Egyptian Journal of Medical Microbiology Volume 30 / No.2 / April 2021 9-14 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Virulence Factors of Pseudomonas aeruginosa Isolated from ICU

[1]Yasser M. Ismail, [1]Sahar M. Fayed, [2]Aliaa H. Wehedy*
[1] Clinical and Chemical Pathology Department, Faculty of Medicine, Benha University
[2] Clinical and Chemical Pathology Department, Fever Hospital, Benha, Egypt

Key words:
P. aeruginosa, phenotypic virulence factors, antibiotic resistance, ICU patients

Corresponding Author:
Aliaa H. Wehedy
Clinical and Chemical Pathology Department, Fever Hospital, Benha, Egypt
Tel.: 01096180773
aliaa.helmy.med@gmail.com

ABSTRACT

Background: *P.aeruginosa has many virulence factors which are the main reason for infection and the emergence of antibiotic resistance leading to an increase of morbidity and mortality. Currently, multidrug resistance is the hardest problem, which made it imperative to search for alternative treatment strategies.* ***Objective:*** *detection of some phenotypic virulence factors of P.aeruginosa isolated from ICU patients and the possibility of any antibiotic resistance related to certain virulence factors released by P. aeruginosa.* ***Methodology:*** *Our study was carried out on patients admitted to ICU Department in Benha University Hospital and infected with P.aeruginosa, the isolates subjected to phenotypic detection of the virulence factors: phospholipase, alkaline protease, lipase, gelatinase, esculin hydrolysis, biofilm formation, hemolysin and DNase production using specific media for each and evaluation of the antibiotic susceptability pattern using Kirby Bauer disk diffusion assay.* ***Results****: P.aeruginosa virulence factors were recorded as follow: hemolysin (70%) followed by alkaline protease (68%), phospholipase (62%), gelatinase & biofilm formation (60%) for each, lipase & bile esculin hydrolysis (54%) for each and DNase (2%).High antibiotic resistance was detected to mostly all of the used antibiotic discs. Also, presence of invasive device, prolonged hospital stay, ICU stay and higher number of virulence factors were associated with poor outcome.* ***Conclusions****: Production of different phenotypic virulence factors in high amount reflects their important role in spread of infection and pathogenicity with increased antibiotic resistance. Therefore finding anti-virulence factors as adjuvant therapy has an important role in treatment of P. aeruginosa especially MDR isolates.*

INTRODUCTION

P. aeruginosa is a gram negative rod shaped, oxidase positive, motile bacterium with a polar flagellum. It is considered an ubiquitous microorganism widespread in different environments, such as soil, water, plants, animals, and humans. This opportunistic pathogen often colonizes immune-compromised patients. So it is largely involved in hospital-acquired infections, including pneumonia, burns, wounds, urinary tract, gastrointestinal infections, otitis media, and keratitis. [1]

The capability of *P.aeruginosa* to tolerate different environmental conditions and to survive in minimal nutritional needs has allowed this opportunistic bacteria to survive in both general population and hospital units.[2]

During patient hospitalization, colonization rates of this organism may exceed fifty percent 50%, mainly among individuals who have experienced fracture or trauma in the skin or mucosal barriers due to existing surgical interventions, indwelling catheters, ventilation systems, tracheostomy or burns.[3]

This pathogen is a commmon causative agent of many types of pneumonia (nosocomial pneumonia, VAP and health care-associated pneumonia), urinary tract, skin and soft tissue infections. [4]

Biofilms attached to medical equipment or indwelling catheters may be one of the major reasons that made *P.aeruginosa* infections are highly transmissible among hospitalized patients.[5] According to The International Nosocomial Infection Control Consortium, *P. aeruginosa* nosocomial infections have become a worldwide health care issue. [6]

P.aeruginosa has many virulence factors which are the main reason for the incidence of infections and the emergence of resistant strains to different antibiotics constantly leading to an increase of morbidity and mortality among patients. Therefore it is critical to develop therapeutic interventions to treat Pseudomonas infections. Currently, multidrug resistance is the hardest problem associated with the treatment of *P.aeruginosa* infections, which made it imperative to search for alternative treatment strategies. [7]

