Available online on www.ijpqa.com
International Journal of Pharmaceutical Quality Assurance 2019; 10(2); 334-341
doi: 10.25258/ijpqa.10.2.21

ISSN 0975 9506

Research Article

Phenotypic Detection and Biofilm Formation among *Pseudomonas aeruginosa* Isolated from Different Sites of Infection

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Received: 22nd Jan, 19; Revised: 30th Mar, 19, Accepted: 30th Apr, 19; Available Online: 25th Jun, 2019

ABSTRACT

In The present study, included the collection of (100) samples from different clinical sites. Clinical samples were collected from patients who were visit and admitted All-Hilla teaching hospital at the period from November (2017) to February (2018). Cultural, biochemical and VITEK$_2$ system were used for identification, and depending on the VITEK$_2$ system (VITEK-$_2$ GN Kit), revealed that twenty one (21) *Pseudomonas aeruginosa* isolates were recovered, The percentage conformational identification of *P. aeruginosa* was performed using VITEK$_2$ system of (21) *P. aeruginosa* was (99%). Nine(42.8%) samples were isolated from burns, 5(23.8%) samples from wound, 3(14.2%) from urine, 2(9.5%) from ear swab, and 1(4.7%) sample was isolated from both blood and sputum. The phenotypic detection of some virulence factors for all isolates were detected. Detection of capsule was done by using capsule staining technique was carried out for *P. aeruginosa* isolates; it was found that all *P. aeruginosa* isolates (100%) have a capsule surrounding the bacterial cell. Hemolysin production by *P. aeruginosa* was studied; it was found that 12(57.1%) isolates were able to produce extracellular hemolysin on blood agar. *P. aeruginosa* isolates were also investigated for their ability to produce siderophores. The results showed that 9(42.8%) isolates of *P. aeruginosa* were able to produce siderophores. Protease production by *P. aeruginosa* isolates was studied; it was found that all these isolates (100%) have this enzyme as appear as a zone around the colony when being grown on (M$_9$) media after adding of (3ml) of (5%) Trichloroacetic acid and incubation for (24 hrs.). Ability of *P. aeruginosa* to produce lipase has been investigated; it found that all these isolates (100%) were able to produce lipase after incubation for (48 hrs.) on egg yolk agar. Also, bacterial biofilms cause chronic diseases that are difficult to control and in the present study, differentiation of bacteria as biofilm producers and non-biofilm producers was done by using (ELISA) TCP method, a total of (21) isolates were tested for their ability to produce biofilm. From these isolates, (19) isolates were form strong biofilm, (2) isolates were form moderate biofilm.

Keywords: *Pseudomonas aeruginosa,* Hemolysin production, siderophores, biofilm.

INTRODUCTION

Pseudomonas aeruginosa is a gram-negative opportunistic bacterium that lives in soil, water, and even in environments like hot tubs. A common bacterium can cause disease in mammalians. It is found in soil, water, skin flora, and most man-made environments throughout the world[1]. *P. aeruginosa* is a non-fermentative, aerobic Gram-negative rod, measuring (0.5) to (0.8) µm by (1.5) to (3.0) µm, almost all strains are motile by means of a single polar flagellum. It normally lives in moist environments, and uses a wide range of organic compounds for growth, thus giving it an exceptional ability to colonize ecological niches where nutrients are limited, from water and soil to plant and animal tissues. Typical biochemical features of *P. aeruginosa* isolates are positive oxidase test, growth at 42 °C, hydrolysis of arginine and gelatine, and nitrate reduction. *Ps. aeruginosa* isolated from respiratory tracts produces large amounts of a substance usually referred to as alginate, an exopolysaccharide consisting of mannuronic and guluronic acids that gives rise to strikingly mucoid colonies[2]. Alginate is a heterogeneous mixture of hexoses produced by all *Ps. aeruginosa* strains on prolonged incubation in media with high carbon and low nitrogen content. The mucoid polysaccharide alginate represents the capsule, which seems to enable the bacteria to resist natural defenses and to protect it from phagocytosis and antibiotics[3]. Proteases play a crucial role in numerous pathologic processes. Arthritis, tumor invasion and metastasis, infection and a number of degenerative diseases have been linked with the involvement of one or more proteolytic enzymes[4]. Microbial proteases have been proposed as virulence factors in a variety of diseases caused by microorganisms. The virulence of *Ps. aeruginosa* is multifactorial, but it is partly determined by exo-products such as alkaline protease and elastase that are responsible for the damage of tissues by degrading elastin, collagen and proteoglycans. These enzymes have been also shown to degrade proteins that function in host defense *in vivo*[5]. *Ps. aeruginosa* has been investigated intensively during past decays regarding its medical and industrial importance. It will establish in different wastes treatment processes such as oil waste bioremediation. Lipase was characterized by

