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The Impact of Methicillin-Resistant Staphylococcus aureus (MRSA) Decolonization Protocols on its Mupirocin and Chlorhexidine Susceptibility

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Abstract

Development of bacterial resistance to the antibiotics constituted a major health problem. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is considered a prominent example. MRSA tends to develop resistance to multiple antibiotic classes rapidly. Furthermore, MRSA colonization was proven to be a major risk factor for infection and subsequent transmission. MRSA decolonization by nasal mupirocin application and chlorhexidine whole-body washing are now recommended. This study aimed to screen for the development of mupirocin and chlorhexidine resistance among MRSA isolated from health facilities that applied decolonization protocols. A total of 272 clinical staphylococcal isolates were investigated. 115 MRSA isolates were collected before application of any of decolonization protocols, 81 isolates were collected from the facilities that applied the targeted decolonization protocol and 76 isolates were collected from the facilities that applied universal decolonization protocol. The isolated strains were identified phenotypically and subjected to PCR for MRSA confirmation. The used PCR assay simultaneously enabled the identification of mupirocin and chlorhexidine resistance genes. The results showed that the baseline mupirocin resistance rate among MRSA isolates was considered moderate (13.9%). While, the baseline chlorhexidine resistance was rare (3.5%).The resistance prevalence rates for both agents did not change significantly during the decolonization practice. In conclusion, the application of the decolonization strategies did not result in a significant change in MRSA susceptibility to either mupirocin or chlorhexidine. However, these results should be interpreted with caution due the small sample size and the relative short period passed since stating the decolonization protocols.

Keywords: Chlorhexidine; Decolonization; MRSA; Mupirocin

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Introduction

Despite decades form its discovery and years of interventions, Methicillin-Resistant Staphylococcus Aureus (MRSA) is still a leading cause of Health Care Associated Infections (HAI) worldwide. MRSA infections are associated with increased rates morbidity and mortality and representan intensive load on the health care system [1]. The Centers for Disease Control and Prevention (CDC) reported more than 80,000 invasive infections and about 11,000 deaths caused by MRSA in 2011 [2]. In Saudi Arabia, MRSA prevalence accounted for 14.8% of all HAI [3]. Colonization with MRSA typically precedes the clinical infection and plays an essential role in its dissemination in hospitals [4]. The MRSA colonization rate in hospitalized patients ranges from 1.3% to 7.6% [5,6] and about 10% to 30% of those carriers subsequently develop infections [7]. The anterior nares are the commonest colonization site but, other sites such as the throat, perineum, skin and skin lesions are frequently colonized [8]. Prevention and control measures for MRSA should be implemented on a large scale, as the interventions at one facility may have a referred effect on MRSA prevalence in other nearby facilities [9]. Control of MRSA is multi-factorial, single intervention measure mostly has non-significant impact on MRSA infection rates. Contact precaution and hand hygiene are of importance [2], however, intended decolonization became a rapidly growing strategy to prevent MRSA infections. Decolonization involves two main protocols; targeted decolonization and non-targeted decolonization through application of antimicrobials to the patient's skin and mucosal surfaces [10]. Targeted decolonization means decolonization of patients who are identified as carrying MRSA while, universal (non-targeted) decolonization means decolonization of

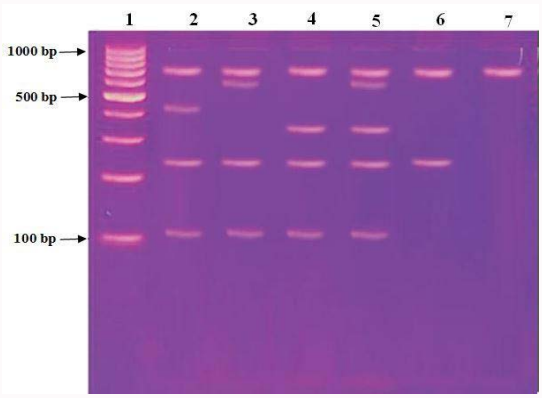

Figure 1: PCR results of the studied genes. Lane 1, DNA ladder. Lane 2, mupA positive methicillin resistance Staphylococcus aureus (MRSA). Lane 3, mupB positive MRSA. Lane 4, qacA/B positive MRSA. Lane 5, mupA&qacA/B positive MRSA. Lane 6, methicillin sensitive Staphylococcus aureus (MSSA) and Lane 7, coagulase-negative staphylococci (CoNS).

