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ORIGINAL ARTICLE

Mupirocin Resistance among Methicillin Resistant *Staphylococcus aureus* Causing Surgical Site Infections

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ABSTRACT

Background: *Methicillin Resistant Staphylococcus aureus (MRSA) is a major cause of serious infections. MRSA is a devastating complication, leading to increased mortality rates, increased hospital stay and costs. Topical mupirocin is used to eradicate nasal carriage and treat local infections with MRSA. Emergence of mupirocin resistance seriously adds to the problem of MRSA infections.* ***Objective****: This study aimed to determine the prevalence of mupirocin resistance and MupA gene among MRSA isolates causing surgical site infections.* ***Methods****: A total of 30 MRSA isolates from 150 patients with surgical site infections were identified. Mupirocin resistance was assessed using the E-test and polymerase chain reaction targeting mupA gene. Biofilm formation was tested.* ***Results****: Out of the 30 MRSA strains, 16.7% were mupirocin resistant and 10% were mupirocin resistant and carrying MupA gene. All of these isolates were biofilm producers and generally, the biofilm producers showed more resistance to antibiotics than non producers.* ***Conclusion****: MRSA infection along with mupirocin resistance represent a warning sign. Thus, it is advisable to test for MRSA colonization and infection among health care staff to control its spread.*

INTRODUCTION

Surgical site infection (SSI) is still an important challenge of healthcare units because it is associated with increased morbidity, mortality and healthcare costs [1]. *Staphylococcus aureus* is a leading cause of SSI. *Staphylococcus aureus* is an important pathogen causing a wide spectrum of infections [2]. The organism usually colonizes the skin and mucous of humans and several animal species. Multiple body sites can be colonized in humans, however, the anterior nares of the nose are the most frequent carriage site for *S. aureus*. Other sites for carriage include the skin, perineum, and pharynx [3].

It has been shown that there is an increased prevalence of staphylococcus infections, which may be attributed to its carriage in anterior nares and hands of health care workers and patients [3]. SSI caused by Methicillin Resistant *Staphylococcus aureus* (MRSA) is a devastating complication, leading to increased mortality rates, increased hospital stay and costs [4].

High case fatality rates have been observed for certain MRSA infections, especially SSI and MRSA bacteremia. Because MRSA can resist many other antibiotics, it has risen to the level of public health threat, both in the hospital and in the community [5]. The pathogenic mechanisms enabling *S. aureus* to cause serious infections could include: biofilm which protects organisms from host immune response; opsonophagocytosis and antimicrobial agents, thus leading to chronic and persistent infections [6].

Alarming is the antibiotic resistance associated with *S.aureus* infections, which is a great concern for the clinicians to prevent spread of infections. Methicillin was commonly used for these infections before the emergence of MRSA [7]. Risk factors for the development of MRSA include irrational use of antibiotics, prolonged hospital stay, nasal and hand carriage in health care staff[8].

MRSA infections are treated with vancomycin and linezolid, while for treating skin and soft tissue infections,as well as decolonization of carriers, mupirocin is used [9].

Mupirocin inhibits the protein synthesis by binding specifically to isoleucyl-tRNA synthetase enzyme. The irrational use of Mupirocin in MRSA infections among patients and its carriage in health care staff has led to the emergence of resistance to this antibiotic. Mupirocin resistance phenotypes include low-level (MuL) and high-level (MuH) resistance according to the minimum inhibitory concentrations (MIC) [10]. MupA, plasmid-mediated gene, had a supplementary modified isoleucyl-tRNA synthetase which leads to the high level resistance to Mupirocin. The mupA gene has the ability to facilitate and disseminate the resistance mechanism in different patterns [11].

Implementation of the basic SSI prevention strategies in hospitals, screening of healthcare staff and

patients for *S.aureus* nasal colonization and decolonization by treating patients with an anti-staphylococcal antimicrobial and/or antiseptic agents within the pre-operative setting has proved to be an important factor in an effort to reduce postoperative complications [12].

Mupirocin resistance in MRSA is a major concern; it can lead to loss of the major treatment option for controlling MRSA. This study was conducted to detect mupirocin resistance among MRSA causing SSI in order to rationalize the use of mupirocin in decolonization and treatment of patients in a tertiary care hospital through applying a proper antibiotic policy to avoid emergence and spread of antibiotic resistant strains.

