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ORIGINAL ARTICLE

Assessment of Mupirocin Resistance among Clinical Isolates of Methicillin Resistant *Staphylococcus aureus*

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ABSTRACT

Background: *Methicillin-resistant* Staphylococcus aureus *(MRSA) colonization is considered a major risk factor for nosocomial infections and its decolonization has reduced these infections. Mupirocin (MUP) is the topical antibiotic of choice for decolonization. MUP decolonization failure is attributed to MUP resistance.* ***Objective:*** *The aim of the current study is to assess MUP resistance among MRSA isolates phenotypically and genotypically.* ***Methodology****: Fifty MRSA isolates were identified in Microbiology Department in the Medical Research Institute hospital, Alexandria University. Antibiotic susceptibility to different classes of antibiotics by disk diffusion method was done. MUP minimum inhibitory concentration (MIC) was determined phenotypically by MUP Ezy MIC™ Strips. MUP resistance was determined genetically by multiplex PCR detection of mupA and mupB.* ***Results:*** *Of all MRSA isolates, 6% exhibited high level and none showed low level MUP resistance. Only mupA was detected in all resistant isolates.* ***Conclusion:*** *Despite low prevalence of MUP resistance, it is appropriate to test MUP resistance prior nasal decolonization.*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most commonly implicated agents in nosocomial infections with significant morbidity and mortality [1]. Colonization with such resistant strains is considered as a high risk for acquiring MRSA infection during hospital stays. Different body sites can be colonized by MRSA with the anterior nares being the most frequently colonized. There is great interest in reducing risk for transmission of infection by decolonization of persons who harbor these bacteria[2].

Different agents have been studied to decolonize MRSA with mupirocin (MUP) being the topical antibiotic of choice for decolonization, with a 5-days course with or without bathing using a topical antiseptic e.g. chlorhexidine. MUP (pseudomonic acid A) is a narrow-spectrum topical antibiotic active predominantly against Gram-positive pathogens, particularly staphylococci, including MRSA, and streptococci [3,4]. MUP is an isoleucine analogue that competitively binds to isoleucyl t-RNA synthetase (IRS) required for the transfer of this amino acid thus interfering with bacterial protein synthesis [5].

The increased burden of MRSA infections among patients and its carriage among health care personnel resulted in unselective use of MUP leading to emergence of its resistance. Two resistance patterns for MUP are noticed [6-8]. First: Low-level MUP resistance (minimum inhibitory concentration (MICs) of 8 µg/ml to lower than 256 µg/ml) which is due to point mutations in the native *ileS* gene. Second: High-level resistance (MIC of ≥512 µg/ml) that is mediated by acquisition of a conjugative plasmid encoding either *mupA* or *mupB* genes, both encode an alternate IRS for which MUP has no affinity[9].

Various studies have suggested that treatment of infections with low-level resistant strains is still possible with normal dosage schedule of 2% topical MUP[10]. Whereas, high-level resistant strains are frequently associated with failure of decolonization and treating skin and soft tissues infections [11,12]. Up to our knowledge, limited data concerning MUP resistance in Egypt evaluating the efficiency of its use in decolonization is available, therefore the aim of the present study was to assess MUP resistance among clinical isolates of MRSA by phenotypic and genotypic methods.

METHODOLOGY

After approval of the ethical committee of Medical Research Institute, a total of 50 clinical isolates of MRSA out of 150 *S. aureus* were collected from Inpatients and Outpatients attending the Medical research institute hospital, Alexandria University and received at the Microbiology Department. Based on the topical activity of MUP, surgical wound swabs, swabs from superficial and deep skin and subcutaneous tissue infections and nasal swabs from carriers were included in this study.