Serious infections caused by *P.aeruginosa* are predominantly hospital-acquired. It is principally problematic for critically ill patients in intensive care units (ICUs) and burn victims.[8]

The aim of our study was detection of some phenotypic virulence factors secreted by *P. aeruginosa*

DOI: https://doi.org/10.51429/EJMM30202

strains isolated from I.C.U hospitalized patients in Benha University Hospital; detection of phospholipase, lipase, alkaline protease, gelatinase, biofilm formation assay, esculin hydrolysis, hemolysin and DNase using the specific media for each and to detect if there is a possibility of antibiotic resistance related to certain virulence factors released by the organism.

METHODOLOGY

Our study was conducted at Microbiology Laboratory of Clinical Pathology Department in Benha University Hospital at the period from January 2018 to January 2019. Different samples were taken from ICU Department of Benha University Hospital, 50 *P.aeruginosa* isolates were collected and identified by convenentional methods then examined for the presence of some phenotypic virulence factors and their antimicrobial susceptability pattern.

Approval of the ethics committee was obtained from Faculty of Medicine, Benha University.

Full history taken: name, age, sex, history of any present underlying diseases like: lung disease (cystic fibrosis), D.M , other immunosuppressive diseases e.g (cancer, organ transplantation) and type of device implanted, others.

Culture of different samples: on routine bacteriological media

Isolation and identification of *P. aeruginosa*:

- This was done by the standard bacteriological methods as Gram staining.
- Oxidase Test.
- Presence of characteristic pigment.
- Biochemical Reaction as Lysine Iron Agar (LIA), Motility Indole Ornithine (MIO), Triple Sugar Iron (TSI), Citrate and Urease agars.
- Semi-automated " VITEK 2 Compact 15" (BioMerieux).

Phenotypic detection of virulence factors:

Detection of phospholipase: Egg yolk agar was inoculated with colonies& was incubated at 35° C for 24-48h. Appearance of a milky white opaque halo around colony means positive test.[9]

***Detection of alkaline protease*:** A single colony of an overnight growth from a nutrient agar was cultured on skim milk agar& was incubated at 37°C for 24-48 h. The appearance of cleared hydrolysis zone indicates of positive test, as described by **Benson et al.** [10]

Detection of gelatinase: The specific media for detecting gelatin liquefaction inoculated with a single colony of overnight culture from nutrient agar and was incubated for 24 h at 37°C. A positive test indicated if the media show no solidification which refer to the ability of bacteria to produce gelatinase. [11]

Detection of DNAase activity: Colonies were inoculated in a DNase agar plates and were incubated at 37°C for 24-48h. Clearance around the bacterial growth considered positive test**.** [12]

Detection of Biofilm production: The *P.aeruginosa* isolates were analyzed for their ability to produce biofilm using a suitable method. Biofilm formation occurs when bacteria switch from a planktonic (Free-swimming) state to a surface attached state. [13]

***Detection of lipase*:** Strains were spotted on Tween agar &were incubated at 37° C for 24h, opaque zone around spots considered positive test. [14]

***Detection of esculin hydrolysis*:** The medium containing Fe 3^+ citrate was inoculated by spotting, then was incubated for 24 h at 37°C. A black precipitate around colonies due to esculetol release considered positive test**.** [14]

Detection of hemolysin: Blood agar plates with colonies were inoculated at 37° C for 24h then checked for zone of hemolysis present around the colonies[15].

Evaluation of the antibiotic susceptibility pattern:

According to clinical and laboratory standards Institute (CLSI) guidlines[16], using Kirby Bauer disk diffusion assay on Muller-Hinton Agar using the following antibiotics discs; norfloxacin (10 µg/disk), imipenem (10u/disk), gentamycin (10 µg/disk), (piperacillin+tazobactam) (100/10 µg/disk), ciprofloxacin (5 µg/disk), meropenem (10 µg/disk), amikacin (30u/disk), ceftazidime (30 µg/disk), aztreonam (30µg/disk) and tobramycin (10ug). (Oxoid UK).

