

Mupirocin resistance in nasal carriage of *Staphylococcus aureus* among healthcare workers of a tertiary care rural hospital

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Abstract

Introduction: Mupirocin (pseudomonic acid A) is a topical antimicrobial agent with excellent antistaphylococcal and antistreptococcal activity. A nasal formulation is approved by the United States Food and Drug Administration for eradicating nasal carriage in adult patients as well as in health care personnel. Resistance to mupirocin has already been reported worldwide. The increasing prevalence of mupirocin resistance among *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CoNS) species could be an important threat to the future use of mupirocin against methicillin-resistant *S. aureus* (MRSA). Thus, this study was carried out to find the prevalence of mupirocin resistance in *S. aureus* and CoNS by disc diffusion and to determine the rates of high-level and low-level mupirocin resistance in *S. aureus* and CoNS by disc diffusion. **Materials and Methods:** A total of 140 healthcare workers (HCWs) (doctor, nursing staff, housekeeping staff) were randomly selected. *S. aureus* and CoNS isolates were tested for mupirocin resistance by the disk diffusion method using 5 µg and 200 µg mupirocin discs. MRSA isolates were tested for antibiotics by Kirby-Bauer disc-diffusion method as per Clinical and Laboratory Standards Institute guidelines. **Results:** Out of 140 nasal swabs collected from HCWs, *S. aureus* was isolated in 38 (27.14%), and CoNS was isolated in 73 (52.14%). MRSA was isolated in 20 (14.28%) and methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS) in 34 (24.29%). Methicillin-sensitive *S. aureus* (MSSA) and MSCoNS isolates were 100% sensitive to mupirocin, but two isolates from MRSA (1.43%) and five from MRCoNS (3.57%) were mupirocin resistant. **Conclusion:** The presence of mupirocin resistance in MRSA and MRCoNS is a cause for concern. It could be limited by regular surveillance and effective infection control initiatives so to inform health care facilities to guide therapeutic and prophylactic use of mupirocin.

Keywords: Antibiotic resistance, methicillin-resistant coagulase-negative *Staphylococci*, methicillin-resistant *Staphylococcus aureus*, mupirocin resistance

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Introduction

Nasal carriage of *Staphylococcus aureus* plays a key role in the epidemiology and pathogenesis of infection and is a major risk factor for the development of both community-acquired and nosocomial

infections.^[1] Currently, the health problems associated with this microorganism have become more serious due to the increasing incidence of methicillin-resistant *S. aureus* (MRSA).^[2] Several studies worldwide have reported the rate of nasal carriage of *S. aureus* strains, varying from 16.8% to 90%.^[3-6] A causal relationship between *S. aureus* nasal carriage and infection is supported by the fact that the nasal strain and the infecting strain share the same genotype.^[7]

In recent years, nosocomial outbreaks of MRSA have become a major infection control problem. MRSA

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strains may spread readily in hospitals from colonized or infected persons. Colonized employees are generally asymptomatic, although they are a potential reservoir of infections acquired by patients.^[8] Colonized or infected hospital personnel (HCWs) may serve as reservoir and disseminator of MRSA in hospitals.^[9]

Nasal mupirocin has an important role to play in the eradication of MRSA carriage. It acts by binding specifically to the bacterial isoleucyl-tRNA synthetase (IRS) enzyme and inhibits its protein synthesis. With the increased use of mupirocin, both low and high level resistance has been reported during treatment with nasal mupirocin.^[10] Mupirocin was first introduced in the UK in 1985 and was used to treat staphylococcal and streptococcal wound infections and to eradicate nasal carriage of *S. aureus* including MRSA.^[11] Within 2 years after its introduction, mupirocin resistance among MRSA isolates emerged in the UK and since then in Ireland 2%, New Zealand 12.4%, the USA 24%, and in Trinidad and Tobago 44.1%.^[12-16]

Although no performance standards or interpretive criteria have been published for mupirocin susceptibility testing, two mupirocin resistance phenotypes namely low level (MuL) and high level (MuH) mupirocin resistance are defined in *Staphylococci*.

Low-level resistance (minimal inhibitory concentration [MICs], 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^[17] 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*.^[18]

Various studies suggest that during mupirocin prophylaxis transfer of mupA gene from normal commensal flora of the skin such as *Staphylococcus epidermidis* to MRSA is responsible for the emergence of mupirocin resistance.^[19] The risk of the emergence of such resistance appears to be greater among methicillin-resistant strains of *S. aureus* than among methicillin-susceptible strains.

Detection and differentiation of both types has important clinical implications. The presence of high-level

mupirocin resistance (MuH) excludes its clinical use, however low-level mupirocin resistance (MuL) can be overcome by recommending higher than usual dosage.

