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ORIGINAL ARTICLE

Mupirocin resistance in methicillin-resistant *Staphylococcus aureus* isolates from anterior nares of healthcare workers of a tertiary care hospital

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Abstract

Background: Nasal colonization of *Staphylococcus aureus* is very common among health care workers, as part of a comprehensive Methicillin Resistant *Staphylococcus aureus* (MRSA) decolonization strategy, Mupirocin (Pseudomonic acid) is a topical antibiotic largely used to eradicate staphylococcal nasal carriage. Increased mupirocin use predisposes to mupirocin resistance, which is significantly associated with persistent MRSA carriage. This resistance is both low level as well as high level among the isolated strains. *Aim and Objectives:* To estimate the nasal carriage of MRSA in Healthcare Workers (HCWs) and to detect level of Mupirocin resistance in isolated MRSA strains. *Material and Methods*: A total 670 nasal swabs of HCWs (doctors, nursing staff and housekeeping staff) from various high risk areas were tested. High level and low level Mupirocin resistance among the isolated MRSA strains was detected by Kirby Bauer disc diffusion method. Minimum Inhibitory Concentration (MIC) of Mupirocin resistance was determined by E test. *Results:* Among 670 nasal swabs, 280 (41.79 %) showed growth of *Staphylococcus aureus* and 353 (52.68%) were Coagulase Negative Staphylococci (CONS). Of 280 *Staphylococcus aureus* strains, 61 (21.78%) strains were methicillin-resistant (MRSA). Mupirocin resistance both low level and high level was observed in 1 (1.63%) MRSA carrier only. Conclusion: The present study showed a high incidence of nasal carriage of MRSA among health care workers. Therefore we suggest MRSA screening of HCWs as a routine practice and insist on Mupirocin resistance detection so that in case if resistance detected alternative treatment can be given.

Keywords: Antibiotic resistance, Methicillin Resistant *Staphylococcus aureus*, Low-level Mupirocin Resistance, High-level Mupirocin Resistance

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is an increasingly common pathogen associated with both nosocomial and community-acquired infections [1]. Colonization of the anterior nares with Staphylococcus aureus is common and reported in various studies [2-3]. Mupirocin is an antimicrobial agent that inhibits the synthesis of bacterial proteins by competitive inhibition of bacterial Isoleucyl-tRNA Synthetase (IRS) enzyme [4]. Mupirocin resistance in clinical isolates of MRSA is reported worldwide [5-6]. S. aureus in the nose is a risk factor for endogenous staphylococcal infection. Intranasal application of mupirocin is used widely to eliminate S. aureus colonization. With the increased use of mupirocin, both low and high level resistance has been reported during treatment with nasal mupirocin [7]. Low-level resistance [Minimal Inhibitory Concentration (MIC) 8-256 μg/ml] is usually associated with point mutations in the chromosomally encoded ileS

gene whereas high-level resistance (MICs, ⩾ 512 µg/ml) is generally due to a plasmid-mediated gene, MupA (also referred to as ileS2), which encodes an additional modified IRS and is typically located on mobile genetic elements, which likely facilitates the dissemination of this resistance mechanism. The MupA gene is typically plasmid mediated, and some of these plasmids are conjugative. MupB is a new high level mupirocin resistance mechanism in S. aureus [8]. Detection and differentiation of low-level and high-level resistance has important clinical application. The presence of high-level Mupirocin resistance (MuH) excludes its clinical use as has been associated with decolonization failure and increased use leads to increased resistance rate. Low-level Mupirocin resistance (MuL) can be overcome by using higher than usual dosage [8-10]. It is therefore essential not only to differentiate between susceptible and resistant strains but also to determine the level of resistance.

The extent of mupirocin-resistance and level of resistance among Healthcare Workers (HCWs) in our area is unknown. So, with this background this study was undertaken to detect mupirocin resistance and also to determine the level of resistance in MRSA isolates from healthcare workers in our setup. This study was designed with following objectives to find the prevalence of nasal carriage of MRSA from the nasal swabs of HCWs, to find the prevalence of mupirocin resistance amongst the nasal isolates of MRSA from the healthcare workers of a tertiary care hospital, to determine the rates of MuH and MuL in nasal isolates of MRSA by disc diffusion.

