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Effect of Disinfectants on Antibiotics Susceptibility of *Pseudomonas aeruginosa*

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Abstract

Disinfectants are widely used to get rid of microorganisms whether in hospitals, health centers or for normal domestic use. Some suggested that when disinfectants are incorrectly diluted the disinfectant might promote the growth of antibiotic-resistant bacteria, therefore, in this study pathogenic bacterium (*Pseudomonas aeruginosa*), isolated from patient with urinary tract infection, treated with two locally popular disinfectants (Claradone and Sarttol). Results showed that the bacterial growth was affected by both disinfectants. The lowest concentration of Claradone that inhibit the growth of this bacterium is considered as the minimum inhibitory concentration (MIC), this was 30%, while the lowest effecting concentration of Sarttol was 3%. A number of survival colonies after treated with high concentration of Calarodone and Sarttol were investigated for their susceptibility to antibiotics, using standard disc diffusion method. Results indicated that these colonies of *P. aeruginosa* resisted antibiotics they were sensitive to before treatment. So it can be concluded that using Claradone and sarttol can make the pathogenic bacterium (*P. aeruginosa*) resist some antibiotics.

Keywords: *Pseudomonas aeruginosa*, Disinfectants, Minimum Inhibitory Concentration, Claradone, Sarttol

1. Introduction

Pseudomonas aeruginosa is an important opportunistic pathogen (de Bentzmann and Plesiat, 2011). It is one of the main causes of hospital-acquired infections (Machado et al., 2013). Effective antibiotics are limited because infections by *P. aeruginosa* are hard to defy, as it is virulent, acquires antibiotic resistance and resists disinfectants (Higgins et al., 2001; Zavascki et al., 2005). Furthermore, the contaminated disinfectant solutions are the source of nosocomial infections (David & Moore, 1997; Odjadjare et al., 2012).

Many methods have been contrived to decrease the population and prevalence of causative agents of infectious diseases. They include chemotherapy, immunization, sterilization and disinfection (Kim et al., 2007). Subsequently, decontamination, disinfection and sterilization became main components of any infection control program (Rutala et al., 2001).

Onaolapo (2001) and Aboh et al. (2013) defined antiseptics as biocides or products that destroy or inhibit the growth of microorganisms in or on living tissue (e.g. health care personnel hand washes and surgical scrubs); and disinfectants are also products or biocides that are used on inanimate objects or surfaces.

The formulation of disinfectants, level of organic charge, synergy, temperature, dilution rate and examination methods influence the antimicrobial activity of disinfectants (Russel et al., 1995; Russel, 2002). The mechanisms of action of antiseptics and disinfectants on bacteria include examination of uptake (Russell & Chopra, 1996), lysis and leakage of intracellular constituents (Christopher et al., 2007), perturbation of cell homeostasis (Kroll and Patchett, 1991), effects on model membranes (Denyer & Stewart, 1998), inhibition of enzymes, electron transport, and oxidative phosphorylation (Kuyyakanond & Quesnel, 1992; McDonnell & Russell, 1999).

Pseudomonas aeruginosa was extensively studied for its high incidence and extraordinary potential to form biofilms in clinical equipment, medical devices and wounds (Hill et al., 2010; de Bentzmann & Plésiat, 2011). Biofilms represent a reservoir of pathogenic bacteria that can disconnect, proceed their planktonic state, and contaminate new surfaces and patients. Moreover, microbial biofilms are well known for their high level of resistance towards antibiotic and biocide treatments (Gilbert et al., 2001). Bacteria within biofilms can easily live in the presence of high antibiotic concentrations similar to the ones that are prescribed during the course of therapies (Russell, 2003; Dorr et al., 2009). Biofilm resistance mechanisms include not only the reaction-diffusion limitation of antimicrobial access to the biofilm-entrapped bacteria (Gilbert et al., 2003), but also the expression of spatially heterogeneous, less susceptible phenotypes, caused either by growth as a biofilm per se, or through the expression of high cell density, or starvation phenotypes. The use of certain active substances in biocides in various settings may contribute to the increased occurrence of antibiotic resistant bacteria (Machado et al., 2013).

