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ORIGINAL ARTICLE

Resistance to Mupirocin among Methicillin Resistant Staphylococcus Aureus Isolates from Community Acquired Infections, Hospital Acquired Infections, and colonized Health Care Workers

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ABSTRACT

Background: *Mupirocin is prescribed as a topical treatment of Staphylococcus aureus infections as well as in decolonization of methicillin-resistant S. aureus (MRSA) in both patients and health care workers (HCWs). Availability and increased use of this drug has led to emergence of resistance especially among MRSA compared to methicillin sensitive S. aureus (MSSA).* ***Objectives:*** *Our study aimed to evaluate the prevalence of mupirocin resistant MRSA isolated from clinical infections and from colonized HCWs.* ***Methodology:*** *Between January to August 2017, 61 MRSA isolates were collected. Mupirocin MICs were detected using mupirocin E-test. mupA PCR was performed for resistant isolates.* ***Results:*** *86.9%of MRSA were isolated from clinical infections; 22.9% were Community acquired (CA-MRSA) and 64% were Health Care acquired (HCA-MRSA). 13.1% of total isolated MRSA were from HCWs. 23% of all MRSA were mupirocin resistant. The percentages of mupirocin resistance in CA-MRSA, HCA-MRSA, and MRSA nasal carriers of HCWs were 1.6%, 18.1%, 3.3% of the total Mupirocin resistant MRSA, respectively. 14.8% of MRSA showed low level resistance, while 8.2% were high level Mupirocin resistant. MupA gene was detected in 42.9% of strains with high level mupirocin resistance.* ***Conclusions:*** *Routine MRSA testing for mupirocin resistance is recommended for early detection and control of the spread of resistance.*

INTRODUCTION

Staphylococcus aureus especially Methicillin Resistant *Staphylococcus aureus* (MRSA) is a common cause of community acquired and hospital-acquired infections causing infections ranging from mild skin and soft-tissue infections to life-threatening infections with high morbidity, mortality and costs [1,2]. MRSA Carriers especially among HCWS are responsible for spread of infection. The main site of colonization is the anterior nares [3].

Mupirocin is a product of Pseudomonas fluorescens. Since late 1980s, Mupirocin ointment has been proven to be a successful topical antibiotic for nasal decolonization of S. aureus [4,5,6]. Also, Mupirocin is prescribed for the treatment of skin and soft tissue infections caused by S. aureus and streptococci species. Widespread use of this drug has led to the emergence of resistance [7].

There are two types of mupirocin resistance, low-level resistance (MICs from 8 to 64 mg/mL) and high-level resistance (MIC ≥ 512 mg/mL) [8]. Resistance among isolates with high-level resistance is mainly due to plasmid-mediated *mupA* gene, which encodes a novel isoleucyl RNA synthetase [9,10]. However, isolates with low-level resistance are often due to acquired base changes in the native isoleucyl RNA synthetase gene, *ileS* [11]. Nonetheless, *mupA* gene have also been detected in low-level mupirocin resistant isolates. The *mupA* gene in these isolates was found on the chromosome not a plasmid [12]. Also, some mupirocin susceptible isolates were reported to have *mupA* gene. This has been related to a frameshift mutation in the gene which inactivates the product [13]. Determinants of resistance to other antibiotics, including tetracycline, macrolides, gentamicin, and trimethoprim may also be carried on the plasmid carrying the *mupA* gene. This means that mupirocin use could select not only for mupirocin resistance, but also for other antibiotics [14-17].

In the clinical field, MRSA with high-level mupirocin resistance are crucial because they may transfer resistance genes to other bacteria by conjugation. Thus, decreases the effect of mupirocin on these bacteria [18].

METHODOLOGY

Study design and Data collection:

The present study was conducted in the Microbiology Department, Tanta University Hospital during the period from January to August 2017. The

study included 69 MRSA isolates that were collected from:

1. clinical specimens taken from inpatients in the hospitals and patients attending Outpatient Clinics. These included skin, pus, blood, central catheter tips, sputum, tracheal tip, urine, and wound. The presence of infection caused by MRSA was diagnosed using standard definitions [19]. Health care acquired MRSA (HCA-MRSA) or community acquired MRSA (CA-MRSA) infections were defined by using previously published criteria [20].
2. Nasal swabs from HCWs were taken after obtaining consent, using sterile cotton swabs moistened with sterile distilled water and transported immediately to Microbiology Laboratory [21].

Antimicrobial susceptibility testing

After identification of *S.aureus* strains using standard microbiology methods, the susceptibility to antimicrobial agents was determined initially by modified Kirby-Bauer's disk diffusion method as per CLSI guidelines [22]. To determine minimum inhibitory concentration (MIC) for oxacillin, E-test strips (LIOFILCHEM® - ITALY) were used. Strains with MIC of ≥4µg/mL were considered MRSA [23]. Mupirocin MICs were determined by Mupirocin E-test strips (AB Biodisk, Sweden). Low level resistance was defined with MICs from 8 to 64 mg/mL and high-level resistance with MICs ≥ 512 mg/mL[8].

Detection of *mupA* gene

Genomic DNA was extracted by the MagNA pure compact nucleic acid isolation kit I in combination with MagNA pure bacteria lysis buffer and DNA bacteria purification protocol (Clinilab). PCR assay was done using species specific forward primer (5'-CCCATGGCTTACCAGTTGA-3') and reverse primer (5'-CCATGGAGCACTATCCGAA-3') with a fragment of 1.6 kb were used [24]. All PCR amplifications were performed in a final volume of 20 µL containing one ρmol of the primer (Forward and Reverse), 0.17 mMdNTPs, 2.5 mM MgCl2, one U of Taq DNA polymerase, buffer of Taq, and 10 µL template DNA. An initial denaturation cycle of (94 °C for two min), was followed by 30 denaturation cycles of (94 °C for one min), annealing at an appropriate temperature for 1 min and elongation (72 °C for 10 min). Biometra T personal thermocycler was used to perform the PCR reactions. PCR products were analyzed using gel electrophoresis in 1.5% agarose stained with ethidium bromide (0.5 g.mL-1), observed under UV transillumination (Illuminator UV star 312nm Biometra) and then photographed (canon).

