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Major Article

The effect of exposure to sub-inhibitory concentrations of hypochlorite and quaternary ammonium compounds on antimicrobial susceptibility of *Pseudomonas aeruginosa*

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Background: *Pseudomonas* is a group of medically important species that inhabit a wide range of niches, including hospital environments. Controversies have emerged about the possible link between improper use of disinfectants and the emergence of antibiotic resistance in bacteria. The aim of this study was to assess the effect of exposure of antibiotic-susceptible *Pseudomonas* isolates to sub-inhibitory concentrations of 2 disinfectants—didecyldimonium chloride and sodium hypochlorite—on their antibiotic susceptibility patterns.

Methods: This study involved 50 *Pseudomonas* isolates. The antibiotic susceptibility patterns of the isolates were assessed using broth microdilution method. The minimal inhibitory concentrations (MICs) of each antibiotic were compared before and after exposure to sub-inhibitory concentrations of didecyldimonium chloride and sodium hypochlorite.

Results: After overnight incubation with sub-inhibitory concentrations of sodium hypochlorite, a statistically significant increase was observed in the MICs of colistin ($P = .012$), ceftazidime ($P < .001$), amikacin ($P < .001$), meropenem ($P < .001$), gentamicin ($P < .001$), piperacillin-tazobactam ($P = .003$), and ciprofloxacin ($P = .004$). In contrast, exposure to sub-inhibitory concentrations of didecyldimonium chloride showed a statistically significant increase in the MICs of amikacin ($P < .001$), gentamicin ($P < .001$), meropenem ($P = .041$), and ciprofloxacin ($P = .008$).

Conclusions: The use of suboptimal concentrations of sodium hypochlorite and didecyldimonium chloride can lead to the evolution of antibiotic-resistant *Pseudomonas* strains.

Clinical settings provide an ideal environment for the growth and proliferation of infectious organisms. The risk of infection and occurrence of nosocomial disease is strongly associated with medical-surgical procedures, with the introduction of pathogenic organisms from contaminated invasive devices, surfaces, and personnel.[1]

Pseudomonas is a rod-shaped, aerobic, Gram-negative bacterium belonging to the family *Pseudomonadaceae*.[2] *Pseudomonas aeruginosa* easily adapts to the environment it inhabits and can also colonize and invade a human host to cause serious infections.[3] It is a common cause of community-acquired and hospital-acquired infections. The development of resistance of *P. aeruginosa* to antibiotics is increasing globally.[4] *P. aeruginosa* shows resistance to the following antibiotics: penicillin G; aminopenicillin, including those combined with beta-lactamase inhibitors; first- and second-generation cephalosporins; piperacillin; piperacillin and tazobactam; cefepime; ceftazidime; aminoglycosides; the quinolones; and the carbapenems; as well as colistin and fosfomycin.[5] Furthermore, a 1.3–2-fold increase in mortality, morbidity, and cost has been reported for patients with resistant versus susceptible infections.[6] Additionally, *P. aeruginosa* is capable of forming complex structures called biofilms. Resistance to antimicrobial agents is the most important feature of biofilm development, which is a complex process that contributes to bacterial persistence in chronic infections.[7]

Biocides are used extensively in hospitals and other healthcare settings to control the growth of microbes on different applications, such as medical device sterilization and surface and water disinfection, as well as preservation of various formulations.[8] They are essential parts of infection control practices and aid in the

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prevention of healthcare-acquired infections.[9] The level of disinfection or sterilization is dependent on the intended use of the object: critical items that contact sterile tissues, semicritical items that contact mucous membranes, and noncritical devices that contact only intact skin. These items require sterilization, high-level disinfection, and low-level disinfection, respectively.[1]

Chlorine-based compounds are widely used in healthcare facilities in a variety of settings, such as spot-disinfection of countertops and floors. This wide use of chlorine products is due to several factors: their broad-spectrum antimicrobial activity, absence of toxic residues, effectiveness even with hard water, low price, fast killing, and ability to remove dried or fixed organisms and biofilms from surfaces.[10,11] The exact mechanism by which free chlorine destroys microorganisms has not been elucidated; however, several factors have been proposed, including oxidation of sulfhydryl enzymes and amino acids, ring chlorination of amino acids, loss of intracellular contents, decreased uptake of nutrients, inhibition of protein synthesis, decreased oxygen uptake, oxidation of respiratory components, decreased adenosine triphosphate production, and breaks in DNA and depressed DNA synthesis.[12,13]