Attention is being directed to the susceptibility of different types of microorganisms to antiseptics and disinfectants. Susceptibility vary in bacteria from one to another, the bacterial spores are the most resistant, followed by mycobacteria, then Gram-negative bacteria, and generally cocci are the most sensitive (Russell, 1999). There is a need to establish linkage between antibiotic and disinfectant resistance in bacteria and whether disinfectants can cause

antibiotic resistance. Some researchers referred that the effect of disinfectants on microorganisms is concentration–dependent, and due to the importance of disinfectants in the prevention of hospital-acquired infections, this study was carried out to confirm whether using of disinfectants could make bacteria resist some antibiotics.

2. Materials and Methods

2.1 Bacterial Isolate

The bacterium used in this study is clinical isolate of *Pseudomonas aeruginosa*, isolated from patient with urinary tract infection and obtained from Department of Biotechnology at Alnahrain University.

2.2 Disinfectants

Disinfectants used in this study are common and commercial, Claradone (40% w/v), which contain povidone iodine and Sarttol (5% w/v), contain dichloro-meta xylen and isopropyl alcohol.

2.3 Antibiotic Susceptibility Test (Atlas et al., 1995; CLSI, 2006)

The susceptibility of the isolate to antibiotics was determined by the disc diffusion test. A sterile cotton swab was dipped into the inoculum's of freshly culture (18 hrs. old) and the entire surface of the Muller Hinton agar plates was swabbed three times by rotating the plate approximately 60º between streaking to ensure even distribution, then the discs of antibiotics were applied and incubated at 37 ºC for 24 hrs. The diameter of the inhibition zone (clear area around discs) indicates the sensitivity of bacteria to that antibiotic.

2.4 Treatment of P. aeruginosa with Disinfectant (Turnidge et al., 2003)

The response of *P. aeruginosa* to Claradone and Sarttol was detected by the minimum inhibitory concentration (MIC), this bacterium was grown in 10 ml of nutrient broth to mid log phase. Then 0.1 ml inoculums of the culture were inoculated in a series of 10 ml fresh nutrient broth tubes containing various concentrations of each one of the disinfectant, serial concentration of Claradone are 5%, 10%, 15%, 20%, 25% and 30% (w/v), and the serial concentration of Sarttol are 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2% and 3% (w/v). All tubes were incubated at 37 ºC for 24-48 hrs.

Bacterial growth was determined by measuring the optical density of the liquid culture in spectrophotometer (Spectronic 20) at 600 nm and compared with the control (without disinfectant) to determine the effect of disinfectant on bacterial growth. The lowest concentration of disinfectant that inhibited the growth of the bacterium considered as the minimum inhibitory concentration (MIC).

Samples were taken from tubes containing the highest concentration of disinfectant that still allow bacterial growth, and 0.1 ml samples were spread on nutrient agar plates and incubated overnight at 37 ºC to score the survived colonies. Survivors were analyzed for antibiotics resistance as a result of disinfectant effects.

3. Results and Discussion

3.1 Bacterial Treated with Disinfectants

The bacterium used in this study is clinical isolate of *Pseudomonas aeruginosa,* which was isolated from patient with urinary tract infection. This isolate was candidate to investigate the effect of disinfectants on its susceptibility to antibiotics, using broth dilution methods.

This bacterium was grown in nutrient broth supplemented with serial dilutions of disinfectant (Claradone and Sarttol). There were two controls, control (1) was a culture broth and control (2) was a culture broth with the disinfectant.

Results presented in Tables 1, 2 show that the bacterial growth was affected by both disinfectants. The lowest concentration of Claradone that inhibited the growth of this bacterium, which is considered as the minimum inhibitory concentration (MIC) was 30% w/v, while the lowest concentration of Sarttol was 3% w/v.