METHODOLOGY

Samples

One hundred and fifty wound swabs and wound fluid specimens were collected from 150 patients diagnosed to have clinical surgical site infection (SSI). The design of the research was approved by the ethics committee, Faculty of Medicine, Suez Canal University.

Specimen culture and processing:

Wound swabs and fluid specimens were inoculated onto blood agar (LAB M Limited, UK) and mannitol salt agar (LAB M Limited, UK), incubated aerobically at 35^{o}C for 24-48 hours. Conventional methods were used to identify *S.aureus* isolates eg: colonial morphology, gram staining characteristics, and biochemical tests.

- **Antibiotic susceptibility test:**

Disc diffusion method using modified Kirby-Bauer technique on Muller Hinton agar (MHA) (Himedia, India) was carried out for antimicrobial susceptibility of *S. aureus* isolates. The following antibiotics were tested: Tetracyclin 30 µg (TE), Chloramphenicol 30 µg (C), Gentamycin 10µg (CN), Erythromycin 15µg (E),Clindamycin 2 µg (DA), Ciprofloxacin 5 µg (CIP), Trimethoprim/sulphamethoxazole 1.25/23.75 µg (SXT), Rifampin 5µg (RA), Linezolide 30µg (LZD); zone diameters interpretation were done according to Clinical and Laboratory Standard Institute [13], *Staphylococcus aureus* ATCC 25923 was used for the quality control in the disc diffusion testing.

Methicillin resistant *S. aureus* (MRSA) were tested by disc diffusion method using cefoxitin disc (FOX 30 µg), any growth for *S.aureus* strains around the disk with ≤21 mm was considered as resistance.

- **Detection of biofilm formation by MRSA strains:**

1-Congo Red Agar method (CRA):

CRA plates were prepared by adding 0.8g of Congo red (Lobal chemie) and 36 g of sucrose (El nasr chemical), to one Liter of BHIB. The plates were inoculated with the test organism from blood agar medium and incubated at 35^{0}C for 24 to 48 hours aerobically. Biofilm producers give black colonies, while non-producers give red colonies.

II- Modified Tissue Culture Plate method (TCP):

All isolates were screened for their ability to form biofilm by the TCP method [14] with a modification in duration of incubation, which was extended to 24 hours[15]. A micro ELISA auto reader (STAT FAX-2100) at wavelength 540 NM was used to determine Optical density (OD) of the samples as an index of bacteria adhering to surface and forming biofilm. The isolates were classified into three categories, non-adherent, moderately adherent, and strongly adherent. When mean OD values ≤ 0.111, it's the cutoff for non-adherent, the isolates were considered as negative; and when the cutoff corresponded to moderately (mean OD values >0.111 or ≤ 0.222) or strongly adherent (mean OD values > 0.222), the isolates were considered as positive[16] .

Mupirocin susceptibility testing:

The MIC susceptibility test to mupirocin was assessed using the E-test mupirocin strips (AB-BIODISK, Solna, Sweden) according to the manufacturer's recommendation, and interpreted based on the CLSI breakpoints, 2011[17]. Strains were considered to be susceptible if the MIC value was ≤ 4 mg/L and low-level mupirocin resistance is considered when MIC 8–256 mg/L, and high-level mupirocin resistance when MIC ≥ 512 mg/L [18]. For quality control in the MIC testing, Staphylococcus aureus ATCC 29213 was used.

Detection of *mup*A gene by PCR

Nucleic acid Extraction:

All MRSA strains were tested by PCR for detection of the *mup*A gene. Genomic DNA was extracted by boiling method. Briefly, several colonies from an overnight-grown culture on nutrient agar were resuspended in 250 µl ddH2O and placed in a boiling water bath for 20 min before being centrifuged at 12000 x g for 5 min. The supernatant containing the extracted DNA was frozen at 20 °C for later PCR amplification.

