Investigation of *Pseudomonas aeruginosa* bacteria in a number of Baghdad schools and extent of their resistance to disinfectants and sterilizers

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Abstract

Purpose – This study was design to investigate of *P. aeruginosa*, an example of Gram-negative bacteria, in seven primary and secondary schools of Baghdad city, and the effects of Ethanol and Dettol of *P. aeruginosa* biofilm.

Design/methodology/approach – Seventy swabs were collected from seven primary and secondary schools of Baghdad city, Iraq, during November -December 2022. Swabs were collected from classes desk, doors handles, students hands and water taps. Standard microbiological testing methods were used on the samples for isolation and identification. The ability of bacteria to form biofilm and the effects of Ethanol and Dettol on"preformed" biofilms was examined by microtiter plate with the use of an ELISA reader.

Findings – In 70 swabs from seven primary and secondary schools, growth was observed in 33 swabs as *P. aeruginosa*. Primary schools were higher contaminated than secondary and water taps and door handles represented the main source of this contamination. The ability of bacteria to produce biofilm was observed in 19 (57.6%) isolates and 14 (42.4%) nonbiofilm producers. As well as, Ethanol (70%) treatment of preformed biofilms led to enhance biofilm formation and revealed significantly greater staining after 4 and 24h than Dettol (3%) compared to an untreated control (tryptic soy broth (TSB) incubation).

Originality/value – Studies on *P. aeruginosa* in Iraqi schools are quite rare. This work is considered distinctive because it drew attention to the presence of pathogenic bacteria within primary and secondary schools, which are not considered their natural environment.

Keywords *P. aeruginosa*, Preformed biofilms, Ethanol, Dettol

Paper type Research paper

Introduction

People spend more than 90% of the day in indoor environments, therefore indoor air quality, or Indoor Air Quality (IAQ), is a growing concern for public and occupational health (Nasir, Colbeck, Sultan, & Ahmed, 2012). Children are one particularly vulnerable category. Because their developing lungs make them less able to handle harmful substances, children are more susceptible to environmental toxins than adults because they breathe more air proportional to their body weight (Branco *et al.*, 2016).

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In school classrooms, the typical characteristics that contribute to the efficient transmission of germs are present: extended close contact between individuals, a large number of often touched surfaces and separated cleaning. Maintaining high-quality interior settings is a challenging issue in school buildings because of their crowded population (Oleiwi, 2017). Since various germs are often encountered during the school day, pathogenic microbes pose a severe hazard in schools. Numerous bacteria reside in the human body, however they are mostly benign and would only become hazardous if they moved to another part of the body (Maori, Okemena, & Wasa, 2013). A bacterial infection can also result from an overabundance of germs in the body, which can cause the bacteria to multiply quickly and physically disrupt the heart, lungs or any other organ's ability to operate. Numerous studies have demonstrated that a variety of harmful microorganisms may be found on contaminated hands, and that hands are a major factor in the spread of fecal-oral illnesses (El-Kased & Gamaleldin, 2020). Additionally, the majority of nursery and primary schools see a consistent pattern of illness transmission through contaminated hands of students. The World Health Organization has acknowledged that improper hand hygiene practices have a major role in the spread of infectious illnesses (World Health Organization guidelines on hand hygiene in health care, 2013).

Furthermore, it is thought that human bacteria that may cause dangerous illnesses like shigellosis find their basic home in human feces. As a result, using toilets in an unsanitary manner and failing to wash your hands after using the restroom might spread bacteria to nearby areas. As a result, bacteria will easily grow on doorknobs, desks, water taps and other surfaces, spreading to anybody who comes into touch with them, including teachers, cleaners, and pupils (Ramakrishna, 2007). It is a widespread conclusion that schoolchildren's hands contain microbial flora, such as *Staphylococcus aureus*, *Klebsiella* sp., *Enterococci* sp., *Escherichia coli*, *Pseudomonas aeruginosa* and some yeast (Japka, Campbell, Esther, & Sheri, 2011). *P. aeruginosa* may thrive in different host conditions and produce a wide range of infections according to its diverse set of virulence factors like biofilm (shrout *et al.*, 2006). Biofilm-forming bacteria become resistant to antibiotics, UV radiation, chemical biocides, the human immune system and other external stimuli because they are embedded in a matrix. Today, it is believed that more than 80% of chronic infectious diseases are caused by biofilm, and it is widely known that conventional antibiotic therapies are inadequate for treating these biofilm-mediated illnesses (Li & Lee, 2017).

