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MOLECULAR DETECTION OF INTEGRON CLASS 1 GENE IN *PROTEUS MIRABILIS* ISOLATED FROM DIABETIC FOOT INFECTIONS

Sddiq Ghani Al-Muhanna[1*] Shaima R. Banoon[2*] and Israa Abdul Ameer Al-Kraety[1]

[1]Department of Medical Laboratory Techniques, Faculty of Medical and Health Techniques, University of AlKafeel, Najaf, Iraq.
[2]Department of Biology, College of Science, University of Misan, Maysan, Iraq.

Abstract

The study aim to isolation and identification of aerobic bacteria from diabetic foot ulcers in diabetic patients, investigation the resistance of bacteria to antibiotic agent. The present study included 50 specimens collected from patients suffering from diabetic foot ulcer for both sexes with an age ranged between (20-70) years. The results revealed that the frequency among males more than 40(80%) female 10(20%). The results showed that G-ve bacteria revealed a high rate 45(90%), 5(11%) belonged to *E. coli*, then *P. aeruginosa* 10(22%) *followed* by *K.pneumonia* with 13(28%) and *proteus mirabillis* 17(37%). Out of the 50 specimens, only 5(11%) isolates belong to *S. aureus.* Among bacterial isolates, *proteus mirabillis* 17(37%) was isolated and diagnosed from clinical specimens of diabetic foot ulcer and detect the antibacterial resistance to some antibacterial agent phenotypically. On the other hand to study the sensitivity of isolates to different antibiotics were tested by Kirby-Bauer disc diffusion method. The date revealed that the antimicrobial resistance rates of the *P. mirabilis* isolates to Colistin (100%), Levofloxacin (60%), Gentamicin (60%), Trimethoprim- sulfamethoxazole (60%) and Piperacillin-tazobactam (60%) were all high. The moderate resistance rate was observed for Ciprofloxacin (50%). The lowest resistance rate was observed for Ceftazidime (30%), Amikacin (20%), Tobramycin (20%), Ticarcillin (20%), Cefepime (10%), Meropenem (0%) and Impenem (0%) and Aztreonam (0%). In molecular research, 15(88%) of Integron gene was used to detect Integron gene. These data indicated a high prevalence of Class 1 integrons among Foot Diabetes Ulcer patients with *P. mirabilis* isolates. Based on these findings, integrons that play an important role in transmitting resistance genes to the clinical bacteria as possible.

Key words: *P.mirabilis*, diabetic foot infection, Integrons gene, class I.

Introduction

Diabetes mellitus (DM) is a chronic condition caused by hereditary and/or acquired insulin development deficiency induced by the pancreas, or by insulin ineffectiveness. Such a deficiency contributes to increased bloodsglucose concentrations, which in turn damagessmany of the body's systems, especially the blood vessels and nerves and makes up the most common diabetes-rely (Spichler *et al.,* 2015). Diabetic foot ulcers are one of the major causes of death and morbidity among diabetes sufferers. It involves damage to all tissue, necrosis or gangrene layers that typically occur on the feet's sole due to peripheral neuropathy or peripheral arterialsdisease (PAD) in patients with diabetes. (Wang *et al*., 2012).

The genus Proteus is a Gram-negative bacillus that belongs to the Enterobacteriaceae family. Members of the genus Proteus are widespreadsin the environment and the gastrointestinal tract of human and animals (Hegazy, 2016; Chen *et al.,* 2017). Proteus is known as a nosocomial, opportunisticspathogen and is more common in community-acquired infections. *Proteus mirabilis* and

Author for correspondence : E-mail: Shimarb@uomisan.edu.iq, Shimarb@uomisan.edu.iq.

P. vulgaris have been reported to cause wounds infections, respiratory tract infections and both community-acquired and catheter-associated urinary tract infections (UTI) (Trivedi *et al.*, 2015).

Bacterial resistance to antimicrobial agents is a serious problem worldwide with regard to treatment of infectious diseases. Understanding of the molecular basissof how resistance genes are acquiredsand transmitted may contribute to the creation of new antimicrobialsstrategies. The spread of antibiotic resistance is usually associated with either the clonal spread of an epidemic strainsor through independent acquisition of the resistance genes on plasmids, transposons or integrons (Martínez *et al.*, 2007). Naturally occurring gene expressionselements called "integrons" have been described as vehicles for the acquisition of resistance genes carried by mobile elements.

