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MOLECULAR SEARCH OF SOME ANTIBIOTIC-BORNE GENES OF THE CLINICAL PSEUDOMONAS AERUGINOSA PLASMID

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Abstract
Clinical samples which included wounds, burns, ear infections, urinary tract infections (UTI), and skin from 300 patient from a different hospital in Thi-Qar province, and 100 skin and stool samples from (healthy people) as control, for investigating the presence of *pseudomonas spp*. Where carried out through October 2019 till January 2020 .The samples were identified based on the morphological , microscopical characteristics of the colonies and biochemical tests, then confirmed by API-20 NE, VITEK2- compact system and the molecular detection based on the 16SrRNA gene, final diagnosis showed that 60 isolates belong to target bacteria were distributed as 30(50%) of burns, 5(8.3) isolates of wounds, 7(11.6) isolates of ear infection, 4(6.6) isolates (sputum),4(6.6)isolates of urinarytract infection, and (8 (13.3),2(3.3)) isolates stool samples and skin swabs respectively, as control samples). All isolates were tested for susceptibility test toward 13 antibiotics using the disc diffusion method, the results showed a high resistance among isolates against Tetracycline, Gentamycin, Amikacine. moderate resistance against Cefepime, Ceftazidime, and Imipenem. And low resistance towards Meropenem, and Ciprofloxacin. The results of antibiotic-resistance genes was diagnostic by Polymerase chain reaction (PCR) amplify based on specific sequences for (MBLs genes)bla VIM ,bla IMP, and bla NDM genes, showed that 26 (43.3%) of isolates have bla VIM gene, 16 (26.6%) of isolates have bla IMP gene, and 11 (18.3%)of isolates have bla NDM gene. while the results of (AME-genes) indicated that 30 (50%) of isolate have aac (6)-I gene and 8(13.3%) of isolate have aph (3)-VI gene. A comparison is made between the presence of genes on the plasmid or the chromosome,the results was increased the antibiotic- resistance genes on the plasmid. DNA partial sequencing was done for four isolates revealed some variants in different samples while some isolates showed 100% similarity with the referencing genome.

Keywords: clinical samples; healthy people; antibiotics

1. INTRODUCTION

Pseudomonas is a gram-negative, non-fermenting, rod-shaped bacterium, and aerobic bacterium. That can cause several types of infection including wound, urinary tract, and respiratory tract infections. As one of the major pathogenic bacteria in hospital acquired disease, it is an opportunistic pathogen causing critically significant diseases in people who are immunocompromised, such as cancer and AIDS. It also involved ear infections, mucosal fibrosis, eye infections as well as becoming the third and fifth common cause of hospital-acquired UTIs and others (Wang *et al.,*2019) Incidences of *Pseudomonas aeruginosa* infections are on the rise worldwide due to its mechanisms of survival, adaptation, and resistance to different types of antimicrobials *P. aeruginosa* was the sixth most common cause of hospital-acquired infections at 7.3% of all cases. (Moradali et al., 2017). Burn patients are at risk for acquiring infection with *Pseudomonas* strains (khan *et al*.,2015). Plasmids have been shown to confer advantageous traits upon *Pseudomonas* clinical isolates. It is an important factor powerful bacterial evolution. Plasmid-encoded functions such as virulence, resistance, metabolism and/or other beneficial functions can support bacterial suitability. (Schulte *et al*.,2019). Antimicrobial resistance is widespread and has expanded among *Pseudomonas* over the years, as a variety of strains are now resistant to almost all widely used antibiotics. Multidrug tolerance amongst these species makes it difficult and costly to manage infections induced by it, such bacteria are known to produce widespread (ESBLs) and (MBLs). The fact that *P. aeruginosa* is both intrinsically resistant and can acquire resistance to a number of antibiotics during therapy limits the available therapeutic options. Therefore, knowledge of the local resistance patterns is essential in order to establish the appropriate treatment strategies (Memish *et al.,2012)*. MBL produce *Pseudomonas* have become a growing therapeutic concern worldwide. The rapid detection of MBL positive isolates is essential to control infection and to stop their distribution (Hodiwala *et al.,2013)* . *Pseudomonas* has also developed a series of ways around antibiotics besides the exo polysaccharide alginate

like drug efflux pumps, modified surface receptors, and beta-lactamases. These enhancements have been either acquiring foreign plasmid DNA or mutations that enhance resistance (Moradali *et al*.,2017). Acquired resistance a horizontal or vertical acquisition of antibiotic resistance is likely. Vertically (mutationally) acquired resistance genes can slowly emerge, while horizontal acquisition is rapid and may occur by various means, including conjugation, transduction, or translation. Mobile DNA components include plasmids, transposons, integrons, prophets and islands of resistance (Breidenstein *et al.,* 2011). Pseudomonas spp. also acquires plasmids encoding aminoglycoside-modifying enzymes (AMEs) and (MBLs), AMEs can be attached to a phosphate, acetyl or adenyl reactive group to the antibiotic molecule (Krause et al.,2016).

2.MATERIALS AND METHODS

A-Collection of bacterial isolates

Clinical isolates of P.auroginosa (60 isolates)were obtained from patients suffering various infections from different teaching hospital in Thi-Qar \Iraq. between August 2019,and January 2020.These samples were distributed as [30 (50%)] isolates from burn infection, , [4 (6.6)] urinary tract infections, [4(6.6)] isolates from wounds infections, [7(11.6)] isolates from otitis media, [8 (13.8)] isolates from stool, [5(8.3)] isolates Sputum from patients suffering from respiratory tract infection ,and [2(3.3)] isolate from skin.

B. Culture Media

Ready culture media include Muller-Hinton agar, Brain heart infusion agar & broth, MacConkey agar with crystal violat, Nacl, and Bile salts, Peptone Water, Cetrimide Agar, Nutrient agar and broth, Methyl red-Voges Proskauer were prepared according to the manufacturer companies instructions, which are fixed on the container. Bacterial diagnosis was performed API-20 NE System and Vitek-2 Compact, and then the isolates were stored at freezing in brain heart infusion broth media and adding 15% of glycerol followed by the genotyping detection using PCR technique. (Boyd *et*

al.,2019)

C.Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines15 for three aminoglycosides [gentamicin (10 mg), amikacin (10 mg), and tobramycin (10 mg)] and for nine other antibiotics [imipenem (10 mg), Cefepime (30mg) , Aztreonam (30mg), Ofloxacin(5mg), ,piperacillin (100 mg), Levofloxacin(5mg), Meropenem(10mg), ceftazidime (30 mg), Tobramycin(10mg), and ciprofloxacin (10 mg)]. All drugs were obtained from Mast laboratories (Merseyside, United Kingdom).

D.Polymerase chain reaction amplification

Polymerase chain reaction (PCR) was used to screen all 60 isolates for the presence of the modifying enzyme genes, aac(6)-I, aph(3)-VI, bla IMP,blaVIM, blaNDM,and l6SrRNA gene was used for bacterial diagnosis .The total template DNA for the PCR amplification was extracted from the supernatant of a mixture of P. aeruginosa isolates produced by plasmid Extraction Kit (Column-Pure plasmid mini-prep kit),and DNA Extraction (Genomic DNA Mini Kit) which provided by (FAVORGEN, Taiwan).

E.Characterization of strains harboring MBL and AMG genes

sixty clinical P. aeruginosa MBL, and AMG-resistant isolates were obtained from patients, with various clinical infections (Burn,sputum,stool,ENT Swabs,skin,urinary infection, and wound infection).