populations of hospital patients regardless of the MRSA colonization status. Both of the two strategies have been demonstrated to decrease cross-transmission and MRSA infection rate [11,12]. The most common decolonization protocol is a 5-day regimen of twice-daily intranasal mupirocin ointment and daily chlorhexidine baths [13]. Furthermore, several studies recommended three consecutive negative swabs to confirm MRSA eradication [2]. Mupirocin is an antibiotic produced by Pseudomonas fluorescens. It was clinically approved in the late 1980's as a topical antibiotic for the clearance of nasal carriage of *Staphylococcus aureus* (*S. aureus*) [14,15]. Mupirocin interferes bacterial protein and RNA synthesis through competitive inhibition of bacterial isoleucyl-tRNA-synthetase [16]. Mupirocin 2% ointment is applied to the anterior nares 2 to3 times daily. Nasal carriage is then normally cleared within 5 to 7 days with excellent efficacy (up to 90%). Mupirocin is also used for the treatment of local skin and soft tissue infections [16]. The mupirocin widespread use for decolonization has been thought to be associated with the development of Mupirocin Resistance (MR) by *S. aureus* as well as other *Staphylococcal* species [16]. Several studies reported MR among MRSA in a wide range of variation, from 1% to 81% [17,18,19]. *Staphylococcal* susceptibility to mupirocin is determined according to the Minimal Inhibitory Concentration (MIC) breakpoints, strains with MIC ≤ 4mg/L are considered susceptible. While, those with MIC ≥ 512mg/L are considered High-Level Mupirocin Resistance (HLMR) strains, in between these two levels (8-256mg/L) are called Low-Level Mupirocin Resistance (LLMR) strains [1]. HLMR is mediated by either mupA or mupB genes, both of which encode a novel isoleucyl-tRNA-synthetase [16]. These genes are carried on two conjugative plasmids, enabling their cross-transmission within *S. aureus* and to other *Staphylococcal* species. LLMR results from mutations in the native chromosomal isoleucyl-tRNA-synthetase gene; these mutations are typically stable and non-transferable [20]. Emergence of HLMR among *S. aureus* leads to MRSA decolonization failure while, LLMR may predispose to its early re-colonization [17,21]. Both of mupA or mupB genes were also detected among Methicillin-sensitive *S. aureus* (MSSA) and Coagulase Negative Staphylococci (CoNS) with a regional variation in the prevalence rates [22]. Chlorhexidine is a biguanide cationic broad-spectrum bactericidal agent. It covalently binds to the bacterial cell membrane and disrupts its integrity and subsequent leakage of the intracellular components and cell death [13]. Chlorhexidine is often used in various forms as a part of oral care, skin antiseptic. Also, it used for whole body bathing as a part of MRSA decolonization strategies along with nasal mupirocin application [23]. Although, chlorhexidine susceptibility testing methods have not been standardized [13], bacterial resistance to chlorhexidine was reported since 1995 [24]. Resistance to chlorhexidine as well as quaternary ammonium compounds is mediated by three genes carried on plasmids; qacA/B that confers high-level resistance and smr that confers low-level resistance [25,26]. These plasmids encode for proton-dependent multidrug efflux pumps [17,27]. Concomitant chlorhexidine resistance to other antiseptics and/or systemic antibiotics was reported and presents additional challenges during the decolonization strategies [28]. In consistent with the recent universal trend CDC and recommendations [29,30], many of our local health facilities have shifted from MRA screening and contact precaution to decolonization strategies (either targeted or universal decolonization). The aim of this study was to monitor the prevalence and the development of mupirocin and chlorhexidine resistance among MRSA isolated from those health facilities.

