

Screening of Nasal Carriage of Mupirocin Resistant *Staphylococcus aureus* among Health Care Providers of Tertiary Care Hospital in Central India

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Abstract

Introduction: Nasal carriage of Mupirocin resistant *Staphylococcus aureus* amongst Health Care Providers (HCP) is a matter of concern for Hospital acquired infection due to Methicillin resistant stains of *S. aureus*. CDC recommends use of Mupirocin for decolonization only in outbreaks or other high prevalence situations. This Cross-sectional Analytical study was carried out to assess the burden of Mupirocin resistance in nasal carriage isolates of *Staphylococcus aureus* in Health care providers from Tertiary care hospital in central India.

Methods: Thirty non repetitive samples each from four groups of health care provider i.e. Consultants, Residents, Nursing staff & Cleaning Staff were collected after informed consent and ethical clearance. Samples from anterior nares were processed for isolation of *S. aureus* and screened for Methicillin resistance with MIC of Mupirocin. Data maintained in Microsoft office Excel was analyzed with statistical tools like tests of proportion & Chi Square test for significance.

Results: Thirty five *S. aureus* strains were isolated of 120 samples collected of which 15 were MRSA. A total of 11 strains were found to be having High level Mupirocin resistant (MupH) of which 9 were Methicillin resistant while 2 were Methicillin sensitive. MupH strain colonization was more in Residents group but Low level Mupirocin Resistant was not found in any group. Statistically no difference was found between Clinical & Non Clinical Groups for MupH.

Conclusion: MupH is on rise in MRSA as well as MSSA strains. Regular screening of HCP for nasal carriage of MRSA & use of Mupirocin only in outbreaks or critical areas in view of developing Mupirocin resistance is of prime importance.

Key-words: Mupirocin resistance, Nasal colonization, MRSA, MIC, Health care provider, Hospital Acquired Infections

Key Messages: The control and prevention of the infection ascribed to MRSA can only be achieved when there is a regular screening of carriers among healthcare providers thus preventing the spread of MRSA in hospital settings as well as community. Large scale multi-centric trials for nasal carriage of *Staphylococcus aureus* and also the CONS with assessment of Mupirocin resistance should be done restricting the use of Mupirocin to decolonization of nasal carriage of MRSA stains in outbreaks or other high prevalence situations amongst Health Care Professionals.

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Introduction

Staphylococcus aureus is now widely recognized as the most common microorganism in community-acquired & hospital-acquired infections causing variety of infections ranging from mild skin and soft-tissue infections to serious life-threatening infections. *S. aureus* harboring various resistance mechanisms causing emergence of multi drug resistant strains have been reported with increasing frequency everywhere.¹

It is not uncommon that Methicillin Resistant *Staphylococcus aureus* (MRSA) has a high rate of associated infections. This is because MRSA strains are resistant to β -lactam antibiotics; hence are not treatable by antibiotics like penicillin, Methicillin, oxacillin, cloxacillin etc. Moreover Hospital Acquired Methicillin Resistance *Staphylococcus aureus* (HA-MRSA) and

Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) is of deep distress and apprehension because they are responsible for post-operative wound infection and also worsening of health conditions of patients.^{2,3,4}

Carriers in hospital settings and public places are responsible for the spread of both HA-MRSA & CA-MRSA. The anterior nares are the main area of colonization for *S. aureus*. Approximately 20% of individuals are persistently nasally colonized with *S. aureus*, and 30% are intermittently colonized. Though numerous other sites like axillae, groin, and gastrointestinal tract can be colonized but nasal colonization appears to play a significant role in the epidemiology and pathogenesis of infection. Colonization provides a reservoir from which bacteria can be introduced when host defenses are breached by activities like shaving, aspiration, insertion of central lines/ catheters or surgery. Colonization clearly increases the risk for subsequent infection. Decolonization strategies are recommended for outbreak and includes cutaneous antiseptics and nasal application of a topical antimicrobial agent such as Mupirocin and includes cutaneous antiseptics and nasal

application of a topical antimicrobial agent such as Mupirocin.^{5,6,7}

Mupirocin (pseudomonic acid A) is derived from *Pseudomonas fluorescens* and is an analogue of isoleucine that inhibits protein synthesis by competitively binding to the enzyme isoleucyl-tRNA synthetase.

