Egypt. J. Vet. Sci. **Vol. 51,** No. 1, pp. 105 - 110 (2020)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Pseudomonas aeuroginosa Biofilm and Antimicrobial Resistance

Farajolah Maleki[1], Saeed Hemati[2,3] and Zahra Mahdavi*[2,3]
[1.] *Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran.*
[2.] *Student Research Committee, Ilam University of Medical Sciences, Ilam, Iran*
[3.] *Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.*

INTRODUCTION: It has been shown that biofilm formations are responsible for many human infections, especially in chronic infections. *Pseudomonas aeruginosa* (*p.a*) is an opportunistic human pathogen, its biofilm has serious negative effects on the health of patients with immune-suppresser e.g. cystic fibrosis individuals. *p.a drug* resistance, a worldwide concern is increasing and biofilm-forming potential is an increasing factor. **Materials and Methods:** One-hundred-fifty clinical *P.a* isolates were collected from Mustafa hospital in Ilam, then their resistance against several β-lactam antibiotics that high consumption in the medical prescription was assessment. Also, biofilm formation was tested by microdilution and microtiter plate methods, respectively. **Results:** There are abilities biofilm formations among the resistances isolates to the most tested antibiotics. But, we observed only a significant correlation between resistance to ceftazidime (P=0.003) and meropenem (P=0.002) with biofilm formation, both in resistance and sensitive isolates. **Conclusion**: Biofilm caused by Increasing drug resistance in the world, as β-lactam antibiotics, will be caused a creating complication *P.a* infection treatment. Moreover, biofilm can form antibiotic-resistant strains and increases many infections in the future. As a result, more studies for founding new drugs against biofilm formation mechanisms are necessary.

Keywords: *Pseudomonas aeruginosa*, Biofilm formation, β-lactam antibiotics.

Introduction

Biofilms are aggregates of multilayer microorganisms that organizing in a self-produced extracellular matrix (EPS) [1, 2]. The biofilm formations are responsible for at least 65% of human infections [3]. The U.S. National Institutes of Health reported that more than 80% of chronic infections caused by biofilm formations [2]. Moreover, the biofilm prevented bacterial damaging by covering them from the host immune system attacking [4]. Another complication related to biofilm is the forming of an implant that is similar to catheter and ventilator tubes. These tubes see in the pathogenic microorganism as *P.a* [5]. The *P.a* biofilm causes an infection that is very dangerous in cystic fibrosis (CF) patients and other immune-suppressed patients. The *P.a* is an opportunistic pathogen that involves humans, animals, and plants [6-8]. *The P.a* has native resistance against some antimicrobial agents. However, antibiotic resistance rapidly has been increasing. They are worldwide concerning. The biofilm-forming is partner important to causing this increased resistance [9]. Antibiotic resistance is an important health concern. Recently in the U.S., about 70% of all hospital-acquired infections are resistant to at least one antibiotics family [10]. In other places, the recent using of β-lactams antibiotics in medicine and agriculture is more than already used [11]. Also, many studies have conducted for the clear mechanism behind biofilm resistance. But, the result of them contradicted. So, our aim of this study was to understand the questions; (1) how much the *P.a* was resistance to the six β-lactam antibiotics including; ceftazidime, piperacillin, ticarcillin, carbenicillin,

Corresponding auther, Zahra Mahdavi, E-mail: zahramahdavi9165@gmail.com. Phone and fax; +98832227101.
(*Received* 21/10/2019, *accepted* 10/12/2019)
DOI. 10.21608/ejvs.2019.18523.1113

aztreonam, and meropenem?. (2) Was there a significant correlation between biofilm-forming, and antibiotic-resistant among *P.a* isolates?.

Materials and Methods

From hospitals, bacterial identification conducted by biochemical tests explanted by Owlia et al. [12]. Determination of minimum inhibitory concentrations (MICs): MIC did according to CLSI protocol with the microdilution method explanted by Percival et al. [13].Biofilm formation assay: Biofilm formation assayed by the microtiter plate method mentioned by Amaguchi et al. [14]. Data analysis: Data expressed by percentage, mean and standard deviation (SD). For studied correlation between our data X2 and Fisher's exact test used. A P values < 0.05 were considered to indicate statistical significance.

