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The Role of Caspases in Inflammatory Responses against Gram Negative Infections

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Abstract--- *Pseudomonas aeruginosa is emerged as one of the utmost difficult nosocomial pathogens. The present study aims to estimate the role of caspases in inflammatory responses against Pseudomonas aeruginosa infection. This study was performed on 50 isolates infected patients. the diagnosis isolates depending on the macroscopic, microscopic,biochemical and API20E tests, results show that the percentage of isolates according to clinical sources were (38%) from burn, (24%) from urine,(6%) from ear swabs,(20%) from sputum and (12%) from wounds. A different abilities to resist antibiotics included Amoxicillin- clavulanic acid, Trimethoprim-sulfamethoxazole and Erythromycins how effectiveness against P.aeruginosa. The ability of biofilm formation as a virulence factor by Micro-titer plate method (MTP), results revealed that (92%) of P. aeruginosa have ability to form biofilm. Molecular diagnosis of isolates implemented by 16SrDNA gene in addition to detect the virulence gene (Flic gene), the results showed (90%) of isolates were positive. the results of the casp-1 patients sequencing showed 70% have heterozygous nucleotide and the level of IL-1β showed no significant differences between infected and non-infected individuals. Comparison between IL-1β level with patients age group where the F, G and H group revealed a significant differences. The comparison between sources infection recorded higher significant differences between cystic fibrosis compared with wound and UTI source.*

Keywords--- *Pseudomonas Aeruginosa, Flagillin, Flic, Caspase-1, IL-1β.*

I. INTRODUCTION

Pseudomonas aeruginosa is one of the nosocomial pathogens, considered as opportunistic bacteria which reasons contagion in excepted dejected subjects (Brooks et al., 2007).It is the major pathogen wound contagion, urinary tract, surgical wound and ear contagion (Todaret al., 2008). A fast and careful method of the concurrence of Pseudomonas is significant to patients isolate and block further pervasion of the illness. (Minion et al., 2010).Some studies contented via utilizing the API20E or classical biochemical test for bacterial dignosis (Capuzzo et al., 2005), the PCR is greatly sensitive specific method to detection P. aeruginosa by 16SrRNA (Xu et al., 2004, Porteous et al., 2002). This bacteria is motile through a single flagellum polar, used in swimming motility, environment spreading, translocate to preferred host cells, optimal colonization sites access, and the Toll-like receptor 5 (TLR5)-dependent inflammatory response (McIsaac, 2012).The flagellum considered as one of the virulence parameter, involved in bacterial pathogenicity (Haiko and Wikstrom 2013).

FliC flagellin is an vital part of bacterial chemotaxis, during infection, its used to adhere to host epithelial cells

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by the binding to the asialyated glycolipid asialoGM1 and can make a strong nuclear factor kappa-light-chain enhancer of activated B cell NF-kB-mediated inflammatory response by signaling through TLR5 and a caspase-1-mediated response through the Nod-like receptor (NLR), Interleukin-1β-converting enzyme (ICE) ICE protease-activating factor (Ipaf) (Miao et al., 2007).Caspases are evolutionarily conserved cysteine proteases that induce apoptosis. Although apoptosis is generally considered to be immunologically silent, caspase-1 (Casp-1) processes the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 to their mature forms (Fantuzzi and Dinarello1999). Casp-1 is unique because it is both pro-apoptotic and pro-inflammatory molecules (Martinon and Tschopp 2004; Martinon et al., 2009).IL-1β is an inflammatory cytokine mediates a diverse range of effects, including T-cell polarization, antibody production, fever and activation of endothelial and phagocyte (O'Neill, 2008; Dinarello, 2009, Auron et al., 1984, Thornberry et al., 1992).

The purpose of the study was to link the infections with the bacterium P.aeruginosa, which possesses the flicgene for the virulence factor of the astronomy and the activation of caspase-1, leading to the high level of IL-1βin infected patients' serum.

II. MATERIALS AND METHODS

Seventy three blood samples and bacterial isolates were collected from different clinical sources using sterile swabs, (n=12)urine cultures, (n=10) sputum, (n=9) wounds, (n=19) burns and(n=3) external otitis from from nine hospitals in Baghdad including: AL-Imam Ali, AL-Kindi General Teaching, Ibn-El Balady, Teaching laboratories in medical city, Fatima Al-Zahraa, Education Baghdad, Burns Specialist, Ghazi Al-Hariri for Specialized Surgery and Child protection hospital in medical city, during (October 2016 till April 2017) with age ranging from 3months to 67 years in nine different Baghdad hospitals. The patients divided into 2 groups according to their ages and type of infection according to their ages. The antimicrobial susceptibility of P. aeruginosa isolates was studied utilizing according to (Ferraro et al. 2000).

Detection of Quantitative Biofilm Formation by the Microtiter Plate Method (MTP)

Biofilm-forming ability was determination by estimated of adhesion to polystyrene microtiter plates (Bose, et al., 2009). Fleetingly, isolates were inoculated in Luria-Bertani (LB) and incubated at 37 °C. of 18 hour 300 µL of LB broth were distributed in flat-bottom and immunized by 10 µL of bacterial broth, after that, plates were incubated at 37 °C of 18 hour, washed by adjusted P.B.S(pH 7.0), then plates were air-dry at 60 °C for 1 hour and stained by crystal violet 0.25% for 1 minnext, acetic acid 33 % was utilized to de-stain samples, finally each well measured at 570 nm, the Cut-off OD (O.D.c) was defined of microtiter- plate test as 3 values of standard deviation above the mean of O.D of negative control, an adherence capability of tested isolates was classified based on O.D. Wholly tested was carry out3 times and the data was averaged (Bose, et al., 2009).