Statistical analysis:

The collected data were analyzed using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Analytical statistics as:Mann Whitney Test (U test),Chi-Square test , Fisher's exact test, Regression analysis (p is significant if <0.05) at confidence interval 95%. OR, odds ratio; CI, confidence interval.

RESULTS

Clinical Isolates

Fifty *P.aeruginosa* isolates were isolated and identified, including sputum (n=13), ETT aspirate (n=7), blood (n = 15), urine (n = 8) and wound (n = 7).

According to the demographic data the median age of studied cases in our study was 45, ranged from 1 month to 75 years; 34% were children, 30% aged from 18 to 30 and 36% aged more than 50 years There were 62% males and 38% females.

Phenotypic Virulence Factors

Our study revealed that the frequency of evaluated virulence factors & the percentage distributed in samples as follow: the highest percent detected in hemolysin (70%) followed by alkaline protease (68%), phospholipase (62%), gelatinase (60%) as biofilm

formation (60%), lipase (54%), bile esculin hydrolysis (54%), and DNAse (2%). (fig. 1,2,3)

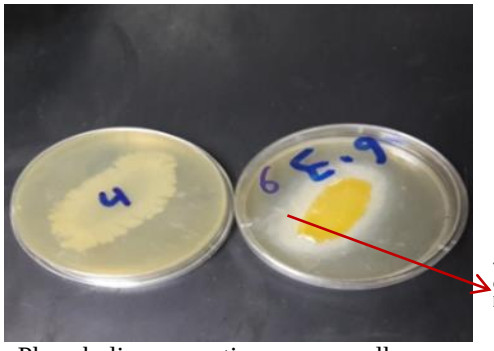

Fig. 1: Phospholipase reaction on egg yolk agar appears as white opaque halo around the colony

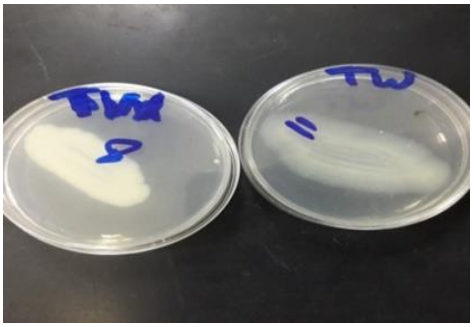

Fig. 2: Tween agar plate shows on Rt side positive lipase reaction appeared as opaque halo around the incubated culture while on Lt side shows negative reaction with no halo

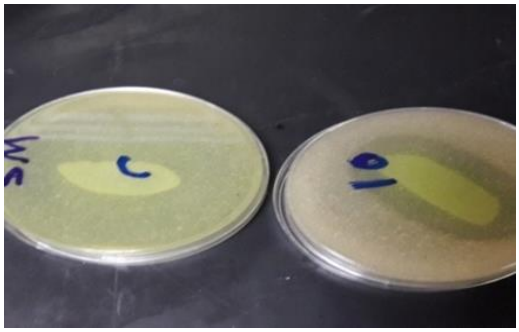

Fig. 3: Skimed milk agar shows on Rt side positive alkaline protease reaction (shows hydrolysis) appeared as clearance zone around the incubated culture while on Lt side shows negative reaction with no change.

Susceptibility Pattern:

In the present study, the highest resistance was for gentamycin (CN) 82% then tobramycin (TOB)74%, cefatizidime (CAZ) 70%, norfloxacin (NOR)70%, ciprofloxacin (CIP) 68%, amikacin (AK) 68%, meronam (MEM)68%, imipenam (IPM) 60%, tazobactam (TZP) 58%, azetronam (ATM) 56%).

In the present study, the highest frequency of resistance was observed with CN and ciprofloxacin, while the highest frequency of sensitivity was observed with imipenim and meropenim. Also, high MDR present in *P. aeruginosa* isolates.