Results

Antimicrobial susceptibility results: Our finding from antibiotic resistance was ceftazidime (95, 67.85%), piperacillin (83,59.28%), ticarcillin (86,61.42%), carbenicillin (82,8.57%), aztreonam (102,72.85%), andmeropeem (84,60%) (Fig.1).

Biofilm forming results: Biofilm formation assay conducted by microtiter plate method. Our findings were as follow: 18 (12.85%) no producer, while 13 (19.28%) weak producer, 30 (21.42%) moderate producer and 79 (56.42%) strong producer for biofilm formation (Fig. 2).

Correlation between the antibiotic resistance and biofilm formation: Our results showed that there is significant correlation between resistance to ceftazidime (P=0.003) and meropenem (P=0.002) with biofilm formation (Tables 1 and 2).

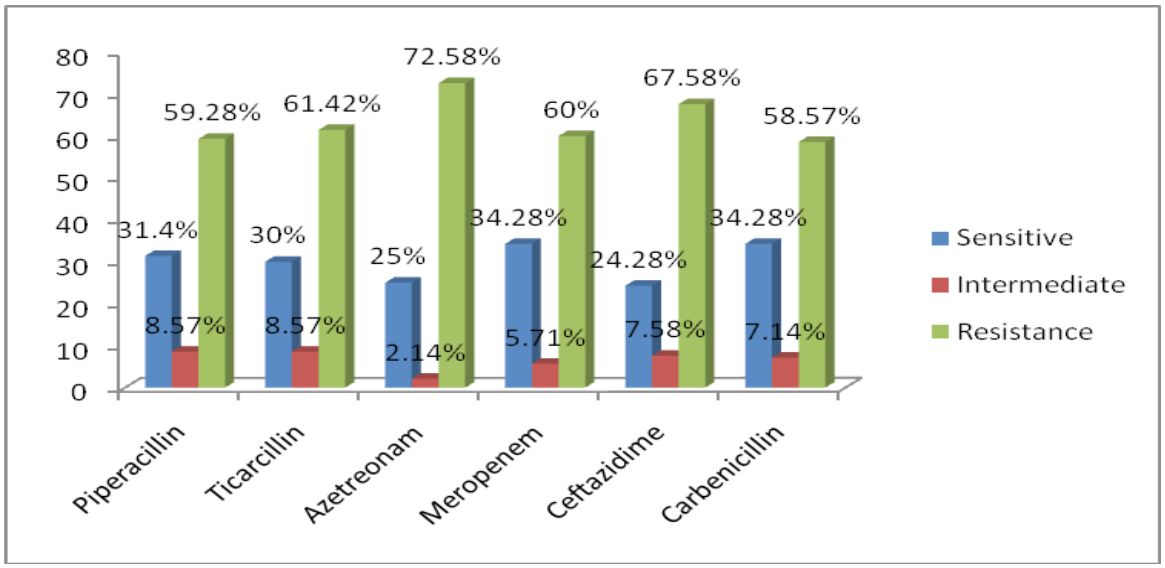

Fig. 1. Antimicrobial susceptibility results for clinical *P.a* isolates

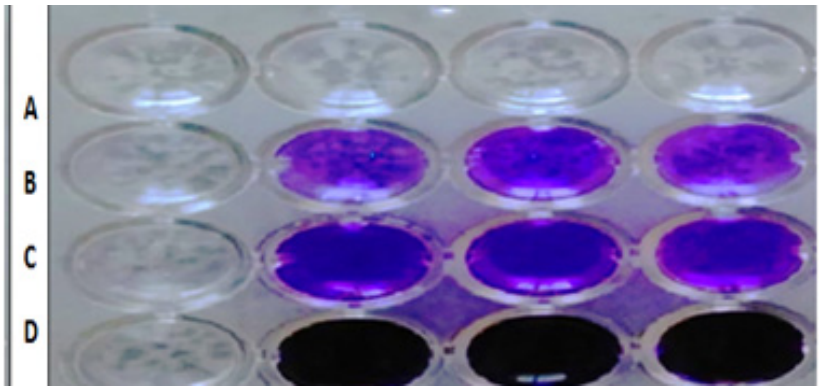

Fig. 2. Biofilm formation by microtiter plate method: A, B, C and D rows showed no producer, weak, moderate and strong producer of biofilm formation.