Of the two mupirocin-resistant phenotypes, the low-level resistant 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 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. High level mupirocin resistance has been associated with failure to clear the organism from patients on mupirocin therapy.^{8,9}

In addition to this there are alarming reports of high resistance of Mupirocin in Methicillin Resistant *Staphylococcus aureus* (MRSA) strains and emergence of a new mobile resistance gene (*mupB*) in MRSA strains for Mupirocin. In addition there are reports of mupirocin resistance developing in Coagulase Negative *Staphylococci* which may act as a reservoir of *mupA* resistance gene.⁸

Today, a Mupirocin resistant strain has been reported from various parts of the world. The prevalence of these strains in Korea, India, South Africa and Nigeria has been reported 5%, 14.6%, 7% and 0.5% respectively.¹⁰

The CDC does not recommend routine use of Mupirocin for decolonization. Mupirocin use should be limited to outbreaks or other high prevalence situations. Therefore this study was undertaken to assess the burden of Mupirocin resistance by determination of Minimum inhibitory concentration (MIC) in nasal carriage isolates of *Staphylococcus aureus* in health care providers of Tertiary care hospital in Bhopal.

Materials and Methods

This Cross-sectional Analytical study was carried out in the Department of Microbiology, in college associated with tertiary care hospital, Bhopal during a time period of 2 months from August to September 2015 as an ICMR- STS project. After obtaining the Ethical Clearance from the Institutional Ethics Committee, Informed Written Consent were taken from the Health Care Providers of legal age and any sex and nasal swabs were collected. One hundred and twenty (120) Non-Repetitive samples from Health Care Providers (HCP) of tertiary care hospital were collected.

Nasal swabs were taken with the help of sterile cotton swab moistened with sterile Distilled Water from anterior nares of Health Care Providers (HCP) and transported immediately to Microbiology Laboratory.¹¹ Health Care Providers were divided into 4 groups – Group I (30 Participants) : Consultants

Group II (30 Participants) : Junior & Senior Residents

Group III (30 Participants): Nursing Staff

Group IV (30 Participants): Cleaning Staff/ Ward-boys/ Mauai bai etc.

Culture & Identification: The samples were inoculated immediately on Blood Agar and smear was prepared for Direct Examination by Gram staining. Inoculated Blood agar plates were incubated for 18-24 Hrs. at 37°C. The Beta hemolytic colonies on blood agar were further identified as *Staphylococci* by Gram staining which shows Gram positive cocci in clusters. All these colonies were subjected to Slide coagulase test & Tube Coagulase test to confirm it to be *Staphylococcus aureus*.¹¹

Slide Coagulase Test: 2 to 3 colonies of isolate were emulsified in a drop of normal saline over a glass slide with the help of inoculating loop to form a milky suspension. Fresh plasma was taken with the help of straight wire and mixed in milky suspension. The slide was rocked to and fro with hand to look for clumps. The visible clumping was taken as slide coagulase positive.¹¹

All Slide Coagulase negative & isolates showing auto agglutination were further tested using Tube Coagulase Test.

Tube Coagulase test: The isolates were inoculated in the peptone water and were incubated for 18-24 Hrs. at 37°C. The 0.5 ml of the overnight broth was mixed with 0.5 ml of 1:9 saline diluted fresh plasma and incubated at 37°C. The tubes were then observed by tilting at 1 hrs. 2 hrs. and 6 hrs. for formation of coagulum. The formation of coagulum was taken as tube coagulase positive.¹¹

The identified strains of *Staphylococcus aureus* were further tested for Methicillin Resistance by Cefoxitin Disk Diffusion Test.

Procedure for MRSA detection by Cefoxitin Disk Diffusion Method & MIC of Mupirocin: For convenience both the test were done simultaneously on the same plate.

