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Molecular Detection of IntegronclassI Gene in Pseudomonas Aerginosa Isolated from Otitis Media

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Abstract--- *The study aimed to investigating the Pseudomonas aerginosa from patients suffering otitis media infection. The study included of the bacterial diagnosis based on relied diagnostic procedures. A total of 40 specimens collected from (ear swab) in Al-Najaf province within period from September to December 2019.These patients ranged from30-75years. The male more than female. Primary identification was depended on Gram stain and biochemical tests. Finally identification with vitek 2 system was done, the results demonstrate the 30(75%) to Pseudomonas aeruginosa. Genetically, study for gene encoding integrin of P. aeruginosa by using PCR technique. The results indicated, all P. aeruginosa isolate contain IntClassIgene that PCR amplification revealed that 30(75%) of P. aeruginosa carried int1gene.*

Keywords--- *P.aerginosa, IntclassI, Otitis Media*.

I. INTRODUCTION

Otitis media (OM) is a collective term to discribe a group of inflammatory and infective conditions moving the middle ear. Its includes pathology of the middle ear and middle mucosa ear. Otitis media is one of the leading causes of healthcare visits and the complications of OM are significant bases of preventable hearing loss (Sign, 2003). Otitis media have many types which include Acute Otitis media (AOM), (OME) and (CSOM). (AOM) is acute inflammation of the middle ear which could be affected via several kinds of infectious microorganisms like bacteria, fungi and viruses. (Hall Stoodley *et al*.2004). *Pseuedomonasaerugenosa* is a non-fermenting aerobic gram negative bacillus, opportunistic pathogen well-known as significant pathogen leading to many infections in hospitals (xu et al., 2009).

Resistance bacterial clarify via any the genes mutation or genetic exchangeable elements like transposons, plasmids, and integrons. Integrons are genetic transportable elements that can transfer the antibiotic genes resistance. The elements can be placed in several accessories of chromosomes and plasmids. Integrin's are capable to capture drug external gene resistance cassettes and mix them utilizing site specific recombination. It is potentially a great agent in the fast diffusion of bacteria resistance, specially between the gram negative bacteria (Bennett, 2008, Hansson *et al*., 2002).

II. MATERIALS AND METHODS

Specimens collection and bacterial identification

A total of 40 clinical specimens (ear swab) composed from patient suffering of otitis media infection attended to AlSadder City Medical, Al Hakeem General Hospital in province Al- Najaf of September to December 2019. The

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specimens were collected from both gender and (30-75) years age. The samples clinical was processed granted for techniques standard (Cheesbrough, 2000). Following collection, clinical specimens were transported to the laboratory wanting delay, ear swabs was inoculated on suitable media, which included Mac Conkey's agar, agar blood and salt mannitol agar plates and incubated at 37°C for 18to24 hr. Primary isolates diagnosis was made on the footing of staining Gram's, morphology of the colonial on several media and biochemical test. In addition the final identification of bacteria isolates biochemically certain with V.I.T.E.K2-automated way.

DNA Extraction

The DNA Genomic was extracted via utilizing a commerce extraction way (DNA Genomic promega Kit).

Molecular Identification

The P.C.R test were performed to discover intI gene of P.aerginosa as appear on table(2). This primers were designed via Alpha DNA company, Canada as on table (1). magnify products was assured, utilizing 1% agarose electrophoresis gel to determine the P.C.R size of the products. The gel was stained with 4 µLof 10mg/mL ethidium bromide (Sigma, USA) and it run at 80v for 1.5h. A band single was observed at the desirable put on light ultraviolet, bands was photographed utilizing gel registration method. A 100bp ladder (Bioneer, Korea) was utilized to measure the Mwut of produces of the amplified.

Table 1: Primers utilized in the study

Primer type	Primer seq.	Amplicon size
intI1-F intI1-R	5′- GGTGTGGCGGGCTTCGTG-3′ 5′- GCATCCTCGGTTTTCTGG-3′	480

Table 2: P.C.R program of intIprimers that apply in the thermocycler

Gene	Primary denaturation	No.of cycles	Denaturation	Annealing	Extension	Final extension
IntI1	5 min, 94°C	35	One min., 94°C,	One min, 50°C	One min,72°C	5 min, 72°C

III. RESULTS AND DISCUSSION

Isolation of the Bacterial about (40) clinical specimens was together from patients suffering from otitis media signs. The bacterial growth were appear highly in male 30 (75%) than female was 15(25%) as show in table (4-2). This results agree with the study by (Kumar &Seth, 2011), resultantly, the findings obtained might be different than those obtained by (Shrestha *et al.*,2011) their study researches revealed that the females were most commonly affected than males.