Quaternary ammonium compounds (QACs) are widely used in ordinary environmental sanitation of noncritical surfaces, such as floors, furniture, and walls. Environmental Protection Agency-registered QACs are appropriate to use for disinfecting medical equipment that contacts intact skin (e.g., blood pressure cuffs).[14] Their bactericidal action has been attributed to the inactivation of energy-producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane.[15] The heavy use of QACs has been blamed for the dissemination of *qac* genes and the spread of efflux pumps.[16]

The possible link between biocide and antibiotic resistance in bacteria and the role of biocides in the emergence of such resistance has provided more controversies regarding their extensive and indiscriminate use. When used appropriately, biocidal products have a very important role to play in the control of healthcare-associated infections.[8] However, exposure of bacteria to biocides, as a part of the infection control policy, can select for mutants with decreased biocide susceptibility that often display a decreased susceptibility to antibiotics;. Thus, biocides may act as an agent of antibiotic resistance. Moreover, it has been reported that exposure of bacteria to biocides, at concentrations below those required to arrest growth, can also select for antibiotic-resistant strains.[17] Indeed, experimental and observational evidence shows that exposure to these non-antibiotic antimicrobial agents can induce or select for bacterial adaptations that result in decreased susceptibility to 1 or more antibiotics. This may occur via cellular mechanisms that are protective across multiple classes of antimicrobial agents or by selection of genetic determinants for resistance to non-antibiotic agents that are linked to genes for antibiotic resistance antibiotics[18]—for example, efflux pumps that may be upregulated by exposure of *P. aeruginosa* to chlorhexidine or benzalkonium chloride.[19] Chlorine tolerance has been found to be correlated with increased minimal inhibitory concentrations (MICs) against various antibiotics.[20]

The aim of this study was to detect the effect of exposure to sub-inhibitory concentrations of the QAC didecyldimonium chloride and sodium hypochlorite on antimicrobial susceptibility patterns of *Pseudomonas* hospital isolates.

METHODS

This experimental study involved 50 *Pseudomonas* isolates collected from the Department of Clinical Pathology at Kasr Al-Ainy Hospital in Cairo, Egypt, during a 4-months period from August to November 2016. Data concerning clinical sources of isolates were obtained from the Department of Clinical Pathology, which receives clinical specimens for culture and sensitivity testing from all of the hospital units. The study was approved by the Research Ethics Committee of the Institutional Review Board, Faculty of Medicine, Cairo University. All isolates were collected on glycerol broth and transferred to the Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University.

Table 1
The exact indication for minimal inhibitory concentrations (MICs) for *Pseudomonas* of the individual antibiotic agents, according to the Clinical and Laboratory Standards Institute (2016).

	MIC Interpretive Criteria (μg/ml)		
Antibiotic	Susceptible	Intermediate	Resistant
Colistin	≤ 2	4	≥ 8
Amikacin	≤ 16	32	≥ 64
Ceftazidime	≤ 8	16	≥ 32
Meropenem	≤ 2	4	≥ 8
Gentamicin	≤ 4	8	≥ 16
Piperacillin-tazobactam	≤ 16/4	32/4 – 64/4	≥ 128/4
Ciprofloxacin	≤ 1	2	≥ 4

NOTE: CLSI, 2016.[22]

Identification of *Pseudomonas* was performed according to *UK Standards for Microbiology Investigations* (2015).[21] *Pseudomonas* was identified as non-fermentative, motile, Gram-negative, oxidase-positive bacilli. Forty-seven of the 50 isolates cultured on blood agar were presumptively identified by a positive oxidase reaction, characteristic pigment production, and grape-like odor as *P. aeruginosa*. The remaining 3 isolates were identified as *Pseudomonas* spp.