Reference Strains: Reference Bacillus. Subtilis, strain PY79 as negative control was obtained from Laboratory of molecular Bacteriology intercollegiate Faculty of Biotechnology.

Blood Sample Collection: Blood samples were collected from patient that are suffering from *P. aeruginosa* infections. For DNA extraction and biomarkers study.

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DNA Extraction: Bacterial Genomic DNA was extracted a wizard Genomic DNA Purification Kit (Promega, Madison, WI).as per the manufacturer instructions then it stored at -20◦C as a template DNA stock. Blood Genomic DNA was extracted via utilizing the Genomic DNA purification Kit (Promega, USA) it's also stored at -20◦C.

PCR Programs: Fifty samples of P. aeruginosa was selected for diagnosis by PCR. the sequence of the primers used for 16S rDNA are PA-SS-F (5'GGGGGATCTTCGGACCTCA 3') and PA-SS-R (5' TCCTTAGAGTGCCCACCCG 3') at One pre cycle of 95°C for 2 min., 30cycles (95°C of 40 sec, 58 °C of 1 min. and 72°C of 2 min.)and One final extension cycle of 72°C for 10 min. the Detection of flagellin gene flic-F- CW45 (5' GGCAGCTGGTTNGCCTG 3') 1.02 kb (type a) flic-R-CW46 (5' GGCCTGCAGATCNCCAA 3') 1.25 kb (type b), the amplification program was run as follow: One pre cycle of 95°C for 2 min., 30cycles (95°C of 40 sec, 55 °C of 1 min. and 72°C of 2 min.) and One final extension cycle of 72°C for 10 min.

theCasp-1gene amplification by casp-1-F (5' CAAGGTCCTGAAGGAGAAGAG 3') and casp-1-R (5' TGAGAGTCCCAGCGTCCCT 3') the amplification program was One pre cycle at 3 min, of 95°C, 30cycles (94°C of 30 sec, 58 °C of 20 sec and 72°C of 20 sec)and One final extension cycle at 72°C of 5 min. Then the amplified products were electrophoresis at 1.5% agarose gel with ethidium bromide

DNA Sequencing : Sequencing of amplified product of *casp-1* gene was done by (National Instrumentation center for Environmental management (NICEM) through using forward and reverse primer for *casp-1* gene.

Homology search was conducted utilizing Basic local alignment search tool BLAST. (NCBI) online and Bio Edit program. The result was compared by reference sequence of the gene obtained of gene bank as control.

Determination of Serum IL-1β Level: Serum was collected and analyzed for secreted IL-1β using ELISA, System. Each sample was assayed in duplicate. The density of two wells and comparison with a standard curve.

Statistical Analysis: Statistical analysis, S.P.S.S 18.0 software was utilized data exhibits as mean. ± SD. one-method analysis of variance (A.N.O.V.A) and Student's *t*- test (Stat view). The p-values less than 0.05 was considered to be statistically.

III. RESULTS AND DISCUSSION

P. aeruginosa is found widely distributed in the environment and is considered an opportunistic pathogen. In this study we selected 50 clinical P. aeruginosa isolated from hospitalization patients from October 2016 to April 2017.

The P. aeruginosa strains isolated from the different clinical sources were identified: 38% (19/50) from Burn's, 24% (12/50) from urine, 20% (10/50) from sputum, 12% (6/50) from wound and 6% (3/50) from otitis.

From 50 isolate (48) isolates gave a positive results on citrimide agar that containing nalidixic acid, it is a differential antibiotic for isolation and identification of P.aeruginosa from other species of Pseudomonas(Kodaka et al., 2003). and API 20E test recorded positive results for all types of isolates except 3(6%) isolates from wound infections that may be caused by some mucoid strains of P.aeruginosa (Fadhel et al., 2013), which are difficult to distinguish by API 20E test (table 1).

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Table 1: The Results of Cultural Tests on Different Media and API 20E Test

Type of isolates	MacKonky agar	Pseudomonas agar	Blood agar	Citrimide agar	Nutrient agar	API 20E test
otitis	3/3	3/3	3/3	1/3	3/3	3/3(100%)
urine	12/12	12/12	12/12	12/12	12/12	12/12(100%)
wounds	6/6	6/6	6/6	6/6	6/6	3/6(50%)
burns	19/19	19/19	19/19	19/19	19/19	19/19(50%)
sputum	10/10	10/10	10/10	10/10	10/10	10/10(100%)

Antimicrobial Susceptibility Test

The results showed isolates a different ability to resist 24 antibiotics table (2).

Table 2: Results of Susceptibility Tests of P. aeruginosa

symbol	Antibiotics	Number of bacterial isolates and their percentage		
		R	I	S
AK	**Amikacin**	21(42%)	4(8%)	25(50%)
AMC	**Amoxicillin- clavulanic acid**	50(100%)	-	-
AMP	**Ampicillin**	36(72%)	-	14(28%)
ATM	**Aztreonam**	34(68%)	7(14%)	9(18%)
CAZ	**Ceftazidime**	14(28%)	-	36(72%)
CIP	**Ciprofloxacin**	28(56%)	-	22(44%)
CLT	**colistin**	47(94%)	-	3(6%)
CPM	**Cefepime**	45(90%)	-	5(10%)
CTR	**Ceftriaxone**	37(74%)	2(4%)	11(22%)
CTX	**Cefotaxime**	44(88%)	-	6(12%)
ERY	**Erythromycin**	50(100%)	-	-
GEN	**Gentamycin**	43(86%)	2(4%)	5(10%)
IPM	**Imipenem**	15(30%)	5(10%)	30(60%)
LEV	**Levofloxacin**	12(24%)	-	38(76%)
MEM	**Meropenem**	1(2%)	-	49(98%)
NA	**Nalidixic acid**	48(96%)	-	2(4%)
NiT	**Nitrofurantoin**	9(96%)	1(2%)	1(2%)
PG	**Penecillin G**	48(96%)	-	2(4%)
PI	**Piperacillin**	31(62%)	3(6%)	16(32%)
TCC	**Ticarcillin-clavulanic acid**	37(74%)	-	13(26%)
TE	**Tetracycline**	49(98%)	-	1(2%)
TI	**Ticarcillin**	38(76%)	4(8%)	8(16%)
TOB	**Tobramycin**	39(78%)	-	11(22%)
TS	**Trimethoprim-sulfamethoxazole**	50(100%)	-	-

