



## Prevalence of Some Virulence Factors among Gram Negative Bacteria Isolated from Patients with Lung Infection and Their Antimicrobial Susceptibility Patterns

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**B**ACTERIAL lung infections can be serious and life-threatening diseases in people of all ages. Recently, bacterial resistance to antibiotics has increased rapidly and the most serious concern is that some bacteria have become resistant to almost all of the easily available antibiotics.

The present work aimed to study the relation between some of the expressed virulence factors and antibiotic resistance among local Gram negative bacteria isolated from infected lungs of patients attended Tanta University hospital, Egypt. Out of 70 different clinical specimens, 30 Gram negative isolates were obtained; 46.6% and 53.3% of total isolates were *Pseudomonas aeruginosa* and Enterobacteriaceae, respectively. All bacterial isolates were tested for their susceptibility against 24 antimicrobial agents as well as the ability to express some virulence factors including hemolysin, gelatinase, protease, lipase, pigment and biofilm formation. The results showed the predominance of *P. aeruginosa* isolates resistance against many of the used antibiotics, with significantly higher mean percent of drug resistant ( $74.11 \pm 7.7$ ) compared to that of Enterobacteriaceae ( $42.97 \pm 9.09$ ). Also, all *Pseudomonas* isolates were extensively drug resistant (XDR), while Enterobacteriaceae were multi-drug resistance (MDR). Although, most virulence factors were expressed at higher rates in *P. aeruginosa* than other bacterial species, no relation between the studied virulence factors and antibiotic resistance except for biofilm production, as the results indicated a direct correlation between resistance of antibiotics and the production of biofilm from tested Gram negative bacteria. There was a significant association between biofilm formation and the extended drug resistant of *Pseudomonas* isolates compared to Enterobacteriaceae ( $P = 0.02$ ).

**Keywords:** Gram negative, Virulence factors, Antibiotic resistance, Lung infection.

### Introduction

Gram-negative bacilli, including the family of Enterobacteriaceae (e.g., *Enterobacter*, *Citrobacter*, *Escherichia coli*, *Klebsiella*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella* and *Yersinia*) and non-lactose fermenting bacteria such as *Pseudomonas* and *Acinetobacter* species, are the most common causes of pneumonia, urinary tract infections, bloodstream and surgical site infections

(Rosenthal et al., 2012; Mehrad et al., 2015). Those pathogens cause life-threatening infections in people of all ages (Pati et al., 2018).

The dilemma is that Gram-negative bacteria show multiple resistance to most available antibiotics with rapid increasing rates of resistance among these pathogens in recent years (Robert & Siegel, 2008; Mehrad et al., 2015). According to the Centers for Disease Control and Prevention (CDC), this situation presents one the most

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important threats to human health worldwide (Atlanta, 2013; Bakour et al., 2016). Moreover, Gram-negative bacilli possess multiple modes of antibiotic resistance and are highly efficient in horizontally transferring them between species (Bakour et al., 2016; Schroeder et al., 2017).

The harmful effects of pathogenic bacteria on their hosts are dependent on bacterial virulence factors which enable them to survive and become more virulent. Bacterial virulence factors ranging from the membrane to secretory proteins (toxins, exoenzymes such as lipases and proteases), biofilm-formation, siderophores as well as polysaccharides that compose the capsules and exhibit anti-phagocytic properties (Hacker & Kaper, 2000). In addition, Gram-negative bacteria have lipopolysaccharide (LPS). These equipments enable bacterial pathogens to establish infection by invading cells, overcoming host defense mechanism and proliferate (Gal-Mor & Finlay, 2006). Bacterial virulence factors are often acquired by horizontal transfer and are thus localized on specific genomic loci (Gal-Mor & Finlay, 2006).

While the spread of multidrug resistant Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus*, routinely capture headlines. Gram-negative pathogens on the other hand attract less attention, even though their emergence and spread are associated with serious public health concerns (Robert & Siegel, 2008). In the 1990s, when Gram positive pathogens were largely responsible for antimicrobial resistance, antimicrobial agents were developed to treat them while, few new effective antibiotics were developed and approved for the treatment of Gram-negative infections (Rice, 2006).

