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Molecular detection of two virulence factors of *Pseudomonas aeruginosa* isolated from burn patients

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Abstract:-

The present study aimed to identify two virulence genes in *P. aeruginosa* isolated from infected burn wound. Out of 70 burn swabs, 54 (77%) showed positive growth on enriched media which obtained from burn wound at Al-Mussan Teaching Hospital in Mussan province, Iraq. Only 13 isolates (35%) identified as *P. aeruginosa*, and those isolates examined using Polymerase Chain Reaction (PCR) for detection the *exo*S and *phz*S genes. The results recorded that 46% and 77% of isolates have *exo*S, *phz*S genes, respectively. We concluded a *P. aeruginosa* harbor diverse percentages of both genes as virulence factors, and those factors must be increased the pathogenicity of this pathogen.
Keywords: *P. aeruginosa*, burn, *exo*S, *phz*S genes, virulence factors.

Material and Methods:-

Samples collection:-

Seventy swabs were collected from different locations of burns patients in burn unit of AL-Mussan Teaching Hospital of Mussan province/Iraq in the period from July to November, 2014 by moistened sterile swabs with normal saline. Swabs were transported for the laboratory for diagnosis, these swabs directly inoculated on Blood agar and MacConkey agar (LAB/ United Kingdom) and incubated at 37ºC for 24 hours.

Identification of *P. aeruginosa*:-

P. aeruginosa was identified based on the morphological properties in culture media and biochemical tests included (catalase test, oxidase test, indol test, methyl red test, voges-proskaure test) which done according to Bergeys manual (MacFaddin, 2000; Murray *et al.*, 2003). VITEK 2 system (BioMerieux, France) was done to confirmed identification of *P. aeruginosa.*

Preparation of bacterial DNA:-

All *P. aeruginosa* isolates were inoculated aerobically on Brain Heart Infusion broth (LAB/ United Kingdom) and incubated for 18–24 h at 37° C. Bacterial DNA extraction was performed using Genomic DNA Extraction kit (Geneaid/Korea).The *exoS* and *phzS* genes were identified using primers described in Table (1). The final volume of PCR reaction tubes is 20µl, consist of 5µl Master Mix (Bioneer/Korea), 1µl of each forward and reverse of the primers specific for each gene, 5µl of DNA template and the volume was completed by adding nuclease free water. The thermocycling conditions of *exoS* gene were set at 94 °C for 3 min followed by 30 cycles of 94 °C for 30 sec, 55 °C for 60 sec, and 72 °C for 90 sec, and final extension at 72 °C for 5 min (Fazeli and Momtaz, 2014), while the thermocycling program of *phzS* gene was set at 96°C for 5 min, followed by 30 cycles of 94

°C for 30 sec, 63 °C for 30 sec, 72°C for 1 min, and final extension at 72 °C for 4 min.

Table (1): Oligonucleotide primers sequences for PCR amplified of *phzS* and *exoS* genes

Gene name	Primer Sequences (5´- 3´)	Product size	Reference
exoS	F:CGT CGT GTT CAA GCA GAT GGT GCT G R: CCG AAC CGC TTC ACC AGG C	444 bp	Fazeli and Momtaz, (2014)
phzS	F: TCG CCA TGA CCG ATA CGC TC R: ACAACCTGAGCCAGCCTTCC	1752 bp	Fazeli and Momtaz, (2014)

Results and Discussion:-

Out of 70 burn swabs, 54 (77%) showed positive growth on blood agar, only 13 isolates (35%) were *P. aeruginosa*, *P. aeruginosa* is the common frequent bacterial agent of burn wound. The results of current study are comparable to other studies, such as the results of Alkaabi, (2013) reported a *P. aeruginosa* was the commonest pathogen, with a percentages of 48.14%, also Kanagapriya *et al*., (2015) documented the predominant bacteria isolated from the infected wounds were *P. aeruginosa* (28%).

The results showed that the prevalence of *exo*S gene harbored in about 46% of all isolates. The band size was 444 bp corresponds to amplification of *exoS* gene, Fig. (1). The *exoS* gene may be associated with increasing the pathogenesis of this bacteria, (Olson *et al*., 1999) revealed that the *exoS* gene is directly translocated into eukaryotic cells by the contact-dependent type III secretory process, it provides the bacterium with a mechanism for manipulating the eukaryotic cells it encounters, *exoS* gene contributing to pathogenicity of this bacteria, bacterial translocation of *exoS* into epithelial cells results in a general inactivation of cellular function, as known by the inhibition of DNA synthesis, loss of focal adhesion, microvillus effacement and cell rounding.