DNA extraction was performed using a PureYield™ Plasmid Miniprep System (Promega, Brazil). Amplification of the MBL and AMG markers (blaIMP, blaVIM, bla NDM, aac(6')-I, aph(3)-IV) were performed using primers .The reaction mixture (25 µL) contained 1.0 µL DNA template (10 ng), 12.5 µL GoTaq1 Green Master Mix (Promega) and 0.5 µLof each primer. Amplifications were performed in Mastercycler Personal (Eppendorf) using the following program: initial denaturation at 95°C for 2 min followed by 30 cycles of 30 seconds at 95°C, 1 min at annealing temperature (52°C for blaIMP, blaVIM,and blaNDM; 51°C for aph(3)-IV ; 55°C for aac(6)-I, 1 min at 72°C and a final extension steap of 5 min at 72°C. Multiplex PCR was performed for genotypic character ization of different MBL (blaIMP, blaVIM,and blaNDM) and AMG (aac(6)-I ,and aph(3)-IV) genes .The amplified PCR products were visualized by electrophoresis in 1.5% agarose gel by the photo documentation System ,table (3-7)

F.Pulsed-Field Gel Electrophoresis (PFGE)

Isolates were typed according to the protocols described by Galetti [40] with modifications, DNA fragments were separated on 1% (w/v) agarose gels in 0.5x TBE [Tris–borate–ethylene diamine tetra-acetic acid (EDTA)] buffer using a CHEF DRIII apparatus (Bio-Rad, USA) with 6 V/cm, pulsed from 5 s to 40 s, for 21 h at 12°C. Gels were stained with safe red and photographed under ultraviolet light. Computer-assisted analysis was performed using BioNumerics 5.01 software (Applied Maths, Belgium). Comparison of the banding patterns was accomplished by the unweighted pair- groupmethod with arithmetic averages (UPGMA) using the Dice similarity coefficient.

G.Statistical analysis

The results in the present study were evaluated statistically via Chi-square using Statistical Package for Social Sciences (SPSS) program version 23 at a probability of ($P < 0.05$) as a significant level between the parameters of the present study like gender, age, type of infection, residency, occupation, and some of virulence factors.

Chi-Square

$$X^2 = \sum_{\lambda=1}^{k} \frac{(O_i - E_i)^2}{E_i}$$

Degree of freedom df = (number of Colum – 1) * (number of rows – 1)

Table (3-7): Sequence of PCR primers and the molecular size of PCR product.

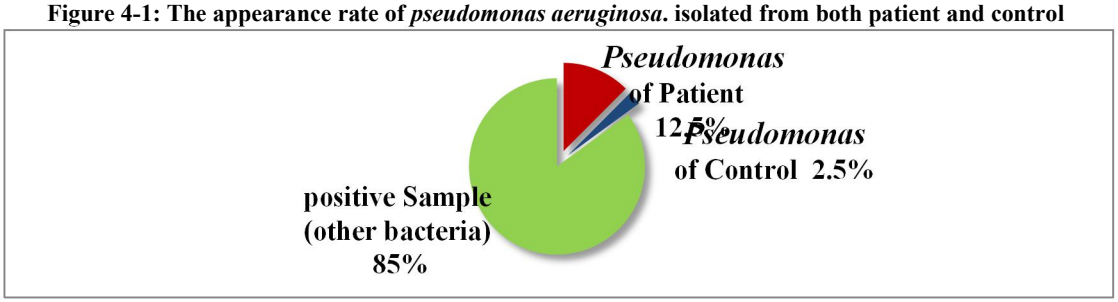
No.	Primer*	Product size	Primer Sequence (5'-3')		Reference
1	bla-IMP	232bp	F	GGAATAGAGTGGCTTAAYTCTC	Fakhri et al., 2017) Dogonchi et al., 2018
			R	GGTTTAAYAAAACAACCACC	
2	bla-VIM	390bp	F	GATGGTGTTTGGTCGCATA	
			R	CGAATGCGCAGCACCAG	
3	bla-NDM-1	621bp	F	GGTTTGGCGATCTGGTTTTC	
			R	CGGAATGGCTCATCACGATC	
4	16S rRNA	956bp	R	GGGGGATCTTCGGACCTCA	Spilker et al.,2004
			F	TCCTTAGAGTGCCCACCCG	
5	aac (6')-I	400bp	R	5-GACATAAGCCTGTTCGGTT-	Akers et al,201

			F	3` 5-TCCGAACTCACGACCGA -	
6	aph (3')-vi	716bp	R	` 5-TTCCTTTTGTCAGGTC-3	Akers et al,2010
			F	5-CGGAAACAGCGTTTTAGA-3	

3. RESULTS

A total of 400 clinical samples from 300 patients and 100 (healthy people) ,as control, only 60 (12.5 % clinical source and 2.5% as control) isolates were belonged to Ps. aeruginosa, while other 340(85) positive to other bacteria and no growth. The most isolates were obtained from burns 30 (60%), wound 4 (8%), urin 4(8%), ear swab 7(14%),and sputum 5(10%),while control 8,2(80%,20%),stool and skin respectively. The primary cultural diagnosis by selective media as citramid agar to differential P.A , was appear circular mucoid smooth colonies with emits sweat grape odor , and then cultured on blood agar . Most isolates appear β-hemolysis on blood agar while others isolates were non hemolysis. All isolates grew on MacConkey agar, but did not ferment lactose sugar. All the isolate grew on the Muller-Hinton agar which produce the diagnostic pigment. The pigment varied from yellowish-green to bluish- green and also the isolates produced a sweat grape-like odor,figure 4-1

Figure 4-1: The appearance rate of *pseudomonas aeruginosa*. isolated from both patient and control



Antibiotic susceptibility of P. aeruginosa

All *isolates* were examined for antibiotic susceptibility tests against 13 antibiotics. The results were a difference in resistance degrees of the confirmed bacteria. The maximum ratio of resistance of *P. aeruginosa* isolates were for Ticarcillin-Clavulanic acid 83.3%, Amikacin 75%, and Aztreonam 73.3%, However, Meropenme was the highest sensitive antibiotic 63.3% followed by Cefepime 56.7%, and Levofloxacin 46.7%. Finally, Gentamicin had intermediate activity 28.3%, followed Levofloxacin with 25.0%, and Imipenem 20.0% .Also, the current results indicated that 46% resistant for four antibiotic Imipenem, Ticarcillin-Clavulanic acid, Gentamycin, Amikacin, and Ceftazidime as Multi-Drug Resistance(**MDR**).The susceptibility test of *P.aeruginosa* isolates was summarized in table 4-6.

Table 4-6: Antibiotics susceptibility for isolated *P. aeruginosa* isolation from clinical source of patients with burns, wound, urin, sputum and stool.

Activity Antibiotics	**Sensitive**		**Intermediate**		**Resistance**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Amikacin AMK	8.0	13.3	7.0	11.7	45.0	75.0
Aztreonam ATM	12.0	20.0	4.0	6.7	44.0	73.3
Cefepime CIP	34.0	56.7	5.0	8.3	11.0	35.0
Ceftazidime CAZ	24.0	40.0	7.0	11.7	29.0	48.3
Ticarcillin-Clavulanic acid TTC	8.0	13.3	2.0	3.3	50.0	83.3
Gentamycin GEN	10.0	16.7	17.0	28.3	33.0	55.0
Imipenem IPM	24.0	40.0	12.0	20.0	24.0	40.0
Levofloxacin LEV	28.0	46.7	15.0	25.0	17.0	28.3
Meropenem MEM	38.0	63.3	8.0	13.3	16.0	26.7
Ofloxacin OFX	24.0	40.0	9.0	15.0	27.0	45.0
Ciprofloxacin CPF	36.0	60.0	10.0	16.7	14.0	23.3
Piperacillin PI	18.0	30.0	10.0	16.7	32.0	53.3
Tobramycin	10	16.7	16	26.7	34	56.7
CalX^2= 248.207	**TabX^2= 36.42**		**Df = 24**		**P. Value= < 0.0001**	

