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Incidence of low and high level mupirocin resistance in staphylococcus aureus isolates from clinical specimens

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Abstract

Aim: Determine the incidence of high-level mupirocin resistance strains, low-level mupirocin resistance strains and mupirocin sensitive phenotypes.

Material and Method: The present study was a prospective observational study carried out in the Department of Microbiology. MRSA isolates recovered from clinical specimens such as pus, blood, urine, tracheal aspirate, wounds swab, surgical pus and synovial fluid from patients who attended various outpatient departments or admitted to various wards of the Institute during one year period from May 2018 to April 2019 were included in the study.Methicillin resistance among *S. aureus* was determined using cefoxitin 30 µg discs by Kirby–Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) 2016 guidelines. E-test was performed by Kirby Bauer disc-diffusion method as per CLSI guidelines by using Hi Comb mupirocin strip. Isolates with MICs > 512 µg/ml were considered as MuH, those with MICs 8-256 µg/ml were considered as MuL and with <4 µg/ml were considered as mupirocin sensitive.

Results: Among 187 Staphylococcus aureus, 43 isolates were MRSA. MRSA isolates were obtained in highest number from pus (69.8%) followed by sputum (9.3%), urine (7%) and blood (4.7%). Out of 43 MRSA isolates, mupirocin resistance were seen in 8 (18.61%) isolates by both E-test and agar dilution method.

Conclusion: The study has demonstrated a higher prevalence of both MuH and MuL in MRSA isolates. Thus it is advis able to routinely perform nasal decolonization of healthcare workers to prevent spread of infections among hospitalized patients.

Keywords: high-level, MRSA, MuH, MuL, mupirocin resistance

Introduction

Staphylococcus aure us infections are one of the most common and serious hospital-acquired infections seen in developing countries [1, 2]. Various studies have shown an increased prevalence of staphylococcus infections which may be attributed to its carriage in anterior n are sand hands of health care workers and patients [3, 4]. Along with that drug resistance seen in cases of Staphy lococcusaureus infections is a great concern for the Clinicians to prevent spread of infections. Methicillin an important drug of penicillin group was commonly used for these infections before the emergence of methicillin-resistant Staphylococcus aureus (MRSA) strains. The important risk factors for development of MRSA are irrational use of antibiotic, prolong duration of hospital stay, nasal and hand carriage in health care staff [5, 6]. Mupirocin (pseudomonic acid A) is one of the structurally related antibiotics of Pseudomonic acids A, B, C and D. It isan analogue of amino acid isoleucine and it is derived from Pseudomonas fluor escens. The mechanism of its action is byinhibition of protein synthesis by competing with isoleucine-transfer RNA (tRNA) syn thetase (IleS). Thus, Preven ting the formation of isoleucy lt RNA halts the incorporation of isoleucine into the nascent polypeptide chain. As Mupirocin is preferentially active against Gram-positive organisms, it is widely used as a topical antibiotic to treat Staphy lococcusaureus infection and for decolonisation. Irrational usage and over-the-counter availability has led to the develop men to f resistance to this drug [7]. Mupirocin susceptibility is categorised into three types:

1. Mupirocin susceptible with minimum inhibitory concentration (MIC) of <4 µg/ml [Mup^{s}]
2. Low-level Mupirocin resistance (Mup^{RL}) with MIC of >8–256 µg/ml
3. High-level Mupirocin resistance (Mup^{RH}) with MIC of >512 µg/ml[7].

The increased pressure of MRSA infections among patients and its carriage in health care staff has led to in dis criminate use of mupirocin which has resulted in emergence ofits resistance [8].Various studies [9, 10] have suggested that treatment of infections with low-level resistant strains is still possible with normal dosage schedule of 0.2% mupirocin ointment. Whereas, high-level resistant strains are frequently associated with failure of decolonization as well as treatment of skin and soft tissues infections. Thus the present study was aimed to determine the incidence of high-level mupirocin resistance strains, low-level mupirocin resistance strains and mupiroc insensitive phenotypes.

Material and method

Study design: The present study was a prospective observational study carried out in the Department of Microbiology.

Ethical considerations: The proposed study was approved by the Ethical Committee of the institute.

Study population: Indoor and outpatient departments patient of tertiary care hospital set up were included for the present study.