Determination of antibiotics minimal inhibitory concentrations (MICs)

The MICs of tested antibiotics, listed in Table 1, were obtained by the broth microdilution method. Interpretation of the results was performed according to Clinical and Laboratory Standards Institute breakpoints as susceptible, intermediate, or resistant, as shown in Table 1.[22]

Determination of disinfectants MICs

Two disinfectants were included in this study: sodium hypochlorite (Clorox 5.25%; Clorox, Oakland, California) and the QAC didecyldimonium chloride (Virusolve+ concentrate 100%; (Amity International Healthcare, South Yorkshire, England). These 2 disinfectants were chosen because they are widely used in Kasr Al-Ainy Hospital for environmental cleaning and disinfection of spills of blood and tissue fluid. The antimicrobial effect of the tested disinfectants against *Pseudomonas* spp. was assessed according to Lotfi et al. (2011) and Al-Jailawi et al. (2013).[23,24] Briefly, 100 μl of 2-fold dilutions of each of the tested disinfectants were distributed in wells from 10 to 1, starting from the highest dilution to the lowest dilution. An inoculum of 100 μl of each tested isolate ($1x10^6$ CFU/ml) was added to wells 10 through 1 in each row. Column 11 was the sterility control, containing broth only, and column 12 was the growth control, containing broth plus inoculum. After overnight at 37°C, the MIC was determined as the highest dilution of the disinfectant that visually inhibited bacterial growth, as demonstrated by turbidity and confirmed by reading the optical density using Micro ELISA autoreader Stat Fax-2100 (Awareness Technology, Palm City, Florida) at wavelength 600 nm.

Effect of exposure to disinfectants on antibiotic susceptibility of the isolates

Bacterial suspensions from wells containing the highest concentration of the disinfectant that still allowed bacterial growth (sub-inhibitory concentration) were inoculated onto nutrient agar plates and incubated overnight at 37°C to isolate the survived organisms. MICs of those survived organisms were retested using the broth microdilution method. Only antibiotic-susceptible or intermediate isolates were included in this step. The effect of sub-inhibitory concentrations of the used disinfectants on the susceptibility of *Pseudomonas* spp. to different antibiotics was assessed by comparing the antibiotics MICs before and after exposure to the disinfectants.

Statistical analysis

Data were statistically described in terms of median, minimum, and maximum, or frequencies and percentages, when appropriate. Comparison of the antibiotics' MIC values before and after exposure of *Pseudomonas* isolates to the 2 tested disinfectants was performed using the non-parametric Wilcoxon signed-rank test. *P* values less than .05 were considered statistically significant. All statistical calculations were performed using SPSS version 22 software (IBM Corp., Armonk, New York).

RESULTS

The 50 *Pseudomonas* spp. were isolated from the following types of infection: 18 (36%) from urinary tract infections, 17 (34%) from wound infections, 8 (16%) from respiratory tract infections, 6 (12%) from ear infections, and 1 (2%) from a case of septicemia.

Antibiotics MICs for the tested isolates

The antibiotic susceptibility pattern, using the broth microdilution method, of the 50 *Pseudomonas* isolates is shown in Table 2.

Determination of disinfectants MICs

For sodium hypochlorite, the MICs ranged from 0.01% to 0.02% (100-200 ppm); for didecyldimonium chloride, the MIC was 0.012% (120 μg/ml) for all isolates.

Effect of exposure to sub-inhibitory concentrations of the disinfectants on *Pseudomonas* antibiotic susceptibility pattern

The measurement of antibiotics' MICs was repeated after overnight incubation with each of the 2 disinfectants, only for antibiotic-susceptible and intermediate-resistant isolates. MIC values before exposure to disinfectants were compared to those after exposure. The exact indication of the antibiotics' MIC values before and after exposure to each of the 2 investigated disinfectants is presented in Tables A1-A7.

The Wilcoxon signed-rank test was performed to determine the effect of overnight incubation of the susceptible and intermediate-resistant *Pseudomonas* isolates with each of the tested disinfectants on the antibiotics' MICs. This was done by comparing the mean ranks of the antibiotics' MICs before and after exposure of *Pseudomonas* isolates to the 2 tested disinfectants.

After overnight incubation with sodium hypochlorite, a statistically significant increase was observed in MICs of colistin in the susceptible and intermediate isolates ($P = .012$), ceftazidime ($P < .001$), amikacin ($P < .001$), meropenem ($P < .001$), gentamicin ($P < .001$), and piperacillin-tazobactam ($P = .003$), as well as ciprofloxacin ($P = .004$).