Integrons are one of the mobile genetic elements which can carry genessof resistance to different antibiotics, which contain integrase gene, two conserve areas of *sul1* and *int1* and one variable area of gene cassettes. A gene cassette includes an open readings frame and at the 3'-end, a recombination site *attC*. Integration or excision of cassettes occurs by a site-specific recombination mechanism catalyzed by the integrase (Mirnejad *et al.*, 2013). An integron is a gene capture system found on plasmids, on chromosome and in transposon. Integron are genetic elements that contain the genetic determinant for the components of asite-specific recombination system that recognizes and captures mobile gene cassettes. Integron themselves are not mobile. "Integrons are 'assembly' platforms that incorporate exogenous open reading frames by site-specific recombination and convert them to functional genes by ensuring their correct expression" as show fig. 1 (Gillings, 2014).

Integron can be divided into two major group: resistance integrons and super-integrons Although not independently mobile, integrons are widespread versatile DNA elements and can be divided into two distinct subsets: the mobile integrons and the chromosomal integron (Corrêa *et al.*, 2014). Class 1 integral gene cassettes normally do not have their own promoters and their transcription depends on the promoters Pc and P2 (Sunde, 2005).

These structures have also been found to be involved in the genetic reassortment of resistance determinants frequently observed in multiple-antibiotic-resistant bacterial pathogens (Martínez *et al.*, 2007).

The aim of the present study to performs a molecular analysis of Integron gene class 1 and to determine their antibiotic resistance pattern as well as comparing these recent findings with those reported in our previous study.

Materials and Methods

Specimens collection and bacterial identification

Collection 50 samples swab specimens from diabetic foot infection ulcer, The specimens were transported by sterile transport swabs to the bacteriology laboratory for culture on MacConkey agar, Mannitol agar and blood agar, the plates were incubated at 37°C for 24 hours then a single pure isolated colony was transferred to trypticase soya agar for the preservation and to carry out other biochemical tests and VITEK 2 compcat system that confirmed the identification of isolates.

Genomic and plasmid (DNA Extraction)

Genomic and plasmid DNA was successfully extracted from *P. mirabilis* isolates by boiling method, briefly, colonies were suspended in 100 microliters of sterile distilled water and boiled at 100°C in the water bath for 15 minutes than rapidly cooled at -20°C for one hour, then centrifugation and the supernatant were preserved for the used in the amplification-processes (Ali *et al.*, 2009). The concentration and purity of the extracted

Table 1: Primers used in this study.

Primer Type	Primer sequence (5'-3')	Amplicon size (bp)	Reference
IntI	F: CAGTGGACATAAGCCTGTTC R: CCCGAGGCATAGACTGTA	160	Zareei, Y. *et al.*, 2014

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

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
IntI	94°C for 5 min	35	94°C for 30 sec	55°C for 30 sec	72°C for 1min	72°C for 7min

DNA were calculated directly by spectrophotometry, the purity of DNA extracted varied between (1.8-2). Gel electrophoresis validated and analyzed the extracted DNA.

Molecular Identification

The PCR assay was performed to detect the integron genes for *P.mirabilis* shown in table 2. Alpha DNA Company, Canada developed these primers as shown in table 1. Agarose gel electrophoresis was observed to the amplified PCR products by staining with ethidium bromide. The product of electrophoresis was detected by the use of a gel documentation device. The positive findings were separated when sample base pairs of the DNA band were equal to the target product size (Bartlett and Stirling, 1998). Finally, the gel was shot using a documentation method for the Biometra gel.

Results and Discussion

Demographic profile of patients with diabetic foot ulcer

The research was carried out on 50 specimens of suspected patients' diabetic foot wounds between October, 2019 and February, 2020. Results showed that 50 clinical specimens were distributed according to the report patient's age ranged from (20-70) years old. The lowest incidence was among the (20-30) and more than (60) age group 3.3% and 1.6% respectively, while the highest incidence was among the (51-60) age group (39.1%).

