Mupirocin Resistance in Coagulase Negative *Staphylococcus* Isolated from Rural Population - An Underestimated Threat?

Dinesh Kumar¹, Dakshina Bisht², Sameer Singh Faujdar³

^{1, 2} Department of Microbiology, Santosh Medical College & Hospital, Ghaziabad Uttar Pradesh, India. ³ Department of Microbiology, Maharishi Markandeshwar Medical College & Hospital, Solan, Himachal Pradesh, India.

ABSTRACT

BACKGROUND

CoNS when exposed to repeated or prolonged treatment with mupirocin ointment, may become a reservoir of high-level resistance determinants and then pass on this resistance to *S. aureus*. CoNS usually tends to be reservoirs of antimicrobial resistance factors, hence they generally lead to recurrence of multi-drug resistance. Hence, it is imperative to identify and discriminate the strains of *S. aureus* and CoNS. We wanted to analyse mupirocin resistance in coagulase negative *Staphylococcus* isolated from a rural population.

METHODS

This study was performed in the Department of Microbiology, SMCH (Ghaziabad) among indoor as well as OPD patients of a tertiary care hospital. All coagulase negative *Staphylococcus* strains were taken from patient's clinical specimens visiting the OPD or from patients getting treatment from the hospital. CoNS were identified by standard biochemical tests. An inhibition zonal area < 21 mm was considered as resistant.

RESULTS

Mupirocin resistance was found to be 7 % in MRCoNS and 5 % in MSCoNS. MupRH and MupRL were 5 % and 7 % respectively. Only 5 (5 %) isolates showed MIC more than 512 µg / mL as described.

CONCLUSIONS

In case of emergence of mupirocin resistance, other decolonization options ought to be considered.

KEY WORDS

CoNS, Methicillin-Resistant, High-Level, Low-Level, Mupirocin Resistance

Corresponding Author:

Dr. Dakshina Bisht.

Department of Microbiology, Santosh Medical College & Hospital, Ghaziabad Uttar Pradesh, India.

E-mail: dakshinabisht@gmail.com

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BACKGROUND

Coagulase-Negative *Staphylococcus* (CoNS) until recently was considered as a non-harmful commensal microflora of human body but now is emerging as an important reason of hospital acquired as well as community acquired infections. CoNS is the leading cause of medical implant device-related infections, nosocomial blood-stream infections, and account substantially for infections in the preterm and low-birth-weight newborns.¹⁻² They give rise to various clinical infections including infective endocarditis especially when the species and strains are unusually virulent.³ The significance of CoNS posing as a greater nosocomial micro-flora in regards to prosthesis and indwelling device-associated infections is identified. CoNS also is the most usually isolated flora in microbiological laboratories.⁴ Of importance, CoNS usually tends to be reservoirs of antimicrobial resistance factors, hence they generally present a great recurrence for multi-drug resistance.⁵ Hence, it is imperative to identify and discriminate the strains of *S. aureus* and CoNS.

The incidence of infections caused by this group of organisms is mostly under-reported because of the difficulty in identifying its pathogenic role; even by using molecular techniques, colonization, and infection cannot be differentiated.⁶ Methicillin resistance has contributed to the emergence of CoNS as a common cause of nosocomial infections. Methicillin resistance in CoNS may not only lead to treatment failure but also spread this resistance to *Staphylococcus aureus* which may pose a challenge to clinicians.⁷ "These strains show confrontation to penicillins, clindamycin, tetracyclines, macrolides, linezolid, methicillin (MET) and now a days to mupirocin (MUP) and vancomycin. This resistance constraints therapeutic choices for intervention and principally enhances case morbidity and mortality".

