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Study of the Effect of Acetic Acid on Biofilm Formation of Pseudomonas Aeruginosa Isolated from Different Clinical Cases

Suaad Khalil Ibrahim
College of Education for Pure Sciences / Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq

Abstract: 98 samples from various clinical sources (burns, wounds, urine, blood, and sputum) were gathered for this investigation from hospitals in Baghdad. Following microscopic and biochemical analysis, 52 Pseudomonas aeruginosa isolates were discovered. The microtiter plate method was used to determine if the bacterial isolates could create biofilm. The outcomes revealed that 80% of the detaches could shape biofilm to varying degrees, with 28.9% of the disengages framing frail biofilm, 24.4% medium biofilm, and 26.7% solid biofilm. Acetic acid shown anti-biological activity, hence a progressive effect of the acid (0.009 – 2.5 %) on biofilm formation was also investigated.

Keywords: Pseudomonas aeruginosa, biofilm, acetic acid, antiseptic, antagonist agents.

Introduction :

Pseudomonas aeruginosa is an important bacterial species because it is widespread in the environment. It lives in wet places, including moist soil, on the surface of vegetables and streams, as well as parasitizing on humans and animals, causing many diseases such as Wounds and burns infections, urinary tract infections, bacteremia, meningitis, endocarditis (Bhasin*et al*., 2015; Hameed and Abdulhay, 2016),*Pseudomonas aeruginosa* has many virulence factors including hemolycin, exotoxin A, leukocidin, flagella, alginate layer, and biofilm (Jimenez *et al*., 2012; Hakman and Al-Daraghi, 2022).

A biofilm is a mini-environment that generally consists of two components: microbial cells andexopolymeric substance which constitutes 90% of the total biomass(Al-Mathkhury*et al*., 2011; Saxena*et al*., 2019). Biofilm provides protection from high temperatures, drought(Scher *et al*., 2005) and host defenses(Leid *et al*., 2002). In addition to protection, it enables interaction between populations. Proximity to cells facilitates horizontal gene transfer and the sharing ofsecondary metabolic products(Dekievit, 2009)

The difficulty of treating infections caused by *Pseudomonas aeruginosa* bacteria is due to its resistance to many antibiotics such as overuse or non-compliance of patients with treatment as well as poor quality antibiotics (Odonkor and Addo , 2011; Hameed*et al.* 2019) . Biological control of biofilm formation is a new strategy instead of killing bacteria, as it focuses on impeding the biofilm formation process as well as reducing the production of antibiotic-resistant bacteria(AL-Fridawy*et al.*, 2020; Obaid and Abdulwahhab, 2021).

Acetic acid is a colorless, weak organic acidand its chemical formula(CH_3COOH) it is present in vinegar in a concentration (3-5)% and can be used as an antiseptic for wounds and burns caused by bacteria (Al-Muslih*et al.*, 2009; Nagoba *et al*., 2013).The researcher Hajati(2018) confirmed that organic acids have antimicrobial activity, just like antibiotics, as they can penetrate the bacterial cell wall causing a change in the cellular pH gradient and then the

collapse of the proton gradient necessary for the process of making ATP (AL-Naemi, 2016; Pande *et al*., 2018) .

Materials and methods

Collection of bacterial samples :

Between September 30, 2020, and January 1, 2021, 98 samples were taken from various clinical sources, such as 26 burns, 25 wounds, 26 urine, 9 blood, and 12 sputum samples from the Medicine City and Central Child Hospitals in Baghdad.

Isolation and identification :

Bacterial isolates were diagnosed by culturing them on blood agar, cetrimide agar, and macConkey agarand studied its phenotypic characteristics in terms of colony shape, smell and color (Baron *et al*., 2007; Ibrahim, 2020) Together with biochemical testing (Oxidase, Indole synthesis, Citrate utilization, Hemolysin, Catalase, and Gelatin hydrolysis) and microscopic analysis (Gram stain).

Stock acetic acid:

Milipolar filter (0.22 µm) was used to sterilize it after 5 milliliters of concentrated acetic acid were added to 95 milliliters of distilled water (Halstead et al., 2015). Make the necessary dilutions using this solution to examine how acetic acid affects the production of biofilms.

Detection of biofilm formation :

The microtiter plate strategy (Cirkovic et al., 2017) was utilized to identify the capacity of P. aeruginosa to frame biofilm. The bacterial suspension was arranged utilizing clean saline and contrasted and the McFarland standard arrangement (0.5). 180 µL of Tryptone soy broth (TSB) enhanced with extra 1% glucose and 20 µL of arranged bacterial suspension was added to each well of the microtiter plate (three replications); the negative control addressed just the medium. Following 24 hours of hatching at 35 5C, the contents of the wells were disposed of and tenderly washed multiple times with 200 µL of clean phosphate cradled saline (PH 7.2) and permitted to dry at room temperature. Cells were fixed with 150 µL of Methanol liquor for 20 minutes after methanol was discarded.Following drying, color joined to the biofilm on the very much was delivered utilizing 150 µL of 96% ethanol liquor per well, and the optical thickness was surveyed at 570 nm utilizing a microtiter plate peruser for 20 minutes at room temperature.

