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ORIGINAL ARTICLE

Detection of Mupirocin Resistance in Methicillin- Resistant *Staphylococcus aureus* Isolates in an Egyptian Hospital

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ABSTRACT

Background: *Nasal carriage of methicillin-resistant Staphylococcus aureus (MRSA) can be eradicated by topical mupirocin application. Mupirocin resistance, on the other hand, is becoming more widespread.* ***Objective:*** *The present work aims to compare conventional and molecular approaches to detect the prevalence of mupirocin resistance in MRSA isolates from clinical and nasal samples, as well as to investigate their susceptibility to other antibiotics.* ***Methodology:*** *Our study included 60 MRSA non-duplicate isolates, 14 from surgical wounds, 16 from urinary tract infections from patients hospitalized to Suez- Canal University Hospital in Ismailia, Egypt, and 30 nasal swabs from health care workers. The minimum inhibitory concentrations (MICs) for MRSA isolates to mupirocin were determined using the E-test method, and PCR targeting the mupA gene was performed.* ***Results:*** *Six isolates out of 60 MRSA isolates (10%) showed high-level mupirocin resistance, while just one strain (1.6%) showed low-level mupirocin resistance. Four of the six MRSA isolates with high levels of mupirocin resistance carried the mupA gene. All seven mupirocin-resistant isolates (11.6%) were isolated from nasal swabs. MRSA strains resistant to mupirocin were more resistant to tetracycline, chloramphenicol, gentamycin, ciprofloxacin, and trimethoprim-sulfamethoxazole than mupirocin-susceptible ones.* ***Conclusion:*** *The high prevalence of mupirocin resistance in MRSA strains at our hospital is alarming. As a result, frequent testing of MRSA for mupirocin resistance is recommended even in settings where mupirocin is not used prophylactically.*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as the main etiological agent and the most frequent microorganism in community- and hospital-acquired infections [1, 2]. Nasal colonization with MRSA is common and it is an important step in the pathogenesis and development of both community acquired and nosocomial infections, these strains provide a reservoir for infection in other sites such as surgical-site and bloodstream infections [3, 4].

Mupirocin (an isoleucine analogue) is a protein synthesis inhibitor; acts by binding irreversibly to isoleucyl t-RNA synthetase (IleS) [5]. Topical mupirocin application possesses a potent antimicrobial activity against staphylococci causing skin and surgical wound infections as well as for preventing nasal colonization of MRSA [6, 7].

Mupirocin resistance develops in two forms; Low-level resistance (MIC, 8–256 µg/ml) which is normally caused by a mutation in the target enzyme gene, whereas high-level resistance (MIC of ≥ 512 µg/ml) is caused by a plasmid harboring the ileS2 gene, which encodes an extra isoleucyl-tRNA synthetase enzyme [8]. As mupirocin use becomes more common, such transmissible resistance has prompted concerns about the propagation of mupirocin resistance [9,10]. Furthermore, the emergence of mupirocin resistance has been associated to a rising risk of staphylococcal infections in patients receiving long-term peritoneal dialysis [11].

The objective of our study was to investigate the prevalence of mupirocin resistance in MRSA isolates from clinical and nasal samples in our hospital, and to discover the resistance patterns of the mupirocin-resistant MRSA against various antibiotics widely used for treatment.

METHODOLOGY

Setting and data collection*:*

This research work was done at Suez-Canal University Hospital in Ismailia, Egypt, for six months (January 2018 to June 2019). The study included 60 MRSA non-duplicate isolates, 14 from surgical wounds, 16 from UTIs, as well as 30 nasal swabs taken from healthcare personnel by gently rotating a sterile cotton swab, soaked with sterile saline, in the vestibule of both anterior nares.

In our institution, mupirocin is frequently used to eradicate *S. aureus* colonization in both patients and healthcare personnel in response to outbreaks of staphylococcal infection. Patients and healthcare personnel included in this study denied routine or sustained mupirocin use. Before the study began, all the participants and staff members gave their agreement for collecting specimens.