Material and Methods

A prospective observational study was conducted for the period of two years, from February 2018 to January 2020 in the Microbiology department of MGM Medical College and Hospital, Aurangabad, Maharashtra. This study was started after obtaining the institutional ethical committee's approval (Letter no –MGM-ECRHS/2017, Date-07/04/2017).

A total of 670 nasal swabs were collected from HCWs including doctors, nursing staff and housekeeping staff after obtaining their informed consent. The age, sex, designation and other relevant information was obtained. Healthcare workers from various intensive care units and operation theatres were included in this study. HCWs having rhinitis, pharyngitis, upper respiratory tract infection, and who were on oral antibiotics were excluded from the study.

Nasal swabs were collected using a sterile cotton swab with transport tube. The sample was collected by rotating the sterile, normal saline moistened swab five to six times in the anterior nares of both nostrils. The swabs were immediately reinserted in transport tubes, labeled properly and transported to the Microbiology laboratory for further processing.

All nasal swabs were inoculated on 5% sheep blood agar and incubated at 37°C for 24 hours. After incubation, identification of *Staphylococcus aureus* was done on the basis of colony morphology, Gram stain and standard biochemical reactions [11].

Detection of MRSA

All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance by Kirby-Bauer disk diffusion method using 30 µg

cefoxitin disc (Himedia ltd, Mumbai) as per Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. The isolates were considered methicillin-resistant if the zone of inhibition was 21 mm or less.

Mupirocin susceptibility testing

The methicillin-resistant *S. aureus* isolates were then tested for mupirocin resistance. Mupirocin resistance was detected by Kirby-Bauer disk diffusion method using 5 µg and 200 µg mupirocin discs (Himedia ltd, Mumbai) which differentiate between low and high level resistance respectively. Criteria of zone diameter breakpoints for susceptible and resistant isolates were set at > 14 mm and < 13 mm respectively [12-13]. The isolate is mupirocin sensitive when a zone diameter of ⩾ 14 mm is obtained for both 5 µg and 200 µg discs.

Isolates that showed zone diameters < 14 mm in the 5 µg disc but ⩾ 14 mm in the 200 µg disc were considered MuL.

Isolates with zone diameter <14 mm for both 5 µg and 200 µg disc were considered MuH. MIC of mupirocin was also determined by Epsilometer-strip (E-Strip) for the isolate of MRSA showing resistance to mupirocin by disc diffusion test.

All HCWs who were MRSA carriers were advised to apply mupirocin ointment locally twice daily for seven days along with relocation from duty. Nasal swab was repeated after seven days for detection of carrier stage persistence. In those with persistence of carrier stage even after application of mupirocin for seven days, we further advised them to extent application for seven more days. Repeat swab was collected after total 14 days of application of mupirocin. Data were entered in Microsoft excel and analyzed using SPSS version 24.0th mean and SD was calculated for quantitative variables and proportions was calculated for categorical variables, chi-square test was applied to check significance association between attributes: Value of p was checked at 5% level of significance.

Results

Among 670 nasal swabs, *S. aureus* was isolated in 280 (41.79%) and Coagulase negative *Staphylococci* (CONS) in 353 (52.68%) swabs. Other organisms were isolated in 23 (3.43%) nasal swabs and there was no any growth in 14 (2.08%) swabs (Table 1). Of 280 *S. aureus* strains, 61(21.78%) strains were MRSA. Over all, MRSA nasal carriage rate was 9.10 % in our study.

Table 1: Nature of organisms isolated from 670 nasal swabs

Isolates	**Number (%)**
Staphylococcus aureus	280 (41.79%)
Coagulase negative Staphylococcus (CONS)	353 (52.68%)
Others	23
No growth	14
Total	**670**

In female HCWs the prevalence of the nasal *S. aureus* colonization rate was 214(76.42%) and MRSA carriage rate was 38(17.75%). In male HCWs the prevalence of the nasal *S. aureus* colonization rate was 66(23.57%) and MRSA carriage rate was 23(34.84%)) and that is statistically significant (Table 2).

Critical area wise total number of samples examined and the rate of colonization of *S. aureus*, MRSA carriage along with mupirocin resistance has been shown in Table 3 and that is statistically significant. The highest prevalence of *S. aureus* colonization was observed in SICU 39 (68.42%) followed by CCU 33 (60%) and PICU16 (59.2%) whereas the MRSA carriage rate was highest in MICU 14 (70%) followed by endoscopy 04 (57.1%) and COT 02 (33.3%).

