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Molecular study of bacterial infection from hemodialysis patients in Wasit centre, Iraq

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ABSTRACT

This study was conducted at the College of Medicine – Wasit University in Cooperation with the Wasit centre for dialysis from the period of October -2017 to February -2018. It has been carried out on 100 urine samples and 100 blood samples from 100 hemodialysis patients referred to the Wasit centre of dialysis from both sex [male and female]. This study showed that from both 100 urine samples, and 100 blood samples, 40% UTIs, and 36% bacteremia. The main bacterial isolates from urine samples represented *Escherichia coli* (40%), and *Proteus merabilis*(11.1), *Staphylococcus aureus* (12.5%), *Entero cloacae* (12.5%), *Pantoea spp* (2.5) *Klebsiella pneumonia* (11.1), while bacterial isolates from blood samples are *Pseudomonas aeruginosa* (33.33%), and *Staphylococcus aureus* (30%), *Pasteurella mulocida* (19.44%), *Entero cloacae* (11.11%), and, *Streptococcus spp* (2.55%). Antimicrobial sensitivity for *Pseudomonas aeruginosa* appeared sensitivity for Amikacin, Ciprofloxacin, Ceftazidime, and imipenem while *Pasteurella mulocida* showed sensitivity with Ciprofloxacin, Imipenem, aztreonam, and nalidixic acid. *Escherichia coli* observed sensitivity with Imipenem, Amikacin, Cefepime, Ciprofloxacin, and Nitrofurantoin. Bacteremia can be seen in a significant number of hemodialysis patients, and *Pseudomonas aeruginosa* is the most universal organism isolated which is resistant to commonly used antibiotics, a significant number of patients had asymptomatic urinary tract infection in which *E.coli* is the most frequent microorganism isolate by using culture and, bacterial isolates detected 100% by PCR technique.

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INTRODUCTION

Renal failure refers to a condition where the kidneys lose their normal function, which may be due to various factors including infections, autoimmune diseases, diabetes, other endocrine disorders, cancer, and toxic chemicals (Meyer and Hostetter, 2007). Renal failure occurs when a sufficient number of nephrons are damaged and therefore the kidneys unable to perform their functions. It can be divided into two main types, namely acute renal failure and chronic renal failure (Stevens and Lowe, 2000; Tortora and Grabowsky, 2000). Acute renal failure (ARF) is a common condition in hospitalised patients, characterised by a rapid fall in glomerular filtration rate (GFR), carries high morbidity and mortality, often preventable, so raped recognition and treatment may prevent irreversible loss of nephrons (Salih et al., 2009).

Chronic renal failure (CRF) is growing among all population groups worldwide, and the incidence of end-stage renal disease (ESRD) continues to increase (Lysaght, 2002). It is defined as kidney damage for more than three months as evidenced by structural or functional abnormalities with or

without decreased glomerular filtration rate (GFR) and manifested either as pathological abnormalities or kidney damage markers in blood or urine or the imaging test. Many people are unaware of the problem until more than 70% of the kidney function has been lost (OShaughnessy, 2005; Norula and Hooda, 2007).

Dialysis (hemo-and peritoneal) is regarded as one of the most common strategies of renal replacement therapy and the main sole for saving the life (Rubin, 2007). It is defined as a procedure that removes excess fluids, and toxic end products of metabolism such as urea from the plasma and correct electrolytes balance by dialysing the patients' blood against fluid containing no urea but with appropriate concentrations of electrolytes free ionised calcium and other plasma constituents (Zilva et al., 1994).

This study aims to investigate the most prevalent causes of bacteremia and urinary tract infections in hemodialysis patients, and genetic study for more predominant bacteria isolates from urine and blood by PCR technique.

MATERIAL AND METHODS

Samples were collected from 100 participants of both male and female with age group. The study was conducted at the College of Medicine – Wasit University in cooperation with the dialysis centre in Wasit Provence from the period of October - 2017 to February -2018 population constituted hemodialysis patients who reported at Wasit centre. This number was made up of 100 urine samples and 100 blood samples. The study designed into two main steps. The respectively first step involved the collection of samples, isolation of bacteria, identification, and antibiotics sensitivity test. The second step included genetic analysis by PCR for more predominates isolation.