Microbiological Methods:

Clinical Specimens were first plated onto Columbia blood agar plates and incubated at 37°C for 24 h. Standard procedures including colony morphology, catalase reaction and coagulase activity were used to identify *S. aureus* isolates [12].

Nasal swabs were put directly onto mannitol salt agar (MSA) (Merck, Germany) and sent to the laboratory and incubated at 35º C in a humidified incubator for 48 h. For further characterization, strains that produced yellow colonies on the MSA plate were sub-cultured onto blood agar plates (Merck, Germany). To confirm methicillin resistance, the *mecA* gene was detected using a conventional PCR method using previously known primers [13].

Antimicrobial susceptibility testing (AST) was performed using Muller-Hinton agar (Oxoid, UK) and McFarland 0.5 standard in accordance with the Clinical and Laboratory Standards Institute (CLSI) standards [14]. The following antimicrobial agents were included in the panel: cefoxitin (30µg), clindamycin (2µg), linezolid (30µg), tetracycline (10µg), ciprofloxacin (5µg), trimethoprim/sulphamethoxazole (2.5µg), gentamycin (10µg), chloramphenicol (30µg), rifampin (5µg) and erythromycin (15µg) (Oxoid). Phenotypic antibiogram patterns were used to exclude duplicate isolates.

Mupirocin susceptibility testing:

Mupirocin minimum inhibitory concentrations (MICs) for *S. aureus* isolates were determined using E-test® mupirocin strips (AB-BIDISK, Solna, Sweden) as directed by the manufacturer. with a sterile forceps, the E-test strip was placed on each plate of Mueller-Hinton agar inoculated with a suspension of isolates to the optical density of a 0.5 McFarland standard. After a 24-hour incubation period at 35° C, E-test MIC values were interpreted by the operator at the point where the bottom of the inhibition zone intersected with the E-test strip.

The MIC breakpoints were correlated with previous studies of mupirocin resistance [15-17]. Mupirocin susceptibility was defined as an MIC of <8 µg /ml, low-level resistance as an MIC of 8 to 256 µg /ml, and high-level resistance as an MIC of ≥ 512 µg /ml.

PCR detection of the *mupA* gene:

The presence of the *mupA* gene was investigated in all strains using PCR. To extract the DNA, Fresh, well-isolated test colonies grown on blood agar plates following overnight incubation were removed and resuspended in 250 µl of sterile distilled water and the suspension incubated in a 90°C heat block for 15 min. Centrifugation followed (7500 x g, 5 min) and the supernatant containing the staphylococcal DNA extract was transferred into new test tubes and frozen for later PCR amplification. Five microlitres of extracted DNA were added to 20 µl of the PCR amplification mix consisting of; 2.5 µl of 10 X buffer, 1.5 mM of $MgCl_2$, 1.5 U of *Taq* polymerase, 1.25 µl of dNTPs and 1.5 µl of each primer. To detect the *mupA gene*, a 456-bp region was amplified by PCR, using a previously published primer pair [15], mup1 (5'-TAT ATT ATG CGA TGG AAG GTT GG-3') and mup2 (5'-AAT AAA ATC AGC TGG AAA GTG TTG-3').

A negative control consisting of the reaction mixture and water (in place of template DNA) was added in each run. The reference strain *S. aureus* ATCC 25923 was utilized as a mup quality control during susceptibility testing and DNA *S. aureus* jj1 from Marcia Giambiagi-deMarval (Brazil) was mupirocin resistant and used as the reference strain quality control for PCR [18]. The cycling settings were 94°C for 5 minutes, then 30 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final 5-minute incubation at 72°C. The amplified PCR products were analyzed on an agarose gel electrophoresis stained with ethidium bromide and the amplicons were seen using a UV light box.

RESULTS

The current study included 60 non-duplicate MRSA isolates (based on distinct phenotypic antibiogram patterns), 30 of which were clinical specimens (14 MRSA isolates from surgical wound infections, 16 MRSA isolates from patients with UTI infections who admitted to Suez- Canal University Hospital), and 30 isolates from health care workers' nasal swabs.