The present study aimed to intensify attention to Gram-negative resistance among local isolates from patients with lung infections who attended Tanta University Hospital, Tanta, Egypt, besides studying the relation between the produced virulence factors of them and antibiotic resistance pattern to facilitate discovery of new drugs.

## **Materials and Methods**

### *Collection of specimens*

A total of 70 Sputum samples were collected from patient attending Tanta Chest Disease Hospital during April 2016 to March 2017. According to Maciel et al. (2009), Samples were

collected early morning after fasting for 8-10hr on a period of two consecutive days. Collection of samples was carried out under direct supervision and using standardized guidance as follows; each patient was asked to use a toothbrush (provided by the study), without toothpaste, to gently remove any food residue from teeth and gums. Patients were instructed to cough deeply to produce a lower respiratory specimen into a sterile previously capped container. This process should be repeated several times to collect at least 2-5ml of sputum if possible. All samples were later examined to make sure it contains thick mucus (yellow to green color). The samples collected on the first day were stored in the refrigerator until the second sample collection in the next day. After collection, all samples were delivered immediately to the laboratory of Bacteriology in Botany Department, Faculty of Science, Tanta University, where they were neutralized with saline and centrifuged at 4000g for 15min. The pellet was cultured on nutrient broth medium.

### *Identification of bacterial isolates*

This work was carried out in Mabaret El-Asafra laboratories, Alexandria, Egypt.

According to Marco et al. (2002), the bacterial isolates were confirmed phenotypically by using VITEK2C system (Biomérieux, France) then the results were interpreted by using VITEK software (version 06.01).

### *Detection of some virulence factors*

#### *Cell-secreted virulence factors*

**Haemolysin production:** The bacterial isolates were tested for their ability to produce haemolysin using blood agar media (Pavlov et al., 2004). Plates were incubated at 37°C for 24hr and then checked for haemolysis around colonies. The results were recorded as follows:  $\alpha$ -haemolysis (greenish zones),  $\beta$ -haemolysis (clear zone) or  $\gamma$ -haemolysis (no haemolysis).

**Protease activity:** All bacterial isolates were assayed for their ability to produce protease enzymes (casein hydrolysis) by using skimmed milk agar as described by Madigan et al. (1999). The isolates were streaked on the 2% skimmed agar and incubated at 37°C for 24hr, plates were checked for halo-regions around the streaks. A zone of clearing of more than 1mm around the streaks was recorded as positive for protease production.

*Gelatinase activity:* Gelatinase (an enzyme that breaks gelatin) production character was tested via straight-line stab inoculation of tubes containing nutrient gelatin medium (MacFaddin, 1985). The tubes were then incubated at 37°C for 24-48hr. Un-inoculated tubes were run besides the inoculated ones as negative control. At the end of incubation period, the liquefaction of the culture medium was observed after placing the culture at 4°C over night.

*Lipase activity:* Egg yolk agar plates were inoculated by each tested isolate and incubated at 37°C. The plates were checked every day for 1-5 days for the appearance of opaque zones surrounding the inocula which indicate positively polytic activity (Sutter et al., 1975).

*Pigment production:* Fourteen *Pseudomonas aeruginosa* isolates were streaked on *Pseudomonas* isolation agar and incubated at 37°C for 24-48hr in order to detect the characteristic fluoresce in (PIA F) and pyocyanin (PIA P) pigment production (Collee et al., 1996; Brooks et al., 2007).

#### *Cell associated virulence factors*

*Biofilm production:* Biofilm production of investigated isolates was measured using microtitre plate (MTP) assay as described by Christensen et al. (1995). All tested organisms isolated from fresh agar plates were inoculated in 10ml of trypticase soy broth with 1% glucose. Broths were incubated at 37°C for 24hr. The cultures were then diluted 1:10 with fresh medium. Individual wells of sterile 96 well microtitre plates were filled with 200µL of the diluted cultures. Negative control wells contained inoculated sterile broth. The plates were incubated at 37°C for 24hr. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2ml of phosphate buffer saline (pH 7.2) four times. This removed free floating bacteria. Biofilm formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed by using 33% Glacial acetic acid and plates were kept for drying. Optical density (OD) of stained adherent biofilm was obtained by using micro ELISA autoreader (model EMR500, Labomed, USA) at wavelength 570nm. The experiment was performed in triplicate and repeated three times. The interpretation of biofilm production (Table 1) was done according to the criteria of Stepanovic et al. (2007).