Indiscriminate use of mupirocin, a topical antibiotic that is employed for eradication of staphylococcal nasal colonization to prevent healthcare-associated methicillin-resistant staphylococcal infections has already led to the emergence of its resistance. CoNS on nasal mucosal surfaces, when exposed to repeated or prolonged treatment with mupirocin ointment, may become a reservoir of high-level resistance determinants and then pass on this resistance to *S. aureus*. The events of gene transfer are most prone to show up at locations colonized by CoNS and coagulase positive staphylococci such as the normal nasal mucosa.⁸

"The resistance issue prevalent globally make laboratories to establish quick, precise, method for characterizing as well as distinguishing MRSA, MUP-resistant *S. aureus*, and MUP-resistant CoNS. PCR is the most common technique used for the same but scarce literature is available".⁹ Hence the current study was done to know about mupirocin resistance status in CoNS, isolated from rural population of UP.

METHODS

This is a prospective study was performed in the Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, with authorization from the Institutional Ethical

Committee (F. No. SU / 2017 / 683 (15); 26 / 05 / 2017) in indoor and outpatient departments.

All *S. CoNS* strains were taken from patients clinical specimens visiting the OPD or getting treatments from the hospital. Samples acquired in the laboratory were taken care for isolation and identification of CoNS isolates. Samples eliminating urine inoculated on 5 percent blood agar and MacConkey agar media, while urine specimens were inoculated on cysteine-lactose electrolyte deficient (CLED) agar media and incubated at 37°C aerobically. The growth was recognized as CoNS with the help of standard biochemical methods".¹⁰

Antimicrobial Susceptibility Testing

"Checking of antimicrobial susceptibility (AST) on all isolates as per the Clinical and Laboratory Standards Institute (CLSI) guidelines isolates were made on Mueller-Hinton agar media (HiMedia) by Kirby-Bauer disc diffusion technique. Isolate inoculum with turbidity of 0.5 McFarland norm (1.5 some 108 CFU / ml) was prepared in peptone water and lawn-culture was prepared on Mueller Hinton agar (MHA) and allowed to dry then sterile forceps placed on MHA antibiotic disks with different potency. Resistance to methicillin was observed using cefoxitin 30 µg disks while conducting antibiotic susceptibility tests (Hi-Media Labs, India), and a size inhibition zone of < 21 mm was considered immune. In vitro antibiotic susceptibility test was done, various antibiotic discs used were penicillin (10 units), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), levofloxacin (5 µg), linezolid (30 µg), rifampin (5 µg), tetracycline (30 µg), and co-trimoxazole (1.25 / 23.75 µg). The reference strain used for standardizing antibiotics susceptibility determination was *S. aureus* ATCC 29213. Diameters of the zone of inhibition were measured, and interpretation for determining sensitive, intermediate, or resistant was done according to standard CLSI guidelines".¹¹

Determination of Mupirocin-Resistant (MupR) CoNS

"Mupirocin resistance among CoNS was assessed using 5 µg and 200 µg mupirocin discs by Kirby-Bauer disc diffusion method. Isolates with zone > 14 mm for 5 µg as well as 200 µg discs was considered as sensitive, isolates with zone < 14 mm for 5 µg but more than 14 mm for 200 µg disc were considered as MupRL, and isolates with zone less than 14 mm for both 5 µg and 200 µg discs was considered as MupRH".¹²

Epsilon meter (E) Test for Determination of Minimum Inhibitory Concentration

"Kirby Bauer's disc-diffusion technique performed an e-test with HiComb mupirocin strip according to CLSI guidelines. Lawn cultivation was prepared on a medium surface of MHA. HiMedia E strip with mupirocin antibiotic ranged from 0.064 - 1240 µg / mL by softly pressing sterile forceps to perfection. The slabs were then aerobically incubated for 24 hours at 35°C. Checking for the minimum inhibitory concentration (MIC) after incubation plates. MIC isolates > 512 µg / mL were treated as MupRH, MICs 8 - 256 µg / mL were treated as MupRL and < 4 µg / mL were treated as mupirocin responsive".¹³

Statistical Analysis

The collected data was tabulated in an Excel Sheet. Data was analysed using IBM SPSS Statistics Windows, version 20.0 IBM Corp., (Armonk, NY, USA) for the generation of descriptive and inferential statistics.

