

IJMA

INTERNATIONAL JOURNAL OF MEDICAL ARTS



Volume 3, Issue 3 [Summer (July-September) 2021]



J-Gate
INDEX

<http://ijma.journals.ekb.eg/>

Print ISSN: 2636-4174

Online ISSN: 2682-3780

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- ✓ International Journal of Medical Arts is the Official Journal of the Damietta Faculty of Medicine, Al-Azhar University, Egypt
- ✓ It is an International, Open Access, Double-blind, Peer-reviewed Journal
- ✓ Published four times a year
- ✓ The First Issue was published in July 2019
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Original Article

Detection of Biocides Resistance Genes among Clinical Isolates of Both Methicillin-Resistant and Methicillin-Sensitive Strains of *Staphylococcus Aureus*

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Submission date: February 08, 2021; Revision date: June 08, 2021; Acceptance date: June 11, 2021



DOAJ

DOI: [10.21608/IJMA.2021.62186.1260](https://doi.org/10.21608/IJMA.2021.62186.1260)

ABSTRACT

BACKGROUND: Biocides compounds are widely used in infection control programs, including hand washing and skin decolonization before invasive procedures. However, its overuse leads to the emergence of *Staphylococcus aureus* with decreased biocides susceptibility, which became a problem in hospitals in different parts of the world.

AIM OF THE WORK: The present work aims to study the susceptibility of methicillin-resistant isolates of staphylococci aureus, obtained from different clinical samples to commonly used biocides, determines the prevalence of the biocide resistance genes, *qacA/B*, and *qacC*, among these isolates.

METHODS: Biocide's resistance genes [*qacA/B*, *qacC*] in Both Methicillin-Resistant and Methicillin-Sensitive Strains of *Staphylococcus aureus* were detected by PCR method. The minimum inhibitory concentrations [MICs] of Biocide agents were determined by broth dilution method, whereas antibiotic susceptibility was analyzed by disk diffusion and MICs methods according to Clinical and Laboratory Standards Institute [CLSI] criteria.

RESULTS: The frequency of Biocides resistance genes [*qacA/B*, *qacC*] was found to be high in our clinical *staphylococci* isolates. The *qacA/B* gene was detected in [63.3%] of MRSA isolates compared with only [23.3%] of MSSA isolates. Simultaneously, the *qacC* gene was detected in [63.3%] of MRSA isolates compared to only [13.3 %] of MSSA isolates., with the significant difference between both groups [P<0.05]. We also found an association between the presence of antiseptic resistance genes *qacA/B*, and increased MIC values to both savlon, hypochlorite, and Phenol [P<0.05]. We also showed that an association between the presence of antiseptic resistance genes *qacC* and the MICs to both savlon, hypochlorite, and Phenol [P<0.05].

CONCLUSION: Our study detects biocide resistance genes [*qacA/B*, *qacC*] in *Staphylococcus aureus* isolates in Egypt; more studies are needed to correct the biocide uses and to support infection control programs to avoid the development of new resistance mechanisms.

Keywords: Resistance; Biocides; *Staphylococcus aureus*; Methicillin-Resistant; Biocides' resistance genes.

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Citation: Sabry W, Al-Dakhakhni MYS, Al-hoseinyIM, Tawab A. Detection of Biocides Resistance Genes among Clinical Isolates of Both Methicillin-Resistant and Methicillin-Sensitive Strains of *Staphylococcus Aureus*. IJMA 2021;3(3): 1602 -1610. [DOI: [10.21608/IJMA.2021.62186.1260](https://doi.org/10.21608/IJMA.2021.62186.1260)].

* Main subject and any subcategories have been classified according to the research topic.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* [MRSA] is an important nosocomial pathogen infecting individual in hospitals worldwide [1]. Many studies detect the important role of *staphylococci aureus* in disease in immune-compromised patients and the increased prevalence of multidrug-resistant strain, the emergence and rise of antibiotic resistance among *staphylococci aureus* is a burden in health care facilities and communities [2]

Biocides are chemical agents applied to inactivate or destroy microorganisms in different sites. Large amounts of biocides are used within different sites, including the medical environment used for disinfection, antiseptics, and cleaning. A wide variety of biocidal agents are commonly used in hospitals and healthcare facilities to decontaminate surfaces, disinfect the hands of health care personnel, and treat hospital-acquired infections [3].