Amplifying the target mupA genes

Amplification of *mup*A genes was carried out in a thermal cycler (Techneprogene INC). The primer sequences for *mup*A genes **(GenBank accession No. X75439)** [19] used in this study were (5'-TGA CAA TAG AAA AGG ACA GG-3 '), (5'-CTA ATT CAA CTG GTA AGC C-3). Five micro liters of the extracted DNA were transferred to 20 µl of the PCR amplification mix consisting of; 1.5 µl of each primer, 1.5 mM of MgCl2, 1.5 U of Taq polymerase, 1.25 µl of dNTPs , 2.5 µl of 10 X PCR buffer (10 Mm Tris-HCl pH9.0, 50 mM KCl, 0.1% Triton x-100). The reaction was carried out at 94 ° C for 5 minutes and then 35 times at 94 ° C 30, 52 ° C, and 72° C for 1 minute.

Gel electrophoresis

After amplification, 10µl of the PCR reaction solution was analyzed by agarose gel electrophoresis

(1.5%-2% agarose in Tris-borate-EDTA and stained with 1 µg / mL ethidium bromide). A 100 bp DNA ladder was used as a molecular weight marker, and expected band of amplicon (190-bp region) were visualized using a UV light box.

Statistical methods

All collected data were in the form of qualititative data expressed as categorical variables and presented in numbers and percentages for testing isolates. Pearson Chi-square test and Fisher's man test were utilized to test the statistical significance of the differences between the study groups. The significant statistical difference was considered when P value < 0.05. Data entry and analysis were carried out using the program statistical package for social sciences (SPSS, version 20.0) (IBM Corporation, New York, USA).

RESULTS

Out of the 30 MRSA strains recovered from 150 SSI, 18 (60%) were biofilm producers and 12 (40%) were non-producers using the CRA method compared to 24 (80%) biofilm producers and 6 (20%) non-producers using the TCP method.

Regarding the antibiotic susceptibility profile of the isolated MRSA strains, all isolates (100%) were sensitive to Linezolide and Vancomycin, twenty four out of 30 (80%) isolates were sensitive to Clindamycin, Rifampin and Chloramphenicol, also 73.3%, 56.7% were sensitive to Ciprofloxacin and tetracycline respectively. While higher resistance rates were detected to both Penicillin G, Erythromycin (96.7%, 29 isolates), followed by resistance to Gentamycin and TMP-SMX (56.7%, 17 isolates); (50%, 15 isolates) respectively (Figure 1).

Regarding the relationship between biofilm production and antibiotic resistance, the biofilm producing isolates showed more resistance than non-biofilm producing isolates to the following antibiotics; Chloramphenicol (83.3% versus 16.7), Penicillin G and Erythromycin (82,8% versus 17.2%), Tetracycline (76.9% versus 23.1%), Ciprofloxacin (75% versus 25%), TMP-SXT (73.3% versus 26.7%), and Gentamycin (70.6% versus 29.4%). otherwise all Clindamycin, Rifampin resistant strains were biofilm producers. All biofilm producers and non biofilm producers were sensitive to Linezolide and Vancomycin as shown in table (1).

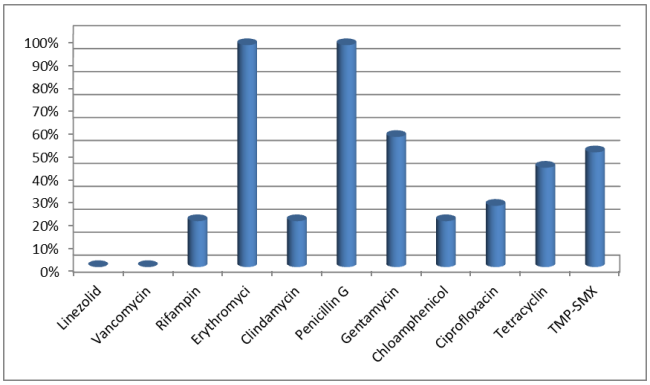

Fig. 1: Resistance rates of isolated MRSA strains to different antibiotics

Table 1: Antibiotic resistance pattern in biofilm producing and non producing MRSA

Antibiotic Resistance	Biofilm production (TCP method)				Total	
	Biofilm producers 24		Biofilm non-producers 6			
	Frequency	%	Frequency	%	Frequency	%
Penicillin G	24	82.8%	5	17.2%	29	100%
Erythromycin	24	82.8%	5	17.2%	29	100%
Clindamycin	6	100%	0	_	6	100%
Rifampin	6	100%	0	_	6	100%
Ciprofloxacin	6	75%	2	25%	8	100%
Gentamycin	12	70.6%	5	29.4%	17	100%
Chloramphenicol	5	83.3%	1	16.7%	6	100%
Tetracycline	10	76.9%	3	23.1%	13	100%
TMP-SMX	11	73.3%	4	26.7%	15	100%