Therefore, it is essential not only to discriminate between susceptible and resistant strains but also to determine the level of resistance. The true extent of mupirocin-resistant among HCWs in our area is unknown. Thus, this study was carried out with the following aims and objectives.

Aims and objectives

- To find the prevalence of *S. aureus* from the nasal swabs of HCWs
- To find the prevalence of mupirocin resistance in *S. aureus* and coagulase-negative *Staphylococcus* (CoNS) spp. by disc diffusion
- To determine the rates of MuH and MuL in *S. aureus* and CoNS spp. by disc diffusion
- To know the antimicrobial susceptibility patterns of *S. aureus*.

Materials and Methods

A prospective cross-sectional study was carried out from the period of December 2013 to April 2014. Approval was obtained from the Ethical Committee for carrying out the study. Written informed consent was obtained from participants.

A total of 140 HCWs (doctor, nursing staff, housekeeping staff) were randomly selected. The age, sex, designation, duration of working in the critical care unit and other relevant information were obtained in a proforma which was made for this purpose. Participants were HCWs in the intensive care unit and operation theatre for more than 1 year. Eight HCWs were not included as they were having upper respiratory tract infection.

Nasal swabs from both nostrils were collected by rotating a sterile cotton swab pre-wetted with sterile saline 5 times on the vestibule of both anterior nares. The swabs were immediately placed in test tubes for further processing in the laboratory.

Nasal swabs from both nostrils were streaked on blood agar for 24 h at 37°C. Identification of *S. aureus* was done by standard biochemical techniques.^[20]

All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance using

cefoxitin disc (30 µg). The isolates were considered methicillin-resistant if the zone of inhibition was 21 mm or less.^[20]

The isolates of *S. aureus* were then tested for mupirocin resistance. This was done by the disk diffusion method using 5 µg and 200 µg mupirocin discs to determine low and high level resistance respectively.^[20]

Criteria of zone diameter breakpoints for susceptible and resistant isolates were set at >14 mm and <13 mm respectively.^[21] Three different phenotypes are:

- Mupirocin susceptible: A zone diameter of ≥14 mm for both 5 µg and 200 µg discs
- Low-level resistance: Isolates that showed zone diameters <14 mm in the 5 µg disc but more than or equal to 14 mm in the 200 µg disc
- High-level resistance: Isolates with zone diameters <14 mm for both 5 µg and 200 µg.

Staphylococcus aureus isolates was then subjected to antimicrobial susceptibility testing by modified Kirby-Bauer’s disc diffusion method on Mueller-Hinton agar plates using as per Clinical and Laboratory Standards Institute guidelines^[20] except for fusidic acid where the French Society of Microbiology recommendations were used.

The following antibiotics were tested amikacin (30 µg), erythromycin (15 µg), clindamycin (2 µg), linezolid (30 µg), gentamycin (30 µg), fusidic acid (10 µg), trimethoprim-sulfamethoxazole (25 µg), teicoplanin (30 µg), rifampicin (10 µg) and vancomycin (30 µg), mupirocin (5 µg) and mupirocin (200 µg) (discs were procured from Hi-media Laboratories, Mumbai, India and Mast group, UK).

Results

Out of 140 HCWs, *S. aureus* was isolated in 38 (27.14%) out of which MRSA and methicillin-sensitive *S. aureus* (MSSA) were 20 (14.28%) and 18 (12.86%) respectively. CoNS was isolated in 73 (52.14%) workers, among them methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS) was found in 34 (24.29%) and methicillin-sensitive coagulase negative *Staphylococci* (MSCoNS) 39 (27.86%) respectively [Table 1]. The prevalence of the *S. aureus* nasal carriage was higher among female HCWs 21 (55.26%) than males 17 (44.74%). There is statistically no significance between colonization of female HCW and male HCW [Table 2].

In relation to the professional category, Doctors have presented the lowest prevalence of colonization (21.05%), followed by the Housekeeping staff (31.58%) and the high prevalence was found in nursing staff (47.37%) [Table 3].

In our study among the 140 health care workers, MSSA and MSCoNS isolates were 100% sensitive to mupirocin but two isolates from MRSA (1.43%) and five from MRCoNS (3.57%) showed mupirocin resistance. [Table 4 and Figure 1].

Discussion

Methicillin-resistant *S. aureus* has been recognized as an important nosocomial pathogen worldwide because of the increased rate of multidrug resistant strains among the hospital acquired MRSA. MRSA colonization precedes infection, anterior nares being the ecological niches of *S. aureus*.

