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ASSOCIATION BETWEEN ANTIBIOTIC RESISTANCE, BIOFILM FORMATION AND *LASB* GENE IN *PSEUDOMONAS AERUGINOSA* ISOLATED FROM DIFFERENT CLINICAL SPECIMENS

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A microorganism's capacity to form a biofilm is seen as a sign of a clinically significant infection. Pseudomonas aeruginosa infections are challenging to treat since the majority of isolates have high levels of innate resistance to numerous antibiotics and a propensity to develop biofilms. A total of 350 specimens were collected from patients. 125 isolates of P. aeruginosa were recovered from different clinical samples. The antibiotic susceptibility of these isolates against Ciprofloxacin, Amikacin, cefepime, Norfloxacin, imipenem, Genamicin, Tobramycin, Aztreonam, Piperacillin-tazobactam and Colistin was determined using disk diffusion method. The TCP technique assay was chosen to identify the development of biofilm. Elastase gene detection was carried out using polymerase chain reaction (PCR) (lasB). The antibiotics against which there was the greatest resistance were cefepime (92.8%) and ciprofloxacin (67.2%). TCP technique identified 15 as weak or non-biofilm producers, 32 as moderate, and 78 as robust producers of biofilm. In 89.6% of P. aeruginosa isolates, LasB was found. P. aeruginosa's pattern of antibiotic resistance was more prevalent in biofilm producers than in non-producers. We come to the conclusion that P. aeruginosa biofilm development and drug resistance have a positive association.

Keywords: *Biofilm, Pseudomonas aeruginosa, TCP, Antibiotic resistance, lasB.*

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a remarkably one of the most adaptable prevalent nosocomial pathogens. They have been linked to serious and potentially fatal infections[1]. Infections caused by *P. aeruginosa* are associated with a higher mortality rate specially in clinical settings[2]. They frequently cause a variety of opportunistic infections, both acute and chronic[3]. Another significant feature of *P. aeruginosa* is the ability to create biofilms, which lower an infection's susceptibility to antimicrobials and, as a result, its therapeutic options[1]. It is now universally recognized that the most prevalent mode of bacterial growth is the creation of biofilms[4]. Biofilm is defined as an collection of microbial cells that is attached (not removed by gentle rinsing) with a surface and covered by an exopolysaccharide matrix (slime)[5&6]. Their transformation from a planktonic to a surface-attached colony involves a number of modifications. When exposed to particular environmental stimuli, they display different phenotypes in terms of their rate of growth and gene transcription[6&7]. Depending on the species of bacteria, these phenotypic changes take different forms. Over 60% of all illnesses are linked to biofilms, according to a National Institutes of Health report[6]. Because they are typically well protected from environmental challenges, antibiotics, desiccation, disinfectants, and the host immune system, microorganisms developing in a biofilm are notoriously difficult to eliminate. The

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fundamental medicinal significance of biofilm formation is that it decreases the sensitivity to antimicrobial medicines. Additionally, the proximity of cells within a biofilm can encourage the spread of antibiotic resistance and plasmid exchange[8].

When *P. aeruginosa* forms biofilms, extracellular enzymes like elastase lasB are produced [9]. Elastase is a zinc metalloprotease that is involved in host colonisation and tissue injury and is encoded by the *lasB* gene. The degradation of elastin, collagen, and immunological components by *lasB* is thought to be a significant virulence factor for bacterial survival from the host immune system. Previous studies have shown that *P. aeruginosa* exhibits a high prevalence of the *lasB* gene regardless of where it was isolated from[10&11]. The findings show that directly or indirectly, *lasB* can affect the formation and architecture of *P. aeruginosa* biofilms[9]. The goal of the study was to look into *P. aeruginosa's* ability to produce biofilms after being isolated from clinical samples using the tissue culture plate (TCP) method. Additionally, to look into the distribution of the *lasB* gene among biofilm-producing *P. aeruginosa* isolates and the profile of antimicrobial resistance among those isolates.