TABLE 1. Correlation between ceftazidime resistance and biofilm formation.

Antimicrobial susceptibility		Biofilm formation producer				Total
		No producer	Weak producer	Moderate producer	Strong producer	
Sensitive	Count	9	5	10	18	42
	% of total	6.4%	3.6%	7.1%	12.9%	30.0%
Intermediate	Count	2	1	9	5	17
	% of total	1.4%	0.7%	6.4%	3.6%	12.1%
Resistance	Count	7	7	11	56	81
	% of total	5.0%	5.0%	7.9%	40.0%	57.9%
Total		18	13	30	79	140
		12.9%	9.3%	21.4%	56.4%	100.0%

TABLE 2. Correlation between meropenem resistance and biofilm formation.

Antimicrobial susceptibility		Biofilm formation producer				Total
		No producer	Weak producer	Moderate producer	Strong producer	
Sensitive	Count	11	6	14	19	50
	% of total	7.9%	4.3%	10.0%	13.6%	35.7%
Intermediate	Count	3	3	3	6	16
	% of total	2.1%	2.1%	2.1%	4.3%	11.4%
Resistance	Count	4	4	12	54	74
	% of total	2.9%	2.9%	8.6%	38.6%	52.9%
Total		18	13	30	79	140
		12.9%	9.3%	21.4%	56.4%	100.0%

Correlation between the antibiotic resistances with each other: Our finding showed that there is significance correlation between the resistances of most antibiotics with each other. These results summarized in the Table 3 ($P<0.05$).

TABLE 3. Results of correlation between the antibiotic resistances with each other.

Antibiotic	Meropenem	Ceftazidime	Piperacillin	Ticarcillin	Carbenicillin	Aztreonam
Meropenem	-	0.00	0.00	0.036	0.00	0.00
Ceftazidime	0.00	-	0.00	0.011	0.00	0.00
Piperacillin	0.00	0.00	-	0.001	0.00	0.00
Ticarcillin	0.036	0.011	0.001	-	0.003	0.671
Carbenicillin	0.00	0.00	0.00	0.003	-	0.00
Aztreonam	0.00	0.00	0.00	0.671	0.00	-

*The numbers represent P value that a P values <0.05 considered to indicate statistical significance.

Discussion

The β-lactam antibiotics are blocking bacterial peptidoglycan (PG) biosynthesis agents including different groups as penicillins (i.e., ticarcillin, piperacillin), cephalosporins (i.e., ceftazidime, cefepime), monobactams (i.e., aztreonam), and carbapenems (i.e., imipenem, meropenem, and doripenem) that commonly used for treatment of pseudomonas infections [15].Therefore, we studied the resistance of some β-lactam antibiotics in 140 clinical P.a isolates that have widespread use. Antibiotic resistance results were ceftazidime (95, 7.85%), piperacillin (83, 59.28%), ticarcillin (86, 61.42%), carbenicillin (82, 58.57%), aztreonam (102, 72.85%), and meropenem (84, 60%). In other studies, results were as follows; Wang et al. (2013) ceftazidime (41.8%), piperacillin/tazobactam (24.7%), imipenem (25.9%), meropenem (20%), azithromycin (33.5%) [16];Estahbanati et al. (2002) carbenicillin (94.8%) [17]; Nikokar et al. (2013) piperacillin (69.9%), imipenem (23.3%) and carbenicillin (74.4%)[18]; Aghamiri et al. (2014) imipenem (47.16%) [19].

Therefore, we concluded that these antibiotics maybe not effective against such infection and need another drug regime. The β-lactams antibiotics recently widespread use in the medical and agriculture, it causes more genetic exchange and spread of resistance genes within bacteria [11]. Moreover, resistance β-Lactam antibiotic microorganisms cause spread antibiotic resistance genes in other bacteria via horizontal gene transfer [20]. We also observed a significant correlation between resistance to antibiotics with each other (Table 3). So, it is likely that resistance against one antibiotic can induce resistance to this antibiotic or other antibiotics in microorganisms by mechanism mentioned above.