Amoxicillin- clavulanic acid, Trimethoprim- sulfamethoxazole and Erythromycin were the effectiveness antibiotic against P.aeruginosa 100%.

Detection of Quantitative Biofilm Formation by the Microtiter Plate Method (MTP): The present research showed different ability to form biofilms by using MTP Method, showed in table (3).

Table 3: Biofilm Results of P. aeruginosa in MTP Method

Biofilm formation	Percentage% and number
High producer	76%(38/50)
Moderate	16%(8/50)
Non-producer	8%(4/50)

The present results showed that most of the isolates have ability to produce biofilm were resistant to antibiotics

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it's consistent with (Kaur et al., 2013). They were found that 65% of the isolates resistant to antibiotics have the ability to form the biofilm, unlike the isolates that cannot form This biofilm indicates the importance of the biofilm and its role in the emergence of high resistance to antibiotics by many of the bacterial species that produce it (Bacalso, et al., 2011), that help it to adhere to host cells (Valletet al., 2004) and gave it the protection of bacteria from external conditions is not appropriate, which helps to stay on hard surfaces, especially in the hospital environment and this leads to the occurrence of injuries acquired from hospitals (Nosocomial infections) (Ramoset al.,2013).

Genotypic Identification

The DNA of bacterial isolates show in figure 1,

Fig. 1: Agarose Gel Electrophoresis of Bacterial Genomic DNA Using 0.7% Agarose, TBE (1x) and Visualized by Ethidium Bromide Stain

PCR Analysis for Bacterial Isolates

Two primer pairs was utilized in this study; the pair Pa SS-F and Pa SS-R that specific to P. aeruginosa (figure 2). 16S rRNA sequence gene offered a utilizedway of the identification of bacteria. It had long been utilized as a taxonomic way in estimation the phylogenies of bacterial species (Drancourt et al., 2000), the data appear wholly 50 isolates gave positive results.

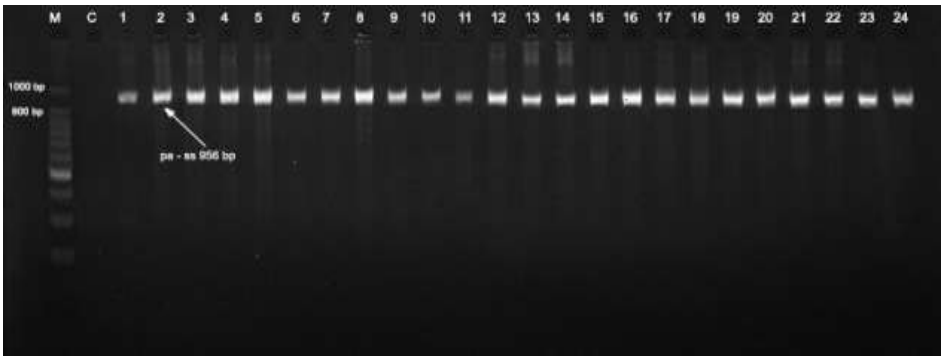

Fig. 2: The PCR Products (956 bp) of 16S rDNA that Specific of P. aeruginosa were Identified in Wholly Samples in 1.5% (1 h /70 vol) Agarose Gel Electrophoresis. M 1500kb DNA Ladder, c: Negative Control and 1- 24 was Various Sample of P. aeruginosa Isolates

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The flic gene asserts that the bacteria are contained on the virulence gene of activating and caspase-1 which in turn leads to the production of Interleukin-1 beta. Flicgene is consist of two types: type A, which has a molecular weight of 1020 base pairs and type B 1250 base pairs (Faeziet al., 2016).

The results of present research showed all isolates were positive, 28(56%) isolate had type a and 17(34%) isolate had type b of the gene, but 5(10%) there were isolates that did not have a flic gene, these were isolates which were cystic fybrosis infection 4 sample, and 1 uti infection of isolate this is agreement with (Nehaet al., 2014).