Table 1. Effect of Claradone on *Pseudomonas aeruginosa* grown in nutrient broth at 37 ºC for 48 hrs

Isolate	Serial concentration of Claradone disinfectant							
	30%	25%	20%	15%	10%	5%	Control 1	Control 2
Pseudomonas aeruginosa	--	±	+	++	++	++	+++	--

(+++): Very good growth (OD600= 1.25-2.0), (++): Good growth (OD600= 0.81-1.2), (+): Moderate growth (OD600= 0.51-0.8), (±): Slight growth (OD600= 0.2-0.5), (-): No growth.

Table 2. Effect of Sarttol on *Pseudomonas aeruginosa* grown in nutrient broth at 37 ºC for 48 hrs

Isolate	Serial concentration of sarttol disinfectant								
	3%	1%	0.5%	0.4%	0.3%`	0.2%	0.1%	Control 1	Control 2
Pseudomonas aeruginosa	--	±	+	++	++	++	++	+++	--

(+++): Very good growth (OD600= 1.25-2.0), (++): Good growth (OD600= 0.81-1.2), (+): Moderate growth (OD600= 0.51-0.8), (±): Slight growth (OD600= 0.2-0.5), (-): No growth.

It was clear that, when the concentration of Claradone is increased the growth of bacteria decreased. Slightly growth of *Pseudomonas aeruginosa* was noticed when treated with 25% of Claradone, while moderate growth and good growth of bacteria was noticed when treated with 20% and 15%, 10% and 5% of Claradone respectively (Table 1).

In addition, the results depicted in Table 2 show that there was no growth of *Pseudomonas aeruginosa* when treated with 3% of Sarttol disinfectant, but the bacterial growth was slight when treated with 1 %. Moderate and good growth of bacteria was noticed when treated with 0.5% and 0.4%, 0.3%, 0.2% and 0.1 of Sarttol respectively, compared with bacterial growth

in control (1) and (2).

Samples were taken from tubes containing the highest concentration of Calarodon (25%) and Sarttol (1%) that still allow bacterial growth, and 0.1 ml samples were spread on nutrient agar plates and incubated overnight at 37 ºC to score the survived colonies. Twenty of survivor colonies for each treatment of Claradone (25%) and Sarttol (1%) were selected and checked for their antibiotic susceptibility.

3.2 Antibiotic susceptibility of Pseudomonas aeruginosa

3.2.1 Before Treatment with Disinfectants

The standard disc diffusion method was used to determine the sensitivity of *P. aeruginosa* isolate to several antibiotics. Table 3 showed that the isolate was resistant to Amikacin, Ampicillin, Azithromycin, Cefotaxime, Cephradine, Chloramphenicol, Novobiocin, Penicillin, Pipracillin, Rifampin , Vancomycin and tetracycline as illustrated in figure 1, and the isolate was sensitive to Cephalothin, Gentamicin, Ciprofloxacin, Nalidixic acid, Cloxacillin, Doxycycline, Streptomycin and Norfloxacillin. The multidruge resistance of bacteria could be due to the permeability of the outer membrane, which might prevent the entry into the cell of most of these antibiotics or due to certain mutations that occur as a result of overuse and misuse of antibiotics, in addition, plasmids and transposons that carrying resistance gene(s) play an important role in spreading the multidruge resistance between bacteria (Aversion et al., 2000; Stock & Wiedmann, 2001; Robicsek et al., 2006).

Table 3. Antibiotic sensitivity of *Pseudomonas aeruginosa* isolate before and after treatment with disinfectants

Antibiotic Disk	Sensitivity of *Pseudomonas aeruginosa*	
	before treatment with disinfectants	After treatment with disinfectants
Amikacin AK (30µg)	R	R
Ampicillin AM (25 µg)	R	R
Azithromycin AZM (15 µg)	R	R
Cefotaxime CTX (30 µg)	R	R
Cephradine CE (30 µg)	R	R
Cephalothin KF (30 µg)	S	S
Chloramphenicol C (10 µg)	R	R
Ciprofloxacin CF (5 µg)	S	S
Cloxacillin CLX (30 µg)	S	R
Doxycycline DO (30 µg)	S	R
Gentamicin CN (10 µg)	S	S
Nalidixic acid NA (30 µg)	S	R
Novobiocin NV (30 µg)	R	R
Norfloxacillin Nor (10 µg)	S	R
Penicillin P (10 µg)	R	R
Pipracillin PC (100 µg)	R	R
Rifampin RA (5 µg)	R	R

Streptomycin S (10 µg)	S	S
Tetracycline TE (10 µg)	R	R
Vancomycin VA (30 µg).	R	R

R: Resistance; S: Sensitive.