Table 1: Prediction of occurrence of MDR in *P.aeruginosa* isolates.

	Univariable				Multivariable			
	P	**OR**	**95% CI**		**P**	**OR**	**95% CI**	
Age	0.463	0.995	0.980	1.009				
Gender	0.180	2.207	0.910	5.350				
Associated comorbidities	**0.041**	1.455	1.207	1.802	0.267	1.670	1.331	2.357
Invasive device	**0.022**	2.539	1.143	5.639	0.555	1.271	0.573	2.818
Length-of hospitalization stay	0.218	1.047	0.973	1.126				
Length of ICU stay	**0.025**	1.052	1.006	1.101	**0.038**	1.549	1.185	1.942
Presence of any virulence factor	0.704	1.343	0.294	6.136				

OR, odds ratio; CI, confidence interval.

Logistic regression analysis was conducted for prediction of MDR using age, gender, associated comorbidities, invasive device, length of hospitalization, ICU stay, virulence factors as covariates. Presence of associated comorbidities, invasive device, ICU stay were associated of MDR occurrence in univariable analysis. However, in multivariable analysis, longer LOH, ICU stay were considered independent predictors of MDR occurrence in pseudomonas isolates. (table1)

Table 2: Prediction of poor outcome

	Univariable				Multivariable			
	P	**OR**	**95% CI**		**p**	**OR**	**95% CI**	
Age	0.235	1.008	0.995	1.022				
Gender	0.424	0.744	0.360	1.536				
Associated comorbidities	0.311	1.443	0.710	2.934				
Invasive device	**<0.001**	5.075	2.100	12.265	**0.016**	3.728	1.277	10.886
Length-of hospitalization stay	**0.003**	1.178	1.057	1.313	0.632	1.004	0.987	1.022
Length of ICU stay	**0.001**	1.126	1.052	1.205	0.543	1.077	0.849	1.365
Presence of any virulence factor	0.700	1.346	0.297	6.095				

OR, odds ratio; CI, confidence interval

Logistic regression analysis was conducted for prediction of poor outcome using age, gender, associated comorbidities, invasive device, length of hospitalization, ICU stay, virulence factors as covariates. Presence of invasive device, longer LOH, ICU stay were associated of poor outcome in univariable analysis. However, in multivariable analysis, presence of invasive device was considered independent predictor of poor outcome in ICU patients with pseudomonas infection. (table 2)

DISCUSSION

P. aeruginosa can cause life threatening infections in patients with the compromised immune system. Hence, it is a leading cause of clinical infections all over the world especially in patients admitted in critical care units recovering from post-operative surgical wounds, burns, traumas and pre-exiting lung diseases such as cystic fibrosis. According to Centre for Disease Control more than 51,000 clinical infections are reported each year in the USA with 400 deaths per year. [16]

It is considered a common pathogen in hospitals and particularly in intensive Care Units. It is involved in various life threatening infections in ICU such as endocarditis, septicemia, urinary tract infections, cystitis, pneumonia and surgical wound infections. Various mechanisms are involved in drug resistance of Pseudomonas.[17]

The current study recorded the percentage of *P.aeruginosa* virulence factors as follows: hemolysin (70%) followed by alkaline protease (68%), phospholipase (62%), gelatinase & biofilm formation (60%) for each, lipase & bile esculin hydrolysis (54%) for each and DNase (2%).

Pramodhini et al.[11] reported slightly higher percentage in some virulence factors expressed by Pseudomonas aeruginosa than our results as hemolysin was 80.3% , 70% positivity for phospholipase, 71.4% for gelatinase. Hamood et al.,[18] suggested that production of high level of phospholipase C is important in all types of infections.

Mihaela et al.[19] study was in agreement with our results as the most frequently expressed soluble virulence factors were haemolysin & lipase (83.33%), gelatinase & phospholipase (66.67%) as examples of the pore forming enzymes which play an important role in dissemination of infection and wound extent, while esculin hydrolysis was (16.67%) lower than in our study which recorded (54%). Production of proteases by the majority of the tested strains indicates the ability of these strains to induce tissue lesions and to delay the wound healing.