In 50 patients, 40(80%) were male and 10(20%) were female as in fig. 2. Increasing infected male more than female frequency similar with the result of Bansal *et al*., (2008) they observed that male more frequency than female (78.64% vs 21.36%) from the total 270 diabetic patients (180 male and 90 female) suffering from foot infections. Predominance of malesover female patients as shown in the study can be explained by the fact that in our country males are exposed more to the outsides environment factors. The predominance male in DFU could be linked to factors such as gender-related differences in life styles and professional roles that require the feet to tolerate more pressure as a result of work, increased level of outdoor work and poor compliance to foot care practices compared to females. The wounds healing in females more better than in male, this may be due to differences in hormones and explain that increase estrogen receptor in female increase healing wound which act as endogenous enhancers of healing process while in male increased the level of androgen was considered harmful for wound healing since androgenic species decrease repair of dermis (Wilkinson and Hardman, 2017).

Table 3: The biochemical features of *P. mirabilis.*

Test	*P. mirabilis*
Oxidase test	+
Catalase test	+
Motility	+

Isolation of pathogenic bacteria of diabetic foot ulcer (DFU)

Culture investigation depending on morphological and biochemical test shows that Gram negative bacteria reveals a high rate 45(90%), 5(11%) belonged to E. *coli*, then *P. aeruginosa* 10(22%) followed by *K. pneumonia* with 13(28%) and *proteus mirabillis* 17(37%). Out of the 50 specimens, only 5(11%) isolates belong to *S.*

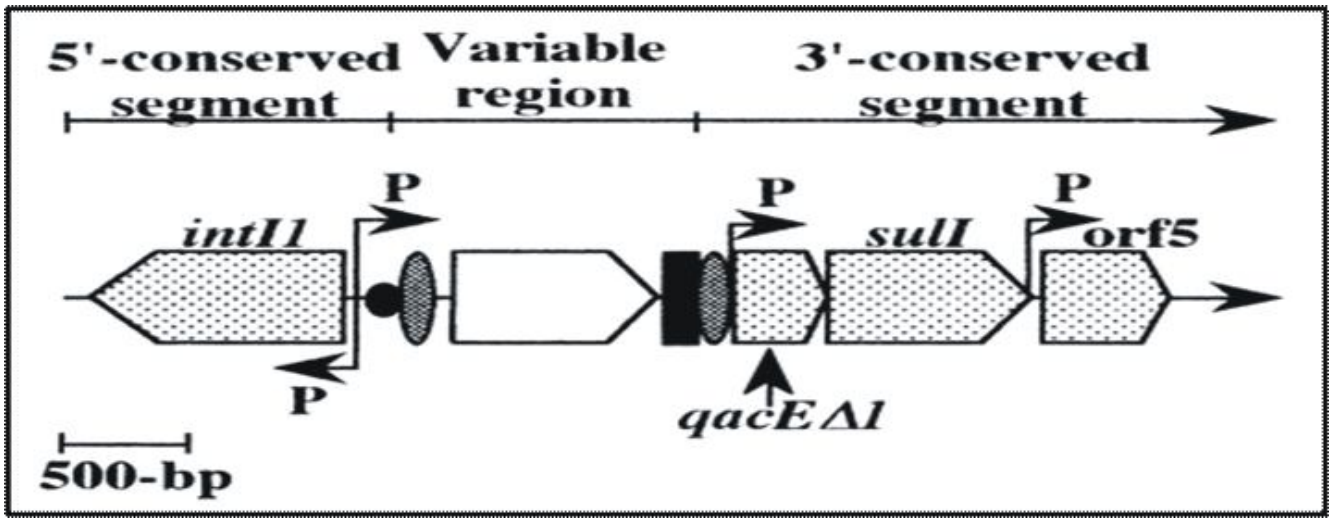

Fig. 1: General structure of class 1 integrons. (Gillings, 2014).