Disinfectants and antiseptics are frequently employed as agents to kill or eradicate germs, notably in hospitals, care facilities for other people and animals, and microbiological laboratories (MacDonnell & Russell, 1999). The selection of disinfectants and antiseptics against pathogenic microorganisms has become a widespread challenge as a result of the extensive usage of these chemicals to regulate and inhibit the growth of bacteria in both living tissue and inanimate objects (Russell & Russell, 1995). Ethanol, Dettol, chlorohexidin and soap are the most often used disinfectants in microbiology labs. As a dehydrating agent, ethanol damages cell membranes, causes protein denaturalization and induces cell lysis (AL-Dabbagh, Ali, Khalil, & Hamad, 2015). It is used in a number of settings to disinfect skin, medical equipment and cooking equipment since it is volatile, leaves little residue and is safe even if used orally. Although ethanol is often utilized, it is uncertain how bacteria would behave when exposed to low levels of ethanol. Environmental factors have been discovered to cause biofilm development thus far, according to many researches. Antibiotics, for instance, aminoglycosides, tetracyclines and chloramphenicol, which are protein synthesis inhibitors, enhance the production of biofilm by *P. aeruginosa* and Escherichia coli (Tashiro *et al.*, 2014). Dettol also affects the cytoplasmic membrane of bacteria by causing denaturation of protein (Manivannan, 2008). It has been revealed that the disinfection agent has antibacterial activity against some harmful microorganisms. Additionally, bacteria are becoming increasingly resistant to modern antiseptics and disinfectants (Wisplinghoff, Schmitt, Wöhrmann, Stefanik,

& Seifert, 2007). In this study, we investigated *P. aeruginosa*, an example of Gram-negative bacteria, in numbers of Iraqi schools and the effects of ethanol and Dettol on their biofilm.

Materials and methods

Samples collection

Current study carried out in seven randomly selected primary and secondary public schools of Baghdad city, Iraq. During November and December 2022. All selected schools were built more than 40 years ago, with dilapidated classes and toilets that lack sanitary conditions and ventilation. Out of 70 swabs were collected from classes' desk, doors handles, students hands and water taps as shown in Table 1. Before taking samples from the students' hands, their consent was obtained. Swabs were taken using a sterile cotton swab, placed in sterile tubes with normal saline and then transported immediately to the microbiology lab in the Department of Biology, College of Science, University of Baghdad for bacteriological identification.

Isolation and identification

Standard microbiological testing methods were used on the samples, including a range of enrichment, selective and differential media (MacConky, Cetrimide and Nutrient agar), (colony morphology analysis using Gram's stain, and several biochemical studies as oxidase and catalase test) (Collee, Miles, & Watt, 1996) and the VITEK 2 system, which was employed to complete the identity.

Ability to form biofilm

P. aeruginosa was grown overnight at 37 °C in tryptic soy broth (TSB), which contains 0.25% glucose, to determine its propensity to produce biofilms. Following that, broth cultures were evaluated using a microtiter plate biofilm formation assay using the same medium as a diluent in comparison to McFarland standard tube No. 0.5. Each well on a 96-well flat-bottomed polystyrene plate with three wells inoculated with 125 L of an isolate suspension before being incubated for 24 hours at 37 °C as previously mentioned (Stepanovic, Vukovic, Dakic, Savic, & Svabic-Vlahovic, 2000). These "preformed" biofilms were then rinsed three times with 300 mL of distilled water. The wells were dyed for 10–15 minutes with 125 L of a 0.1% crystal violet solution in water after being dried inverted at room temperature. The wells were washed three times to eliminate remaining crystal violet after it was extracted. The stain dissolves in 125 L of 30% acetic acid and measures optical density (OD) 540 nm. With the use of an ELISA reader (Stat Fax-2100), the absorbance of the de-staining solution was measured at 540 nm. Each test was performed in triplicate. The un-inoculated medium was used as a control to calculate the background OD. Three standard deviations more than the mean OD of the negative control was designated as the cutoff OD (ODc). The isolates were grouped into four groups based on the microtiter plate results, as indicated in Table 2.

