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Study Effect of Some Detergents on Biofilm Formation By *Pseudomonas aeruginosa*

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Abstract :
Four samples were taken from patients with burns and wounds, MacConky agar was prepared for isolation and identification the samples , after inoculated and incubated at 37C for 24 hr. the results were pale colonies because Pseudomonas aerugenosa is lactose non-fermenter .The isolated bacteria was cultured on congo red agar to tested its ability for biofilm formation after incubated at 37c for 24 hr. its give positive result by forming black colonies with adhesion . The detergents were used to study their ability to inhibit biofilm formation are zahi al-wazer , qasir brayt , emlaq and lifebuoy by diluted them and add 1 ml to congo red agar after that inoculated with bacteria and incubation at 37c for 24 hr. the results were indicated that all detergents were inhibited the biofilm formation by forming red to black colonies with loss adhesion but Zahi-al-wazer is more effects and lifebuoy is little effect on it.

Keywords: *Pseudomonas aeruginosa, biofilm, congo red, detergents.*

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I. Introduction

P.aerugenosa is a gram – negative bacillus , non- sporing ,non-capsulate and usually motile by virtue of one or two polar flagella measuring about 0.6*2mm[1], growing well at 37-42 C , its growth at 42 C helps differentiate it from other *Pseudomonas* species[2] . *P.aerugenosa* in a culture can produce multiple colony types [2] the most common colonial form is relatively large , low – convex with an irregular surface , an edge that is translucent and an oblong shape with the long axis parallel to the line of inoculum [1] . When it is grown on MacConkey agar it forms pale colonies because its lactose –non fermenter , grow well on cetrimide agar 0.03 % [3]. Most strains produce being greenish-blue due to production of soluble blue phenazine pigment pyoverdin [1], which gives a greenish color to the agar [2] Some strains produce the dark red pigment pyorubin or black pigment pyomelanin [2] ,*P.aeruginosa* is catalase and oxidase-positive[2] its obligate aerobe , that grows readily on many types of culture media[2]. *Pseudomonas* infections often have a characteristic sweet odor and have become a substantial cause of infection in patients with immunodeficiencies. It is one of the main agents of hospital – acquired infections such as pneumonia , urinary tract infections and bacteremia [4]. Because they feed on the wet surfaces , these bacteria can be found in medical equipment such as catheter tube . Therefore , it is one of the infective infection , which is a cause of the rash of hot water bath . It is also capable of analyzing hydrocarbons , it has been used to crack tar and oil when oil leaks out[5] [6].

P.aerugenosa has a variety of virulence factors that contribute to its ability to grow in various host environments and cause many different types of infection . these virulence factors include: adhesins, secreted toxins, proteases , effector proteins , and pigments, which disrupt or control host cellular pathways and target the extracellular matrix .

p.aerugenosa is pathogenic only when introduced into areas devoid of normal defenses , such as when mucous membranes and skin are disrupted by direct tissue damage as in the case of burn and wounds ; when intravenous or urinary catheters are used; or when neutropenia is present , as in cancer chemo-therapy(opportunistic pathogen) . The bacterium attaches to and colonize the mucous membranes or skin , invades locally , and produces systemic disease . these processes are promoted by pili , enzymes and toxins described earlier . Lepopolysaccharide plays a direct role in fever , shock , oliguria, leukocytosis and leukopenia , disseminated intravascular coagulation and adult respiratory distress syndrome [7].

P.aeruginosa has an ability to develop incredibly hardy biofilms on many seemingly uninhabitable surfaces . After attachment to a surface movement across that surface by twitching motility leads to the formation of micro colonies . Evolution of mature biofilm architecture depends on biofilm matrix , which consist of the polysaccharides pel, psl, and alginate , extracellular DNA, and proteins, including cupA, cupB ,

and cupC fimbriae and LecB . These fimbriae mediate bacterial attachment during the initial stages of biofilm formation . Depending on *P. aeruginosa* strain and \ or nutritional conditions , different biofilm phenotypes can be developed [8] . for instance , in glucose minimal media , biofilm lifestyle cycle of *P. aerugenisa* PAOI can be subdivided into five major phenotypic steps. The process begins by the reversible adhesion of planktonic bacteria onto a surface suitable for growth , followed by irreversible attachment of bacteria , which thereafter form microcolonies in EPS matrix. progressively , bacterial microcolonies expand and their confluences lead to a more structured phenotype with noncolonized space . then , noncolonized spaces are filled with bacteria which finally cover entire surface .

II. Materials And Methods

1- Isolation :

Four specimens were isolated from patient with skin wounds and skin burns on MacConky agar .

