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Research article

Isolation, identification of *pseudomonas aeruginosa* from a dental unit water line and effect of disinfectants on its antibiotic susceptibility

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Abstract

Many clinical isolates are pathogenic strains that can be transmitted from one individual to another if health safety measures are not followed. Resistance of pathogenic bacteria to some antibiotics can be acquired due to use of sub-MIC levels of disinfectants. In this study, we isolated a *pseudomonas sp.* strain from biofilm lining the suction tube of a dental unit in a public hospital. The isolation medium was *pseudomonas*-base agar and the total count was 3 CFU/ ml. The isolated strain was gram negative and when examined according to API 20 NE tests was identified to be *pseudomonas aeruginosa.* The MIC of the disinfectant "Nanofix" to *p. aeruginosa* was determined. The antimicrobial susceptibility of this strain was tested before and after the disinfectant "Nanofix" treatment against 7 antibiotics namely; ciprofloxacin, tazobactam, aztreonam, meropenem, clavulanic acid, ceftizidime and vancomycin by agar diffusion method. Before disinfectant treatment the *p. aeruginosa* isolate showed resistance towards meropenem and ceftizidime while it showed a weak susceptibility to tazobactam and aztreonam showing inhibition zones of 1.2 cm and 1.2 cm. A moderate susceptibility to vancomycin with an inhibition zone of 1.5 cm and the strongest susceptibility was towards ciprofloxacin and clavulanic acid giving inhibition zones of 2.6, 2.2 cm respectively. After sub-MIC nanofix treatment the *p. aeruginosa* isolate showed no inhibition zones against tested antibiotics except for ciprofloxacin 1.8 cm and clavulanic acid 1.2 cm indicating induced resistance.

Introduction

Opportunistic pathogens were detected in various parts of dental machine units. Especially in the absence of the proper hygiene and safety precautions. The presence of these pathogens results in the formation of a biofilm. This biofilm is considered a source of infection to other patients. Among the pathogens reported to inhabit such biofilms is, *pseudomonas aeruginosa,* one of the major nosocomial infection pathogens [1]. Immuno compromised patients showing diseases like, cancer, chronic bronchitis and cystic fibrosis are subjected to high mortality rates because of the infection with this pathogen. *P. aeruginosa* is found where humidity is found in places like humidifiers, water baths, sinks and suction tubes of dental units and hospitals [2].

Biofilms harbour bacteria that can survive the application of antibiotics prescribed during the treatment course [3]. The inaccessibility of antibiotic to bacteria within biofilm can be referred to; the polysaccharide matrix [4], high cell density and starvation cell phenotype. Also, certain active ingredients in disinfectants can increase the occurrence of antibiotic resistant bacteria [5] or the cells contained in a biofilm express a less susceptible phenotype.

Antiseptics or disinfectants are biocide products that destroy and inhibit the growth of microorganisms in or on living tissue. The disinfectants composition, dilution, organic charge, and temperature affect the antimicrobial activity of disinfectants [6]. On the other hand, if the disinfectant is used in a concentration below the one required to inhibit or kill the bacterial cell, it might cause bacterial cells to develop resistance to some antibiotics.

Dental units and dental equipments like; Hand pieces, suction tubes, scalers and syringes are places where opportunistic pathogens can inhabit if the sterilization procedure is not efficient. Aerosols can be a source of

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infection to the pulmonary system of humans due to dispersion of biofilm fragments [7]. The treatment of *p. aeruginosa* infections is not an easy task because of their acquired resistance to many disinfectants and antibiotics, like cephalosporins [8]. Aminoglycosides and fluoroquinolones latest generations proved to be efficient in the elimination of *p.aeruginosa* infection [2].

Disinfectants are used on a large scale in hospitals to prevent the spread of different pathogenic infections. That's why we have to elucidate the relationship between antibiotic resistance and disinfectant use on one side and whether disinfectants can cause antibiotic resistance on the other side. Researchers proved that, the effect of disinfectants on microorganisms depends upon their concentration [9].

The aim of this study was to confirm whether using of disinfectants could make bacteria resistant to some antibiotics, emphasizing the need for effective means of reducing the pathogenic bacteria within dental units and elucidating the risk of cross-infection in dental practice if hygiene procedures are not properly followed. Particularly in the case infections caused by *p. aeruginosa*.

Materials and Methods

Collection of samples

A sterile curette was used to collect biofilm samples from the inner part of the suction tube of a dental unit in the National Research Centre public hospital. Each sample was placed in 10 ml phosphate buffer glass tubes, adjusted to pH 6.5-7.0.The tubes were kept at 4°C for less than 3h until they reached the lab.

