American Journal of Infection Control ■■ (2018) ■■-■■

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major Article

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

Aya Mahmoud Nasr MSc, Marwa Salah Mostafa MD *, Heba Hamed Arnaout MD, Amal Ali Ali Elshimy MD

Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo, Egypt

Key Words:
Pseudomonas
Antibiotic resistance
Disinfectant
Sodium hypochlorite
Didecyldimonium chloride

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

* Address correspondence to Marwa Salah Mostafa, MD, Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo, Egypt.
E-mail address: marwa75_jan@yahoo.com (M.S. Mostafa).
Conflicts of Interest: None to report.

0196-6553/
https://doi.org/10.1016/j.ajic.2018.04.201

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.