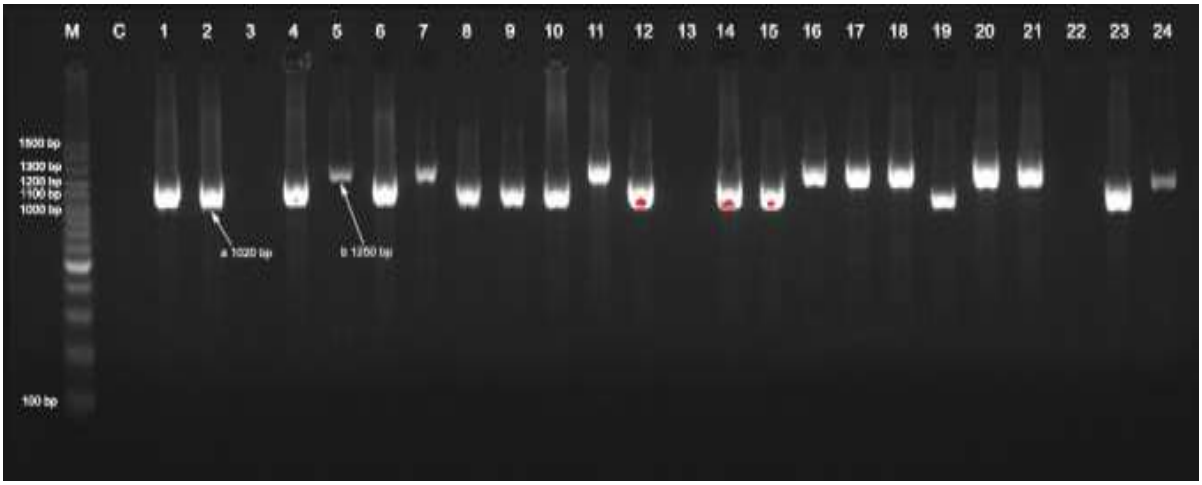

Fig. 3: Agarose Gel Electrophoresis of PCR Product Amplified from flic Gene, the DNA Fragments of Type a 1020 bp and Type b 1250bp, were Amplified from Flic Gene using Ladder; Lane M (100-1500 bp), Lane C is Control Negative. The Figure shows all Samples Gave Positive Result (lanes from 1 to 24) Except Five Isolates Gave Negative Results

Apart from extracellular parameter, the primary attachment mediator (flagella) appear a important kind in initiation of infection. 2kind of flagallant proteins has been identified in P. aeruginosa, kind ‘a’ and kind ‘b’ that can be distinguished on the basis of molecular size and reactions by kind -specific polyclonal and monoclonal antibodies (Allison 1985).

Kind ‘a’ and ‘b’ flagellant of P. aeruginosa do not exhibit phase variation; a single strain produces single kind of flagellant, and no switching among kinds ‘a’ and ‘b’ has been observed.

Oligonucleotide primers specific of N-terminal (C.W.46) and C-terminal (C.W.45) conserved regions of flagellant gene was utilized of PCR amplification of the flagellant gene of P. aeruginosa P.A.O1. In a physical genome analysis of the virulence-associated fliC locus in P. aeruginosa strains, mapping and sequencing revealed groups of heterologous a-kind (1164 bp; 1185 bp) and greatly conserved ‘b’-kind (1467 bp) flagallin genes [Spangenberg C, 1996]. Percentage fliC occurrence was find to be 90% (37.77% ‘b’-kind flagellin, 62.23% ‘a’-kind) (Lenaet al., 2016).

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Genomic DNA Isolation from Human Blood

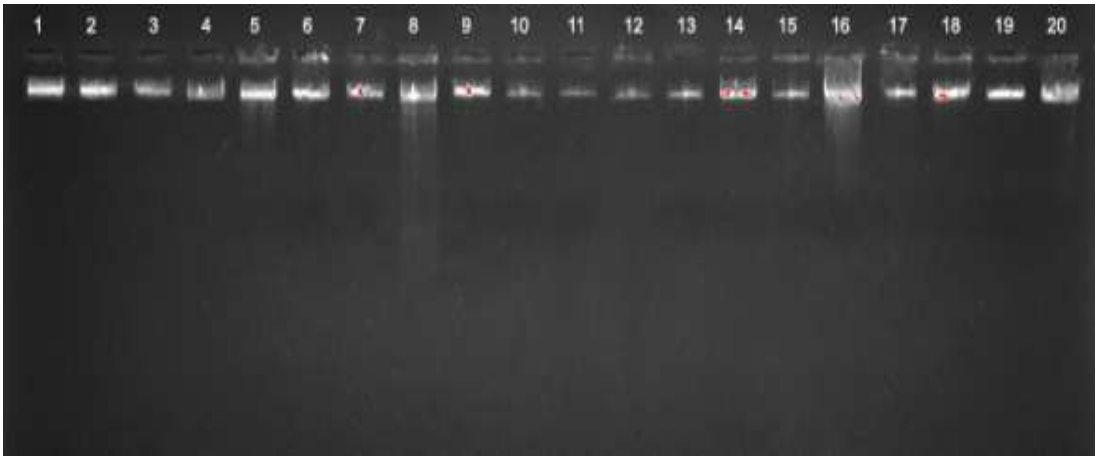

Fig. 4: The Electrophoresis Pattern of Human Genomic DNA Using 0.7% Agarose, TBE (1x), Ethidium Bromide Stain

The study of caspase-1 in terms of inflammatory and stimulate the immune system, according to the present research is the first studied in this field deal with the relationship between caspase-1 and p. aeruginosa infection.

The result for the caspase-1amplification in figure 4.