Isolation of *pseudomonas sp.* strain

The sample tubes were vortexed for 1 min. then, centrifuged at 5000 rpm for 5min. and residue was discarded. Serial dilution (10^{-1} – 10^{-3}) was made to each sample in phosphate buffer pH 6.5-7.0. Pseudomonas base agar medium was poured in sterile petri-dishes after seeding with 0.1 ml of each sample dilution separately. The plates were incubated at 35°C for 48h. The produced *pseudomonas sp.* colonies were counted and preserved on nutrient agar slants.

Gram staining

The smear was prepared from the culture and flooded with crystal violet solution for two minutes. Then the slide was washed with distilled water and gram's iodine was applied for one minute. After that 95% alcohol was applied until the colour runs off. Finally dilute fuchsin solution was applied for about one minute. Then the slide was washed with distilled water and microscopically examined under oil immersion.

Identification of *p. aeruginosa* by API 20 NE

The chemical tests of API 20 NE system were performed on 24h old culture grown at 35°C on nutrient agar.

Disinfectants and antibiotics

Disinfectant used in this study was nanofix, a product of the Egyptian detergent industries company (EEC1336/2008 1272/2008). The active ingredient is alcohol ethoxylates 2% (w/v). The antibiotics used were ciprofloxacin, tazobactam, aztreonam, meropenem, clavulanic acid, ceftizidime and vancomycin. The concentration of each antibiotic was 500 mg/ml. All antibiotics were manufactured by the Egyptian int. Pharmaceutical industries company.

Minimum inhibitory concentration test (MIC)

The minimum inhibitory concentration is the highest dilution which fails to show growth. In this test, the required dilutions of the antibiotics were prepared in sterile distilled water (5%, 10%, 15%, 20% and 25%). The liquefied nutrient agar medium was inoculated by1 ml of the culture suspension of *p. aeruginosa,* poured in Petri- dishes and left to solidify. Wells of 1 cm diameter were made. Each well was inoculated with 0.1 ml of each of the antibiotic sample dilutions separately. Dishes were incubated at 35°C for 24h. The inhibition zones produced if any were measured in cm and MIC was determined.

Test of antibiotic susceptibility before disinfectant treatment

Susceptibility to antibiotics was tested by the agar diffusion method where a volume of 0.1 ml of each antibiotic was inoculated in 1.0 cm diameter wells made in nutrient-agar medium, in sterile Petri dishes. The nutrient agar medium was seeded by 1.0 ml of a 24h old *pseudomonas aeruginosa* cell suspension. The Petri dishes were incubated at 35°C for 24 h and inhibition zones were measured.

Test of antibiotic susceptibility after disinfectant treatment

The surviving colonies after sub-MIC disinfectant treatment (5%) were used for agar diffusion method like described in the previous test.

Results and Discussion

The isolated *pseudomonas sp.* strain was gram –ve and was identified by API 20 NE tests according to table 1 to be *pseudomonas aeruginosa.* The colony count was 3 CFU/ml.

Minimum inhibitory concentration (MIC) of nanofix to *p. Aeruginosa*

The MIC of the disinfectant under test which is nanofix against *p. Aeruginosa* was found to be 10% according to table 2.

Table 1. Identification of *pseudumonas aeruginosa* strain by API 20 NE

Test	Reaction of the strain
Oxidase reaction	+
Catalase reaction	+
Growth at 42°C	-
Production of fluorescent pigment	+
Indole production on tryptophane	-
Glucose acidification	-
Argenine dehydrolase	+
Urease	+
Esculin hydrolysis	-
Gelatine hydrolysis	+
β-Galactosidase	-
D-Glucose	+
L-Arabinose	-
D-Mannose	-
D-Mannitol	+
N-Acetyl-D-Glucosamine	+
Maltose	-
Gluconate	+
Caprate	+
Adipate	+
L-Malate	+
Citrate	+
Phenylactate	-

Table 2. Minimum Inhibitory Concentration (MIC) of Nanofix

Concentration (%)	5	10	15	20	25
Inhibition zone (cm)	-	1.8	2.2	2.9	3.5

Antibiotics susceptibility

Infections caused by *p.aeruginosa* can be fatal. They are usually difficult to treat [10]. Their antimicrobial susceptibility is limited to only a few drugs [11]. And the increased use of antibiotics can cause the emergence of antibiotic resistant strains [12].

Figure 1 and 2 demonstrate the susceptibility of *p. aeruginosa* isolate to seven antibiotics used for the treatment of infections caused by this bacteria. Indicating that, the *p. aeruginosa* was susceptible to 5 antibiotics (Ciprofloxacin, Tazobactom, Aztreonam, Clavulanic acid and Vancomycin with inhibition zones 2.6, 1.2, 1.2, 2.2, 1.5 cm respectively) before nanofix treatment while it was susceptible to only two antibiotics (Ciprofloxacin and Clavulanic acid with inhibition zones 1.8 and 1.2 cm respectively) after treatment with sub-MIC level of the disinfectant nanofix.