DIAGNOSIS

Standard procedures for identification & characterisation of bacterial pathogens included. Cheesbrough.

1. In this study, the number of males is 57 patients, and females 43 patients.
2. Isolation of bacteria by [blood agar, MacConky agar, chocolate agar, brain heart infusion broth], and the infections in UTIs is males (23) cases, and in female (17) cases.while the incidence of bacteremia is (16) males and (20) females.
3. Biochemical test by [set of sugar, API system include (API 20 E., API staph.)
4. Bacterial isolation
   - ***E.coli*** is a Gram-negative bacillus appeared as pink colonies on MacConky agar due to its ability to ferment lactose, gave a positive result for Indol test, and motility. It showed acid slant and acid button Triple Sugar Iron medium, with gas, and without H_2S, and gave a negative result for oxidase test also do not utilise citrate. A biochemical reaction of *E. coli* on API 20 E strip after 24 hr. At 37°C.
   - ***Pseudomonas aeruginosa*** was G-ve rods, motile and colonies on MacConky agar appeared as small pale colonies due to lactose non-fermenting. This bacterium produced Pyocyanin pigment. Figure [3-10], capable of growing at 42°C and also at 4°C. It gave a positive result for oxidase, catalase, and Simmon's citrate, not ferment glucose on Triple Sugar Iron medium [alkaline for both slant and butt], and not produce H2S. Indol test for this bacterium was negative.
   - ***Pasteurella multocida*** is a gram-negative, non-motile, penicillin sensitive coccobacillus belonging to the pasteurellaceae family. Pasteurella multocida (*P. multocida*) can be present as a commensal in the nasopharynx of apparently healthy animals and as a primary or secondary pathogen in several animal species. Toxigenic strains of the bacteria have been isolated from humans with tonsillitis, rhinitis, sinusitis, pleuritis, appendicitis, and septicemia, suggesting a potential zoonosis. Most human isolates belong to capsular types A and D, similar to types isolated from swine.
   - ***Proteus mirabilis*** G-ve bacilli on Gram-stained smear, rapidly motile by flagella. This is indicated by swarming phenomena on a blood agar plate. It is non-lactose fermenter, so it gives pale colonies on MacConky agar, produces urease that hydrolyses urea, and liberate ammonia caused change urea agar base medium from yellow to pink.
5. Sensitivity test by [Muller Hinton agar]

Molecular Detection [Polymerase Chain Reaction]

PCR assay was performed for detection of *E. coli, P.mirabilis*, *P.aeruginosa*, and *P. multocida* based on 16S rRNA gene as well as detection of virulence factor gene in these bacteria. This assay was carried out according to the method described by Memmeri, & Pourzer, as the following steps:

1. Genomic DNA extraction:- Genomic DNA was extracted from bacterial isolates by using Genomic DNA Mini Bacteria Kit (Genaied – USA)
2. Primers: The primers were designed in this study by using NCBI Gene-Bank and Primer 3 online and provided by (Bioneer Company, Korea) as following
3. Estimation of DNA extracted

Table 1: list of sequence primer gene

Primer	Sequence (5'-3')		Product Size
16S-rRNA gene *E.coli*	F	GGAACTGAGACACGGTCCAG	589bp
	R	TTTAACCTTGCGGCCGTACT	
E.coli Pap gene	F	CGCAGTCAGTAGCCCGTATT	416bp
	R	ACGCAGGTAAGCAGAATCGT	
16S-rRNA gene *P.mutocida pneumonae*	F	TGATAAATCGACCGCCTGGG	454bp
	R	AGTTGCAGACTCCAATCCGG	
P.mutocida pneumonae toxA gene	F	GCCTTTTCGCGGATATGCTG	300bp
	R	TCATAAGGCCCTCCAACCTG	
16S-rRNA gene *P. mirabilis*	F	CTCCTTTGAGTTCCCGCCAT	366bp
	R	ATACCCTGGTAGTCCACGCT	
UreC *P. mirabilis*	F	CAAGCCCAAGAAGGTCTCGT	453bp
	R	ATTGGGCTCTCCTACCGACT	
16S-rRNA gene *P. aeruginosa*	F	TCAACCTGGGAACTGCATCC	698bp
	R	CAGACTGCGATCCGGACTAC	
lasB gene *P. aeruginosa*	F	ACCTACAAGCAGGTCAACGG	524bp