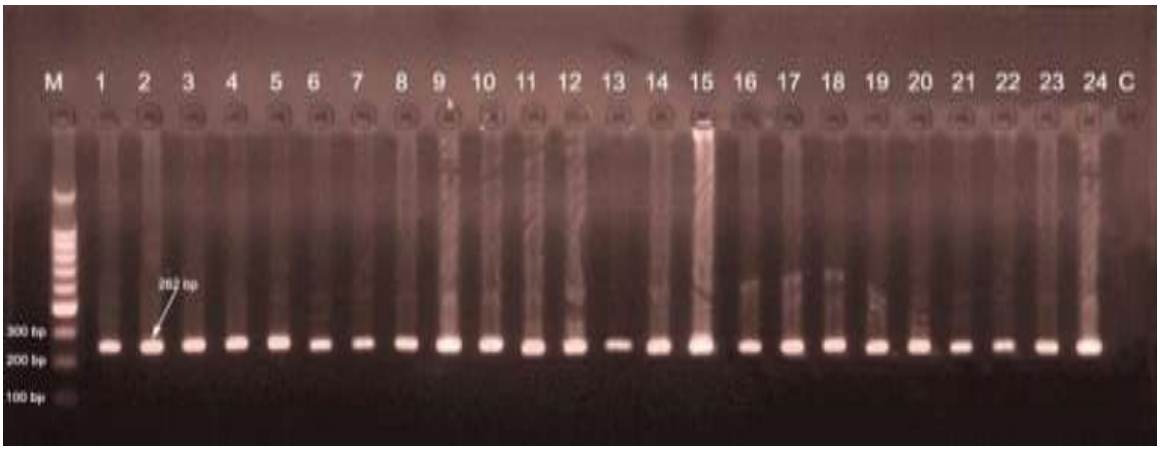

Fig. 5: Agarose Gel Electrophoresis of PCR Product Amplified from casp-1 Gene, the DNA Fragments of 262 bp were Amplified from casp-1 Gene Using Ladder; Lane M (100bp), Lane C is Control Negative. The figure shows all Samples Gave Positive Result (lanes from 1 to 24)

Casp-1 Gene Sequencing

Twenty samples of amplified PCR-products (forward and reverse strand) for casp-1 gene from patients were further analyzed by direct sequencing for detecting SNPs within these sequences. Present sequences were compared with reference sequence of casp-1 in national center biotechnology information (NCBI) Gene Bank. Primer set covers exon 2 region (5658 – 5919bp). All SNPs are located in STS "sequence tagged site" of exon2.

The genetic variance were found in 70% of patients samples that had a heterozygous nucleotide.

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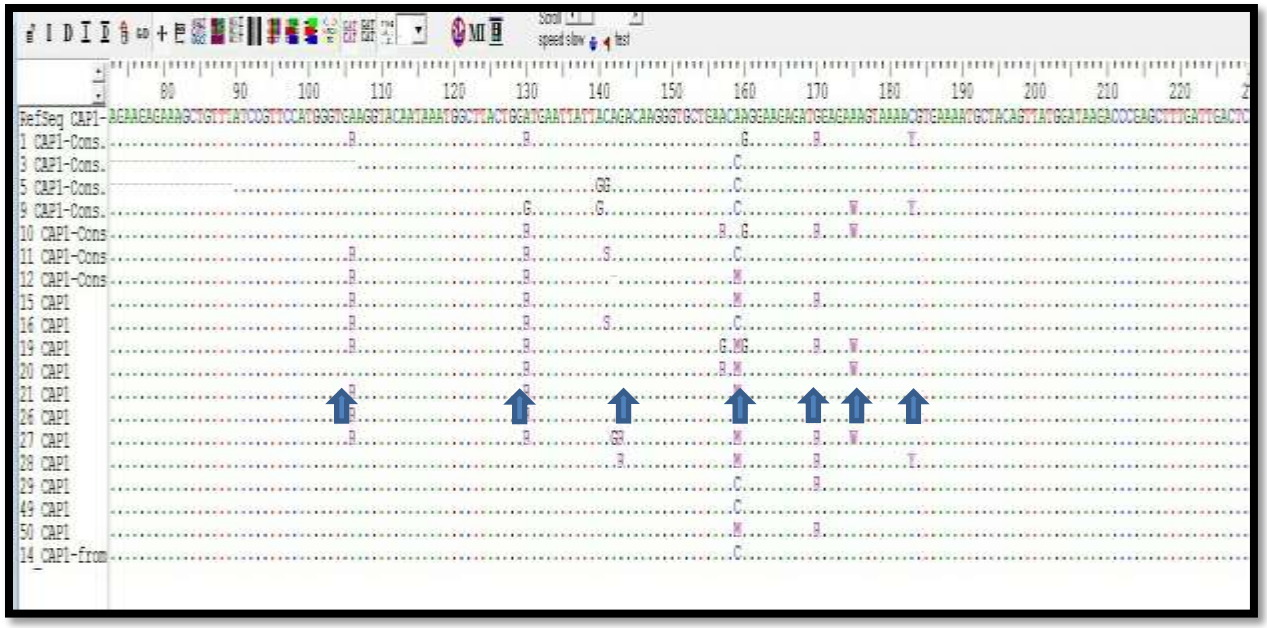

Fig. 6: Sequencing of casp-1 Gene with Heterozygous R, M, W, SAND Y Compared with Wild Type Casp-1obtained From Gene Bank