MATERIALS AND METHODS

Specimen collection

In the current study, 350 different clinical specimens (urine, sputum, ear discharge, wound exudate and stool) were collected from patients attending Minia university hospital.

Bacterial identification

Biochemical tests and the conventional microbiological approach were both used to confirm the *P. aeruginosa* strains.Until employed, the isolated bacteria were kept in trypticase soy broth (TSB) with 40% glycerol at -70°C.

Detection of biofilm formation by Tissue culture plate method (TCP)

The gold-standard technique for biofilm identification is the tissue culture plate (TCP) assay, which was first published by Christensen et al. in 1995. In 10 mL of TSB with 1% glucose, isolates from freshly prepared agar plates were injected. Broths were incubated for 24 hrs at 37°C. Then fresh medium was added and the cultures were diluted 1:100.

200 L of the diluted cultures were placed in each of the 96 wells of sterile flat-bottom tissue culture plates. For the purpose of testing media sterility and non-specific binding, only sterile broths were offered as a blank. Likewise, control organisms were diluted and incubated as well. The tissue culture plates contained the three controls as well as blanks.

The culture plates underwent a 24-hrs incubation period at 37°C. The wells were cleaned four times with 0.2 mL of phosphate buffer saline (pH 7.2) before being let to dry naturally. The wells were next stained for 30 minutes at room temperature with 200 L of 0.1% crystal violet. To get rid of the excess colour, the plates were rinsed with distilled water and then left to dry. The use of 200 l of 95% ethanol helped to dissolve the adherent stain. A micro ELISA auto reader operating at a wavelength of 630 nm was used to measure the optical densities (OD) of stained adherent biofilm. Three times of the experiment were carried out in duplicate. All test results were computed, and the average of the OD values of the sterile medium was subtracted. Non-biofilm producers were judged to have ODs below 0.120, moderate biofilm producers to have ODs between 0.120 and 0.240, and more than 0.240 as strong biofilm producers[4-7].

Antimicrobial Susceptibility Testing

Kirby–Bauer disc diffusion method was used for Antimicrobial susceptibility testing of *P. aeruginosa* strains according to Clinical & Laboratory Standards Institute guidelines[12]. The used antibiotics were Ciprofloxacin (CIP, 5 μg), Amikacin (AK, 30 μg), cefepime (CPM, 30 μg), Norfloxacin (NX, 10 μg), imipenem (IPM, 10 μg), Genamicin (GEN, 10μg), Tobramycin (TOB, 10 μg), Aztreonam (ATM, 30 μg), Piperacillin-tazobactam (PIT 100/10 μg) and Colistin (CL, 10 μg). Inhibition zone was recorded in mm. The susceptibility pattern was determined using the CLSI interpretation chart as susceptible (S), intermediate (I) and resistant (R).

DNA extraction and PCR

According to the manufacturer's instructions, DNA from *P. aeruginosa* isolates

was extracted using a DNA extraction kit (Qiagen kit, Germany). Using a specific pair of primers (Metabion, Germany) F: GGAATGAACGAAGCGTTCTC and R: GGTCCAGTAGTAGCGGTTGG[13]. PCR amplification was carried out in a 50 µL reaction mixture which contained 16 µL master mix including PCR buffer, MgCl2 , dNTPs, Taq DNA polymerase, 0.5 µLof each forward and reverse primer, and 4 µL of DNA template. About 29 µL ultra-pure water was then added to make up a final volume to 50 µL. The procedure for the PCR test was as follows: Initial denaturation at 95 °C for five minutes, followed by 30 cycles of denaturation at 94 °C for one minute each, one minute of annealing at 63 °C, one minute of extension at 72 °C, and finally seven minutes of final extension at 72 °C. After being stained with 1% ethidium bromide, PCR products were placed onto a 1.5% agarose gel and then seen under UV light.