Table 1. Collected swabs from surfaces

Type of swab	Numbers of swabs
Classes desks	13
Door handles	31
Students hands	16
Water taps	10

Effect of disinfectants and sterilizers on biofilm

The effects of ethanol and Dettol on *P. aeruginosa* biofilm were completed in two stages: First off, biofilms of *P. aeruginosa* were performed as described in above step. Secondly, 300 µL of distilled water were used to wash these "preformed" biofilms three times. The wells were allowed to dry at ambient temperature while inverted. We examined how ethanol (70%) and Dettol (3%), as given in Table 3, affected biofilm. Plates were incubated at 37 °C for 4 and 24 h after the addition of the disinfectants and in the control wells that did not contain any disinfectants (TSB incubation). These periods of time, respectively, were used to assess the effects of both short- and long-term disinfectants exposure. The supernatant in each well was removed after disinfectants treatment. The plates were washed twice in water, stained for 10 minutes in 0.1% crystal violet and then washed four times in water to determine the presence of biofilms. Biofilms might be seen on the bottom of wells as purple-stained films. By dissolving the dye in 30% acetic acid and measuring OD540 nm, biofilms were quantified (Conover, Mishra, & Deora, 2011).

Statistical analysis

The impacts of the variables in this study were examined using the statistical package for social science (SPSS) 2018 software. Categorical data were created using count and percentage. *t*-test was used to evaluate how ethanol and Dettol affected the biofilm (SAS, 2018).

Results and discussion

Isolation and identification of P. aeruginosa

Recent results indicated out of 70 swabs (classes desk, door handles, students hands and water taps) obtained from seven primary and secondary schools, growth was observed in 33 samples as shown in Table 4. The cetrimide agar, which is known to be poisonous and to suppress other microbial flora, is the medium on which these isolates are grown. Colonies featured elevated centers, flat edges, a creamy color and a fruity odor. They also had a mucoid, smooth appearance. *P. aeruginosa* growth was identified by pyocyanin production results in a shiny green pigment (Figure 1). In accordance with Markey, Leonard, Archambault, Cullinane, and Maguire (2013), the investigation also revealed that

Table 2. Microtiter plate classification of bacterial biofilm formation

Biofilm intensity	Cut-off value
None producer	OD ≤ ODc*
Weak	ODc < OD ≤ 2*ODc
moderate	2*ODc < OD ≤ 4*ODc
Strong	4*ODc < OD

Note(s): *OD = optical density, ODc (Cut off value) = average OD of negative control + (3*Standard Deviation)

Source(s): Stepanovic *et al.* (2000)

Table 3. Shown disinfectants and sterilizers that used in this study

No.	Commercial name	Formula and chemical name	Concentration	Origin
1	Ethanol alcohol	Ethanol alcohol CH3CH2OH	(%70)	China
2	Dettol	chloroxylenol (C8H9ClO)	(%3)	Iraq

Pseudomonas species were represented in these isolates since they presented as Gram-negative, nonspore-forming rods. The 33 isolates were frequently stained with Gram stain, during the microscopic examination for target isolates. Straight or slightly rod shapes were observed.

The results of current study revealed that the primary schools higher contaminated than secondary schools with *P. aeruginosa*. Also, water taps and door handles represented the main source of this contamination. It was discovered that hand contamination of places like desks and other classroom items significantly predicted the likelihood of developing diarrhea. It is necessary to investigate ways to lessen these contaminations, such as emphasizing hygiene education and keeping track of hand washing (Kaltenthaler, Elsworth, Schweiger, Mara, & Braunholtz, 1995). Current results agreed with Oleiwi (2017) that found Baghdad official schools higher contamination with *P. aeruginosa* than private ones. Also, Badri, Alani, and Hassan (2016) could isolated Pseudomonas sp. from the air samples of Baghdad schools. In primary schools in Malaysia and Poland also, found the most frequently isolated Gram-negative bacteria were Pseudomonas spp. (Hussin, Sanna, Shamsudinb, & Hashima, 2011; Bragoszewska, Mainka, Pastuszka, Lizonczyk, & Desta, 2018).

Biofilm formation

According to recent findings, each isolate had a varied capacity for forming biofilm under the same conditions. According to the criteria shown in Table 5 and Figure 2, the findings were

Values biofilm intensity	Calculation mean of OD	Cut-off value
None producer	OD ≤ 0.064	OD ≤ ODc
Weak	0.064 < OD ≤ 0.128	ODc < OD ≤ 2 * ODc
moderate	0.128 ≤ OD ≤ 0.256	2 * ODc < OD ≤ 4 * ODc
Strong	0.256 < OD	4 * ODc < OD

