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ORIGINAL ARTICLE

Cross-resistance between Antibiotics and Disinfectants in Staphylococcus *aureus*

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ABSTRACT

Background: *Disinfectants play an important role in the control of nosocomial infections. Appearance of MRSA resistant to disinfectants used in hospitals is alarming.* ***Objectives:*** *We aimed to detect cross resistance between antibiotics and disinfectants among MRSA and MSSA, and to compare the efficacy of three disinfectants; (Clorox®, ULTRASOL AF ®0.25%, Virusolve +® 0.5%) against MRSA and MSSA.* ***Methodology:*** *Twenty-five MSSA and 25 MRSA isolates were collected from Cairo University Hospital laboratories Antimicrobial susceptibility pattern was compared, and susceptibility to the three mentioned disinfectants was carried out.* ***Results:*** *With Clorox, 44% of MRSA isolates versus 68% of MSSA isolates showed no growth. With Ultrasol AF and Virosulve+ 36% of MRSA isolates showed no growth with both agents in comparison to 56% and 44% of MSSA isolates respectively.* ***Conclusions:*** *No relationship between antibiotic resistance and non-susceptibility to disinfectants was found. Both hypochlorite and QACs couldn't completely eradicate MRSA and MSSA isolates tested.*

INTRODUCTION

Hospital-acquired infections (HAIs) represent a main problem throughout the world associated with increased morbidity, mortality and horrible health care costs[1].

Disinfectants represent an essential part of any infection control program and aid in the prevention of healthcare associated infections (HAI) [2]. However, researchers have raised concerns by suggesting that disinfectant overuse may induce mutations in microorganisms leading to cross-resistance between disinfectants and antibiotics [3]. Moreover, a disinfectant is almost never 100% effective due to the resistance of some bacteria to certain chemical compounds and due to inadequate cleaning protocols. When such a disinfectant is removed, the surviving bacterial population can regrow [4].

Several points have to be concerned when choosing a disinfectant, as its efficiency, compliance with regulations, infection rates, the types of surfaces to be disinfected and the virulence of the microorganisms circulating [5].

Disinfectants used in healthcare facilities are many, and are used either alone or in combinations. These include chlorine compounds, quaternary ammonium compounds hydrogen peroxide and phenolics [6].

In spite of our knowledge to risk factors, prevention and control measures, the incidence of HAI has not decreased and outbreaks caused by multidrug-resistant pathogens are still seen[7].

This is probably due to environmental contamination that plays a major role in transmission of infection through many bacterial pathogens [8]. Such microorganisms are resistant to most of the available antibiotics and to many disinfectants [9]. Among those superbugs comes *Staphylococcus aureus*, in particular methicillin-resistant *S. aureus* (MRSA). While strains of methicillin -susceptible *S. aureus* (MSSA) are generally susceptible to commonly used hospital disinfectants, some MRSA strains have been reported to have reduced susceptibility to chlorhexidine, quaternary ammonium compounds (QACs) and cetrimide [10].

Available data show that decreased susceptibility of MRSA strains to QACs may be due to *qac* gene determinants, which confer resistance through efflux pumps [11]. These genes predominate in staphylococci, in which the *qac*A/B genes were most frequently reported followed by the *qac*C/D genes [12,13]. More seriously, it has been demonstrated that these genetic determinants are carried on plasmids that harbour various antibiotic resistance genes (pSK1 and β-lactamase/heavy metal resistance plasmids) [14].

The aim of the present work was to detect cross resistance between antibiotics and disinfectants among MRSA and MSSA isolates, as well as to compare the efficacy of three disinfectants; one chlorine containing compound and two QACs available in the Egyptian market (Clorox®, ULTRASOL AF ®0.25% , Virusolve +® 0.5%) against MRSA and MSSA.

METHODOLOGY

Collection and Identification of *Staphylococcus aureus* isolates:

The study included a total number of fifty (50) *Staph aureus* isolates divided into 25 MSSA isolates and 25 MRSA isolates. All isolates were collected from Cairo University Hospital Laboratories between January and May 2015 from different clinical specimens including urine, blood, pus and sputum. Isolates were identified by conventional bacteriological methods

Antimicrobial susceptibility testing:

Antimicrobial susceptibility profile of the 50 collected isolates was performed by Kirby- Bauer disk diffusion method using commercial antibiotic discs (Oxoid, UK). Cefoxitin disk was used to differentiate between MRSA and MSSA isolates Other discs used included penicillin, erythromycin, clindamycin, ciprofloxacin, doxycycline, chloramphenicol, tetracycline, gentamycin, cotrimoxazole, rifampin and linezolid. Results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute [15].