The number and percentage of isolates that showed increase in MICs after overnight incubation with sodium hypochlorite are shown in Table 3. Briefly, an increase was observed in MICs of colistin in

Table 2
Antibiotic susceptibility pattern of the *Pseudomonas* isolates to the tested antibiotics using the broth microdilution method before exposure to disinfectants.

	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Colistin	47 (94)	3 (6)	0 (0)
Amikacin	27 (54)	0 (0)	23 (46)
Ceftazidime	24 (48)	0 (0)	26 (52)
Meropenem	25 (50)	1 (2)	24 (48)
Gentamicin	25 (50)	0 (0)	25 (50)
Piperacillin-tazobactam	24 (48)	0 (0)	26 (52)
Ciprofloxacin	24 (48)	0 (0)	26 (52)

Table 3
Antibiotics' minimal inhibitory concentration (MIC) change before and after incubation with sodium hypochlorite.

Antibiotics (no. of susceptible and intermediate-resistant isolates)		MIC Before	MIC After	Isolates with MIC increase, no (%)	Isolates without MIC increase, no (%)	*P value*
Piperacillin-tazobactam (24)	Median	4	6	11 (45.83%)	13 (54.17%)	.003
	Minimum	2	4			
	Maximum	8	128			
Ceftazidime (24)	Median	0.5	2	20 (83.33%)	4 (16.67%)	<.001
	Minimum	0.25	1			
	Maximum	8	64			
Gentamicin (25)	Median	0.125	0.25	22 (88%)	3 (12%)	<.001
	Minimum	0.125	0.25			
	Maximum	0.5	2			
Amikacin (27)	Median	0.125	0.5	25 (92.6%)	2 (7.4%)	<.001
	Minimum	0.125	0.25			
	Maximum	8	16			
Ciprofloxacin (24)	Median	0.125	0.125	10 (41.67%)	14 (58.33%)	.004
	Minimum	0.125	0.125			
	Maximum	0.125	4			
Meropenem (26)	Median	0.125	0.5	20 (76.9%)	6 (23.1%)	<.001
	Minimum	0.125	0.125			
	Maximum	4	64			
Colistin (50)	Median	1	5	37 (74%)	13 (26%)	.012
	Minimum	0.25	1			
	Maximum	4	16			

Table 4
Antibiotics' minimal inhibitory concentration (MIC) change before and after incubation with didecyldimonium chloride.

Antibiotics (no. of susceptible and intermediate-resistant isolates)		MIC Before	MIC After	Isolates with MIC increase, no (%)	Isolates without MIC increase, no (%)	*P value*
Piperacillin-tazobactam (24)	Median	4	4	4 (16.67%)	20 (83.33%)	.811
	Minimum	2	2			
	Maximum	8	8			
Ceftazidime (24)	Median	0.5	1	14 (58.33%)	10 (41.67%)	.711
	Minimum	0.25	1			
	Maximum	8	64			
Gentamicin (25)	Median	0.125	0.25	22 (88%)	3 (12%)	<.001
	Minimum	0.125	0.25			
	Maximum	0.5	1			
Amikacin (27)	Median	0.125	0.5	24 (88.9%)	3 (11.1%)	<.001
	Minimum	0.125	0.5			
	Maximum	8	16			
Ciprofloxacin (24)	Median	0.125	0.125	7 (29.17%)	17 (70.83%)	.008
	Minimum	0.125	0.125			
	Maximum	0.125	0.25			
Meropenem (26)	Median	0.125	0.25	17 (65.4%)	9 (34.6%)	.041
	Minimum	0.125	0.125			
	Maximum	4	64			
Colistin (50)	Median	1	1	32 (64%)	18 (36%)	.173
	Minimum	0.25	1			
	Maximum	4	16			

37/50 (74%) of the susceptible and intermediate isolates, ceftazidime in 20/24 (83.33%), amikacin in 25/27 (92.6%), meropenem in 20/26 (76.9%), gentamicin in 22/25 (88%), and piperacillin-tazobactam in 11/24 (45.83%), as well as ciprofloxacin in 10/24 (41.67%).

After overnight incubation with didecyldimonium chloride, a statistically significant increase was observed in MICs of amikacin ($P < .001$), gentamicin ($P < .001$), meropenem ($P = .041$), and ciprofloxacin in the susceptible and intermediate isolates ($P = .008$), whereas no statistically significant increase was observed in MICs of piperacillin-tazobactam ($P = .811$), ceftazidime ($P = .711$), or colistin ($P = .173$).