Materials and Methods

MRSA isolates and identification

MRSA strains were collected from clinical samples referred from different clinical departments of the main hospitals in Taif region, Saudi Arabia. 115MRSA isolates were collected before application of any of decolonization protocols (August, 2016) and157MRSA isolates were collected later during the period from February to December 2017(i.e.6 months after starting the decolonization protocols). 81 isolates were collected from the facilities that applied the targeted decolonization protocol and 76 isolates were collected from the facilities that applied universal decolonization protocol. *S. Aureus* was identified by the standard microbiological methods (Colony morphology, Gram staining and tube coagulase test). The standard MRSA surveillance method amoxicillin containing mannitol salt agar was preformed after enrichment in trypticase soy broth for 24 h [31]. Positive isolates were consequently subjected to molecular assay and both mupirocin and chlorhexidine susceptibility testing.

Mupirocin and chlorhexidine susceptibility testing

Mupirocin susceptibility was determined by the E-test method (bioMérieux, Durham, NC) according to the manufacturer's instructions. HLMR was defined as a MIC ≥ 512 mg/L while, LLMR was defined as a MIC of 8 256mg/L to 256 mg/L [1,32]. Chlorhexidine susceptibility was performed by broth micro-dilution methods using chlorhexidine solution (Sigma-Aldrich, St. Louis, MO). MRSA isolate was considered as non-susceptible to chlorhexidine if the MIC was 4 µg/ml [33].

Molecular assay

A recently published heptaplex PCR assay was applied [29]. This assay enabled MRSA confirmation by detecting *Staphylococcus* genus specific gene (staph 16 rRNA to distinguish staphylococci from other bacteria), *Staphylococcus aureus* species specific (nucto distinguish *S. aureus* from CoNS) and methicillin resistance gene (mecA to distinguish MRSA from MSSA). The assay and simultaneously screens for the presence of chlorhexidine resistance genes (qacA/B and smr) as well as mupirocin resistance genes (mupA and mupB) [29]. Bacterial DNA was extracted by rapid method where, 1-5 colonies from sub-cultured tryptic soy agar (Becton, Dickinson, Franklin Lakes NJ) were suspended in 50 µl of distilled water in a hot water bath (99°C for 10 min), followed by high-speed centrifugation

Table 1: The primers sequences and the products sizes of the studied genes.

Primer	Sequence	Product size (bp)
Staph-16SrRNA	F-AACTCTGTTATTAGGGAAGAACA	756
	R-CCACCTTCCTCCGGTTTGTCACC	
Nuc	F-GCGATTGATGGTGATACGGTT	279
	R- AGCCAAGCCTTGACGAACTAAAGC	
MecA	F-GTGAAGATATACCAAGTGATT	112
	R-ATCAGTATTTCACCTTGTCCG	
MupA	F-TATATTATGCGATGGAAGGTTGG	456
	R- AATAAAATCAGCTGGAAAGTGTTG	
MupB	F-CTAGAAGTCGATTTTGGAGTAG	674
	R-AGTGTCTAAAATGATAAGACGATC	
qacA/B	F-GCAGAAAGTGCAGAGTTCG	361
	R-CCAGTCCAATCATGCCTG	
Smr	F-GCCATAAGTACTGAAGTTATTGGA	195
	R-GACTACGGTTGTTAAGACTAAACCT	

Table 2: The patients population and the clinical source of the isolated strains.

Variable	BLS	TDS	UDS
	No. = 115	No. = 81	No. = 76
Age (M ± SD in years)	59 ± 23	52 ± 19	61 ± 12
Gender (female/male)	60/55	58/23	50/26
Nationality (Sudi/Non-Saudi)	78/37	61/20	55/21
Hospital stay (M ± SD in days)	10 ± 5	12 ± 7	9 ± 6
The strain source, No.(%)			
Wound/burn	47(40.9%)	41(50.6%)	39(51.3%)
Blood stream	29(25.2%)	15(18.5%)	16(21.1%)
UTI	8(7.0%)	5(6.2%)	6(7.9%)
LRT	12(10.4%)	9(11.1%)	6(7.9%)
URT	4(3.7%)	4(4.9%)	1(1.3%)
Others	15(13.1%)	8(9.9%)	8(10.5%)

(20,000 x g) for 1 min. Primers and 2 µl of extracted DNA were added to PCR mixture and subjected to cycling conditions as described before [29]. The PCR primers sequences and the expected product sizes are shown in Table 1.