Our study recorded alkaline protease in 68% of the isolates; In harmony with our result. Karatuna et al.,[21] reported the presence of alkaline protease in 73% of lower respiratory samples, another study done by Ciragil et al.,[22] found that alkaline protease in 52% of Cystic Fibrosis (CF) patients and 65% of other non-Cystic Fibrosis.

The biofilms decrease the antimicrobial penetration, give protection from host immune system and provide tolerance against antimicrobials by inducing persistence[23]. In clinical settings, biofilms are formed mostly on indwelling and implanted medical devices used in immunocompromised patients due to improper handling.

In the current study 60% of the studied isolates were recorded as biofilm producers, Pramodhini et al.,[11] disagreed with our results and reported only 34% biofilm producers this may be due to variable conditions like site of infection and presence of device. Several studies in agreement with our results as they reported higher percentage of biofilm production 76% [24,25]and 80%.[31]

DNases are useful virulence factors for bacteria in certain conditions, as for escaping viscous secretions and NETs (neutrophil extracellular traps), which are chromatin formations involved in trapping and killing *P. aeruginosa* in respiratory infections [26]. Because of its anatomy and physiology it is unusual to find thick mucus and NETs within the urinary tract and this may explain the lack of DNases production in UTI isolates, In our study only one isolate gave positive DNase test in sputum sample this may be due to the organism did not depend on this virulence factor in its pathogenicity.

The rise in MDR pathogen infection rates has galvanized efforts to identify new ways to prevent or

treat bacterial infections. One promising new therapeutic approach is to target bacterial virulence factors, with the goal of limiting or preventing pathology rather than outright killing the bacteria. In many cases, the bacterial products that cause host damage are dispensable for colonization or even growth, and their absence leads pathogens to develop a more commensal profile**.** [27]

High antibiotic resistance was detected in our study which recorded as follow: gentamycin (CN 82%) then tobramycin (TOB 74%), cefatizidime as norfloxacin (70%), ciprofloxacin as amikacin (68%), meronam (MEM 68%), imipenam (IPM 60%), azobactam (TZP58%), azetronam (ATM 56%) .

It detected resistance to cefatizidime (70%) and ciprofloxacin (68%), Zafer et al.,[28] reported that several studies showing resistance rate of ceftazidime above 60% that are considered nearer to ours ,while ciprofloxacin from 48-59% which had lower resistance than our results**.**

In contrast to our results Joseph et al.[29] observed significant reduction in resistant rate of ceftazidime from 50% to 33% and of ciprofloxacin from 49% to 33%. The higher resistance to most of the used antibiotics in our study may be due to all our isolates were obtained from ICU**.**

Finlayson et al.,[30]reported that the ability of the isolates to produce pigment and other virulence factors such as elastase, protease, siderophore and DNAse activity appears to be more significantly associated with multi drug resistance (MDR).

In the present study MDR was significantly associated with bile esculin hydrolysis, phospholipase was significantly associated with higher frequency of resistance to ATM. While lipase was significantly associated with higher frequency of resistance to AK and ATM Otherwise, no significant association was found between MDR versus other type or number of virulence factors.

In our study, presence of associated comorbidities, invasive device, longer LOH, ICU stay that detected were associated with MDR occurrence in univariable analysis. However, in multivariable analysis, longer LOH, ICU stay were considered independent predictors of MDR occurrence in pseudomonas isolates.