Sample and Data collection: MRSA isolates recovered from clinical specimens such as pus, blood, urine, tracheal aspirate, wounds swab, surgical pus and synovial fluid from patients who attended various outpatient departments or admitted to various wards of the Institute during one year period from May 2018 to April 2019 were included in the study. Informed consent was obtained from each patient and a detailed clinical history and demographic profile was taken and recorded from the patients whose culture was positive for MRSA.

Sample processing: Clinical specimens obtained in the micro biology laboratory were processed as per routine micro biological procedures for isolation and identification of Staphylococcus aureus. All the clinical specimens excluding urine were inoculated on 5% blood a garand Mac Conkey agar media, whereas urine samples were inoculated on Cysteine-lactose electrolyte deficient (CLED) agar media and incubated at 37ºC aerobically. The growth was identified as Staphylococcus aureus by using conventional biochemical methods according to standard microbiological techniques.

Determination of methicillin-resistant *Staphylococcus aureus:* Methicillin resistance among *S. aureus* was determined using cefoxitin 30 µg discsby Kirby–Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) 2016 guidelines [11]. Inoculum was prepared in 4 ml sterile saline to obtain a suspension of 0.5 McFarland standards. Mueller Hinton agar (MHA) was inoculated and incubated at 37ºC for 24 h. After incubation, zone of inhibition was measured by unaided eye and size of ≤21 mm was taken as resistant and ≥22 mm as sensitive.

Determination of mupirocin-resistant *Staphylococcus aureus (MupRSA):* Mupirocin resistance among MRSA strains was determined by using 5 µg and 200 µg mupirocin discsby Kirby–Bauer disc diffusion method. Isolates to be tested was inoculated on MHA culture medium and both the discs was applied and incubated at 37ºC for 24 h. After incubation, zone of inhibition was measured and the isolates were categorized as sensitive, low-level resistant and high-level resistant. Isolates with zone ≥ 14 mm for both 5µg and 200 µg discs was considered as sensitive, those with zone < 14 mm for 5 µg but ≥ 14 mmfor 200 µg disc was considered as low-level resistant (MuL), and those with zone < 14 mm for both 5 µg and 200 µg discs was considered as high-level resistant (MuH).

Determination of antibiotic susceptibility pattern of MRSA and MupRSA: It was done by Kirby–Bauer disc diffusion method with the help of various antibiotic discs i.e. Penicillin (10 units), oxacillin (1 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), vancomycin (5 µg), linezolid (30 µg), tetracycline (30 µg), doxycycline (30 µg) and cotrimoxazole (1.25/23.75 µg). After incubation, zone of inhibition was measured by unaided eye and interpretation was determined as sensitive, intermediate or resistant as per CLSI guidelines [11].

Epsilometer test (E-test) for determination of minimum inhibitory concentration for mupirocin:
E-test was performed by Kirby Bauer disc-diffusion method as per CLSI guidelines by using Hi Comb mupirocin strip. Lawn culture was made on the surface of MHA medium. Hi Comb strip with mupirocin antibiotic ranges from 0.1-240 µg/ml was applied perfectly by gently pressing using a sterile forceps. The plates were then incubated aerobically at 35ºC for24 hours. After incubation plates were examined for the minimum inhibitory concentration (MIC). Isolates with MICs > 512 µg/ml were considered as MuH, those with MICs 8-256 µg/ml were considered as MuL and with <4 µg/ml were considered as mupirocin sensitive.

Statistical analysis: Data so collected was tabulated in an excel sheet, under the guidance of statistician. Data was analyzed using IBM SPSS. Statistics Windows, Version 20.0. (Armonk, NY: IBM Corp) for the generation of descriptive and inferential statistics. The statistical significant difference among groups was determined by the Chi square test and the level of significance was set at $p < 0.05$.

Results: A total of 187 Staphylococcus aureus were obtained from 3492 different clinical samples. Among 187 Staphy lococcusaureus, 43 isolates were MRSA. MRSA isolates were obtained in highest number from pus (69.8%) followed by sputum (9.3%), urine (7%) and blood (4.7%) as shown in Table 1.
Out of 43 MRSA isolates, mupirocin resistance were seenin 8 (18.61%) isolates by both E-test and agar dilution method (Table 2).
Table 3 shows the antimicrobial susceptibility profile of MRSA isolates. In this study, it was observed that MRS Aisolates were resistant to 86.05%, 55.81%, 44.19%, and 39.53%of penicillin, cotrimoxazole, cephalothin, and cefuroximere spectively. Vancomycin and linezolid were uniform ly sensitive to all MRSA isolates.