The prevalence of *S. aureus* in a nasal carriage of HCWs in our study was 38 (27.14%). Similar were the findings of Golia *et al.* (24.84%)^[22] and Rongpharpi *et al.* (22.22%).^[23]

The prevalence of MRSA and MSSA in our study was 20 (14.28%) and 18 (12.86%) Golia *et al.* noted to the tune of 13.37% and 11.46%.^[22] Other workers

Table 1: Culture results of 140 nasal swabs of HCW

Isolates	Number (%)
Coagulase positive <i>Staphylococcus</i> (n=38)	
MRSA	20 (14.28)
MSSA	18 (12.86)
CoNS (n=73)	
MRCoNS	34 (24.29)
MSCoNS	39 (27.86)
Other organism	17 (12.14)
Sterile culture	12 (8.57)
Total	140

MRSA of 14.28% and MRCoNS of 24.29%. HCW: Healthcare worker; MRSA: Methicillin-resistant *S. aureus*; MSSA: Methicillin-sensitive *S. aureus*; MRCoNS: Methicillin resistant coagulase negative *Staphylococcus*; MSCoNS: Methicillin-sensitive coagulase-negative *Staphylococcus*; CoNS: Coagulase-negative *Staphylococcus*; *S. aureus*: *Staphylococcus aureus*

Table 2: Gender-wise prevalence of *S. aureus* and MRSA isolates

Gender	Total number	<i>S. aureus</i> n=38 (%)	P	MRSA n=20 (%)	P
Male	67	17 (25.37)	0.055	9 (13.43)	0.1
Female	73	21 (28.37)	0.05	11 (15.06)	0.1
Total	140	38 (27.14)		20 (14.28)	

This table depicts that gender has no association on prevalence of *S. aureus* and MRSA isolates (Chi-square test P is significant if value is more than 3.041). *S. aureus*: *Staphylococcus aureus*; MRSA: Methicillin-resistant *S. aureus*

reported the prevalence of MRSA of 11.43%, and 28.6% respectively.^[23-25]

Recent studies have shown that the prevalence of MRSA colonization among health staff changes according to the location and with the characteristics of each institution.

In this study, female HCWs were more colonized 21 (55.26) when compared to male HCWs 17 (44.74). Similar were the findings of Shakya *et al.* who observed males HCWs (42%) and females HCWs (57%).^[25]

In relation to the professional category, doctors have presented the lowest prevalence of colonization (21.05%), followed by the housekeeping staff (31.58%) and the high prevalence was found in nursing staff (47.37%) [Table 3]. Other workers reported the prevalence of colonization among doctors to the tune of 25% and 22.7%.^[23,24]

Silva *et al.* observe that to develop activities like respiratory therapists or laboratory technicians represent a risk factor for the colonization by *S. aureus*. These individual possesses 4.57 times greater probability of being colonized when compared to doctors. And the author believe that the high prevalence among nursing staff probably occurs because these professionals are less provided with information related to risks, for the team and for the patients, caused by health staff, colonized by pathogenic microorganisms.^[26]

Mupirocin is the cornerstone of decolonization regimens, a successful strategy to prevent healthcare-associated staphylococcal infections. However, new MRSA colonization has been reported even after the use of mupirocin.

Shakya *et al.* observed 0% resistance to mupirocin in the HCW's whereas in our study among the 140 HCW's, MSSA and MScONS isolates were 100% sensitive to mupirocin but two isolates from MRSA (1.43%) and five from MRCoNS (3.57%) were mupirocin resistance [Table 4 and Figure 1].^[25]

Prolonged, widespread or uncontrolled use and multiple courses of mupirocin are all associated with the development of mupirocin resistance^[14] exposure of CoNS on skin surfaces during prolonged or repeated topical application of mupirocin may lead to the development of a reservoir of high-level resistance determinants in CoNS which may then be transferred to *S. aureus* in patients on mupirocin therapy.

Oommen *et al.* observed the rate of mupirocin resistance in *S. aureus* as 1.02% but the rate of mupirocin resistance in CoNS (16%). In a study by Gadepalli *et al.* which first documented the extent of mupirocin resistance in an Indian hospital, it was found that 1% of 200 *S. aureus* isolates (including 0.9% of MRSA and 1.1% of MSSA) showed low-level resistance and 5% showed high-level resistance (8.2% of MRSA and 1.1% of MSSA isolates).^[27,28]

In a study that included *Staphylococci* from 19 European hospitals, the prevalence of MuH was found to be 1.6% in *S. aureus* and 5.6% in CoNS.^[29]