A similar research proved that, the development of resistant bacterial strains can be a result of use of sub-inhibitory concentrations of the disinfectants [13]. The disinfectant accumulation and efflux mechanisms can cause the resistance of microorganisms to antibiotics as a result of the change in their cells and rarely from mutations [14]. Researchers confirmed that the continuous usage of disinfectants lead to the evolution of some nosocomial microorganisms that became resistant to antibiotics [15]. In a similar study the theory of adaptation to disinfectants which can develop antibiotic resistance. In this study, the development of *p. aeruginosa* resistance to antibiotics may be a result of adaptation to the disinfectant used [16,17].

The findings of a similar research confirmed that the *pseudomonas sp.* isolates were susceptible to ciprofloxacin and norfloxacin. Ciprofloxacin should be given when necessary as an alternative therapeutic agent for resistant isolates [18]. In another study, gentamicin was found to be effective in the treatment of infections caused by gram negative bacteria. However, other reports showed increased percentage of resistance to this drug where, a number of isolates were showed resistance [19]

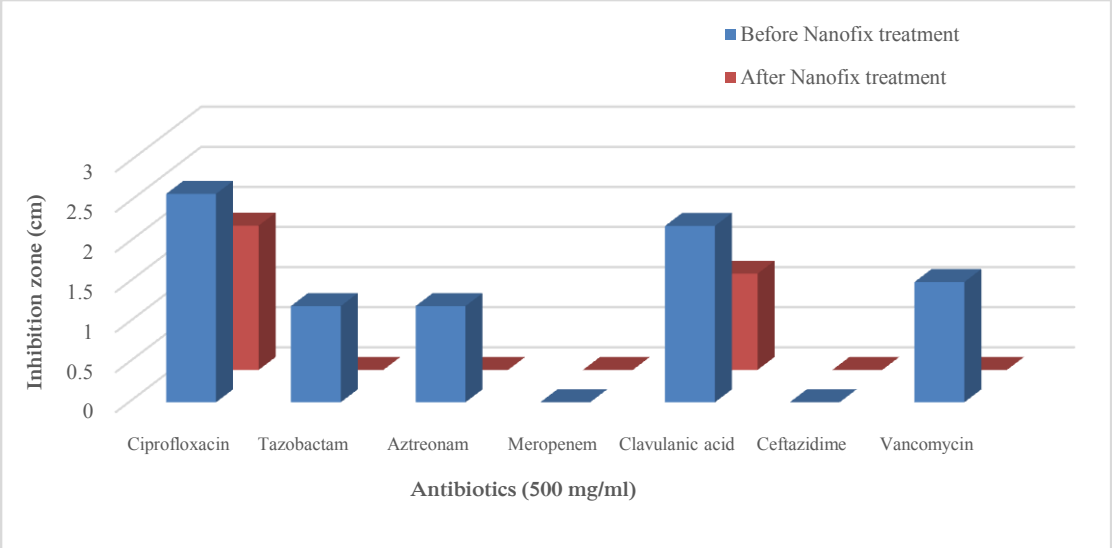
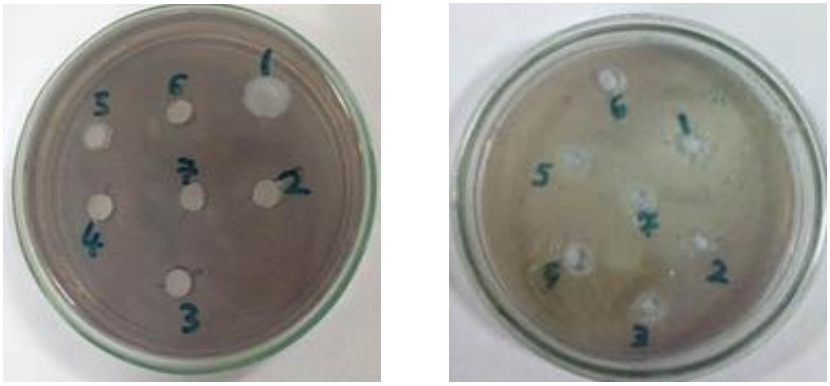


Figure 1. Effect of antibiotics on *pseudomonas aeruginosa* before and after nanofix treatment



After Nanofix treatment *Before Nanofix treatment*

1:Ciprofloxacin, 2: Tazobactam, 3:Aztreonam, 4: Meropenem, 5:Clavulanic acid, 6:Ceftazidime, 7:Vancomycin

Figure 2. Inhibition zones of antibiotics against p. aeruginosa

Conclusion

It can be concluded that some disinfectants can cause induced resistance in *pseudomonas aeruginosa,* causing it overcome the action of some antibiotics and become multidrug resistant (superbug), if these disinfectant products are used in an incorrect dilution.

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