Concerning mupirocin resistance, all MRSA strains were tested for Mupriocin resistance using the MIC method (E-test mupirocin strips) and conventional PCR to detect the presence of mupA (190 bp) gene. Using the MIC method, 5 isolates (16.7%) were shown to be mupirocin resistant, on the other hand three isolates (10%) were positive for *mupA* gene by PCR (figure 2), additionally these strains were biofilm producers.

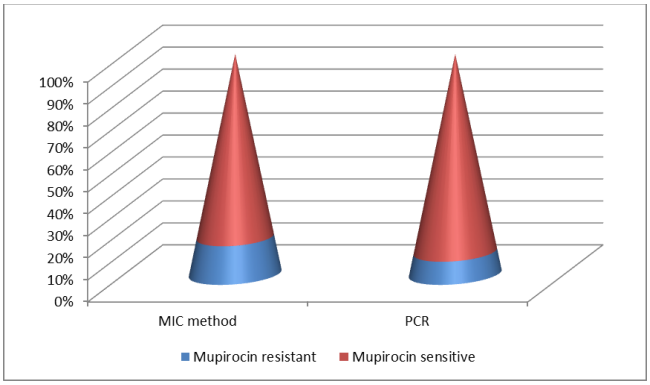

Fig. 2: MIC and PCR detection methods for mupirocin resistance

DISCUSSION

SSI is the most common healthcare associated infection, SSIs was defined as wound infections that occur following a surgical procedure [20]; Also Kathju and his colleague [21] reported that it occurred due to the contamination of a wound by micro-organisms derived from the patient's own skin flora. The predominant organism causing SSIs was *Staphylococcus aureus*, especially MRSA. Its needs more attention due to its resistance to commonly used antibiotics in the hospital and regular monitoring of the incidence MRSA causing SSI by proper antibiotic prophylaxis [20].

In our study percentage of MRSA causing SSI was 20%, matching with the results of Esmat *et al.* in Sohag, who found that *Staphylococcus aureus* was responsible for 20% of SSIs; all of which were MRSA [22]. In many American and European hospitals, the percentage of MRSA has ranged from 29% to 35%; while in India it ranged from 30% to 87% [23,24]. Higher rates were reported by Sibabrata *et al.* [25] and Shazia Parveen *et al.*[26]; where the rate of MRSA was 47% and 48% respectively.

According to our study, Twenty four out of thirty MRSA isolates (80%) were biofilm producers using the TCP method as a gold standard method [27]; while 60% of isolates were biofilm producers by CRA method.

Comparatively, Hashem *et al.*[28] found that modified TCP detected 70% of *staphylococci* isolates as biofilm producers. Lower rates were detected by Mathur *et al.* [29] and El Hadidi [30], Ankit Belbase *et al.* [31] who reported detection rates of 53.9%, 48%, 46.1% respectively. A study conducted by Sibabrata *et al.*[25] showed that biofilm production was 53.2% among MRSA strains in comparison to 28.3% among MSSA strains.

Biofilm have been implicated in numerous acute and chronic infections. Several reports have linked biofilm to the induction and persistence of inflammation and delayed healing in wound infections. The ratio of the planktonic to biofilm phenotypes are shifted to the biofilm phenotype in chronic wounds, thus resulting in delayed wound healing [32].

Our susceptibility profile of MRSA strains showed that all isolates were sensitive to Linezolide and Vancomycin, 80% of isolates were sensitive to clindamycin, rifampin and chloramphenicol, also 73.3%, 56.7% were sensitive to ciprofloxacin and tetracycline respectively .

Similarly, Ankit Belbase *et al.* [31], Hashem *et al.* [28] and Susmita Bhattacharya *et al.*[20] reported in their studies that all *S. aureus* including MRSA isolates were linezolid and vancomycin sensitive. Also, El Hadidi,[30] reported that all isolates were sensitive to vancomycin. Furthermore, Ankit Belbase *et al.* [31] demonstrated that susceptibility of MRSA to clindamycin was (88.9%), to tetracycline and chloramphenicol was (97.2%). Also Susmita Bhattacharya *et al.* [20] reported that highly sensitive drugs against MRSA were mupirocin (88.39%), levofloxacin (75.66%) and doxycycline (72.28%).