Results

A total of 272 MRSA strains were identified during the study period;115 isolates were collected before application of any of decolonization protocols and was termed baseline strains (BLS) group, 81 isolates were collected from the health care facilities that applied the targeted decolonization protocol and was termed Targeted Decolonization Strains (TDS) group and 76 isolates were collected from the facilities that applied the universal decolonization protocol and was termed Universal Decolonization Strains (UDS) group. All of the studied isolates were recovered from samples obtained from clinical infections that developed three days after hospitalization (the screening isolates recovered during targeted decolonization were excluded). Most of the MRSA isolates were collected from blood stream and wound infections in the three groups (Table 2). Mupirocin MIC results of 272 MRSA isolates revealed that the overall prevalence rate of LLMR was 5.5% with no significant differences between the three groups. HLMR ranged between 7.9% (in UDS group) to 11.1% (in TDS group) with no significant differences compared to BLS group (P = 0.574 and 0.845 respectively). Chlorhexidine resistance showed no significant change in the prevalence rates due to either types of decolonization compared to the base line prevalence rate (3.5% in BLS group, 2.5% in TDG group and 5.3% in UDS group) (Table 3). PCR results confirmed all phenotypically identified MRSA isolates (all of the isolates were positive for Staph-16S rRNA, nuc and mecA) (Figure 1). The mupA gene was found to be the main responsible for HLMR in the three study groups (7.8% in BLS group, 11.1% in TDS group and 6.6% in UDS group). While mupB was detected only in two strains, one in BLS group and the other in UDS group, both of the two strains were recovered from the same hospital. Also, qacA/B gene was the only detect for chlorhexidine resistance (2.6% in BLS group, 2.5% in TDS group and 3.9% in UDS group) as no smr gene was detected (Table 4).

Discussion

S. aureus is still accounting for a large percent of HAI at a rat higher than any other pathogen [34-36]. Furthermore, *S. aureus* as a prominent history in rapidly developing resistance to multiple antibiotic classes [36]. MRSA colonization was proven to be a major risk factor for infection as well as subsequent transmission. According

Table 3: The mupirocin and chlorhexidine MIC results of the isolated strains.

MIC results	BLS	TDS	UDS
	No. = 115	No. = 81	No. = 76
Mupirocin:			
- S:	99 (86.1%)	68(84.0%)	65(85.5%)
- LLMR:	6(5.2%)	4(4.9%)	5(6.6%)
- HLMR:	10(8.7%)	9(11.1%)	6(7.9%)
Chlorhexidine:			
- S:	111(96.5%)	79(97.5%)	72(94.7%)
- R:	4(3.5%)	2(2.5%)	4(5.3%)

Table 4: The positive PCR results of the investigated genes among the isolated strains.

The gene	BLS		TDS		UDS	
	No. = 115		No. = 81		No. = 76	
	No.	%	No.	%.	No.	%
Staph-16S rRNA	115	100%	81	100%	76	100%
Nuc	115	100%	81	100%	76	100%
mecA	115	100%	81	100%	76	100%
mupA	9	7.80%	9	11.10%	5	6.60%
mupB	1	0.90%	0	0%	1	1.30%
qacA/B	3	2.60%	2	2.50%	3	3.90%
Smr	0	0%	0	0%	0	0%

CDC recommendations, infection control committees worldwide started to implement MRSA decolonization strategies in addition to contact precautions [29]. Intranasal mupirocin application and chlorhexidine whole-body washing are the recommended practices for decolonization [30]. A major drawback that suspected to rise due this approach is the selection of resistance to mupirocin and chlorhexidine. So, screening for the increased rate of resistance to these agents is recommended [30].

