Journal of Microbiology and Infectious Diseases / JMID
2013; 3 (2): 56-60
doi: 10.5799/ahinjs.02.2013.02.0081

RESEARCH ARTICLE

Extended Spectrum β-lactamases and antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* in the West Bank, Palestine

Tamer Essawi, Israr Sabri, Mohammad A. Farraj

Master Program in Clinical Laboratory Science, Birzeit University, Birzeit, Palestine

ABSTRACT

Objectives: Class D oxacillinases are frequently acquired by gram negative bacteria in general and *P. aeruginosa* in particular. *P. aeruginosa* is commonly implicated in causing nosocomial infections. The evolution of antibiotic resistance in *P. aeruginosa* and the acquisition of blaOXA genes interfere with successful treatment.

Methods: A total of 49 clinical isolates of *P. aeruginosa* were obtained from Rafidia Hospital, West Bank, Palestine. Antimicrobial susceptibility testing of the isolates was performed by the standard disc diffusion method following the guidelines of CLSI. The prevalence of class D β-lactamases (OXA groups I, II and III) as well as the pseudomonas specific enzymes (CARB-3) were determined by PCR.

Results: Susceptibility of *P. aeruginosa* to carbapenems was the highest 89%, and lowest to ticarcillin/clavulanic acid 70%. This study revealed that *P. aeruginosa* produced oxacillinase enzymes at rates of: OXA-10 (40.8%), OXA-2 (20.4%) and OXA-1 (18.4%). All ceftazidime resistant strains expressed OXA-1 and OXA-2, 18.4%. PSE group was expressed in 10.2%.

Conclusions: This is the first research conducted to investigate the correlation between OXA genes (blaOXA-1, blaOXA-2 and blaOXA-10) and antimicrobial resistance among *P. aeruginosa* clinical isolates in Palestine. The results obtained could contribute to better treatment and reduction of the evolution of resistant strains. In addition, it will provide important information regarding the geographical distribution of class D β-lactamases. *J Microbiol Infect Dis 2013; 3(2): 56-60*

Key words: *P. aeruginosa*, β-lactamase, susceptibility, oxacillinases, blaOXA genes.

Filistin, Batı Şeria'da izole edilen Pseudomonas aeruginosa izolatlarında β-laktamaz varlığı ve antibiyotik duyarlılıkları

ÖZET

Amaç: D sınıfı oksasilinazlar gram negatif basiller içinde ve de özellikle *P. aeruginosa* kökenlerinde yaygındır. *P. aeruginosa* hastane enfeksiyonlarının sık nedenlerinden biridir. *P. aeruginosa* kökenlerinde antibiyotik direncinin ve *blaOXA* genlerinin kazanılması tedavi başarısını etkiler. Bu çalışmada *P. aeruginosa* kökenlerinde antibiyotik direnci ve blaOXA gen varlığının tedavi başarısı üzerine etkileri araştırıldı.

Yöntemler: Toplam 49 klinik *P. aeruginosa* izolatı Filistin'de bulunan Rafidia Hastanesinden elde edildi. Antimikrobiyal duyarlılık testleri standart disk difüzyon metodu ile CLSI standartlarına göre yapıldı. D grubu β-lactamaz (OXA grop I, II ve III) sıklığı ve pseudomonasa özgün enzimlerin (CARB-3) varlığı PCR yöntemiyle araştırıldı.

Bulgular: *P. aeruginosa* kökenlerinde en yüksek duyarlılık karbapenemlere (%89) ve en düşük duyarlılık ise tikarsilin/klavulanik aside (%70) karşı saptandı. Bu çalışmanın sonuçlarına göre; *P. aeruginosa* kökenlerinde OXA-10 oranı %40,8, OXA-2 %20,4 ve OXA-1 %18,4. Seftazidime dirençli kökenlerin hepsi OXA-1 taşırken %18,4'ü OXA-11 taşıyorlardı. PSE grubu salgılanması ise %10,2 idi.