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Table 1: Classification of bacteria biofilm formation by TCB method.

Mean of O.D. value at 630 nm	Biofilm formation
<0.120	Non
0.120-0.240	Moderate
>0.240	High

Table 2: Distribution of *Pseudomonas aeruginosa* isolates among clinical samples.

Sources of isolates	No. of *P. aeruginosa* isolates	Isolates %
Burns	9	42.8%
Wound	5	23.8%
Urine	3	14.2%
Ear swab	2	9.5%
Blood	1	4.7%
Sputum	1	4.7%
Total number	21	100%

its ability to hydrolyse long chain triglycerides[6]. Lipases have been used for degrading castor oil either for production of new products or for technical lipases production. Part of these enzymes was the thermo stable enzymes, which were better, suited for harsh industrial processes and constitute more than 65% of the global market[7]. Protease, lipase, urease and asparaginase were important enzymes produced by *P. aeruginosa* as well as many other products like alginate, which used in many biotechnological applications. There were an increasing interest in *Ps. aeruginosa* lipase and protease[8]. Hemolysin of *Ps. aeruginosa* has a cytopathic action on blood and tissue culture cells. Lysis and disintegration of the architecture of the cell involving membrane and cytoplasm were demonstrated by morphological changes. The hemolytic activity of hemolysin is inhibited by normal sera and by albumin. The hemolytic and cytopathic actions are explained by assuming that they alter the molecular architecture of the membranes. The variability may relate to the differing availability of reactive sites on the cells, or indicate that the two activities are associated with two different enzymes. *Ps. aeruginosa*, hemolysin is responsible for the colonization of lung and other tissues. There are two hemolysins; one is a phospholipase C and the other is a lecithinase. They appear to act synergistically to break down lipids and lecithin. Genes encoding phospholipase C are chromosomal genes as a part of operon. The cytotoxin and hemolysins contribute to invasion through their cytotoxic effects on eukaryotic cells[9]. Many microorganisms produce Siderophores, it is Low-molecular-mass molecules that have a high specificity for chelating or binding iron, More than (500) different siderophores have been identified from microorganisms. Some bacteria produce more than one type of siderophores. Aerobic bacteria and other living organisms for a variety of biochemical reactions in the cell require iron. Although iron is the fourth most abundant element in the Earth's crust[10]. It is not readily available to bacteria, iron is found in nature mostly as insoluble precipitate that is part of hydroxide polymers. Bacteria live in the soil or water, must have a mechanism to solubilize iron from these precipitates in order to assimilate iron from the environment[11]. Bacteria in biofilms are markedly different from their planktonic counterparts. One of the prominent characteristics of biofilm bacteria is that cells grow in multicellular aggregates encased in an extracellular matrix produced by the bacteria[12]. Extracellular matrices protect constituent cells from external aggression and act as a diffusion barrier to small molecules, resulting in a bacterial community in which some of the cells are metabolically inactive[13]. Sessile biofilm communities can also give rise to no sessile individuals that can rapidly multiply and disperse. Therefore, biofilms can not only protect bacteria from host-defense mechanisms including phagocytosis but also serve as a recalcitrant source of bacteria during antimicrobial therapy[14]. In the developed world, more than (60%) of bacterial infections currently treated by physicians are thought to involve biofilm formation[15].

