Print ISSN: 2321-6379
Online ISSN: 2321-595X
DOI: 10.17354/ijssl/2017/106

Original Article

Antibiotic Resistance Patterns of Biofilm-Forming *Pseudomonas Aeruginosa* Isolates from Mechanically Ventilated Patients

Mina Yekani[1,2], Mohammad Yousef Memar[1,3], Naser Alizadeh[1,3], Nasser Safaei[4], Reza Ghotaslou[1,2]*

[1]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, [2]Microbiology Department, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, [3]Infectious and Tropical Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, [4]Department of Cardiothoracic Surgery, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Introduction: *Pseudomonas aeruginosa* (*P. aeruginosa*) is one of the most commoncauses of difficult-to-treat lung infections. The aim of study wasto evaluate susceptibility patterns of biofilm-forming *P. aeruginosa* atmechanically ventilated patients.

Materials & Methods: Totally,50 *P. aeruginosa* isolates obtained from endotracheal aspirate specimensin patients of cardio-surgical intensive care unitswho were intubated for more than 48h. Detection of biofilm-forming carried out by tube and microtitre assaysandsusceptibility testing was performed by Kirby-Bauer method.

Results: Resistance to piperacillin, gentamicin, ciprofloxacin, aztreonam, ceftizoximeand levofloxacin has been more than 60%. Resistance to colistinwas not seen.Multidrug resistant (MDR) was detected in 65% of the isolates. In the present study, 28 of 50(56%) and 19 of 50 (38%) isolates were biofilm-forming by microtitreand tube methods, respectively. Overall,biofilm-forming isolates were more resistant than non-biofilm-forming *P. aeruginosa* to antibiotics. The biofilm formation was significantly higher instrains that were MDR($p < 0.05$).

Conclusion: Themost effective drug against *P. aeruginosa*was colistin, followed by carbapenems, amikacin and cefepime.*P. aeruginosa* biofilms were extensively more resistant to furthermost antibiotics tested. Therefore, biofilm formation may need more attention when antibiotic treatment is selected for intubated patients.

Key words: Antibiotic resistance, Biofilm, Mechanically ventilated patients, *Pseudomonasaeruginosa*

INTRODUCTION

Biofilms are microorganism accretions used by single or multiple bacterial species to survive in natural environments and play animportant role in infectious diseases(1, 2).About80% ofhumaninfectionsare causedbybiofilmsparticularly hospital infections(3, 4).Biofilm-forming bacteria are resistanttodifferent antibiotics,andlead tochronic infectionthateradication therapy isdifficult. One of the most medical important biofilm-forming bacteria is *Pseudomonas aeruginosa (P. aeruginosa)*, which is usually associated with human nosocomial infections,sever opportunist infections, ventilated-associated pneumonia (VAP) andinfections in the lungs of patients suffering from cystic fibrosis (1, 5-7).*P. aeruginosa* is a one of main respiratory tract pathogen. Rapid colonization of biofilm-forming pathogenic bacteria as *P. aeruginosa* on the outside of inserted endotracheal tubes is an important cause of pneumoniaand septicemia inmechanically ventilated patients (MVP)(8).

Current treatment of *P. aeruginosa* biofilms focusses on the use of antibiotics but the development of antibiotic resistance has led to the ineffectiveness of current therapies. Understanding the bacterial drug resistance due to biofilm-forming is necessary to know the potential drug goals for future studies. While facts about antibiotic sensitivity can assist select the suitable antimicrobial agents in addition to control nosocomial infections. Regarding the different

Access this article online

www.ijss-sn.com

Month of Submission : 07-2017
Month of Peer Review : 07-2017
Month of Acceptance : 08-2017
Month of Publishing : 08-2017

Corresponding Author: Dr. R. Ghotaslou, Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran and Department of Microbiology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Azerbaijan, Iran. E-mail: rzgotaslo@yahoo.com

reports about increasing worldwide drug resistance of Pseudomonas, this study was done to evaluate susceptibility patterns of biofilm-forming *P. aeruginosa*isolated from MVPandthe association between biofilm formation potential and antibiotic resistance.

METHODS AND MATERIALS

Patients

Regular endotracheal aspirate investigation cultures were accomplished in patients who were intubated for more than 48h in the Shaheed MadaniHospital(Cardiac surgery center), Tabriz University of Medical Sciences, Iran. The current study was approved by the local ethics community [No:5/4/8214, Date:2014/07/17].