In relation to the professional category, housekeeping staff 54 (80.59%) have presented the highest rate of colonization followed by Doctors 52 (44.06%) and the lowest rate of colonization was found in Nursing staff 174 (35.87%) which is statistically significant (Table 4). The MRSA carrier rate was highest among doctors16 (30.76%) followed by housekeeping staff 11 (20.37%) and lowest in nursing staff i.e. 34 (19.54 %) (Table 4) which is statistically not significant.

In our study among the 61 MRSA isolates, only one (1.63%) isolate from nursing staff of MICU showed both low level and high level Mupirocin resistance by disk diffusion method. We have confirmed the result with Etest and this test also showed the same results (Table 4).

We have noted that decolonization was achieved in 47 (77%) HCWs within seven days of application of Mupirocin twice daily regularly. In 13 (21.31 %) HCWs colonization persists even after seven days application of Mupirocin and decolonized thereafter by extending application for seven more days. One HCW who showed Mupirocin resistance there was no decolonization even fourteen days application of Mupirocin, advised to take alternative method of eradication.

Table 2: Gender wise rate of *S. aureus* colonization, MRSA carrier and Mupirocin resistance (MuH and MuL)

Gender	Total Number	*S. aureus* n=280 (41.79%)		MRSA n=61 (21.78%)		MuH and MuL resistance
Male	157	66 (23.57%)	Chi-square =8.52 p=0.04	23 (34.84%)	Chi-square =8.65 p=0.03	00
Female	513	214 (76.42%)		38 (17.75%)		01 (2.12%)
Total	670	280 (41.79%)		61		

Methicillin resistant Staphylococcus aureus: MRSA, Mupirocin high level resistance: MuH, Mupirocin low level resistance: MuL

Table 3: Critical area wise rate of *S. aureus*, MRSA and Mupirocin resistance (MuH and MuL)

Area	Total Number of samples tested	Prevalence of *S. aureus*	p	Prevalence of MRSA	p	MuH and MuL resistance
EICU	50	27 (54%)	Chi-square =19.4 p=0.013	04 (14.3%)	Chi-square =17.1 p=0.029	
RGY ICU	23	11 (47.82%)		02 (18.1%)		
CCU	55	**33 (60%)**		02 (6.0%)		
PICU	27	**16 (59.2%)**		03 (18.7%)		
NICU	58	32 (55.17%)		03 (9.3%)		
SICU	57	**39 (68.42%)**		08 (20.5%)		
OBGY ICU and LR	91	36 (39.56%)		11 (30.5%)		
KT ICU and Dialysis	42	25 (59.52%)		03 (12%)		
Endoscopy	22	07 (31.81%)		**04 (57.1%)**		
OT General	117	28 (23.93%)		05 (17.8%)		
COT	59	06 (10.16%)		**02 (33.3%)**		
MICU	69	20 (28.98%)		**14 (70%)**		**0191.63%)**
Total	**670**	**280**		**61**		**01**

Emergency ICU: EICU), Rajiv Gandhi Yojna ICU: RGY ICU, Cardiac Care Unit: CCU, Paediatric ICU: PICU, Neonate ICU: NICU, Surgical ICU: SICU, Obstretic & Gynec ICU: OBGY ICU, Labour Room: LR), Kidney Transplant ICU: KT ICU, Operation theatre: OT, Cardiac OT: COT, Medical ICU: MICU

Table 3: Category wise rate of *S. aureus*, MRSA carriage status and high, low level Mupirocin resistance (MuH and MuL)

Category	Total No of Nasal Swabs	*S. aureus* Grown		MRSA positive		MuH and MuL resistance
Doctor	118	52 (44.06%)	Chi-square =48.7 p<0.0001	16 (30.76%)	Chi-square =3.04 p=0.219	
Nursing staff	485	174 (35.87%)		34 (19.54%)		01
Housekeeping	67	54 (80.59%)		11 (20.37%)		
Total	670	280 (41.7%)		61 (21.78%)		01 (1.63%)

Methicillin resistant Staphylococcus aureus: MRSA, Mupirocin high level resistance: MuH, Mupirocin low level resistance: MuL

Figure 1: Demonstrating resistance of *S. aureus* to Cifoxitin 30 µg Mupirocin 5 µg and 200 µg