**TABLE1. Interpretation of biofilm production.**

Average OD value	Biofilm production
$\leq \text{ODc} / \text{ODc} < \sim \leq 2x \text{ODc}$	Non/weak
$2x \text{ODc} < \sim \leq 4x \text{ODc}$	Moderate
$> 4x \text{ODc}$	Strong

Optical density cut-off value (ODc)= Average OD of negative control + 3x standard deviation (SD) of negative control.

#### *Antibiotic susceptibility testing*

Antibiogram was performed using commercially available antibiotic discs. All bacterial isolates were screened for their susceptibility against 24 different antibiotic discs using the agar disc diffusion method (Cursino et al., 2005).

Bacterial isolates were sub-cultured on Muller Hinton (MH) agar plates for 18hr at 37°C. A suspension was prepared using few separate colonies for each isolate in 1-2ml of normal saline. Each suspension was diluted using sterile normal saline to obtain cell count of about  $10^7$  CFU/ml. one hundred microliters of each of the previous suspensions was dropped on the center of two well dried plates of MH agar and was then spread homogeneously using sterile cotton swab and left to dry for 15min. at 37°C. The antibiotic discs were then applied to the prepared plates using sterile forceps, and they pressed gently in site and incubated at 37°C for 18hr. The diameters of the growth inhibition zones were interpreted as susceptible, intermediately resistant or resistant by referring to the Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement (CLSI, 2015).

#### *Extensive drug (XDR) and multidrug resistance (MDR) selection*

According to Magiorakos et al. (2012) MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, while XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories)

#### *Statistical methods*

The data were collected, tabulated and statistically analyzed using Minitab 17.1.0.0 (Minitab, 2013) for windows. All tests were two sided. P-value <0.05 was considered significant. Data normality was checked for using Shapiro-Wilk test. Continues data represent by mean and stander deviation (SD) and categorical data as number and (%), comparison between two continues data was

done using student t-test, while between two or more categorical data via *chi* square test.

## Results

### *Isolation of Garm negative bacteria from clinical sputum samples*

Thirty isolates out of atotal of 70 clinical sputum specimens showed growth on MacConkey agar media to select gram negative organisms.

### *Identification of the investigated bacterial isolates by VITEK2*

All thirty bacterial isolates were identified phenotypically using VITEK2 system which revealed that 14 (46.6%) out of total isolates were identified as *P. aeruginosa*, with 95-99% probability, while the remaining 16 (53.4%) isolates belonged to Enterobacteriaceae were identified as follows; seven (23.33%) isolates were *Enterobacter cloacae* complex with 95-99% probability, four (13.33%) isolates were *Klebsiella pneumonia* with 99% probability, three (10%) isolates were *Citrobacter* species with 99% probability. One (3.33%) isolate was *Providencia stuartii* with 99% probability and the remaining single isolate (3.33%) was identified as *Serratia rubidaea* with 99% probability. The identification results and probability percentage are all shown in Fig. 1.

### *Phenotypic expression of some virulence factors among investigated bacterial isolates*

All recovered thirty isolates were tested for their virulence-forming capacity through studying their ability to produce haemolysin, protease, lipase, gelatinase and biofilm.

As shown in Table 2, All *Pseudomonas*

isolates showed their ability to produce gelatinase, protease and pigment. In addition, 92.8% and 64.29% of isolates produced haemolysin and lipase, respectively. On the other hand, Enterobacteriaceae showed variation in their virulence ability, as 87.5, 6.25 and 6.25 of isolates produce protease, haemolysin and gelatinase, respectively and none of isolates produce lipase.

Also, all *Pseudomonas* isolates were able to form biofilm, as 42.85% and 57.14% produce strong and moderate biofilm, respectively, while 25% and 31% of Enterobacteriaceae formed strong and moderate biofilm respectively and 31.25% were non-biofilm producer (Table 3).