The worldwide increase in antimicrobial resistance in bacterial, leading to increased mortality and morbidity in humans, highlights the importance of infection control practices and the key role of healthcare biocides. However, the widespread use of biocides in hospitals has led to the emergence of disinfectant resistant bacteria. Excessive use may also result in the appearance of cross-resistance between widely used biocides and antibiotics [4].

Epidemiological data on antiseptic susceptibility and the Distribution of resistance genes are useful for nosocomial infection control. Multidrug efflux systems are possibly the most described resistance mechanism regarding acquired resistance to biocides. Antiseptic resistance genes, *qacA/B*, *qacC* have been identified in *Staphylococcus species* [5].

The *qacA/B*, *qacC* are found mainly in *staphylococci aureus* clinical isolates and could be responsible for reduced susceptibility to certain antiseptic agents. The incidence rate of MRSA in our hospital has increased to more than 80% of clinical isolates, and the prevalence of methicillin-resistant coagulase-negative *staphylococci* is also rising [6].

AIM OF THE WORK

The present work aims to study the susceptibility of methicillin-resistant isolates of *staphylococci Aureus*, obtained from different clinical samples, commonly used biocides [antiseptic and disinfectant agents] to determine the prevalence of the biocide resistance genes, *qacA/B*, and *qacC* among these isolates.

PATIENTS AND METHODS

The present study was conducted on 600 strains isolated from hospital admitted patients, healthcare workers [HCWs], and environmental samples from different Clinical Departments of Al-Azhar University Hospital, Damietta Faculty of Medicine. From which 60 isolates of methicillin-resistant *staphylococci Aureus* [MRSA] as well as 60 isolates of methicillin-sensitive *staphylococci Aureus* [MSSA] were taken to study the susceptibility to commonly used biocides and to determine the prevalence of the biocide resistance genes, *qacA/B*, and *qacC*, among these isolates. This work was conducted at the Microbiology Department of Damietta Faculty of Medicine from March 2019 to Jul 2020.

1- Data collection

Special questionnaires were prepared, including sex, age, health status, work type, medical history of the patients. Other data were also recorded for each HCWs. The samples were obtained from admitted patient's samples, Healthcare workers [HCWs] and environmental samples. Then, each sample was undergone to the following:

I- Isolation and identification of *staphylococci aureus* by the following:

[A] Direct Smear: Gram-stained smears: Typical *staphylococci* appear as gram-positive cocci in clusters.

[B] Culture and confirming the result:

- by inoculating them on blood agar and mannitol salt agar plates, given rise to typical colonies after 18-24 hours at 37°C in aerobic incubation [7].

- Colonies suspected to be *staphylococci aureus* were confirmed by gram-stain, catalase, slide and tube coagulase, and deoxyribonuclease [DNase] tests [7]

II- Detection of Methicillin Resistance:

A] Determination of the susceptibility to Oxacillin and vancomycin antibiotics by the disc diffusion and minimal inhibitory concentration [MIC] methods: according to the recommendations of the [CLSI,2010, CLSI 2020] [8].

B] Detection of penicillin-binding protein 2a by the SlidexR MRSA detection: MRSA isolates were tested for the presence of [PBP2a] [product of the *mecA* gene] by the Slidex®MRSA agglutination test [9].

III-Detection of *staphylococci Aureus* Susceptibility of both [MRSA] and [MSSA] isolates to Biocides by the Broth Dilution Method. The procedure was done by the serial two-fold broth dilution method as described by Mazzola *et al.* [10]. Biocides tested in the present study were selected from the chemical agents used to disinfect patient care items and instruments in the hospital. They were glutaraldehyde or cidex [2.0% w/v] alkalized with sodium bicarbonate [pH= 8.3] supplied by Johnson and Johnson's; Sodium hypochlorite [10% w/v] [pH 7.0] supplied by Elnasr Chemical Co.; Chlorhexidine digluconate [0.3%]-Cetrimide [3%] mixture [savlon] supplied by Cairo Pharma; aqueous polyvinyl-pyrrolidone-iodine [10% w/v, PVP-I₂] solution supplied by Nile Co.; and Phenol [10% w/v] solution supplied by El-Nasr Chemical Co. The MIC for each biocide was identified as the minimum concentration at which there was no visible growth.