In our study, the resistance rate of MRSA isolates to both Penicillin G, Erythromycin was (96.7%), followed by Gentamycin and trimethoprim-sulphamethoxazole (TMP-SMX) (56.7%); (50%) respectively.

Similar resistance pattern of MRSA was detected by Ankit Belbase *et al.*[31] to penicillin (100%), ciprofloxacine (77.2%), both erythromycin, cotrimoxazole (72.2%), and for gentamicin (38.8%).

We found that antibiotic resistance among biofilm producers was significantly higher than non-biofilm producers to Clindamycin, Rifampin, Chloramphenicol, penicillin G, Erythromycin, Tetracycline, Ciprofloxacin, TMP-SXT, and Gentamycin .

Both Sasirekha *et al.* [23] and Rezaei *et al.* studies [33] found higher antibiotic resistance among biofilm producers for ciprofloxacin, co-trimoxazole, rifampicin, erythromycin and clindamycin. Likewise Belbase A *et al.* [31] and Ghasemian *et al.* [34] reported that multidrug resistance and methicillin resistance were more frequently found among biofilm producing strains in comparison to non producers.

These findings are attributed to the protective nature of the biofilm, the bacteria growing in it is intrinsically resistant to many antibiotics.

Additionaly, Croes et al.[35] reported that biofilm is one of the defense mechanism of *S. aureus; it makes* bacteria resistant to host defense mechanisms and show resistance to standard antibiotic therapy.

Many mechanisms are involved in rendering biofilm producers more antibiotic resistant such as difficulty in antibiotic penetration, presence of antibiotic degradation mechanisms,in the center of biofilm, and slow growth rate of the bacteria [36].

Mupirocin resistance was tested phenotypically and genotypically. Five isolates (16.7%) were detected as resistant by MIC method (E-test strips), while conventional PCR detected three isolates out of the

thirty MRSA strains (10%) as positive for *mup*A gene; additionally these strains were biofilm producers.

Resistance to mupirocin, reduces the effectiveness of decolonizing strategies for *S. aureus* or MRSA. Unrestricted overuse has been associated with emergence of resistance through enhanced selective pressure and cross-transmission[37]. The association of high-level resistance in *S. aureus* with plasmids is a major threat with clinical use of the antibiotic[38].

A study in Spain found that 12% of MRSA isolates possessed high-level mupirocin resistance (*mupA* gene); this represented both a clonal expansion of *S. aureus* strains and the spread of a *mupA* allele, this allele is present on a pSK41-type conjugative plasmid, the transfer of which could create a new, potentially pandemic *S. aureus* strain [11].

Our results are also consistent with those of Sibabrata *et al.* [25], demonstrating that prevalence of MupH among MRSA was 6.4%, trends quite similar to studies like Schimtz *et al.* and Oommen *et al.* [39,40]. In contrast, some studies by shalaby and Elshahat, Hadadi *et al.* and Rashidi *et al.*, reported that mupA gene could be detected in approximately forty percent of strains that were with high-level mupirocin resistance [41-43]

In Egypt, the prevalence of mupirocin resistance among MRSA strains was 11.6% in Suez Canal University Hospital, Ismailia. MRSA strains were isolated from surgical wound and urinary tract infections of the patients and from nasal swabs of the healthcare workers [44]. Also Barakat & Nabil reported 17.8% Mupirocin-resistance among MRSA strains [45].

In our study, we found that all isolates which carry mupH gene (*mup* A) were biofilm producers; additionally Sibabrata *et al.*, [25] demonstrated a higher prevalence of mupriocin resistance among the biofilm producing strains, all strains expressing mupH and 50% of strains expressing MupL were biofilm producers.

CONCLUSION

MRSA is a serious nosocomial organism causing surgical site infections. Extensive use of mupirocin to treat skin, postoperative wound infections as well as control the nasal carriage of MRSA in health care institutions have contributed to the emergence of resistant strains. Thus screening for mupirocin-resistant *S. aureus* is important in order to allow appropriate antibiotic choice and control spread of these resistant strains.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- 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.

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