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Phenotypic detection

S. aureus isolates were identified by their morphology and culture characteristics upon culturing on blood agar, using standard tests: catalase, and slide coagulase test and growth and fermentation of mannitol on Mannitol salt agar. MRSA isolates were identified by Cefoxitin disc diffusion test according to the CLSI. All MRSA isolates were tested for their susceptibility to different antibiotics by Kirby Bauer disc diffusion method. Since Kirby Bauer disc diffusion method is not recommended for susceptibility testing of *S. aureus* to Vancomycin, determination of MIC was done by broth dilution method. MUP resistance was tested by both MUP Ezy MIC™ Strips and disc diffusion method with 200 µg disc to detect high level resistance according to CLSI [13].

Genotypic detection

DNA of MRSA isolates was extracted using boiling method [14]. The isolates were confirmed to be MRSA by amplification of the *femA* and *mecA* genes by multiplex PCR. Multiplex PCR for *mupA* and *mupB* genes was done for detecting high level MUP resistance. Primers and annealing temperature used are listed in table 1. Amplified samples were analysed by gel electrophoresis and visualized by using UV transillumination.

Table 1: Primers for the detection of target gene

Primer	Nucleotide sequence	Amplicon size (bp)	Annealing Temperature	Reference
femA	Forward: CTTACTTACTGCTGTACCTG	686	52˚C	[15,16]
	Reverse: ATCTCGCTTGTTATGTGC			
mecA	Forward: TGGCTATCGTGTCACAATCG	304		[15, 17]
	Reverse: CTGGAACTTGTTGAGCAGAG			
mupA	Forward: TATATTATGCGATGGAAGGTTGG	457	50˚C	[9, 18]
	Reverse: AATAAAATCAGCTGGAAAGTGTTG			
mupB	Forward: CTAGAAGTCGATTTTGGAGTAG	674		[9]
	Reverse: AGTGTCTAAAATGATAAGACGATC			

RESULTS

The majority of MRSA isolates 23 (46%) were obtained from wound swabs followed by nasal swabs 18 (36%) and pus swabs 9 (18%). All the isolates were sensitive to Vancomycin, Tigecycline and Linezolid. 68% of the isolates were sensitive to Trimethoprim-Sulfamethoxazole and Rifampin. While 66% of the isolates were sensitive to Chloramphenicol, 58% were sensitive to Fusidic acid, and 50% were sensitive to Levofloxacin. The isolates were most resistant to Gentamicin (62%), Doxycycline (58%), while 50% were resistant to Erythromycin and Ciprofloxacin.

Regarding MUP, 3 isolates (6%) had high level resistance showing no zone of inhibition around the MUP 200 µg disc with MIC > 1024 µg/ml while, 47 (94%) isolates were susceptible to MUP with MIC ≤ 2 µg/ml. None of the 50 isolates showed low level MUP resistance. Interestingly, the 3 high level MUP resistant isolates came from nasal swabs collected from ICU patients who had a previous history of MUP exposure, showing also resistance to Clindamycin, Gentamicin, Ciprofloxacin, Levofloxacin, Trimethoprim-Sulfamethoxazole, Chloramphenicol, Rifampin (100%) but they were sensitive to Vancomycin, Tigecycline, Linezolid and Fusidic acid.

The 3 phenotypically high MUP resistant samples were genotypically positive for only *mupA* gene, but none of the 50 MRSA samples was positive for *mupB* gene. Figure (1).

Figure 1: Detection of amplified *mupA*, *mupB* genes by multiplex PCR:

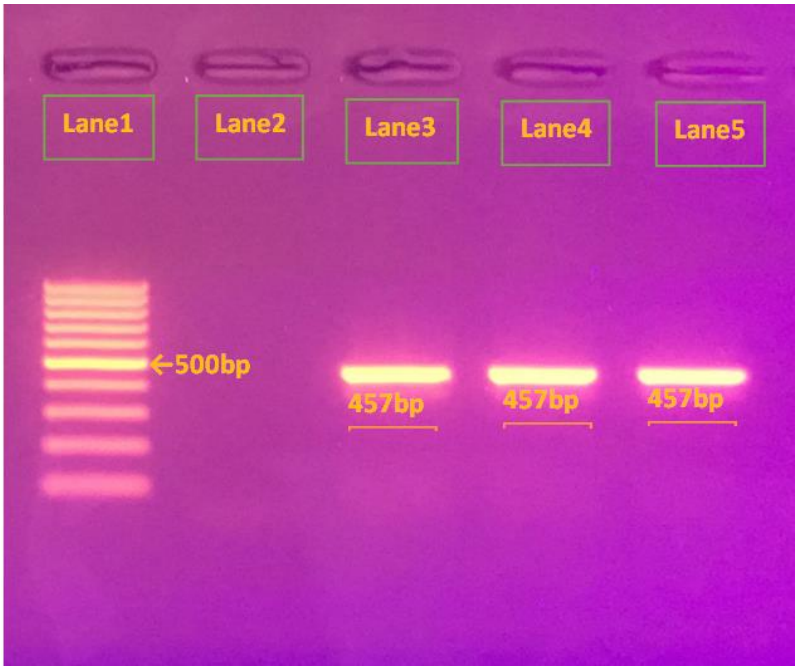

Fig. 1: Ethidium bromide stained agarose gel electrophoresis for *mupA* and *mupB* detection. Lane 1: contains a 100 bp DNA ladder. Lane 2: shows negative bands for the amplification of *mupA* gene at 457 bp and *mupB* gene at 674 bp. lane 3-5: show positive bands for the amplification of *mupA* gene at 457 bp and negative bands for the amplification of *mupB* gene at 674 bp. The gel was visualized by ultraviolet light at 302 nm.

DISCUSSION

MRSA has led to high rate of morbidity and mortality as well as high costs of health care services. Having a gene, *mecA*, makes it resistant to the entire class of β-lactam drugs except for 5th generation cephalosporins: ceftaroline and ceftobiprole. The identification of MRSA was confirmed in all isolates in this study by detection of *mecA* and *femA* genes. Similarly Veloso et al. detected both *femA* and *mecA* in all their MRSA isolates.[19].

In this study, all MRSA isolates were sensitive to vancomycin and linezolid similar to recent studies [20, 21] and tigecycline as similarly found by others [22,23], while 50% were resistant to erythromycin, levofloxacin and ciprofloxacin similar to other studies [24, 25]

The difference in antimicrobial susceptibility between countries may be explained by the difference in the infection control policies and antimicrobial stewardship between these countries and the abuse of over the counter antibiotics in countries of low socio-economic level. Another possible explanation is the shift in selective antimicrobial pressures from beta-lactam therapy to other groups of antimicrobials causing the resistant organisms to be replaced by susceptible ones.

Although MRSA colonized patient does not need treatment, a course of decolonization treatment with topical MUP, is the cornerstone for eradication to prevent future infections or transmission.[26].

In this study, we screened all 50 MRSA isolates for MUP resistance using MUP (200µg) disc by disc diffusion method according to CLSI [13] and by Ezy MIC™ strip test. 3 isolates (6%) were positive for high level MUP resistance and had an MIC > 1024 µg/ml. The MIC for the remaining MRSA isolates 47 (94%) was < 2 µg/ml (from 0.5 -2 µg/ml). None of the 50 isolates showed low level MUP resistance. *mupA* was detected by conventional PCR in the three isolates, while *mupB* was not detected.

Previous study from Egypt by Barakat and Nabil[27] reported higher MUP resistance in 13 (17.8%) among their MRSA isolates. Of them 5 (38.5%) were low level MUP-resistant and 8 (61.5%) were high level MUP-resistant. The *mupA* gene was detected by conventional PCR in 75.0% (6/8) of the high level MUP resistant strains and in none of low level MUP resistant strains. *mupA* was not detected in two isolates that were phenotypically high level MUP resistant, this may be attributed to the presence of the *mupB* gene, which was outside the scope of their study. Also Agarwal et al.[25], detected 4 (14.3%) MUP resistant isolates out of 28 MRSA isolates by E-test; 3 (75%) isolates were high level MUP resistant while only one isolate (25%) isolate was low level MUP resistant. On the other hand, McNeil et al.[28], collected a total of 108 MRSA isolates, 11 isolates (10.2%) showed high level MUP resistance,

while 3 isolates (2.8%) showed low level MUP resistance. *mupA* was detected in only 9 of the 11 high level MUP resistant isolates. Unexpectedly, they reported the presence of one isolate with a low level MUP phenotype possessed *mupA* gene, sequencing data revealed a single nucleotide deletion that caused a frameshift and a nonfunctional peptide, such findings was not reported in our study.