The isolated *Staphylococcus aureus* strain were inoculated in Peptone water and incubated. The turbidity of broth thus prepared was matched with 0.5 McFarland Turbidity standard using Densimat (Bio Meraux, India). Lawn culture was made on Muller Hinton Agar (MHA).

The Cefoxitin 30 µg disk from Hi-Media India was placed on the MHA on one side & on the other side Ezy- MIC test strips of Mupirocin from Hi-Media India having concentration gradient from 0.064 µg/ml to 1024 µg/ml was applied on the plate surface, and the plates were incubated for 18-24 Hrs. at 37°C.

The zone of inhibition was measured using zone scale. The zone of inhibition of <21 mm for Cefoxitin

was considered as Cefoxitin Resistant indicating the strain to be MRSA. All strains showing zone of inhibition of equal to or more than 22 mm were considered as MSSA as per CLSI 2014 guidelines.¹²

The plates were also observed for zone of inhibition intersecting the graduated strip of Ezy-MIC and readings was noted and was divided into –^{11,12}

1. Mupirocin Sensitive (S): MIC of <4 µg/ml,
2. Low Level Mupirocin Resistance (MupL): MIC of 8–256 µg/ml,
3. High Level Mupirocin Resistance (MupH): MIC of > 512 µg/ml.

Procedure for Data Management & Analysis: All data was maintained in Microsoft office Excel. All statistical analysis were carried out using Excel and Statistical tools like tests of proportion & Pearson's Chi Square test for significance were applied.

Result

Out of a total of 120 nasal samples collected (30 in each group) and cultured on Blood agar, 52(43.33%) were having growth as small round beta hemolytic colonies. The Gram stain of the growth revealed around 49(40.83%) to be gram positive cocci in clusters whereas 3 (2.5%) were aerobic spore bearers (ASB). [Table 1]

Table 1: Nasal samples showing growth and coagulase test positive stains of *Staphylococcus aureus*

Groups	No. of Samples n (%)	Growth on Blood Agar n (%)	Gram Stain showing Gram Positive cocci n (%)	Coagulase positive	
				Slide n (%)	Tube n (%)
Group I	30 (25)	08(6.66)	08(6.66)	06 (5.00)	0(0.00)
Group II	30 (25)	11 (9.16)	11 (9.16)	8 (6.66)	1(0.83)
Group III	30 (25)	13 (10.83)	13 (10.83)	8 (6.66)	1 (0.83)
Group IV	30 (25)	20 (16.66)	17 (14.16)	10 (10.00)	1(0.83)
Total	120 (100)	52 (43.33)	49 (40.83)	32 (26.66)	3(2.50)

Out of 49 such strains which were subjected to coagulase test. A total of 32 (26.66 %) were positive by slide coagulase whereas 03(2.5%) were positive by tube coagulase test performed in strains which were negative for slide coagulase. 35 out of 49 grown i.e. 71.42% were found to be *Staphylococcus aureus*. [Table 1]

All the 35 *Staphylococcus aureus* strains were subjected to cefoxitin disk diffusion test as a confirmatory test for testing Methicillin resistance as per CLSI 2014 guidelines. 15 out of 35 (42.85%) were found to be Methicillin Resistant (MRSA) whereas 20 out of 35 strains (57.14%) strains were found to be Methicilline Sensitive (MSSA). [Table 2]