RESULTS

Overall 100 CoNS isolates were obtained. Antibiotic susceptibility test of these isolates using cefoxitin 30 µg disc showed that 24 (24 %) isolates were methicillin-resistant CoNS (MRCoNS) and 76 (76 %) isolates were methicillin-sensitive CoNS (MSCoNS).

Isolate (100)	Mupirocin Sensitive (MupS)		Low-Level Mupirocin Resistance (MupRL)		High-Level Mupirocin Resistance (MupRH)	
	N	%	N	%	N	%
MRCoNS (24) (24 %)	17	70.8	4	16.6	3	12.5
MSCoNS (76) (76 %)	71	93.4	3	4.2	2	66.6

Table 1. Distribution of Mupirocin Resistant CoNS

E-test MIC Range (µg / ml)	No. of Isolates (100)	
	N	%
Sensitive (< 4)	88	88
Low-Level Resistance (8 - 256)	7	7
High-Level Resistance (> 512)	5	5

Table 2. MIC of Mupirocin against CoNS

Overall occurrence of mupirocin resistance was 12 % among all CoNS isolates. Mupirocin resistance was found 7 % in MRCoNS and 5 % in MSCoNS. MupRH and MupRL were 7 % and 5 % respectively (Table 1). Out of 100 isolates, majority isolates (88 %) showed less than 4 µg / mL MIC. 7 (7 %) isolates were found to have MIC between 8 - 256 µg / mL followed by 5 (5 %) isolates were showed MIC more than 512 µg / mL as described in Table 2.

Antibiotic	MSCoNS, N = 76 (%)		MRCoNS, N = 24 (%)	
	N	%	N	%
Penicillin	23	30.26	24	100
Erythromycin	13	17.11	13	54.17
Clindamycin	15	19.74	17	70.83
Cefoxitin	0	0.00	24	100.00
Ciprofloxacin	6	7.89	7	29.17
Levofloxacin	3	3.95	3	12.50
Linezolid	0	0.00	0	0.00
Rifampin	3	3.95	21	87.50
Co-Trimoxazole	22	28.95	15	62.50
Tetracycline	15	19.74	11	45.83

Table 3. Antibiotic Resistance Pattern of Methicillin-Sensitive Coagulates Negative Staphylococcus and Methicillin-Resistant Coagulates Negative Staphylococcus Isolates

The strains which were significantly coexisting with major antibiotic resistance were MRCoNS strains with 100 % resistance to penicillin, and a higher resistance to rifampin (87.50 %), clindamycin (70.83 %), co-trimoxazole (62.50 %), and erythromycin (54.17 %). All CoNS isolates were equally susceptible to linezolid (Table 3).

DISCUSSION

Advancement in medical sciences with development and the use of medical implants and devices has prolonged the human life span on one hand, although it led CoNS, commensal

bacteria, on the other hand, to become a source of hospital-acquired infections. A significant etiological agent of severe life-threatening blood stream infections, the central nervous system shunts prosthetic joints, and preterm babies. *S. Aureus* and CoNS are significant causes of nosocomial and community-acquired infections.¹⁴ They are more frequently present in clinical microbiology laboratories as isolated bacterial flora.¹⁵ CoNS has an extraordinary capability to gather elements of antibiotic resistance, such as penicillin, clindamycin, macrolide, tetracycline, and methicillin, and lately, mupirocin (MUP), and vancomycin. It results in the development of multidrug-resistant strains which reduce the treatment options for infections caused by these species.

