DOI: 10.7860/JCDR/2015/15230.6678

Original Article

Microbiology Section

Screening for Mupirocin Resistance in *Staphylococcus*

AVR JEYA SANJU[1], SRIDHARAN SATHYAMOORTHY KOPULA[2], KENNEDY KUMAR PALRAJ[3]

ABSTRACT

Introduction: Mupirocin is widely used topical antibiotic for the treatment of skin and soft tissue infections caused by *Staphylococcus* and *Streptococcus*. In addition nasal formulations are approved for the use in nasal eradication of methicillin-resistant *Staphylococcus aureus* in patients and health care workers. Wide usage of mupirocin has resulted in resistance leading to treatment failure.

Aim: To screen for the mupirocin resistance among the *Staphylococcus* isolates using disc diffusion and minimum inhibitory concentration method.

Materials and Methods: A cross-sectional study was done at Microbiology Department of Sri Ramachandra University with 100 strains of *Staphylococcus* spp isolated from skin and soft tissue infections. Methicillin susceptibility was done by disc diffusion method using oxacillin (1 µgm) and cefoxitin (30 µgm) discs. Isolates were screened for mupirocin resistance by disc diffusion method using 5 µgm discs. High level and low level resistance determined by MIC using agar dilution method.

Results: In 100 *Staphylococcus* spp 56 were *Staphylococcus aureus* and 44 were CoNS. Among the 56 *Staphylococcus aureus* 49 (87.5%) were mupirocin susceptible and 7 (12.5%) resistant by 5µg disc diffusion method. However by MIC method 11 (19.6%) were high and low level mupirocin resistant. Out of 44 CoNS 22 (50%) and 18 (41%) were susceptible by disc diffusion and MIC method respectively. Of the 26 resistant CoNS low level and high level mupirocin resistant was observed in 7 (15.9%) and 19 (43.1%) respectively.

Conclusion: Screening for mupirocin resistance by disc diffusion method is important before attempting decolonisation. Mupirocin resistance is more with CoNS. Disc diffusion method may miss low level Mupirocin resistance.

Keywords: High level mupirocin resistance, Low level mupirocin resistance, Methicillin resistant *Staphylococcus aureus*

INTRODUCTION

Mupirocin was first introduced in United Kingdom in 1985 [1], which is *pseudomonic* acid A, an antibiotic produced by *Pseudomonas fluorescens* showing high level of activity against *Staphylococci*, *Streptococci*, certain gram-negative bacteria like *Haemophilus influenzae* and *Neisseria gonorrhoeae* [2]. Mupirocin is widely used topical antibiotic for the treatment of skin and soft tissue infections. In addition nasal formulations are approved for the use in nasal eradication of methicillin resistant *Staphylococcus aureus* in patients and health care workers [3]. Wide usage of mupirocin has resulted in resistance leading to treatment failure. Mupirocin is an analogue of isoleucine which competitively binds to isoleucine-t-RNA synthetase, and thereby inhibits protein synthesis in the bacterium. Three categories of mupirocin susceptibility have been described for *Staphylococcus* spp i) Mupirocin susceptible with minimum inhibitory concentrations (MIC) of ≤ 4 µg/mL, ii) Low-level mupirocin resistance (MuL) with MICs from 8 to 256 µg/mL and iii) High-level mupirocin resistance (MuH) with MICs ≥ 512 µg/mL [4]. However two years after introduction varying rates and degrees (low level and high level) of mupirocin resistance have been reported from various parts of the world. With this background this study was done to look for the prevalence of mupirocin resistance among *Staphylococcus* spp in our centre.

MATERIALS AND METHODS

A cross-sectional study was conducted between January and April 2013 at Clinical Microbiology Laboratory of Sri Ramachandra University, a tertiary care centre with more than 1900 in patients facility after obtaining institutional ethical clearance (Ref: CSP/13/26/39). A non-repetitive clinically significant 100 consecutively isolated *Staphylococcus* spp from exudate specimens of patients with skin and soft tissue infections were included in the study. All the study samples were subjected to tube coagulase test and screening for methicillin resistance with oxacillin (1µg) and cefoxitin (30 µg) disc by disc diffusion method in Muller Hinton Agar and results were interpreted as per CLSI guidelines [5].

Screening for Mupirocin resistance: Test isolates were screened for mupriocin resistance along with ATCC *Staphylococcus aureus* controls using 5 mcg Mupirocin disc obtained from Hi Media Laboratories by Kirby-Bayer disc diffusion method. The zone of inhibition was measured and interpreted based on CLSI guidelines M100-S23 [5]. MIC for Mupirocin was determined by agar dilution method as per EUCAST DEFINITIVE DOCUMENT E. Def 3.1, June 2000 [6] with pure form drug powder procured from Hi Media laboratories. The various dilution of drug powder prepared was 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 512. Test organisms in peptone water matching tube No 2 of Mac Farland's standard was spot inoculated into MHA and incubated at 37ºC along with controls. Plates were read after overnight incubation and the results were interpreted based on CLSI guidelines M100-S23 [5].

RESULTS

A total of 100 *Staphylococcus* isolates were included in the study. Out of which 56 of them were *Staphylococcus aureus* and 44 were CoNS. The results of the Mupirocin susceptibility by disc diffusion method and MIC by agar dilution method are shown in the [Table/Fig-1]. Mupirocin resistance was found to be more associated with CoNS (59%) than *Staphylococcus aureus* (19.6%) based on MIC.