Figure 2: Demonstrating low and high levels of Mupirocin resistance in MRSA

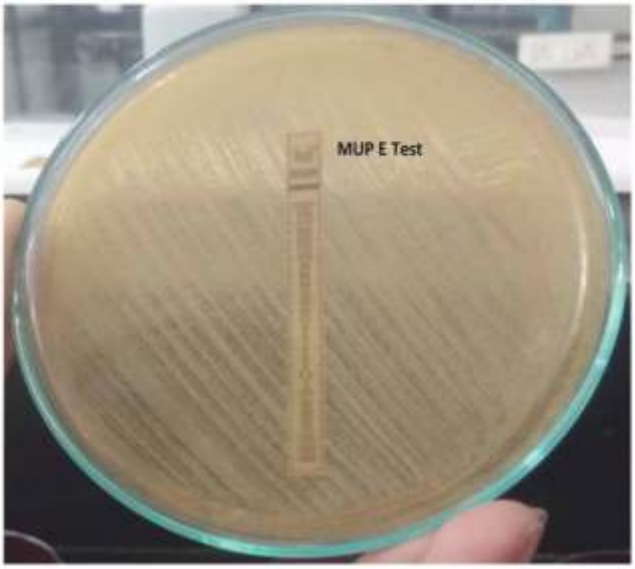

Figure 3: Mupirocin E test showing resistance to Mupirocin

Discussion

Methicillin-resistant *S. aureus* is one of the most common nosocomial pathogen. The main sources of MRSA in the hospital environment are the colonized patients and HCWs who are asymptomatic and they may serve as reservoir and disseminator of MRSA in hospitals. MRSA infections lead to prolong hospital stay, and increase in treatment cost.

In present study the rate of nasal carriage of *S. aureus* in HCWs was 280 (41.79%) which was higher than 14.44% in the studies conducted by Moghadam *et al*. [9], 14.28% by Kaur and Pandey [10], 17.5% in Radhakrishna *et al*. [3], and has been reported as 27.5 % and 28.91% by various authors [14-15]. The results of present study are comparable with the studies 48% and 47.5% conducted by Agrawal *et al*. [2] and AlAbdli and Baiu [14] respectively. Sing *et al*. have detected colonization at two different sites ,in nasal swab only it was 28.91%, in hand swab only it was 9.63% and both nasal swab and hand swab 7.22% total colonization rate was 45.7% [15].

Among 280 *S. aureus* methicillin resistance were seen in 61 isolates. Thus 21.78% of the HCWs were harboring the MRSA. Our results are in accordance with the study conducted by AlAbdli and Baiu (21.4%) [14]. Little lower rates were reported by the studies conducted in different hospital setting worldwide which has been reported in the range of 9.7% to 14.28% [2, 10, 14-15]. Higher percentage of MRSA carriage has been reported by Moghadam *et al*. 43.58% [9]. This difference in rate of nasal carriage of *S. aureus* and MRSA in various hospitals may be due to difference in effectiveness of hospital infection control measures.

In the present study, in relation to the professional category, housekeeping staff (80.59%) have presented the highest rate of nasal carriage of *S. aureus* followed by doctors (44.06 %) and the lowest rate of colonization was found in nursing staff (35.87%). Radhakrishna *et al*. [3] reported MRSA carriage rate 13.3% in housekeeping staff and 2.7% in nursing staff. In majority of studies the colonization rate was higher amongst the nursing staff, followed by housekeeping staff and then in doctors [2, 3, 10].

MRSA carriage rate was particularly high among the doctors 16 (30.76%) which was similar with the findings of Al Abdli and Baiu (30.6%) [14]. In our study, only one case (1.63%) of Mupirocin resistance (MuH and MuL) was reported. Kaur and Pandey [10] reported 1.43 % and Agarwal *et al*. [2] reported 2% Mupirocin resistance. Solmaz *et al*. [9] reported 1.85% Mupirocin resistance with one MRSA strain showed high level mupirocin resistance.

Conclusion

The present study showed a high prevalence of nasal carriage of MRSA among health care workers, these HCWs can be the source of MRSA infections. Therefore we suggest MRSA screening of HCWs as a routine practice. We also insist on mupirocin resistance detection so that in case if resistance detected alternative treatment can be given.

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