Table 2: Results of blood culture

Blood samples N=100 number of isolate		Urine samples N=100	
Type of bacteria	Number & percentage	Type of bacteria	Number & percentage
Pseudomonas	12(33.33)	*E.coli*	16(40)
Past.multo	7(19.44)	*P.mirablis*	12(30)
Staphylococcus	11(30.55)	*staphylococcus*	5(12.5)
Ent.clo	4(11.11)	*E.cloa*	5(12.5)
Streptococcus	2(5.55)	*Pant.*	1(2.5)
-------	----------	*Kleb.*	1(2.5)
Total	36	total	40
X^2	12.924*		33.360*
P value	0.012		<0.0001

The extracted DNA was checked by using anodrop (THERMO. USA) that measured DNA concentration (ng/µL) and checked the DNA purity by reading the absorbance at (260 /280 nm) as following steps:

- After opening up the Nanodrop software, choose the appropriate application (Nucleic acid, DNA).
- A dry chem-wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.
- The sampling arm was lowered and clicking OK to initialised the Nanodrop, then cleaning off the pedestals and 1µl of DNA were added to measurement.

4. PCR master mix preparation
   - (MyGene, Bioneer. Korea)
5. PCR Thermocycler Conditions
   - Bioneer/ Korea
6. DNA sequencing method
   - DNA sequencing method was performed for the study of confirmative identification of bacterial isolates based on multiple sequence alignment analysis and phylogenetic tree relationship analysis studies of local bacterial isolates with NCBI-Genbank Global isolates and determination of genetic changes based on single nucleotide substitution analysis, as well as submission the local isolates in NCBI-GenBank database. The sequencing of the PCR product 16S rRNA gene was purified from agarose gel by using (EZ EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic. Canada)

RESULTS

The results of urine culture showed that 60urine samples [60%]out of 100 samples appeared with no growth, but 40 samples [40%] revealed growth of *E.coli* 16 [40 %], *Proteus mirabilis* 12 [30 %] , *S.aureus* 5 [12.5%], *E.cloacae*5[12.5%], *Klebsiella pneumonia* 1 [2.5 %], and, *Pantoea spp 1[2.5%]*, in Table [2]. Table [2]: Results of a urine culture. The result of blood cultures among the 100 blood samples, 64samples [64%] shows no growth, but 36

Table 3: Antibiotic sensitivity testing for isolated bacteria

Type of antibiotics	Sensitivity No &% Type of isolated bacteria							
	E.coli (N=16)	Proteus (n=12)	Pseudo monas (n=12	Paste urella n=7	Staphelo coccus n=16	Strepto coccus n=2	Ent clo-acae n=9	Pontoae spp n=1
Imipenem	16(100)	10(83.33)	12(100)	7(100)	-------	-------	9(100)	1(100)
Nitrofu-rantion	5(31.25)	10(83.33)	12(100)	2(28)	12(75)	1(50)	7(77.77)	1(100)
Nalidaxic acid	6(37.5)	9(75)	---------	7(100)	-------	-------	3(33.33)	0(0)
Aztreonam	5(31.25)	6(50)	6(50)	5(71.42)	--------	--------	2(22.22)	0(0)
Gentamicin	8(50)	8(66.66)	2(16.66)	1(14.28)	6(37.5)	0(0)	8(88.88(	1(100)
Amikacin	10(62.5)	12(100)	12(100)	4(57.14)	14(87.5)	2(100)	6(66.66)	1(100)
Ciprofoxa-cin	5(31.25)	11(91.66)	12(100)	5(71.42)	12(75)	1(50)	7(77.77)	1(100)
Amoxicilin	6(37.5)	3(25)	2(16.66)	3(42.85)	-------	-------	4(44.44)	1(100)
Chloram phenicol	7(43.75)	10(83.33)	4(33.33)	0(0)	14(87.5)	2(100)	8(88.88)	0(0)
Cefepime	12(75)	9(75)	9(75)	5(71.42)	10(62.5)	2(100)	5(55.55)	1(100)
Tetracy-cline	2(12.5)	1(8.33)	2(16.66)	1(14)	14(87.5)	2(100)	2(22.22)	0(0)
Trime-thoprim	6(37.5)	6(50)	3(25)	2(28)	12(75)	2(100)	3(33.33)	1(100)
Ceftazidine	----	----- -	-------	-------	2(12.5)	1(50)	--------	------
Vancomy-cin	-------	-------	------	--------	13(81.25)	2(100)	--------	------
X^2	38.937*	44.308*	72.006*	31.399*	37.270*	9.975**	31.602*	11**
P value	<0.0001	<0.0001	<0.0001	0.001	<0.0001	0.353	0.001	0.358