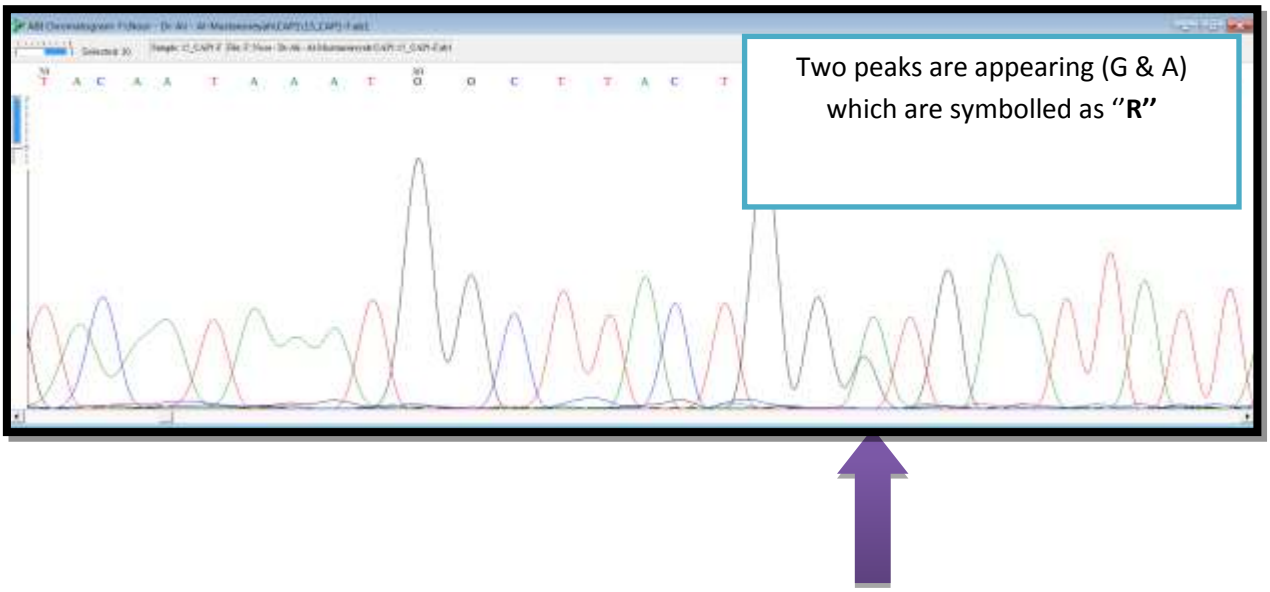

Fig. 7: Histogram of Sequencing of Casp-1 Gene with Heterozygous

SNPs are the most common type of human genetic variation, (Jinet al., 2015). In addition, SNPs that exhibit a high genetic stability may directly affect in protein expression or structure and therefore underlie numerous genetic disorders (Chen et al., 2013). The variation in the immune response is under genetic control and it is associated with an inflammatory pathway, especially influence the outcome of diseases (Noreen and Arshad, 2015). Several single nucleotide polymorphisms (SNPs) of casp-1 can affect genetic susceptibility to infections or even sepsis (Schnetzke et al., 2015).

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So that the genetic variations such as single nucleotide polymorphisms (SNPs) greatly influence innate immune responses towards pathogenic challenges and disease outcome; therefore, a range of susceptibility to infections appears among people, with some of them being predisposed to certain infections while others are being protected (Hill, 2001, Skevakiet al., 2015).

Level of IL-1β in Patients Serum with Different Age Group and Different Infection Sources

According to their ages, the patients has been divided into 8 groups (3month- 3year (group A), 4-9 year (groups B), 10-19 year (groups C), 20-29 year groups D), 30- 39year (group E), 40-49 year (groups F), 50-59 year (groups G), 60-69 year groups H).

IL-1β concentration was significantly increase P-value < 0.005, P-value < 0.000 in some of age groups compared with their controls, the higher level of IL-1β has been shown in age group H (± S.D was 8.7± 1.8 pg/ml) (P=0.00002), as shown in table (4)

Table 4: Statistical Analysis of Conversion the Level of IL-1β between Patient and Kit Standard Control Group

Group of ages	N	Mean	Std. Dev.	P value
Kit standard Con.	7	70.6	89.6	0.06
A. 3m-3yrCon.	8	6.4	1.6	
B.4-9yrCon.	5	11.4	7	0.17
C.10-19 yrCon.	14	45.4	42.6	0.38
D.20-29 yrCon.	5	134.4	62.2	0.20
E.30-39 yrCon.	4	111.7	72.5	0.45
F.40-49 yrCon.	7	31.2	26.7	0.009
G.50-59 yrCon.	3	11.9	1.5	0.0005
H.60-69 yrCon.	4	8.7	1.8	0.00002

P<0.05

According to source of infection, the patients has been divided into 5 groups (Otitis, UTI, Cystic fibrosis, wound and Burn).

IL-1β concentration was significantly increase P-value < 0.005in some of sources groups compared with each other, the higher level of IL-1β has been shown in cystic fibrosis with wound and UTI (± S.D was 10.9± 7.3 pg/ml), (± S.D was 33.9± 12 pg/ml) and (± S.D was 26.5± 29 pg/ml) (P=0.00002), as shown in table (5)

Table 5: Statistical Analysis of Conversion the Level of IL-1β between CYSTIC Fibrosis Infection Source and Other Sources Group

	N	Mean	Std. Dev.	P value
Cystic fibrosis	10	10.9	7.3	0.01
Burn	19	74.8	73.6	
Otitis	3	33	37.6	0.003
UTI	12	26.5	29	0.002
wound	6	33.9	12	0.002

P<0.05

The results of present research showed a high genetic variability in the caspase-1 gene, where 70% of samples had heterozygous gene-altering sequences in the nucleotide sequences (Qinget al., 2009). By measuring the level of

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IL-1β in the patients' serum it be found a correlation between the heterogeneity of samples and the high level of interleukin and this confirms that the caspase-1 is responsible for maturation the secretion of large amounts of IL-1β during bacterial infections (Gross et al., 2011), especially the incidence of P.aerginosa which is the focus of our study.

In humans, early markers of inflammation include IL-1β, IL-6, IL-8, IL-10, and TNF-α. IL-1β and IL-8 typically display the highest levels at the time of patient admission to the hospital (Vindenes, 1998). IL-1β seems to be a key component of the inflammatory mediator cascade, regulating the host response to infection, injury, and inflammation (Kaplan, 1989, Sheu et al., 2007, Castejon and Brough, et al,. 2011).

IV. CONCLUSIONS

The results showed a different ability to resist antibiotics from isolates. Amoxicillin- clavulanic acid, Trimethoprim- sulfamethoxazole and Erythromycin were the effectiveness antibiotic against P.aeruginosa 100%. The present research showed different ability to form biofilms by using MTP Method, 46(92%) was former (strong and moderate producer).

