

# Molecular Investigating Class I, II And III Integrins In Pseudomonas Aeruginosae Isolated From Diabetic Foot Infection Patients In Najaf Province

Israa Abdul Ameer Al-Kraety, Sddiq Ghani Al-Muhanna\* & Enas Jalil Baqer Almayali

*Department of Medical Laboratory Techniques, Faculty of Medical and Health Techniques,  
University of AlKafeel, Najaf, Iraq  
Sddiq.g@gmail.com*

**Abstract:** *The study aimed to isolate and diagnose Pseudomonas aeruginosae from clinical specimens of diabetic foot infection patients, study the resistance of bacteria to antimicrobial agent and molecular detection of class 1, 2 and 3 integron-associated gene cassettes from Pseudomonas aeruginosa in Najaf province. Sensitivity test was examined via disk spread, method antibiotic, the insulated shows 100% resistance to cefepim, amoxiclave, ceftazidime, gentamicin, cefotaxime and cephalaxine though the carry-on of the antibiotics showed various activity against the isolates. At molecular study, the investigated the presence of integrin's genes (intI, intII and intIII) using PCR technique and electrophoresis systems, PCR analysis showed that the integrin IntI, IntII and IntIII were detected in 12/30, 16/30 and 6/30 respectively in Pseudomonas aeruginosa isolates.*

**Keywords.** *Pseudomonas aeruginosa, diabetic foot infection, Integrins gene*

## INTRODUCTION

Pseudomonas aeruginosa is a significant human opportunistic bacterium in the diabetic foot. It is rod-shaped non-fermenting bacterium with unipolar motility consider a gram-negative aerobic [1]. P. aeruginosa is often first specifically through its pearlescent aspect and tortilla-like or grape-like odor. Have the accountable ability to display a wide of disease from surface colonization of ulcers to wide tissue spoilage, septic arthritis, count, osteomyelitis, and bacteremia [2]. Because the dangerous and dreaded pathogen therefore P. aeruginosa is commonly resistant to antibiotics. The clinical P. aeruginosa isolates display orientation to increase resistance towards many a high percentage and antimicrobial agents or multi-drug resistance (MDR) phenotype. Aminoglycosides, fluoroquinolones and anti-pseudomonal agents consider is the most effective beta-lactams, there's a technique of resistance to antimicrobial agents to the production of beta-lactamases, multidrug efflux pumps, presence of integrons, and downregulation of outer membrane porins. Found the large of antibiotic resistance genes found on transposons and plasmids that are located at a unique site named integron [3]. Carry these elements approximately 10% of the sequenced bacterial genomes, also on chromosomes about 90 distinct integrin Classes that most of them located. The primary of integrin's that include functional are Class 1, Class 2, and Class 3 that display a number of features not a model of the large numerically dominant chromosomal integrin Classes. Transposons can be carried utmost usually have on to 6 cassettes towed from a pool of on 100 cassettes and almost every of which encodes antibiotic confrontation determinants [4]. The most common integrons; Classe I found in P. aeruginosa bacteria while the others

integrans Class 2 and Class 3 amidst these pathogens are not commonly reported [5]. This study aimed to investigating class I, II and III Integrans in *Pseudomonas aeruginosa* isolated from Diabetic Foot infection patients.

## MATERIALS AND METHODS

### Specimens Collection and Bacterial Identification

In this Study can be collecting 30 samples swab specimens of diabetic foot infection ulcer, The samples were imparted through sterile transfer swabs to the bacteriology laboratory into culture on MacConkey agar and blood agar, through 24 hr. the plates was incubated at temp. 37°C after that then single isolate was transport to trypticase soya agar for diagnosis by biochemical tests and VITEK 2 compact system that proven the identification of isolates.

### Plasmid DNA Extraction

Isolation of bacterial plasmid according to PureYield™ plasmid Miniprep Kit, the kit designed for isolation of plasmid DNA from cultures bacterial cells. The procedure of DNA extraction was done according to the manufacture instructions.

### Identification

The PCR assay was performed to detect the integron genes for *P. aeruginosa* shown in table (1), these primers were produced by Alpha DNA Company, Canada. The mixture of PCR for each primer with final volume 20 µl/reaction and the protocol used depending on Master Mix (AccuPower® PCR PreMix (Bioneer, Korea) instructions. Each monoplex PCR reaction mixture consisted of 2µl Forward Primer (10 picomole), 2µl Reverse Primer (10 picomole), 9µl De-ionized water, and 7µl the DNA of the isolates were added into the AccuPower® Taq PCR PreMix tubes that contain (Taq DNA polymerase, dNTPs, KCl, MgCl<sub>2</sub>, and buffer). All PCR components were assembled in a PCR tube then the PCR reactions for primers as shown in table 2. The PCR products were resolved by electrophoresis in a 0.9% agarose gel stained with Ethidium bromide staining 0.5% (BioBasic, Canada) and then visualized by Gel Documentation. The 100-bp DNA ladder (Bioneer, South Korea) used as a reference standard [6]. The positive results were distinguished [7]. Lastly, by using Biometra gel documentation system.