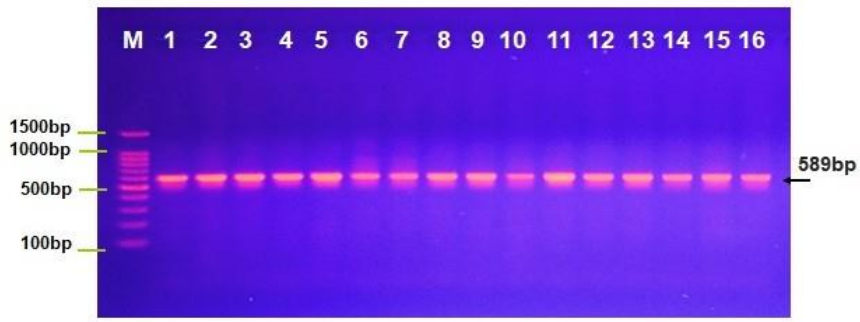

Figure 1: 16S rRNA gene for E. coli; Agarose gel electrophoresis image that show PCR product analysis for 16S ribosomal RNA gene in Escherichia coli isolates. M (Marker ladder 1500-100bp). Lane (1-16) Positive UTI Escherichia coli isolates at 589bp product size.

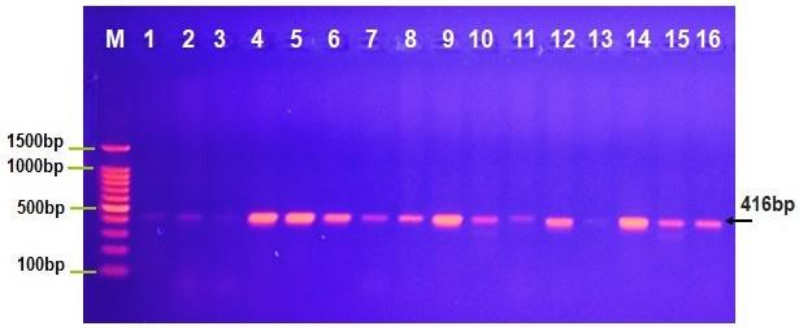

Figure 2: Virulence gene for E. coli; Agarose gel electrophoresis image that show PCR product analysis for pyelonephritis-associated pili (pap) gene in Escherichia coli isolates. M (Marker ladder 1500-100bp). Lane (4-12 and 14-16) Positive UTI Escherichia coli isolates at 416bp product size.