"Mupirocin is used as a topical antibacterial that demonstrates a higher degree of streptococci and staphylococci evading infection, and few gram-negative bacteria, including *Haemophilus influenza* and *Neisseria gonorrhoeae*, but is much less active against most gram-negative bacilli and anaerobes. It is also used in the treatment of skin infection and for the prevention of MRSA. Mupirocin was used in clinical practice in 1985 and is slowly expanded globally in the form of ointment, after its application. Mupirocin-resistant clinical isolates were first recorded in 1987 with upsurge in the rate of resistance".¹⁶ Extended usage and regular usage of mupirocin is thought to be a significant factors correlated with mupirocin resistance rise.¹⁶ Simple and fast defining and discriminating against *S. aureus*, in order to administer effective antimicrobial therapy promptly and to minimize the unnecessary use of a few groups of antibiotics, aureus and CoNS, MET resistance and MUP resistance identification are important. Mupirocin is a topical antibiotic originally isolated from *Pseudomonas fluorescens* and by competitively binding to isoleucyl-tRNA synthetase (IleS), it inhibits bacterial protein synthesis. This drug is especially effective against *streptococci* and *staphylococci* like *Staphylococcus aureus* (MRSA) which is immune to methicillin. Mupirocin is one of the most common topical agents used to treat skin infection and MRSA nasal carry eradication. Increased use of this antibiotic has been followed by mupirocin-resistant MRSA outbreaks.

In the present study, mupirocin resistance was found among 12 % of CoNS isolates i.e. 7 % in MRSA and 5 % in MSSA. MupRH and MupRL were 7 % and 5 % respectively (7 %). Isolates were found to have MIC between 8 - 256 µg / mL followed by 5 (5 %) isolates showed MIC more than 512 µg / mL in the present study. These findings were identical to the investigations of Agarwal L et al,¹⁷ i.e. methicillin resistance was found among 7.6 % CoNS isolates which are higher as reported by Akhtar (2.1 %)¹⁸ and much lower than 18 - 60 % reported elsewhere.¹⁹⁻²⁰ Differences in the prevalence of CoNS, MRCoNS, and MupRCoNS might be due to the discrepancy in the methodology of recognition used in the laboratories, knowledge, practices, and policies of the institution.

CoNS have a potential tendency to cumulate antibiotic resistance determinants such as to penicillin, clindamycin, macrolide, tetracycline, and methicillin and, in recent times, mupirocin (MUP), and vancomycin. This embarked to the formation of multidrug-resistant strains which reduce the options for the therapy of diseases resulting due to these organisms. Authors of different studies across the globe have used different antibiotics to establish the pattern of antibiotic susceptibility of CoNS and found that MRCoNS were

multidrug-resistant and recommend performing antibiotic sensitivity test before treatment of infections caused by these species. MRCoNS is significantly correlated with antibacterial resistance such as penicillin, rifampin, clindamycin, Trimethoprim/sulphamethoxazole, erythromycin, and ciprofloxacin in our study. Rahman et al,²¹ studied the CoNS clinical isolates and found in decreasing order the percentage of isolates resistant to oxacillin (45 %), followed by gentamicin (40 %), cefuroxime (31 %), ceftriaxone (30 %), ciprofloxacin (22 %), and none resistant to imipenem and vancomycin and Shah et al,²² found > 50 % of the clinical CoNS isolates were resistant to cephalosporins, aminoglycosides, quinolones, and no organisms resistant to vancomycin. Mupirocin resistance, especially high-level resistance, provides less treatment options for topical use. Another cause for concern is the existence of comparatively higher levels of mupirocin resistance in CoNS. Studies say that mupA gene known to be encoding for resistance to mupirocin could be transferred from MRSA *S. epidermidis* during prophylaxis of mupirocin, which could pose a significant threat to future use of mupirocin against MRSA.

Higher carriage rate for CoNS, MRCoNS, and MupRCoNS seen in rural population may be partially related to their personal hygiene. Increased use of mupirocin in hospital leads to surge in high-level resistance to mupirocin & resistance to other CoNS antibiotics. It might impact the treatment of prosthetic infections leading to increase in risk of high-level mupirocin resistance among *S. aureus* over the long term.

CONCLUSIONS

Enhanced mupirocin usage is correlated with a faster surge in higher level mupirocin resistance and also resistance to various other antibacterials in CoNS. This might exhibit tangible consequence in treating infections and might, in the long run, enhance the risk of *S. aureus* for greater level of resistance to mupirocin. In case of development of mupirocin resistance, other decolonization options should be considered.

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