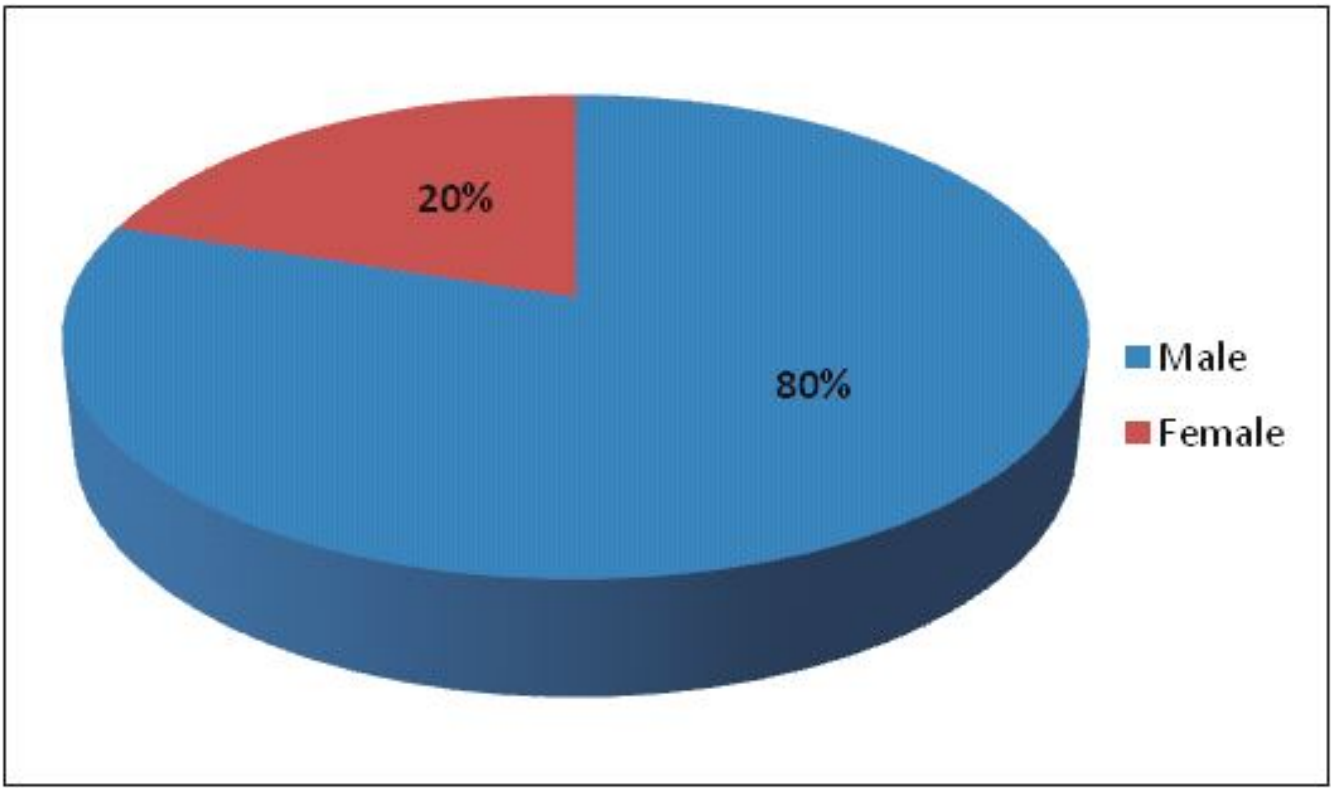

Fig. 2: Total patients of diabetic foot ulcer according to gender.

aureus. Bansal *et al*., (2008) observed that gram negative bacteria were isolated in 76% whereas gram positive bacteria were isolated in 24% of DFU patients. The predominance of gram negative bacteria has been noted in this study agree with (Banu *et al*., 2015).

***Proteus mirabilis* identification**

• Morphologically characterization:

The bacterial isolates collected from clinical samples were initially classified based on cultural morphology, microscopic properties and biochemical studies. Microscopically *P. mirabilis* a gram negative bacilli emerged, *P. mirabilis*' cultural identification was based on the colonial morphology. When the *P. mirabilis* colonies were grown on the blood agar, swarming colonies appear (Fahadawi *et al.,* 2019).

The biochemical tests

Biochemical test results that were reported in table 3 were considered to complement the initial identification of *P. mirabilis* isolates. The isolates have reported general characteristics, the isolates have been positive for oxidase and catalase testing (Khalifa *et al*., 2019).

After extensive bacteriological analysis based on morphological, cultural and biochemical samples, the isolates were tentatively identified as *P. mirabilis.*

The final identification was carried out using an automated compact VITEK-2 Compact system with 47 biochemical tests and one GN-ID card Well performed negative regulation (Appendix 2). Results show that only 17 isolates from diabetic foot wound were identified as *P. mirabilis* with confidence level of ID message was very high (Probability percentage between 88 and 98).