Six isolates out of 60 MRSA isolates (10%) showed high- level mupirocin resistance (MIC of ≥ 512 µg /ml), while just one isolate (1.6%) showed low- level mupirocin resistance (MIC 8µg/ml). All 7 mupirocin-resistant isolates (11.6 %) came from nasal carriers. MRSA isolates from clinical specimens were 100 % susceptible to mupirocin (MIC of <8 µg /ml).

The AST results for mupirocin-susceptible and mupirocin-resistant MRSA isolates are summarized in Table 1. Resistance to vancomycin was not identified. Mupirocin resistant MRSA strains were considerably more resistant to antimicrobials than mupirocin-susceptible ones. They exhibited higher resistance to tetracycline (85.7% versus 35.8%), chloramphenicol (57.1% versus 7.5%), gentamycin (85.7% versus 35.8%), ciprofloxacin (85.7% versus 20.7%), and trimethoprim-sulfamethoxazole (85.7% versus 39.6%). However, susceptibility to erythromycin, clindamycin, rifampin and linezolid showed no difference.

Table 1: Antimicrobial susceptibilities of mupirocin-susceptible and mupirocin-resistant MRSA strains isolated from nasal swabs and clinical specimens:

Antibiotic susceptibility		Mupirocin phenotypes		*p* value
		Susceptible N=53	Resistant N=7	
Tetracycline (TE)	S	34 (64.1%)	1 (14.2%)	≤0.05*
	R	19 (35.8%)	6 (85.7%)	
Chloramphenicol (C)	S	49 (92.4%)	3 (42.8%)	≤0.05*
	R	4 (7.5%)	4 (57.1%)	
Gentamycin (CN)	S	34 (64.1%)	1 (14.2%)	≤0.05*
	R	19 (35.8%)	6 (85.7%)	
Erythromycin (E)	S	16 (30.1%)	1 (14.2%)	(NS)
	R	37 (69.8%)	6 (85.7%)	
Clindamycin (DA)	S	45 (84.9%)	4 (57.1%)	(NS)
	R	8 (15%)	3 (42.8%)	
Ciprofloxacin (CIP)	S	42(79.2%)	1 (14.2%)	≤0.05*
	R	11 (20.7%)	6 (85.7%)	
Trimethoprim/sulphamethoxazole (SXT)	S	32 (60.3%)	1 (14.2%)	≤0.05*
	R	21 (39.6%)	6 (85.7%)	
Rifampin (RA)	S	45(84.9%)	4 (57.1%)	(NS)
	R	8 (15%)	3 (42.8%)	
Linezolid (LZD)	S	53(100%)	6 (85.7%)	(NS)
	R	0 (0%)	1 (14.2%)	

*: Statistically significant result (significant values are considered at p value ≤ 0.05), NS: Not significant

Only 6.6 % (4/ 60) of the MRSA isolates possessed the *mupA* gene (Figure 1) and they were all isolated from the nasal swabs exhibiting high- level mupirocin resistance, whereas none of the 30 isolates from clinical specimens did.

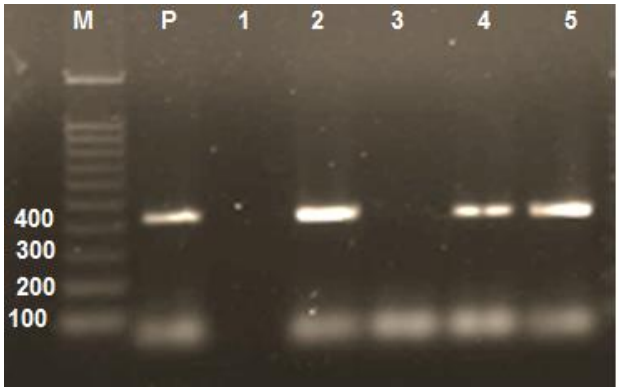

Fig. 1: M (100 bp ladder); P (positive control); 2,4,5 (positive samples); 1,3 (negative samples)

DISCUSSION

With the increased urgency for MRSA infection prevention, it is probable that mupirocin will be used more frequently for MRSA nasal decontamination. Understanding the mechanisms and epidemiology of mupirocin resistance is critical for predicting how changes in mupirocin use will affect bacterial populations and MRSA treatment [5].