Statistical analysis showed that *P. aeruginosa* isolates were significantly associated with haemolytic activity, gelatinase production, lipase production, protease production, and pigment production,  $P = (< 0.001, < 0.001, < 0.001, < 0.001$  and 0.04, respectively). Moreover, *Pseudomonas* showed significant association with biofilm formation; moderate and strong producing form, in comparison with Enterobacteriaceae,  $P = 0.04$  as shown in Fig. 2.

### *Susceptibility of investigated bacterial isolates to different antibiotics*

The antibiotic resistance patterns of both, Enterobacteriaceae and *P. aeruginosa*, isolates showed MDR with no difference between them (Table 4). However, the mean percent of drug resistant (DR) was significantly higher in *P. aeruginosa*; (74.11±7.7) in comparison with Enterobacteriaceae isolates (42.97±9.09) with significant statistical difference at  $P < 0.001$  as shown in Fig. 3.

## Identified bacteria %



Fig. 1. Prevalence of *Pseudomonas* and Enterobacteriaceae in collected sputum samples.

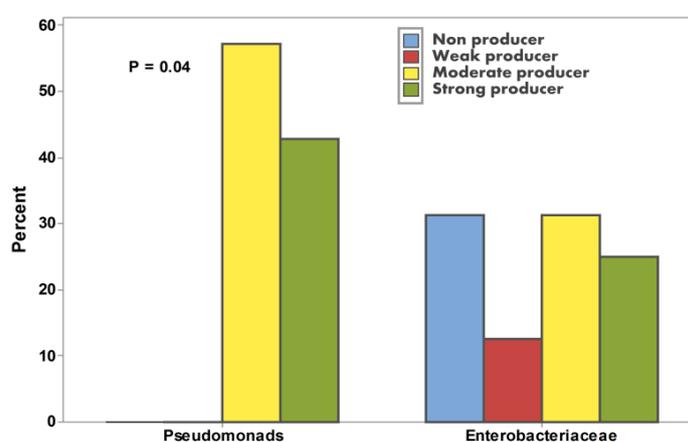
**TABLE 2. Expression of some virulence factors in both *Pseudomonas* and Enterobacteriaceae.**

Virulence factors	Enterobacteriaceae (n= 16)		<i>Pseudomonas</i> (n= 14)		P
Haemolytic activity	1	6.25%	13	92.86%	< 0.001 <sup>#</sup>
Gelatinase	1	6.25%	14	100%	< 0.001 <sup>#</sup>
Lipase	0	0%	9	64.29%	< 0.001 <sup>#</sup>
Protease	14	87.5%	14	100%	0.4 <sup>#</sup>
Pigment production	0	0%	14	100%	< 0.001 <sup>#</sup>

<sup>#</sup>: Chi-square test; P considered significant if < 0.05.

**TABLE 3. Biofilm production in both *Pseudomonas* and Enterobacteriaceae.**

Biofilm production	Enterobacteriaceae (n= 16)		<i>Pseudomonas</i> (n= 14)	
Strong producers	4	25.0%	6	42.85%
Moderate producers	5	31.25%	8	57.14%
Weak producers	2	12.5%	0	0%
Non-producers	5	31.25%	0	0%

**Fig. 2. Biofilm production in both *Pseudomonas* and Enterobacteriaceae.**

Moreover, *P. aeruginosa* showed significant extensive drug resistant activity (XDR) in comparison with Enterobacteriaceae ( $P < 0.001$ ) as shown in Fig. 4.

For  $\beta$ -lactams, all *Pseudomonas* isolates were resistant to all tested antibiotics except Meropenem and Aztreonam with 36% and 64% resistance, respectively. On the other hand, all Enterobacteriaceae were 100% resistant to 7 antibiotics (Ampicillin, Amoxicillin, Pivocillin, Piperacillin, Oxacillin, Amoxicillin/ Clavulanic acid and Ceftazidime). While the resistance towards the other tested antibiotics ranged between 25% for Cefepime and Ceftriaxone and 88% for Meropenem.

In case of aminoglycosides, *P. aeruginosa* isolates recorded the highest incidence of resistance to kanamycin (86%), while

Enterobacteriaceae showed the lowest percentage of incidence to the same antibiotic. Streptomycin was found to be the most active antibiotic against both *P. aeruginosa* and Enterobacteriaceae isolates with low resistance incidence of 5% and 1%, respectively.

