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Molecular Detection of *Pseudomonas aeurogenosa* secreting Exotoxin type A in Post-surgical operation wound inflammations

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Abstract:

Using the PCR for the molecular detection of *P. aeurogenosa* from clinical isolation samples of inflammation wound after surgery , by using Exotoxin A (ETA) gene primers amplification with 396 bp fragment length , study result showed that *P. aeurogenosa* that contain this gene give +ve result neither the *P. aeurogenosa* that not had this gene gives –ve result , the specify & sensitivity of this test was tested with accurate 96% , because that PCR detection for the +ve *P. aeurogenosa* making a noticeable band for 3 µl of reaction mixture , that could be useful for small amount of bacteria in wound infection without needs to bacterial culture or other traditional biochemical test, 33 specimen swap samples from inflammation wound patient cases in periods from 11/2016 to 1/ 2017 , specimen were cultured and diagnosed then harvested for DNA isolation altered by PCR & ETA gene primer preparation followed by agarose gel electrophoresis for ETA gene detection , this study was showed that 23 sample specimen were +ve contain this target gene , indicate that 70%was +ve result from total specimen.

Keywords: ETA gene, *P. aeurogenosa*, wound inflammation

INTRODUCTION

The wounds after surgery is a possible place for the opportunistic bacterial growth where it colonized and spread latterly, wide spreading of this bacteria is one of reasons for infection by this bacteria, it may occur in hospital contaminated floor and might be transferred through bandages that previously used in treating most of this bacteria is opportunistic, their for it easily convert to pathogenicity , infection with it might lead to death as in case of cystic fibrosis, cancer and sever burning [1,2], it noticeable that in abdominal surgery that delayed in curing duo to not accurate diagnostician or staying for long period in hospital or clinics [2,3] .

P. aeurogenosa is commonly spreading and mostly common in clinical infection in blood stream, wound infection, pneumonia, this bacteria is gram –ve aerobic bacilli motile by polar flagellum, free living in water & soil , related to Family pseudomonadaceue [4], virulence factors production such as alkaline-protease , elastease , hemolysin, & pyocyanin that might cause damage in tissue like blood stream [5], this bacteria considered as one of the bacteria causative agent that cause bacteremia in tissue or organ transplantation in recipient patient , it cause e about 28% of bacteremia at all [18], most of previously studies showed that vegetable is the main source for bacterial contaminating with the *P. aeurogenosa* notwithstanding using of hydrochloric acid (concentration 1%) due to it production of exotoxin A that lead to tissue necrosis & death by blocking the protein biosynthesis process at constructed level A at the level of poly peptide chain elongation factor 2 , it consist of tow basic subunit, 1st is fragment is responsible for stimulating & fragment B that is responsible for reaction with cell receptors in eukaryotes [19-21].

The diagnosis of the *P. aeurogenosa* from the wounds is done by culturing , isolation altered by biochemical tests done in most of laboratory , but this is time consuming while infection diagnosing must be done in short time period which is banefully in treating , using of the PCR technique for diagnosis lead to specific and rapid detection for most of pathogenic agent was well documented [6].

In this study we will explain how to use the PCR technique for diagnostic of *P. aeurogenosa* from inflamed wounds after surgery by using the exotoxin A gene (ETA gene) , this gene related to family of bacterial toxins that work as active cellular toxins , the aim of this study is to detect the rate or percentage of this bacteria in Babylon province and also to determine the accuracy of PCR technique in diagnosis in the epidemic study [13,14,17], *P. aeurogenosa* was first named by scientist Schroeler in 1872 A.C. , it was isolated in his culture from skin wounds with blue-green color , also Gessard in 1882 A.C. perform punch of studies from (1892, 1890,1891) noticed that this bacteria produced tow pigments 1st was the polyamine that derivative from pyocyanin (mean ugly blue) that characterize the infection with *P. aeurogenosa* , this pigment is blue-green non- fluorescence dissolved in chloroform & water second is fluorescin pigment which is yellow-green in color [7,9].

Classification by Wilson & Dowling 1998)
Kingdome : Bacteria
Phylum : Proteobacteria
Class : Gammaproteobacteria
Order: Pseudomonadalg
Family : Pseudomonadaceae
Genus : *Pseudomonas aeurogenosa*

MATERIALS & METHODS

Plastics: 10cm petri dishes, 1.5 Eppendorf tubes, polyethylene tubes, tips, PCR tubes, cotton swabs.

Instrument : Eppendorf centrifuge, freezer, GFL waterbath, Memmert Incubator , Memmert oven , Mupid agarose gel electrophoresis system , Atta gel documentation system , Clever Scientific Thermocycler , Eppendorf Micropipette , Sartorius water Deionizer.