Test the effect of acetic acid on biofilm formation :

The Cirkovic et al. (2017) The impact of acidic corrosive on biofilm development was investigated utilizing a microtiter plate strategy. The bacterial suspension was made with clean saline and contrasted and the McFarland standard arrangement (0.5). To make a negative control, 200 µL of tryptone soy broth (TSB) enhanced with an additional 1 % glucose was added to each well of the main segment of the microtiter plate. To make a positive control, 20 µL of the bacterial suspension was added to the well of the second segment, which contains 180 µL of tryptone soy broth and glucose. For each disconnect and concentration, the bacterial suspension was arranged utilizing clean saline, and the outcomes were contrasted and the McFarland standard arrangement (0.5),, Following a 24-hour brooding period at 35°C, the contents of each very much were discarded and delicately flushed multiple times with 200 µL of sterile phosphate cradled saline (pH 7.2). The wells were then permitted to air dry at room temperature. Then, the cells were fixed for 20 minutes utilizing 150 µL of methanol liquor. Following the methanol removal, the cells were dried and stained for 15 minutes utilizing 2% precious stone violet (150 µL per well). At last, the wells were cleaned with refined water. Following drying, color connected to the biofilm on the very much was delivered utilizing 150 µL of 96% ethanol liquor per well, and the optical thickness was evaluated at 570 nm utilizing a microtiter plate peruser for 20 minutes at room temperature. The restraint proportion was determined utilizing the accompanying equation:

$$inhibition\ ratio(\%) = \frac{control - test}{control} \times 100$$

Result and discussion

Following morphological and biochemical analysis, 52 P. aeruginosa isolates were recovered. Since lactose sugar is not used in the fermentation process, the colonies of these isolates were pale on macConkey agar (Forbes et al., 2007). When the colony grew on the blood agar, hemolysin caused a transparent aura to form around it, while the cetrimide agar appeared bluish and greenish due to its ability to secrete the water-soluble pigments bioverdin and biocyanin (Kayser et al., 2005).Under a microscope, P.aeruginosa bacteria were observed to be single, binary, or short chains of gram-negative rod-shaped bacteria (Brooks et al., 2013).

Regarding the biochemical test discoveries, every seclude showed favorable outcomes for the trial of oxidase, catalase, citrate usage, and gelatinase, however negative outcomes for the indol test. These results matched the data that was provided (Todar, 2011).

The ability to form a biofilm

As shown in figure (2), the consequences of the phenotypic identification revealed that, when contrasted with the negative control, 80% of the bacterial secludes created biofilm to varying degrees, with 20% not shaping, 26.7% framing firmly, 24.4% framing reasonably, and 28.9% shaping pitifully.

The findings of this investigation differed from those of Abootaleb et al. (2020), who reported that 24% of P. aeruginosa isolates formed biofilms, and from those of Hemati et al. (2020), who reported that 14% did not.

The variation in the quantity of cells that at first connected to the microtiter plate wells or the amount of auto inducers (majority detecting signal particles), which are created from each confine and are critical to the development of biofilms, could be the reason for this variation in the thickness of the biofilm (Beenken et al., 2010).

Effect of acetic acid on biofilm formation :

Nine concentrations (0.009, 0.019, 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5 %) of acetic acid were used to examine its effects. Three bacterial strains were shown to have high, moderate, and weak biofilm production. The effects of acetic acid on biofilm formation were displayed in Table (1). For the strong bacterial isolate, the percentage of biofilm inhibition was 39, 43, 45, 46, 61.6, 60, 63, 64, and 60.4 %), while for the bacterial isolate that is an intermediate biofilm formation, the percentage of inhibition was 35, 35, 33, 32, 59, 56, 58, 59.6, 65, and for the weak formation, it was 34.6, 39.5, 40, 43, 60, 66.5, 60, 62, and 72).

According to Tawre et al. (2021), the biofilm was completely inhibited at a concentration of 0.156 % of acetic acid, and the minimum inhibitory concentration of the drug-resistant P. aeruginosa was found to be 0.156 %. Additionally, he observed a decrease in the volume of cells treated with acetic acid and their death upon disruption of the extracellular matrix in comparison to untreated cells, indicating damage to the membrane resulting from the disruption of the proton gradient. While Bjarnsholt et al. (2015) noted that acetic acid must have a PH of less than 4.76 to be effective against P. aeruginosa bacteria biofilms and that acetic acid at a concentration of 0.5% completely eradicated the biofilms, Madhusudhan (2016) indicated that acetic acid is good for many gram-positive and gram-negative bacteria, especially P. aeruginosa. It is frequently used for wounds with concentrations between (0.5 – 5%). Furthermore, for the treatment of P. aeruginosa infection, acetic acid is a useful therapy approach when used either alone or in conjunction with other antibacterial medicines including colistin, ciprofloxacin, and tobramycin. In order to eradicate P. aeruginosa infections, Al-ibran and Khan (2010) investigated the effects of 1% acetic acid on wound infections. They discovered that using acetic acid for 10–14 days eliminated bacteria in 90% of instances.