Interestingly, all *Pseudomonas* isolates were 100% resistant to both Fluroquinolones (Ciprofloxacin and Nalidixic acid) and Phenicoles (Chloramphenicol), however none of Enterobacteriaceae isolates was resistant to them.

Both organisms; Enterobacteriaceae and *Pseudomonas* isolates were resistant towards TE and CT groups by 100% for both. SXT and VA resistance insignificantly associated with a particular organism, Finally C resistance was significantly associated with *P. aeruginosa*, ( $P < 0.001$ ).

**TABLE 4. Antibiotic resistance profile of both Enterobacteriaceae and *P. aeruginosa*.**

Antibiotics	Enterobacteriaceae (n= 16)		Pseudomonas (n= 14)		P
	n	%	n	%	
<b>β-Lactams</b>					
Ampicillin (AMP)	16	100	14	100	*
Amoxicillin (AX)	16	100	14	100	*
Penicillin(P)	16	100	14	100	*
Piperacillin(PRL)	16	100	14	100	*
Oxacillin(OX)	16	100	14	100	*
Amoxicillin/ Clavulanic acid (AMC)	16	100	14	100	*
Ceftazidime(CAZ)	16	100	14	100	*
Cefepime(FEP)	4	25	14	100	<0.001
Ceftriaxone(CRO)	4	25	14	100	<0.001
Imipenem(IPM)	6	37.5	14	100	<0.001
Meropenem(MEM)	14	87.5	5	35.71	0.006
Aztreonam(ATM)	4	25	9	64.29	0.03
<b>Aminoglycosides</b>					
Gentamicin (CN)	4	25	10	71.43	0.01
Amikacin(AK)	3	18.75	5	35.71	0.4
Streptomycin (S)	1	6.25	6	42.86	0.01
Tobramycin (TOB)	2	12.5	7	50	0.02
Kanamycin (K)	1	6.25	12	85.71	< 0.001
<b>Fluoroquinolones</b>					
Ciprofloxacin (CIP)	0	0	14	100	< 0.001
Nalidixic acid (NA)	0	0	14	100	< 0.001
<b>Folate pathway inhibitors</b>					
Co-trimoxazole (Sulphamethoxazole/ Trimethoprim) (SXT)	8	50	8	57.14	0.7
<b>Tetracyclines</b>					
Tetracycline (TE)	16	100	14	100	*
<b>Phenicoles</b>					
Chloramphenicol (C)	0	0	14	100	< 0.001
<b>Lipopeptides</b>					
ColistinSulphate(CT)	16	100	14	100	*
<b>Glycopeptides</b>					
(VA)	14	87.5	14	100	0.4

#: Chi-square test; P considered significant if <0.05, \*: Not significant, n: Nnumber, SD: Stander deviation, DR: Drug resistance, MDR: Multidrug resistance, XDR: Extensive drug resistance. %: Percentage of resistance. No: number of isolates.

#### *Relation between biofilm production and extensive drug resistance (XDR)*

As shown in Fig. 5, all *P. aeruginosa* isolates were XDR, also there was a significant association of biofilm production and extensive drug

resistance, as all XDR group (*Pseudomonas*) were biofilm producers, while only 68% of non-XDR group (Enterobacteriaceae) were biofilm producers, (P= 0.02).

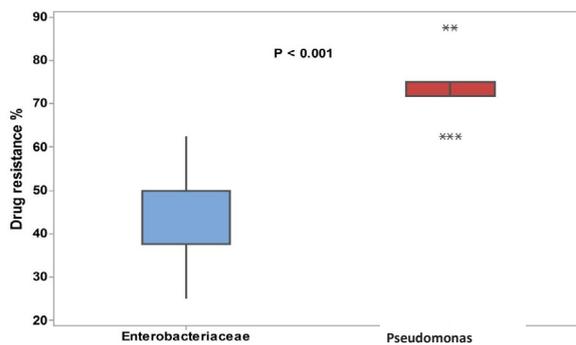


Fig. 3. Drug resistance % in both Enterobacteriaceae and *Pseudomonas*.