Table 1. In this study using Primers.

Type of Primer	Primer sequence (5'-3')	Amplicon size (bp)	Reference
<i>IntI</i>	F: CAGTGGACATAAGCCTGTTC R: CCCGAGGCATAGACTGTA	160	Zareei Y et al., 2014
<i>IntII</i>	F: CACGGATATGCGACAAAAAG R: GATGACAACGAGTGACGAAATG	787	Zareei Y et al., 2014
<i>IntIII</i>	F:GCCTCCGGCAGCGACTTTCAG R: ACGGATCTGCCAAACCTGACT	980	Odumosu BT et al., 2013

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Last extension
<i>IntI, II, III</i>	95°C at 6 min	35	95°C at 45 Sec	51°C at 45 Sec	72°C at 1 Min	72°C at 7Min

Table 2. PCR programs of primers that apply in the thermocycler

## RESULTS AND DISCUSSION

Diabetic individuals tend to evolve various complications like peripheral arterial disease and neuropathy that participate to foot ulceration [8]. In this study *P.aeruginosae* connected with diabetic foot ulcers, this consequence correspond with previously studies shown *S. aureus*, *PROTEUS MIRABILIS* and *P.aeruginosa* as common bacterial species frequently connected with wound fluids in diabetic foot ulcers [9, 10].

In this study according to diagnosis by bacteriological culture and VITEK method, *P.aeruginosae* was found to be the most common isolate 30 (30%), this result agrees with a previous study *P.aeruginosae* was isolated in 16% , other authors reported *P.aeruginosae* in 17.5% of cultures from diabetic ulcers [5].

Pappu K et al. [11] also found correlate good with results they reported about 76% gram negative bacilli of the organisms which were isolated, *Pseudomonas* being the predominant pathogen (23%), then *S.aureus* (21%). Zubair et al [12] reported *E.coli* the predominant gram negative isolates, about (26.6%) and (10.6%) in the center while the rest acquired the infection and from large than half of the patients already had *pseudomonas* infection. *P.aeruginosa* this opportunistic bacteria show the mist resistance to Cefepim, Amoxiclave, Cefotaxime, Cephalexine, and Gentamicin (00 % ), followed by Timethoprime ( 91.6 % ) , Ciprofloxacin ( 25 % ) and very low resistance to Imipenime, Amikacin and Pipracillin – Tazobactam ( 8.3 % ).

This results agree with [13] who found *P. aeruginosa* were showed highest resistance against Amoxicillin/clavulanic acid, Cefotaxime and Trimethoprim. The lower result resistance to Ciprofloxacin was (33.3%).

Antibiotic predisposition, manner *P. aeruginosa* appears of isolates about 53% were renitent to further than five antibiotics. In Thailand, Poonsuk et al. appaers resistance of *P. aeruginosa* isolates increase in Cefotaxime (96%), Gentamicin (99%), Ciprofloxacin and (95%) Amikacin (92.1%). Fazeli et al. reported the *P. aeruginosa* isolates were renitent to Gentamicin (32.2 %) and Ciprofloxacin (29 %) [14].

The results showed that the integron Class1 *IntI*, *IntII* and *IntIII* were detected in 12 out 30, 16 out 30 and 6 out 30 respectively from *P.aeruginosae* isolates as shown in figure (1, 2 and 3 respectively).

In this study the results appear that the spread of Class 1, Class 2 and Class 3 integron genes in some *P. aeruginosa* isolates, class II integron was large spread than other integrons. *P. aeruginosa* is an opportunistic pathogen that contain a large ambit of human contagion, especially resistant to numerous antibiotics that makes it hard to cure. The transfer of resistance genes is appeared recent studies through integrons has significant role in acquiring resistant in bacteria. These genes can be originated by plasmids and transposons [15]. In a

study conducted in 2010 by Yosefi [16], the prevalence of Integron gene I was reported 56.3%. Moreover, Fonseca [17] reported 41.5%, in China in 2009, it was reported 38% [18], and in the Gu study, it was reported 40.8% [15]. In a study by Shibata [19] in Japan, integron I was reported to be more prevalent, whereas integron III was observed to be sporadic [19].