Molecular Diagnosis of *P. aeruginosa*

Conventional PCR was used for the final identification of 60 isolates using 16S rRNA gene. The electrophoresis results showed DNA bands of approximately 956 bp in all *P. aeruginosa* isolates, Table 4-7 figures (4-5)

Table 4-7: appearance rate of 16SrRNA gene in both clinical source and control

source	**Isolate**	**Result** (16SrRNA gene)	**%**
Clinical	**50**	**positive**	**100%**
Control	**10**	**positive**	**100%**

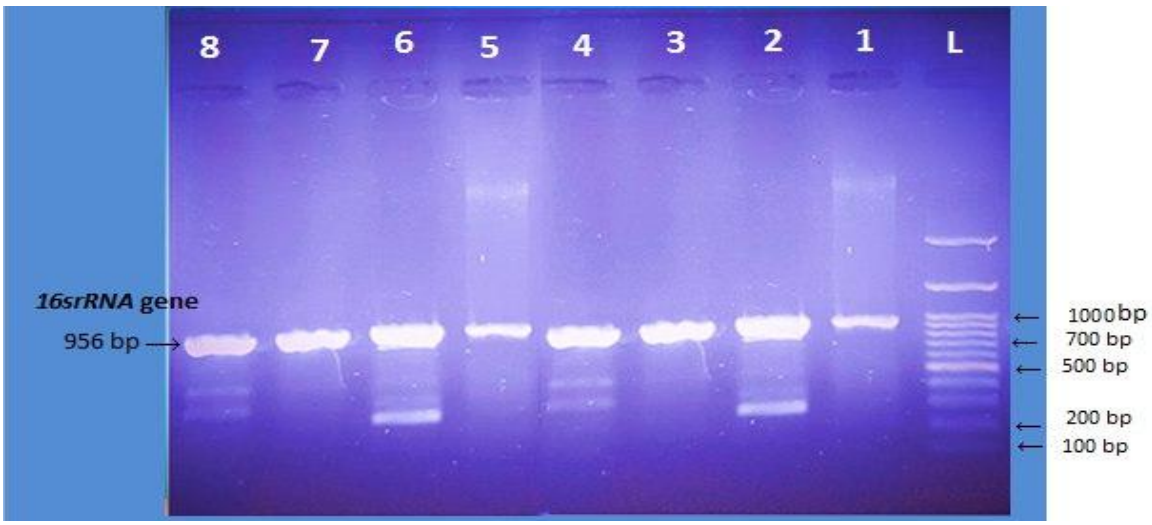
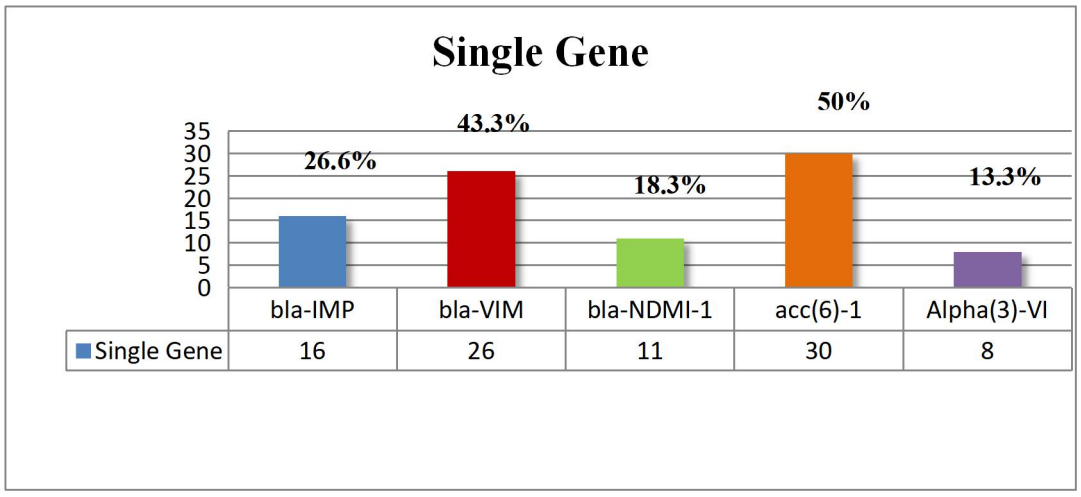


Figure 4-5: Agarose gel electrophoresis of *16S rRNA* gene (956 bp) of PCR Product (L: DNA Ladder 100-10000 bp, Agarose: 1%, Volt: 80v, Lanes 1-8 represent bands of *pseudomonas* isolates

Molecular Diagnosis of antibiotic Resistance genes

The phylogenetic group was determined by using a triplex PCR method for selected the phylogenetic groups of *Pseudomonas* strains according to the PCR identification gene, *bla*-VIM, *bla*-NDMI-1, *aac*(6)-1, and *aph*(3)-VI gene. Based on the presence or absence gene marker. Study results showed 30 (50%) of *p. aeruginosa* isolates presen twith *aac*(6)-1 gene followed by 26 (43.3%) with *bla*-VIM gene , 16 (26.6%) of isolates harbored *bla*-IMP gene, , 11 (18.3%) contained *bla*-NDM-1, and the lowest rate 8 (13.3%) occurred with *aph* (3)-VI , figure 4-6

Figure 4-6: frequencies of genes in isolated *P. aeruginosa*

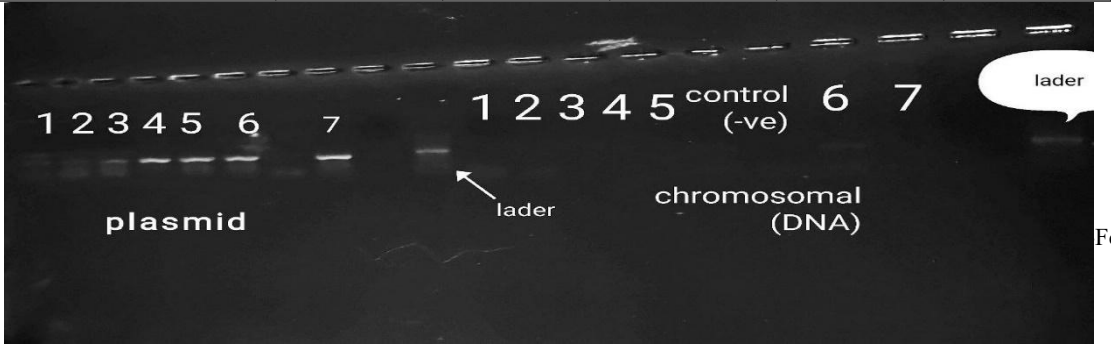


Genes Frequencies Isolated from Chromosome and Plasmid.