Testing the effect of the disinfectants on *Staphylococcus aureus* isolates:

Three disinfectants were used in the present study; sodium hypochlorite solution (5 %) (Clorox, Egypt), QAC (ULTRASOL AF 0.25%, DR.SCHUMACHER GMBH, Germany) and a novel QAC (Virusolve + 0.5%, Amity, England).

The concentrations and contact times chosen in this study were based upon manufacturers' recommendations for each disinfectant. (Table 1).

The method used in this study for testing the efficacy of the selected disinfectants was based on the suspension tests for disinfectants [16,17]. The work was conducted on three successive days.

On day 1; each bacterial isolate was grown on blood agar plates and incubated at 37°C overnight. On day 2, a 0.5 McFarland bacterial suspension (1.5×10^8 cfu/*mL*)] of each bacterial isolate was prepared in broth and disinfectants were freshly prepared according to dilutions mentioned in table 1. In a sterile micro-titre plate, 100µL from the prepared disinfectant was dispensed in a well of the plate. Next, 10µL of the bacterial suspension was inoculated into each disinfectant and contents were mixed to ensure exposure of the bacterial cells to the disinfectants. The bacterial suspensions were exposed to the disinfectants for the chosen contact time after which, 5µL of human albumin was mixed with each disinfectant/bacterial suspension mixture to neutralize the remaining disinfectant. Finally, 10µL from each well was inoculated and spread onto fresh blood agar and incubated at 37°C overnight. A positive control (bacterial suspension in broth) and negative control (diluted disinfectant only) wells were included for every test run to demonstrate adequate microbial growth over the course of the incubation period and media sterility. On day 3, all plates were examined for surviving bacteria. The bacteria was identified using colony morphology and Gram stained film. Results were recorded as presence of growth or no growth.

Table 1: Contact time and concentration for the disinfectants:

Disinfectant	Concentration	Contact time
1.Clorox	500ppm 10 ml per liter.	15 minutes
2.Ultrasol AF	0.25% 2.5 ml per liter	15 minutes
3. Virusolve +	5 ml per liter	10 minutes

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (SD), median and range. Comparison of numerical variables between the study groups was done using Kruskal Wallis test with posthoc multiple 2-group comparisons. *p* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

A total of 50 *Staphylococcus aureus* stains were isolated from different clinical specimens as shown in (table 2).

Table 2: Types of specimens from which isolates were collected:

Specimen type	MRSA	MSSA	Total
Wound infections	17 (68%)	17(68%)	34 (68%)
Chest infections	5 (20%)	4 (16%)	9 (18%)
BSI	3 (12%)	2 (8%)	5 (10%)
UTI	0 (0%)	2 (8%)	2 (4%)
Total	25	25	50

Antimicrobial susceptibility pattern of *S.aureus* isolates:

For MRSA isolates: All isolates were resistant to cefoxitin while 22/25 (88%) were sensitive to linezolid followed by 17/25 (68%) sensitive to doxycycline, 14/25 (56%) sensitive to cotrimoxazole, 13/25 (52%) sensitive to chloramphenicol, 11/25 (44%) sensitive to rifampin, 8/25 (32%) sensitive to clindamycin, 7/25 (28%) sensitive to erythromycin, 6/25 (24%) sensitive to tetracycline, 5/25 (20%) sensitive to ciprofloxacin, and finally 4/25 (16%) sensitive to gentamycin.

For MSSA isolates: All isolates were sensitive to cefoxitin and to chloramphenicol, rifampin, ciprofloxacin and linezolid followed by 24/25(96%) sensitive to clindamycin and doxycycline, then 21/25(84%) sensitive to erythromycin and cotrimoxazole, and 18/25 (72%) sensitive to gentamycin and tetracycline. However, only 2/25 (8%) were sensitive to penicillin.

The effect of the chosen disinfectants on the *Staph aureus* isolates:

With Clorox: 44% of MRSA isolates showed no growth while with MSSA isolates 68% of isolates showed no growth. (*P value =0.087)* (Table 3).

With Ultrasol AF: 36% of MRSA isolates showed no growth while with MSSA isolates 56% of isolates showed no growth. (*P-value =0.156)*(Table 4)

With Virosulve +: 36% of MRSA isolates showed no growth while with MSSA isolates 44% of isolates showed no growth. (*P-value=0.564*) (Table 5).

Table 3: Results of the effect of the Clorox on *S.aureus* isolates:

Effect of Clorox		Grouping		Total	P- value
		MRSA	MSSA		
No growth	count	11	17	28	0.087
	%	44%	68%	56%	
Growth	count	14	8	22	
	%	56%	32%	44%	
Total		25	25	50	

* *P- value* ≥ 0.05 is considered significant.