Sonuç: Bu çalışma Filistin'deki *P. aeruginosa* izolatları üzerinde yapılan ve OXA genleriyle (blaOXA-1, blaOXA-2 ve blaOXA-10) antimikrobiyal direnç arasındaki ilişkiyi araştıran ilk çalışmadır. Sonuçlarımız dirençli kökenlerdeki tedavi prensiplerini anlamaya yardımcı oldu ve D grubu β-laktamazların coğrafi dağılımı hakkında bilgi sahibi olmamızı sağladı.

Anahtar kelimeler: *P. aeruginosa*, β-laktamaz, Duyarlılık, Oksasilinaz, blaOXA genleri.

Correspondence: Tamer Essawi,
Master Program in Clinical Laboratory Science, Birzeit University, Birzeit, Palestine Email: tessawi@birzeit.edu
Received: 27 November, 2012 Accepted: 28 May, 2013

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen commonly involved in infections of the immunosuppressed patients and a major cause of nosocomial infections.[1-4] *P. aeruginosa* is intrinsically resistant to various classes of antibiotics through constitutive expression of various efflux pumps, production of β-lactamases and decreased permeability of the outer membrane.[5-7] Acquired resistance by *P. aeruginosa* is mediated by the acquisition of resistance genes for β-lactamases, mutations and fluoroquinolones, over expression of the efflux pumps and decreased expression of porin proteins. Resistance of *P. aeruginosa* to aminoglycosides involves their inactivation by several modifying enzymes which inhibit the binding of these antibiotics to their target.[2,6-8] *P. aeruginosa* is capable of developing multidrug resistance causing treatment failure and resulting in increased rates of morbidity and mortality.[9,10]

Although extended spectrum β-lactamases (ESBLs) of classes A, B and D have recently been reported in *P. aeruginosa*, OXA and PSE types are the most prevalent β-lactamases encountered.[11,12] Genes encoding oxacillinase enzymes are intrinsic in gram negative bacteria including *P. aeruginosa*.[13] The acquired OXA genes can have a narrow or expanded spectrum of hydrolysis of antibiotics.[14] The prevalence of OXA type β-lactamases in *P. aeruginosa* had never been investigated in Palestine. The aim of this study was to assess the antimicrobial susceptibility of clinical isolates of *P. aeruginosa*, the rate of Ambler group A and Ambler group D β-lactamases in our isolates.

METHODS

Bacterial isolates

A total of 49 isolates of *P. aeruginosa* were obtained in 2010 from various clinical sources including wounds (21), sputum (8), urine (6), sores (4), Ear (4), blood (3), CSF (1) and other sites (2). There were 20 isolates from the burn unit, 13 isolates from the intensive care unit, 7 from surgical ward and 9 from outpatient clinics. The ages of the patients ranged from newborn babies to 77 years with 28 females and 21 males. To avoid duplication, one sample for each patient was obtained. The isolates were obtained from Rafidia Surgical Hospital in Nablus, West Bank, Palestine.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *P. aeruginosa* isolates was determined by disc diffusion following the recommendations and guidelines of the Clinical Laboratory Standard Institute (CLSI).[15,16] The antibiotics tested were: Imipenem (10 ug), meropenem (10 ug), gentamicin (10 ug), ceftazidime (30 ug), ciprofloxacin (5 ug), ticarcillin (75 ug) and ticarcillin/clavulanic acid (75 ug/10 ug), (all from Oxoid, Bakingstoke, United Kingdom). *P. aeruginosa* ATCC 27853 was used as a quality control to ensure the accuracy of the antimicrobial susceptibility results. The zone of inhibition was precisely measured using Vernier Caliper and interpreted according to CLSI.[16] Extended spectrum oxacillinases in *P. aeruginosa* was detected by the double disk synergy test according to Didier et al.[17] using ceftazidime resistant isolates with imipenem.

DNA extraction

Bacterial DNA was extracted by emulsifying two to three colonies taken from an overnight culture in 200 ul PBS and heated at 95°C for 10 minute. The suspension was then centrifuged at 12,000 rpm for two minutes and the supernatant containing the DNA was stored at -20°C to be used for amplification by PCR.