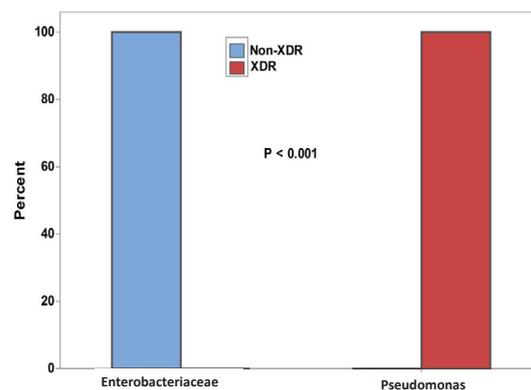


Fig. 4. XDR in both Enterobacteriaceae and *Pseudomonas* (Chi-square test; P considered significant if <0.05.)

## Discussion

The dramatic increase in antibiotic resistance among Gram-negative bacteria in recent decades was identified by the Centers for Disease Control and Prevention as among the most important threats to human health worldwide (Mehrad et al., 2015). In a particular, *P. aeruginosa* infections demonstrate high morbidity and mortality. In addition, chronic pseudomonal lung infections are one of the most common pathogens causing respiratory infections of hospitalized patients (Shaan et al., 2013). For example, in the United States, *P. aeruginosa* is among the most common hospital pathogens and is the second most common pathogen isolated from patients with ventilator-associated pneumonia (Hidron et al., 2008).

In this study, the antibiotic resistance pattern as well as distribution of Gram-negative bacteria from patients with lung infections attended Tanta University hospital was studied. Also, the relation between some expressed virulence factors and antibiotic resistance profile was carried out.

The present study revealed that the most common bacteria isolated from the infected lungs was from Enterobacteriaceae family followed by *P. aeruginosa* isolates (53.3-46.6%, respectively). Mehrad et al. (2015) stated that the most common causes of nosocomial Gram-negative infections are members of the family Enterobacteriaceae followed by *Pseudomonas* and *Acinetobacter* species. In addition, the results showed that the most studied virulence factors were secreted by *P. aeruginosa* isolates more than other species. Several virulence factors are secreted by *P. aeruginosa* and

Enterobacteriaceae which lead to destruction of lung function due to hyper inflammatory response, possibly exacerbated by bacterial toxins, causes the progressive deterioration of lung function and ultimately makes these lung infections fatal (Shaan et al., 2013).

Furthermore, the present data illustrated the significant association of biofilm production in *Pseudomonas* isolates, strong and moderate producing form, in comparison with Enterobacteriaceae ( $P < 0.04$ ). However, all *Pseudomonas* isolates were biofilm producers, while 31% of Enterobacteriaceae isolates were non-biofilm producers. The correlation between pathogenicity of *P. aeruginosa* and biofilm formation had been clearly demonstrated by Shaan et al. (2013) who explained that *P. aeruginosa* can overcome host response through alginate overproduction, that is widely participate in biofilm formation in cystic fibrosis (CF) lung. Also, soluble lectins of *P. aeruginosa* had been involved in strengthen, establishment of biofilms and adhesion to the airways of cystic fibrosis patients (Shaan et al., 2013; Mewe et al., 2005). Thus, could explain the stronger biofilm production in *Pseudomonas* than Enterobacteriaceae.

The different antibiotic resistance patterns was carried out and it was found that *Pseudomonas* and Enterobacteriaceae isolates were MDR; on the other hand, all *Pseudomonas* isolates were XDR, while none of Enterobacteriaceae was XDR according to Magiorakos et al. (2011). In addition, the mean percent of drug resistance was significantly higher in *P. aeruginosa* in comparison with Enterobacteriaceae ( $P < 0.001$ ). *P. aeruginosa*

possess intrinsic resistance that makes them less susceptible to a number of antibiotics than other Gram-negative bacteria (Hancock & Brinkman, 2002). Intrinsic resistance in *P. aeruginosa* is due to the low permeability of its outer membrane, the constitutive expression of membrane efflux (Mex) pumps and the natural occurrence of an inducible chromosomal  $\beta$ -lactamase, AmpC (Strateva & Yordanov, 2009). Also, the emergence of resistance to these agents is mostly due to the production of  $\beta$ -lactamases by Gram-negative bacteria which is the most prevalent mechanism of resistance to  $\beta$ -lactam antibiotics (Robert & Siegel, 2008).