Phenotypic resistance of Isolates

• Antibiotic Susceptibility

This test was conducted to all isolates by using disc diffusion test. The results were interpreted according to the recommendation of CLSI, (2019). The date revealed that the antimicrobial resistance rates of the *P. mirabilis* isolates to Colistin (100%), Levofloxacin (60%), Gentamicin (60%), Trimethoprim-sulfamethoxazole (60%) and Piperacillin-tazobactam (60%) were all high. The moderate resistance rate was observed for Ciprofloxacin (50%). The lowest resistance rate was observed for Ceftazidime (30%), Amikacin (20%), Tobramycin (20%), Ticarcillin (20%), Cefepime (10%), Meropenem (0%) and Impenem (0%) and Aztreonam (0%). Some Proteus mirabilis isolates show an elevation in resistance to imipenem due to several reasons: loss of outer membrane porins, reduced PBP1 a expression or

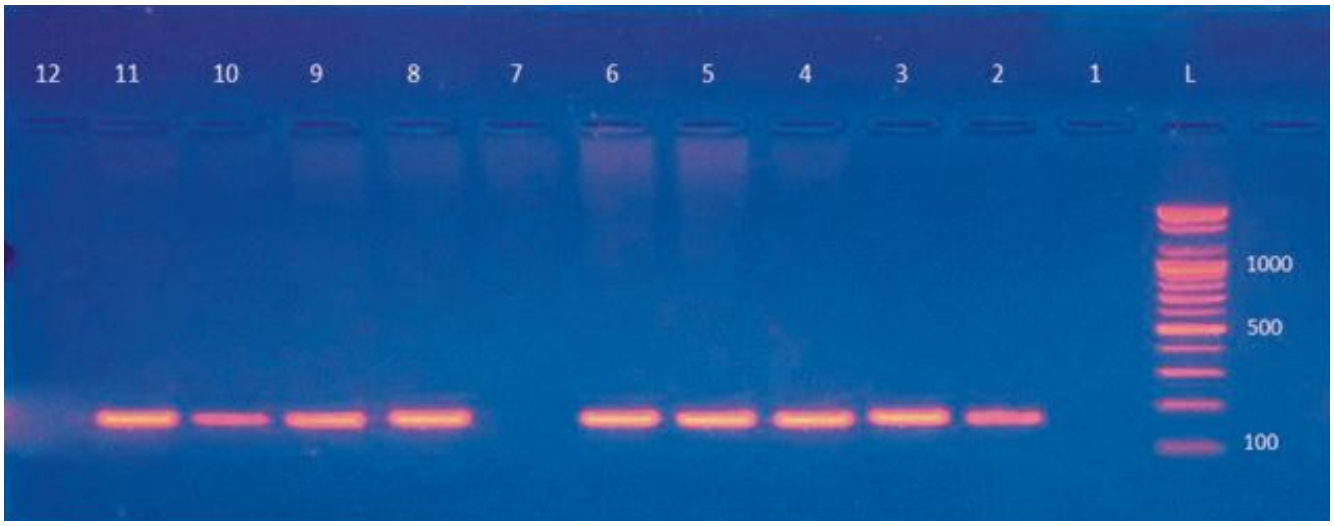

Fig. 3: *P. mirabilis* isolate PCR amplification products amplified with intI1 gene primers of product 160 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1-6)(8-17) display positive results with *intI1* gene3.

reduced PBP2 imipenem binding (Girlich *et al.*, 2014). That the production of imipenem resistant in Proteus mirabilis is due to a lack of OMP 24 kDa. Amikacin is an amino-glycoside antibiotic used to treat bacterial infection of different forms. This works by binding to the ribosomal subunit of the bacterial 30s which causes misreading of mRNA and leaving the Bacterium which cannot synthesize proteins that are vital to its development. Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the class of fluroquinolone drugs and is a part of a broad variety of antimicrobial agents that inhibits bacterial DNA and protein synthesis and is considered an effective medication for urinary care tract infection. A Study in Taiwan by Wang *et al*., (2014) reported that the susceptibility to this antimicrobial decreased (from 80.1% to 53.8%). That is considered roughly in agreement to the outcome of this report, on resistant growth. Because of their synergistic impact on bacteria, trimethoprim and sulfamethoxazole work together. It is antimicrobial bactericidal agent for gram positive as well as gram negative bacteria abroad. Trimethoprim is a diaminopyrimidine, while sulfamethoxazole is a sulfonamide and co-trimoxazole inhibits the synthesis of tetrahydrofolic acid, essential for the synthesis of bacterial nucleic acid, along with two components of the drug that inhibit specific steps in the pathway of folate synthesis (Ramlakhan *et al.*, 2014). In this study *P. mirabilis* isolates developed high resistance (72%) against trimethoprim-sulfamethoxazole, which was approximately confirmeds with Nahar *et al.*, (2014) reported a resistant percentage of (66.7%).