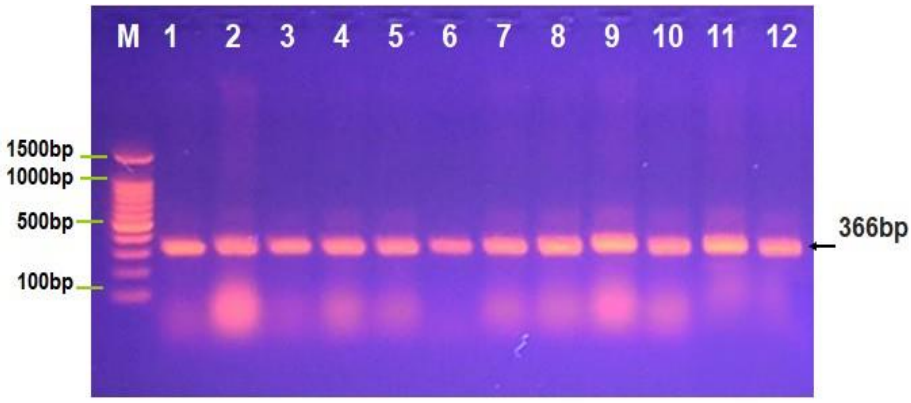

Figure 3: 16S rRNA gene for P. mirabilis; Agarose gel electrophoresis image that shows PCR product analysis for 16S ribosomal RNA gene in Proteus mirabilis isolates. M (Marker ladder 1500-100bp). Lane (1-12) Positive UTI Proteus mirabilis isolates at 366bp product size.

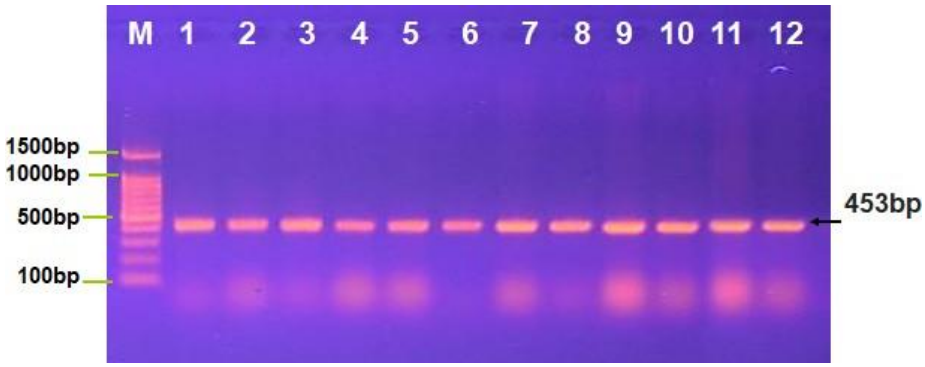

Figure 4: Virulence gene for P. mirabilis; Agarose gel electrophoresis image that shows PCR product analysis for UreC gene in Proteus mirabilis isolates. M (Marker ladder 1500-100bp). Lane (1-12) Positive UTI Proteus mirabilis isolates at 453bp product size.

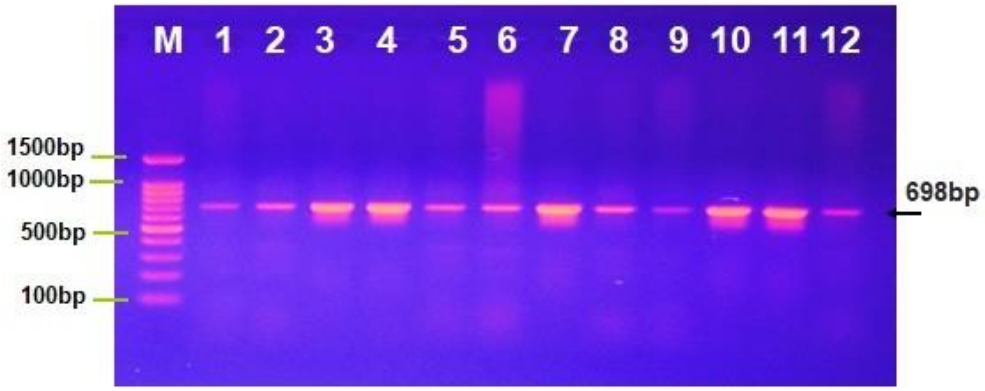

Figure 5: 16S rRNA gene for P. aeruginosa; Agarose gel electrophoresis image that shows PCR product analysis for 16S ribosomal RNA gene in Pseudomonas aeruginosa isolates. M (Marker ladder 1500-100bp). Lane (1-12) Positive UTI Pseudomonas aeruginosa isolates at 698bp product size.

samples [36%] appear bacterial growth, which included: - Pseudomonas aeruginosa 12[33.33%], & *Staphylococcus aureus 11[30.55] Pasteurella multocida* 7 [19.44%], Entero cloacae 4[11.11%], *Streptococcus spp* 2 [5.55%] in table [2].