Table 4.
Biofilm production capacity (OD540 nm) of bacterial isolates

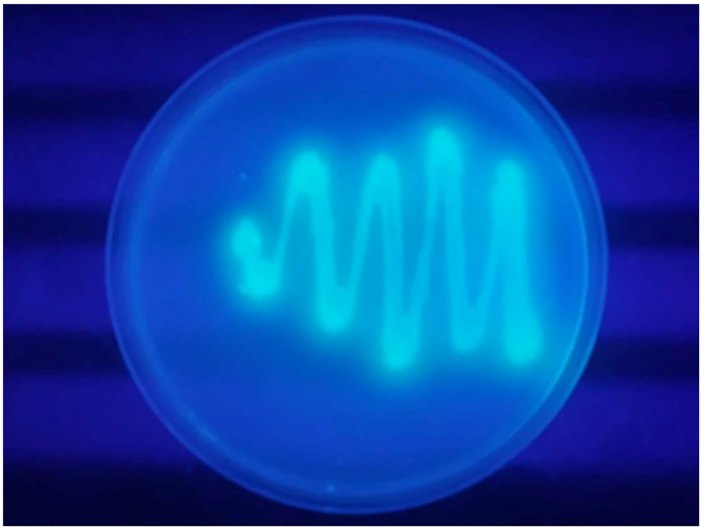


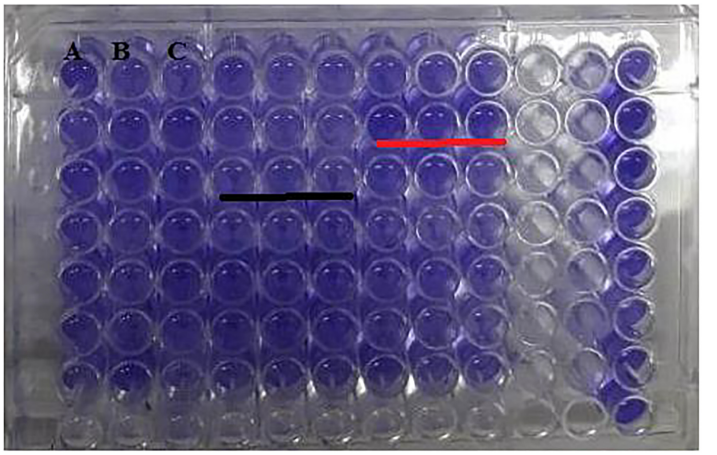
Figure 1.
P. aeruginosa colonies on Cetrimide agar after 24 hours of incubation at 37 ℃

divided into four groups: nonproducer, weak, moderate and strong biofilm producer (Stepanovic *et al.*, 2000).

According to the findings, out of 33 isolates of *P. aeruginosa*, 19 (57.6%) were biofilm producers, with 7 (36.8%) as strong biofilm producers, 3 (15.8%) moderate producers, 9 (47.4%) weak producers, and 14 (42.4%) non-biofilm producers as shown in Figure 3.

Table 5. numbers of bacteria isolates according to their source

Source	No. of isolates (%)	Type of schools	
		Primary	Secondary
Classes desks	4 (30.8%)	3 (75%)	1 (25%)
Door handles	18 (58.1%)	13 (72%)	5 (73%)
students hands	3 (18.8)	3 (100%)	0
Water taps	8 (80%)	5 (62.5%)	3 (37.5%)



Note(s): Strong biofilm development was seen by the red line

Figure 2. A micro titer plate A, B and C represented isolates were tested in triplicate, and the black line denoted a weak biofilm development

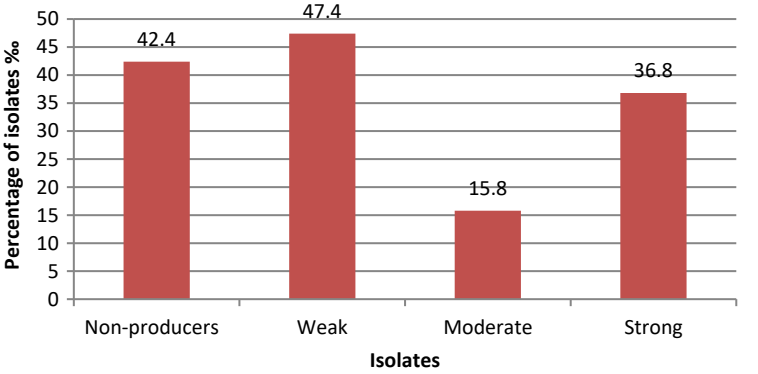


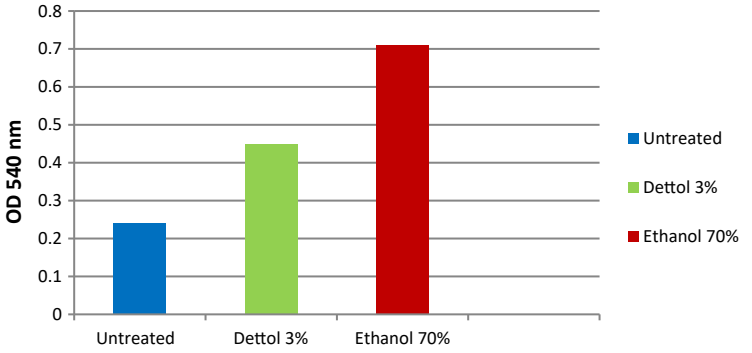
Figure 3. Distribution of *P. aeruginosa* isolates, among nonproducers, weak, moderate and strong groups of biofilm production

