Research Article

Virulence Genes in *Pseudomonas aeruginosa* Clinical Isolates

Mohammed S. Mohammed, Ebtisam S. Mohamed, Wedad M. Abdel-Rahim and Alyaa E. Abdelkader
Department of Microbiology, El-Minia Faculty of Medicine

Abstract
Introduction: *Pseudomonas aeruginosa (P. aeruginosa),* which is a non-fermentative Gram negative opportunistic pathogen, is the leading cause of many infections including pneumonia, wound and urinary tract infection. **Aim of the work:** To determine the ability of *P. aeruginosa* clinical isolates to produce biofilm. To detect expression of Lec A gene and biofilm development. **Materials and methods:** Sample Collection and Microbiological Processing: Samples were collected and sent directly to the microbiology laboratory of Medical Microbiology and Immunology Department, Faculty of Medicine, Minia University for bacteriological study. It was done in the period from April 2017 to December 2017. Pus from wound was collected by using sterile ordinary swab, then plates of Cetramide agar media were inoculated and incubated under aerobic conditions at 37°C and a Gram stained film was done. **Results:** Patients and bacterial isolates: This study was conducted at Minia University Hospitals during the period from April 2017 till December 2018. Two hundred and twenty two samples were collected from surgical wounds from outpatient clinic and in-patient surgery department. Out of 222 bacterial isolates, one hundred isolates of *P. aeruginosa* were detected. They showed growth on cetramide agar. They were citrate and oxidase positive. They were indole test negative, methyl red test negative and VP test negative. TSI test showed red butt and red slant. **Conclusion:** 1- The degree of biofilm formation differs between different *P. aeurginosa* strains. 2- *Lec A* gene has a significant role in biofilm formation. 3- Imipenem is highly effective against *P. aeurginosa in vitro*. Biofilm producing strains have higher resistance to antibiotics even in their planktonic form than non-biofilm producing strains. **Recommendations:** Large scale studies on a large number of *P. aeruginosa* clinical isolates to study their abilities to form biofilm and its relation to *Lec A* gene and antibiotics resistance.

Keywords: Virulence Genes, Pseudomonas aeruginosa, biofilm

Introduction
Pseudomonas aeruginosa *(P.aeruginosa),* which is a non-fermentative Gram negative opportunistic pathogen, is the leading cause of many infections including pneumonia, wound and urinary tract infection[(1)]**.**

It has become an important cause of community-acquired and nosocomial infections, especially in immune compromised patients and patients with indwelling medical devices[(2)]**.**

Eradication of infections caused by *P. aeruginosa* is difficult, as the majority of strains exhibit intrinsic resistance to many antibiotics[(3)]**.** This intrinsic resistance is due to expression of beta-lactamases, efflux pumps and low permeability of the outer-membrane[(3)]

Aim of the work
1- To determine the ability of *P. aeruginosa* clinical isolates to produce biofilm.
2- To detect association between expre-ssion of Las R, Lec A and Pel A genes and biofilm development.

Materials and methods
1- Sample Collection and Microbiological Processing:
Samples were collected and sent directly to the microbiology laboratory of Medical Micro-

biology and Immunology Department, Faculty of Medicine, Minia University for bacteriological study. It was done in the period from April 2017 to December 2017. Pus from wound was collected by using sterile ordinary swab,

then plates of Cetramide agar media were inoculated and incubated under aerobic conditions at 37°C and a Gram stained film was done**.**

2- Identification of the isolated organisms:

Culture on cetrimide agar (Biolife, Italy):-

Cetrimide Agar is used for the selective isolation of *Pseudomonas aeruginosa.*

Preparations

45.7 grams in 1000 ml purified/distilled water containing 10 ml glycerol were suspended. They were heated to boiling to dissolve the medium completely. Then Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle, cooled to 45-50°C, mixed well and poured into sterile petri dishes.

Results

Patients and bacterial isolates:

This study was conducted at Minia University Hospitals during the period from April 2017 till December 2018. Two hundred and twenty two samples were collected from surgical wounds from outpatient clinic and in-patient surgery department. Out of 222 bacterial isolates, one hundred isolates of *P. aeruginosa* were detected. They showed growth on cetramide agar. They were citrate and oxidase positive. They were indole test negative, methyl red test negative and VP test negative. TSI test showed red butt and red slant.