Sensitivity test

The Kirby baure method is used to determine the effectiveness of various antibiotics by comparing results to an interpretive standard. In this study, the sensitivity test for all isolated was performed as follows;

Extraction of chromosomal DNA: The DNA was extracted from 47 isolates of [*E. coli* (16), *P.mirabilis* (12). *P.aeruginosa* (12), *P.multocida* (7)], by DNA extraction kit (Genaied – USA) and then detected by 16s rRNA gene by gel electrophoresis.

16S rRNA gene: The 16s rRNA gene was detected in all isolates [100%] percentage by gel electrophoresis.

A DNA sequence (phylogenic tree analysis): In this study two strains for each isolated bacteria include {E*. coli, P. mirabilis, P.aeruginosa, P.multocida }*, making these trees to compare the sequence

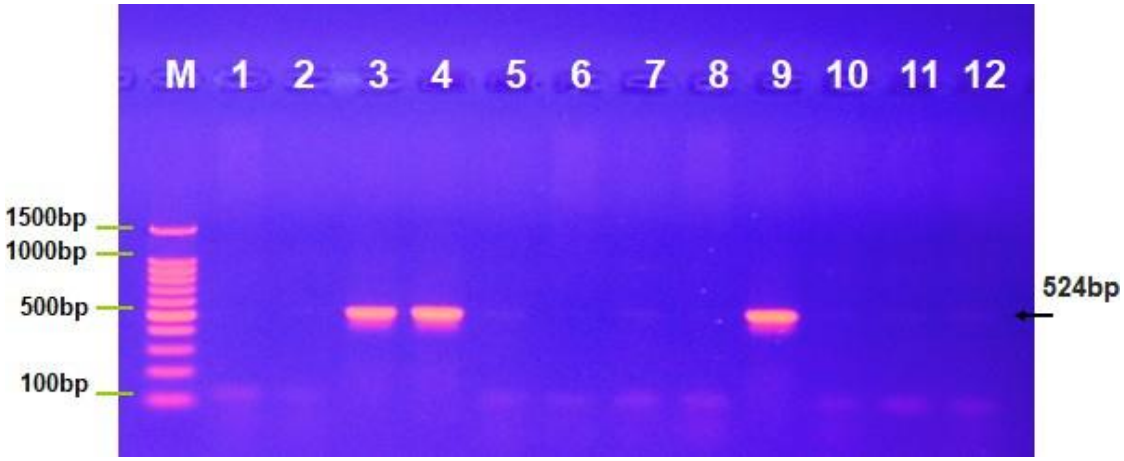

Figure 6: Virulence gene for P. aeruginosa; Agarose gel electrophoresis image that shows PCR product analysis for elastase LasB precursor (lasB) gene in Pseudomonas aeruginosa isolates. M (Marker ladder 1500-100bp). Lane (3, 4, and 9) Positive UTI Pseudomonas aeruginosa isolates at 524bp product size.