Statistical analysis

SPSS, 17 statistical software, was used for statistical analysis (SPSS Inc., Chicago, IL (.Chi-square (X2) and Fisher's exact tests were used to compare the frequencies of biofilm formation, the *lasB* gene, and antibiotic resistance. Pearson's correlation coefficient (r2) was used to establish bivariate linear correlations (P 0.05). If the P-value is less than 0.05, it is considered significant.

RESULTS AND DISCUSSION

Results

Specimen collection

In this study, 125 *P. aeruginosa* strains were recovered from 350 different clinical specimens. Table (1) showed the prevalence of *P. aeruginosa* according to the source of clinical specimens. Data in the table indicated that *P. aeruginosa* was the most prevalent in wound exudates (41.2%), followed by ear discharge samples (38.6%), stool samples (35.6%), sputum specimens (31%) and urine samples (30%).

Biofilm formation

Biofilm production by TCP method was seen in 110(88%) isolates, while 15(12%) were negative as seen in Table2 which shows that biofilm formation was strong in 60% of urine isolates, 57.1% of wound exudate isolates, 66.7% of sputum samples, 68.7% of ear discharge samples and 56.2% of stool samples. As shown in Figure 1 among 125 *P. aeruginosa* isolates, 78 isolates were detected as strong, 32 as moderate and 15 as weak or non- biofilm producers.

Table1: Number of *P. aeruginosa* isolates recovered from different clinical sources.

Source	No. of specimens	*P. aeruginosa* isolates	
		No.	%*
Urine	50	15	30
Wound exudate	85	35	41.2
sputum	87	27	31
Ear discharge	83	32	38.6
Stool	45	16	35.6

*****percentage is correlated to no. of specimens of the same type.

Table2: The percentage of biofilm formation among *P. aeruginosa* isolates (n=125).

Source(no.)	Strong		Moderate		Weak/Non		P value
	No.	%*	No.	%*	No.	%*	
Urine(15)	9	60	4	26.7	2	13.3	0.976
Wound exudate(35)	20	57.1	10	28.6	5	14.3	0.743
Sputum(27)	18	66.7	6	22.2	3	11.1	0.871
Ear discharge(32)	22	68.7	7	21.9	3	9.4	0.685
Stool(16)	9	56.2	5	31.3	2	12.5	0.842
Total(125)	78	62.4	32	25.6	15	12	

* percentage is correlated to total no. of isolates of the same source.