Conclusion :

Our results demonstrate that Pseudomonas aeruginosa exhibits very significant biofilm inhibition at low concentrations of acetic acid, with 90% efficacy against bacterial growth and biofilm formation. Because it works well both as a standalone antiseptic and as a therapeutic agent when combined with other antagonist compounds, acetic acid is regarded as an excellent antiseptic for wounds and burns. Bacteria are 90% removed after 10 days of use with acetic acid.

Conflict of interests

According to the writers, there are no conflicting interests.

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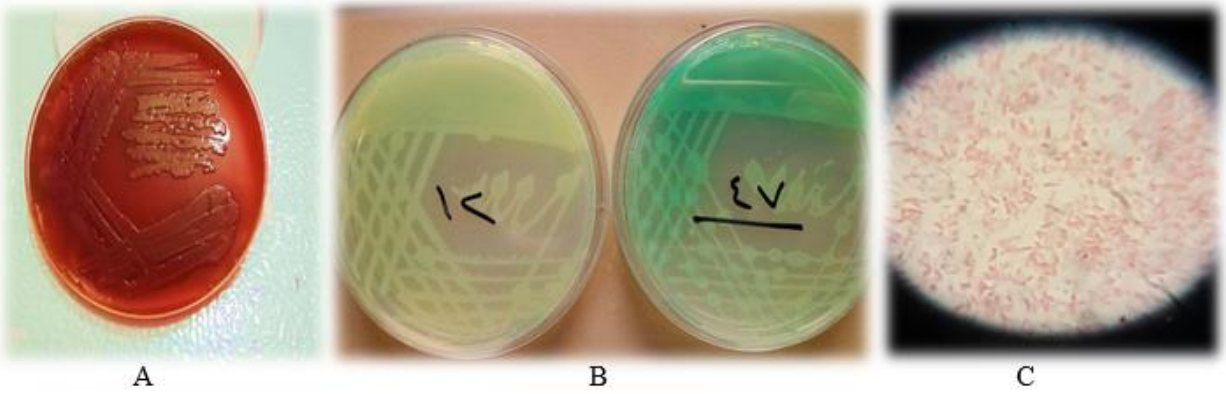
Table (1) effect of acetic acid on biofilm formation

inhibition ratio %			Acetic acid concentration %
Weak formation	medium formation	Strong formation	
34.6	35	39	0.009
39.5	35	43	0.019
40	33	45	0.039
43	32	46	0.078
60	59	61.6	0.156
66.5	56	60	0.312
60	58	63	0.625
62	59.6	64	1.25
72	65	60.4	2.5



A B C

Figure 1 : shape of *P. aerugenosa* : A, Blood agar. B, cetrimide agar. C, Microscope.

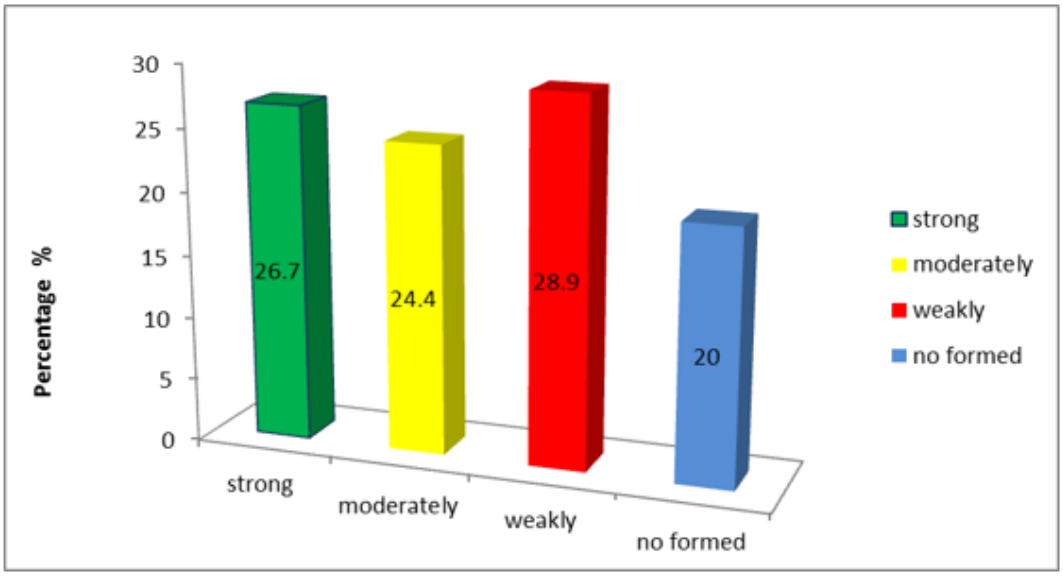


Figure (2) : percentage of *P. aeruginosa*isolates on biofilm formation