The number and percentage of isolates that showed increase in MICs after overnight incubation with didecyldimonium chloride are shown in Table 4. Briefly, an increase was observed in MICs of amikacin in 24/27 (88.9%), gentamicin in 22/25 (88%), meropenem in 17/26 (65.4%), and ciprofloxacin in 7/24 (29.17%) of the susceptible and intermediate isolates.

DISCUSSION

Improved understanding is needed of the effectiveness of environmental surface cleaning and disinfection, to reduce the incidence of infectious diseases and colonization in healthcare workers and patients as well as the adverse health effects caused by improper use of disinfection.[25]

In Kasr Al-Ainy Hospital, *Pseudomonas* infection is a serious problem that accounts for 15%-40% of hospital-acquired infections in various departments, according to the monthly infection control surveillance program. Approximately 50% of these infections are multidrug resistant that exhibit resistance to several classes of antibiotics, including anti-pseudomonal penicillins, cephalosporins, aminoglycosides, quinolones, and carbapenems. In our hospital, carbapenems such as meropenem and imipenem are currently the drug of choice for treatment of *Pseudomonas* infections; however, the resistance rate to carbapenems is increasing. In cases of carbapenem resistance, a combination of a carbapenem and an aminoglycoside or colistin is prescribed.

This study showed that the inhibitory concentration of didecyldimonium chloride for all *Pseudomonas* isolates was 120 μg/mL. This concentration was much higher than that determined by Langsrud et al. (2003),[26] in which the MIC of *Pseudomonas* spp. ranged from 5 to 40 μg/mL, whereas the minimal bactericidal concentration was in the range of 10-60 μg/mL. Furthermore, they reported that 2 passages of *Pseudomonas fluorescence* in gradually higher concentrations of disinfectant were sufficient to increase the MIC of didecyldimonium chloride from 10 to 50 μg/mL. On the other hand, a commercial product of didecyldimonium chloride at a concentration of 10,000 μg/mL killed only 88.3% of multidrug-resistant *P. aeruginosa* colonies at a 0.5-minute contact time.[27]

In this study, the MIC of sodium hypochlorite on *Pseudomonas* isolates ranged from 100 to 200 ppm after overnight incubation. In 2008, the Centers for Disease Control and Prevention stated that 100 ppm of free chlorine was able to kill 10^6–10^7 of *P. aeruginosa* in <10 minutes.[14,28] Several studies have examined the antimicrobial effect of chlorine-based disinfectants using various concentrations and different contact periods, ranging from 30 seconds to 24 hours. Deshaies et al. (2012) showed that, after a contact time of 5 minutes, *P. aeruginosa* was killed by a concentration of 82 ppm of chlorine-based commercial disinfectant, which was much less than the manufacturer's recommended concentration (5250 ppm).[29] A study by Mitiku et al. (2014) confirmed that 5000 ppm of sodium hypochlorite showed lethal effect on 91.4% of *P. aeruginosa* isolates.[30]

These findings show that despite the ability of chlorine-based disinfectants of inhibiting the growth of *Pseudomonas* isolates in concentrations as low as 0.01%, as reported in the present study, it is questionable if these low concentrations can be safely recommended. In fact, the use of low disinfectant concentrations can be a crucial cause of the emergence of antibiotic-resistant *Pseudomonas* strains.

The dramatic increase in the number of hospital-acquired infections is currently linked to the pandemic of multidrug resistance. Clinical environments provide an ideal reservoir for the growth, proliferation, and transmission of pathogenic organisms.[31] Moreover, concerns have been raised recently regarding co-selection for antibiotic resistance among bacteria exposed to biocides used as disinfectants and antiseptics. Indeed, experimental and observational evidence shows that exposure to these non-antibiotic antimicrobial agents can induce or select for

bacterial adaptations that result in decreased susceptibility to 1 or more antibiotics.[18]