The prevalence of Integron II in the study of Keramati was reported 9% in 2014 in Zanjan [20]. Khosravi also reported, 5.3% in 2011 [21]. Coding genes of antibiotic impedance are often imparted via mobile genetic elements called integrons [20] that can be placed in, transposons, chromosomes, and plasmids. These elements are very important in the evolution of several drug resistance, such as plasmids and transposons. The overall structure of integrons, resistance genes are on determined gene cassettes. The transfer of resistance genes occurs due to the connection ability of cassette in the integron set during a particular recombination process [22]. At the end of 3' and 5' integrons, two nucleotide sequences are protected. Essential components of area 5 in all integrons consist of: 1. integrase gene that is site-specific for recombinase enzymes, 2. attI sequence is a particular recombination place situated in the vicinity of intI, used as a receiver for the gene cassette, 3. The promoter is required for expression of available gene cassette, integrated between sector 3' and 5' integrons [23].

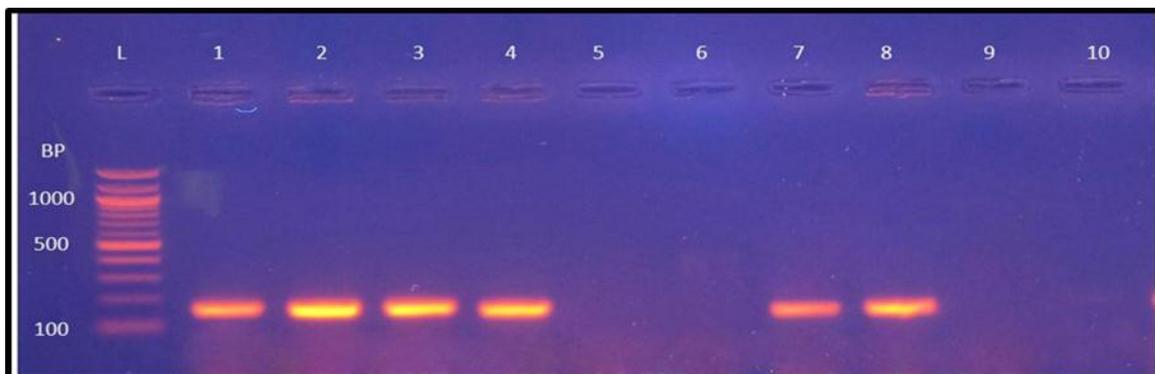


Figure 1. Yields of PCR amplification to *P.aeruginosa* isolates that amplified from IntI gene primer by way of produce 160 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1 to 10) appear positive results with intI gene.

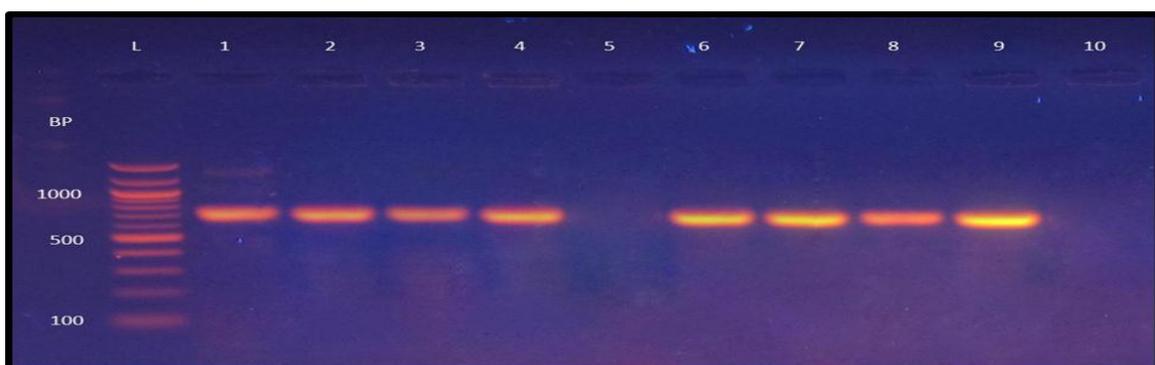


Figure 2. Yields of PCR amplification to *P.aeruginosa* isolates that amplified from IntII gene primer for produce 787 bp. Lane (L), Lanes (1 to 10), DNA molecular size marker (100-bp ladder), appear the int II gene positive results.