CONCLUSION

Serious infections with *P. aeruginosa* are problematic for critically ill patients in ICU. Presence of risk factors as invasive device, prolonged hospital stay, ICU stay and higher number of virulence factors were associated with poor outcome. Production of different phenotypic virulence factors by *P.aeruginosa* is the main weapon used to overcome the host immunity and increased resistance, specifically the biofilm causing colonization &spread of infection. Therefore further studies are recommended to find anti-virulence factors as adjuvant therapy with antibiotic treatment of *P.aeruginosa* infection especially those showing MDR.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Sousa AM, Pereira MO.: Pseudomonas aeruginosa diversification during infection development in cystic fibrosis lungs: a review. Pathogen 2014;3:680–703. http://dx.doi.org/10.3390/pathogens3030680.
2. Schwartz T, Armant O, Bretschneider N, Hahn A, Kirchen S, Seifert M, et al.: Whole genome and transcriptome analyses of environmental antibiotic sensitive and multi-resistant Pseudomonas aeruginosa isolates exposed to waste water and tap water. MicrobBiotechnoly.2015; 8(1):116–130.
3. Anvarinejad M, Japoni A, Rafaatpour N, Mardaneh J, Abbasi P, Amin Shahidi M, et al.: Burn Patients Infected With Metallo-Beta- Lactamase-Producing Pseudomonas aeruginosa: Multidrug-Resistant Strains. Arch Trauma Res.2014; 3(2) :e18182.
4. Soltani J, Poorabbas B, Miri N, Mardaneh J. : Health care associated infections, antibiotic resistance and clinical outcome: A surveillance study from Sanandaj, Iran. World Journal of Clinical Cases.2016; 4(3) :63–70.
5. Russotto V., Cortegiani A., Raineri S.M.,Giarratano A: Bacterial contamination of inanimate surfaces and equipement in the intensive care unit. J. Intensive care2015; 3:54.10.1186/s40560-015-0120-5.
6. Rosenthal VD, Al-Abdely HM, El-Kholy A, Alkhawaja SA, Leblebicioglu H, Mehta Y et al.: International nosocomial infection control consortium report,data summary of 50 countries for 2010-2015:device-associated module.Am.J. Infect. Control 2016; (44) 1495-1504. 10.1016/j.ajic.2016.08.007
7. Fairoz Al-Wrafy, Ewa Brzozowska1, Sabina Górska1, Andrzej Gamian1(2016): Pathogenic factors of Pseudomonas aeruginosa – the role of biofilm in pathogenicity and as a target for phage therapy , Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland 2016.
8. Poorabbas B, Mardaneh J, Rezaei Z, Kalani M, Pouladfar G, Alami MH, et al.: Nosocomial