DNA for the PCR amplification was extracted from the supernatant of a mixture of P. aeruginosa isolates produced by plasmid Extraction Kit (Column-Pure plasmid mini-prep kit), and DNA Extraction (Genomic DNA Mini Kit) which provided by (FAVORGEN, Taiwan). The current results indicated that the most virulence genes isolated from *P. aeruginosa* were located on the plasmid, the most of them was aac(6)-1 with a percentage 32.6 %, while the lowest rate was 13% aph (3)-VI. The most isolates genes on chromosome were involved (8) aac(6)-1 with a percentage 28.6 %. While the lowest percentage 0.0% included gene. The results also showed there was a significant statistical difference in gene frequencies between plasmid and chromosome at P. value < 0.05. Table (4-14), figure (4-15)

Table 4.14: Gene frequencies isolated from chromosome and plasmid

Gene Frequencies	Chromosome		Plasmid		Total	
	No.	%	No.	%	No.	%



***bla*-IMP**	6.0	28.6	16.0	17.6	22.0	19.6
***bla*-VIM**	5.0	23.8	26.0	28.6	31.0	27.7
***bla*-NDM-1**	4.0	19.0	11.0	12.1	15.0	13.4
***aac* (6)-1**	6.0	28.6	30.0	33.0	36.0	32.1
***aph* (3)-VI**	0.0	0.0	8.0	8.8	8.0	7.1
Total	21.0	100.0	91.0	100.0	112	100.
CalX²= 13. 885	**TabX²=9.49**		**Df = 4**	**P. Value= 0.008**		

Figure 4.15: Agarose gel electrophoresis of *aph*(3)-IV gene (716 bp) of PCR Product Gene isolated from chromosome and plasmid, Agarose: 1%, Volt: 80v, Lanes 1-7 (Plasmid band +ve) ,lader , 1-7 lanes (chromosomal DNA –Ve) represent bands of pseudomonas isolates

comparison rate of resistance genes

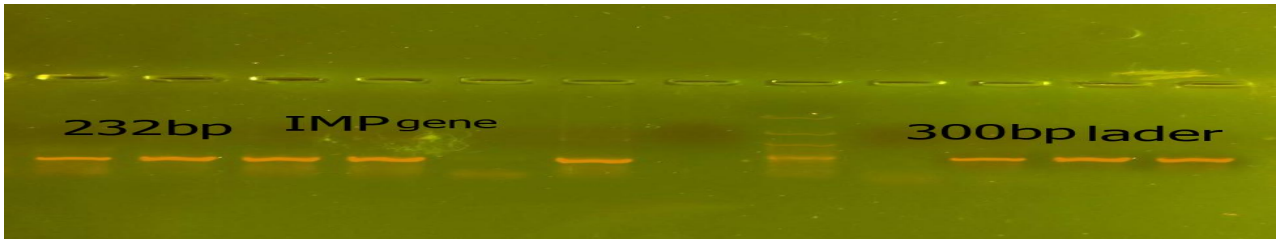
***bla* IMP gene.**

Figure (4-12) referred to the results of the genes electrophoresis that showed the existence of the *bla* IMP gene in both the chromosome and the plasmid of isolates, including the control. It was the elevated rate in the burning sample (14/50 isolates,63%), it was highest on the plasmid (16/60 isolates, 100%) than in the chromosome (6/60 isolates, 100%),but it declined in the control isolates that was (1/10 isolates 9.1%) in both plasmid and chromosome, there was a significant difference between these groups (P1<0.001) as shown in table (4-9), figure (4-10)

Table 4.9: comparison appearance rate of *bla*.IMP gene in both chromosome and plasmid of *P. aeruginosa* isolates from clinical sample

Overall total		**Appearance**				**Clinical Samples**
		Plasmid		**Chromosome**		
%	No.	%	No.	%	No.	
63.6	14.0	68.8	11.0	50.0	3.0	**Burns**
0.0	0.0	0.0	0.0	0.0	0.0	**wound**
0.0	0.0	0.0	0.0	0.0	0.0	**Urine**
4.5	1.0	6.3	1.0	0.0	0.0	**Sputum**
22.7	5.0	18.8	3.0	33.3	2.0	**Ear Swab**
9.1	2.0	6.3	1.0	16.7	1.0	**Healthy control**
100	22.0	100	16.0	100	6.0	**Total**
P. value = <0.001		Df = 3		TabX2 = 7.81		CalX2 = 18.064

Figure 4-10: Agarose gel electrophoresis of *bla* IMP gene (232 bp) of PCR Product (L: DNA Ladder 100-1500 bp, Agarose: 2%,



Volt: 100v, Lanes 1-7 represent bands of *Pseudomonas* isolates).

***bla* VIM gene**.

Experimental analysis results of the electrophoresis occurred the *bla* VIM gene in both the chromosome and the plasmid of isolates, including the control that pointed out to high over all rate in burning isolates (18/ 30 isolates, 69.2%) than others, it was high on a plasmid (26/60 isolates,100%) but in chromosome was (5/60 isolates ,100%), and decreased in the control to (0/10 isolates 0%), and(1/10 ,isolates, 3.2%) in plasmid and chromosome respectively ,there was a significant difference between these groups (P1<0.001) as shown in table (4-10)

Table 4.10: comparison appearance rate of *bla*.VIM gene in both chromosome and plasmid of *P. aeruginosa* isolate from clinical sample

Overall total		**Appearance**				**Clinical samples**
		Plasmid		**Chromosome**		
%	NO.	%	No.	%	No.	
74.2	23.0	69.2	18.0	100	5.0	**Burns**
6.5	2.0	7.7	2.0	0.0	0.0	**wound**
3.2	1.0	3.8	1.0	0.0	0.0	**Urine**
3.2	1.0	3.8	1.0	0.0	0.0	**Sputum**
9.7	3.0	11.5	3.0	0.0	0.0	**Ear Swab**
3.2	1.0	3.8	1.0	0.0	0.0	**Healthy control**
100	31.0	100	26.0	100	5.0	**Total**
P. value = <0.001		Df = 5		TabX2 = 11.07		CalX2 = 37.682

***bla* NDM gene.**

the data of statistical analysis found the *bla* NDM gene in both the chromosome and the plasmid of isolates, including the control. It was the reached highest rate in the burning sample (11/30 isolates , 90.9%) than the other clinical

samples it was high on the plasmid (11/60 isolates,100%) but decreased to chromosome (4/60 isolates,100%), when in the control sample it was drop to (0/10 isolates 0%) and (1/10 isolates 10%) in plasmid and chromosome respectively . There was a significant difference between these groups (P1<0.001) As shown in table (4-11), figure (4-14)

Table 4.11: comparison appearance rate of *bla*.NDM gene in both chromosome and plasmid of *P. aeruginosa* isolate from clinical sample

Overall total		Appearance				Clinical Samples
		plasmid		Chromosome		
%	NO.	%	No.	%	No.	
86.7	13.0	90.9	10.0	75.0	3.0	**Burns**
0.0	0.0	0.0	0.0	0.0	0.0	**wound**
0.0	0.0	0.0	0.0	0.0	0.0	**Urine**
0.0	0.0	0.0	0.0	0.0	0.0	**Sputum**
6.7	1.0	9.1	1.0	0.0	0.0	**Ear Swab**
6.7	1.0	0.0	0.0	25.0	1.0	**Healthy control**
100	15.0	100	11.0	100	4.0	**Total**
P. value = <0.001		Df = 2		TabX2 = 5.99		CalX2 = 35.542

***aac*(6)-I gene.**

Gene expermintal electrophoresis explained the *bla* aac(6)-I gene in both the chromosome and the plasmid of isolates, including the control. It (23/30 isolates, 76.7%), raised rate in the burning sample than other, where it was on a plasmid (30/60 isolates,100%) when compared with the chromosome (6/60 isolates ,100%), but in the control sample it fall to (1/10 isolates, 10%) and (2/10 isolates 20%) in plasmid and chromosome respectively, there was a significant difference between these groups (P1<0.001) as in table (4-12),figure(4-15)

Table 4.12: comparison appearance rate of *aac*(6)-I gene in both chromosome and plasmid of *P. aeruginosa* isolate from clinical sample

Overall total		Appearance				Clinical Samples
		Plasmid		Chromosome		
%	NO.	%	No.	%	No.	
75.0	27.0	76.7	23.0	66.7	4.0	**Burns**
0.0	0.0	0.0	0.0	0.0	0.0	**wound**
0.0	0.0	0.0	0.0	0.0	0.0	**Urine**
5.6	2.0	6.7	2.0	0.0	0.0	**Sputum**
11.1	4.0	13.3	4.0	0.0	0.0	**Ear Swab**
8.3	3.0	3.3	1.0	33.3	2.0	**Health control**
100	36.0	100	30.0	100	6.0	**Total**
P. value = <0.001		Df = 3		TabX2 = 7.81		CalX2 = 46.694