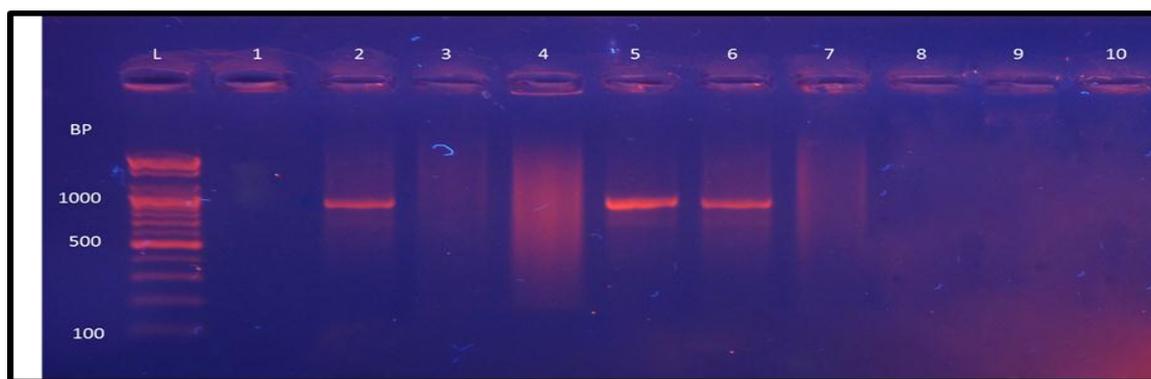


Figure 3. Yields of PCR amplification to *P. aeruginosa* isolates that amplified from *IntIII* gene primer by way of produce 980 bp. Lane (L), Lanes (1 to 10), DNA molecular size marker (100-bp ladder), appear positive results with *int III* gene.

## REFERENCES

- [1] Martínez C, Pons E, Prats G, León J. (2004). "Salicylic acid regulates flowering time and links defence responses and reproductive development". *Plant J* 37: 209-217.
- [2] Driver VR, Fabbi M, Lavery LA, Gibbons G. (2010). The costs of diabetic foot: the economic case for the limb salvage team. *J Vasc Surg* 52: 17S-22S. 7.
- [3] Mbunda F, Mchembe DM Chalya LP, Rambau P, Mshana ES, et al. (2012). Experiences with Surgical treatment of chronic lower limb ulcers at a Tertiary hospital in northwestern Tanzania: A prospective review of 300 cases. *BMC Dermatol* 12-17. 10.
- [4] Chalya LP, Mabula BJ, Dass MR, Kabangila R, Jaka H, et al. (2011). Surgical management of Diabetic foot ulcers: A Tanzanian university teaching hospital experience. *BMC Res Note* 4: 365. 13.
- [5] Sharma VK, Khadka PB, Joshi A, Sharma R. (2006). Common pathogens isolated in diabetic foot infection in Bir Hospital. *Kathmandu Univ Med J (KUMJ)* 4: 295-301.
- [6] Sambrook J. and Russell R.W. (2001). *Molecular cloning: A laboratory manual*, 3rd ed. Cold spring harbor laboratory press, cold spring harbor, N.Y.
- [7] Bartlett, J. S. and Stirling D.(1998). *PCR Protocols: Methods in Molecular and Biology*. 2th. Humana Press Inc. Totowa. NJ.
- [8] Dinh, T., Tecilazich, F., Kafanas, A., Doupis, J., Gnardellis, C., Leal, E., Tellechea, A., Pradhan, L., Lyons, T. E. & other authors .(2012). Mechanisms involved in the development and healing of diabetic foot ulceration. *Diabetes* 61, 2937–2947.
- [9] Wang, S.-H., Sun, Z.-L., Guo, Y.-J., Yang, B.-Q., Yuan, Y., Wei, Q. & Ye, K.-P. (2010). Meticillin-resistant *Staphylococcus aureus* isolated from foot ulcers in diabetic patients in a Chinese care hospital: risk factors for infection and prevalence. *J Med Microbiol* 59, 1219–1224.
- [10] Zubair, M., Malik, A. & Ahmad, J. (2011). Clinico-microbiological study and antimicrobial drug resistance profile of diabetic foot infections in North India. *Foot (Edinb)* 21, 6–14.
- [11] Pappu AK, Sinha A, Johnson A. (2011) .Microbiological profile of diabetic foot ulcer. *Calicut Med Journal* e1-4. 20.
- [12] Zubair M, Malik A, Ahmad J. (2010). Clinico-bacteriology and risk factors for the diabetic foot infection with multidrug resistant microorganisms in North India. *Biol Med*, 22-34.
- [13] Peng CF, Lee MF, Fu HT, et al.(2007). Characterization of class 1 integrons and antimicrobial resistance in CTX-M-3-producing *Serratia marcescens* isolates from southern Taiwan. *Jpn J Infect Dis*;60:250e256.