In this study, after overnight incubation with sodium hypochlorite, a statistically significant increase was observed in MICs of the tested antibiotics. After overnight incubation with didecyldimonium chloride, a statistically significant increase was observed in MICs of only amikacin, gentamicin, meropenem, and ciprofloxacin. Some disinfectants are reported to share the same mechanism of action with some antibiotics, and this can cause resistance to disinfectants used in environmental cleaning.[32] Cross-resistance between antibiotics and disinfectants may occur via cellular mechanisms that are protective against multiple classes of antimicrobial agents or by selection of genetic determinants for resistance to non-antibiotic agents that are linked to genes for antibiotic resistance.[18] Interestingly, multidrug-resistant *P. aeruginosa* has been shown to have significantly higher MICs of sodium hypochlorite compared to antibiotic-susceptible isolates.[33]

The association between antibiotic resistance and exposure to disinfectants can be also attributed to the relevant effects of these antimicrobial agents on bacterial community structure, such as mobilization of genetic elements or mutagenesis. Some studies have also suggested a potential molecular link between reduced susceptibility to some disinfectants and antibiotic resistance. Increased resistance to antiseptics and disinfectants has been associated with mutation and/or presence of plasmids,[34] and both have been observed in some strains of *P. aeruginosa*.[35]

Comprehensive efforts, including basic infection control education, improved selection and use of products, and practical training, are required to minimize harmful cleaning and disinfection exposures without reducing the effectiveness of infection prevention.[25] To our knowledge, no previous studies have examined the effect of exposure to these 2 disinfectants on *Pseudomonas* resistance to antibiotics; however, the evolution of antibiotic-resistant healthcare-acquired microorganisms after treatment with sub-MICs of different disinfectants has been evaluated and confirmed.[36] The results of previous studies, together with the present study, suggest that exposure to sub-inhibitory doses of various disinfectants can induce antibiotic resistance in clinical *Pseudomonas* isolates through either natural selection process or enforcement of acquiring resistance mechanisms to antibiotics as an adaptation to the new environment. Thus, the use of appropriate bactericidal concentrations of various disinfectants should be emphasized by the infection prevention and control specialist as a part of infection control program standards in healthcare settings. Sodium hypochlorite and didecyldiminum chloride are still recommended for low- and intermediate-level disinfection in our hospital. However, precautions are in place regarding the use of appropriate concentrations as recommended by the manufacturers, particularly for prevention of infections caused by antibiotic-resistant bacteria such as *P. aeruginosa*.

CONCLUSIONS

This study showed that the use of suboptimal concentrations of sodium hypochlorite and didecyldimonium chloride can lead to the evolution of antibiotic-resistant *Pseudomonas* strains. This study emphasizes the need to adhere strictly to standard disinfection policy in hospitals to achieve proper prevention and control of healthcare-associated infections. Rotational use of different disinfectants is recommended to avoid development of resistance or selection of resistant strains in the hospital environment. Further studies are recommended to study the effect of exposure to sub-inhibitory concentrations of sodium hypochlorite and didecyldimonium chloride on antibiotic susceptibility of *Pseudomonas* spp.

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APPENDIX

Table A1
Indications of amikacin minimal inhibitory concentrations (MICs) before and after exposure of susceptible and intermediate-resistant *Pseudomonas* isolates to sub-inhibitory concentrations of disinfectants.

Isolate no.	Before disinfectant	After Clorox	After Virusolve	Isolate no.	Before disinfectant	After Clorox	After Virusolve
1	S	S*	S*	**31**	S	S*	S*
2	S	S*	S	**32**	S	S*	S*
3	S	S	S*	**33**	S	S*	S*
4	S	S*	S*	**34**	S	S*	S*
9	S	S*	S*	**35**	S	S*	S*
10	S	S*	S*	**36**	S	S*	S*
11	S	S*	S*	**37**	S	S*	S*
12	S	S*	S*	**40**	S	S*	S*
18	S	S*	S*	**42**	S	S*	S*
21	S	S*	S	**44**	S	S*	S*
24	S	S*	S*	**45**	S	S*	S*
26	S	S*	S*	**47**	S	S*	S
28	S	S*	S*	**48**	S	S	S*
29	S	S*	S*				

NOTE. After Clorox: 25/27 showed increase in MIC values ($P < .001$); none showed change of the MIC indication.
NOTE. After Virusolve: 24/27 showed increase in MIC values ($P < .001$); none showed change of the MIC indication.
S, Susceptible.
*MIC values increased after overnight incubation with the disinfectant.