Also, we studied the ability of biofilm formation. It structures formed by several processes embedded in the self-produced extracellular matrix called EPS [21]. Many infections (such as sinusitis, otitis media, tonsillitis, and cholesteatoma, etc.) reported by a biofilm of pathogen_bacteria [22]. Chronic biofilm infections are an important health concern, it needs more hospital visits that sometimes treatment fails [23].Among our isolates, 87.15% formed a biofilm, while 12.85% not producing any biofilm.

Therefore, these findings demonstrated that *P.a* has a high ability for biofilm forming. In agreement, previous studies also showed the tendency of these isolates for biofilm formation [24]. Our data analysis determined that there is a significant correlation between resistance to ceftazidime (P=0.003) and meropenem (P=0.002) with biofilm formation. For it's high resistant there are multiple hypotheses described in previous studies (7, 25, 27-29). Azuma et al. in consistence with our finding showed that antibiotic resistance correlated with biofilm formation among *P.a* isolates [30]. Here, we observed that a susceptible organism similar to resistance organisms capable of biofilm forming. With biofilm formation, they can encounter with unsuitable environmental conditions and cause diverse infections.

In addition, there are rather complex interactions between biofilm and antibiotics resistance, some antibiotics can induce biofilm formation at the sub minimal concentration (sub-MIC) as signaling molecules [31-33]. Thus, the widespread use of β-lactam antibiotics can expose bacteria with low levels f antibiotic concentrations. For encounter, we must have enough niceties in various aspects of antibiotic utilization including their correct application, suitable concentration use, no excessive and unnecessary prescribing and etc. Finally, studies demonstrated that biofilms are responsible for many important infections, especially chronic infections. Recently, they related to important health care problems worldwide. Because of high bacterial ability for its synthesis (resistance and susceptible organisms), it is critical proceedings for their forming preventing or eradication. We emphasis that hospitals and medical centers use from artificial implants (such as Urinary catheter, ventilator and etc.) contained with antimicrobial agents for prevention bacterial attachment. Moreover, due to the lack of efficient antibiotics against medical biofilms [34], we also suggest that further studies about the production of new anti-biofilm drugs or optimized current drugs against organisms or biofilms.

Acknowledgments

Current study supported by instruments of Ilam University of Medical Sciences, authors acknowledges from Clinical Microbiology Research Center staff.

Conflict of interest

The authors declare not conflict of interest.

Funding statement

All funds for this study provided by Ilam University of Medical Sciences.

References

1. Gupta, K., Marques, C.N., Petrova, O.E. and Sauer, K. Antimicrobial tolerance of Pseudomonas aeruginosa biofilms is activated during an early developmental stage and requires the two-component hybrid SagS. *J. Bacteriol*., **195** (21), 4975-4987 (2013).

2. Christensen, L.D., van Gennip, M., Rybtke, M.T., Wu, H., Chiang, W-C. and Alhede, M. Clearance of Pseudomonas aeruginosa foreign-body biofilm infections through reduction of the cyclic di-GMP level in the bacteria. *Infect. Immun*., **81** (8), 2705-2713 (2013).

3. de la Fuente-Núñez, C. and Hancock, RE., Using anti-biofilm peptides to treat antibiotic-resistant bacterial infections. *Postdoc J*., **3** (2), 1-8 (2015).

4. Savage, V.J., Chopra, I. and O'Neill, A.J. *Staphylococcus aureus* biofilms promote horizontal transfer of antibiotic resistance. *Antimicrob. Agents CH*., **57** (4), 1968-1970 (2013).

5. Mulcahy, L.R., Isabella, V.M. and Lewis, K. *Pseudomonas aeruginosa* biofilms in disease. *Microb. Ecol*., **68** (1), 1-12 (2014).