The results of Genotypic detection for virulence gene (Flic gene) showed that the 45(90%) were positive then the isolates.so, we concluded that the isolates that have the virulence gene are very effective at the high level of interleukin-1 beta in infected patients. Primer design for human caspase-1 gene enzyme %100 samples was positive. Sequencing for 20 PCR product of casp-1 gene to detect variations of Iraqi population 14 of it has a heterozygous nucleotides. Detect the level of serum interleukin -1β with different age group of patients and made a comparison between different infection sources the highest significant (P= 0.00002) is in 60-69 years age group compared with kit IL-1β level and the highest significant (P=0.002) recorded when compared cystic fibrosis with wound and UTI source.

When the study was completed and the results were collected, the results were interrelated as the bacterial isolates that were strongly resistant to antibiotics had high virulence factors, such as biofilm formation. The isolates possessed the flic gene responsible for activating the caspase-1 which in turn synthesized the IL-1β at high level in patients and all these results confirmed by the result of the sequencing, where the samples conducted by the test of the sequence were highly heterogeneous genetic sites of certain gene and this result was compared with the reference sequence of caspase-1approved by NCBI.

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REFERENCES

[1] Brooks, G. F.; Carroll, K. C.; Butel, J. S.; Morse, S. A. (2007). Jawetz, Melnick and Adedberg's Medical Microbiology. (24thed).The *McGraw-Hill. USA*, pp.818.

[2] Capuzzo, C.; Firrao, G.; Mazzon, L.; Squartini, A. and Girolami, V. (2005). „Candidatus Erwinia dacicola", a coevolved symbiotic bacterium of the olive fly Bactrocera oleae (Gmelin). *International Journal of Systematic and Evolutionary Microbiology.* 55: 1641–1647.

DOI: 10.37200/IJPR/V24I5/PR2020222
Received: 14 Mar 2020 | Revised: 30 Mar 2020 | Accepted: 06 Apr 2020

[3] Haiko J, Westerlund-Wikstrom B. The role of the bacterial flagellum in adhesion and virulence. *Biology* 2013;13:1242-67.

[4] Xu J, J. E.; Moore, P. G.; Murphy, B. C.; Millar, and Elborn, J. S. (2004). Early detection of Pseudomonas aeruginosa comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis (CF). *Ann Clin Microbiol Antimicrob.*, 3(21).

[5] McIsaac SM, Stadnyk AW, Lin TJ. Toll-like receptors in the host defense against Pseudomonas aeruginosa respiratory infection and cystic fibrosis. *Journal of leukocyte biology* 2012;92:977-85.

[6] Cheesbrough, M. (1991).Medical Laboratory Manual for Tropical Countries. 2nd ed., ELSB, Cambridge., 11:PP. 508-511.

[7] Ramos, G. ; Rocha, J. and Tuon, F. (2013). Seasonal humidity may influence *Pseudomonas aeruginosa* hospital acquired infection rates. *International J. Infectious Disease*. 17: 757-761.

[8] Drancourt, M.; Bollet, C.; Carlioz, A.; Martelin, R.; Gayral, J. P. and Raoult, D. (2000). 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J. Clini. Microbiol*, 38: 3623-3630.

[9] Faezi, S.; Bahrmand, A.R.; Mahdavi, M. ; Siadat, S. D.; Nikokar, I.; Sardari, S. and Holder, I. N. (2016). High Yield Overexpression, Refolding, Purification and Characterization of *Pseudomonas aeruginosa* Type B-Flagellin: An Improved Method without Sonication. *Int J Mol Cell Med,5:1-12.*

[10] Neha, S.; Shriya, D.; Sanjay, C. and Kusum, H. (2014). Molecular detection of virulence genes as markers in Pseudomonas aeruginosa isolated from urinary tract infections. *Int J Mol Epidemiol Genet*, 5(3): 125–134.

[11] Jin, S.B.; Zhang, X.F.; Lu, J.G.; Fu, H.T.; Jia, Z.Y. and Sun, X.W. (2015). Genetic analysis of QTL for eye cross and eye diameter in common carp (Cyprinus carpio L.) using microsatellites and SNPs. *Genet Mol Res*, 14:3557-3569.

[12] Chen, H.Y.; Huang, W.; Leung, V.H.; Fung, S.L.; Ma, S.L.; Jiang, H. and Tang, N.L.(2013). Functional Interaction between SNPs and Microsatellite in the Transcriptional Regulation of Insulin-Like Growth Factor 1. *Human mutation*, 34(9):1289-1297.

[13] Skevaki, C.; Pararas, M.; Kostelidou, K.; Tsakris, A. and Routsias, J.G. (2015). Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious diseases. *Clinical and Experimental Immunology*, 180(2):165-177.

[14] Noreen, M., and Arshad, M. (2015). Association of TLR1, TLR2, TLR4, TLR6, and TIRAP polymorphisms with disease susceptibility. *Immunologic research*, 62(2):234-252.

[15] Hill, A.V. (2001). The genomics and genetics of human infectious disease susceptibility. *Annual review of genomics and human genetics*, 2(1):373-400.

[16] Qing, L.; Lindsay, M. M.;[1] Bruce, A.; Patricia, H.; Idan, M.; Tongzhang, Z.; Mark, P. P.; James, R. C..; Yawei, Z.; Andrew, G.;Wendy, C.; Meredith, Y.; Theodore, R. H.; Claire, M. V.; Scott, D.; Brian, L.; Anne, K.; Maryjean, S.; Shelia, H. Z.; Nilanjan, C.; Stephen, J. C.; Nathaniel, R. and Sophia, S. W. (2009).Genetic variation in caspase genes and risk of non-Hodgkin lymphoma: a pooled analysis of 3 population-based case-control studies. *Blood*, 114(2): 264–267.