IV- Detection of Biocides Resistance Genes by Polymerase Chain Reaction [PCR]: The *qacA/B*, *qacC*, resistance genes were detected in all isolates [both MRSA and MSSA isolates showed biocides resistance] by multiplex PCR according to Ignak *et al.* [2017] [11].

VI-Statistical analysis: Data was tabulated and analyzed using the Statistical Package for Social Sciences [SPSS] software, version 27 (IBM® SPSS® Inc., Armonk, USA). Data were presented as frequencies, with [P values <0.05] considered statistically significant.

RESULTS

The present study showed that the incidences of *staphylococci aureus* strain isolated from different samples of the study patients, HCWs and environmental strains isolated were 350 out of 600 [58.3%] among all isolated strains, 269 out of 400 strains [67.2%] were isolated from patients and 48 out of 100 strains [48%] were isolated from HCWs. In comparison, 33 out of 100 [33%] were isolated from environmental samples, and the incidence of *staphylococci aureus* in patients was 269 out of 400 [67.2%], 53 out of 400 [13.2%] was MRSA, and 217 out of 400 [54.2%] of them was MSSA. The incidence of *staphylococci aureus* isolates in HCWs was 48 out of 100 [48%], of them 7 out of 100 [7%] was MRSA, and 41 out of 100 [41%] of them was MSSA. The incidence of *staphylococci aureus* in environmental samples was 33 out of 100 [33%], all of them. And there was no significant difference between the three different methods, disc diffusion, MIC, and PBP2a production in patients, HCW, and environmental samples.

Comparison between MRSA and MSSA isolates regarding the MIC to the different studied biocides shows a highly significant difference between both MRSA and MSSA

isolates as regards the MICs to betadine [PVP-I₂], Savlon, hypochlorite, and Phenol [P<0.05]. However, no significant difference was found between both groups as regards the MICs of the glutaraldehyde [Cadex] [P>0.05] [Table 1]. The Comparison between MRSA and MSSA isolates as regards the detection of biocides resistance genes [*qacA/B* and *qacC*] shows; the *qacA/B* gene was detected in 38 out of 60 [63.3%] of MRSA compared with only 14 out of 60 [23.3%] of MSSA isolates. And the *qacC* gene was detected in 38 out of 60 [63.3%] of MRSA and only in 8 out of 60 [13.3 %] of MSSA isolates. There was a highly significant association between both [P<0.05]. By the results of the PCR test, all isolates were classified into 4 groups: Group 1: Included 18 isolates [were +ve to *qacA/B* only]. Group 2: Included 12 isolates [were +ve to *qacC* only]. Group 3: Included 56 isolates [were -ve to both *qacA/B* and *qacC*]. Group 4: Included 34 isolates [were +ve to both *qacA/B* and *qacC*].

The comparison between group1 [*qacA/B* +ve] and group 3[*qacA/B* -ve and *qacC* -ve] as regards the MIC to the different studied biocides shows, there was no significant difference between group1 [*qacA/B* +ve] and group 3 [*qacA/B* -ve and *qacC* -ve] as regards the MIC to betadine [PVP-I₂] and glutaraldehyde [Cidex] [P>0.05]. However, there was an association between both groups regarding the MICs to savlon, hypochlorite, and Phenol [P<0.05] [Table 2]. The comparison between group2 [*qacC* +ve] and group 3 [*qacA/B* -ve and *qacC* -ve] as regards the MIC to the different studied biocides shows, there was no significant difference between group2 [*qacC* +ve] and group 3 [*qacA/B* -ve and *qacC* -ve] as regards the MICs to betadine [PVP-I₂] and glutaraldehyde [Cidex] [P>0.05]. However, a highly significant difference between both groups was detected regarding the MICs to savlon, hypochlorite, and Phenol [P<0.05] [Table3]. The comparison between group 4 [*qacA/B* +ve, *qacC* +ve] and group 3 [*qacA/B* -ve and *qacC* -ve] as regards the MIC to the different studied biocides shows, there was no significant difference between group 4 [*qacA/B* +ve, *qacC* +ve] and group 3 [*qacA/B* -ve and *qacC* -ve] as regards the MIC to glutaraldehyde [Cidex] [P>0.05]. However, a highly significant difference between both groups was detected as regards the MICs to betadine [PVP-I₂], savlon, hypochlorite, and Phenol [P<0.05] [Table 4]. Comparing group1 [*qacA/B* +ve] and group 2 [*qacC* +ve] regarding the MIC to the different studied biocides, shows there was no significant difference between group1 [*qacA/B* +ve], and group 2 [*qacC* +ve] as regards the MICs to betadine [PVP-I₂], hypochlorite, and phenol [P>0.05]. However, there was a significant difference between both groups regarding the MICs to savlon [P<0.05]. However, a significant difference between both groups was detected regarding the MICs to savlon [P<0.05] [Table 5].