Microbial Identification and Biofilm Detection

Totally,50non-repetitive*P. aeruginosa* isolates obtained from endotracheal specimens and were identified by standard tests in Microbiology Department of Tabriz University of Medical Sciences during 2014-2015(9). Detection of biofilmcarried out by tube and microtiter assays(10, 11). All biofilm experiments were done in triplicates and the data were averaged and *P. aeruginosa* PAO1 was used as a positive control.

Antimicrobial Susceptibility Testing

Susceptibility testing was performed by Kirby- Bauer method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines(12). Antibiotic discs used in this study included ciprofloxacin, levofloxacin, ceftizoxime, amikacin, gentamicin, cefepime, imipenem, meropenem, piperacillin, aztreonam and colistin (Mast, England). Resistant to more than one agent in three or more classes of antibiotics is defined multiple drug resistant (MDR).

Statistical Methods

Results were entered into the SPSS software version 16 and the data were analyzed by Fisher's exact tests and Pv≤0.05 was regarded statistically significant. The figure was built using Microsoft Excel.

RESULTS

We analyzed 50 clinical isolates of *P. aeruginosa*from endotracheal aspirate.In the present study, 28 of 50(56%) and 19 of 50 (38%) isolates were biofilm-forming relative to a standard *P. aeruginosa*strain PAO1by microtitre andtube methods,respectively. The mean age of patients was47±14including 21 females and 29 males.Resistance to imipenem, meropenem, amikacin, cefepime, piperacillin, gentamicin, ciprofloxacin, levofloxacin, aztreonam, and ceftizoxime were41.94%, 49.27%, 55.48%, 55.86, 61.58%, 67.41%, 67.5%, 68.02%, 69.42% and 70.98%, respectively. Most important observation was in case of colistin that resistance to colistin was not found. MDR isolates were detected in 65% of the isolates. Additionally, among the MDR isolates, the highest prevalence of resistance was related to ceftizoxime, and followed by aztreonam,levofloxacin andciprofloxacin, and the lowest resistance was observed against imipenem. Remarkably, allMDR isolates were sensitive to colistin.The biofilm formation was significantly higher in strains that were MDR ($p< 0.05$). According to results,biofilm-forming isolates were more resistant than non-biofilm-forming *P. aeruginosa* to β-lactams and aminoglycosides. But,noteworthy difference was not detected among biofilm-forming and non-biofilmforming *P. aeruginosa*in resistance to quinolones (Figure 1).

Figure 1, The frequency of antibiotic resistance in biofilm-forming and no biofilm-forming *P. aeruginosa* (IMI= imipenem, CIP= ciprofloxacin, AZT= aztreonam, GEN= gentamicin, PIP= piperacillin, CO= colistin, CTZ= ceftizoxime, LEVO= levofloxacin, MERO= meropenem, AMK= amikacin, FEP= cefepime).

DISCUSSION

Due to restricted oxygen, slow-growing nature of the biofilm, biofilm formation has a crucial role in the establishment and persistence of infectionsand tolerance to antibiotics. Eradication therapy of bacterial biofilm-forming is difficult(10, 13-15).

In this study, the biofilm-forming potential of bacterial strains was assessed using qualitative and quantitative methods. The prevalence of biofilm-forming of *P. aeruginosa* by microtitreand tubemethods was58% and 38%, respectively. It seems microtitreassay was more sensitive than tube method for biofilm detection. Based on three separate studies, the frequency of

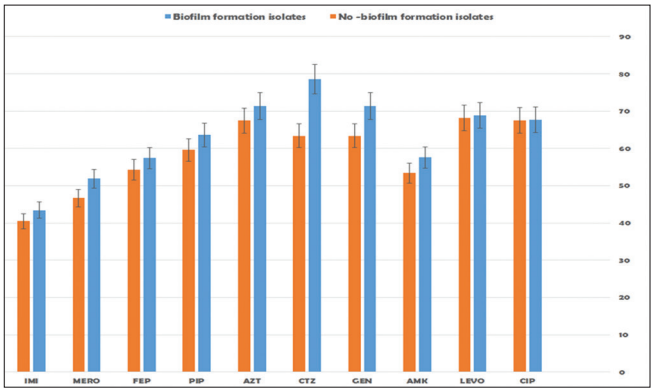

Figure 1: The frequency of antibiotic resistance in biofilm-forming and no biofilm-forming *P. aeruginosa*

biofilm producer*P. aeruginosa*isolates was reported from 23.3% to 35%(16-18).The differences between the various reports about the prevalence of biofilm formation may be attributedto the variation in the sites of infection, multiple subcultures of bacteria, method of biofilmdetection,species-specific and bacterial strain.