2- Identification : The process of identification worked by using MacConky agar . This medium was prepared according to the instruction of company , boild for 1 minute, sterilized , and then poured into sterile petri-dishes. It is a selective and differential medium used for identifying gram negative bacteria and detecting their ability to ferment lactose the collected specimens were inoculated on MacConky agar then incubated at 37c for 24 hr. , the pale colonies (lactose non fermenter) indicated as positive result also OF test was formed by stab the sample in tube contain semi sold media of carbohydrate.

3-Detection of bacterial ability to produce slime layer*:*

It is done by using congo red agar method , medium prepared by solubility 6 gm from Brain heart agar and 5 gm of sucrose in 90 ml of distilled water , boiled for1 minute , sterilized by using autoclave and add congo red pigment that prepared by solubility 0.2 gm from congo red pigment in 10 ml of distilled water , then poured into sterile petri-dishes (figure 3).Congo red agar medium was inoculated with single colony of tested Bacterial strain by streaking incubated at 37 c for 24 hr. a positive result was indicated by black colonies with dry crystalline consistency Non – slime producer usually remained pink [9].

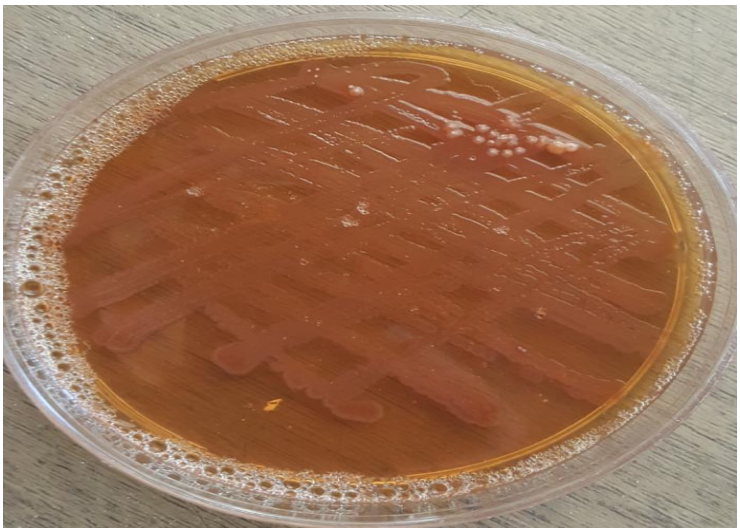
4-Inhibitory biofilm formation by using detergents :

Four detergents were used to determine their inhibitory effect on biofilm formation , these detergents were zahi al –wazer , qasir brayt , lifbouy and eimlaq, these detergents prepared in equal concentrations by diluting them , this done by add 90 ml of distill water to 10 ml of each detergent and add 1ml from each concentration on sterile petri- dishes then pour the dishes in congo red agar medium then move the dishes towards the clock and reverse of clock for homogeneity. The sample was taken from congo red agar after incubation and culture it on congo red agar contain detergent and incubated at 37 c for 24 hr..

III. Results And Discussion

Isolation and Identefication :

MacConkey agar was inoculated with isolated bacteria and incubated at 37 c for 24 hr. , the result was shown pale colonies on MacConkey agar (Fig 1), and it gives positive result in OF test by conversion the color from green to yellow (Fig.2).



Fig(1) . isolation and identification of *P. aerugenosa* on MacConky agar.

Fig. (2). OF test of *P.aerugenosa* give positive result

The ability of adhesion and slime layer formation :

All the isolates of *P. aeruginosa* are tested for their ability to produce slime layer by the congo red agar method . The results in this study show that all isolated give positive result that appeared as black colonies with a dry crystalline consistency. but the fourth isolates was the better for biofilm formation(Fig 3) and we used this isolates for detection ability of detergents for inhibited biofilm formation .

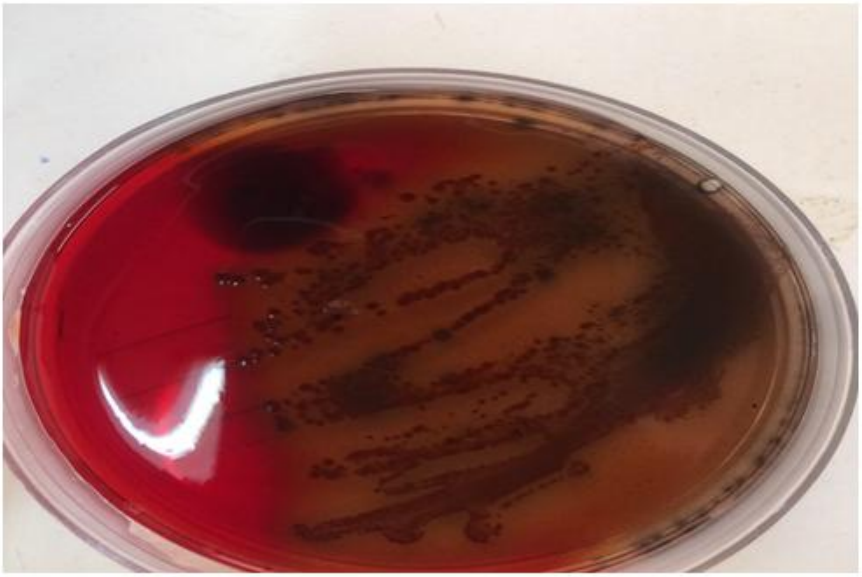


Fig.(3). *P.aeruginosa* adhesion on congo red agar

Inhibiton biofilm formation by using detergents :