Chemicals: agarose, Nutrient Agar, brain heart broth. Ethidium Bromide, Macconkey, TBE Buffer, Ethanol, Isopropanol, , Genomic DNA Extraction Wiz. Of (Promega), Green , Master Mix (Promega) , Deionized Distal Water [22,23]

Stages of work could be divided into:
1- Samples taken from patient that have wounds inflammation in hospitals.
2- Specimen culturing on Nutrient agar or Macconkey agar.
3- Moving the bacterial growth from agar media to broth media
4- DNA extraction
5- Amplification with PCR by using the ETA gene primers
6- Agarose gel electrophoresis for the PCR product

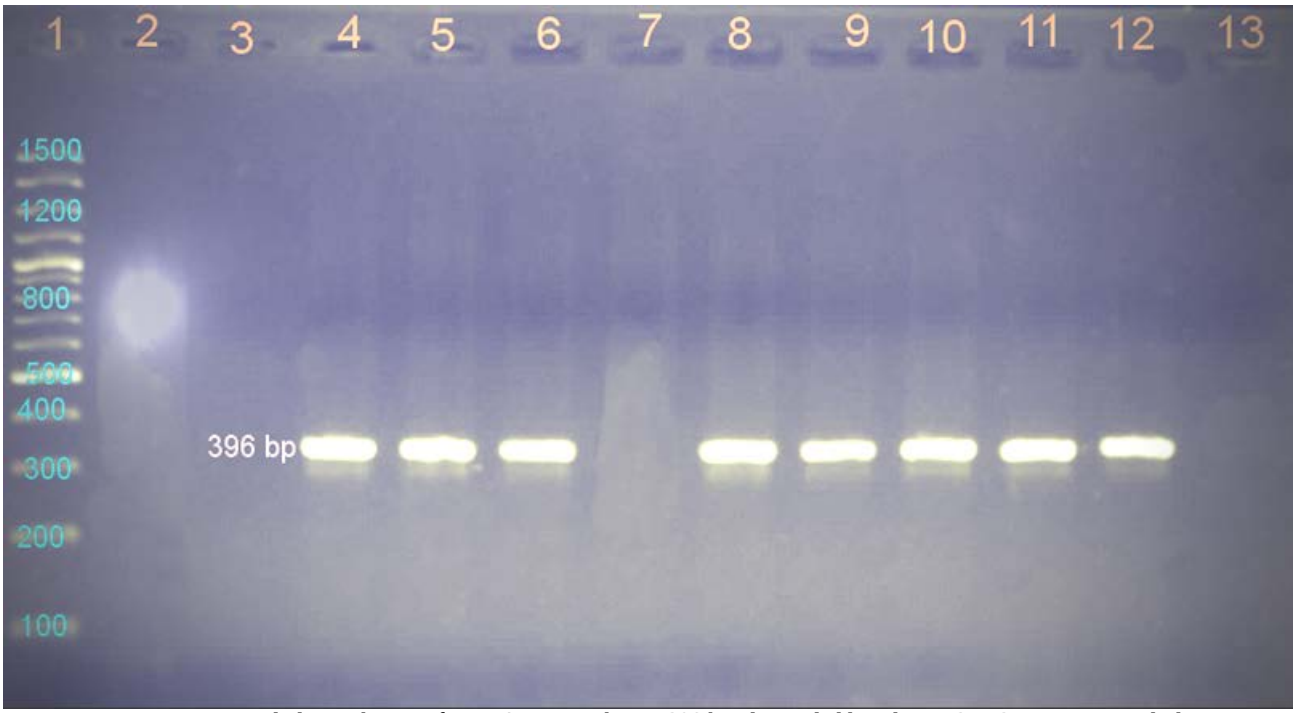

Image 2 agarose gel electrophoresis for ETA gene with size 396 bp , lane 1 ladder , lane 2,3,7,13 negative result, lane 4,5,6,8,9,10,11,12 positive result.

All 34 specimen gated from patient with inflammated wound after they had a surgery from Merjan medical city in Babylon province in period from 2015/ 12 till 2016 / 3 , specimen included male & female, samples taken by cotton swabs from of discharge wounds , then cultured on media depending on manufacture leaflets , nutrient agar was prepared by mixing 20 gm of it with 1000 ml of D.W. , then autoclaved , followed by spreading the specimen swabs on nutrient agar media that casted in 10 cm petri dishes followed by incubation at 37C° for 24-48 hr., then moved to the broth media and incubated at 37C° for 24-48 hr. followed by DNA extraction that done duo to Promega USA corporation protocol Wizard® Genomic DNA Purification Kit ref # A1120, altered by PCR .

PCR Conditions : 20µl protocol , 95 C° pre incubation for 2 min. , 30 cycle , 94 C° for 1 min. , 72 C° for 1 min. , 72 C° for 7 min.
Latterly making a agarose gel electrophoresis (1% , 70V/ 50 min.) for the PCR product [25] .

RESULT & DISCUSSION

Detection for the DNA fragment that amplified by the PCR, in this study fragment size was 396 bp for ETA gene of *Pseudomonas aeurogenosa*

This result of agarose gel electrophoresing showed that if the band in size 396 bp was present that mean positive result or in other word mean the presence of this gene as indicator for *P. aeurogenosa* presence in the infection specimen samples, 21 sample of 33 total samples was recorded as +ve results, the two primers that used in this study was designed & prepared depending on previously publication [6,15,16] , the activity of exotoxin A that produced by *P. aeurogenosa* duo to it ability to block the protein phosphorylation by effecting on elongation factor 2 in eukaryote [6, 12,18,19,20] , leading latterly to cell necrosis & death, ETA gene consist mainly of 3 subunits (domain I, II,III) responsible for binding with cell receptors then to membrane translocations then to ADP ribosylation alternatively [10, 21,22] . the special primers that designed for this study was prepared depending on previously researches that Belonging to the ETA gene which measure the primer specificity for detection of infection duo to present of amplified ETA gene fragment 396 bp or not , most of non *P. aeurogenosa* will failed or give –ve results in this test [19] .

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