Table [1]: Comparison between MRSA and MSSA isolates regarding the MIC to the different studied biocides.

BIOCIDES	CONC	Sensitive isolates		P VALUE [SIG.]
		MRSA	MSSA	
Betadine [PVP-I ₂]	0.625	0	2	<0.05 [S]
	1.25	8	34	
	2.5	52	24	
Cidex	0.250	0	2	>0.05 [NS]
	0.500	60	56	
	1.000	0	2	
Savlon	0.051	0	46	<0.05 [S]
	1.65	34	8	
	3.30	26	6	
Hypochlorite	0.206	0	46	<0.05 [S]
	0.412	2	0	
	0.825	38	12	
	1.65	6	2	
	3.30	14	0	
Phenol	0.078	0	46	<0.05 [S]
	0.312	2	0	
	0.625	16	0	
	1.25	30	6	
	2.50	12	8	

Table [2]: Comparison between group1 [qacA/B +ve] and group 3[qacA/B -ve and qacC -ve] as regards the MIC to the different studied biocides.

Biocides.	Conc	Sensitive isolates		P-value [Sig.]
		Group 3 [AB-ve/C-ve]	Group 1 [AB+ve/C-ve]	
Betadine [PVP-I ₂]	0.625	2	0	>0.05 [NS]
	1.25	32	6	
	2.5	22	12	
Cidex	0.250	2	0	>0.05 [NS]
	0.500	52	18	
	1.000	2	0	
Savlon	0.051	46	0	<0.05 [S]
	1.65	6	8	
	3.30	4	10	
Hypochlorite	0.206	46	0	<0.05 [S]
	0.825	8	10	
	1.65	0	4	
	3.30	2	4	
Phenol	0.078	46	0	<0.05 [S]
	0.625	0	2	
	1.25	0	12	
	2.50	10	4	

Table [3]: Comparison between group2 [qacC +ve] and group 3 [qacA/B -ve and qacC -ve] as regards the MIC to the different studied biocides.

Biocides	Conc	Sensitive isolates		P-value [Sig.]
		Group 3 [AB-ve/C-ve]	Group 2 [AB-ve/C+ve]	
Betadine [PVP-I ₂]	0.625	2	0	>0.05 [NS]
	1.25	32	2	
	2.5	22	10	
Cidex	0.250	2	0	>0.05 [NS]
	0.500	52	12	
	1.000	2	0	
Savlon	0.051	46	0	<0.05 [HS]
	1.65	6	12	
	3.30	4	0	
Hypochlorite	0.206	46	0	<0.05 [HS]
	0.412	0	2	
	0.825	8	10	
	3.30	2	0	
Phenol	0.078	46	0	<0.05 [HS]
	0.625	0	6	
	1.25	0	6	
	2.50	10	0	

NS = non-significant; HS = highly significant

Table [4]: Comparison between group 4 [qacA/B +ve, qacC +ve] and group 3 [qacA/B -ve and qacC -ve] as regards the MIC to the different studied biocides.