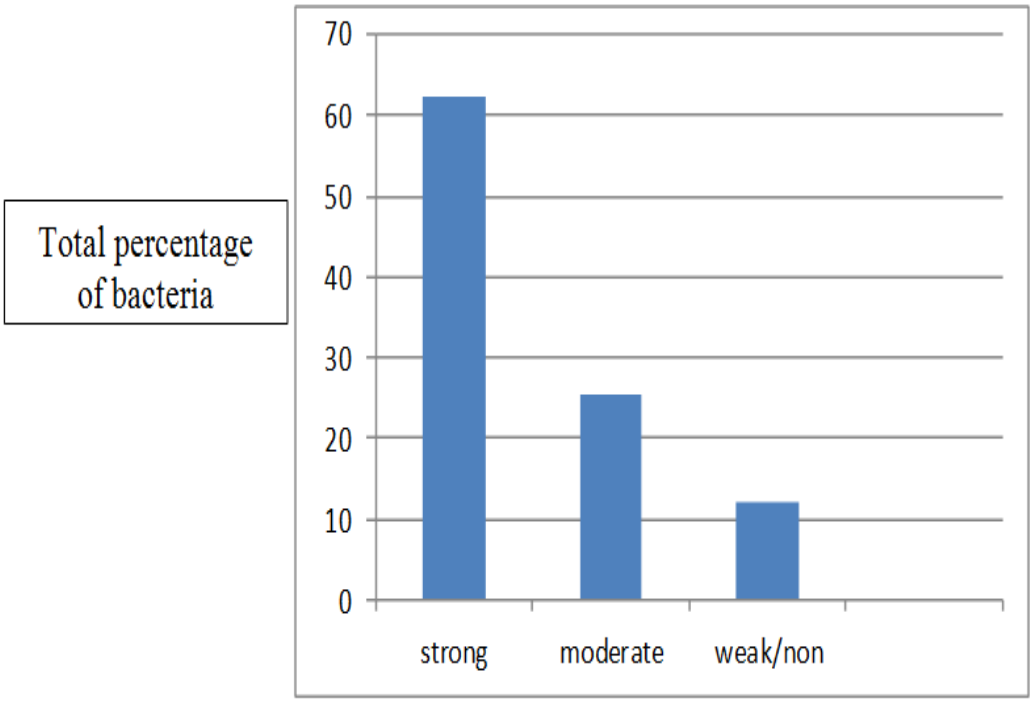

Fig.1: Screening of *P. aeruginosa* isolates for detection of biofilm formation by TCP method.

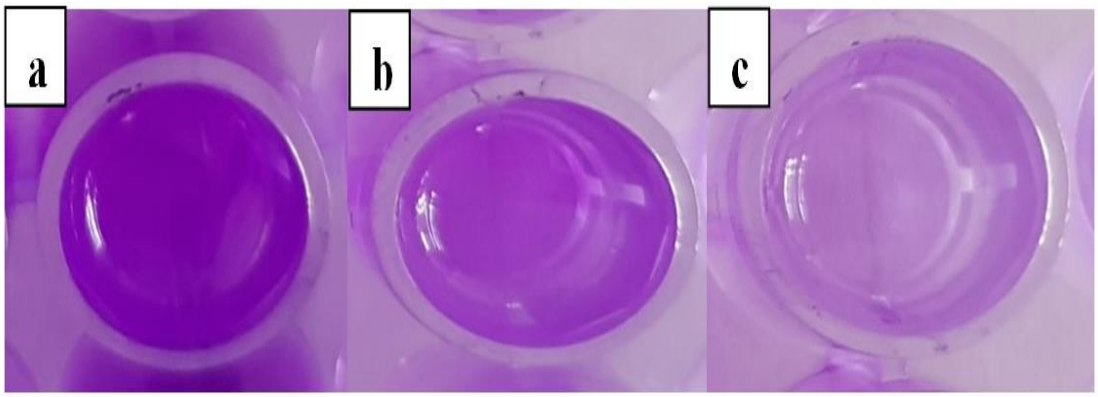

Fig.2: Biofilm production by TCP method, a: strong, b: moderate and c: weak or non- biofilm producer.

Antimicrobial profile

In this study, antimicrobial susceptibility profiles to 10 antibiotics, representing seven different classes, showed high rate of antibiotic resistance. Table (3) showed the effect of different antimicrobial agents on *P. aeruginosa*. Data showed that the most effective antibiotics against *P. aeruginosa* were Imipenem, Piperacillin-tazobactam and Tobramycin where only 12%, 16% and16.8% of the isolates were resistant to these antibiotics respectively. *P. aeruginosa* isolates were more resistant to Cefepime (92.8), Ciprofloxacin (67.2%), Colistin (60%) , Norfloxacin (57.6 %) and Aztreonam (50.4%), while only 38.4% and 28.8 % were resistant to Amikacin and Genamicin respectively. The antibiotic resistance pattern of *P. aeruginosa* was found higher in biofilm producers than in biofilm non-producers as shown in Table3.