***aph*(3)-IV gene.**

The molecular test elucidated the *aph*(3)-IV gene occurred only in plasmids, including the control. The overall was in high rate in the burning sample (6/30 isolates, 75%) compared with other clinical samples (8/60 isolates,100%) but down in the control samples to (1/10 isolates 10%) and (0/10 isolates 0%), in plasmid and chromosome respectively, table (4-13), figure (4-16)

Table 4.13: comparison appearance rate of *aph*(3)-IV gene in both chromosome and plasmid of *P. aeruginosa* isolate from clinical sample

Overall total		Appearance				Clinical samples
		Plasmid		Chromosome		
%	No.	%	No.	%	No.	
75	6.0	75	6.0	0.0	0.0	**Burns**
12.5	1.0	12.5	1.0	0.0	0.0	**wound**
0.0	0.0	0.0	0.0	0.0	0.0	**Urine**
0.0	0.0	0.0	0.0	0.0	0.0	**Sputum**
0.0	0.0	0.0	0.0	0.0	0.0	**Ear Swab**
12.5	1.0	12.5	1.0	0.0	0.0	**Healthy control**
100	8.0	100	8.0	0.0	0.0	**Total**

DISCUSSION

According to clinical source

The results of the present study showed different bacterial species isolated from different swabs of patients with many clinical sources. *P. aerogenosa*, was the most common cause of with the highest ratio of isolation. Most studies refer to *P. aeruginosa* the predominant organism isolated from burns, Ear infection. As a common point of views, *P. aeruginosa* considered a ubiquitous microbe which exists in wide ranges of environments, developing mechanisms to antibiotic resistance persistently, ability to compete with other organisms, and widely distributed in the water (Hailu *et al.,* 2016) The results of the current study recorded that there were the most patients with burn and the ear infection was suffer from *pseudomonas* infect with percentage of 50.0%, 11.7 respectively. The present study results disagreed with a previous study in Kirkuk province (Al-Abideen, Ahmed 2015). who was reported the low rate of burns infection with *P. aeruginosa* , and another study in Nigeria were recorded the percentage of *P. aeruginosa* as following, the wound swab and it is closely (29.87%) has the highest frequency of *P.aeruginosa* infections followed by ear swab

(22.08%).Azeez and Owolabi,(2020). The present study results agree with, Haleem *et al*., (2011) a previous study in Hilla province. Who was reported the most isolates were obtained from burns (55%) and another study in Mosul province (Hamdoon *et al*.,2011), he showed the predominant microorganism was *P. aeruginosa* (50%), the high percentage of burn patients because the skin forms an invasion by bacteria, fungi, and viruses. Any breach in this barrier provides easy access for microbial invasion.

Antimicrobial susceptibility

Antibiogram results for the studied isolates showed high sensitivity to imipenem and meropenem (carbapenems). Carbapenems are the most effective beta-lactams in the treatment of *P. aeruginosa* infections because they exhibit a high affinity for penicillin-binding protein, are stable against broad-spectrum beta lactamases, and easily pass through the outer membrane (Farhan et al.,2019). Carbapenem resistance in *P. aeruginosa* is mediated by the release of MBLs, the excretion of the drug from the bacteria via efflux pumps. The release of carbapenemase from these is important because of the genes encoding to these enzymes are transferred via plasmids, transposons and spread among isolates (Vural *et al*.,2020). The result in the current study showed there was found different in the activity of meropenem and imipenem to *P. aeruginos* , the resistance was 40%,26% respectively, which disagree with (Hussein *et al*.,2018) who reported that both of them have the same percentage 34.95% resistance. Furthermore, current finding indicated that higher resistance against imipenem and meropenem have compared with study in Najaf by Al-Shara, (2013) who reported that the resistance rate was 7.4% and 14.8%, respectively. The current study result reported high sensitivity percentage for meropenem it was 63.3% and resistances was 26.7%. The present study approximated with an earlier study in Turkey showed the percentage of resistances was evaluated, 26.4% (Vural *et al*.,2020) . The sensitivity results of amikacin, gentamicin (aminoglycosides) in the present study showed a low percentage of amikacin sensitivity and a moderate percentage of gentamicin sensitivity by comparison with other studies. The current study results disagree with previous studies in Najaf and Baqubah Cities that showed the percentages of gentamicin and amikacin sensitivity were 48%, 100%, and 80%, 85%, respectively. (Jaloob and Gafil, 2012; Muhammed and Hamood,2016) (Dubois *et al*.,2008). They illustrated that the rate of resistance for gentamicin reached to 55.8% and these results are much compatible with the percentage of this study. In the present study, amikacin percentage resistance disagrees with most previous studies, that revealed high sensitivity to amikacin. Whereas about our study revealed 75.0% were resistance isolates, this means most isolates tend to become more resistant. On the other hand, the present study agrees with a previous study in Brazil province (Araújo *et al*.,2018). who was reported the high rate of P. aeruginosa resistance aminoglycoside were 55%? The present study showed the majority of isolates were resistant to piperacillin (which belongs to penicillins) and aztreonam (monobactams). This result came compatible with a predate study in Najaf province that found from total 40 isolates only 19 and 14 isolates were sensitive to piperacillin and aztreonam, respectively (Al-Zubaidy, 2014)

Multi-Drug Resistance

The current results, high antibiotic resistance against most of the tested antibiotics was demonstrated in this study (46%) of isolates was MDR, and (40%) isolates was extensively drug-resistant XDR, except for 8 isolates (14%) that were susceptible to all applied antibiotic. Recent studies have shown that multi-drug resistance and several virulence determinants are key factors that contribute to the global spread of *P. aeruginosa* in hospitals. (Beceiro *et al*.,2013; Galetti *et al.,*2015) The mean resistance to antimicrobial agents comprised of three or more antipseudomonal groups (aminoglycosides, carbapenems, penicillins / cephalosporine, and fluoroquinolones) are Multidrug-resistant (Magiorakos, 2011). This result is similar to the Asian research and numerous studies have shown significantly higher MDR *P. aeruginosa* (cefalosporins, carbapenems, and aminoglycosides) in Pakistan have isolates. (Khan et al.,2014), India (Gill *et al*.,2016). And Thailand (Suwantarat and Carroll, 2016) with resistant rates of 30%, 50%, and ≥20%, respectively

4.1. Molecular Study

4.1.1. *Pseudomonas aeruginosa* diagnosis.

The 16SrRNA gene is used for phylogenetic studies, and it is used in the precise classification of bacteria. The 16S rRNA gene can be compared not only among all bacteria but also with the 16S rRNA gene of archeobacteria and the 18S rRNA gene of eukaryotes. This gene is highly stable among all organisms. The current study revealed all isolates belong to *P. aeruginosa*. also, using the 16S rRNA gene to diagnose the isolates from the many *Pseudomonas* infections and found this gene in all targeted isolates. This confirms the ability and accuracy of this gene in bacterial diagnosis. (Dekhil, 2017)

Detection of antibiotic-resistance genes

A-MBLs genes identification.

