ISSN: 2383-2568 *Quarterly of International Archives of Health Sciences 2016;3(3):125-129*

Role of Class I, II and III Integrons in Multidrug Resistance in *Pseudomonas aeruginosa* Isolated from Nosocomial Hospital

ARTICLE INFO

Article Type
Descriptive Study

Authors
Nourbakhsh F.* *PhD,*
Momtaz H.[1] *PhD*

How to cite this article
Nourbakhsh F, Momtaz H. Role of Class I, II and III Integrons in Multidrug Resistance in Pseudomonas aeruginosa Isolated from Nosocomial Hospital. International Archives of Health Sciences. 2016;3(3):125-129.

ABSTRACT

Aims The increasing usage of antibiotics can cause resistance to the treatment of infections, which can caused by bacteria, e.g. *Pseudomonas aeruginosa*.The aim of this study was to trace the class I, II and III integrons in isolates of P. aeruginosa of nosocomial infection and determining the antibiotic resistance pattern of the bacteria.

Instrument & Methods In this cross-sectional study, 100 *Pseudomonas aeruginosa* clinical isolates of infected wounds, bedsores, burns, urinary tract infections and respiratory tract infections were collected from patients of 3 Isfahan City hospitals, Iran (Al Zahra, Kashani, Shariati) in 2015. After identification tests and antibiogram, integrons class I, II and III were detected by M-PCR method.Data analysis were performed in SPSS 16 software using Chi-square and Fisher exact tests and the relationship between the presence of class III, II, I was calculated by M-PCR test.

Findings All isolates had multiple antibiotic resistances. The highest antibiotic resistance was to Tetracycline (85%) and the lowest to Norfloxacin (12.5%). There were significant differences between class I and the two other classes of integrons (p=0.036). There was a statistically significant difference between the presence of blaTEM gene in *Pseudomonas aeruginosa* with other coding genes for antibiotic resistance (p=0.029).

Conclusion *Pseudomonas aeruginosa* isolates are multi-drug resistant and almost all isolates from clinical infections have class I, II and III Integrons.

Keywords *Pseudomonas aeruginosa*; Integrons; Multiple Antibiotic Resistance; Hospital Infections

CITATION LINKS

[1] Frequency of classes I and II integrons in clinical isolates of ... [2] Drug resistance of Pseudomonas aeruginosa to ceftazidime and imipenem in ... [3] MIC determination of Pseudomonas aeruginosa strains were isolated from clinical specimens of ... [4] Persistent bacteremia from pseudomonas aeruginosa with in vitro resistance to the ... [5] Relationship between antimicrobial resistance and class I integron in ... [6] Effective antibiotics in combination against Extreme Drug-resistant Pseudomonas aeruginosa with decreased susceptibity to ... [7] Jawetz, Melnick, &Adelberg's medical ... [8] Pseudomonas aeruginosa extracellular products inhibit staphylococcal growth, and disrupt established biofilms produced by Staphylococcus ... [9] Physicochemical characterization and antimicrobial properties of rhamnolipids produced by ... [10] Analysis of integrons and associated gene cassettes in ... [11] Detection of point mutations associated with antibiotic resistance in ... [12] Genetic fingerprinting and antimicrobial susceptibility profiles of Pseudomonas aeruginosa hospital ... [13] Multiple antibiotic-resistant Pseudomonas aeruginosa and lung function decline in ... [14] Most multidrug-resistant Pseudomonas aeruginosa isolates from hospitals in ... [15] Optimal meropenem concentrations to ... [16] The Study of Antibiotic Resistance Pattern and the Frequency of Extended-Spectrum ... [17] Investigation of blaIMP-1, blaVIM-1 and blaSPM-1 MBL Genes among Clinical Strains of ... [18] Antimicrobial resistance among ... [19] Class 1 integrons in Pseudomonas aeruginosa and ... [20] Pseudomonas aeruginosa infections in ... [21] Characterization of Pseudomonas aeruginosa isolates: occurrence rates, antimicrobial ... [22] Antibiotic resistance in clinical isolates of ... [23] Antibiotic resistance in Pseudomonas ... [24] Pseudomonas aeruginosa: A survey of ... [25] Molecular mechanisms of ... [26] Class 1 integron and Imipenem ... [27] Class 1 integrons in Pseudomonas ... [28] Identification and characterization of ... [29] Prevalence and characterization of ... [30] PCR typing of genetic determinants for ... [31] Analysis of integrons and associated gene cassettes ...