Biocides	Conc	Sensitive isolates		P-value [Sig.]
		Group 3 [AB-ve/C-ve]	Group 4 [AB+ve/C+ve]	
Betadine [PVP-I ₂]	0.625	2	0	<0.05 [HS]
	1.25	32	2	
	2.5	22	32	
Cidex	0.250	2	0	>0.05 [NS]
	0.500	54	34	
	1.000	2	0	
Savlon	0.051	46	0	<0.05 [HS]
	1.65	6	16	
	3.30	4	18	
Hypochlorite	0.206	46	0	<0.05 [HS]
	0.825	8	22	
	1.65	0	4	
	3.30	2	8	
Phenol	0.078	46	0	<0.05 [HS]
	0.312	0	2	
	0.625	0	8	
	1.25	0	18	
	2.50	10	6	

NS=non-significant; HS = highly significant

Table [5]: Comparison between group1 [qacA/B +ve] and group 2 [qacC +ve] as regards the MIC to the different studied biocides.

Biocides	Conc	Sensitive isolates		P-value [sig.]
		Group 1 [Ve+ AB/C-ve]	Group 2 [Ve-AB/C+ve]	
betadine [PVP-I ₂]	1.25	6	2	>0.05 [NS]
	2.5	12	10	
Savlon	1.65	8	12	<0.05 [S]
	3.30	10	0	
Hypochlorite	0.412	0	2	>0.05 [NS]
	0.825	10	10	
	1.65	4	0	
	3.30	4	0	
Phenol	0.625	2	6	>0.05 [NS]
	1.25	12	6	
	2.50	4	0	

NS=non-significant; S = significant

DISCUSSION

The present work aimed to study the susceptibility of methicillin-resistant *Staphylococcus aureus*, MRSA, and MSSA strains, obtained from different samples, to commonly used biocides and detect the presence of the biocide's resistance genes, qacA/B and qacC, among these isolates. The present study showed that all incidences of *staphylococci aureus* strain isolated from different samples of the study patients, HCWs and environmental strains isolated were 350 out of 600 [58.3%] among all isolated strains, 269 out of 400 strains [67.2%] were isolated from patients and 48 out 100 strains [48%] were isolated from HCWs, while 33 out of 100 [33%] were isolated from environmental samples. Our results demonstrated that a difference of the incidence of *staphylococci aureus* isolated from different samples of the study, being [67.2%] as regard to patients, and [48%] as regard to HCWs, and [33%] as regard to environmental samples, with statistically significant difference. In the study conducted between August 2011 and June 2012, at Al- Kadhamia teaching Hospital and AlNuman hospital, Baghdad, Iraq [12]. One hundred and six *staphylococci aureus* were isolated from

patients and Healthcare workers. The incidence of *staphylococci aureus* in this study was 42.4%. which was lower than our results [58.3%]. Other study conducted at Al-Zahraa University Hospital included admitted 300 female patients to identify the causative organism[s] responsible for surgical site infection following Cesarean section. The most common organism isolated was *staphylococci aureus* 31/300 [10.3%] [13], which was lower than our results [58.3%].

In our study, the *staphylococci aureus* isolated strains were 350 out of 600 [58.3%]. This result compared to those obtained by [14] with *staphylococci aureus* percentages of 31.27%. The high rates of *staphylococci aureus* contamination in our study are likely to reflect both poor hand hygiene practices and compromised hygienic measures and increased duration of hospital stay.

As regard to patients' group, MRSA were isolated 53 out of 400 [13.2%] compared 216 out of 400 [54%] of MSSA. While HCWs group MRSA strains were isolated was 7 out of 100 [7%] compared to 41 out of 100 [41%] of MSSA, and environmental group, all environmental isolates 33 out of