The present study is carried out to achieve the following objectives

Isolation of *P. aeruginosa* from different sites of infection.

Identification of *P. aeruginosa* by microbial, biochemical and $VITEK_2$ system.

study of some virulence factors by phenotypic detection ex; capsule, hemolysin, siderophore, lipase, protease and lecithinase

Study the ability of *P. aeruginosa* to biofilm formation.

MATERIALS AND METHODS

Patients and study design

This study included (100) patients (aged 5 year-70 years), the specimens were collected from different sits of infections burns, wounds, urine, seputum, ear swab, blood from patients who were visit All-Hilla teaching hospital laboratory in AL-Hilla city at the period from November (2017) to February (2018).

Ethical Approval

A valid consent was achieved from each patients before their inclusion in the study.

Identification of P. aeruginosa

Microscopic Properties

Gram's stain was used to examine the bacteria for studying the microscopic properties as initial identification of *P. aeruginosa*[16].

Cultural Characteristics

Morphological colonies characteristics were recorded on the specific media for primary identification of *P. aeruginosa*[2].

Biochemical tests of *P. aeruginosa* :were done by 1-2 colony tested for oxidase, catalase, Simmone Citrate, Kligler Iron agar and Indole tests and these entire tests positive[16].

Definitive Identification by $VITEK_2$ – Compact

using the identification of *Pseudomonas aeruginosa* was confirmed using of $VITEK_2$-Compact, which represent an advanced colorimetric technology for bacterial

Table 3: Biochemical tests for Identification of *Pseudomonas aeruginosa.*

Test	Gram stain	Indole	MR	VP	Citrate	Oxidase	Motility	Catalase	TSI Slant/ butt	H2S	Gas
Results	Gram -ve	-	+	-	+	+	+	+	K/ K	-	-

(+) positive; (-) negative; (Alk) alkaline; (A) acid; (TSI) triple sugar iron; (MR) methyl red; (VP) voges-proskauer

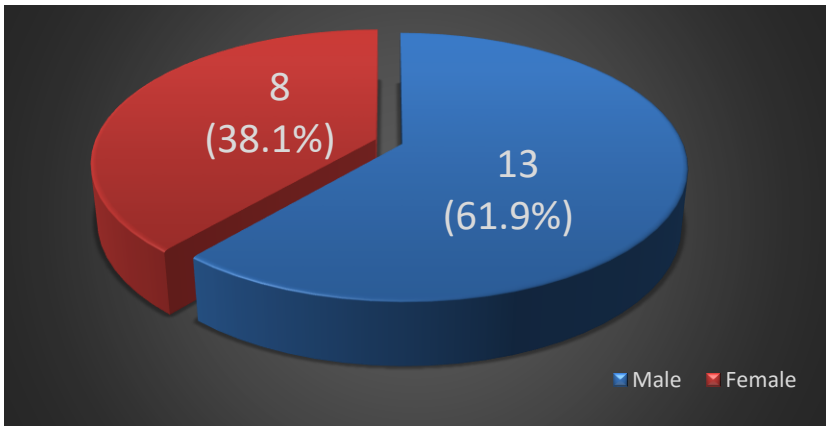

Figure 1: Distribution of infected patients according to gender.

Figure 2: production of capsule by *P. aeruginosa.*

identification; gram-negative (GN) card was used for this purpose for *Pseudomonas aeruginosa* Identification. All the following steps were done according to the manufactures instructions (Biomerieux, France). Three ml of normal saline was placed in plane test tube and inoculated with a lope full of isolated colony. The test tube was inserted into the Dens Check machine for standardization of the colony to McFarland standard solution ($1.5X10^{8}$cfu/ml). The standardized inoculum was placed into the cassette and a sample identification number entered into the computer software via barcode. The VITEK-$_2$ card type was then read from barcode placed on the card during manufacture and the card was thus connected to the sample identification port. The cassette was placed in the filter module, when the card were filled, transferred the cassette to the reader/ incubator module. All the subsequent steps were handled by the instruments, the instrument controls the incubation temperature, the optical reading of the cards and continuously monitors and transfers test data to the computer for analysis. When the test cycle was completed, the system automatically ejected the cards into a waste container.