Mupirocin resistance is common among MRSA strains, which makes nasal decolonization difficult. We used both phenotypic and genotypic approaches to determine the prevalence of mupirocin resistance in our tertiary-care hospital.

The overall rate of mupirocin resistance was not low in our institution (11.6%), despite the lack of a policy at our institution to use mupirocin to decolonize patients who screen positive for MRSA at admission. and lacing use of mupirocin to decolonize hospital staff, except for outbreak situations. Mupirocin is available without a prescription over the counter in Egypt, which could explain widespread of mupirocin resistance, particularly among health-care personnel.

Using the E-test, 10% of MRSA isolates had high-level mupirocin resistance, while only one isolate (1.6%) had low-level mupirocin resistance. A study done documented that mupirocin resistance was 19% in MRSA and 9% in MSSA. High-level and low- level mupirocin resistance were 9% and 4% respectively [19].

In Kuwait hospitals, MRSA strains expressing high-level resistance to mupirocin showed a declining trend from 85 (9.3%) in 2011 to 50 (3.6%) in 2013 and increased slightly to 66 (4.0%) in 2014 [20].

Poovelikunnel and colleagues [21] found a substantial link between previous mupirocin exposure and both low- and high-level mupirocin resistance in their study. Falling of rates of high-level mupirocin resistance had

occurred after restriction of mupirocin prescribing. Mupirocin resistance is often related to its widespread uses. Both low-level and high-level mupirocin resistance have been documented among MRSA isolates, but the rate of resistance varies by geographic area [17].

Also, Ohadian Moghadam and colleagues [22] conducted a survey of 270 medical personnel to determine the prevalence of S. aureus nasal colonization, which was 14.4%, with 43.58 % being MRSA and the rest being MSSA, and five isolates (1.85 %) being mupirocin-resistant, three of which showed high level resistance.

In the current study, mupirocin resistant MRSA strains were also resistant to tetracycline, chloramphenicol, gentamycin, ciprofloxacin, and trimethoprim-sulfamethoxazole compared to mupirocin-susceptible ones, This could be explained by the fact that the plasmid-encoded *mupA* gene mediating high-level mupirocin resistance in *S. aureus* can be found on conjugative plasmids that carry numerous antimicrobial resistance determinants [23].

Contrary to our findings, an Indian investigation compared three groups' antibiotic sensitivity patterns (Mupirocin sensitive, Low-level mupirocin resistance, High-level mupirocin resistance) and revealed that Teicoplanin, Linezolid, and Vancomycin sensitivity was 100% in all three groups. Except for Penicillin, all antibiotics exhibited good sensitivity against all group isolates [19].

In a study done on 291 strains of *S. aureus* isolated in Kuwait hospitals, 30.6 % were MRSA, and 100% of all isolates were sensitive to mupirocin, rifampicin, vancomycin, and linezolid, but showed varied degrees of resistance to aminoglycosides, macrolides, tetracycline, trimethoprim, fusidic acid, and fluoroquinolones [24].

CONCLUSION

Our research had some limitations. First, only the patient's first positive MRSA isolate was included in the study. Additional isolates from the same patients may have increased rates of mupirocin resistance. Second, because we did not use pulsed-field gel electrophoresis to type our isolates as it is not provided in our lab; the breakdown of individual MRSA clones among our samples is unknown. This is significant since pervious research has documented that different clones have varied rates of mupirocin resistance.

Recommendation:

We recommend that, all MRSA isolates should be routinely tested for high-level mupirocin resistance and institutions considering broad mupirocin usage should evaluate these resistance issues and devise ways to track the impact of mupirocin use. The monitoring plan should not only focus on mupirocin resistance, but also on how mupirocin use may contribute to the spread of multidrug resistance through its interactions with other resistance determinants.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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