Isolated samples were inoculated by streaking on congo red agar contain 1 ml from diluted detergents and incubated at 37 c for 24 hr. The results was appeared as Red to black colonies with lost adhesion in the center on medium that containing zahi al-wazer (fig. 4) . Red colonies with less adhesion in petri – dish that containing qasir brayt (fig .5) . Red colonies with more adhesion than in medium that containing qasir in petri-dish containing eimlaq as in (fig. 6) . Red colonies with adhesion in petri – dish that containing lifebuoy (fig. 7)

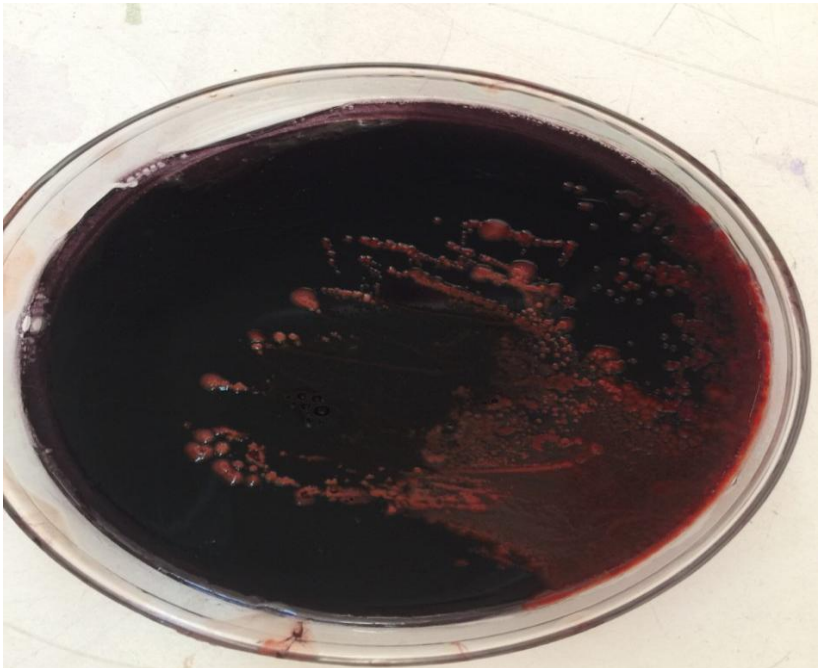


Fig. 4 . Inhibitory effect of Zahi AL- wazer on Adhesion ability of *P.aerugenosa*