Relation between biofilm production and antibiotic resistance:

Table: Antibiotic resistance among biofilm producing and non-biofilm producing strains

antibiotic resistance	Biofilm production		Chi-squared	
	Non-biofilm producer N=73	Biofilm producer N=27	X^2	p-value
	Freq.(%)	Freq.(%)		
Imipenem (IMP)	7(9.6%)	0(0%)	3.240	0.198
Amoxicillin-clavulanate (AMC)	22(30.1%)	8(29.6%)	0.012	0.994
Amikacin (AK)	34(46.6%)	14(51.9%)	0.969	0.616
Ceftazidime (CAZ)	25(34.2%)	10(37%)	1.547	0.461
Levofloxacin (LEV)	25(34.2%)	8(29.6%)	0.198	0.906

Data analyzed by chi-squared and Fischer exact test

This table (table 6) shows that antibiotic resistance to Amikacin and Ceftazidime was higher among biofilm producing strains than non-biofilm producing strains, while resistance to Imipenem, Amoxicillin-Clavulanate and Levofloxacin was higher among non-biofilm producing strains than biofilm producing strains.

Discussion

Pseudomonas aeruginosa (*P.aeruginosa*) has become an important cause of gram-negative infections, especially in immunocompromised patients. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week (Friedrich et al., 2016).

The majority of *P. aeruginosa* strains exhibit intrinsic resistance to several antibiotics[(5)]**.** They also have a high number of virulence

factors including capability to produce biofilm that are responsible for many persistent and chronic infections[5].

Many antibiotics, believed to be the only treatment for *P. aeruginosa* infections, are becoming obsolete as drug-resistant strains are on the rise. So, knowledge about the resistant profile of current strains is very important to target this bacterium[6].

The present study included 100 isolates of *P. aeurginosa* from patients admitted to Minia University Hospitals. The isolates obtained from surgical wounds.

Pseudomonas resistance to most antibiotics is due to a combination of multiple factors. It is intrinsically resistant to antimicrobial agents due to low permeability of its cell wall, presence of different efflux pumps and constitutive expression of β-lactamases[8]. It can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages[9].

Recommendations

1. Large scale studies on a large number of *P. aeruginosa* clinical isolates to study their abilities to form biofilm and its relation to *Lec A* gene and antibiotics resistance.
2. Study the effect of Las R, Lec A and Pel A inhibition on biofilm control.
3. Many researches are needed to find easier methods for diagnosing biofilm infection and to develop more specific antimicrobial agents and methods for eradication or inhibition of biofilm.

References

1. Abdel-Mawgoud A., Lépine F., and Déziel E. (2010): Rhamnolipids: diversity of structures, microbial origins and roles. Appl. Microbiol. Biotechnol. 86 (5):1323-36
2. Al-Mathkhury H. and Assal S. (2012): Inhibitory effect of lactobacilli filtrate on *Klebsiella pneumoniae* biofilm. The Iraqi Postgraduate Med. J., 11(2): 168-179.
3. Beenken K., Mrak L., Griffin L., Zielinska A., Shaw L. and Rice K. (2010): Epistatic relationships between *sar A* and *agr* in *Staphylococcus aureus* biofilm formation. PLoS ONE. 5: e10790.
4. Brooks G. and Carroll K. (2010): Pseudomonads, Acinetobacters and uncommon Gram-negative bacteria. Jawetz., Melnich and Adelberg's medical micro-biology. 25th edition. Chapter 16.P.P. 227-234.
5. Cheesbrough M. (2006): Microbiological tests In District laboratory practice in tropical countries, 2nd ed., part 2., chapt.7: 1-130.
6. Cotter J., Maguire P., Soberon F., Daniels S., O'Gara J., and Casey E. (2011): Disinfection of meticillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms using a remote non-thermal gas plasma. J. Hosp. Infect., 78:204-207.
7. Donlan R. and Rodney M. (2001): Biofilm Formation: A Clinically Relevant Microbiological Process. Clinical Infectious Diseases. 33(8): 1387–1392.
8. Fiorillo L., Zucker M., Sawyer D., and Lin A. (2001): The Pseudomonas hot-foot syndrome. N. Engl. J.Med.,345(5): 335-338.
9. Gassab E., Krifa N., Sayah N., Kaireddine N., and Koubaa J. (2011): Necrotizing otitis externa: report of 36 cases. Tunis Med., 89(2):151-6.