*Department of Pharmacodynamy & Toxicology, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[1]Department of Microbiology, Faculty of Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Correspondence
Address: Department of Pharmacodynamy and Toxicology, Faculty of Pharmacy, Mashhad University of Medical Sciences, Shahid Fakouri Street, Mashhad, Iran. Postal Code: 9177899191
Phone: +98 (31) 42662153
Fax: +98 (31) 42662153
fahimeh_nourbakhsh@yahoo.com

Article History
Received: May 18, 2016
Accepted: July 13, 2016
ePublished: September 8, 2016

Introduction

Pseudomonas aeruginosa is a non-fermentative and gram-negative bacilli that causes nosocomial infection in the natural host [1]. Pseudomonas is found in large quantities in water, soil, plants and animals. This bacterium can live as normal flora on the skin, nose and respiratory systems of humans too [2]. Because the bacteria have low needs to grow, it remains in the environment and can be easily transmitted to susceptible patients and infect them [3].

Pseudomonas has an outer membrane with low permeability. It also has multidrug discharger pumps, lactamase and the outer membrane purine degradation set that could be the important reason for resistance of these microorganisms to treatment [2]. Coding genes of antibiotic resistance are often transported by mobile genetic elements called integrons [1].

Integrons are mobile elements that can be placed in plasmids, chromosomes or even transposons. These elements are important in the development of multiple drug resistance in Pseudomonas. The overall structure of integrons, resistance genes are on determined gene cassettes. The transfer of resistance genes occur due to the connection of cassette in the integron set during specific recombination process [4]. At the end of the 3' and 5' integrons, 2 nucleotide sequences are protected. The role of these elements in the development of multiple resistance and resistance to a wide range of antibiotics, especially antibiotics used in hospitals, makes finding appropriate treatment and infection control tools difficult [4, 5].

Given the important role of this bacterium in nosocomial infections and the role of integron gene cassettes based on the transfer of antibiotic resistance, the aim of this study was to trace the class I, II and III integrons in isolates of *P. aeruginosa* of nosocomial infection and determining the antibiotic resistance pattern of the bacteria.

Instrument & Methods

In this cross-sectional study, 100 *Pseudomonas aeruginosa* clinical isolates of infected wounds (24 isolates), bedsores (18 isolates), burns (38 isolates), urinary tract infections (12 isolates) and respiratory tract infections (8 isolates) were collected from patients of 3 Isfahan City hospitals, Iran (Al Zahra, Kashani, Shariati) in 2015. The patients were all infected after hospitalization at Orthopedics (21 cases), Internal ward (54 cases), ICU (15 cases) and ICU special (10 cases).

Isolates have been transferred to a microbiology laboratory in Isfahan and were identified by biochemical tests [1]. Studied Isolates were re-identified after the restoration and re-cultivation on blood agar medium using biochemical tests such as Gram stain, catalase test and oxidase (Merck; Germany) [6, 7]. Bacteria grown in the TSB in 1.5ml microtubes in 9000rpm were deposited for 3 minutes; DNA was extracted according to manufacturer's instructions (Fermentas, Germany). Agarose gel electrophoresis was used to assess the quality of extracted DNA from the analyzed samples. Bio photometer devices were used to quantify purified DNA and amount of DNA in the sample was determined by measuring DNA content of each sample at a light wave length of 280nm.

P. aeruginosa isolates were also confirmed by PCR [8]. At all stages of PCR testing (Figure 1), the standard strains of *P. aeruginosa* (ATCC 27853) was used as positive control [9].