The percentage of *exoS* gene in present study showed slightly percentage in comparison with the results of other studies which documented a high percentages such as Fazeli and Momtaz, (2014) recorded that 67.6% of human clinical samples which isolated from burn, respiratory and urinary tract infections had *exoS* gene as the most commonly detected virulence genes. Afterward, Wolfgang *et al*., (2003) showed that 13 out of 19 strains of *P. aeruginosa* contained *exoS* gene, also Finnan *et al*., (2004) revealed that the massive majority of *P. aeruginosa* isolates contain *exoS* gene, because this genes are involved in phagocytosis and lung injury in the human host.

The *exoS* gene must be related to some diseases, Sun *et al*., (2012) recorded that the *exoS* gene contributed to dissemination in burn, keratitis and lung diseases. Exotoxin S and other exotoxin such as *exoU*, and *exoT* genes were importance in the pathogenicity of lung and other diseases (Shaver and Hauser, 2004).

Some studies like Feltman *et al*., (2001) suggested that all *P. aeruginosa* isolates harbor some genes which encoding the type III secretion apparatus but that three of the four type III effector proteins (ExoT, ExoS, ExoU, and ExoY) are variable and that the huge majority of isolates contain either the *exo*S or the *exo*U gene but not both.

The results of PCR assay showed that 77% of *P. aeruginosa* isolates ported *phzS* gene, the band was 1752 bp size corresponds to amplification of goal gene. The results of present study was agreed with study of Fazeli and Momtaz, (2014) depicted that a high percentage of clinical isolates of *P. aeruginosa* had *phz*S gene. On other hand, the most of the clinical isolates carried all the genes involved in phenazine production, with the exception of CF194, which lacked both *phzI* and *phzS*, and CF242, which lacked *phzS* (Finnan *et al*.,2004).

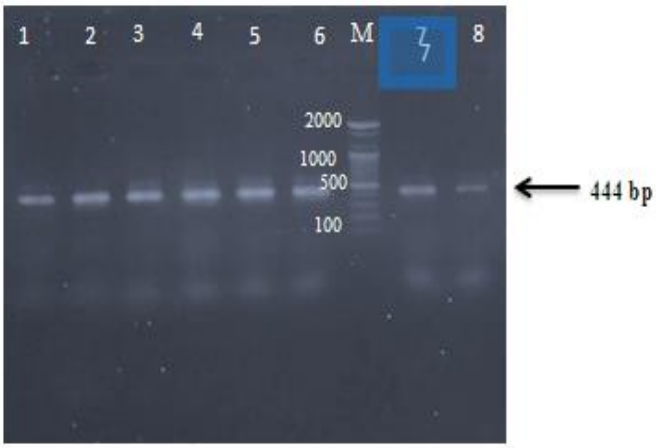

Fig. (1): Agarose gel electrophoresis of *exo*S gene amplification, M: ladder, 1-8: positive results

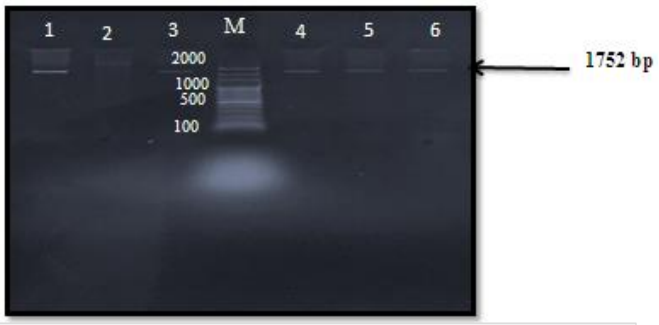

Fig. (2): Agarose gel electrophoresis of *phz*S gene amplification, M: ladder, 1,3,4-6: positive results, 2: negative result

Conclusions:-

We concluded a *P. aeruginosa* have diverse percentage of *exo*S and *phz*S genes as virulence factors, and those factors must be increased the pathogenicity of this pathogen.

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