULTS

This study was carried out on 100 number of HCWs, the swaps samples were collected from three different sites *i.e*., anterior nares, palm and web spaces of each HCWs. The isolates were identified based on the growth characteristics as mentioned in (Table 1). Out of 100 HCWs, 59 (59 %) of them were identified as *Staphylococcus aureus* carriers. Among 59 *Staphylococcus aureus* carriers, the distribution of sites of *Staphylococcus aureus* carriers were 47 (80%) from anterior nares, 3 (5.08 %) from palm and 9 (15.25 %) from web spaces (Fig.1).

All *Staphylococcus aureus* isolates were subjected to routine disc diffusion tests using Cefoxitin 30 µg to detect methicillin resistance. Among the 59 *Staphylococcus aureus* carriers, 36 (61.01%) were found to be Methicillin Resistant *Staphylococcus aureus (MRSA)* and 23 (38.98%) were found to be Methicillin Sensitive *Staphylococcus aureus (MSSA).*(Fig.2). The site wise distribution was 31 (52.54%) from anterior nares, 04 (44.44%) from web spaces and 01(33.33%) from palm (Table.1).

In these current studies, next part of the investigation was determination of Mupirocin resistance isolates from Methicillin Resistant *Staphylococcus aureus*, which were determinate by both disk diffusion and PCR method. Among the 36 MRSA, 27 (75%) showed mupirocin Resistance and 09 (25%) showed mupirocin sensitive (Fig.3). Among them, 15 (55.55%) showed as High level Mupirocin resistance and 12 (44.44%) were low level resistance. The highest percentage of high level Mupirocin resistance were observed in anterior nares (36%) and followed by web spaces and none isolated from palm (5.5%) (Table.1).

DISCUSSION

In recent years *S. aureus* infections have been rising, especially MRSA has become a global problem. MRSA strains are associated with longer hospital stay, prolonged antibiotic administration and higher costs than infections caused by methicillin – susceptible *S. aureus* (MSSA) strains (Kumar *et al*., 2011). In recent years, dissemination of MRSA has been increasingly recognized in other healthcare settings, including primary care. The previous studies of Radhakrishna *et al*., (2013) and Kumar et al., 2011 were observed MRSA isolates from health care workers. This phenomenon was similar to our observation. The data obtained from this study revealed that there were reservoirs or carriers of MRSA in healthcare workers. Among the 100 samples, 59% of HCWs was carrier of *S.aureus.* In 2013 Radhakrishna *et al*., observed 14.2% of *S.aureus* from HCWs, which were low percentage comparative to our studies.

In this study highest prevalence were obtained from anterior nares and second most by web spaces and followed by palm. Our observation was similar to Kalyani *et al*., 2012 studies. They were also observed the highest occurrence of *S.aureus* in anterior nares. This finding is of great concern as carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection. Prevalence of nasal carriage of *S. aureus* in other countries is also different. This difference may be due, in part, to differences in geographical distribution, differences in the quality and size of samples and the culture methods used to detect *S. aureus*.

The role of health care workers in the transmission of infections has been extensively described. Most often, spread is from patient to patient on the hands of health care workers, person to person through direct contact and on medical devices. The importance of hands in the transmission of hospital infections is worldwide accepted. However, it may not be the regular practice to include hand washing (HW) as routine behavior in health-care workers, since microorganisms are invisible or there are no adequate elements to carry out this practice (Edem *et al*., 2013).

Since MRSA strain are resistant to all β-lactam antibiotics and the treatment options are limited significantly. The incidence of nosocomial infection caused by MRSA continues to increase worldwide (Kumar *et al*., 2011). In the present study 61.01% of isolates were MRSA. The site wise distribution was 52.54% from anterior nares 44.44% from web spaces and 33.33% from palm. Previous studies have shown the role of clinical staff as nasal carriers of MRSA. Nares and the anterior nare are the most important sites of staphylococcal colonization and potential sources of MRSA (Ahmed *et al*., 2012).

Fadeyi *et.al* 2010 from Nigeria in 2011 screened 198 Health Care workers (HCWs) for isolation of MRSA in Anterior naries and Hand. Among them 104 had MRSA either in the nose, hand or both giving a carriage rate of 52.5%. In this study Nasal carriage was higher than hand, similar observation was obtained from previous studies of Kalyani *et al*., 2012. In our present study, the MRSA colonization rate (61.01%) in health care workers was more when compared to (8.5%) previous studies of Mathanraj (2009). In 2000, Preetha *et al.* (2000) screened healthcare workers of the burn unit of a tertiary care hospital and found that 71% of the healthcare workers were positive for nasal carriage of MRSA; this is very much in line with our findings.