In this study a multiplex assay was applied to test a total of 272 clinical staphylococcal isolates. The assay enabled the differentiation of *S. aureus*, MRSA and simultaneous identification of mupirocin and chlorhexidine resistance genes. The results of this study showed that the prevalence rate of MR among BLS group was considered moderate (5.2% LLMR and 8.7% HLMR) and this rate did not change significantly during the decolonization practice. A possible explanation for these findings may be the success-until now at least-of decolonization process to reduce the prevalence of MRSA sufficiently to prevent the selection of resistance. HLMR was found to be more prevalent the TDS group than in the UDS group (11.1% and 7.9% respectively) compared to the BSL group. This difference (although statistically non-significant i.e. p = 0.574) may be explained by the superior efficacy of UDS not only in MRSA decolonization but also decolonization of MSSA and CoNS. Both of MSSA and CoNS harbor mupA and mupB carrying plasmids that have the ability to transfer among different *staphylococcal* species [29]. These results are consistent with Hayden et al. [13] who studied the prevalence of MR after decolonization and reported that the baseline rate of LLMR was 7.1% while, the rate of HLMR was 7.5%. Other studies reported higher rates of MR (both LLMR and HLMR) after using of mupirocin for MRSA decolonization and suggested that mupirocin exposure is considered an important risk factor for HLMR colonization. Those studies claimed the spread of MR to its widespread and sustained use over long periods and also, to its extra-nasal use such as on vascular catheter exit sites and wounds [13,37,38].

The global distribution of chlorhexidine susceptibility varies [39]. In the present study, a relatively low prevalence of qacA/B and chlorhexidine resistance was detected. The difference of chlorhexidine resistance between BLS group and either of the two other study groups was statistically non-significant (p = 0.686 and 0.547 respectively). These results are in consistence with previously published studies that reported low rates of phenotypic and/or genotypic chlorhexidine resistance among MRSA isolates [39,40]. On the other hand, other studies reported a higher prevalence of chlorhexidine resistance and/or qacA/B gene among MRSA isolates. For example McNeil JC, et al., [41] reported that more than 22% of MRSA isolates carried qacA/B. Also, Warren and colleagues [42] identified a statistically significant increase in the annual prevalence of qacA/B among MRSA isolates from surgical ICU [42]. In the present study, the results showed a discrepancy between chlorhexidine phenotypic susceptibility testing and the presence of qacA/B gene among the studied isolates. Where, MIC results revealed 10 chlorhexidine resistant isolates (4 strains in the baseline group and 6 in the decolonization groups) while PCR results revealed 8 isolates carrying qacA/B (3 in the baseline group and 5 in the decolonization groups). This discrepancy may be due to the fact that qacA/B is not a specific predictor of chlorhexidine resistance. This resistance may be achieved by another efflux pump mediated by genes other than qacA/B. Also, Horner et al. [43] reported that only three out of five qacA/B-positive isolates were phenotypically susceptible to chlorhexidine [43]. The results of present study revealed a rare co-existence of mupirocin and chlorhexidine resistance. Only one strain was positive for both mupA and qacA/B genes. This co-existence represents a crucial risk factor for decolonization failure [17].These findings are in consistence with a previous study where the co-existence of chlorhexidine and mupirocin resistance among MRSA was not detected. Although the same study reported a significant occurrence of this co-existence among MSSA and CoNS isolates [29]. These results, together with other descriptions of decolonization failure due to reduced mupirocin and chlorhexidine susceptibility [44,45,46] justify the necessity of their susceptibility monitoring as a complementary part of decolonization programs. Two main limitations of this study should be mentioned. First, the small sample size that may affect the statistics analysis so it is difficult to be generalized. Second, the intervention period of decolonization program was still relatively short (14 months). So, repeating of this study on a larger number of isolates and after a longer period of intervention is recommended.

Conclusion

In Conclusion, MR was found to be moderate while chlorhexidine non-susceptibility and carriage of qacA/B were rare among MRSA isolates recovered from our health care facilities. Application of the decolonization strategies did not result in a significant change in the prevalence rates of either mupirocin or chlorhexidine resistances among MRSA isolates. However, these results should be interpreted with caution due to the fact that bacterial resistance may require longer period to develop and spread. So, periodic surveillance should be done in health care facilities that apply any of decolonization protocols.

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