Molecular detection of class 1 integron gene

Integrons are mobile genetics elements that could be importants in the dissemination and accumulation of resistance genes in bacteria that usually located within transposonssor conjugative plasmids (Mazel, 2006). Integrons are genetic elements with a specific functional configuration that has evolved in bacteria, catching and expressing cassettes of exogenous genes through site-specific recombination (Xiao *et al.*, 2019). The result showed that the integron gene was detected in 15(88%) *p. mirabilis* isolates as in fig. 3.

Class 1 integrons are the most common and widely distributed among Gram-negative bacteria isolated from clinical samples and often are associated with lateral transfer of antibacterial resistance genes (Al-Sanouri *et al*., 2008). Integrons are mobile genetic elements that could be important in the dissemination and accumulation of resistance genes in bacteria. Integrons are usually located within transposons or conjugative plasmids (Mazel, 2006). In a previous study, 65% of isolates harbored class 1 integron (Bashir *et al*., 2015), whereas in another study class 1 integron was found in 49% of uropathogenic isolates (Ajiboye *et al*., 2009). However, these strains needs to be sequenceds by 16S rRNA or RAPD-typing for further studies as recommended by Salih and Shafeek, 2019; Banoon *et al.*, 2019).

Conclusions

1. The high frequency of specimens collection from patients infected with diabetic foot ulcer G-ve isolated are higher than G +ve.

2. Male is more frequent than Female
3. Most samples isolated from clinical infections had Integrons gene as one of the important mechanisms for acquisition and dissemination of antibiotics resistance mechanisms in *p. mirabilis.*

References

Al-Sanouri, T.M., B. Paglietti, A. Haddadin, M. Murgia, D. Bacciu, M. Youssef and S. Rubino (2008). Emergence of Plasmid-Mediated Multidrug Resistance in Epidemic and Non-Epidemic Strains of *Salmonella Enterica Serotype Typhi* from Jordan. *J. Infect. Dev. Ctries.*, **2:** 295-301.

Bansal, E., A. Garg, S. Bhatia, A. Attri and J. Chander (2008). Spectrum of microbial flora in diabetic foot ulcers. *Indian J Pathol Microbiol*., **51:** 204-208.

Bartlett, J.S. and D. Stirling (1998). PCR Protocols: Methods in Molecular and Biology. 2nd. Humana Press Inc. Totowa. NJ.

Bashir, D., M.A. Thokar, B.A. Fomda, G. Bashir, D. Zahoor, A. Shabir Ahmad and A.S. Toboli (2011). Detection of metallo-beta- lactamases (MBL) producing *Pseudomonas aeruginosa* at a tertiary care hospital in Kashmir. *African Journal of Microbiology Research*., **5:** 164-172.

Banoon, S.R., Z.K. Kadhim, Z.S. Aziz and R.M. E-Wadh (2019). Using Random Amplified Polymorphic DNA (RAPD) Fingerprinting Technique to Analyze Genetic Variation in Staphylococcus Aureus Isolated from Different Sources in Babylon Province Hospitals. *Indian Journal of Public Health Research & Development.*, **10(9):** 1300-1305.

Corrêa, F.E.L., F.G. da-Silva Dantas, A.B. Grisolia, B. do Amaral Crispim and K.M.P. Oliveira (2014). Identification of class 1 and 2 integrons from clinical and environmental Salmonella isolates. *J. Infect. Dev. Ctries*., **8:** 1518-1524.

Chen, C.M., C.H. Lai, H.J. Wu and L.T. Wu (2017). Genetic characteristic of class 1 integrons in proteus mirabilis isolates from urine samples. *Bio Medicine.*, **7(2)**.

Fahadawi, A., A. Mohammed, A. Obadi, I. Waleed and A.S. Hasan (2019). Antibiogram of Pseudomonas aeruginosa Isolated from Burn & Wound Infections Among Inpatients and Outpatients Attending to Ramadi Teaching Hospital in Ramadi, Iraq. *Egyptian Academic Journal of Biological Sciences, G. Microbiology.*, **11(1):** 13-22.