[17] Sheu, JN.; Chen, MC.; Cheng, SL.; Lee, IC.; Chen, SM. And Tsay, GJ. (2007). Urine interleukin-1beta in children with acute pyelonephritis and renal scarring. *Nephrology (Carlton)*, 12(5): 487-93.

[18] Kaplan, E.; Dinarello, CA. and Gelfand, JA. (1989). Interleukin-1 and the response to injury. *Immunol Res*, 8(2):118-129.

[19] Gross, O., C. J. Thomas, G. Guarda, and J. Tschopp. 2011. The inflammasome: an integrated view. *Immunol. Rev*, 243: 136–151.

[20] Vindenes, H.A.; Ulvestad, E. and Bjerknes, R. (1998). Concentrations of cytokines in plasma ofpatients with large burns: their relation to time after injury, burn size, inflammatoryvariables, infection, and outcome. *Eur J Surg*, 164(9):647-656.

[21] Schnetzke, U.; Spies-Weisshart, B.; Yomade, O.; Fischer, M.; Rachow, T.; Schrenk, K.; Glaser, A.V.; von Lilienfeld-Toal, M.; Hochhaus, A. and Scholl, S. (2015). Polymorphisms of Toll-like receptors (TLR2 and TLR4) are associated with the risk of infectious complications in acute myeloid leukemia. *Genes and immunity*, 16(1): 83.

[22] Lena, F.; Ali, H.; Al-Marzoqi.; Zahraa, M.; Al Taee. And Ammar, and A. Shalan. (2016). Molecular and Phenotypic Study of Virulence Genes in a Pathogenic Strain of *Pseudomonas aeruginosa* isolated from various clinical origins by PCR: Profiles of genes and Toxins. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 7(1):590-598.

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[23] Allison, J. S.; Dawson, M.; Drake, D. And Montie. T. C. (1985). Electrophoretic separation and molecular weight characterization of *Pseudomonas aeruginosa* H-antigen flagellins. *Infect. Immun*, 49:770–774.

[24] Vallet, I. ; Diggle, S.P. ; Stacey, R.E. ; Camara, M. ;Ventre, I. ; Lory, S. ; Lazdunski, A. ; Williams, P. and Filloux, A. (2004). Biofilm formation in *Pseudomonas aeruginosa:* fimbrial *cup* gene clusters are controlled by the transcriptional regulator. *J. Bacteriology*, 186(9): 2880-2890.

[25] Bacalso, M. ; Xu, T. ; Yeung, K. and Zheng, D. (2011). Biofilm formation of *Pseudomonas aeruginosa* PA14 required *lasI* and was stimulated by the *Pseudomonas* quinolone signal although salicylic acid inhibition is independent of the *pqs* pathway. *JEMI*, 15: 84-89.

[26] Kaur, D.C. and Wankhede, S.V. (2013). A study of biofilm formation and metallo beta-lactamases in *Pseudomonas aeruginosa* in a tertiary care rural hospital. *Intern. J. Sci. Res. Pub*., 3(10): 1-10.

[27] Kodaka, H.; Iwata, M.; Yumoto, S. and Kashitani, F. (2003). Evaluation of a new agar medium containing cetrimide, kanamycin and nalidixic acid for isolation and enhancement of pigment production of *Pseudomonas aeruginosa* in clinical samples. *Journal of basic microbiology*, 43(5):407-413.

[28] Bose, S.; Khodke, M.; Basak, S. and Mallick, S.K. (2009). Detection of Biofilm Producing Staphylococci: Need of The Hour. *J. Clin. Diag. Res*, 3: 1915-1920.

[29] Ferraro, M. J.; Craig, W. A.; Dudley, M. N.; Eliopoulos, G. M.; Hecht, D. W.; Hindler, J.; (2000). Performance standards for antimicrobial disk susceptibility tests. Approved Standard M2-A7. *Wayne, Pa: National Committee for Clinical. Lab.* Standards. 7

[30] MacFaddin, J. F. (2000). Biochemical Tests for Identification of Medical Bacteria. 2nd ed., Waverly Press, *Inc., Baltimor, USA.* PP. 64-67.

[31] Porteous, L. A.; Widmer, F. and seidles, R. T. (2002). Multiple enzyme restriction fragment length polymorphism analysis for high resolution distinction Pseudomonas 16S r RNA genes. *J. Microbiol. Meth.,* 51:733-843

[32] Thornberry, N.A., Bull, H.G., Calaycay, J.R., Chapman, K.T., Howard, A.D., Kostura, M.J., *et al*. (1992) A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature* 356: 768–774.

[33] Martinon, F., Mayor, A., and Tschopp, J. (2009) The inflammasomes: guardians of the body. *Annu Rev Immunol* 27: 229–265.

[34] Martinon, F., and J. Tschopp.2004. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. Cell 117: 561–574

[35] Fantuzzi, G., and C. A. Dinarello. 1999. Interleukin-18 and interleukin-1 beta: two cytokine substrates for ICE (caspase-1). *J. Clin. Immunol.* 19:1–11.).

[36] Miao, EA.; Andersen-Nissen, E.; Warren, SE. and Aderem, A. (2007). TLR5 and Ipaf: dual sensors of bacterial flagellin in the innate immune system. *Semin Immunopathol*, 29: 275-288.

[37] Todar, K. (2008). "Pseudomonas aeruginosa." *Online Text Book for Bacteriology.* 2008.

[38] Minion, S. A. (2010). Investigation into the introduction of clonal strains of Pseudomonas aeruginosa to the cystic fibrosis population by consumption of raw salad vegetables. *Thesis, Queensland university of technology, Brisbane, Australia.*

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