Lower prevalence of MUP resistance was reported by Hosseini et al.[29] who found only 2 isolates (1.06%) with MUP resistance among their 188 MRSA isolates. Both isolates carried both *mupA* and *mupB* by PCR.

Similar to the present work Doudoulakakis et al.[30] showed that only 3 (2.9%) high level MUP resistant MRSA strains were detected among 102 *S. aureus* and no low level MUP resistance was tested by using 5µg and 200µg discs, also *mupA* gene was detected in all three isolates by conventional PCR.

Several studies reported higher level of MUP resistance. Joshi et al.[31] revealed that 15/29(51%) MRSA isolates isolated from nasal swabs showed high level MUP resistance by E-test, 14(93.3%) harbored the *mupA* gene and one isolate carried *mupB* gene. An Iranian study reported by Shahsavan et al. [32] detected 42/62 (67.7%) among their MRSA isolates showed high level MUP resistance by disc diffusion method. Of which only 27 carried the *mupA* gene. This may be attributable to the presence of other high level MUP resistance genes (*mupB)*, but detection of *mupB* was not performed in this study as it was discovered lately in 2012.

MUP resistance in some countries may be caused by prior use and overconsumption. A strong association between MUP resistance and prior MUP use was reported by Antonov et al.[33] This is consistent with previous published reports[34-36]. It should be noted that the three high level MUP resistant isolates in this study all came from ICU patients' nasal swabs with prior use of MUP for MRSA nasal decolonization

MUP resistance may also aid in the spread of multidrug resistance through co-selection with other resistance genes. Both Fritz et al. and McNeil et al. [28,37] reported that high rates of clindamycin resistance have been observed in MUP resistant *S. aureus* isolates.. Also Chaves F et al reported the co-resistance of MUP with ciprofloxacin, gentamicin, and clindamycin[6]. This is consistent with the present work as the three high level MUP resistant MRSA isolates were also clindamycin, ciprofloxacin, and gentamicin resistant.

CONCLUSION

Clearance of MRSA nasal colonization can reduce the subsequent risk of development of infection. This study correlates the prevalence of MUP resistance and its usage in clinical practice. Since MUP is an effective therapy for decolonization of MRSA among carriers, MUP treatment should therefore be used cautiously to avoid the emergence of MUP resistance and the spread of resistance in hospitals in which MRSA is frequently isolated. Consequently, it is appropriate to test for MUP resistance prior to nasal decolonization.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

Ethical approval

The study was approved by the ethics committee of the Medical Research Institute – Alexandria University which is constituted and operating according to ICH GCP guidelines and applicable local and institutional regulations and guidelines which govern IRB operation.

REFERENCES

1. Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999–2005. Emerging infectious diseases 2007;13(12):1840
2. McConeghy KW, Mikolich DJ, LaPlante KL. Agents for the decolonization of methicillin-resistant Staphylococcus aureus. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 2009;29(3):263-80
3. Van Rijen MM, Bonten M, Wenzel RP, Kluytmans JA. Intranasal mupirocin for reduction of Staphylococcus aureus infections in surgical patients with nasal carriage: a systematic review. Journal of antimicrobial chemotherapy 2008;61(2):254-61
4. Bradley SF. Eradication or decolonization of methicillin-resistant Staphylococcus aureus carriage: what are we doing and why are we doing it?: The University of Chicago Press, 2007.
5. Sanju AJ, Kopula SS, Palraj KK. Screening for mupirocin resistance in staphylococcus. Journal of clinical and diagnostic research: JCDR 2015;9(10):DC09
6. Chaves F, García-Martínez J, de Miguel S, Otero JR. Molecular characterization of resistance to mupirocin in methicillin-susceptible and-resistant isolates of Staphylococcus aureus from nasal samples. Journal of clinical microbiology 2004;42(2):822-24