In the present study, aminoglycosides seem to be the most effective class of antibiotics against the investigated *P. aeruginosa* isolates comparing to other classes. However, there appears to be a trend to increase resistance by *P. aeruginosa* to these antibiotics, particularly Gentamicin. We found that 71.4%, 35.7% and 85.7% of the isolates were resistant to the aminoglycosides gentamicin, amikacin and kanamycin, respectively. In CF patients, repeated administration of aminoglycosides to combat chronic lung colonization by *P. aeruginosa* tends to select subpopulations of mutants exhibiting an increasing resistance over time (Bolard et al., 2018). This can be achieved by the horizontal transfer of mobile elements carrying a variety of genes encoding either stereospecific aminoglycoside-modifying enzymes or 16S rRNA methyltransferases, which both prevent the interaction of antibiotics with their target (Poole, 2005). Aminoglycoside resistance may also result from nonenzymatic, mutation-driven intrinsic mechanisms that affect translation machinery (Wilcox et al., 2001) or that tend to reduce the intracellular accumulation of the drug molecules by limiting their diffusion across the bacterial membranes or by promoting their extrusion outside the bacterial cell (Poole, 2005). Moreover, as with any antimicrobial there are geographical variations in resistance rates that likely reflect differences in aminoglycoside prescription patterns and/or the quality of infection control practices (Poole, 2005). For Enterobacteriaceae, fluoroquinolones (FQ) were the most active antibiotics against them, as none of the isolates was resistant to ciprofloxacin and nalidixic acid. These results are in agreement with several studies reported that FQ antibiotics are currently the most effective treatment of nosocomial pneumonia especially infections caused by bacteria from family Enterobacteriaceae (Oliphant et al., 2002; Guillard et al., 2015).

The relationship between antibiotic resistance and the production of previously tested virulence factors of investigated isolates was also studied in the present work. Although most tested virulence factors (haemolysin, lipase, gelatinase, protease and pigment) were expressed at higher rates in *P. aeruginosa* than other bacterial species, still MDR bacterial isolates belonging to both *Pseudomonas* and Enterobacteriaceae gave positive results for the tested virulence factors. Consequently, there was no direct correlation between some produced virulence factors and antibiotic resistance. On the same context, Edge & Enabulele (2014) reported that there was no relationship between hemolysin production and antibiotic resistance among several bacteria isolates from blood culture. Furthermore, another study suggested that antibiotic resistance might not be associated with pigment production in *P. aeruginosa* (Finlayson & Brown, 2011). The obtained data revealed that these virulence factors might increase the ferocity of the bacteria and increase their ability to invade and infect the host cell causing progressive deterioration in host cell.

Regarding biofilm production, the present data indicated that there was a direct correlation between biofilm production and antibiotic resistance, as the results explored that; all *P. aeruginosa* isolate were XDR, also there were significant association of biofilm production and extensive drug resistance as all XDR group, *Pseudomonas*, was biofilm producer, while only 68% of non-XDR, Enterobacteriaceae, group was biofilm producer, ( $P= 0.02$ ). Hence, the present study suggested that the higher biofilm production, the greater the resistance of bacteria to antibiotics. Besides, the biofilm composition might play role in antibiotic resistance, for example, the presence of alginate in *Pseudomonas* biofilms enhances the antibiotic resistance by binding and inactivating the antimicrobials and acting at the same time as an impermeable barrier (Drenkard, 2003). These data are on the same line with those obtained from Allam (2017) who studied the relation between biofilm formation and antibiotic resistance in bacterial urinary tract infection concluded that the most antibiotics resistant uropathogenic isolates were biofilm producers.