Table 3: Category-wise prevalence of *S. aureus* and MRSA isolates

Category	Total number of nasal swabs	<i>S. aureus</i> (number (%))	P	MRSA (number (%))	P
Doctor	38	8 (21.05)	1.3	3 (15)	0.8
Nursing staff	62	18 (47.37)	1.92	9 (45)	0.5
Housekeeping staff	40	12 (31.58)	0.08	8 (40)	0.14
Total	140	38 (27.14)		20 (14.28)	

This table depicts that profession has no association on prevalence of *S. aureus* and MRSA isolates (Chi-square test P is significant if value is more than 3.041). *S. aureus*: *Staphylococcus aureus*, MRSA: Methicillin-resistant *S. aureus*

Table 4: Mupirocin resistance in *Staphylococcus spp*

Category	Mupirocin resistance				Total n=54
	MRSA n=20		MRCoNS n=34		
	MuL	MuH	MuL	MuH	
Doctor	1	0	0	0	1
Nursing staff	0	1	0	3	4
Housekeeping staff	0	0	0	2	1
Total (%)	1 (0.71)	1 (0.71)	0	5 (3.57)	7

This table depicts two isolates from MRSA and five isolates from MRCoNS were mupirocin resistant. MRSA: Methicillin-resistant *S. aureus*, MRCoNS: Methicillin resistant coagulase negative *Staphylococcus*, MuH: High-level mupirocin resistance; MuL: Low-level mupirocin resistance

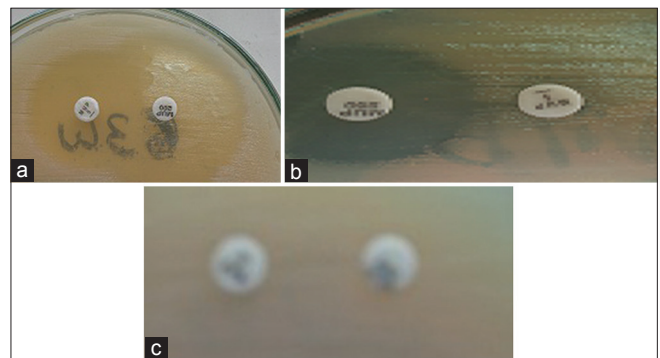


Figure 1: (a) Mupirocin susceptible (b) Low-level resistance (c) High-level resistance

In our study higher percentage of antibiotic resistance was noted to erythromycin (66.67%), gentamicin (50%),

clindamycin (47.61%), trimethoprim-sulfamethoxazole (42.85%) and lower resistance was observed for rifampicin (2.38%) and fusidic acid (11.90%). None of our isolates showed resistance to teicoplanin, vancomycin and linezolid [Table 5].

O'Neill *et al.* reported that MRSA strains with mupirocin resistance were often more susceptible to other antimicrobial agents, such as tetracycline and trimethoprim-sulfamethoxazole. In contrast, mupirocin-resistant isolates were more likely to be resistant to fusidic acid. It is speculated by the author that the *fusB* determinant, which is responsible for fusidic acid resistance is on the same plasmid as the *mupA* gene in isolates with MuH.^[30] However, there are studies that demonstrate that some isolate bacterial of *S. aureus* already present genes resistant to mupirocin, compromising the therapeutic value of the latter.

If stable chromosomal MuH were to become prevalent, reducing the antibiotic selection pressure may not lead to a reduction in rates of resistance and control measures will have to rely on prevention of transmission.

Conclusions

Mupirocin is a topical antibiotic used for treating MRSA associated skin and soft tissue infections and eliminating nasal colonization of MRSA among patients and medical staff.

The presence of mupirocin resistance in MRSA and MRCoNS is a cause for concern. Hospitals need to develop more stringent hospital infection control policies, but also to create awareness among housekeeping and nursing staff by educating them to eradicate MRSA carriage.

Mupirocin nasal ointment should be reserved for the eradication (in both patients and staff) of nasal carriage

Table 5: Antibiotic resistance pattern of *S. aureus* isolates

Antibiotic	<i>S. aureus</i> n=42 (%)
Erythromycin	28 (66.67)
Gentamycin	21 (50)
Clindamycin	20 (47.61)
Trimethoprim-sulfamethoxazole	18 (42.85)
Amikacin	11 (26.19)
Fusidic acid	5 (11.90)
Rifampicin	1 (2.38)
Vancomycin	0
Teicoplanin	0
Linezolid	0

This table depicts higher resistance to erythromycin (66.67%) followed by clindamycin (50%). No isolates were resistant to vancomycin, teicoplanin, and linezolid. *S. aureus: Staphylococcus aureus*

of MRSA. Alternative preparations such as chlorhexidine and neomycin cream (Naseptin-Manufactured by Alliance Pharmaceuticals) should be considered if colonization persists after two courses of mupirocin or if swabs confirm mupirocin resistance.

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