100 [33%] were MSSA. There was a significant difference between both MRSA and MSSA isolates in the three groups. In the study conducted in north Jordan, the incidence of *staphylococci aureus* in patients were [19.8%], [5.8%] were found to be MRSA [15]. There is a difference in the incidence of both *staphylococci aureus* [19.8%], and MRSA [5.8%] between this study and our study. In our study a higher incidence of both *staphylococci aureus* [67.2%], and MRSA [13.2%], with statistically significant difference. In another study, conducted at Fahd hospital, Al-Madinah Al-Munawarah, KSA on patients, [HCWs] and environment, samples isolated from 117 patients, 25 HCWs and 12 environmental sites [16]. The rate of MRSA was 5.98 % [7/117] in patients. which was lower than our results [13.2%], the rate of MRSA was 36% [9/25] in HCWs which was higher than our results [7%]. Two MRSA strains were isolated from the 12 environmental samples compared to no environmental MRSA in our results. In another trial was undertaken between August 2011 and June 2012, at Al-Kadhamia teaching Hospital and Al-Numan hospital, Baghdad, Iraq [11]. One hundred and six *staphylococci aureus* were isolated from patients and Healthcare workers. The incidence of *staphylococci aureus* in this study was 42.4%. which was lower than our results [58.3%]. The rate of MRSA in patients was 97 % which was higher than our results [13.2%], the rate of MRSA in HCWs was 82%% which was higher than our results [7%], which confirm the higher incidence rate of MRSA in both patients and HCWs in this study thank our study.

In another study was conducted at MNH in Dar es Salaam, Tanzania, between May and June 2018, on environmental samples, the results of this study showed that *staphylococci aureus* incidence was [20%] from environmental samples, which was lower than our results [33%], these results including 19.5% for MRSA, compared with our results [0%] for MRSA [17].

Another study was conducted at Al Zahraa University Hospital on 300 females admitted patients to identify the most causative organism[s] responsible for surgical site infection after Cesarean section. The most common organism isolated was *staphylococci aureus* [10.3%] [13], which was lower than our results [58.3%].

Staphylococci aureus isolates were then identified according to sensitivity to oxacillin, revealing 21 isolates were MRSA 21/300 [7%] among isolates of *staphylococci aureus* strains. The rate of MRSA was 7 % in patients, which was lower than our results [13.2%], and from [HCWs] & environment collected swabs, [26%] were positive on culture, 18 isolates [5.6%] were obtained from HCWs. 65 isolates were isolated from environmental samples [20 %].

The most common organism isolated from both sources [clinical & environmental specimens] was [MRSA] isolates [5.6%] [13], which was lower than our results in both [HCWs] 7 out of 100 [7%] and & environmental samples [0%].

Other study conducted at Al-Azhar University Hospital, Damietta branch. The results obtained in Egyptian study by Alaa Eldeen *et al.* [18] revealed MRSA nasal carriage rate of [2.3%] among medical residents in Gynecology department which is lower than our study results about MRSA isolates in [HCWs] [7%]. Our study result is higher than the study result conducted by Siegel *et al.* [19], who reported an MRSA colonization rate of 2.1% in the nasal cavity and axilla of HCWs.

The high rates of MRSA contamination in [HCWs] are likely to be a source of infection and transmission of pathogens within the hospital and remains a hazard for hospitalized patients. Also, cross-transmission of microorganisms from patients and the environment may have an important role.

The high MRSA contamination rates reflect both poor hand hygiene practices and compromised hygienic measures. Increased duration of stay in hospitals. The difference in MRSA rates in different studies might be due to the difference in locations and periods of the studies, the difference in hygienic conditions maintained in different hospitals, the hospital's healthcare facilities, and the implementation of the program for infection control, and rational use of antibiotics.

As regarding the MIC of both [MRSA and MSSA] isolates to the different studied biocides, both MRSA and MSSA isolates were effectively inhibited by the user's concentrations of betadine [PVP-I₂] [2.5%], glutaraldehyde [0.5%], Savlon [0.825%], hypochlorite [3.3%] and Phenol [1.25%]. However, the MRSA isolates had statistically higher MICs to betadine [PVP-I₂] [$P < 0.05$] as compared to MSSA isolates. This finding goes in agreement with Zhang *et al.* [20], who also found that MRSA isolates had increased MIC to povidone-iodine [betadine] as compared to the MSSA isolates. By contrast, Bamber and Neal [21], reported no differences between the sensitivity of MRSA and MSSA to betadine. Also, a highly significant difference was observed between both [MRSA and MSSA] regarding the MICs to Savlon [$P < 0.05$]. This finding goes in accordance with the previous finding of Irizarry *et al.* [22], who also found the increased value of MICs of MRSA strains for some biocides, including cetrimide and chlorhexidine. Also, Wootton *et al.* [23] observed that the MICs for QACs for MRSA were 1.5-3-fold higher than those for MSSA. However, Al-Masaudi *et al.* [24] found that there was no

important difference between MRSA and MSSA regarding their susceptibility to savlon.