The increased prevalence of carbapenem-resistance in Pseudomonas *aeruginosa*. caused by Metallo-β- lactamase (MBL) is worrisome in clinical settings worldwide. The mortality rate associated with infections caused by MBLs producing organisms ranging from 18 to 67%.(Adam and Elhag, 2018).The present study aimed to determine the prevalence of Metallo-β-lactamase genes among some *P. aeruginosa* (Gram-negative) clinical isolates (Carbapenems susceptible and resistant) and this study carried out to detect MBL genes such as (*bla*VIM, *bla*IMP, and *bla*NDM) by multiplex PCR mixture reaction among 60 *pseudomonas spp* . clinical isolates. Verona integron Metallo-beta-lactamase (VIM)was the most frequent gene represent 26 (43%) among 60 *pseudomonades* isolate positive MBL genes, imipenemase (IMP) represent 16 (26.4%), and the NDM was the least detected in 11(18%). Our results agree with studies done in Uganda, Tanzania, Egypt, and Iran by Okoche *et al*., (2015). and Aghamiri (Aghamiri *et al.,*2014). they reported the frequencies of (VIM, IMP, and NDM) as (38.9%),(25%) ,(20.9%), respectively. However, our findings were differing with that create in Iraq by Anoar et al,(2014) they reported IMP was the maximum frequently identified gene 33(18.6%), VIM 19(10.7%), and NDM 2(1.12%) isolates, and in another study in Egypt, Zafer *et al*.,(2014):was reported *bla*VIM-2, *bla*IMP-1, *bla*NDM, and in *P. aeruginosa* while screening for MBLs in 122 *P. aeruginosa* isolates. The prevalence of *bla*VIM- was found to be 58.3%, , *bla*NDM was 4.2%, and *bla*IMP-1-like gene was 2.1% .

B-AMEs genes identification

In the cases of the presence or absent single gene the results of the current study indicated that 30 of 60 isolated bacteria contained(aminoglycoside acetyltransferase [AAC]): *aac*(6')-I, and 8 of 60 isolated bacteria contain (aminoglycoside phosphoryl transferase [APH]) *aph*(3)-VI. All isolates that were resistant to one or more aminoglycoside antibiotics were subjected to PCR analysis to detect the presence of the following resistance genes: *aac*(6')-

I , *Aph*-(3)-VI . The results of this study showed that the *aac*(6)-I was the most prevalent AMEs genes since it was found in (50%) of the isolates followed aph(3)-VI when the rates were(13.3%) The present data discrepancy with a former study performed in India province. by Odumosu et al.,(2015.were reported aac (6)-I in 10 (18.5%) and aph (3)-VI in was found in 8% of the resistant isolates. The less percentage of last AME genes (*aph*(3)- VI) relatively close with the rate reported by Kim et al., (2008) who showed that the prevalence of *aph*(3)- VI gene was 5.5%, while (Akers *et al*.,2010) in Korea and Vaziri et al.(2011) in Iran reported a higher percentage of *aph*(3)- VI prevalence when their rate reached 14.8% and 11% respectively. This resulted was agree with our studies. the *aac*(6)-I gene was detected in 12 isolates and all these isolates were resistant to gentamicin. The *aac*(6) was the most prevalent AMEs encountered in 63.7% of isolates which is similar to those showed a remarkable high of other reports from India and abroad (Chaudhary and Payasi, 2014) .On the other hand, , Incompatible to our results, a Korean nationwide study of 250 isolates of P. aeruginosa reported that *aph*(3)-VI, and *aac*(6)-I were all prevalent. Vaziri et al.,(2011).And in the other study was reported the percentage of *aph*(3)-VI was (13.3%) .The difference in the distribution of modifying enzymes may derive from differences in aminoglycoside prescription patterns, the selection of bacterial population, or geographical differences in the occurrence of aminoglycoside resistance genes.

Gene frequencies isolate from chromosome and plasmid

PCR analysis confirmed the identification of plasmid in all study clinical isolations of *P. aeroginosa* with (4/10 isolates 40%) in the control sample was carried resistance antibiotic genes .Plasmids, as extrachromosomal genetic elements, are the main vectors mediating the supper of antimicrobial resistance (AMR) genes. (Koonin *et al*.,2016).The genes encoding the carbapenemases and aminoglycosides in pseudomonas sp. are usually located in plasmids, which significantly increase the risk of their dissemination .(Araújo , 2016).The present study agrees with a previous study in Nigeria province done by Thomas *et al*.,(2015) . who was reported from the 74 strains analyzed; 32 (43.2%) had one plasmid, 37(50%) had two plasmids while the remaining five carried three plasmids each. And another study in china by Li *et al*.,(2020). The current results indicated that the most antibiotic resistance genes isolated from P. aeruginosa were located on the plasmid. The present data agree with a former study performed in China done by Wu *et al*.,(2019) this study documented the involvement of plasmids as factors responsible for antibiotic resistance.

comparsion appearance rate of resistance genes

A. *bla*.IMP gene

The current study has shown that there is a clear difference in the high percentage of the *bla*IMP gene in the burn samples (11/30 isolates 68.8%) and also its high presence compared to its presence in the control it was 6.3% .There is a clear statistical difference in the direction of the plasmid. The present study results were inconsistent with other studies in Tehran province by Fallah *et al*.,(2013). who was reported that Imipenemase (IMP) Metallo-β-lactamase genes on *P. aeruginosa* isolated from hospitalized burn patients were positive to isolates 6 (28.6%), they have shown that IMP-1 producing *P. aeruginosa* strains is an emerging threat in burn care parts and should be contained by the implementation of timely identification and strict isolation methods

B.*bla.*VIM gene

The current study revealed a high occurrence of *bla*.VIM gene in Burns sample and also its high presence (18/30 isolates 69.2%) compared to its presence in the control, it was one isolate (3.2%). Also, its high in plasmid compared with chromosomal genes. The present study results agree with a previous study in Lebanon province by Dagher *et al*.,(2019) they have shown that The main finding in the study was the emergence of *P. aeruginosa* harboring the VIM plasmid, which has never been detected before in Lebanon, or the studies did not specify the genetic location of the *bla*VIM gene. This finding poses a serious public health problem especially in burn patients because the plasmid containing this β lactamase is a major source of dissemination of this enzyme

C.*bla*.NDM gene

The present study showed the existence of the NDM gene in a high percentage of burn infection it was (10/30 isolates 90.1%) and increased in plasmid compared to its presence in the chromosome and control. Our current study is similar to previous studies conducted in Iraqi hospitals by Ismail and Mahmoud, (2018). They found that the proportion of the presence of NDM within the chromosome is very low, as well as its presence within the control.

D.*aac*(6)-IV gene

Hospitalized burned patients are at high risk of multidrug-resistant infections mainly due to a disruption of the skin barrier, alterations in the specific and nonspecific components of the immune system (Kashfi *et al*.,2017). Aminoglycosides modifying enzyme AME-genes that encode the aminoglycosides acetyl-transferase (AAC) (Abo-State *et al*.,2018)0The current study revealed a high occurrence of *aac*(6)-I gene in burn sample, where it reached (23/30 isolates, 76.7%), compared to its presence in the control, it was (2/10 isolates 33.3%). Also, its high in plasmid compared with chromosomal genes. The present study results disagree with a previous study in Nigeria province by Odumosu *et al*., (2015) .They are reported 22.2% *P. aeruginosa* isolates harboring *aac*(6′)-I. and this study performed that the *aac*(6′)-I are often located on plasmids, and encoding resistance. The unabated spread of AMEs in developed countries due to the use of aminoglycoside has been a clinical challenge for over two decades.

E. *aph*(3)-IV gene

little is known about the presence of genes for AMEs in colonized isolates. In China, one study described the presence of aph(3')-VI associated with another carbapenemase, (Grazziotin *et al*.,2016). The current study revealed a high occurrence of *aph*(3)-VI gene in the Burn sample, it was (6/30 isolates 75%), compared to its presence in the control, it was (1/10 isolates 12.5%), Also it's high in plasmid compared with chromosomal genes, it was (8/0). Our current study is similar to previous studies in-AL.Diwaniya province by Alshammari *et al.,* (2019) .they reported the presence aph (3)-IV at a high rate with burn samples. The high resistance of *P. aeruginosa* isolated from burn centers may be attributed to the antibiotic selective pressure of the antibiotic (Lachiewicz *et al*.,2017). On the other hand, there is no study showing the presence or absence of that gene on the chromosome.