- [14]Fazeli H, Akbari R, Moghim S, Narimani T, Arabestani MR, Ghoddousi AR.(2012). Pseudomonas aeruginosa infections in patients, hospital means, and personnel's specimens. J Res Med Sci; 17: 332-7.
- [15]Gu B, Tong M, Zhao W, Liu G, Ning M, Pan S, et al. (2007). Prevalence and characterization of class 1 integrons among Pseudomonas aeruginosa and Acinetobacter baumannii isolates from patients in Nanjing, China. Journal of Clinical Microbiology; 45(1): 241-43.
- [16]Yousefi S, Nahaei M, Farajnia S, Ghojzadeh M, Akhi M, Sharifi Y, et al.(2010). Class 1 integron and Imipenem Resistance in Clinical Isolates of Pseudomonas aeruginosa: Prevalence and Antibiotic Susceptibility. Iranian Journal of Microbiology;2(3):115-21.
- [17]Fonseca EL, Vieira VV, Cipriano R, Vicente AC.(2005). Class 1 integrons in Pseudomonas aeruginosa isolates from clinical settings in Amazon region, BrazilWeily Online library; 44: 303-309.
- [18]Chen J, Su Z, Liu Y, Wang S, Dai X, Li Y, et al.(2009). Identification and characterization of class 1 integrons among Pseudomonas aeruginosa isolates from patients in Zhenjiang, China. International Journal of Infectious Diseases;13(6):717-21.
- [19]Shibata N , Doi Y, Yamane K, Yagi T, Kurokawa H, Shibayama K,et al.(2003). PCR Typing of Genetic Determinants for MetalloLactamases and Integrases Carried by GramNegative Bacteria Isolated in Japan, with Focus on the Class 3 Integron. J Clin Microbiol;41(12):5407-13.
- [20]Keramati N, Zeighami H, Haghi F.(2014). lots of classes I and II integrons in clinical isolates of Pseudomonas aeruginosa producing metallo-lactamase. Journal of Zanjan University of Medical Sciences ;22 (94):111-19.
- [21]Khosravi Y, Tee Tay S, Vadivelu J.(2011). Analysis of integrons and associated gene cassettes of metallo- $\beta$ -lactamase-positive Pseudomonas aeruginosa in Malaysia. Journal Med Microiol;60:988-94.
- [22]Peymani A, Naserpour Farivar T, Rahimi H, Ranjbar M, Najafipour R.(2014). the frequency of class I integron in Pseudomonas aeruginosa isolates with multi-drug resistance patterns in hospitals in the city of Qazvin and Tehran. Journal of Qom; 8 (3):61-69.
- [23]Zareei Y, Islami G, Zandi H, Mousavi M, Koosha H, Akhavan F, et al.(2014). The relationship between antibiotic resistance and integrons class in Pseudomonas aeruginosa clinical isolates from 2012 to 2013 in the city of Yazd. Bimonthly Journal of grace;18(1): 60-67.
- [24]Khanolkar MP, Bain SC, Stephens JW. (2008). The diabetic foot. QJM 101: 685- 695.
- [25]Lim T, Mwipatayi B, Murray R, Sieunarine K, Abbas M, et al. (2006). Microbiological profile of chronic ulcers of the lower limb: a prospective observational cohort study. ANZ J Surg 76: 688-692. 11.
- [26]Odumosu BT, Adeniyi BA and Chndra R.(2013). Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant Pseudomonas aeruginosa from Southwest Nigeria. Ann Clin Microbiol. Antimicrob;12(29):1-7.
- [27]Poonsuk K, Tribuddharat C, Rungtip C. Class 1 integrons in Pseudomonas aeruginosa and Acintobacter baumannii isolated from clinical isolates. Southeast Asian Journal of Tropical Medicine and Public Health 2012; 376-48.
- [28]Priyadarhini, S., Jeya, M., Linda Susan, S.( 2013). "The Bacteriology of Diabetic Foot Ulcers, with a Special Reference to Multidrug Resistant Strains", J. Clin. Diagn. Res., 7(3): 441-445.
- [29]Shanmugam P, MJ, Susan SL.(2013). The bacteriology of diabetic foot ulcers, with a special reference to multidrug resistant strains. J Clin Diagn Res 7: 441- 445.

- [30] Sivanmaliappan TS, Sevanan M. (2011). Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* from Diabetes Patients with Foot Ulcers. *Int J Microbiol* 2011: 605195. 12.
- [31] Zahedi Bialvaei A, Samadi Kafil H, Ebrahimzadeh Leylabadlo H, Asgharzadeh M, Aghazadeh M.(2015). Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. *Iran J Microbiol*; 7: 226-46.