Figure 1) Sequencing primers [5, 10-12] related to detecting genes in *Pseudomonas aeruginosa*

Gene	Sequences (5'→3')	Length (bp)
GyrA	F: 5'-GTGTGCTTTATGCCATGAG-3' R: 5'-GGTTTCCTTTTCCAGGTC-3'	287
ParC	F: 5'-CATCGTCTACGCCATGAG-3' R: 5'-AGCAGCACCTCGGAATAG-3'	267
Bla TEM	F: 5'-ATGAGTATTCAACATTTCCG-3' R: 5'-CTGACAGTTACCAATGCTTA-3'	867
Bla SHV	F: 5'-GGTTATGCGTTATATTCGCC-3' R: 5'-TTAGCGTTGCCAGTGCTC-3'	867
Bla OXA	F: 5'-ACACAATACATATCAACTTCGC-3' R: 5'-AGTGTGTTTAGAATGGTGATC-3'	814
Bla CTX-M	F: 5'-ATGTGCAGYACCAGTAARGT-3' R: 5'-TGGGTRAARTARGTSACCAGA-3'	593
Bla DHA	F: 5'-CACACGGAAGGTTAATTCTGA-3' R: 5'-CGGTTARACGGCTGAACCTG-3'	970
Bla VEB	F: 5'-CGACTTCCATTTCCCGATGC-3' R: 5'-GGACTCTGCAACAAATACGC-3'	642
IntI	F: 5'-CAGTGGACATAAGCCTGTTC-3' R: 5'-CCCGAGGCATAGACTGTA-3'	160
IntII	F: 5'-CACGGATATGCGACAAAAAG-3' R: 5'-GATGACAACGAGTGACGAAATG-3'	787
IntIII	F: 5'-GCCTCCGGCAGCGACTTTCAG_3' R: 5'-ACGGATCTGCCAAACCTGACT-3'	980

Antibiogram was performed using disk diffusion method (Kirby Bauer) according to

CLSI tables. Isolates of *P. aeruginosa* were saved in BHI medium and then were prepared for a density equivalent to 0.5 McFarland dense to be presented to the Mueller Hinton medium in the presence of antimicrobial discs, e.g. Tetracycline (30mg/disc), Streptomycin (10mg/disc), Sulfamethoxazole (25mg/disc), Gentamicin (10mg/disc), Norfloxacin (5mg/disc), Cephalotin (3mg/disc), Ciprofloxacin (5g/disc), Trimethoprim (5mg/disc), and Ampicillin (10IU/disk) they were grown and after 24 hours of incubation at 37°C, sensitivity or resistance of bacteria to different antibiotics were determined and measuring the diameter of growth inhibition around each disc. In this experiment, the standard strains of *P. aeruginosa* (ATCC 10145) were used as positive control in determination of antibiotic susceptibility of isolates [10-12].

Data analysis were performed in SPSS 16 software using Chi-square and Fisher exact tests and the relationship between the presence of class III, II, I was calculated by M-PCR test.

Findings

58% of isolates was detected from women and 42% from men. All isolates had multiple antibiotic resistances. The highest antibiotic resistance was to Tetracycline (85%) and the lowest to Norfloxacin (12.5%); other resistances were to Streptomycin (25.6%), Sulfamethoxazole (18.3%), Gentamicin (65.1%), Cephalotin (42.2%), Ciprofloxacin (59.8%), Trimethoprim (17.2%), and Ampicillin (52.3%).

Figure 2) Distribution of coding genes of antibiotic resistance in *P. aeruginosa* isolates from nosocomial infection

Site	Infected wounds	Respiratory infections	UTI	Bedsore	Burn	Total
Number	24	7	13	18	38	100
Bla_{TEM}	27	4	6	9	22	68
bla_{SHV}	14	2	3	1	3	23
Bla_{OXA}	1	2	1	3	3	10
Bla_{CTX-M}	1	2	3	8	4	18
Bla_{DHA}	1	2	1	2	4	10
bla_{VEB}	-	-	-	3	7	10
GyrA	2	3	-	3	4	12
parC	1	-	-	2	2	5

The highest incidence of antibiotic resistance in *Pseudomonas aeruginosa* isolates was related to Integrons I with a frequency of 95% and the lowest was related to Integrons III with a frequency of 10%. There were significant differences between class I and the two other classes of integrons (p=0.036). Except the respiratory tract isolates, other integron classes were present in all studied isolates.

