

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

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Abstract

Introduction: *Pseudomonas aeruginosa* (*P. aeruginosa*) is one of the most common causes of difficult-to-treat lung infections. The aim of study was to evaluate susceptibility patterns of biofilm-forming *P. aeruginosa* at mechanically ventilated patients.

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

Results: Resistance to piperacillin, gentamicin, ciprofloxacin, aztreonam, ceftizoxime and levofloxacin has been more than 60%. Resistance to colistin was 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 microtitre and tube methods, respectively. Overall, biofilm-forming isolates were more resistant than non-biofilm-forming *P. aeruginosa* to antibiotics. The biofilm formation was significantly higher in strains that were MDR ($p < 0.05$).

Conclusion: The most 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, *Pseudomonas aeruginosa*

INTRODUCTION

Biofilms are microorganism accretions used by single or multiple bacterial species to survive in natural environments and play an important role in infectious diseases (1, 2). About 80% of human infections are caused by biofilms particularly hospital infections (3, 4). Biofilm-forming bacteria are resistant to different antibiotics, and lead to chronic infection that eradication therapy is difficult. One of the most medically important biofilm-forming

bacteria is *Pseudomonas aeruginosa* (*P. aeruginosa*), which is usually associated with human nosocomial infections, severe opportunist infections, ventilated-associated pneumonia (VAP) and infections in the lungs of patients suffering from cystic fibrosis (1, 5-7). *P. aeruginosa* is one of the main respiratory tract pathogens. Rapid colonization of biofilm-forming pathogenic bacteria as *P. aeruginosa* on the outside of inserted endotracheal tubes is an important cause of pneumonia and septicemia in mechanically ventilated patients (MVP) (8).

Current treatment of *P. aeruginosa* biofilms focuses 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

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

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reports about increasing worldwide drug resistance of *Pseudomonas*, this study was done to evaluate susceptibility patterns of biofilm-forming *P. aeruginosa* isolated from MVP and the 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 Madani Hospital (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, 50 non-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 biofilm carried 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 $P \leq 0.05$ 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 PAO1 by microtiter and tube methods, respectively. The mean age of patients was 47 ± 14 including 21 females and 29 males. Resistance to imipenem, meropenem, amikacin, cefepime, piperacillin, gentamicin, ciprofloxacin, levofloxacin, aztreonam, and ceftizoxime were 41.94%, 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 and ciprofloxacin, and the lowest resistance was observed against imipenem. Remarkably, all MDR 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-biofilm-forming *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 infections and 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 microtiter and tube methods was 58% and 38%, respectively. It seems microtiter assay was more sensitive than tube method for biofilm detection. Based on three separate studies, the frequency of

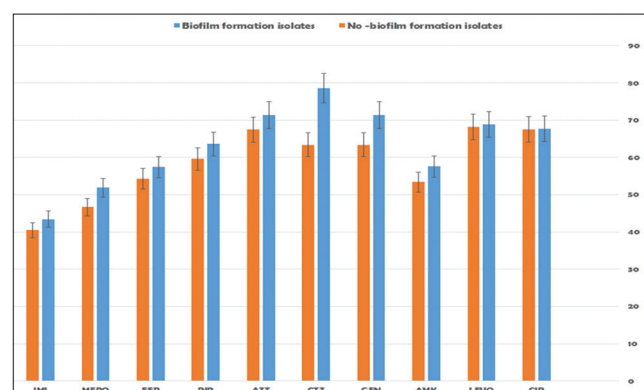


Figure 1: The frequency of antibiotic resistance in biofilm-forming and no biofilm-forming *P. aeruginosa*

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 attributed to the variation in the sites of infection, multiple subcultures of bacteria, method of biofilm detection, 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* infectious frustrating because *P. aeruginosa* infections happen in compromised host and the infected patient not responding well 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 was high (61.45%); these bacteria are more sensitive to amikacin than gentamicin. The prevalence of resistance to aminoglycosides previously has been reported 24 to 76.7% (20, 21). There are geographical differences in resistance rate that likely reflect variation in aminoglycoside use patterns. Despite the high rate of resistance to aminoglycoside, this antibiotic is still considered an 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 fluoroquinolone and cephalosporin. The augmented frequency 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 probably will 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 about half 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 carbapenems decreases effectiveness of these antibiotics for empirical therapy.

Interestingly, all isolates were susceptible to colistin in agreement with Akhiani and 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, colistin is toxic and clinical experience about the use of colistin in patients is limited, it seems further 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 ($P < 0.05$). 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 quinolone resistance. 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 clear such 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 be suitable 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 by Immunology 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.

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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):1-5.

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