PCR

The primers used for the amplifications were obtained from Invitrogen and specified in Table 1. The sequences and the size of each amplicon have been specified previously by Bert et al.[18] Amplification was conducted in a total volume of 25 µL using C-1000 thermal cycler (BioRad, USA). PCR conditions were as follows: 3 minutes at 95°C, followed by 40 cycles of 20 seconds at 95°C, 30 seconds at primer specific annealing temperature (Table 1), 30 second at 72°C and a final extension at 72°C for 5 minutes. The PCR products were run on a 1.5% agarose gel in 1x TAE buffer containing 2.0 µg/ml ethidium bromide. Samples were electrophoresed at 92 V/cm for 30 minutes. The size of the DNA amplicons was determined by comparing it with a 100 bp DNA ladder (Gene Dire) included with each gel. Samples were visualized under UV and then photographed using the Gel Doc system (BioRad, USA).

Table 1. Primer sets used and amplicon sizes to determine the presence of bla OXA genes and PSE group in the clinical isolates of *P. aeruginosa*

Primer pair	Target	5' - 3' sequence	Product size (bp)	Annealing (°C)
OXA10-F OXA10-R	OXA group I	5-TCAACAAATCGCCAGAGAAG-3 5 - CCACTCAACCCATCCTACCC -3	276	56
OXA2- F OXA2- R	OXA Group II	5- AAGAAACGCTACTCGCCTGC -3 5 - CCACTCAACCCATCCTACCC -3	478	58
OXA1-F OXA1-R	OXA Group III	5-TTTTCTGTTGTTTGGGTTTT-3 5-TTTCTTGGCTTTTATGCTTG-3	427	51.5
CARB3-F CARB3-R	PSE	5-ACCGTATTGAGCCTGATTTA-3 5-ATTGAAGCCTGTGTTTGAGC-3	321	52.7

RESULTS

Antimicrobial susceptibility testing

The results of the antimicrobial susceptibility testing for *P. aeruginosa* are shown in Table 2. The carbapenems (imepenem and meropenem) showed the highest activity of 89.8% against this organism followed by ciprofloxacin, gentamicin, ceftazidime, ticarcillin and ticarcillin/clavulanic acid. Multidrug resistance is defined as the ability to resist three different classes of antibiotics or more. In the present study 22.4% (11/49) were multidrug resistant. Six of the multidrug resistant isolates were resistant to all antibiotics tested except two of them were susceptible only to ciprofloxacin. The rate of extended spectrum oxacillinases in *P. aeruginosa* was 8.2% (4/59).

Table 2. Antimicrobial susceptibility results for *P. aeruginosa*; Susceptible (S), Intermediate (I), Resistant (R), percentage (%).

Antibiotic	S (%)	I (%)	R (%)
Ceftazidime	39 (79.6)	2 (4.1)	8 (16.3)
Ciprofloxacin	41(83.7)	1 (2)	7 (14.3)
Gentamicin	39 (79.6)	2 (4.1)	8 (16.3)
Imipenem	44(89.8)	0	5 (10.2)
Meropenem	44 (89.8)	0	5 (10.2)
Ticarcillin	37 (75.5)	1(2)	11 (22.5)
Timentin	35 (71.4)	0	14 (28.6)

PCR results

PCR was performed to detect the expression of Ambler group D β-lactamases by *P. aeruginosa*. The strains that were positive for the different β-lactamase genes tested were subsequently used as positive controls. Primers were selected to determine the carriage of genes for OXA-groups I (blaOXA-10), II (blaOXA-2) and III (blaOXA-1) and the PSE group (PSE-1, PSE-4, CARB-3). A representative photograph of the gel for the PCR results is shown in Figure 1.

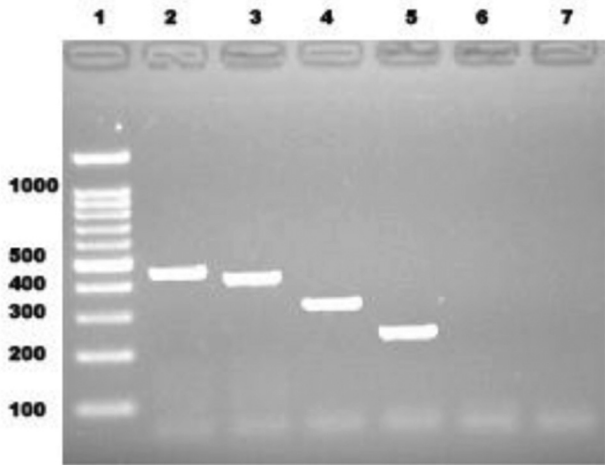

Figure 1. Agarose gel electrophoresis for the amplification of blaOXA genes and CARB3 genes. Lane 1, 100 bp DNA ladder; Lane 2, blaOXA-1; Lane 3, blaOXA-2; Lane 4, CARB3; Lane 5, blaOXA-10; Lane 6, negative control, Lane 7, blank.