DISCUSSION

Mupirocin resistance is being reported in many parts of the world *viz*- Spain 11.3%, USA 13.2%, Trinidad Tobago 26.1%, China 6.6%, India 6%, Turkey 45% and Korea 5% [7] due to its indiscriminate

Isolates	Mupirocin Disc		MIC		
	S	R	S	MuL	MuH
MRSA(35)	28	7	24	4	7
MSSA(21)	21	0	21	0	0
MRCoNS (40)	19	21	15	7	19
MSCoNS (4)	3	1	3	0	1

[Table/Fig-1]: Comparison of Mupirocin susceptibility by disc diffusion and MIC

usage. In our study 11(39.2%, n=28) MRSA strains showed mupirocin resistance by MIC out of which 4(14.2%, n=28) and 7(25%, n=28) were MuL and MuH resistance respectively. Among the 44 CoNS 26(59%) was resistant to Mupirocin by MIC, showing 7(15.9%, n=44) and 19 (43.1%, n=44) MuL and MuH resistance respectively. An earlier study done in 1885 when mupirocin was just introduced Sutherland R [2] and his team on their evaluation of antimicrobial activity of mupirocin reported 100% susceptibility for both coagulase positive and negative *Staphylococcus* with MIC range falling between 0.06 to 0.12 µg/l. The correlation between disc diffusion and MIC value for the drug was appropriate in our study; most of the strains with 6 mm zone size had an MIC value of 512 µg/ml. This has been comparable with Finlay J E et al., [8] who had evaluated the relations between disc diffusion and agar dilution and E-testing. South Indian study by Oommen S K et al., [4] reported 2% MRSA and 28% coagulase negative *Staphylococcus* showing high level mupirocin resistant, Similarly Jayakumar S et al., [9] in his study has reported 2.2% mupirocin resistance in MRSA and 2.9% in MSSA, while in CoNS 14.3% had Mupirocin resistance stating the increased prevalence of resistance in CoNS. It is well proven from our study and other studies that screening of mupirocin resistance by 5 µg cannot differentiate among the MuL and MuH strains and it needs the concomitant use of both the 5 and 200 µg disc. As evident from our study mupirocin resistance is more in CoNS which remains a threat as they play major contributory role especially *Staphylococcus epidermidis* by transferring mupA gene to MRSA while attempting for decolonization with mupirocin [4].

LIMITATIONS

The limitations of our study are high level mupirocin resistance by disc diffusion with 200 µg disc were not done and the molecular study for the presence and absence of the genes conferring mupirocin resistance would have given even more information.

CONCLUSION

Screening for mupirocin resistance before decolonization treatment is better practice for avoiding treatment failure mainly in patients undergoing major surgeries. Mupirocin resistance is seen more common with CoNS; they also play an etiological role in causing post-operative infections and also contribute for the transfer of resistant gene. Mupirocin resistant strains are also multi drug resistant, so pre-operative decolonization failure cases may result in increase in hospital stay and financial burden to the patients and hospital. While screening for mupirocin resistance better to screen for both MuL and MuH by disc diffusion or E test for better appropriate results.

REFERENCES

[1] Oorett F A. The emergence of mupirocin resistance among clinical isolates of methicillin resistant *staphylococcus aureus* in trinidad: a first report. *Jpn J Infect Dis*. 2008; 61(2):107-10.

[2] Sutherland R, Boon R J, Griffin K E, Masters P J, Slocombe B, White A R. Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. *Antimicrob Agents Chemother*.1985; 27(4):495-98.

[3] Critchley I A, Young C L, Stone K C, Ochsner U A, Guiles J, Tarasoe T et al. Antibacterial activity of REP8839, a new antibiotic for topical use". *Antimicrob Agents Chemother.* 2005;49(10):4253–62.

[4] Oommen S K, Appalraju B, Jinsha K. Mupirocin resistance in clinical isolates of *staphylococci* in a tertiary centre in south india. *Indian J Med Microbiol*. 2010; 28(4): 372-75.

[5] CLSI. Performance standards for antimicrobial susceptibility testing; twenty –third international supplement. CLSI document M100-S23. Wayne P A: Clinical and laboratory Standard Institute; 2013.

[6] EUCAST Definitive Document E.DEF 3.1, June 2000: Determination of Minimum Inhibitory Concentrations (MICs) of Antibacterial Agents by Agar Dilutions. European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases. *Clin Microbiol Infect*. 2000; 6(9):509-15.

[7] Nonika R, Purva M, Nidhi B, Gunjan G, Rajrani D et al. Resistance pattern of mupirocin in methicillin-resistant *staphylococcus aureus* in trauma patients and comparison between disc diffusion and e-test for better detection in low resource countries. *J Lab Physicians*. 2014; 6(2): 91-95.

[8] Finlay J E, Miller L A, Poupard J A. Intrepretive criteria for testing susceptiblity of *staphylococci* to mupirocin. *Antimicrob. agents Chemother*.1997; 41(5): 1137-39.

[9] Jayakumar S, Meerabai M, Banu A S, Mathew R, Kalyani M, et al. Prevalence of high and low level mupirocin resistance among *staphylococcus* isolates from skin infection in a tertiary care hospital. *Journal of Clinical and Diagnostic Research*. 2013; 7(2): 238-42.

PARTICULARS OF CONTRIBUTORS:

1. Student, Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai, India.
2. Associate Professor, Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai, India.
3. Associate Professor, Department of Microbiology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Kennedy Kumar Palraj,
No. 25 First School Street, Chattiram, Pattabiram, Chennai-600072, India.
E-mail: kennychennai1973@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 15, 2015**
Date of Peer Review: **Jul 01, 2015**
Date of Acceptance: **Jul 01, 2015**
Date of Publishing: **Oct 01, 2015**