Emergence of MRSA has lead to the development of multi-drug resistant strains. Increase in the reports of strains fully resistant to most of the currently available antimicrobial agents in the hospital settings. Infections caused by MRSA strains are associated with longer hospital stay, prolonged antibiotic administration and higher cost than infections caused by methicillin-susceptible *S. aureus* strains.

Mupirocin (pseudomonic acid A) is a topical antibiotic with good activity against S. aureus. mupirocin has been used to eradicate nasal *S. aureus*. However, indiscriminate use of mupirocin can select resistant strains, and its use for nasal eradication is controversial (Ramsey *et al*., 1996; Lowy, 2003). According to Gilbart *et al*. (1993), *S. aureus* strains resistant to mupirocin can be divided into two groups such as low-level and high-level resistance. In this present investigation both high and low level mupirocin resistance isolates were observed. Totally 75% of isolates were resistance to mupirocin, among them 55.55% showed as High level Mupirocin resistance and 12 (44.44%) were low level resistance.

In this study, high level mupirocin resistance was found among HCWs MRSA isolates and minimal number of isolates showed low level resistance. These findings are comparable to the Indian studies of Kaur *et al.*, (2014). They were observed only 3.5% of mupirocin resistance isolates from HCWs. In this study there was perfect correlation between the phenotypic (conventional) and genotypic (PCR) results for identification of *S. aureus* and detection of mupirocin resistance. Perez-Roth *et al.* (2002) also reported complete consistency between the results of phenotypic tests and PCR used to identify *S. aureus* and assess its resistance to mupirocin.

CONCLUSION

Our study shows a high incidence of *MRSA* isolation from pus samples in patients and an increased incidence of nasal carriage in health care workers. Prevalence of MuH resistance among *MRSA* isolates was significantly high in health care workers. There is need for Healthcare workers to wash their hands regularly with antiseptic soap, or to disinfect the hand by rubbing with alcohol solution. Proper infection control measures should be adopted and further research on multidrug resistant organisms and surveillance of nosocomial infection should be carried out. Public enlightenment against the abuse of antibiotics should be carried out.

Our study suggests periodic screening of health care workers for nasal carriage for MuH resistant *MRSA*, as they are significant reservoirs for transmission of MuH resistant *MRSA* to patients and to other health care workers. Detection of MuH resistant *MRSA* by phenotypic disc diffusion method is a better alternative to the genotypic detection method, as our study suggests and moreover, it is a cost effective method and does not require any instrumentation or skilled personnel to perform.

Figure 1 Site wise Distribution of *Staphylococcus aureus* in Health Care Workers

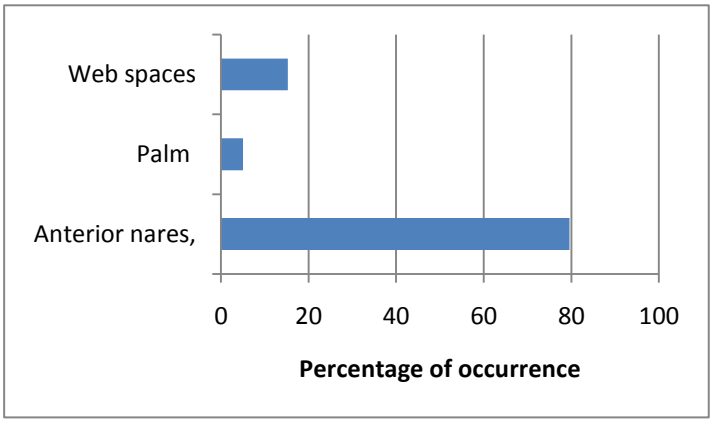

Figure 2 Prevalence of MRSA and MSSA in HCW'S

Fig.3 Prevalence of Mupirocin Resistance in MRSA isolated from HCW's

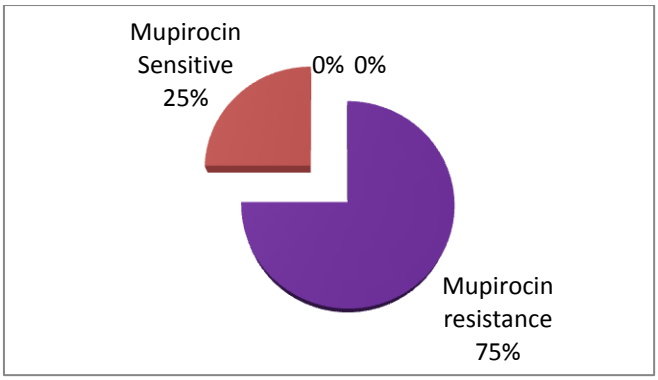

Table 1 Distribution of MRSA in different sites in HCW's

Sr. No	Types of isolates	Different sites in HCW's (%)		
		Anterior nares	Palm	Web spaces
1.	MRSA	52.5	33.3	44.4
2.	Mupirocin R^+ in MRSA	36	0	5.55

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