7. Shalaby MM, El Shahat DA. Resistance to Mupirocin among Methicillin Resistant Staphylococcus Aureus Isolates from Community Acquired Infections, Hospital Acquired Infections, and colonized Health Care Workers. Egyptian Journal of Medical Microbiology 2018;27(2):65-69
8. Mostafa MS, Awad AR. Localization and Characterization of MupA Gene in High and Low-Level Mupirocin Resistant Methicillin Resistant Staphylococcus Aureus. Egyptian Journal of Medical Microbiology 2020;29(3):171-77
9. Seah C, Alexander DC, Louie L, et al. MupB, a new high-level mupirocin resistance mechanism in Staphylococcus aureus. Antimicrobial agents and chemotherapy 2012;56(4):1916-20
10. Hudson I. The efficacy of intranasal mupirocin in the prevention of staphylococcal infections: a review of recent experience. Journal of Hospital Infection 1994;27(2):81-98
11. Robicsek A, Beaumont JL, Thomson Jr RB, Govindarajan G, Peterson LR. Topical therapy for methicillin-resistant Staphylococcus aureus colonization: impact on infection risk. Infection control and hospital epidemiology 2009;30(7):623
12. Simor AE, Phillips E, McGeer A, et al. Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant Staphylococcus aureus colonization. Clinical Infectious Diseases 2007;44(2):178-85
13. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI Supplement M100 Wayne PCaLSI. 2020
14. Mullis KB. The unusual origin of the polymerase chain reaction. Sci Am 1990;262(4):56-61, 64-5 doi: 10.1038/scientificamerican0490-56[published Online First: Epub Date]|.
15. Vannuffel P, Gigi J, Ezzedine H, et al. Specific detection of methicillin-resistant Staphylococcus species by multiplex PCR. Journal of clinical microbiology 1995;33(11):2864-67
16. Ardic N, Sareyyupoglu B, Ozyurt M, Haznedaroglu T, Ilga U. Investigation of aminoglycoside modifying enzyme genes in methicillin-resistant staphylococci. Microbiological research 2006;161(1):49-54
17. Shokravi Z, Mehrad L, Ramazani A. Detecting the frequency of aminoglycoside modifying enzyme encoding genes among clinical isolates of methicillin-resistant Staphylococcus aureus. BioImpacts: BI 2015;5(2):87
18. Antonio M, McFerran N, Pallen MJ. Mutations affecting the Rossman fold of isoleucyl-tRNA synthetase are correlated with low-level mupirocin resistance in Staphylococcus aureus. Antimicrobial Agents and Chemotherapy 2002;46(2):438-42
19. Veloso JO, Lamaro-Cardoso J, Neves LS, et al. Methicillin-resistant and vancomycin-intermediate Staphylococcus aureus colonizing patients and intensive care unit environment: virulence profile and genetic variability. Apmis 2019;127(11):717-26
20. Bride LdL, Pereira MF, Barbosa MC, et al. Differences in resistance profiles and virulence genes among methicillin-resistant and methicillin-susceptible Staphylococcus aureus of different lineages at a public tertiary hospital. Revista da Sociedade Brasileira de Medicina Tropical 2019;52
21. Coombs GW, Daley DA, Lee YT, Pang S. Australian Group on Antimicrobial Resistance (AGAR) Australian Staphylococcus aureus Sepsis Outcome Programme (ASSOP) Annual Report 201&. Communicable Diseases Intelligence 2019;43
22. Zhanel GG, Adam HJ, Low DE, et al. Antimicrobial susceptibility of 15,644 pathogens from Canadian hospitals: results of the CANWARD 2007–2009 study. Diagnostic microbiology and infectious disease 2011;69(3):291-306
23. Rodloff AC, Dowzicky MJ. Antimicrobial susceptibility among european gram-negative and gram-positive isolates collected as part of the tigecycline evaluation and surveillance trial (2004-2014). Chemotherapy 2017;62(1):1-11
24. Enström J, Fröding I, Giske CG, et al. USA300 methicillin-resistant Staphylococcus aureus in Stockholm, Sweden, from 2008 to 2016. PloS one 2018;13(11):e0205761
25. Agarwal L, Singh AK, Sengupta C, Agarwal A. Nasal carriage of methicillin-and mupirocin-resistant S. aureus among health care workers in a tertiary care hospital. Journal of research in pharmacy practice 2015;4(4):182
26. Hetem DJ, Bonten MJ. Clinical relevance of mupirocin resistance in Staphylococcus aureus. Journal of Hospital Infection 2013;85(4):249-56
27. Barakat GI, Nabil YM. Correlation of mupirocin resistance with biofilm production in methicillin-resistant Staphylococcus aureus from surgical site infections in a tertiary centre, Egypt. Journal of global antimicrobial resistance 2016;4:16-20
28. McNeil JC, Hulten KG, Kaplan SL, Mason EO. Mupirocin resistance in Staphylococcus aureus causing recurrent skin and soft tissue infections in