Table 1: Staphylococcus aureus and MRSA distribution on the basis of source

Specimen	No. of Samples	S. aureus		MRSA		p value
		N	%	N	%	
Pus	582	87	46.52	30	69.8	0.001*
Sputum	414	23	12.30	4	9.3	0.47
Urine	1681	6	3.21	3	7.0	0.11
Blood	607	54	28.88	2	4.7	<0.01*
Surgical pus	42	7	3.74	1	2.3	0.32
Tracheal aspirates	41	2	1.07	1	2.3	0.71
Synovial fluid	39	3	1.60	1	2.3	0.38
Wound swab	86	5	2.67	1	2.3	0.82
Total	3492	187		43		

*: statistically significant

Table 2: Mupirocin resistant Staphylococcus aureus among MRSA isolates (n = 43)

E-test MIC range	Agar dilution method MIC range	MRSA	
		N	%
<4µg/ml	<4µg/ml	35	81.40
8-256 µg/ml	8-256 µg/ml	3	6.98
>512 µg/ml	>512 µg/ml	5	11.63

Table 3: Antimicrobial resistance pattern of MRSA and Mupirocin-resistant MRSA isolates (n = 43)

Antibiotics	MRSA (43)		Mupirocin-resistant MRSA (8)	
	N	%	N	%
Penicillin	37	86.05	8	100
Oxacillin	39	90.70	8	100
Cephalothin	19	44.19	3	37.5
Cefuroxime	17	39.53	4	50
Clindamycin	5	11.63	5	62.5
Erythromycin	10	23.26	4	50
Chloramphenicol	7	16.28	2	25
Cotrimoxazole	24	55.81	2	25
Vancomycin	0	0.00	0	0.00
Linezolid	0	0.00	0	0.00

Discussion

MRSA is one of the leading cause of infections among health care staff and hospitalized patients. It also causes community associated infections. It causes a wide range of infections such as abscesses, impetigo, cellulitis, deep seated pyogenic lesions, meningitis, septicaemia, and pneumonia [12]. Prevalence of MRSA infections may increase because of improper hand hygiene and hand ling of MRSA carrier patients. Mupirocin is a commonly use dantibiotic for decolonization of MRSA in carriers and for treatment of skin and soft tissue infections caused by MRSA. Emergence of mupirocin resistance due to its irrational use for treatment of skin and soft tissue infections is further worsening the problem of MRSA infections [13].

Studies also suggest that mupirocin resistan cemay be transferred from the commensal flora of sk into MRSA during mupirocin therapy [14]. In this study, a total of 43 (22.99%) MRSA isolates were obtained from 187 Staphylococcus aureus. This result was in accordance with the study reported by Parul Chaturve di*et al* [15]. The prevalence is quite low as compared to several other studies conducted in this region [16, 17]. This may be attributed to lesser exposure to anti biotics due to low-level of healthcare facilities in this region in past years. Among 43 MRSA isolates, 8 (18.61%) isolates were mupirocin resistant which is high as compared to other studies [18, 19] conducted in this region, but similar to study done by Parul Chaturvedi *et al* [15]. The reason for higher prevalence of mupirocin resistance may be an increased use of mupirocin ointment for skin and soft issue infections. Five (11.63%) MuH and 3 (6.98%) MuL MRSA isolates determined by two different MIC methods were comparable. Whereas MuL isolates may be treated with normal dosage schedule of mupirocin ointment, but MuH has been found to be associated with treatment and de colonization failure. Antibiotic susceptibility pattern of MRSA and mupirocin resistant MRSA isolates showed no significant association of methicillin resistance and mupirocin resistance with resistance to other antibiotics in this study.

Conclusion: The study has demonstrated a higher prevalence of both MuH and MuL in MRSA isolates. This poses a serious challenge to the clinicians to deal with increasing problem of MRSA transmission in the hospital. This May be due to an increased prevalence of MRSA infections in the health care setup and over the counter sale of drugs. Thus it is ad Visable to routinely perform nasal decolonization of healthcare workers to prevent spread of infections among hospitalized patients and to detect mupiroc in resistance in MRSA strains isolated from the carriers so that alternative decolonization methods may be used.