This could be explicated via the variances among females and males in, lifestyle, behavior, anatomic, socioeconomic status and the part of hormones sex in the reguletion of the immune way (Mohanna&Bahannan 2016).

ago the studies utilizer indiscriminate selection of subjects, a male dominance may be accidental result and yet not recognized genetic and anatomical several among females and male pertaining exists of the ear (Afolabi *et al.*,2012).

Pseudomonas aeruginosa Isolation and Identification thatinvolved cultural, biochemical and morphology, tests.

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The last identefication was included with the automated vitek-2 compact way utilizing G.N-I.D. Results appeared that *Pseudomonesaaruginosa* constitute 30 isolates (75%) of these isolates, The other bacterial isolates *Klebsiellapnaumoniae, Enterobecteraeroganes and Streptococcus spp*. all *P. aeruginosa* isolate contain *IntClassI*gene that P.C.R amplification revealed that 30(75%) of *P. aaruginosa* carried int1geneas figure1. This result agree with Mohadeseh Zarei-Yazdeli *et al*.,2018and agree with AL-Kraety, & Al-Ammar,2017thusclass1 integrin gene was discovered in 14 / 30 The has a number of reports describing the prevalence of class 1 integrininside gram-negative isolates clinical, Class 1 integrin were perceived in 119 (82.6%) isolatesIn our study, class 1 integron were perceived in 82.6% of the isolates. Reports of *P. aerugenosa* clinical isolates transport class 1 integron in Iran vary between 39.4% and 56.3% (Nikokar *et al*., 2013).Other studies carried out on various clinical samples in Amazon region in Brazil (Fonseca *et al*.,2005), Malaysia (Khosravi *et al*.,2011), Nanjing (Gu*et al*.,2007) and Zhenjiang (Chen et al.,2009) in China showed that 41.5%, 63%, 38% and 40.8% of *P. aeruginosa* isolates carried the class 1 integron gene, respectively. Comparing the results of our study with those from other studies, the increased prevalence of integrase 1 is obvious which can be the result of geographical differences and indiscriminate use of antibiotics. In theis study, the class 1 integron on strains M.D.R have rise diffusion as compared with the strains non-M.D.R; the data coordinated with the data Yousefi and Zhenjiang. us also study detected that class 1 integron was significantly connected with antibiotics resistance, inclusive β-lactam, quinolones, aminoglycosides. In think of the truth that much resistance antibiotic gene cassettes encoding resistance to a broad range of antibiotics in P. *aeruginosa* are carried via class 1 integrin, this is not wonder. Resistance antibiotics against was too observed in other integrin-negative isolates, though. Chromosomal encoded enzymes or other mobile elements might account for the acquisition of the resistance antibiotic genes of the isolates (chen *et al*., 2009).