children. Antimicrobial Agents and Chemotherapy 2011;55(5):2431-33

29. Mahmoodzadeh Hosseini H, Kiyani N, Amin M, Hedayati Ch M, Imani Fooladi AA. Distribution of high-level mupirocin resistance among clinical MRSA. J Chemother 2017;29(4):215-19 doi: 10.1080/1120009X.2016.1201257[published Online First: Epub Date]|.
30. Doudoulakakis A, Spiliopoulou I, Spyridis N, et al. Emergence of a Staphylococcus aureus clone resistant to mupirocin and fusidic acid carrying exotoxin genes and causing mainly skin infections. Journal of clinical microbiology 2017;55(8):2529-37
31. Joshi PR, Acharya M, Aryal R, et al. Emergence of staphylococcal cassette chromosome mec type I with high-level mupirocin resistance among methicillin-resistant Staphylococcus aureus. Asian Pacific Journal of Tropical Biomedicine 2017;7(3):193-97
32. Shahsavan S, Emaneini M, Khoshgnab BN, et al. A high prevalence of mupirocin and macrolide resistance determinant among Staphylococcus aureus strains isolated from burnt patients. Burns 2012;38(3):378-82
33. Antonov NK, Garzon MC, Morel KD, Whittier S, Planet PJ, Lauren CT. High prevalence of mupirocin resistance in Staphylococcus aureus isolates from a pediatric population. Antimicrobial Agents and Chemotherapy 2015;59(6):3350-56
34. McNeil JC, Hulten KG, Kaplan SL, Mason EO. Decreased susceptibilities to retapamulin, mupirocin, and chlorhexidine among Staphylococcus aureus isolates causing skin and soft tissue infections in otherwise healthy children. Antimicrobial agents and chemotherapy 2014;58(5):2878-83
35. Lee A, Macedo-Vinas M, Francois P, et al. Trends in mupirocin resistance in meticillin-resistant Staphylococcus aureus and mupirocin consumption at a tertiary care hospital. Journal of Hospital Infection 2011;77(4):360-62
36. Talon D, Marion C, Thouverez M, Bertrand X. Mupirocin resistance is not an inevitable consequence of mupirocin use. Journal of Hospital Infection 2011;79(4):366-67
37. Fritz SA, Hogan PG, Camins BC, et al. Mupirocin and chlorhexidine resistance in Staphylococcus aureus in patients with community-onset skin and soft tissue infections. Antimicrobial Agents and Chemotherapy 2013;57(1):559-68