There was a statistically significant difference between the presence of bla_{TEM} gene in *Pseudomonas aeruginosa* with other coding genes for antibiotic resistance (p=0.029; Figure 2).

Discussion

Pseudomonas aeruginosa is an important pathogen that causes different ranges of human infections. It is especially resistant to many antibiotics and over time, it is somehow impossible to treat its infections. In this way, *Pseudomonas aeruginosa* is known as a multi-drug resistance bacterium, especially in hospital acquired infection.

In this study, we have 3 main components with the aim of analyses the frequency of isolates, antibiotic susceptibility patterns and distribution of class I, II and III integrons in *P. aeruginosa* isolates from nosocomial infection in Isfahan. Similar Studies were conducted by Ren *et al.* in 2012 in America [13], Cholly *et al.* in 2011 in France [14], Taccone *et al.* in 2012 in Brooklyn [15] and also in Iran, by Taghvaei *et al.* in 2013 in Arak [16].

In our study, the most isolates were related to infected wounds (24%) and burns (38%). Zareei Yazdeli *et al.* have reported the highest number of infections with *Pseudomonas aeruginosa* at burns (43.8%) [5] and Shahcheraghi *et al.* have reported the frequency of *Pseudomonas aeruginosa* in the wound infections as 37% [17]. In Babay study in Saudi Arabia, the most isolates were separated from the wounds [18].

In the first part of our study, the antibiotic pattern of *Pseudomonas aeruginosa* was examined and we were found that it has over 50% resistance to more than 5 antibiotics. One of the main reasons for the emergence of this resistance is intractable consumption of some antibiotics including tetracycline. In Thailand, Poonsuk *et al.* have shown that an increase in resistance of *Pseudomonas aeruginosa* strains to Amikacin (92.1%), Ceftazidime (96%), Gentamicin (99%) and Ciprofloxacin (95%), while, the least

resistance were observed to Streptomycin (84%) and Tetracycline (58.8%) [19]. Fazeli *et al.* have shown that *Pseudomonas aeruginosa* isolates were resistant to Ciprofloxacin (29%) and Gentamicin (32.2%) [20]. Ciprofloxacin is one of the strongest medications available for the treatment of infections caused by *Pseudomonas aeruginosa*, particularly in treatment of urinary tract infections [21]. Resistance of *Pseudomonas aeruginosa* to Ciprofloxacin in Latin America have been reported 26.8% and in Europe 10-32% [22-25].

The main 3 classes of integrons were detected with a frequency of 95% for I, 54% for II and 10% for III. The prevalence of Integron I was reported by Yousefi *et al.* as 56.3% [26], Fonseca *et al.* as 41.5% [27], Chen *et al.* as 38% [28] and GU *et al.* as 40.8% [29]. Shibata *et al.* have found Integron I the most common and Integron III was observed sporadically in Japan [30]. The prevalence of Integron II was reported 9% in Zanjan [2] and 5.3% in Malaysia [31].

There was no limitation on selecting samples or other part of study. In state hospitals, it is necessary to use management practices in order to optimize the use of antibiotics and the correct administration of antibiotics, preferably based on the results of antibiogram and tracking coding genes for antibiotic resistance.

Conclusion

Pseudomonas aeruginosa isolates are multi-drug resistant and almost all isolates from clinical infections have class I, II and III Integrons.

Acknowledgements: We would like to express our gratitude to Professors who provided assistance at all levels of the research project, especially in Islamic Azad University of Shahrekord.

Ethical Permission: None declared by the authors.

Conflicts of Interests: we certify that there is no conflict of interest in this manuscript.

Funding/Support: The study was supported as a research project (No. 94/6532) in Islamic Azad University of Shahrekord.