References

1. Dubey D, Rath S, Sahu MC, Pattnaik L, Debata NK, Padhy RN. Surveillance of infection status of drug resistant Staphylococcus aureus in an Indian teaching hospital. Asian Pac J Trop Dis**,**2013:3:133-42.
2. Kaur H, Purwar S, Saini A, Kaur H, Karadesai SG, Kholkute SD *et al*. Status of Methicillin-resistant Staphylococcus aureus Infections and Evaluation of PVL Producing Strains inBelgaum, South India. JKIMSU,2012:1:43-51.
3. Sivaraman K, Venkataraman N, Cole AM. Staphylococcus aureus Nasal Carriage and its Contributing Factors. FutureMicrobiol,2009:4:999-1008.
4. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA *et al*. The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis,2005:5:751-62.
5. Klutymans J, van Balkum A, Verbrughi H. Nasal carriage of Staphylococcus aureus: Epidemiology, underlying mechanisms and associated risk. ClinMicrobiol Rev,1997:10:505-20.
6. Graffunder EM, Venezia RA. Risk factors associated with nosocomial methicillin-resistant Staphylococcus aureus (MRSA) infection including previous use of anti microbials. J Antimicrob Che mother,2002:49:999-1005.
7. Patel JB, Gorwitz RJ, Jernigan JA. Mupirocin resistance. Clin Infect Dis,2009:49:935-41.
8. Hogue JS, Buttke P, Braun LE, Fairchok MP. Mupirocin resistance related to increasing mupirocin use in clinical isolates of methicillin-resistant Staphylococcus aureus in apediatric population. J ClinMicrobiol,2010:48:2599-600.
9. Hudson IR. The efficacy of intranasal mupirocin in the prevention of staphylococcal infections: A review of recent experience. J Hosp Infect,1994:27:81-98.
10. Walker ES, Vasquez JE, Dula R, Bullock H, Sarubbi FA. Mupirocin-resistant, methicillin-resistant Staphylococcus aureus: Does mupirocin remain effective? Infect Control HospEpidemiol,2003:24:342-6.
11. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition and Performance Standards for Antimicrobial Susceptibility Testing: Twenty- Third Informational Supplement. CLSI Document M02-A11 and M100-S23. Wayne, PA: Clinical and Laboratory Standard Institute, 2013.
12. Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant Staphylococcus aureus infection. Clin Infect Dis,2008:46:S350-9.
13. Rodvold KA, Mc Coneghy KW. Methicillin-resistant Staphylococcus aureus therapy: Past, present, and future. ClinInfect Dis,2014:58:S20-7.
14. Hurdle JG, O Neill AJ, Mody L, Chopra I, Bradley SF. In vivo transfer of high-level mupirocin resistance from Staphylococcus epidermidis to methicillin-resistant Staphylococcus aureus associated with failure of mupirocinprophylaxis. J Anti `microb

Chemother,2005:56:1166-8.
15. Chaturvedi P, Singh AK, Singh AK, Shukla S, Agarwal L. Prevalence of mupirocin resistant staphylococcus aureus isolates among patients admitted to a tertiary care hospital. North Am JMed Sci,2014:6:403-7.
16. Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis DS, Gautam V, *et al*. Methicillin-resistant Staphylococcus aureus (MRSA) in India: Prevalence and susceptibility pattern. Indian J MedRes,2013:137:363-9.
17. Arora S, Devi P, Arora U, Devi B. Prevalence of methicillin resistant Staphylococcus aureus (MRSA) in a tertiary carehospital in northern India. J Lab Physicians 2010;2:78-81.
18. Singh AK, Venkatesh V, Singh M. Mupirocin resistance in clinical isolates of Staphylococcus aureus in a tertiary care hospital set upin north India. Int J Med Res Health Sci,2013:2:840-7.
19. Gadepalli R, Dhawan B, Mohanty S, Kapil A, Das BK, Chaudhry R *et al*. Mupirocin resistance in Staphylococcus aureus in an Indian hospital. DiagnMicrobiol Infect Dis,2007:58:125-7.