Detection of virulence factors

Hemolysin production

Hemolysin production was carried out by Inoculating bacterial isolate on blood agar medium at (37˚C) for (24-48) hours, An appearance of clear zone around the colonies referred to complete hemolysis (β- hemolysis), greenish zone around the colonies referred to partial hemolysis (α- hemolysis), while no clear or greenish zone referred to non-hemolysis (γ- hemolysis)[3]

Iron uptake by siderophore

Medium used in this test was M_9 medium to determining the ability to uptake iron by using siderophore. The media was inoculated with bacteria and incubated anaerobically at (37°C) for (48) hrs. If there was bacterial growth, it demonstrated that the bacteria could utilize the $FeSO_4$ as an iron source by using siderophores[17].

Lecithinase (phospholipase) production

Lecithinase test was carried out in egg-yolk agar medium to determine the ability of *P.aeruginosa* to produce these enzymes. After inoculation of the medium agar, the plates

were incubated anaerobically for 48 hrs. at (37°C). Lecithinase break down lecithin to insoluble diglyceride and phosphorylcholine, which results in a white opaque zone of precipitation, which spreads beyond the edge of the colony[2].

Extracellular protease production

M_9 media was used for the detection of protease enzyme. After sterilization in autoclave and cooling at (50°C), and (0.25) gm/L glucose (sterilized by filtration) was added, the medium was then supplemented by (1%) casein. Pores was made in agar medium and inoculation of this media with (20µl) from bacterial broth in each pores and incubation at (37°C) for (24) hours; (3ml) of (3%) trichloroacetic acid was added to precipitate the protein. The formation of transparent area around the colony indicated a positive result[18].

Capsule production

The solutions (India ink) were prepared according to the required microbiological methods. A smear slide is prepared from bacterial suspension on glass slide without fixing and is left to dry. Floot gently with (1%) of india ink and left for one minutes, allowed to dry in air, and examined under the microscope. The organism should be deep purple, and the capsule a faint blue against a light purple background[19].

Biofilm Production

Tissue culture plate method (TCP) assay (also called semi quantitative micro titer plate test (biofilm assay) described by[20] was considered as standard test for detection of biofilm formation as follow: Isolates from fresh agar plates were inoculated in tryptcase soya broth (TSB) containing 1% glucose an incubated anaerobically for (72) hrs. at (37°C) and then diluted (1:100) with TSB. Individual wells of sterile, polystyrene, (96) well-flat bottom tissue culture plates wells were filled with (150µl) of the diluted cultures and only broth served as control to check non-specific binding of media. Each isolate was inoculated in triplicate. The tissue culture plates were incubated for (24) hrs. at (37°C). After incubation, content of each well was gently removed by tapping water. The wells were washed four times with phosphate buffer saline (pH7.2) to remove free-floating bacteria. Biofilms formed by adherent sessile organisms in plate were fixed by placing in oven at (37°C) for (30) minutes. All wells stained with crystal violet (0.1 % v/v). Excess stain was rinsed off by through washing with deionized water and plates were kept for drying. One hundred fifty (150µl) of acetone/ethanol (20:80, v/v) mixture was added to dissolve bounded crystal violet. The optical density (O.D.) at (570nm) was recorded and the results were interpreted according to the Table (1).