REFERENCES:

1- Abo-State, M. A. M., Saleh, Y. E. S., & Ghareeb, H. M. (2018). Prevalence and sequence of aminoglycosides modifying enzymes genes among E. coli and Klebsiella species isolated from Egyptian hospitals. Journal of radiation research and applied sciences, 11(4), 408-415.

2- Akers, K. S., Chaney, C., Barsoumian, A., Beckius, M., Zera, W., Yu, X., ... & Murray, C. K. (2010). Aminoglycoside resistance and susceptibility testing errors in Acinetobacter baumannii-calcoaceticus complex. Journal of clinical microbiology, 48(4), 1132-1138.
3- Alshammari, M. M. M., Alkhudhairy, M. K., & Soghi, A. A. (2019). Prevalence of aminoglycoside modifying enzyme genes in Pseudomonas aeruginosa isolated from Burn Centers in Iraq. Drug Inventi
4- Al-Shara JMR. Phenotypic and molecular detecting of carbapenem resistant Pseudomonas aeruginosa in Najaf Hospitals. PhD Thesis. Faculty of Science. University of Kufa. Iraq; 2013.
5- Al-Zubaidy, B. J., Abdulrahman, T. R., & Ahmed, W. A. (2014). Detection of exo a gene in Pseudomonas aeruginosa from human and dogs using polymerase chain reaction. IOSR, 9, 38-41.
6- Araújo, B. F. (2016). Epidemiologia e caracterização molecular de Pseudomonas aeruginosa, Klebsiella pneumoniae e Escherichia coli multirresistentes carreando determinantes de resistência às quinolonas mediada por plasmídeos.
7- Araújo, B. F. (2016). Epidemiologia e caracterização molecular de Pseudomonas aeruginosa, Klebsiella pneumoniae e Escherichia coli multirresistentes carreando determinantes de resistência às quinolonas mediada por plasmídeos.
8- Beceiro A, Tomás M, Bou G. Antimicrobial Resistance and Virulence: a Successful or Deleterious Association in the Bacterial World?. Clinical Microbiology Reviews 2013; 26: 185–230
9- Boyd, D. A., Lisboa, L. F., Rennie, R., Zhanel, G. G., Dingle, T. C., & Mulvey, M. R. (2019). Identification of a novel metallo-β-lactamase, CAM-1, in clinical Pseudomonas aeruginosa isolates from Canada. *Journal of Antimicrobial Chemotherapy*, *74*(6), 1563-1567.
10- Breidenstein, E. B. M., de la Fuente-Núñez, C., & Hancock, R. E. W. (2011). Pseudomonas aeruginosa: all roads lead to resistance. Trends in Microbiology, 19(8), 419–426.
11- Chaudhary, M., & Payasi, A. (2014). Resistance patterns and prevalence of the aminoglycoside modifying enzymes in clinical isolates of gram negative pathogens. Global Journal of Pharmacology, 8(1), 73-79.
12- Dagher, T. N., Al-Bayssari, C., Diene, S. M., Azar, E., & Rolain, J. M. (2019). Emergence of plasmid-encoded VIM-2–producing Pseudomonas aeruginosa isolated from clinical samples in Lebanon. New Microbes and New Infections, 29, 100521.
13- Dekhil, K. R. (2017). Molecular characterization and phylogenetic analysis of *Pseudomonas aeruginosa* isolated from active chronic otitis media. International Journal of Research in Pharmaceutical Sciences, 8(3): 389-396.
14- Dubois, V.; Arpin, C.; Dupart, V.; Scavelli, A. Coulange, . Andr , C. et al.(2008). β-lactam and aminoglycoside resistance rates and mechanisms among Pseudomonas aeruginosa in French general practice (community and private healthcare centres). J Antimicrob Chemother; 62:316-23
15- Fallah, F., Borhan, R. S., & Hashemi, A. (2013). Detection of bla (IMP) and bla (VIM) metallo-β-lactamases genes among Pseudomonas aeruginosa strains. International journal of burns and trauma, 3(2), 122
16- Farhan, S. M., Ibrahim, R. A., Mahran, K. M., Hetta, H. F., & Abd El-Baky, R. M. (2019). Antimicrobial resistance pattern and molecular genetic distribution of metallo-β-lactamases producing Pseudomonas aeruginosa isolated from hospitals in Minia, Egypt. Infection and Drug Resistance, 12, 2125
17- Galetti, R., Andrade, L. N., Clímaco, E. C., Pitondo-Silva, A., Ferreira, J. C., & Darini, A. L. C. (2015). Genomic diversification and virulence features in SPM-1–producing Pseudomonas aeruginosa 13years later. Diagnostic Microbiology & Infectious Disease, 2(82), 179-180.
18- Gill J, Arora S, Khanna S, Kumar KH. Prevalence of multidrug-resistant, extensively drug-resistant, and pandrug-resistant Pseudomonas aeruginosa from a tertiary level ntensive Care Unit. J Glob Infect Dis 2016; 8(4): 155- 159.
19- Hailu, D., Mekonnen, D., Derbie, A., Mulu, W., & Abera, B. (2016). Pathogenic bacteria profile and antimicrobial susceptibility patterns of ear infection at Bahir Dar Regional Health Research Laboratory Center, Ethiopia. SpringerPlus, 5(1), 466.
20- Haleem, H., Tarrad, J. K., & Banyan, I. A. (2011). Isolation of Pseudomonas aeruginosa from clinical cases and environmental samples, and analysis of its antibiotic resistant spectrum at hilla teaching hospital. Medical Journal of Babylon, 8(4), 618-624
21- Hodiwala, A., Dhoke, R., & Urhekar, A. D. (2013). Incidence of metallo-betalactamase producing pseudomonas, acinetobacter & enterobacterial isolates in hospitalised patients. Int J Pharamcy Biol Sci, 3, 79-83.
22- Hussein, Z. K., Kadhim, H. S., & Hassan, J. S. (2018). 3. DETECTION OF NEW DELHI METALLO-BETA-LACTAMASE-1 (BLANDM-1) IN CARBAPENEM-RESISTANT PSEUDOMONAS AERUGINOSA ISOLATED FROM CLINICAL SAMPLES IN WASIT HOSPITALS. Iraqi Journal of Medical Sciences, 16(3), 239-246.
23- Ismail, S. J., & Mahmoud, S. S. (2018). First detection of New Delhi metallo-β-lactamases variants (NDM-1, NDM-2) among Pseudomonas aeruginosa isolated from Iraqi hospitals. Iranian journal of microbiology, 10(2), 98.
24- Khan, F., Pham, D. T., Oloketuyi, S. F., & Kim, Y. M. (2020). Antibiotics Application Strategies to Control Biofilm Formation in Pathogenic Bacteria. Current pharmaceutical biotechnology
25- Khan, H. A., Ahmad, A., & Mehboob, R. (2015). Nosocomial infections and their control strategies. Asian pacific journal of tropical biomedicine, 5(7), 509-514.
26- Kim, S. H.; Kim, M. G.; Kim, S. S.; Cha, S. H. & Yeo, S. G. (2015). Change in detection rate of methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa and their antibiotic sensitivities in patients with chronic suppurative otitis media. The Journal of International Advanced Otology, 11(2): 151-156.
27- Koonin EV. Horizontal gene transfer: essentiality and evolvability in prokar- yotes, and roles in evolutionary transitions. F1000Res 2016; 5: pii=F1000 Faculty Rev-1805. 7
28- Krause, K. M., Serio, A. W., Kane, T. R., & Connolly, L. E. (2016). Aminoglycosides: an overview. Cold Spring Harbor perspectives in medicine, 6(6), a027029.
29- Lachiewicz AM, Hauck CG, Weber DJ, Cairns BA, van Duin D. Bacterial infections after burn injuries: Impact of multidrug resistance. Clin Infect Dis 2017;65:2130-6
30- Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Olsson-Liljequist, B. (2011). Multidrug-Resistant (MDR), Extensively Drug Resistant (XDR) and Pandrug-1 Resistant (PDR) Bacteria in Healthcare Settings. Expert Proposal for a Standardized International Terminology
31- Memish, Z. A., Shibl, A. M., Kambal, A. M., Ohaly, Y. A., Ishaq, A., & Livermore, D. M. (2012). Antimicrobial