Table3: Antimicrobial susceptibility pattern of *P. aeruginosa* among biofilm producers and non-produce**.**

Antimicrobial agent	Biofilm Producers No. (%*)			Biofilm Non producers No. (%**)			Total resistant No. (%***)			P value
	S	I	R	S	I	R	S	I	R	
Ciprofloxacin	23 (20.9)	6 (5.5)	81 (73.6)	11 (73.3)	1 (6.7)	3 (20)	34 (27.2)	7 (5.6)	84 (67.2)	<0.001*
Amikacin	59 (53.6)	9 (8.2)	47 (42.7)	1 (6.7)	13 (86.7)	1 (6.7)	55 (44)	22 (17.6)	48 (38.4)	<0.001*
Cefepime	0 (0)	0 (0)	110 (100)	9 (60)	0 (0)	6 (40)	9 (7.2)	0 (0)	116 (92.8)	<0.001*
Norfloxacin	34 (30.9)	6 (5.5)	70 (63.6)	6 (40)	7 (46.7)	2 (13.3)	40 (32)	13 (10.4)	72 (57.6)	<0.001*
Imipenem	95 (86.4)	0 (0)	15 (13.6)	15 (100)	0 (0)	0 (0)	110 (88)	0 (0)	15 (12.8)	0.127
Genamicin	67 (60.9)	9 (8.2)	34 (30.9)	10 (66.7)	3 (29)	2 (13.3)	77 (61.6)	12 (9.6)	36 (28.8)	<0.001*
Tobramycin	77 (70)	12 (10.9)	21 (19.1)	13 (86.7)	2 (13.3)	0 (0)	90 (72)	14 (11.2)	21 (16.8)	0.179
Aztreonam	50 (45.5)	0 (0)	60 (54.5)	12 (80)	0 (0)	3 (29)	62 (496)	0 (0)	63 (50.4)	0.012*
Piperacillin-tazobactam	85 (77.3)	6 (5.5)	19 (17.3)	11 (73.3)	3 (29)	1 (6.7)	96 (76.8)	9 (7.2)	20 (16)	0.089
Colistin	32 (29.1)	5 (4.5)	73 (66.4)	12 (80)	1 (6.7)	2 (13.3)	44 (35.2)	6 (4.8)	75 (60)	<0.001*

*****percentage is correlated to total biofilm producers (110).

**percentage is correlated to total biofilm non producers (15).

*** percentage is correlated to total *P. aeruginosa* isolates (125).

Correlation between biofilm formation and type of resistant *P. aeruginosa* isolates.

The results in this study showed that 79(63.2%) of isolates were multi-drug resistant (MDR) and 46(36.8%) were Non multi-drug resistant (Non-MDR) as shown in table 4. Our study proved the existence of significant association between antibiotic resistance and biofilm formation as shown in table 4 which shows that 97.5 % of MDR *p. aeruginosa* isolates were biofilm producers and only 2.5% of them were biofilm non-producers.

Table4: Correlation between biofilm formation and type of resistant *P. aeruginosa* isolates.

	MDR		Non-MDR		P value
	No.	%*	No.	%**	
Biofilm Producers	77	97.5	33	71.7	<0.001*
Biofilm Non producers	2	2.5	13	28.3	

***** Percentage is correlated to total no. of MDR *P. aeruginosa* isolates(79).

****** Percentage is correlated to total no. of Non-MDR *P. aeruginosa* isolates(46).

Detection of *lasB* gene in isolates by PCR

In this study, *lasB* gene was found in 112 isolates (89.6%), 107 (97.3%) of biofilm producers isolates were *lasB* positive and only 5 (33%) of biofilm non-producers isolates were *lasB* positive and there was a statistically significant relationship between *lasB* gene expression and biofilm formation as shown in table 5.

Comparison of the antimicrobial sensitivity results of *P. aeruginosa* isolates with and without elastase gene

The comparison of antibiotic sensitivity results of *P. aeruginosa* isolates with and without elastase gene showed a statistically significant difference, except in the case of gentamicin, tobramycin, piperacillin/tazobactam and imipenem antibiotics. *P. aeruginosa* isolates without elastase gene compared with isolates with elastase gene. The comparison of the two groups of strains, with and without elastase gene, is shown in Table 6.

Table 5: Distribution of *lasB* gene among biofilm producers and Non-producers *P. aeruginosa* isolates.