References

1- Keramati N, Zeighami H, Haghi F. Frequency of classes I and II integrons in clinical isolates of Pseudomonas aeruginosa producing metallo-lactamase. J Zanjan Univ Med Sci. 2014;22(94):111-9. [Persian]

2- Kanani M, Khadiri T, Khazaei S, Madani H, Melikian Zadeh A. Drug resistance of Pseudomonas aeruginosa to ceftazidime and imipenem in Imam Reza Kermanshah 2006-2010 years. Yafte. 2013;15(4):52-60. [Persian]

3- Rajabpour M, Arabestani MR, Yousefi Moshoof R, Alikhani M. MIC determination of Pseudomonas aeruginosa strains were isolated from clinical specimens of patients admitted to educational hospitals in Hamedan. Iran Med J Microbiol. 2013;7(3):18-25. [Persian]

4- Gangcuangco LM, Clark P, Stewart C, Miljkovic G, Saul ZK. Persistent bacteremia from pseudomonas aeruginosa with in vitro resistance to the novel antibiotics ceftolozane-tazobactam and ceftazidime-avibactam. Case Report InfectDis. 2016;2016:1520404.

5- Zareei Yazdeli M, Islami G, Zandi H, Mousavi M, Koosha H, Akhavan F, et al. Relationship between antimicrobial resistance and class I integron in Pseudomonas aeruginosa isolated from clinical specimens in Yazd during 2012-2013. Fayz. 2014;18(1):60-7. [Persian]

6- Lim T, Lee W, Tan T, Sasikala S, Teo J, Hsu L, et al. Effective antibiotics in combination against Extreme Drug-resistant Pseudomonas aeruginosa with decreased susceptibity to Polymixin B. Plos One. 2011; Available from:
http://dx.doi.org/10.1371/journal.pone.0028177

7- Brooks G, Carroll KC, Butel J, Morse S, Mietzner T. Jawetz, Melnick, & Adelberg's medical microbiology. 26th edition. North America: McGraw-Hill Medical; 2013.

8- Qin Z, Yang L, Qu D, Molin S, Tolker-Nielsen T. Pseudomonas aeruginosa extracellular products inhibit staphylococcal growth, and disrupt established biofilms produced by Staphylococcus epidermidis. Microbiology. 2009;155(Pt 7):2148-56.

9- Haba E, Pinazo A, Jauregui O, Espuny MJ, Infante MR, Manresa A. Physicochemical characterization and antimicrobial properties of rhamnolipids produced by Pseudomonas aeruginosa 47T2 NCBIM 40044. Biotechnol Bioeng. 2003;81(3):316-22.

10- Odumosu B, Adeniyi B, Chndra R. Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant Pseudomonas aeruginosa from Southwest Nigeria. Ann Clin Microbiol Antimicrob. 2013;12:29.

11- Gorgani N, Ahlbrand S, Patterson A, Pourmand N. Detection of point mutations associated with antibiotic resistance in Pseudomonas aeruginosa. Int J Antimicrob Agents. 2009;34(5):414-8.

12- Lim KT, Yasin RM, Yeo CC, Puthucheary SD, Balan G, Maning N, et al. Genetic fingerprinting and antimicrobial susceptibility profiles of Pseudomonas aeruginosa hospital isolates in Malaysia. J Microbiol Immunol Infect. 2009;42(3):197-209.

13- Ren CL, Konstan MW, Yegin A, Rasouliyan L, Trzaskoma B, Morgan WJ, et al. Multiple antibiotic-resistant Pseudomonas aeruginosa and lung function decline in patients with cystic fibrosis. J Cyst Fibros. 2012;11(4):293-9.

14- Cholley P, Thouverez M, Hoquet 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(7):2578-83.