The percentage of OXA groups I, II and III among the isolates tested was 40.8% (20/49), 20.4% (10/49) and 22.5% (11/49). The percentage of OXA group I and OXA group II carried by the same isolate was 18.4% (9/49). PSE group was carried by 10.2% of the isolates (5/49). All ceftazidime resistant isolates, 16.3% (8/49) carried the gene for OXA group I.

DISCUSSION

The carriage of group D β-lactamase genes (OXA groups I, II and III) among clinical isolates of *P. aeruginosa* was determined by PCR. OXA group I was the most commonly found among these isolates (40.8%). Interestingly, all ceftazidime resistant isolates carried the β-lactamase gene for OXA group I.

P. aeruginosa is an opportunistic pathogen that causes serious infections particularly in intensive care units. It has been reported that significant thermal injuries induce a state of immunosuppression that predisposes burn patients to infections.[19] It has also been reported that septic patients in intensive care units suffer from compromised immune functions.[20] Based on this, and because the majority of patients in this study (33/49) were from burn unit and intensive care unit, it is safe to consider that these patients may have immunosuppressed status, and become prone to infections.

Multidrug resistant strains are frequent and pose serious problems that result in treatment failure and high mortality rates. Antibiotic susceptibility testing and PCR were carried out to determine the degree of resistance and the prevalence of Ambler class D enzymes among 49 clinical isolates of *P. aeruginosa*.

The antibiotic susceptibility results are shown in Table 2. The resistance profile of the clinical isolates is similar to literature published by Heintz and by Lister et al.[7] In addition, our results are comparable with results obtained in neighboring Israel and Turkey.[2] Resistance rates to ceftazidime and carbapenems are similar to results obtained in Israel, while resistance rates to quinolones and aminoglycosides are higher in Israel. The resistance of *P. aeruginosa* to these antibiotics is consistently higher in Turkey.[2]

The prevalence of OXA type β-lactamases is frequently encountered in *P. aeruginosa*. These enzymes have never been investigated in Palestine. The majority of β-lactamases detected in this study belonged to group D as compared to group A (63.3% versus 10.2%). In a Korean study [11], they reported that class D is more frequent than class A indicating geographical distribution of these genes. OXA-10 was the most common (40.8%) followed by OXA-1 (22.5%) and OXA-2 (20.4%). blaOXA-10 is the most frequently encountered gene in *P. aeruginosa*.[11,21] All ceftazidime resistant isolates that carried only the blaOXA-10 (8/49) were obtained from the intensive care unit. This indicates that the isolates that carry blaOXA-10 are more resistant to these antibiotics than the strains that do not carry it.

Four ceftazidime resistant isolates carried blaOXA-2 (4/9) in addition to blaOXA-10. It is interesting to note that all isolates carrying the blaOXA-1 gene (10/49) were susceptible to all antibiotics tested. Most of the multidrug resistant isolates encountered in this study carried both blaOXA-10 and blaOXA-2 or blaOXA-10 alone. Although two of these isolates were resistant to all antibiotics tested, three isolates were only susceptible to imipenem and two isolates were only susceptible to ciprofloxacin.

The acquisition of OXA β-lactamases is frequently identified in gram negative bacteria particularly in *P. aeruginosa*, Enterobacteriaceae and Acinetobacter species. Selective antibiotic pressure that develops in response to over use of β-lactam antibiotics particularly in hospitals can be responsible for the expression and dissemination of these enzymes. The threat of treatment failure is amplified by the evolution of *P. aeruginosa* strains expressing expanded spectrum oxacillinase activity (ES-OXA).