RESULTS AND DISCUSSION

Isolation and Identification of Pseudomonas aeruginosa

The present study included the collection of (100) samples from different clinical sites. Clinical samples were collected from patients who were visit All-Hilla teaching hospital laboratory in AL-Hilla city at the period from November (2017) to February (2018). Cultural, biochemical and $VITEK_2$ system were used for identification, and depending on the $VITEK_2$ system revealed that twenty one (21) *Pseudomonas aeruginosa* isolates were recovered. These results were shown in Table (2). These samples were recovered from patients their age ranged from (5-70) years and the males were more infected than females as shown in Figure (1). The Table (2) indicates that *P. aeruginosa* were isolated mainly from burn samples (42.8%), followed by wound (23.8%) and urine (14.2%), while ear swabs (9.5%), blood and seputum (4.7%). These results are in agreement with study done by Latif in Iraq who showed that *P. aeruginosa* were most common (44.4%) in burn infections, followed by (38.1%) otitis media, (16.6%) wounds and (6.6%) urinary tract infections, while *P. aeruginosa* cannot be isolated from eye infections[21]. However, these results are different from the results obtained by[22] who found that most of *P. aeruginosa* isolates (80.6%) were recovered from burns. In northeastern Nigeria which found that significant proportion of isolates were recovered from wounds (39.6%), followed by ear (otitis media) (30.2%), and UTI (7.5%)[23]. Al-Mamori,[24] who found that most isolates were obtained from burns (8.55%), wounds (3.95%), ear swabs (3.30%) and (1.97%) isolates from each urine and blood in Hilla city. Al-Derzi,[25] in the North of Iraq (Mosul and Duhok) revealed that, the most common *P. aeruginosa* isolates comes from purulent specimens collected from skin wounds and burns (44.4%) followed by isolates from UTI (31.8%) and ear discharge specimens (12.4%). Also, the study of[26] in Kurdistan region of Iraq found highest percentage of *P. aeruginosa* was obtained from burn samples (%10.9) whereas the lowest percentage were obtained from otitis samples (%1.81). There are differences in the percentage of infections between our results and others, and the reasons for these variations in all studies may be due to the percentage of distribution of isolates, which varied according to the place of clinical samples collection, environmental factors, nutrition requirements and virulence factors[27]. To initiate infection, *P. aeruginosa* usually requires a substantial break in the skin, which considered the first-line of defenses against microbes. Such a break can result from breach or bypass of normal cutaneous or mucosal barriers (e.g., trauma, surgery, serious burns). *P. aeruginosa* associated burn infections was common[28]. *P. aeruginosa* is a common cause of infections in burns, established through colonization of the burn wound by the patient's own normal flora or from the environment. Patients with burns infected with *P. aeruginosa* have an increased mortality rate and longer hospital stays compared to non-infected patients. They also have an increased number of surgical procedures and higher associated antibiotic costs[29]. *P. aeruginosa* isolates (21 isolates) were identified using traditional morphological and biochemical diagnostic tests according to the methods of[16]. The results were show in Table (3). *P. aeruginosa* are gram-negative bacilli and the colonies on nutrient agar are approximately (2mm) in diameter with bluish green coloration and irregular, feathered edges[30]. The conformational identification of *P.*

Table 4: Virulence factors of *Pseudomonas aeruginosa* isolates.

Virulence factors	Clinical sample No.	%
Hemolysin	12	57.1%
Siderophore	9	42.9%
Capsule	21	100%
Protease	21	100%
Lipase	21	100%

aeruginosa was performed using $VITEK_2$ system (VITEK-$_2$ GN Kit). The percentage of[21] *P. aeruginosa* was (99 %).

Virulence factors

P. aeruginosa produces extracellular toxins; these toxins include hemolysin, siderophore, lecithinase, protease, elastase, collagenase enzymes, exotoxin A and exoenzyme S[31].