Effect of disinfectants and sterilizers on biofilm formation

As stated in materials and methods, we cultured *P. aeruginosa* biofilms on 96-well plates for 24 hours at 37 ℃. These biofilms were exposed to 70% ethanol and 3% Dettol for 4 and 24 hours at 37 ℃, respectively, and then in contrast to an untreated control group TSB incubation. We discovered that preformed biofilms could not be eliminated by disinfectants treatments. After 4 hours of treatment with ethanol (70%) and Dettol (3%) staining was greatly enhanced, according to quantification of biofilm levels following disinfectants treatment. Figure 4.

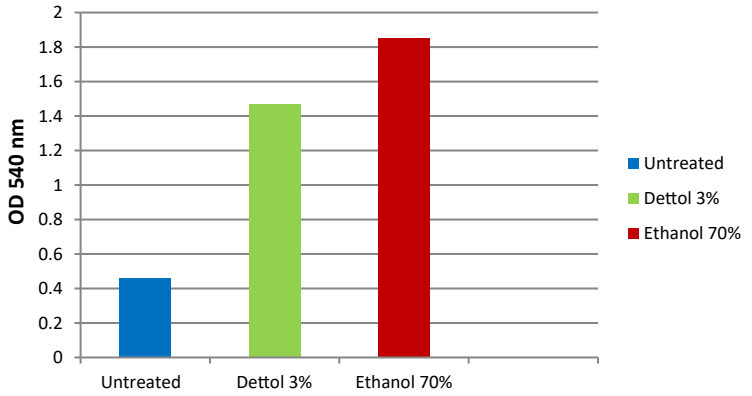
Similarly, disinfectants and sterilizers treatment failed to destroy biofilms after 24 h. But on the contrary, disinfectants and sterilizers treatment of preformed biofilms led to enhance biofilm formation and revealed significantly greater staining after 24 h as shown in Figure 5.

We note that ethanol 70% concentration enhanced biofilm formation higher than Dettol 3%, while untreated isolates had the lowest biofilm formation at both exposure time 4 and 24 h. In general disinfectants treatment of preformed *P. aeruginosa* biofilms resulted enhanced



Note(s): After a 4-h period, biofilm levels significantly increased (*$p < 0.05$)

Figure 4.
P. aeruginosa biofilm levels 4-h after Ethanol 70% and Dettol 3% treatment



Note(s): After a 24-h period, biofilm levels highly significant increased (*$p < 0.05$)

Figure 5.
P. aeruginosa biofilm levels 24-h after Ethanol 70% and Dettol 3% treatment

biofilm formation (Figures 4 and 5). Also, AL-Dabbagh *et al.* (2015) found after 5 minutes of the exposure to the different concentration of Ethanol and Dettol, Dettol was less activity against *P. aeruginosa* than Ethanol. Tashiro *et al.* (2014) revealed low concentrations of Ethanol increase initial biofilm formation in *P. aeruginosa* after Ethanol treatment of planktonic bacteria. In contrast to the results of the current study, on a group of Pseudomonas bacteria isolated from Hospital in Libya, Elrotob, Almahjub, and Abudena (2021) found a delay in the effectiveness of Ethanol and Dettol and none showed any effect during the first 20 minutes of exposure. Twenty four hours later, good efficacy was observed for Dettol, but not on all types of bacteria, while Ethanol showed slight results starting from minute 20.

Conclusion

Used of disinfectants and sterilizers in this study led to an enhancement in the preformed biofilms of *P. aeruginosa*. Ethanol at (70%) and Dettol (3%) concentrations maybe accelerate the formation of biofilms by enhancing auto-aggregation capacity and decreasing swimming motility. Also, Ethanol was shown more effect than Dettol against biofilm formation after 4 and 24 h of exposure. The growing use of these sterilizers and disinfectants has led to a rise in outbreaks and microbial resistance, particularly when microbes are able to form biofilms.

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