	Biofilm Producers		**Biofilm Non producers**		**P value**
	No.	**%***	**No.**	**%****	
***lasB*+**	107	97.3	5	33.3	<0.001*

***** Percentage is correlated to total no. of biofilm producer *P. aeruginosa* isolates(110).
****** Percentage is correlated to total no. of biofilm non-producer *P. aeruginosa* isolates(15).

Table 6: Distribution of lasB gene among sensitive, intermediate and resistant *P. aeruginosa* isolates.

Antimicrobial agent	***lasB*+ N(%*)**			***lasB*- N(%**)**			**P value**
	Sensitive	**Intermediate**	**Resistant**	**Sensitive**	**Intermediate**	**Resistant**	
Ciprofloxacin	24(21.4)	5(4.5)	83(74.1)	10(76.9)	2(15.4)	1(7.7)	<0.001*
Amikacin	44(39.3)	22(19.6)	46(41.1)	11(84.6)	0(0)	2(15.4)	0.007*
Cefepime	0(0)	0(0)	112(100)	9(69.2)	0(0)	4(30.8)	<0.001*
Norfloxacin	28(25)	12(10.7)	72(64.3)	12(92.3)	1(7.7)	0(0)	<0.001*
Imipenem	99(88.4)	0(0)	13(11.6)	11(84.6)	0(0)	2(15.4)	0.692
Genamicin	67(59.8)	10(8.9)	35(31.3)	10(76.9)	2(15.4)	1(7.7)	0.192
Tobramycin	79(70.5)	12(10.7)	21(18.8)	11(84.6)	2(15.4)	0(0)	0.225
Aztreonam	49(43.8)	0(0)	63(56.3)	13(100)	0(0)	0(0)	<0.001*
Piperacillin-tazobactam	86(76.8)	7(6.3)	19(17)	10(76.9)	2(15.4)	1(7.7)	0.372
Colistin	32(28.6)	5(4.5)	75(67)	12(92.3)	1(7.7)	0(0)	<0.001*

***** Percentage is correlated to total no. of *P. aeruginosa* isolates which are *lasB* positive (112).
****** Percentage is correlated to total no. of *P. aeruginosa* isolates which are *lasB* negative (13).

Discussion

The major problem associated with infections formed by biofilm producer bacteria is prevalence of resistance to various antibiotics [2]. Extracellular matrix production is a sign of a developed biofilm and serves as a barrier to antibiotics, increasing resistance to them[3]. In the current study we tested 125 clinical isolates of *P. aeruginosa* for their ability to form biofilm by TCP method which could detect 110(88%) of 125 *P. aeruginosa* as biofilm producers (Table1). Based on our results, there was a high prevalence of biofilm formation in our isolates. Another study reported that out of 34 Gram-negative bacteria including *P. aeruginosa* isolates were 25

(73.5%) isolates produce biofilm by TCP method[14].

Another observation was made by Ahmed (2013) in Erbil, who reported that only 28 (38.4%) of the 73 Gram-negative bacteria isolates tested using the TCP approach generated biofilms of a strong or moderate nature. [15]. In addition, Lima et al. (2017) examined TCP to look for biofilm development in isolates of *P. aeruginosa*. Their findings showed that 75% of the isolates formed biofilms. [16]. Maximum biofilm generation in *P. aeruginosa* (100%) was described by Devaraj and Sajjan (2015) among Gram negative bacilli. 93% of Gram negative bacilli had biofilm development, and the TCP technique was the most reliable.[17].

According to a study published in 2011, urinary tract infections accounted for 50% of *P. aeruginosa's* biofilm generation[18]. Samant and Pai (2012) discovered a very high rate of biofilm generation in staphylococcal isolates (89%), and they advise using the TCP approach for biofilm identification[19]. In this study, the TCP technique produced a strong biofilm at a rate of 62.4%. According to a study by Karthic and Gopinath (2016), 35% and 25% of 20 clinical isolates of *P. aeruginosa* that were taken from various clinical specimens produced moderate or strong biofilms, which was not comparable to our findings.[2]. *P. aeruginosa* does, in fact, contain a number of resistance mechanisms that allow it to survive in the presence of antibiotics or biotoxins as well as make it a significant hazard for nosocomial infections. [20]. Table 3 makes clear that there was a substantial prevalence of resistance to all commonly employed antimicrobial treatments.