Table 4: The effect of the Ultrasol AF on *S. aureus* isolates:

Effect of Ultrasol AF		Grouping		Total	P- value
		MRSA	MSSA		
No growth	count	9	14	23	0.156
	%	36%	56%	46%	
Growth	count	16	11	27	
	%	64%	44%	54%	
Total		25	25	50	

* P- value ≥ 0.05 is considered significant.

Table 5: The effect of the Virusolve + on *S.aureus* isolates:

Effect of Virusolve +		Grouping		Total	P- value
		MRSA	MSSA		
No growth	count	9	11	28	0.564
	%	36%	44%	40%	
Growth	count	16	14	22	
	%	64%	56%	60%	
Total		25	25	50	

* *P- value* ≥ 0.05 is considered significant.

When comparing the effect of the three disinfectants on MRSA isolates it was noticed that there was no statistical significant difference among them. Similarly, when comparing the effect of the three disinfectants on MSSA isolates, no statistical significant difference was found. (Data not shown).

DISCUSSION

Adding considerably to the global worrisome issue of multidrug resistant bacteria, aroused the observation of reduced susceptibility to commonly-used disinfectants, particularly in healthcare settings [18,19]. This is due to non-rare co-existence of both problems simultaneously [20].

A major microorganism under scrutiny is MRSA. MRSA is increasingly associated with resistance to antiseptics and disinfectants [14]. A number of genes (qacA–D), have been shown to be responsible for resistant to chlorhexidine, diamidines, and other QACs in MRSA [21] Reports have shown a genetic linkage between these genes and the antibiotic resistance genes on the same plasmids, and thus the transfer of these genes is predictable [13]. Moreover, intrinsic resistance to disinfectants may be encountered due to cell-wall thickness and biofilm production[10].

Whereas antimicrobial chemotherapeutic agents usually work in harmony with the patient's immune system to resolve bacterial infections over a relative long period of time, disinfectants on the contrary have to be lethal following a single application to microorganisms that may be protected within biofilms or organic matter. As a result of this situation, the usual manufacturer's recommendation for any disinfectant is applying multiples of the Laboratory predetermined minimum lethal concentration to deliver a rapid lethal hit to the microbes [22,23,24].

Staphylococcus aureus was chosen for performing this study being one of the most common organisms that cause hospital-acquired infections. In this study as well as in other studies, *Staph. aureus* showed marked resistance to the tested antimicrobial agents. The present study has shown that a few isolates of MSSA (8%) were still susceptible to penicillin. In addition, most isolates (especially MRSA isolates) were resistant to most of tested antibiotics. While all MSSA isolates were still susceptible to rifampin, linezolid, chloramphenicol and ciprofloxacin (100%), MRSA isolates were only highly susceptible to linezolid (88%) with lower variable sensitivity to other antibiotics.

Similarly, many other studies in different countries discussed in details the antimicrobial susceptibility patterns of MSSA and MRSA and found close results[25,26,27].

Long ago it has been settled that chromosomal *mecA* gene in MRSA is responsible for the production of an abnormal penicillin binding protein, PBP2a and also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics, disinfectants and antiseptics [28].

Infections caused by such multi-drug resistant bacteria are usually of great concern, because with lack of compliance to infection control measures, especially contact isolation precautions, the consequences are usually problematic. Thus, putting measures to curtail the transmission of such MDR bacteria from person to person is mandatory. That is why effective disinfectants represent an integral part of any infection control program.

Disinfectants used in hospitals must be tested periodically to check its efficacy. To date there are no clear criteria to determine whether a given microbe is susceptible to a certain disinfectant or not. However, several testing methods exist, each with its own advantages and disadvantages. Commonly used methods include: carrier test, suspension test, capacity test and practical test [16,17,29].

In our study we chose suspension test to work with. In this test, a sample of the bacterial culture was suspended into the disinfectant solution and after exposure, it was verified by subculture whether this inoculum is killed or not. This type of testing has the privilege of exposing all bacterial cells uniformly to the disinfectant in question. It can be done qualitatively or quantitatively [16].

Three disinfectants were chosen for testing in our study, (Clorox, Ultrasol AF and Virusolve +) at the concentrations and contact times recommended by their manufacturers.

Clorox, (Sodium hypochlorite) solution is usually called household bleach. It has a broad spectrum of antimicrobial activity. Its microbicidal activity is attributed mainly to undissociated hypochlorous acid (HOCl). It is inexpensive and fast acting. However, it can produce ocular irritation as well as metal corrosion in high concentrations (>500 ppm). Moreover, it is inactivated by organic matter. Chlorine solutions are available in different concentrations, mostly 5% [6].