Infections: Multicenter surveillance of antimicrobial resistance profile of Staphylococcus aureus and Gram negative rods isolated from blood and other sterile body fluids in Iran. Iranian Journal of Microbioly2015; 7(3) :127–135.
9. Mittal R, Aggarwal S, Sharma S,Chhibber S,Harjai K.:Urinary tract infections caused by Pseudomonas aeruginosa :a mini review, J Infect Puplic Health.2009; 2(3):101-11.
10. Benson HJ: Microbiological Applications: Laboratory Manual in General Microbiology, (8th ed). Complete version. McGraw-Hill. U.S.A. 2002.
11. Pramodhini S, Umadevi S, seetha KS.: Detection of virulence determinants and its association with drug resistance in clinical isolates of Pseudomonas aeruginosa. Int Journal Research Medical Science. 2016 Sep.;4(9) :3917-3923.
12. Collee AG, Fraser BP, Marmino, Simons A: "Mackin and McCartney Practical Medical Microbiology". 14thed. The Churchil Livingstone, Inc. USA. 1996.; 2(8):658-662.
13. Barbara M. Coffey and Gregory G. Anderson: Biofilm Formation in the 96-well Microtiter Plate. Springer Science+Business Media New York 2014 vol.1149, DOI 10.1007/978-1-4939-0473-0 _48.
14. Georgescul M, Gheorghe I, Curutiu C, Lazar V, Bleotu C, and Chifiriuc M-C: .Virulence and resistance features of pseudomonas aeruginosa strains isolated from chronic leg ulcers. BMC Infectious Diseases 2016.; 16(Suppl 1):92.
15. Pavlov D, De Wet CME, Grebow WOK, Ehlers MM: Potentially pathogenic features of heterophic plate count bacteria isolated from treated and untreated drinking water. Int Journal of Food Microbioly 2004.;92 :275-287.
16. Clinical and Laboratory Standard Institute (CLSI): Performance Standards for Antimicrobial Suseptibility Testing: Twenty-six Informational Supplement, (Wayne, PA, USA) 2016.
17. CDC: *Pseudomonas aeruginosa* in healthcare settings. Atlanta, GA: Centers for Disease Control and Prevention, 2018. https://www.cdc.gov/hai/organisms/pseudomonas.
18. Santajit S, Indrawattana N.: Mechanisms of antimicrobial resistance in ESKAPE pathogens. Biomed Res Int. 2016:1e8.
19. Hamood AN, Griswold JA, Duhan CM. Production of extracellular virulence factors by Pseudomonas aeruginosa isolates obtained from tracheal, urinary tract, and wound infections. J surg Res.1996; 61:425-32
20. Georgescu M, Gheorghe I, Curutiu C, Lazar V, Bleotu C, Chifiriuc M-C: Virulence and resistance features of Pseudomonas aeruginosa strains isolated from chronic leg ulcers From The 11th Edition of the Scientific Days of the National Institute for Infectious Diseases "Prof. Dr. Matei Balş'" Bucharest, Romania. 14-16 October 2015(Georgescu et al. BMC Infectious Diseases 2016, 16(Suppl 1):92 DOI 10.1186/s12879-016-1396-3
21. Karatuna O, Yagci A.: Analysis of quorum sensing-dependent virulence factor production and its relationship with antimicrobial susceptibility in Pseudomonas aeruginosa respiratory isolates. Clin Microbiol Infect 2010; 16: 1770-5
22. Ciragil P, Soyletir G.: Alginate, elastase and alkaline protease production of Pseudomonas aeruginosa strains isolated from various body sites. Mikrobiyol Bul. 2004 Oct; 38(4):341-7.
23. Mulcahy LR, Isabella VM, Lewis K.: *Pseudomonas aeruginosa* biofilms in disease. Microb Ecol. 2014; 68(1):1–12. https ://doi.org/10.1007/s00248-013-0297-x.
24. Khalil MAEF, Sonbol FI, Mohamed AFB, Sameh SA.: Comparative study of virulence factors among ESβL-producing and nonproducing Pseudomonas aeruginosa clinical isolates. Turk J Med Sci. 2015; 45:60-9.
25. Mohammad HH: Phenotypic Investigation for Virulence factors of Pyocine producing Pseudomonas aeruginosa Isolated from Burn Wounds, Iraq. International Journal of Scientific & Engineering Research. 2013;4(7):2114-21.
26. Anghel AM, Holban AM Grumezescu e, Andronescu A, Ficai AG Anghel M, Maganu V, Lazar MC: Modified wound dressing with phyto-nanostructured coating to prevent staphylococcal and pseudomonal biofilm development. NRL 2012, 7, 690.
27. Johnson BK, Abramovitch RB. Small molecules that sabotage bacterial virulence. Trends pharmacol. Sci. 2017 38, 339–362. doi: 10.1016/j.tips.2017.
28. Zafer MM, Al-Agamy MH, El-Mahallawy HA, Amin MA, El-Din Ashour MS.: Antimicrobial Resistance Pattern and Their Beta-Lactamase Encoding Genes among Pseudomonas aeruginosa Strains Isolated from Cancer Patients. Bio Med Research International 2014.
29. Joseph NM, Devi S, Shashikala P, Kanungo R.: Changing Trend in the Antibiotic Resistance Pattern of Pseudomonas aeruginosa Isolated from Wound Swabs of Out-Patients and in-Patients of a Tertiary Care Hospital. Journal of Clinical and Diagnostic Research. 2013; 7(10):2170-2.
30. Finlayson EA. Brown PD: Comparison of antibiotic resistance and virulence factors in pigmented and non-pigmented Pseudomonas aeruginosa. West Indian Medical Journal.2011; 60:24-32.
31. Mona fattoh, Asmaa Nasr El din, Nadia Ahmed et al., EJMM, 2018 : 27(1) :25-33.