Table 2: Result of Cefoxitin Disk Diffusion Test Identifying MRSA & MSSA stains

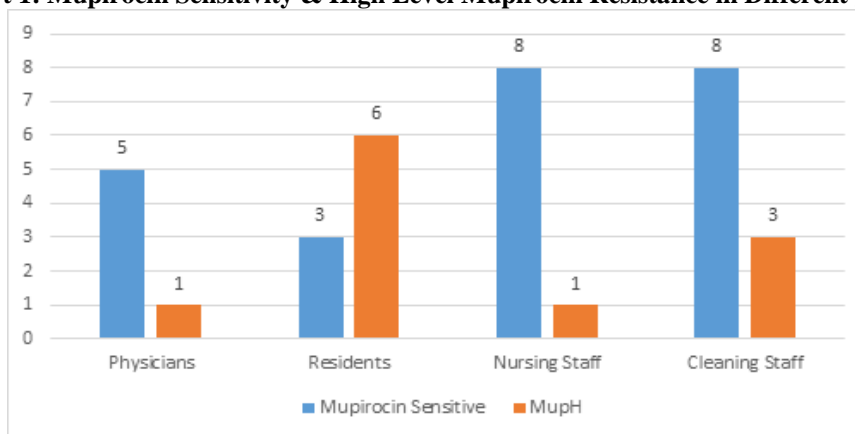
Groups	No. of isolates identified as <i>S. aureus</i>	MSSA (ZOI > 22mm)	MRSA (ZOI < 21mm)
Group I	6	4	2
Group II	9	5	4
Group III	9	4	5
Group IV	11	7	4
Total	35	20	15
ZOI: Zone of Inhibition			

All the 35 strains of *Staphylococcus aureus* were subjected to MIC determination by Ezy MIC strips from HiMedia India for Mupirocin. The group wise distribution of Mupirosin sensitive, Low Level Mupirocin Resistance (MupL) and High Level Mupirocin Resistance (MupH) is as shown in Table 3 & Chart 1.

Table 3: Mupirocin sensitive, Low level & High Level resistance by MIC testing in MRSA & MSSA strains

Staphylococcus aureus	Mupirocin Sensitive				Total G1 to G4	Low Level Mupirocin Resistance (MupL)				Total G1 to G4	High Level Mupirocin Resistance (MupH)				Total G1 to G4
	I	II	III	IV		I	II	III	IV		I	II	III	IV	
MRSA (n=15) 42.85%	1	0	4	1	6 (17.14%)	0	0	0	0	0 (0.00%)	1	4	1	3	9 (25.71%)
MSSA (n=20) 57.14%	4	3	4	7	18 (51.42%)	0	0	0	0	0 (0.00%)	0	2	0	0	2 (5.71%)
Total (n=35) 100 %	5	3	8	8	24 (68.57%)	0	0	0	0	0 (0.00%)	1	6	1	3	11 (31.42%)

Chart 1: Mupirocin Sensitivity & High Level Mupirocin Resistance in Different groups



Noteworthy over here is all Eleven MupH strains was having MIC of >1024 µg/ml whereas all sensitive strains were having MIC of < 2 µg/ml. Two (2) Methicillin sensitive strains were also found to carry high level resistance which is a matter of concern. [Table 3]

When Clinical and Non-Clinical groups (combination of Group 1 & 2 against Group 3 & 4) were compared for MupH against Mupirocin sensitivity for both MRSA and MSSA by Chi square test it was observed that there is no statistically significant difference between the two. (Table 4)

Table 4: Test of significane applied to MRSA & MSSA strains in Clinical & Non- Clinical HCW

MRSA Stains	Clinical (Group I & II)	Non-Clinical (Group III & IV)	Total	Chi-Square Test
MupH	05	04	09	$X^2=2.26$ $P\text{ value}=0.132$ Not Significant
Mupirocin Sensitive	01	05	06	
Total	06	09	15	
MSSA Strain	Clinical (Group I & II)	Non-Clinical (Group III & IV)	Total	
MupH	05	04	09	$X^2=2.71$ $P\text{ value}=0.09$ Not Significant
Mupirocin Sensitive	01	05	06	
Total	06	09	15	

Discussion

Out of 120 samples processed 49 (40.83%) showed growth of *Staphylococcus*. 35 of 49 (71.42%) strains turns out to be *Staphylococcus aureus* and rest 14 (28.58%) were Coagulase Negative *Staphylococcus* (CONS). Overall isolation percentage of *Staphylococcus aureus* from 120 nasal samples turns out to be 35/120 i.e 29.16% which is well in accordance with the isolation rates in different part of the world.