Hemolysin and siderophores production

P. aeruginosa has two pathways to acquisition iron, one of these pathway is hemolysin, and these bacteria produce two hemolysin, it appear to be cytotoxic for most eukaryotic cells, hemolysin contribute to invasion through their cytotoxic effects on eukaryotic cells. Bacteria may use hemolysin as a way to obtain nutrients from host cells. For example, iron may be a limiting factor in the growth of various pathogenic bacteria[32]. Production of hemolysin by *P. aeruginosa* is mostly associated with pathogenic bacteria, therefore it is considered as important factor that participates in their pathogenesis[33]. Hemolysin production by *P. aeruginosa* was studied; it was found that 12(57.1%) isolates were able to produce extracellular hemolysin on blood agar as shown in Table (4), which is in agreement with[34] who stated that hemolysin is produced by (66.7%) *P. aeruginosa* isolated from different sources. Hemolysin is lytic to erythrocytes and it is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury, and impaired host defenses. Exposure of PMNLs and release of leukotriene and ATP; cause marked morphologic alterations; and impair chemotaxis and phagocytosis. Lysis occurs at higher concentrations[35]. Monocytes and granulocytes are highly susceptible to hemolysin cytotoxicity, whereas lymphocytes are relatively resistant[36]. Many hemolysin probably form pores in the plasma membrane of erythrocytes, so hemoglobin and/or iron are released[37]. It has necrotizing effect on red blood skin of injected animals[38]. In present study, *P. aeruginosa* isolates were also investigated for their ability to produce siderophores synthesis. The results show that 9(42.8%) isolates of *P. aeruginosa* are able to produce siderophores. The results were shown in Table (4). Siderophores are metal chelating agents with low molecular masses (200 to 2000 Daltons), which provide the microorganisms with an efficient Fe-acquisition system due to their high affinity for Fe (III) complexation (Schwyn and Neilands, 1987; Kraemer, 2004). Iron (Fe) is an essential element for the growth of almost all living microorganisms since it acts as a catalyst in enzymatic processes, oxygen metabolism, electron transfer, and DNA and RNA synthesis[39,40]. The production of the siderophores could help the microorganisms in their competition for mineral nutrients, in addition to function as a virulence factor to protect the microorganisms against other harmful microorganisms inhabiting in their environment[41]. The isolates of *P. aeruginosa* appear hemolysis on the blood agar did not have siderophores; these results resemble the result obtained by[24]. The bacteria that are able to produce siderophores have no ability to produce hemolysin. So, bacteria are need only one mechanism for obtaining iron that can increase disease risk by functioning as a readily a viable essential nutrient for invading microbial and neoplastic cell to survive and replicate in host, microbial pathogens must acquire host iron, that identical with the results obtained by[42].

Detection of Capsule

Detection of capsule was done by using capsule staining technique was carried out for *P. aeruginosa* isolates; it was found that all *P. aeruginosa* isolates (100%) have a capsule surrounding the bacterial cell. The results were shown in Table (4) and Figure (2). This result was identical with the results obtained by[43] who found that all isolates of *P. aeruginosa* have capsule. Capsule is an important structure of some bacterial cells. It lies outside the cell wall of bacteria, and made as a barrier between the cell wall and the environment it cause of various diseases[44]. Capsule of *Pseudomonas* contains exopolysaccharide layer called alginate associated with the outer membrane. This layer serves to protect the bacteria from adversity and enhances adhesion to solid surfaces. The alginate layer of *P. aeruginosa*, which helps it to escape from host defenses and resist the antimicrobial action of antibiotics, and increase attachment of the bacteria away from the surface, allowing them to spread and colonize new sites[45]. Capsule is often produced only under specific growth conditions, even though not essential for life, Capsules probably help bacteria to survive in nature and help many pathogenic and normal flora bacteria to initially resist phagocytosis by the host's phagocytic cells and prevent killing by bactericidal serum factors. As well as in soil and water, prevent bacteria from being engulfed by protozoans, and help many bacteria to adhere to surfaces and thus resist flushing[46].

Protease production

Protease production by *P. aeruginosa* isolates was studied; it was found that all these isolates (100%) have this enzyme as appears in the zone around the colony when being grown on (M_9) media after adding of (3ml) of (5%) Trichloroacetic acid and incubation for (24 hrs.), the results were shown in Table (4). The results were identical to the results of[34] who reported that all *P. aeruginosa* that isolated from all clinical specimens 21(100%) protease producers, but Gupta *et al*., (2006) found that (80%) of *P. aeruginosa* isolates produced protease. The proteases was responsible for many features of the pathogenesis of *Pseudomonas* in the eye infections. Proteases are degrade the integrity of the host physical barriers by splitting proteins and amino acids[47]. Proteases are considered important virulence factors in burn

Table 5: Biofilm formation by *P. aeruginosa* isolates.