Table A2
Indications of meropenem minimal inhibitory concentrations (MICs) before and after exposure of susceptible and intermediate-resistant *Pseudomonas* isolates to sub-inhibitory concentrations of disinfectants.

Isolate no.	Before disinfectant	After Clorox	After Virusolve	Isolate no.	Before disinfectant	After Clorox	After Virusolve
2	S	R*	S	**31**	S	R*	R*
3	S	S	S	**32**	S	S*	S*
4	S	S	S	**33**	S	S*	S*
9	S	S*	S*	**34**	S	S	S
10	S	S*	S*	**35**	S	S*	S*
11	S	S*	S*	**36**	I	R*	R*
12	S	S*	S*	**37**	S	S*	S*
18	S	S*	S*	**40**	S	S*	S*
21	S	R*	S	**42**	S	S*	S*
24	S	S*	S*	**44**	S	S	S
26	S	S*	S*	**45**	S	S	S
28	S	S*	S*	**47**	S	R*	S
29	S	S*	S*	**48**	S	S	S

NOTE. After Clorox: 20/26 showed increase in MIC values ($P < .001$); 5 became resistant.
NOTE. After Virusolve: 17/26 showed increase in MIC values ($P = .041$); 2 became resistant.
I, Intermediate; R, resistant; S, Susceptible.
*MIC values increased after overnight incubation with the disinfectant.

Table A3
Indications of gentamicin minimal inhibitory concentrations (MICs) before and after exposure of susceptible and intermediate-resistant *Pseudomonas* isolates to sub-inhibitory concentrations of disinfectants.

Isolate no.	Before disinfectant	After Clorox	After Virusolve	Isolate no.	Before disinfectant	After Clorox	After Virusolve
1	S	S*	S*	**29**	S	S*	S*
2	S	S*	S*	**32**	S	S*	S*
3	S	S*	S*	**33**	S	S*	S*
4	S	S	S	**34**	S	S	S
9	S	S*	S*	**35**	S	S*	S*
10	S	S*	S*	**37**	S	S*	S*
11	S	S*	S*	**40**	S	S*	S*
12	S	S*	S*	**42**	S	S*	S*
18	S	S*	S*	**44**	S	S*	S*
21	S	S*	S*	**45**	S	S	S
24	S	S*	S*	**47**	S	S*	S*
26	S	S*	S*	**48**	S	S*	S*
28	S	S*	S*				

NOTE. After Clorox: 22/25 showed increase in MIC values ($P < .001$); none showed change of the MIC indication.
NOTE. After Virusolve: 22/25 showed increase in MIC values ($P < .001$); none showed change of the MIC indication.
S, Susceptible.
*MIC values increased after overnight incubation with the disinfectant.

Table A4
Indications of ciprofloxacin minimal inhibitory concentrations (MICs) before and after exposure of susceptible and intermediate-resistant *Pseudomonas* isolates to sub-inhibitory concentrations of disinfectants.

Isolate no.	Before disinfectant	After Clorox	After Virusolve	Isolate no.	Before disinfectant	After Clorox	After Virusolve
2	S	R*	S	**32**	S	S*	S*
3	S	S	S	**33**	S	S	S
4	S	S	S	**34**	S	S*	S*
9	S	S*	S	**35**	S	S	S
10	S	S	S	**37**	S	S	S*
11	S	S	S*	**40**	S	S	S
12	S	S*	S	**42**	S	S*	S
18	S	S	S	**44**	S	S*	S*
21	S	R*	S	**45**	S	S	S
24	S	S	S*	**47**	S	R*	S
26	S	S*	S*	**48**	S	S	S
28	S	S	S				
29	S	S	S				

NOTE. After Clorox: 10/24 showed increase in MIC values ($P = .004$); 3 became resistant.
NOTE. After Virusolve: 7/24 showed increase in MIC values ($P = .008$); none showed change of the MIC indication.
R, resistant; S, Susceptible.
*MIC values increased after overnight incubation with the disinfectant.

Table A5
Indications of ceftazidime minimal inhibitory concentrations (MICs) before and after exposure of susceptible and intermediate-resistant *Pseudomonas* isolates to sub-inhibitory concentrations of disinfectants.