RESULTS

During the course of the study, 98 *Staphylococcus aureus* strains were recovered from patients with either community acquired, or health care acquired infections and from colonized HCWs. Of these, sixty-one (62.2%)were MRSA as confirmed by Oxacillin E-test. Fifty-three (86.9%)of MRSA strains were from clinical infections; 14 (22.9%) were CA-MRSA and 39 (64%) were HCA-MRSA. Regarding HCWs, 8 MRSA strains (13.1% of total isolated MRSA) were isolated. 16% of screened HCWs (n=50) were nasal carriers of MRSA (table 1). Fourteen of all MRSA strains (23%) were proved to be mupirocin resistant. The percentages of mupirocin resistance in CA-MRSA, HCA-MRSA, and MRSA nasal carriers of HCWs were 1.6%, 18.1%, 3.3% of the total Mupirocin resistant MRSA, respectively (table 2). Nine strains (14.8%) showed low level mupirocin resistance (MICs= 8-64 mg/mL), while, 5 (8.2%) strains were high level mupirocin resistant (MICs ≥ 512 mg/mL) (table 3). MupA gene was detected in 6 (42.9%) of strains that showed high level mupirocin resistance.

Table 1: Percentages of isolated MRSA strains according to their sources.

Source of MRSA	MRSA strains	
	No.	%
Community acquired infections	14	22.9%
Health care acquired infections	39	64%
Colonized HCWs	8	13.1%
Total	61	100%

Table 2: Percentages of Mupirocin resistant strains among total isolated MRSA and individually isolated MRSA from different sources.

MRSA source	Mupirocin R		Mupirocin S		Total	
	No.	%	No.	%	No.	%
CA-MRSA(n=14)	1	1.6%	13	21.3%	14	22.9%
HCA-MRSA(n=39)	11	18.1%	28	45.9%	39	64%
HCWs carriers(n=8)	2	3.3%	6	9.8%	8	13.1%
Total MRSA(n=61)	14	23%	47	77%	61	100%

Table 3: High-level and Low-level Mupirocin resistance among Mupirocin resistant MRSA.

	Low level resistance	High level resistance	Total	
			No.	%
Mupirocin resistant CA-MRSA	0	1	1	1.6%
Mupirocin resistant HCA-MRSA	7	4	11	18.1%
Mupirocin resistant HCWs MRSA	2	0	2	3.3%
Total	9 (14.8%)	5 (8.2%)	14	23%

DISCUSSION

Since mupirocin became available in 1985, it was prescribed for treating many skin infections and for de-colonization therapy of MRSA in both patients and HCWs. Shortly after, Mupirocin resistance was first reported in *S. aureus* from United Kingdom. Currently, there is a worldwide increase in mupirocin resistant *S. aureus*[25]. The risk of emergence of such resistance seems to be greater among MRSA strains than among methicillin-susceptible strains [26,27].

During our study, sixty-one MRSA non-repetitive strains were isolated. Forteen (22.9%) of total isolated MRSA were CA-MRSA, 39 (64%) were HCA-MRSA, and 8 MRSA strains (13.1%) were from colonized HCWs. Also, during the study, Mupirocin resistance was found in 14 (23%) of MRSA strains. Nearly similar results were observed in a study performed by Park et al, 2012 [28] as they reported that 14.1% of MRSA isolated were resistant to mupirocin.

The percentages of Mupirocin resistance in CA-MRSA, HCA-MRSA, and MRSA nasal isolates were 1.6%, 18.1%, 3.3%, respectively. Not surprisingly, the least percentage of mupirocin resistance was among CA-MRSA. Contrarily, Simor et al,[29] reported a higher percentage (14%). The discrepancy between our results and their study may be the bigger number of strains they studied. Also, we found that 3.3% of Mupirocin resistant MRSA were from nasal swabs of HCWs. However, an Indian study[30] that aimed to assess mupirocin resistance in *S.aureus* nasal isolates from HCWs revealed that 25.71% of isolated *Staph. aureus* were mupirocin resistant MRSA. We could contribute this to the increased use of mupirocin.

Clinically, high-level mupirocin resistant strains are more important compared to Low-level resistant strains because the concentration of mupirocin in the ointment (2%) is more than the MICs of the low-level mupirocin resistant bacteria[31]. The prevalence of low-level mupirocin resistance (14.8%) was higher than that of high-level resistance (8.2%) in the current work. Same observation revealed by Dardi, [32]. In their study, high-level mupirocin resistance was observed in 5.99% and low-level resistance in 15.35%. Also, our results agree to much extent with the reports in the literature of 1-13% for low-level and 2.4-14% for high-level resistance[33].

During our work, mupA gene could be detected in 42.9% of strains that were high level mupirocin resistant. Similar results were found by Marine et al, [34] and Rashidi et al,[35]. However, lower prevelance of mupA gene (25%) was reported in the study conducted by Shahsavan et al,[36].

CONCLUSIONS

The prevalence of mupirocin resistance in MRSA should be considered. So, routine MRSA testing of for mupirocin resistance is recommended. This will help in the early detection of resistance and in the controlling the spread of resistance.

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