P. aeruginosa has emerged as a main lung pathogen, responsible for severe respiratory infections such as pneumonia. Increasing use of ventilator in intensive ward of hospitals has significantly increased the risk for obtaining *P. aeruginosa* infections. *P. aeruginosa* is inherently resistant to numerous antibiotics and change to even more resistance mechanisms has been discovered.Therapy of *P. aeruginosa* infectiousis frustrating because *P. aeruginosa* infectionshappen incompromised host and the infected patient notrespondingwell to drugs as well as *P. aeruginosa* are resistant to most antibiotics. A combination of aminoglycosides and beta-lactam usually are used against *P. aeruginosa* infections(19).

Aminoglycosides are bactericidal activity and show synergy with beta-lactam against *P. aeruginosa*. In the present study, the rate of *P. aeruginosa* resistance to aminoglycoside washigh(61.45 %);these bacteriaare more sensitive to amikacin than gentamicin.The prevalence of resistance to aminoglycosides previously has been reported24 to 76.7%(20, 21).There are geographical differences in resistance rate that likely reflect variation in aminoglycoside use patterns. Despitethe high rate of resistance to aminoglycoside,this antibioticis still consideredan essential part of anti-pseudomonas drugs implicated in the management of pulmonary infections(22).

In this study, similar to a previous investigation(23), high level resistance was observed to fluoroquinoloneand cephalosporin. The augmentedfrequency of MDR *P. aeruginosa* can cause limitations in antibiotic therapy. So, it is essential to investigate the occurrence of MDR in the world. The MDR *P. aeruginosa* was reported from many countries. In two separate studies from Iran(24, 25), reported that 30.1% and 58.65% of the *P. aeruginosa*isolates were MDR.

Currently, MDR *P. aeruginosa* have appeared throughout the world and, more than 30% of the strains are MDR(26). In the present study, the prevalence of MDR isolates were 65%. Reported amounts of MDR *P. aeruginosa* varied broadly based on difference in antibiotic use in the region, socioeconomic state, geographical area, sample size, MDR definition and samples source.It seems in comparison with previous studies, MDR rate has increased. At present, there are numerous reports showed the trend of increasing MDR *P. aeruginosa*(27, 28).A few isolates of *P. aeruginosa* were pan-resistant (excluding colistin), and this problem probablywill be increased in the near future. Carbapenems are considered the last-line antibiotic for treatment of MDR *P. aeruginosa* infections(24).Other studies in Iran reported that the prevalence of imipenem resistance varied from 2.9% to 61.83%(29, 30).The findings of this research indicated that abouthalf of *P. aeruginosa*isolates were susceptible to imipenem. This antibiotic seems to be appropriate for empirical treatment of infections. But, emergence resistance of bacterial strains to carbapenemsdecreases effectiveness of these antibiotics for empirical therapy.

Interestingly, all isolates were susceptible to colistin in agreement with Akhiand co-workers, findings(19). Although this study shows the high *in vitro* activity of colistin against MDR *P. aeruginosa*, the data were still unfavorable.Because, colistinis toxic and clinical experience about the use of colistin in patients is limited, it seemsfurther experience with this antibiotic is needed.If novel antimicrobial agents will not be introduced, clinicians may become obliged to experience again older drugs such as colistin without regard to their toxicity(23).

We found a significant difference between MDR and biofilm formation ($Pv<0.05$).Remarkably, antibiotic susceptibility testing showed that total rate of drug resistance among biofilm-forming isolates was higher than non-biofilm-forming isolates. In the present study, biofilm-forming isolates were more resistant than non-biofilm-forming *P. aeruginosa* to β-lactams and aminoglycosides. But, a significant difference was not distinguished among biofilm-forming and non-biofilm forming *P. aeruginosa* in quinolonsresistance. This proposes that physiological features particular to biofilms formation; efflux pumps expression, pharmacologic characteristics, β-lactamase and amino-transferase production might play a role in improve biofilm antimicrobial resistance. However, biofilm-producing bacteria are 10 to 1,000 times more resistant to antimicrobial agents than the planktonic cell(31).This can be one explanation as to why there is a higher failure rate in the eradication of biofilm-related infections. Recently, mechanism of biofilm resistance to antimicrobial agents has become clearsuch as: the greater biomass, inherent resistance, virulence genes exchange, tolerance to antimicrobial agents, restricted antibiotic penetration, inactivation of antibiotics,an adaptive response, the presence of persisting cells, nutrient limitation and a slow-growing or starved state(10, 32).