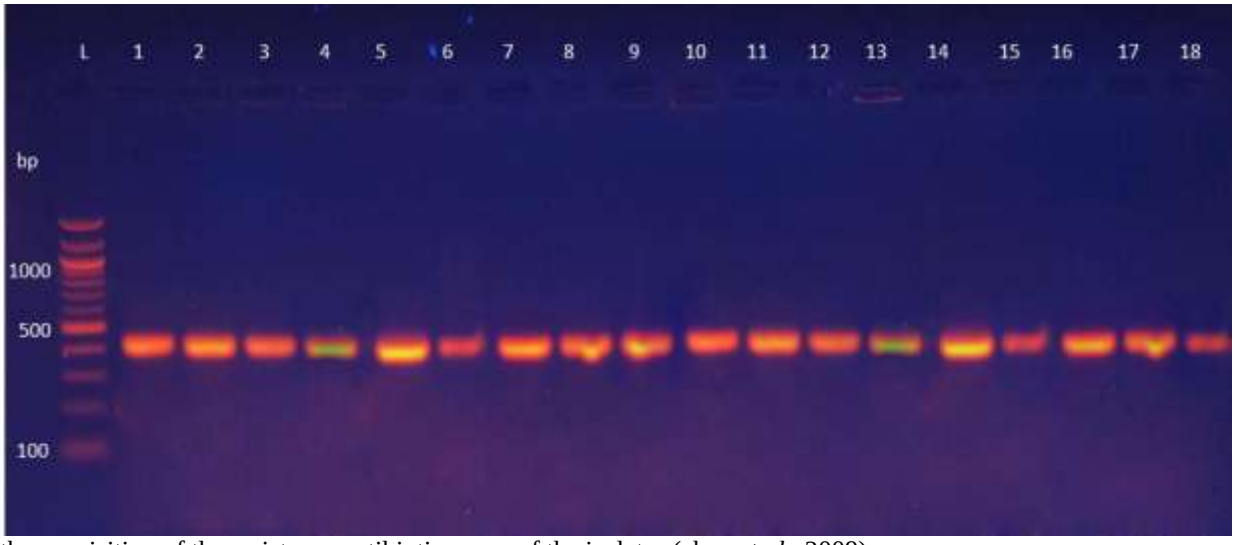

Figure 1: Yields of P.C.R amplification to *P. aeruginosa* isolates that amplified from *IntI*gene primer by way of produce 480bp.Lane (L), Lanes (1 to18), DNA mole. size marker (100-bp ladder), appear results positive with *intI*gene.

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IV. CONCLUSION

In the study for gene encoding integron of *P. aeruginosa* by using PCR technique. The results indicated, all *P. aeruginosa* isolate contain *IntClassI*gene that PCR amplification revealed that 30(75%) of *P. aeruginosa* carried int1gene.

REFERENCES

[1] Afolabi OA; Salaudeen AG; Ologe FE; Nwabuisi C & Nwawolo.(2012).CC Pattern of bacterial isolates in the middle ear discharge of patients with chronic suppurative otitis media in a tertiary hospital in North central Nigeria. *African Health Sciences* Vol 12 No 3.

[2] AL-Kraety, I. A. A., & Al-Ammar, M. (2017). RELATION OF CLASS1 INTEGRON GENE WITH MULTI-DRUG RESISTANCE SALMONELLA TYPI ISOLATES. *Pak. J. Biotechnol.* Vol, 14(4), 537-541.

[3] Bennett PM. (2008).Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *Br J Pharmacol.*

[4] 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. *Int J Infect Dis.*

[5] Fonseca EL, Vieira VV, Cipriano R, Vicente AC. (2005).Class 1 integrons in Pseudomonas aeruginosa isolates from clinical settings in Amazon region, Brazil. *FEMS Immunol Med Microbiol.*

[6] 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. *J Clin Microbiol.*

[7] Hall Stoodley L, Costerton JW, Stoodley P. (2004). Bacterial biofilms: From the natural environment to infectious diseases. *Nat Rev Microbiol* 2(2):95-108.

[8] Hansson K, Sundström L, Pelletier A, Roy PH.(2002).IntI2 integron integrase in Tn7. *J Bacteriol.*

[9] Khosravi L, Tee Tay S, Vadivelu J.(2011). Analysis of integrons and associated gene cassettes of metallo-b-lactamase-positive Pseudomonas aeruginosa in Malaysia. *J Med Microbiol.*

[10] Kumar H. & Seth S. (2011). Bacterial and Fungal Study of 100 cases of Chronic Suppurative Otitis Media. *Journal of Clinical and Diagnostic Research*, Nov; 5(6): 1224 – 1227.

[11] MohadesehZarei-Yazdeli, Gilda Eslami, HengamehZandi, Masoumeh Kiani, Kazem Barzegar, Hanieh Alipanah, SeyedMorteza Mousavi1, Marzieh Shukohifar.(2018). Prevalence of class 1, 2 and 3 integrons among multidrug-resistant Pseudomonas aeruginosa in Yazd, Iran: *Iranian journl of microbiology*: volume 10,number 5.

[12] Mohanna M. A. B. & Bahannan A. A.(2016). bacterial profile and antibiogram of otitis media Among children in yemen. *J Ayub Med Coll Abbottabad*; 28(3).

[13] Nikokar I, Tishayar A, Flakiyan Z, Alijani K, Rehana-banisaeed S, Hossinpour M, et al. (2013).Antibiotic resistance and frequency of class 1 integrons among Pseudomonas aeruginosa, isolated from burn patients in Guilan, Iran. *Iran J Microbiol.*

[14] Shrestha BL., Amatya RCM., Shrestha I., Ghosh I. (2011). Microbiological Profile of Chronic Suppurative Otitis Media. *Nepalese Journal of ENT Head and Neck Surgery*,; 2(2): 6 – 7.

[15] Xu Z, Li L, Shirtliff ME, et al.(2009). Occurrence and characteristics of class 1 and 2 integrons in Pseudomonas aeruginosa isolates from patients in southern China. *J Clin Microbiol.*

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