Girlich, D., L. Dortet, L. Poirel and P. Nordmann (2014). Integration of the blaNDM-1 carbapenemase gene into Salmonella genomic island 1 (SGI1- W) in a Proteus mirabilis clinical isolate. *J. Antimicrobial Chemother*., dku371.

Gillings, M.R. (2014). Integrons: Past, present and future. *Microbiol. Mol. Biol. Rev*., **78:** 257-277.

Hegazy, W.A.H. (2016). Diclofenac inhibits virulence of Proteus mirabilis isolated from diabetic foot ulcer. *Afr. J. of Microbiol. Res*., **10:** 733-743.

Khalifa, M.M., A.A. Elkhawaga, M.A. Hassan, A.M. Zahran, A.M. Fathalla, W.A. El-Said and O. El-Badawy (2019). Highly specific Electrochemical Sensing of Pseudomonas aeruginosa in patients suffering from corneal ulcers: A comparative study. *Scientific reports.*, **9(1):** 1-12.

Martínez, N., M.C. Mendoza, I. Rodríguez, S. Soto, M. Bances and M.R. Rodicio (2007). Detailed Structure of Integrons and Transposons Carried by Large Conjugative Plasmids Responsible for Multidrug Resistance in Diverse Genomic Types of *Salmonella Enterica Serovar Brandenburg*. *Journal of Antimicrobial Chemotheraphy*.

Mazel, D. (2006). Integrons: Agents of Bacterial Evolution. *Nature Reviews Microbiology.*, **4(8):** 608-620.

Mirnejad, R., S. Mostofi and F. Masjedian (2013). Antibiotic Resistance and Carriage Class 1 and 2 Integrons in Clinical Isolates of *Acinetobacter Baumannii* from Tehran, Iran. *Asian Pacific J. of Tropical Biomedicine.*, **3(2):** 140-145.

Ramlakhan, S., V. Singh, J. Stone and A. Ramtahal (2014). Clinical Options for the Treatment of Urinary Tract Infections in Children. *Clin. Med. Insights Pediatr*., **8:** 31-37.

Salih, T.S. and R.R. Shafeek (2019). *In silico* Detection of Acquired Antimicrobial Resistance Genes in 110 Complete Genome Sequences of Acinetobacter baumannii. *Jordan Journal of Biological Sciences.*, **12(5)**.

Spichler, A., B.L. Hurwitz, D. Armstrong and B.A. Lipsky (2015). Microbiology Of Diabetic Foot Infections: From Louis Pasteur To 'Crime Scene Investigation'. Bmc Medicine.

Sunde, M. (2005). Class I integron with a group II intron detected in an Escherichia coli strain from a free-range reindeer. *Antimicrobial agents and chemotherapy.*, **49(6):** 2512-2514.

Trivedi, K.M., A. Branton, D. Trivedi, G. Nayak, S.C. Mondal and S. Jana (2015). Phenotyping and genotyping characterization of Proteus vulgaris; after biofield treatment. *Int. J. Genetics and Genomics*., **3:** 66-73.

Wang, A.H., Z.R. Xu and L.N. Ji (2012). Clinical Characteristics And Medical Costs Of Diabetics With Amputation At Central Kirketerp-Møller Urban Hospitals In China. *Zhonghua Yi Xue Za Zhi*., **92:** 224-7.

Wang, J.T., P.C. Chen, S.C. Chang, Y.R. Shiau, H.Y. Wang, J.F. Lai, L.T. Yassen and I.N. Khelkal (2015). Effect of fatty acids on virulence factors of Proteus mirabilis. *I.J.A.B.R*., **5(2):** 108-117.

Wilkinson, H.N. and M.J. Hardman (2017). The role of estrogen in cutaneous ageing and repair. *Maturitas*., **103:** 60-64.

Xiao, L., X. Wang, N. Kong, M. Cao, L. Zhang, Q. Wei and W.

Liu (2019). Polymorphisms of gene cassette promoters of the class 1 integron in clinical proteus isolates. *Frontiers in Microbiology.*, **10:** 790.

Zareei, Y., G. Islami, H. Zandi, M. Mousavi, H. Koosha and F. Akhavan *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.