Eradication therapy of infections related to biofilm is challenging. A broad understanding of the organization, biofilm genes and structure of the *P. aeruginosa* biofilm

matrix may assist in the development of novel antibiotic therapy aimed at disrupting biofilms. Our data highlight on the importance of: 1) good handling of tracheal tube in order to avoid dangerous infections, 2) selecting accurate and effective antibiotics in MVP infections based microbiology laboratory reports to avoid antibiotic resistance, and 3) according to our results, combination of inhaled antimicrobial agents (e.g. carbapenems and colistin) may besuitable as a way to avoid biofilm formation in the MTP.However, due to the small number of MVP patients examined, further studies examining biofilm formation and antibiotic resistance in larger patients will be required.

In conclusion, we observe a high level of antibiotic resistance among biofilm-forming *P. aeruginosa*strains. This study shows that MDR and biofilm-forming *P. aeruginosa* strains chiefly involved in MVP. Due to high rate of *P. aeruginosa* colonization in the respiratory tract, biofilm formation can increase the toxicity and pathogenicity of this bacterium. All the *P. aeruginosa* even MDR and biofilm-forming strains were sensitive to colistin.

Funding

This work was supported byImmunology Research Center [grant number:93/33], Tabriz University of Medical Sciences, Tabriz, Iran.

ACKNOWLEDGMENTS

The authors are grateful to Miss B. Salahi Eshlaghi for editorial assistance.

REFERENCES

1. Sauer K, Cullen M, Rickard A, Zeef L, Davies D, Gilbert P. Characterization of nutrient-induced dispersion in Pseudomonas aeruginosa PAO1 biofilm. Journal of bacteriology. 2004;186(21):7312-26.
2. Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis. 2002;8(9).
3. Lehman SM, Donlan RM. Bacteriophage-mediated control of a two-species biofilm formed by microorganisms causing catheter-associated urinary tract infections in an in vitro urinary catheter model. Antimicrobial agents and chemotherapy. 2015;59(2):1127-37.
4. Bryers JD. Medical biofilms. Biotechnology and bioengineering. 2008;100(1):1-18.
5. D'Journo XB, Rolain JM, Doddoli C, Raoult D, Thomas PA. Airways colonizations in patients undergoing lung cancer surgery. European journal of cardio-thoracic surgery. 2011;40(2):309-19.
6. Laverty G, Gorman SP, Gilmore BF. Biomolecular mechanisms of Pseudomonas aeruginosa and Escherichia coli biofilm formation. Pathogens. 2014;3(3):596-632.
7. Khan W, Bernier SP, Kuchma SL, Hammond JH, Hasan F, O'Toole GA. Aminoglycoside resistance of Pseudomonas aeruginosa biofilms modulated by extracellular polysaccharide. International microbiology: the official journal of the Spanish Society for Microbiology. 2010;13(4):207.
8. Sharma G, Rao S, Bansal A, Dang S, Gupta S, Gabrani R. Pseudomonas aeruginosa biofilm: potential therapeutic targets. Biologicals. 2014;42(1):1-7.
9. Mahon CR, Lehman DC, Manuselis G. Textbook of Diagnostic Microbiology-E-Book: Elsevier Health Sciences; 2014.
10. Ghotaslou R, Salahi B. Effects of Oxygen on In-vitro Biofilm Formation and Antimicrobial Resistance of Pseudomonas aeruginosae. Pharmaceutical Sciences. 2013;19(3):96.
11. Shakibaie M, Forootanfar H, Golkari Y, Mohammadi-Khorsand T, Shakibaie MR. Anti-biofilm activity of biogenic selenium nanoparticles and selenium dioxide against clinical isolates of Staphylococcus aureus, Pseudomonas aeruginosa, and Proteus mirabilis. Journal of Trace Elements in Medicine and Biology. 2015;29:235-41.
12. Wayne P. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 2007;17.
13. Gallant CV, Raivio TL, Olson JC, Woods DE, Storey DG. Pseudomonas aeruginosa cystic fibrosis clinical isolates produce exotoxin A with altered ADP-ribosyltransferase activity and cytotoxicity. Microbiology. 2000;146(8):1891-9.
14. Patankar YR, Lovewell RR, Poynter ME, Jyot J, Kazmierczak BI, Berwin B. Flagellar motility is a key determinant of the magnitude of the inflammasome response to Pseudomonas aeruginosa. Infection and immunity. 2013;81(6):2043-52.
15. Folsom JP, Richards L, Pitts B, Roe F, Ehrlich GD, Parker A, et al. Physiology of Pseudomonas aeruginosa in biofilms as revealed by transcriptome analysis. BMC microbiology. 2010;10(1):294.
16. Kádár B, Szász M, Kristóf K, Pesti N, Krizsán G, Szentandrássy J, et al. In vitro activity of clarithromycin in combination with other antimicrobial agents against biofilm-forming Pseudomonas aeruginosa strains. Acta microbiologica et immunologica Hungarica. 2010;57(3):235-45.
17. Coban AY, Ciftci A, Onuk EE, Erturan Z, Tanriverdi CY, Durupinar B. Investigation of biofilm formation and relationship with genotype and antibiotic susceptibility of Pseudomonas aeruginosa strains isolated from patients with cystic fibrosis. Mikrobiyoloji bulteni. 2009;43(4):563-73.
18. Hou W, Sun X, Wang Z, Zhang Y. Biofilm-Forming Capacity of Staphylococcus epidermidis, Staphylococcus aureus, and Pseudomonas aeruginosa from Ocular InfectionsBiofilm-Forming Capacity of Human Flora Bacteria. Investigative ophthalmology & visual science. 2012;53(9):5624-31.
19. Akhi MT, Ghotaslou R, Beheshtirouy S, Asgharzadeh M, Pirzadeh T, Asghari B, et al. Antibiotic susceptibility pattern of aerobic and anaerobic bacteria isolated from surgical site infection of hospitalized patients. Jundishapur journal of microbiology. 2015;8(7).
20. Mansoor K, Tanvir SB, Shariq A, Hussain A, Farooqi BJ, Ahmed S, et al. Frequency and susceptibility pattern of Multidrug Resistant Pseudomonas aeruginosa in isolates of patients from a tertiary care hospital of Karachi, Pakistan. 2015.
21. Japoni A, Alborzi A, Kalani M, Nasiri J, Hayati M, Farshad S. Susceptibility patterns and cross-resistance of antibiotics against Pseudomonas aeruginosa isolated from burn patients in the South of Iran. Burns. 2006;32(3):343-7.
22. Poole K. Aminoglycoside resistance in Pseudomonas aeruginosa. Antimicrobial agents and Chemotherapy. 2005;49(2):479-87.
23. Memar MY, Pormehrali R, Alizadeh N, Ghotaslou R, Bannazadeh Baghi H. Colistin, an option for treatment of multiple drug resistant Pseudomonas aeruginosa. Physiology and Pharmacology. 2016;20(2):130-6.
24. Yousefi S, Nahaei M, Farajnia S, Ghojazadeh M, Akhi M, Sharifi Y, et al. Class 1 integron and imipenem resistance in clinical isolates of Pseudomonas aeruginosa: prevalence and antibiotic susceptibility. Iranian journal of microbiology. 2010;2(3):113-9.
25. Ghadaksaz A, Fooladi AAI, Hosseini HM, Amin M. The prevalence of some Pseudomonas virulence genes related to biofilm formation and alginate production among clinical isolates. Journal of Applied Biomedicine. 2015;13(1):61-8.
26. Burjanadze I, Kurtsikashvili G, Tsereteli D, Tsertsvadze E, Kekelidze M, Imnadze P, et al. Pseudomonas aeruginosa infection in an intensive care unit. International Journal of Infection Control. 2007;3(2).
27. Vojtová V, Kolár M, Hricová K, Uvízl R, Neiser J, Blahut L, et al. Antibiotic utilization and Pseudomonas aeruginosa resistance in intensive care units. New Microbiologica. 2011;34(3):291-8.
28. Van Eldere J. Multicentre surveillance of Pseudomonas aeruginosa susceptibility patterns in nosocomial infections. Journal of Antimicrobial