Unlike the results by Al-Masaudi *et al.* [24] who found that both MRSA and MSSA are equally sensitive to phenols, a highly significant difference in the present study, between the MRSA and MSSA isolates regarding the MICs to Phenol [$P < 0.05$] was observed.

Regarding hypochlorite sensitivity in the present work, the MRSA isolates had significantly higher MICs than the MSSA isolates [$P < 0.05$]. The MRSA isolates MIC value exceeded the user-defined concentrations [MIC = 0.825]. This finding supports the finding of Mitchell *et al.* [25], who reported an increased MIC for MRSA than MSSA isolates to hypochlorite.

In our study, no significant difference was reported between both MRSA and MSSA isolates regarding the MICs of the glutaraldehyde and glutaraldehyde concentration as low as 0.5% effectively inhibited all isolates. This finding is in accordance with the findings of Reynaldo *et al.* [26] who reported that MRSA isolates are sensitive to 2% glutaraldehyde as MSSA. Again, Kheiralla *et al.* [27] noted, the highest concentration of glutaraldehyde [2%] was the most concentration effective against the MRSA isolates. Also, the authors noted that the 0.5% concentration was more effective on the MRSA isolates than the concentration of 1%.

The studied MRSA isolates were sensitive to the most used disinfectants/ antiseptics at the user's defined concentrations, and there was an important difference between the MICs of MRSA isolates as compared to the MSSA isolates. This is important as it may mean that certain isolates have an ability to survive at lower concentrations of biocides and that the use of biocides may act as a selective pressure to allow these isolates to predominate.

As regards the presence of biocides resistance genes [qacA/B and qacC] in both MRSA and MSSA isolates. In the current work, the qacA/B gene was present in 43.3% of the studied *staphylococci aureus* isolates. There was a significant difference between the MRSA isolates and the MSSA isolates regarding the presence of the qacA/B gene since the gene was presented in 63.3% of MRSA isolates and in 23.3% of MSSA isolates [$P < 0.05$]. This goes in agreement with the study of Mayer *et al.* [28], who detected the qacA/B genes in 42% of their studied *staphylococci aureus* strains were isolated between 1997 and 1999 from 24 different European university hospitals. The authors noticed significantly more MRSA than MSSA isolates possessed the qacA/B genes [63% versus 12%,

respectively]. Also, Taheri *et al.* [29] detected the qacA/B gene in 76.46% of their studied *staphylococci aureus* strains isolates. The authors noticed that significantly more MRSA than MSSA isolates possessed the qacA/B genes [63.3% versus 25.9%, respectively].

Also, Noguchi *et al.* [30] detected the qacA/B gene in 41.6% of MRSA strains isolated from different Asian countries between 1998 and 1999. Later, Sekiguchi *et al.* [31] reported a higher detection rate of the qacA/B gene [52.3%] among their studied MRSA isolates from a Tokyo hospital. Furthermore, Noguchi *et al.* [30] detected the [qacA/B] gene in 45.9% of MRSA isolates cultured from patients. A much higher rate was reported by Miyazaki *et al.* [32], who found the [qacA/B] gene in 80% of MRSA strains isolated in Brazil. Also, Conceicao *et al.* [33] detected the qacA/B gene among 40.5% of MRSA strains isolates. Also, Wang *et al.* [34] detected the [qacA/B] gene among 61.1% of MRSA strains isolated in China.

On the other side, Vali *et al.* [35] detected the [qacA/B] gene in only 10% of their studied isolated MRSA from hospitals in Edinburgh. Also, Ignak *et al.* [11] detected the [qacA/B] gene among 10.3% of MRSA isolates.