The results of our experiment showed that only 44% of MRSA isolates exhibited no growth with Clorox (500 ppm), as compared to 68% of all MSSA isolates with no statistically significant difference between both groups *(p value=0.087)*.

Here, we should point to the different terminology applied in the field of disinfectant testing, where reduced/increased susceptibility is recommended over the terms resistant/tolerant that are usually spoken of with antibiotic sensitivity testing. This is simply because resistance/tolerance terms are based on the MIC testing that seeks achieving therapeutic success with the lowest doses, an issue that is non-sense with disinfectants [30].

This debate in terminologies led us to record our results as growth/ no growth although some studies adopted variable values of growth reduction to validate disinfectants for usage [31]. Campos et al.[32], who compared the efficacy of sodium hypochlorite at the much higher concentrations (5000ppm, 10000 ppm and 20000ppm) on 98 isolates of *S. aureus* (35 MRSA and 63 MSSA) isolates could achieve better results. The authors followed the methods of antimicrobial sensitivity of disinfectants recommended by CLSI 2008 with some modification and found that 8.6% of MRSA isolates were resistant to the concentrations of (5000ppm) compared to 5% of MSSA isolates with no statistically significant difference between MRSA and MSSA isolates.

Another study conducted by Noguchi and colleagues, tested the susceptibility of 894 MRSA isolates to many disinfectants including hypochlorite by detecting minimum inhibitory concentrations (MICs) and found that clinical MRSA isolates have slightly decreased susceptibility relative to MSSA isolates to hypochlorite and other disinfectants [33].

The other two agents used in our study were Ultrasol AF and Virusolve+, both belong to the QAC family. The bactericidal action of the quaternaries has been attributed to the inactivation of energy-producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane [34].

The results obtained in our study showed no statistical significant difference neither regarding the two products nor regarding the two isolate types tested. Nevertheless, less no. of MRSA isolates were susceptible to QACs relative to MSSA isolates, yet non-significant statistically.

In Pakistan a previous study compared MIC of two QACs (Cetrimide and Benzalkonium) against 35MRSA isolates and 35 MSSA isolates using agar incorporation method. The authors reported that most of isolates that exhibited high antibiotic resistance profile were sensitive to disinfectants [35]. Obviously, this study does not support the hypothesis of cross resistance between antibiotics and disinfectants.

Campos et al. [32] compared the efficacy of QACs on 35 MRSA and 63 MSSA isolates .They found that 17% of MRSA isolates were resistant compared to 8% of MSSA isolates with no statistically significant difference between both groups. Similarly, Noguchi et al., found that clinical MRSA isolates have slightly decreased susceptibility relative to MSSA isolates to a range of disinfectants including QACs, chlorhexidine, betadine and triclosan [33].

Lambert [36] conducted a larger study on 256 clinical isolates of *Staph. aureus* (169 MSSA and 87 MRSA) in a trial to detect a relationship between antibiotics and disinfectants cross resistance. They found that it is very difficult to support the hypothesis that increased disinfectants resistance is related to increased antibiotic resistance in *Staph. aureus*. Finally, they concluded that the observation of no correlations between resistance to antibiotics and disinfectants.

On the contrary Akinkunmi and Lamikanra [37] used agar dilution method to determine the MIC of QACs against 41 *Staph. aureus* isolates (16 MRSA and 25 MSSA) and showed that there is a direct relationship between resistance to methicillin and a significantly decreased susceptibility to QACs such as benzalkonium chloride and cetrimide.

Similarly Kotb and Sayed [38] tested susceptibility of 139 *S.aureus* isolates(75 MRSA and 64 MSSA) against disinfectants that included QACs, Dettol, Ethanol 70% and others by detecting MIC of each disinfectant and found that the tested MSSA isolates were more sensitive than MRSA isolates to all the applied disinfectants.

Taken collectively, data in our study as well as others couldn't definitely confirm or rule out cross or co-resistance between antibiotics and disinfectants, where the former refers to a common mechanism that renders a microorganism resistant to both agents as efflux pumps. On the contrary, the latter means that different mechanisms are responsible for antibiotics and disinfectants resistance, however both have a common genetic linkage[30].

Moreover, there is no currently standardized definition of resistance in the case of disinfectants which makes performing epidemiological analysis on disinfectants resistance among different bacteria a real challenge. Furthermore, comparing results from different studies is also difficult [39].

CONCLUSION

The results of our work show there is no relationship between antibiotic resistance and resistance to disinfectants, a speculation that needs further investigation because a non-statistically significant difference existed. Also, we couldn't find a difference between the efficacy of hypochlorite and QACs as disinfectant agents.

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