In contrast to isolates that did not produce biofilm, biofilm-producing *P. aeruginosa* isolates displayed noticeably high levels of antimicrobial resistance to many different classes of antibiotics. This discovery is significant because biofilm, which is known to prevent the spread of antibiotics, makes it more challenging to treat patients with pseudomonal infections.Other researchers have confirmed this observation[2&21].

The findings of this study demonstrate a strong correlation between the development of biofilms and antibiotic resistance. Similar information has been documented in the literature, with significant levels of biofilm in resistance isolates shown by Karthic and Gopinath (2016)[2]. This occurrence may be related to gene transfer mechanisms in biofilm settings, which are frequently acquired through genetic information transfer from one organism to another and delayed antibiotic diffusion inside the bacterial cell[22]. According to Devaraj and Sajjan's (2015) study, which revealed maximal resistance to penicillin (100%) and cephalexin (100%), third- and fourth-generation cephalosporins were shown to have the highest overall resistance [17]. This result might be the result of the misuse of antibiotics in this circumstance. The current investigation is similar to the report of Karthic and Gopinath since imipenem and ciprofloxacin were observed to be less commonly resisted, in accordance with our observations (2016). In contrast to our findings, which indicated that 75 (60%) of the isolates were resistant to colistin, Rewatkar and Wadher (2013) revealed that all of their isolates were sensitive to colistin[21].

P. aeruginosa produces a number of extracellular proteases, such as elastase *lasB*, which are essential virulence factors that harm host tissue when they infect a host and disrupt the host's antibacterial defence mechanisms[23&24]. Over 75% of the clinical isolates of *P. aeruginosa* secrete elastase *lasB*[25]. Yu H et al. investigated how *lasB* affected the growth of the *P. aeruginosa* PAO1 biofilm and showed that *lasB* could promote biofilm formation in part via controlling rhamnolipids[26]. In a study, Tielen et al. shown that changes in the matrix's composition and characteristics can affect *lasB's* ability to affect the formation and architecture of mucoid *P. aeruginosa* SG81 biofilm[9]. *LasB* and the development of biofilms were statistically significantly correlated in this study. The colony of biofilms made by *P. aeruginosa* strains released elastase *lasB* after being exposed to ciprofloxacin at bactericidal concentrations, and the proteolytic activities of the colony biofilms investigated were significantly reduced in comparison to the control biofilms, according to a different study by Oldak *et al.*[24]. Deptula et al investigation's found that multidrug-resistant *P. aeruginosa* clinical isolates had lasA and lasB activity[27]. Furthermore, Sun et al. and Najafi et al.

reported that all of the -lactam-resistant isolates had minimal lasB activity[28].

In the current study, we found a substantial correlation between isolates with and without the elastase gene's antibiotic susceptibility results. According to our research, the elastase-deficient isolates were more susceptible to the antibiotics aztreonam, norfloxacin, colistin, amikacin, and imipenem. Except for gentamicin, tobramycin, piperacillin/tazobactam, and imipenem antibiotics, there was a statistically significant difference between the results of the assessment of the antibiotic sensitivity of *P. aeruginosa* isolates with and without the elastase gene. In *P. aeruginosa* isolates, Fricks-Lima et al. found a substantial positive connection between biofilm resistance and biofilm formation for imipenem, indicating that resistance to this antibiotic strongly linked with biofilm development [29]. Delissalde et al. reported that *P. aeruginosa* isolates that produce biofilms are more resistant to the antibiotics piperacillin/tazobactam and imipenem than are isolates that don't produce biofilms[30]. The data indicates the possibility that there may be a connection between *lasB* expression, development of biofilm and resistance to antibiotics.