6. Olsen, I. Biofilm-specific antibiotic tolerance and resistance. *Eur. J. Clin. Microbiol. Infect*., **34** (5), 877-886 (2015

7. Zhang, L., Fritsch, M., Hammond, L., Landreville, R., Slatculescu, C. and Colavita, A. Identification of genes involved in *Pseudomonas aeruginosa* biofilm-specific resistance to antibiotics. *PloS one*., **8** (4),e61625(8pages) (2013).

8. Hemati, S., Jalilian, F.A., Pakzad, I., Taherikalani, M., Maleki, A. and Karimi, S. The correlation between the presence of quorum sensing, toxin-antitoxin system genes and MIC values with ability of biofilm formation in clinical isolates of *Pseudomonas aeruginosa*. *IJM*., **6** (3),133-139 (2014).

9. Vincent, M.G., John, N.P., Narayanan, P., Vani, C. and Murugan, S. In vitro study on the efficacy of zinc oxide and titanium dioxide nanoparticles against metallo beta-lactamase and biofilm producing *Pseudomonas aeruginosa*. *J. Appl. Pharm. Sci*., **4** (07), 041-046 (2014).

10. Marti, E., Jofre, J. and Balcazar, J.L. Prevalence of antibiotic resistance genes and bacterial community composition in a river influenced by a wastewater treatment plant. *PloS one*., **8** (10), e78906 (2013).

11. Kaplan, J.B., Izano, E.A., Gopal, P., Karwacki, M.T., Kim, S. and Bose, J.L. Low levels of β-lactam antibiotics induce extracellular DNA release and biofilm formation in *Staphylococcus aureus*. *MBio*., **3** (4), e00198-e00212 (2012).

12. Owlia, P., Nosrati, R., Alaghehbandan, R. and Lari, A.R. Antimicrobial susceptibility differences among mucoid and non-mucoid *Pseudomonas aeruginosa* isolates. *GMS Hyg. Infect. Control*., **9** (2), 1-6 (2014).

13. Percival, S.L., Hill, K.E., Malic, S., Thomas, D.W. and Williams, D.W. Antimicrobial tolerance and the significance of persister cells in recalcitrant chronic wound biofilms. *Wound Repair .Regen.,* **19** (1), 1-9 (2011).

14. Yamaguchi, Y. and Inouye, M. Regulation of growth and death in *Escherichia coli* by toxin–antitoxin systems. *Nat. Rev. Microbiol*., **9** (11), 779-790 (2011).

15. Morita, Y., Tomida, J. and Kawamura, Y. Responses of *Pseudomonas aeruginosa* to antimicrobials. *Front Microbiol*., **4** (422), 1-8 (2013).

16. Wang, H., Tu, F., Gui, Z., Lu, X. and Chu, W. Antibiotic resistance profiles and quorum sensing-dependent virulence factors in clinical isolates of *Pseudomonas aeruginosa*. *Indian J. Microbiol*., **53** (2), 163-167 (2013).

17. Estahbanati, H.K., Kashani, P.P. and Ghanaatpisheh, F. Frequency of *Pseudomonas aeruginosa* serotypes in burn wound infections and their resistance to antibiotics. *Burns*., **28**(4), 340-348 (2002).

18. Nikokar, I., Tishayar, A., Flakiyan, Z., Alijani, K., Rehana-Banisaeed, S. and Hossinpour, M. Antibiotic resistance and frequency of class 1 integrons among *Pseudomonas aeruginosa*, isolated from burn patients in Guilan, Iran. *IJM*., **5** (1), 36-41 (2013).

19. Aghamiri, S., Amirmozafari, N., Fallah Mehrabadi, J., Fouladtan, B. and Samadi Kafil, H. Antibiotic Resistance Pattern and Evaluation of Metallo-Beta Lactamase Genes Including bla-IMP and bla-VIM Types in *Pseudomonas aeruginosa* Isolated from Patients in Tehran Hospitals. *ISRN Microbiol*., 2014, 1-6 (2014).

20. Dupin, C., Tamanai-Shacoori, Z., Ehrmann, E., Dupont, A., Barloy-Hubler, F. and Bousarghin, L.Oral Gram-negative anaerobic bacilli as a reservoir of β-lactam resistance genes facilitating infections with multiresistant bacteria. *Int. J. Antimicrob Agents*., **45** (2), 99-105 (2015).