In a study carried out in Iran by Moghadam S O et al isolated 39 (14.44%) *Staphylococcus aureus* from nasal carriage in Health Care workers. Whereas in a study conducted by Lakshmi S. Kakhandki and B.V. Peerapur in Bijapur, Karnataka 33(23.6%) strains were isolated from the nasal carriage.^{13,14}

Fifteen out of 35 (42.85%) were found to be Methicillin Resistant (MRSA) whereas 20 out of 35 strains (57.14%) strains were found to be Methicilline Sensitive (MSSA) in our study. In a similar study conducted by Kaur

DC & Narayan PA 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.¹⁵

The comparative table about Mupirocin resistance in MRSA & MSSA strains isolated amongst health care workers in our study and other studies is as follows:

Studies	MSSA		MRSA	
	MupLn (%)	MupHn (%)	MupLn (%)	MupHn (%)
Present Study	0(0.00%)	02(5.71%)	0(0.00%)	9 (25.71%)
Hee-Jeong Yun et al ¹⁶¹⁶	-	1(0.3%)	-	15(4.7%)
Franz-Josef Schimitz et al ¹⁷¹⁷	2(0.9%)	2(0.9%)	2(3.5%)	1(1.8%)
Oommen SK et al ¹⁸¹⁸	--	--	--	1(2.08%)
Gadepalli et al ¹⁹¹⁹	--	1.0%	--	5%

It is worthwhile to state over here that in Group 2 of Residents the colonisation of High Level Mupirocin resistance was found to be high i.e 6/9 stains (66.66%)[Table 3, Chart 1]. This may be because residents are backbone of any tertiary care hospitals and may not be following the proper hand hygiene practices.

The principal mode of MRSA transmission within an institution is from patient to patient via the transiently colonised hands of hospital personnel who acquire the organism after direct patient contact or after handling the contaminated materials. Decolonisation of MRSA strains from Health care providers after regular surveillance with the mupirocin is required.

According to CDC Mupirocin use should be used only in outbreaks or other high prevalence situations. Health care workers colonized with MRSA, but who have not been linked epidemiologically to transmission, do not require treatment.

In our study 2 MSSA strains were found to possess High level resistance. Nasal application of mupirocin at clinically effective concentrations may result in the presence of low levels of antibiotic in the pharynx, which could induce or select for the emergence of mupirocin resistant MRSA & MSSA with likelihood of recolonization also.²⁰ Other topical agents like Chlorohexidine and Naseptin have been less effective than Mupirocin.²¹

Recolonisation may occur after discontinuation of the therapy. It may be possible to give long term intermittent therapy with mupirocin which may prove more effective in suppression & eradication of MRSA colonisation. But this again warrants strict vigilance as it may also lead to increased resistance to Mupirocin.²²

Moreover the present study showed that the group IV and group III had high positive culture. But MRSA colonization was more observed in Group II. Statistically no difference was found between Clinical & Non Clinical Groups for High level Mupirocin resistance against Mupirocin Sensitive strains. [Table 4, Chart 3].

All these groups Clinical & Non-Clinical are the people to be in contact with patients for a longer period

of time, more so with nursing, ward boys & cleaning staff, therefore surveillance through regular screening for Nasal carriage & treatment of the carriers should be obligatory for prevention of HAI. The study shows the need for a periodic screening of all the hospital personnel and measures which are taken to treat the carriers.

Conclusion

The control and prevention of the infection ascribed to MRSA can only be achieved when there is a regular screening of carriers among healthcare providers thus preventing the spread of MRSA in hospital settings as well as community. Sensitization and training of all Health care providers regarding hand hygiene and implementation of the same by all is need of the hour. Large scale multi-centric trials for nasal carriage of *Staphylococcus aureus* and also the CONS with assessment of Mupirocin resistance should be done restricting the use of Mupirocin to decolonization of nasal carriage of MRSA stains in outbreaks or other high prevalence situations amongst Health Care Professionals.

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