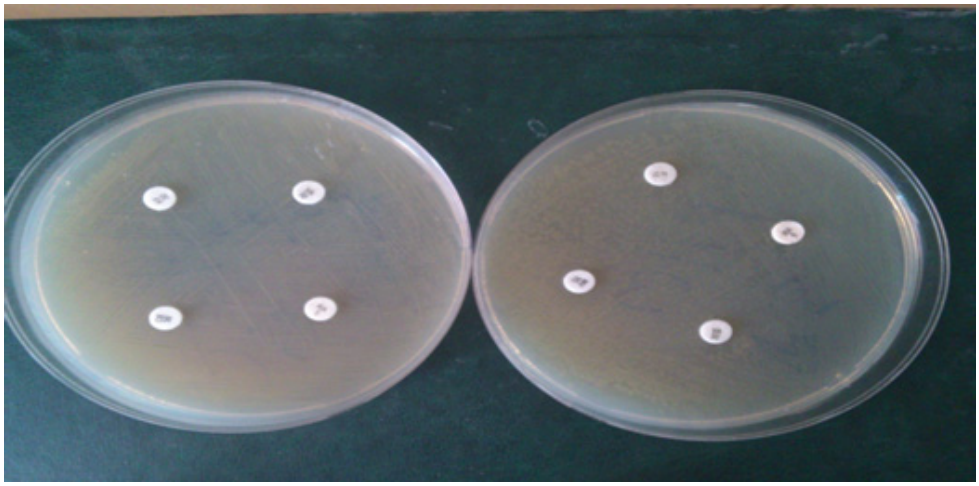

Figure 1. Shows *Pseudomonas aeruginosa* resistance to some antibiotics

3.2.2 After Treatment with Disinfectants

The selected survivor colonies after treated with Claradone and Sarttol were investigated for their susceptibility to antibiotics. It was noticed that some of these colonies (from both treatments) of *Pseudomonas aeruginosa* became resistant to Nalidixic acid, Norfloxacillin, Cloxacillin and Doxycycline, which was sensitive to them before treatment. As shown in Figures 2, 3. And Table 3.

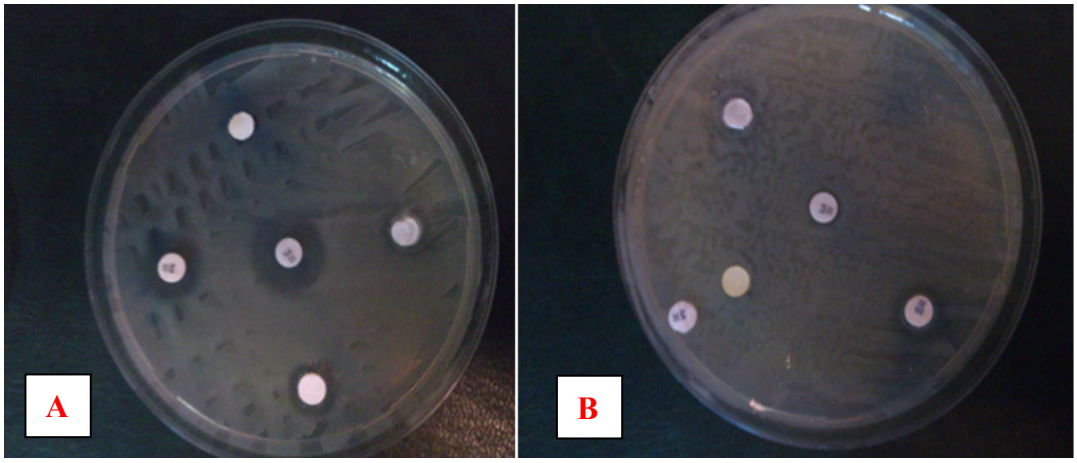

Figure 2. Shows Antibiotics susceptibility of *Pseudomonas aeruginosa* (A) before treatment to disinfectant, (B) after treatment to disinfectant