In conclusion, the present study is the first to determine the prevalence of b blaOXA-10, blaOXA-2 and blaOXA-10 among clinical isolates of *P. aeruginosa*. The emergence of extended spectrum group D β-lactamases among *P. aeruginosa* isolates must be taken seriously. Hospitals should take the necessary measures to limit the spread of this pathogen. This would contribute to better treatment and prevent their spread.

REFERENCES

1. Giamarellou H, Kanellakopoulou K. Current therapies for *Pseudomonas aeruginosa*. Crit Care Clin 2008;24:261-278.
2. Souli M, Galani I, Giamarellou H. Emergence of extensively drug-resistant and pandrug resistant Gram negative bacilli in Europe. Eurosurveillance 2008;13:1-11.
3. Wirth FW, Picoli SU, Cantarelli VV, et al. Metallo-β-lactamase-producing *Pseudomonas aeruginosa* in two hospitals from Southern Brazil. Braz J Infect Dis 2009;13:170-172.
4. Mahmood F, Hakimiyan A, Jayaraman V, et al. A novel human antimicrobial factor targets *Pseudomonas aeruginosa* through its type III secretion system. J Med Microbiol 2013;62,531-539.
5. Hancock EWR, Speert DP. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and impact on treatment. Drug Resistance Updates 2000;3:247-255.
6. Mesaros N, Nordmann P, Plesiat P, et al. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect 2007;13:560-578.
7. Lister PD, Wolter DJ, Hanson ND. Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 2009;22:582-610.
8. Hirsch EB, Tam VH. Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes. Expert Rev Pharmacoecon Outcomes Res 2010,10:441-451.

9. Cholley P, Thouverez M, Hocquet D, van der Mee-Marquet N, Talon D, Bertrand X. Most multidrug-resistant *Pseudomonas aeruginosa* isolates from hospitals in Eastern France belong to a few clonal types. J Clin Microbiol 2011;49:2578-2583.
10. Khosravi Y, Tay ST, Vadivelu J. Analysis of integrons and associated gene cassettes of metallo-β-lactamase-positive *Pseudomonas aeruginosa* in Malaysia. J Med Microbiol 2011; 60:988-994
11. Lee S, Park YJ, Kim M, et al. Prevalence of Ambler class A and D β-lactamases among clinical isolates of *Pseudomonas aeruginosa* in Korea. J Antimicrob Chemother 2005;56:122-127.
12. Strateva T, Ouzounova-Raykova V, Markova B, Todorova A, Marteva-Proevska Y, Mitov I. Problematic clinical isolates of *Pseudomonas aeruginosa* from the university hospitals in Sofia, Bulgaria: current status of antimicrobial resistance and prevailing resistance mechanisms. J Med Microbiol 2007;56:956-963.
13. Strateva T, Yordanov D. *Pseudomonas aeruginosa* - a phenomenon of bacterial resistance. J Med Microbiol 2009;58:1133-1148.
14. Doi Y, Poirel L, David L, Paterson DL, Nordmann P. Characterization of a Naturally Occurring Class D β-Lactamase from Achromobacter xylosoxidans. Antimicrobial agents and chemotherapy 2008;52:1952-1956.
15. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests; Approved standard - 8th edition 2009; MO2-A10,29:4-27.
16. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 20th information supplement 2010; M100-S20,30:52-53.
17. Didier H, Dehecq B, Bertrand X, Plesiat P. Strain-Tailored Double-Disk Synergy Test Detects Extended-Spectrum Oxacillinases in *Pseudomonas aeruginosa*. J Clin Microbiol 2011;49:2262-2265.
18. Bert Frederic, Branger Catherine, Lambert-Zechovsky Nicole. Identification of PSE and OXA β-lactamase genes in *Pseudomonas aeruginosa* using PCR-restriction fragment length polymorphism J Antimicrob Chemother 2002;50:11-18.
19. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn Wound Infections. Clin Microb Rev 2006;19:403-434.
20. Venet F, Lepape A, Monneret G. Clinical review: flow cytometry perspectives in the ICU - from diagnosis of infection to monitoring of injury-induced immune dysfunctions. Crit Care 2011;15:231.
21. Heintz BH, Halilovic J. Lessons learned from surveillance of antimicrobial susceptibilities of *Pseudomonas aeruginosa* at a large academic medical center. Pharmaceuticals 2010;3:1070-1083.