Chemotherapy. 2003;51(2):347-52.
29. Nikbin V, Abdi-Ali A, Feizabadi M, Gharavi S. Pulsed field gel electrophoresis & plasmid profile of Pseudomonas aeruginosa at two hospitals in Tehran, Iran. Indian Journal of Medical Research. 2007;126(2):146.
30. Bahar MA, Jamali S, Samadikuchaksaraei A. Imipenem-resistant Pseudomonas aeruginosa strains carry metallo-β-lactamase gene bla VIM in a level I Iranian burn hospital. Burns. 2010;36(6):826-30.
31. Abidi SH, Sherwani SK, Siddiqui TR, Bashir A, Kazmi SU. Drug resistance profile and biofilm forming potential of Pseudomonas aeruginosa isolated from contact lenses in Karachi-Pakistan. BMC ophthalmology. 2013;13(1):57.
32. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. The lancet. 2001;358(9276):135-8.

How to cite this article: Yekani M, Memar MY, Alizadeh N, Safaei N, Ghotaslou R. Antibiotic Resistance Patterns of Biofilm-Forming Pseudomonas Aeruginosa Isolates from Mechanically Ventilated Patients. Int J Sci Stud 2017;5(5):84-88.

Source of Support: Nil, **Conflict of Interest:** None declared.