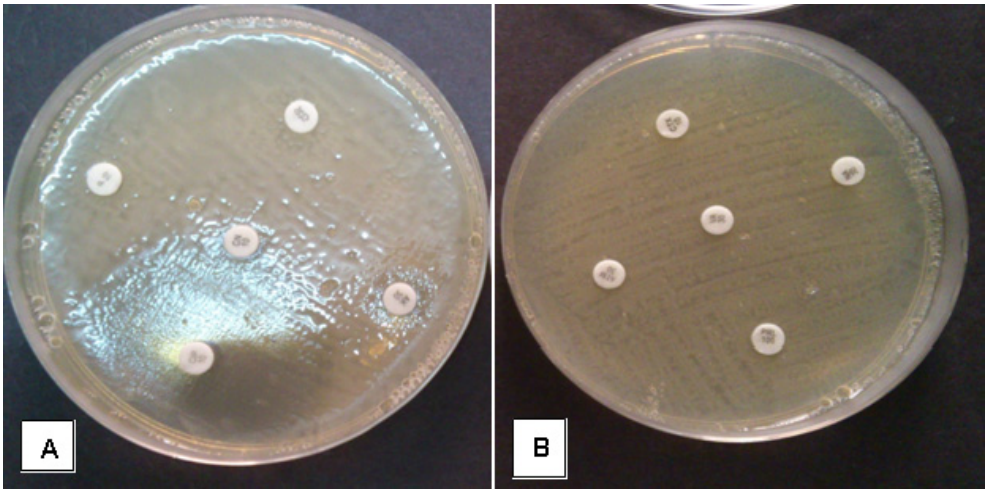

Figure 3. Shows Antibiotics susceptibility of *Pseudomonas aeruginosa* (A) before treatment to disinfectant, (B) after treatment to disinfectant

These results pointed that treatment of *Pseudomonas aeruginosa* with sub-MIC of Claradone and Sarttol make this bacterium to become resistant to some antibiotics. These results were consistent with those of Olukemi and Funmilayo (2011), who found that the use of sub-inhibitory concentrations of the disinfectants causes a development in resistance and virulence of bacterial strains. They concluded that using lower concentrations of disinfectants than advised by the manufacturers might have serious influence on bedridden patients.

Poole (2002) reported that the resistance of microorganism to disinfectants typically results from a change in their cells that is affected by disinfectant accumulation, expression of efflux mechanisms and rarely from mutations.

Iroha et al. (2011) elucidated in their study that the continuous usage of disinfectants lead to the evolve of some nosocomial microorganisms and became resistant to antibiotics.

In the study of Mc Cay et al. (2010) they investigated, the theory of adaptation to disinfectants can develop antibiotic resistance. They related it to the type of the antibiotic, mainly the disinfectant and to the organism. Further to this, they concluded that the sub-MIC of disinfectant could keep and choose the coadapted bacteria in the natural environment; the outcome of the selection process is highly affected by disinfectant concentration and the limitation of nutrients.

In this study, the development of *P. aeruginosa* resistance to antibiotics may be a result of adaptation to the disinfectant used. The precise role of plasmids in disinfectant resistance and the biochemical, physiological and molecular changes in the bacteria after using disinfectants are unknown and need extensive studies. To shed more light on the relationship between development of antibiotic resistance and constant using of disinfectant, it is recommended to include more examples of opportunistic bacteria, in future.

4. Conclusion

It can be concluded that disinfectants (Claradone and Sarttol) can a train *Pseudomonas aeruginosa* to resist some antibiotics and become multidrug resistant (superbug), when these disinfectants incorrectly diluted.

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