In the present work, the qacC gene was presented in 38.3% of the studied *staphylococci aureus* isolates. The gene was presented in 63.3% of the MRSA strains isolates and in 13.3% of the MSSA isolates [$P < 0.05$]. These results are much higher than those reported by Alam *et al.* [36] and Noguchi *et al.* [29], who reported the qacC gene in 3.3% and 2.5% only of their studied MRSA isolates, respectively. Also, our results are much higher than those reported by Conceicao *et al.* [33], and Taheri *et al.* [29], who detected the qacC gene in 3.7%, and 6.7% only of their studied MRSA isolates, respectively. However, Vali *et al.* [35] noted that the qacC gene was more spread [44.2%] than the [qacA/B] gene [10%] among their studied MRSA strains isolated from hospitals in Edinburgh. Also, Ignak *et al.* [11] noted that the qacC gene was more spread [13.8%] than the [qacA/B] gene [10.3%] among their studied MRSA strains isolated from hospitals. The high spreading of these genes is likely to be due to selective pressure by the various disinfection agents used in hospitals. Moreover, it is noted that more MRSA than MSSA isolates was positive for the [qacA/B] gene. This may be due to [the location of the qacA/B genes on multi-resistance plasmids like the pSK qacA] and the β -lactamase/heavy metal resistance families], such as pSK23. These plasmids harbor a few determinants responsible for resistance to a range of antimicrobial agents. This finding plays an important role in qacA/B genes in biocide resistance.

In the present trial, a highly significant difference was

found between group 1 [qacA/B +ve] and group 3 [qacA/B and qacC -ve] regarding the MICs to savlon, hypochlorite, and Phenol. Yet, no significant difference was observed between both groups regarding the susceptibility to betadine [PVP-I₂] and glutaraldehyde. Behr *et al.* [37] also observed a significant difference between these isolates that carry qacA/B and those that do not carry qacA/B regarding MICs of QACs and chlorhexidine.

In our study, a highly significant difference was found between group 2 [qacC +ve] and group 3 [qacA/B -ve and qacC -ve] regarding the MICs to savlon, hypochlorite, and Phenol. Yet, no significant difference was detected between both groups regarding to the susceptibility to betadine [PVP-I₂] and glutaraldehyde. Similarly, Mycock *et al.* [35] reported the increased MICs for isolates carrying the qacC gene of biocides, including chlorhexidine, cetrimide, benzalkonium chloride, and hypochlorite. Also, Sekiguchi *et al.* [31] reported a significant difference between the isolated strains that carry the qacC gene and those that do not carry the qacC gene regarding the MICs of QACs.

In the current study, a highly significant difference was found between group 4 [qacA/B +ve, qacC +ve] and group 3 [qacA/B-ve and qacC -ve] regarding the MICs to betadine [PVP-I₂], savlon, hypochlorite and Phenol. In agreement with Al-Masaudi *et al.* [24] who reported increased MICs against plasmid-carrying MRSA strains for biocides including chlorhexidine, cetrimide, benzalkonium chloride, hypochlorite, and betadine. In addition, a significance difference was found between group1 [qacA/B +ve] and group 2 [qacC +ve] regarding the MICs to savlon with the qacA/B +ve group being more resistant than the qacC +ve group to savlon. No significant difference was detected between both groups regarding their susceptibility to betadine [PVP-I₂], glutaraldehyde, hypochlorite and phenol. This finding goes in accordance with Russell *et al.* [39] who reported that the qacA/B gene specifies resistance to chlorhexidine and QACs whereas the qacC gene carry resistance to some QACs as previously reported by Leelaporn *et al.* [40]

From the current study we concluded that although the studied MRSA isolates strains were susceptible to most of the commonly used disinfectants/antiseptics at the user's defined concentrations, there was a significant difference between the MICs of MRSA isolates strains as compared to the MSSA isolates. This is of importance as it may mean that certain isolates will have an ability to survive at lower concentrations of biocides, the use of biocides act as a selective pressure to allow these isolates to predominate. Thus, we recommend eradicating MRSA colonization in both patients and HCWs to prevent its spread to the

community. Further studies on larger number of isolates to confirm our findings. Other studies on other multi-drug resistant organisms particularly gram-negative organisms are also recommended.

Financial and Non-financial Relationships and Activities of Interest

None

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