Conclusion

In this study, a high frequency of biofilm manufacturing among *P. aeruginosa* isolates was shown. Our *P. aeruginosa* isolates were shown to be resistant to the majority of commonly used antimicrobials, which is significant. This showed a greater propensity for biofilm formation among *P. aeruginosa* clinical isolates and that there is a significant association between biofilm formation and antibiotic resistance. These isolates formed biofilms through a variety of ways, and *lasB* was thought to be a key contributor to biofilm formation.

Declaration section

I hereby declare that all the information given above is true and correct to the best of my knowledge.

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نشرة العلوم الصيدلية
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الارتباط بين مقاومة المضادات الحيوية وتكوين الأغشية الحيوية وجين lasB في Pseudomonas aeruginosa المعزولة من عينات سريرية مختلفة

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تعد بكتريا *Pseudomonas aeruginosa* أحد الأسباب الرئيسيه للإصابات المكتسبة في المستشفيات في جميع أنحاء العالم، كما أنها لها القدرة على مقاومة العديد من المضادات الحيوية والمطهرات. نتجت هذه الظاهرة من مقاومة البكتريا للمضادات الحيوية في مصر نتيجة كثرة وسوء استخدام المضادات الحيوية دون استشارة الطبيب والرجوع إليه. لذلك أصبح الاحتياج إلى نوع جديد من الأدوية ذات تركيب كيميائي جديد للتغلب على مقاومة البكتريا من الضروريات. أجريت هذه الدراسة للتعرف على مدى انتشار بكتريا *Pseudomonas aeruginosa* المقاومة للمضادات الحيوية وتعيين حساسية البكتريا المفصولة للمضادات الحيوية المختلفة وقدرتها على تكوين الغشاء الحيوي وتعيين الجين *lasB* وعلاقته بمقاومة المضادات الحيوية وتكوين الغشاء الحيوي. أجريت هذه الدراسة على ٣٥٠ عينة إكلينيكية مختلفة تم تجميعها من بعض مرضى مستشفى المنيا الجامعي. وقد تم فصل ١٢٥ سلالة من *Pseudomonas aeruginosa* (٣٥,٧ %). وبينت نتائج هذه الدراسة أن أعلى نسبة للعزلات تم فصلها من عينات الجروح (٤١,٢%) يليها عينات إلتهابات الأذن الوسطى (٣٨,٦%) يليها عينات البراز(٣٥,٦%) يليها عينات البصاق(٣١ %) يليها عينات البول(٣٠ %). تم إجراء اختبار حساسية للعديد من المضادات الحيوية على السلالات المعزولة من سودوموناس إرجنوزا ووجد أن السلالات المعزولة أكثر مقاومة للسيفيبيم بنسبة (٩٢,٨%) يليه السبروفلوكساسين (٦٧,٢%) يليه الكولستين (٦٠%) يليه النورفلوكساسين (٥٧,٦%) يليه الأزيترونام (٥٠,٤%) يليه الأميكاسين (٣٨,٤%) يليه الجنتاميسين (٢٨,٨%) يليه التوبراميسين (١٦,٨%) يليه البيبراسيللين–تازوباكتام (١٦%) وأكثرهم كفاءة وأقلهم مقاومة كان الإميبنيم (١٢%). تم الكشف عن قدرة العزلات المختارة من *Pseudomonas aeruginosa* على تكوين الغشاء الحيوي. وجد أن ٦٢,٤ % من العزلات كانت عالية الإنتاج للغشاء الحيوي، و ٢٥,٦ % كانت منتجة معتدلة للغشاء الحيوي، و ١٢% كانت ضعيفة الإنتاج أو غير منتجة للغشاء الحيوي .على المستوى الجزيئي، أوضح تفاعل البلمرة المتسلسل أن نسبة انتشار *lasB* في العزلات كان (٨٩,٦%).