21. Chua, S.L., Tan, S.Y-Y., Rybtke, M.T., Chen, Y., Rice, S.A. and Kjelleberg, S., Bis-(3′-5′)-cyclic dimeric GMP regulates antimicrobial peptide resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents CH*., **57** (5), 2066-2075 (2013).

22. Zaïri, A., Ferrières, L., Latour-Lambert, P., Beloin, C., Tangy, F. and Ghigo, J.M. In vitro activities of dermaseptins K4S4 and K4K20S4 against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* planktonic growth and biofilm formation. *Antimicrob. Agents CH*., **58** (4), 2221-2228 (2014).

23. Barraud, N., Buson, A., Jarolimek ,W. and Rice, S.A., Mannitol enhances antibiotic sensitivity of persister bacteria in *Pseudomonas aeruginosa* biofilms. *PloS one*., **8** (12), e84220 (2013).

24. Masadeh, M.M., Mhaidat, N.M., Alzoubi, K.H., Hussein, E.I. and Al-Trad, E.I. In vitro determination of the antibiotic susceptibility of biofilm-forming *Pseudomonas aeruginosa* and *Staphylococcus aureus*: possible role of proteolytic activity and membrane lipopolysaccharide. *Infect. Drug Resist*., **6**, 27-32 (2013).

25. Chiang, W.C., Nilsson, M., Jensen, P.Ø., Høiby, N., Nielsen, TE. and Givskov, M. Extracellular DNA shields against aminoglycosides in *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents CH*., **57** (5), 2352-2361 (2013).

26. Tarquinio, K., Confreda, K., Shurko, J. and LaPlante, K. Activities of Tobramycin and Polymyxin E against *Pseudomonas aeruginosa* Biofilm-Coated Medical Grade Endotracheal Tubes. *Antimicrob Agents CH*., **58** (3), 1723-1729 (2014).

27. Tyerman, J.G., Ponciano, J.M., Joyce, P., Forney, L.J., Harmon, L.J. The evolution of antibiotic susceptibility and resistance during the formation of *Escherichia coli* biofilms in the absence of antibiotics. *BMC Evol. Biol*., **13** (22), 1-7 (2013).

28.Tseng, B.S., Zhang, W., Harrison, J.J., Quach, T.P., Song, J.L. and Penterman, J. The extracellular matrix protects *Pseudomonas aeruginosa* biofilms by limiting the penetration of tobramycin. *Environ. Microbiol*., **15** (10), 2865-2878 (2013).

29. Cook, L. and Dunny, G. Effects of biofilm growth on plasmid copy number and expression of antibiotic resistance genes in *Enterococcus faecalis*. *Antimicrob Agents CH*., **57** (4), 1850-1856 (2013).

30. Azuma, M., Murakami, K., Hirota, K., Ono, T., Miyake, Y. and Nishioka, Y. The relationship between antibiotic tolerance and severity of clinical isolated *Pseudomonas aeruginosa* in respiratory disease patients. *Int. J. Antimicrob. Agents*., **42**, S152-S152 (2013)

31. Redelman, C.V., Chakravarty, S. and Anderson, G.G. Antibiotic treatment of *Pseudomonas aeruginosa* biofilms stimulates expression of the magnesium transporter gene mgtE. *Microbiology*, **160** (1), 165-178 (2014).

32. Hengzhuang, W., Ciofu, O., Yang, L., Wu, H., Song, Z. and Oliver, A., High β-lactamase levels change the pharmacodynamics of β-lactam antibiotics in *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents CH*., **57** (1), 196-204 (2013).

33. Bruchmann , J., Kirchen, S. and Schwartz, T. Sub-inhibitory concentrations of antibiotics and wastewater influencing biofilm formation and gene expression of multi-resistant *Pseudomonas aeruginosa* wastewater isolates. *Environ. Sci. Pollut. Res. Int*., **20** (6), 3539-3549 (2013).

34. Beloin, C., Renard, S., Ghigo, J-M. and Lebeaux, D. Novel approaches to combat bacterial biofilms. *Curr. Opin. Pharmacol*., **18**, 61-68 (2014).