Isolate no.	Before disinfectant	After Clorox	After Virusolve	Isolate no.	Before disinfectant	After Clorox	After Virusolve
2	S	R*	S	**32**	S	S*	S*
3	S	S	S	**33**	S	S*	S*
4	S	S	S	**34**	S	S*	S*
9	S	S*	S	**35**	S	S*	S*
10	S	S*	R*	**37**	S	S*	S*
11	S	S*	S*	**40**	S	S*	R*
12	S	S*	S	**42**	S	S*	S
18	S	S*	R*	**44**	S	S*	S*
21	S	R*	S	**45**	S	S	S
24	S	S*	S*	**47**	S	R*	S
26	S	S*	S*	**48**	S	S	S
28	S	S*	S*				
29	S	S*	S*				

NOTE. After Clorox: 20/24 showed increase in MIC values ($P < .001$); 3 became resistant.
NOTE. After Virusolve: 14/24 showed increase in MIC values ($P = .711$); 3 became resistant.
R, Resistant; S, Susceptible.
*MIC values increased after overnight incubation with the disinfectant.

Table A6
Indications of piperacillin-tazobactam minimal inhibitory concentrations (MICs) before and after exposure of susceptible and intermediate-resistant *Pseudomonas* isolates to sub-inhibitory concentrations of disinfectants.

Isolate no.	Before disinfectant	After Clorox	After Virusolve	Isolate no.	Before disinfectant	After Clorox	After Virusolve
2	S	R*	S	**32**	S	S*	S
3	S	S	S	**33**	S	I*	S*
4	S	S	S	**34**	S	S*	S*
9	S	S	S	**35**	S	S*	S
10	S	S	S	**37**	S	S	S
11	S	S	S	**40**	S	S	S
12	S	S	S	**42**	S	S	S
18	S	S	S	**44**	S	S*	S*
21	S	R*	S	**45**	S	S	S
24	S	S	S	**47**	S	R*	S
26	S	S*	S	**48**	S	S	S
28	S	S*	S				
29	S	I*	S*				

NOTE. After Clorox: 11/24 showed increase in MIC values ($P = .003$). 5 changed; 3 became resistant, 2 intermediate.
NOTE. After Virusolve: 4/24 showed increase in MICs values ($P = .811$); none showed change of the MIC indication.
I, Intermediate; R, Resistant; S, Susceptible.
*MIC values increased after overnight incubation with the disinfectant.

Table A7
Indications of colistin minimal inhibitory concentrations (MICs) before and after exposure of susceptible and intermediate-resistant *Pseudomonas* isolates to sub-inhibitory concentrations of disinfectants.

Isolate no.	Before disinfectant	After Clorox	After Virusolve	Isolate no.	Before disinfectant	After Clorox	After Virusolve
1	S	S*	S*	**26**	S	S*	S*
2	I	R*	S	**27**	S	R*	R*
3	S	R*	S	**28**	S	S*	S*
4	S	R*	S	**29**	S	S*	S*
5	S	S	S	**30**	S	R*	R*
6	S	S	S	**31**	S	S*	S*
7	S	R*	R*	**32**	S	S*	S*
8	S	R*	R*	**33**	S	S*	S*
9	S	S*	S*	**34**	S	S	S*
10	S	S*	S*	**35**	S	S*	S*
11	S	S*	S*	**36**	S	S*	S*
12	S	S*	S*	**37**	S	S*	S*
13	S	R*	R*	**38**	S	S	S
14	S	S	S	**39**	S	S	S
15	S	R*	R*	**40**	S	S*	S*
16	S	S	S	**41**	S	R*	R*
17	S	R*	R*	**42**	S	S*	S*
18	S	S*	S*	**43**	S	R*	R*
19	S	S	S	**44**	S	S	S*
20	S	S	S	**45**	S	R*	S
21	I	R*	S	**46**	S	R*	R*
22	S	R*	R*	**47**	I	R*	S
23	S	S	S	**48**	S	R*	S
24	S	S*	S*	**49**	S	S	S
25	S	S	S	**50**	S	R*	R*

NOTE. After Clorox: 37/50 showed increase in MIC values ($P = .012$); 19 became resistant.
NOTE. After Virusolve: 32/50 showed increase in MIC values ($P = .173$); 12 became resistant.
I, Intermediate;R, Resistant; S, Susceptible.
*MIC values increased after overnight incubation with the disinfectant.