15- Taccone FS, Cotton F, Roisin S, Vincent JL, Jacobs F. Optimal meropenem concentrations to treat multidrug-resistant pseudomonas aeruginosa septic shock. Antimicrob Agents Chemother. 2012;56(4):2129-31.
16- Taghvai R, Shojapour M, Sadeghi A, Babai A. The Study of Antibiotic Resistance Pattern and the Frequency of Extended-Spectrum Beta-Lactamases (ESBL) in Pseudomonas aeruginosa Strains Isolated from Medical Centers in Arak City, Iran. Qom Univ Med Sci. 2013;7(4):36-41. [Persian]
17- Shahcheraghi F, Nikbin and, Shoorj F, Shafei M. Investigation of blaIMP-1, blaVIM-1 and blaSPM-1 MBL Genes among Clinical Strains of Pseudomonas aeruginosa Isolated from Imam Khomeini Hospital, Tehran, Iran. Pejouhandeh. 2009;14(2):67-72. [Persian]
18- Babay HA. Antimicrobial resistance among clinical isolates of pseudomonas aeruginosa from petients in a Teaching Hospital, Riyadh, Soudi Arabia. Jpn J Infect Dis. 2007;60(2-3):123-5.
19- Poonsuk K, Tribuddharat C, Rungtip C. Class 1 integrons in Pseudomonas aeruginosa and Acinetobacter baumannii isolated from clinical isolates. Southeast Asian J Trop Med Public Health. 2012;43(2):376-48.
20- Fazeli H, Akbari R, Moghim S, Narimani T, Arabestani MR, Ghoddousi AR. Pseudomonas aeruginosa infections in patients, hospital means, and personnel's specimens. J Res Med Sci. 2012;17(4):332-7.
21- Gales AC, Jones RN, Turnidge J, Rennie R, Ramphal R. Characterization of Pseudomonas aeruginosa isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the global SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infec Dis. 2001;32(Suppl 2):S146-55.
22- Brown PD, Izundu A. Antibiotic resistance in clinical isolates of Pseudomonas aeruginosa in Jamaica. Rev Panam Salud Publica 2004;16(2):125-30.
23- Bonfiglio G, Carciotto V, Russo G, Stefani S, Schito GC, Debbia E, et al. Antibiotic resistance in Pseudomonas aeruginosa: An Italian survey. J Antimicrob Chemother. 1998;41(2):307-10.
24- Bouza E, Garcia-Garrote F, Cercenado E, Marin M, Diaz MS. Pseudomonas aeruginosa: A survey of resistance in 136 hospitals in Spain. Antimicrob Agents Chemother. 1999;43(4):981-2.
25- Du SJ, Kuo HC, Cheng CH, Fei ACY, Wei HW, Chang SK. Molecular mechanisms of ceftazidime resistance in Pseudomonas aeruginosa isolates from canine and human infections. Vet Med. 2010;55(4):172-82.
26- 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. Iran J Microbiol. 2010;2(3):115-21.
27- Fonseca EL, Vieira VV, Cipriano R, Vicente AC. Class 1 integrons in Pseudomonas aeruginosa isolates from clinical settings in Amazon region, Brazil. FEMS Immunol Med Microbiol. 2005;44(3):303-9.
28- Chen J, Su Z, Liu Y, Wang S, Dai X, Li Y, et al. Identification and characterization of class 1 integrons among Pseudomonas aeruginosa isolates from patients in Zhenjiang, China. Int J Infect Dis. 2009;13(6):717-21.
29- Gu B, Tong M, Zhao W, Liu G, Ning M, Pan S, et al. Prevalence and characterization of class 1 integrons among Pseudomonas aeruginosa and Acinetobacter baumannii isolates from patients in Nanjing, China. J Clin Microbiol. 2007;45(1):241-3.
30- Shibata N, Doi Y, Yamane K, Yagi T, Kurokawa H, Shibayama K, et al. PCR typing of genetic determinants for metallo-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. J Clin Microbiol. 2003;41(12):5407-13.
31- Khosravi Y, Tay ST, Vadivelu J. Analysis of integrons and associated gene cassettes ofmetallo- â lactamase-positive Pseudomonas aeruginosa in Malaysia. J Med Microbiol. 2011;60(Pt 7):988-94.