Bacterial isolates (no.)	Biofilm			
	Strong	Moderate	Weak	% of biofilm formation
P. aeruginosa (21)	19(90.5%)	2(9.5 %)	0%	100%

infections were contributed to the invasiveness of the organisms, which damage host tissues and interfere with host antibacterial defense mechanisms[48]. Protease (included alkaline protease) and elstease in their two types (Las A, and Las B)[49]. Elastase is a metalloprotease, which degrades elastin and collagen and inactivates human immunoglobulin G, serum alpha-1, proteinase inhibitor and several complement components[50]. *P. aeruginosa* elastase activates metalloproteases inducing the destruction of corneal stroma[51]. Alkaline proteases are referring to proteolytic enzymes, which work optimally in alkaline pH[52]. These extracellular proteases is important and enable the cell to absorb and utilize hydrolytic products, at the same time, these extracellular proteases have also been commercially exploited to assist protein degradation in various industrial processes[53,54].

Lipase production

Ability of *P. aeruginosa* to produce lipase has been investigated, it found that all these isolates (100%) are able to produce lipase after incubation for (48 hrs.) on egg yolk agar. These results were showed in Table (4). This result is consistent with[55], who found that all isolates examined in their study gave positive result in a test for lipase production, also[43] found that (90%) of the isolates produced lipase. Lipase is water-soluble enzyme that catalyzes the hydrolysis of ester chemical bonds in water insoluble lipid substrate, most lipases act as a specific position on the glycerol backbone of lipid substrate[56]. This virulence factor is important in burn wound infections[57] and acts on the animal cell membrane by insertion into the membrane forming a pore in cell and cleaves phospholipids, also lyses red blood cells, phagocytes and their granules[58].

Biofilm formation

Bacterial biofilms cause chronic diseases that are difficult to control and in the present study, differentiation of bacteria as biofilm producers and non-biofilm producers was done by using (ELISA), a total of (21) isolates were tested for their ability to produce biofilm. From these isolates, (19) isolates were form strong biofilm, (2) isolates were form moderate biofilm as shown in Table (5). Biofilms are surface-associated communities enclosed within an extracellular matrix[59], mainly composed of polysaccharides, proteins, nucleic acids, lipids and other macromolecules and chemicals[60]. Particularly, extracellular polysaccharides are a crucial component of the matrix, and carry out a range of functions such as promoting attachment to surfaces and other cells, building and maintaining biofilm structure, as well as protecting the cells against environmental assaults and predation, including antimicrobials and host defenses[61]. *P. aeruginosa* produces at least three polysaccharides (alginate, Pel, and Psl) that are determinant for the stability of the biofilm structure[62]. It has been documented that biofilms are more resistant to antimicrobial agents, i.e. antibiotics, surfactants, disinfectants, than planktonic counterpart[63]. In addition, their physiology significantly differs from that of planktonic cells[64]. The biofilm could be formed by *Pseudomonas aeruginosa,* within 10-hours after its taking position in the wound. Biofilm formation in gram-negative bacteria occurs when bacterial cells first swim along a surface, using flagellar-mediated motility, until attachment occurs at a specific site[59]. *Pseudomonas* spp. have been noted to form biofilms in any environment whereas other bacterial species often require a specific temperature or pH[66]. Three global non-microbicidal strategies have been proposed to struggle against pathogenic bacteria with biofilm formation ability by (i) avoiding microbial attachment to a surface. (ii) Disrupting biofilm development and/or affecting biofilm architecture in order to enhance the penetration of antimicrobials and (iii) affecting biofilm maturation and/or inducing its dispersion and degradation[67].

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