The results of this study suggested that the remarkable increase in antibiotic resistance among *P. aeruginosa* isolates was due to the stronger biofilm formation than Enterobacteriaceae. In contrast, there was not direct correlation between

other tested virulence factors and antibiotic resistance. Consequently, the importance of accurate local periodic reports of the resistance pattern is of great importance for providing the most effective antimicrobial prescription patterns. In addition, there is an urgent need to intensify searching for new effective antibiofilm compounds as current treatment regimens are not standardized or broadly effective (Rabin et al., 2015; Webber and Hughes, 2017). Moreover, the commercial antibiotics do not eradicate biofilms effectively, but can also develop resistance (Webber & Hughes, 2017). There is therefore a need for approaches to treat and prevent biofilm-related infections, which do not rely on antibiotics as reported by Webber & Hughes (2017).

### **Conclusion**

In this study, the most common Gram negative bacteria isolated from local clinical samples belongs to family Enterobacteriaceae followed by *P. aeruginosa*. Also, *Pseudomonas* isolates were the most virulent species and XDR. While, Enterobacteriaceae isolates were MDR. However, the results showed that biofilm production by *Pseudomonas* isolates was more associated with antibiotic resistant comparing with the other studied virulence factors. Hence, knowledge of organisms causing lung infections and local resistance pattern is crucial in determining appropriate antibiotic treatment. Also, the resistant isolates should be routinely screened for different kind of easily detectable virulence factors to update the characteristics and new types of resistance mechanisms emerging in local isolates.

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### انتشار بعض عوامل الضراوة بين البكتيريا سالبة الجرام المعزولة من المرضى المصابين بعدوى الرئة و أنماط المقاومة للمضادات الحيوية لديهم

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قد تكون التهابات الرئة الجرثومية من الأمراض الخطيرة والمهددة للحياة عند الأشخاص من جميع الأعمار. و لقد زادت فى الآونة الأخيرة قدرة البكتيريا على مقاومة المضادات الحيوية بشكل سريع و الأشد خطرا هو أن بعض البكتيريا أصبحت تقريبا مقاومة لجميع المضادات الحيوية المتاحة.

ويهدف هذا العمل إلى دراسة العلاقة بين إنتاج بعض عوامل الضراوة ومقاومة المضادات الحيوية فى بعض العزلات البكتيرية سالبة الجرام المعزولة من بعض المرضى المصابين بعدوى فى الرئة من مستشفى جامعة طنطا، مصر. من أصل سبعون عينة مختلفة، تم الحصول على ثلاثون عزلة سالبة الجرام: 46.6% و 53.3% من العزلات كانت من عائلة الانتيروبكتيريا و بكتيريا السيدوموناس اريجينوزا، بالتتابع. كما تم اختبار مقاومة جميع العزلات البكتيرية ضد أربع وعشرون من المضادات الحيوية وكذلك القدرة على إنتاج بعض عوامل الضراوة مثل الهيموليسين، والجيلاتيناز، والبروتياز، والليباز، الصباغات وتكوين الغشاء الحيوى البكتيري. ولقد أظهرت النتائج أن عزلات السيدوموناس كانت مقاومة للعديد من المضادات الحيوية المستخدمة (74.11 ± 7.7)، بنسبة أعلى بكثير مقارنة بعزلات الأنثروبكتيريا (42.97 ± 9.0). كما تم أيضا، دراسة مقاومة هذه الأنواع البكتيرية للمضادات الحيوية ووجد أن جميع عزلات السيدوموناس كانت واسعة المقاومة للمضادات الحيوية (XDR) بينما على الجانب الآخر عزلات الانتيروبكتيريا كانت متعددة المقاومة (MDR). على الرغم من أن معظم عوامل الضراوة انتجت بمعدلات أعلى فى عزلات السيدوموناس اريجينوزا من الأنواع البكتيرية الأخرى، إلا أنه لم يوجد علاقة بين عوامل الضراوة المدروسة ومقاومة المضادات الحيوية باستثناء إنتاج الغشاء الحيوى البكتيري، حيث أظهرت النتائج وجود علاقة مباشرة بين تكوين الغشاء الحيوى البكتيري و قدرة البكتيريا على مقاومة المضادات الحيوية. كما وجد ارتباط كبير بين تكوين الفيلم البكتيري والمقومة الواسعة للمضادات الحيوية (XDR) لعزلات السيدوموناس مقارنة بنظيرتها من عائلة الانتيروبكتيريا (P= 0.02).