resistance among non-fermenting Gram-negative bacteria in Saudi Arabia. Journal of antimicrobial chemotherapy, 67(7), 1701-1705.
32- Moradali MF, Ghods S, Rehm BHA. 2017. Pseudomonas aeruginosa Lifestyle: A Paradigm for Adaptation, Survival, and Persistence. Front Cell Infect Microbiol 7:1113– 29.
33- Moradali MF, Ghods S, Rehm BHA. 2017. Pseudomonas aeruginosa Lifestyle: A Paradigm for Adaptation, Survival, and Persistence. Front Cell Infect Microbiol 7:1113– 29.
34- Muhammed, N. K. & Hamood, H. J. (2016). Role of Bacteria in chronic Suppurative Otitis Media and Sensitivity pattern in Baqubah Teaching Hospital. Diyala Journal of Medicine, 10(2): 25-33.
35- Odumosu, B. T., Adeniyi, B. A., & Chandra, R. (2015). Occurrence of aminoglycoside-modifying enzymes genes (aac (6′)-I and ant (2″)-I) in clinical isolates of Pseudomonas aeruginosa from Southwest Nigeria. African health sciences, 15(4), 1277-1281.
36- Ewaid, S.H.; Abed, S.A.; Al-Ansari, N. Crop Water Requirements and Irrigation Schedules for Some Major Crops in Southern Iraq. Water 2019, 11, 756.
37- Ewaid, S.H.; Abed, S.A.; Al-Ansari, N. Water Footprint of Wheat in Iraq. Water 2019, 11, 535.
38- Ewaid, S.H.; Abed, S.A.; Al-Ansari, N. Assessment of Main Cereal Crop Trade Impacts on Water and Land Security in Iraq. Agronomy 2020, 10, 98.
39- Salah Salman Zain Al-Abideen 1, Zahraa Ayed Ahmed 2. (2015). Investigation of induced beta-lactase enzymes in Pseudomonas aeruginosa isolated from different pathological models in the city of Kirkuk. Kirkuk University Journal - Scientific Studies, 10 (4), 71-92.
40- Schulte, M., Sterzenbach, T., Miskiewicz, K., Elpers, L., Hensel, M., & Hansmeier, N. (2019). A versatile remote control system for functional expression of bacterial virulence genes based on the tetA promoter. International Journal of Medical Microbiology, 309(1), 54-65.
41- Al-Grawi E.D.C. and Al-Awsi G.R.L. Expression of CDKN2A (p16/Ink4a) among Colorectal Cancer Patients: A cohort study. Journal of Pharmaceutical Sciences and Research. 2018; 10 (5): 1145-1147.
42- Shamran AR, Shaker ZH, Al-Awsi GRL, Khamis AS, Tolaifeh ZA. and Jameel ZI., 2018. Rapd-PCR is a good DNA finger-printing technique to detect phylogenetic relationships among Staphylococcus. aureus isolated from different sources in Hilla city, Iraq. Biochem Cell Arch. 2018; 18 (suppl. 1): 1157- 1161.
43- Eqbal Dohan Chalap, and Ghaidaa Raheem Lateef Al-Awsi. 2019. "A General Overview of the Genetic Effects of Extracellular Polymers for Enterococcus Faecium in Cancer Cells". International Journal of Research in Pharmaceutical Sciences 10 (1), 436-43. https://pharmascope.org/index.php/ijrps/article/view/74.
44- Chillab Eqbal Dohan, Talib Ro'a Ali, Al-Awsi Ghaidaa Raheem Lateef, (2019). Genetics of Sickle Cell Anemia Disorders in Baghdad City, Iraq. Indian Journal of Public Health Research & Development, 10 (2): 817-822.
45- Ali A Alsudani et al 2019 J. Phys.: Conf. Ser. 1294 062099
46- Ali A Alsudani and Ghaidaa Raheem Lateef Al-Awsi, 2020. Biocontrol of Rhizoctonia solani (Kühn) and Fusarium solani (Marti) causing damping-off disease in tomato with Azotobacter chroococcum and Pseudomonas fluorescens. Pakistan Journal of Biological Sciences, 23: 1456-1461.
47- Ahmed Ali Obaid et al 2019 J. Phys.: Conf. Ser. 1294 022026.
48- Ahmed Abdulameer Alwan, Ahmed Ali Obaid, & Hussain T. Ajeel. (2019). Assessment of the outcome of the tubularized incised plate (T.I.P) tech-nique in the management of distal hypospadias; prospective single-centre study. International Journal of Research in Pharmaceutical Sciences, 10 (2), 1547-1550. https://doi.org/10.26452/ijrps.v10i2.874.
49- Murad, Mohammed Challoob, and Ahmed Ali Obaid and Falah Mahdi Ali. "Prevalence of Nocturnal Enuresis and Its Associated Ultrasonic Findings in Children of Wasit." Systematic Reviews in Pharmacy 11 (2020), 120-122. doi:10.31838/srp.2020.10.20.
50- Ghaidaa Raheem Lateef Al-Awsi et al 2019 J. Phys.: Conf. Ser. 1294 062077.
51- Mahdi Ali, F., and A. A. Obaid. "PROSTATE VAPORIZATION BY DIODE LASER FOR PATIENTS WITH BENIGN PROSTATIC ENLARGEMENT". Asian Journal of Pharmaceutical and Clinical Research, Vol. 11, no. 12, Dec. 2018, pp. 523-5, doi:10.22159/ajpcr. 2018.v11i12.29572.
52- Suwantarat, N., & Carroll, K. C. (2016). Epidemiology and molecular characterization of multidrug-resistant Gram-negative bacteria in Southeast Asia. Antimicrobial Resistance & Infection Control, 5(1), 1-8.
53- Thomas, B. T., Agu, G. C., Makanjuola, S. O., & Davies, A. (2015). Plasmid profiling and antibiotic resistance of extended spectrum beta lactamases producing Pseudomonas aeruginosa expressing Ampc betalactamase enzyme. Am-Eurasian J Sci Res, 10, 9.
54- Vural, E., Delialioğlu, N., Tezcan Ulger, S., Emekdas, G., & Serin, M. S. (2020). Phenotypic and Molecular Detection of the Metallo-Beta-Lactamases in Carbapenem-Resistant Pseudomonas aeruginosa Isolates from Clinical Samples. Jundishapur Journal of Microbiology, 13(2).
55- Ewaid, S.H.; Abed, S.A.; Al-Ansari, N.; Salih, R.M. Development and Evaluation of a Water Quality Index for the Iraqi Rivers. Hydrology 2020, 7, 67.
56- Ewaid, S.H., Al-Farhani, B.F., Abed, S.A. & Al-Ansari, N. (2020). Modeling of trihalomethane compounds formation in Baghdad water supply network. Scientific Review Engineering and Environmental Sciences, 29 (2), 136-144. doi: 10.22630/PNIKS.2020.29.2.12.
57- Wang, T., Hou, Y., & Wang, R. (2019). A case report of community-acquired Pseudomonas aeruginosa pneumonia complicated with MODS in a previously healthy patient and related literature review. BMC infectious diseases, 19(1), 1-6.
58- Wu, W., Feng, Y., Tang, G., Qiao, F., McNally, A., & Zong, Z. (2019). NDM metallo-β-lactamases and their bacterial producers in health care settings. Clinical microbiology reviews, 32(2).