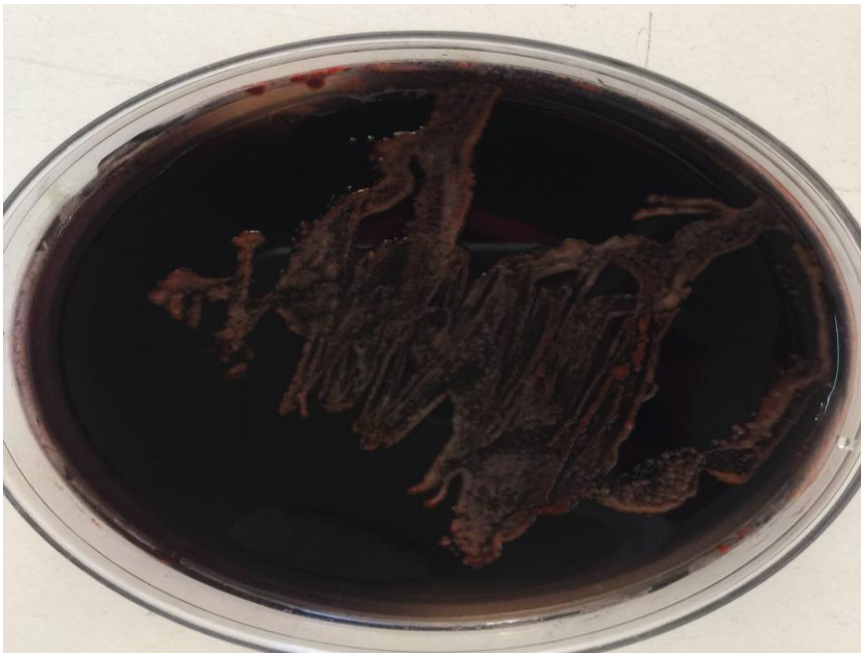


Fig. 5 inhibitor effect of Qasire bryte on Adhesion ability of *P.aeruginosa*

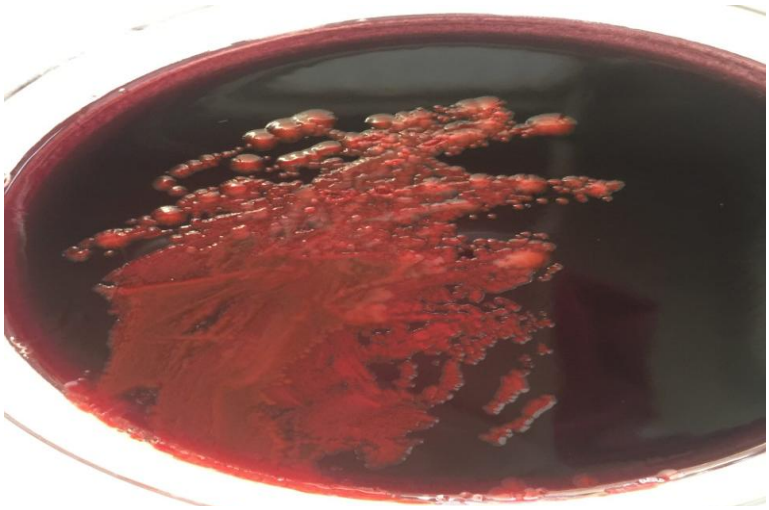


Fig 6. Inhibitor effect of Emlaq on Adhesion ability of *P . aeruginosa*

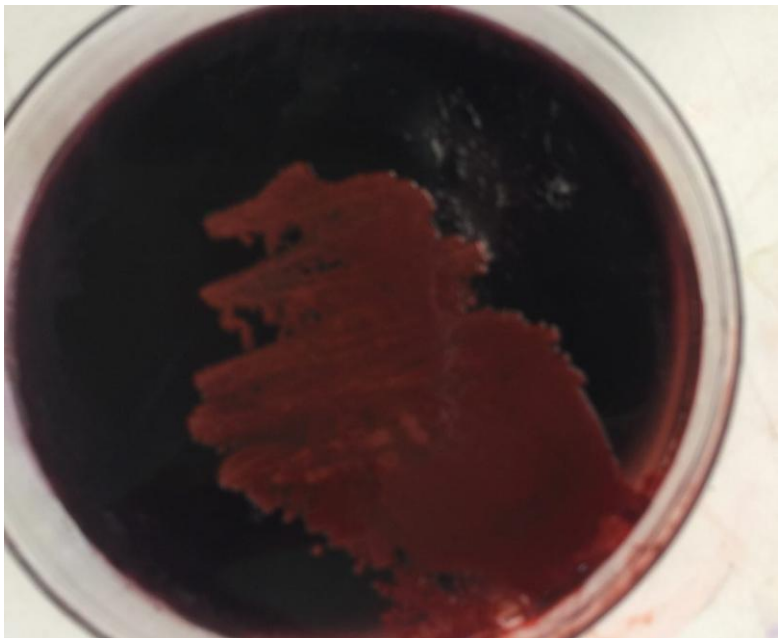


Fig.(7). inhibitory effect of Lifebouy on Adhesion ability of *P. aeruginosa*

Biofilms are sessile microbial communities growing on surfaces that often embedded in a matrix of extracellular polymeric substances [10]. According to National Institutes of Health [11], approximately 80% of foodborne pathogens are able to form biofilm. In food industry,

biofilms can create source of product contamination which leads to massive hygienic problems and economic losses due to food spoilage{ 12]. herefore, the cleaning phase is the most important stage for reducing microbial coloniation and for removing attached microorganism. In food handling operations, these detergents can be used as rinses, sprayed onto surfaces or circulated through equipment in Cleaning in Place (CIP) operations to prevent the microbial biofilm formation [13]. As mentioned by Gibson et al. [14], the use of

detergents in cleaning procedure are not specifically designed as antimicrobial agents but instead to break down food soils, removes surface contamination, then followed by application of disinfectant to minimize the viability of the remaining organisms.

There are many types of detergents and each has different effects, so effective detergent is important to produce effective cleaning, save labour and low cost for cleaning the processing line. In some areas, detergent substances are biodegrade poorly and are associated with allergic reactions and skin and eye irritation. This data demonstrated the importance of choosing an appropriate and an effective detergent that aid removal of attached bacteria and reduce the viability of organisms

IV. Conclusion

The detergents which used in this study was inhibited biofilm formation formation by *P. aerugenosa* and Zahi al-wazer was shown more effect than other detergents and Lifebouy was shown less effect against biofilm formation .

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