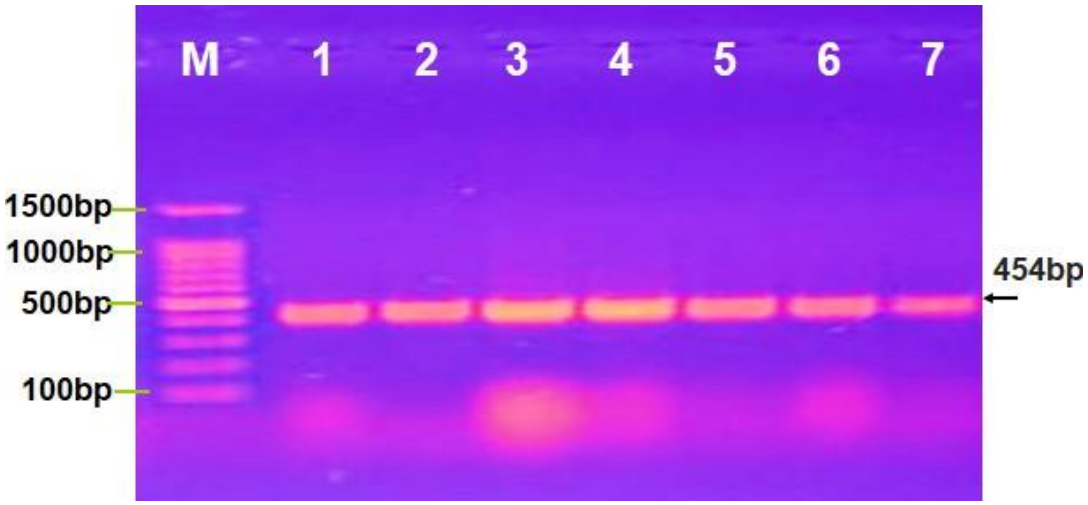

Figure 7: Virulence gene for P. multocida; Agarose gel electrophoresis image that shows PCR product analysis for the toxA gene in P.mutocida pneumonia isolates. M (Marker ladder 1500-100bp). Lane (1-7) Positive UTI P.mutocida pneumonia isolates at 300bp product size.

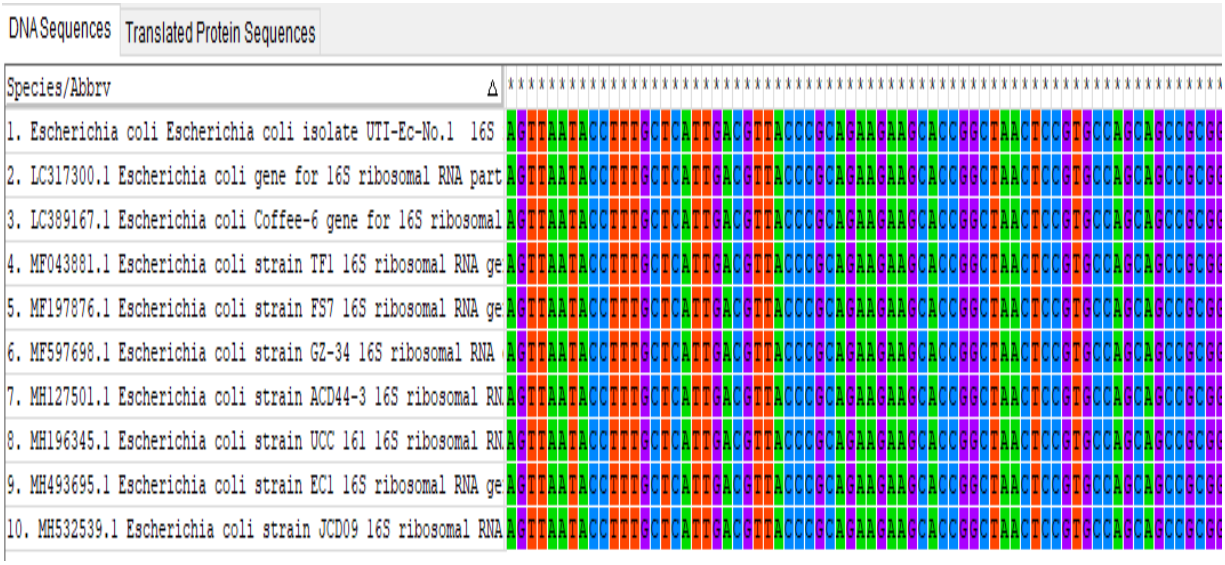

Figure 8: Multiple sequence alignment analysis of the partial 16S ribosomal RNA gene sequence for local Escherichia coli isolates (UTI-No.1) with NCBI-Blast Escherichia coli 16S ribosomal RNA gene by using (MEGA 6.0, multiple alignment analysis tools). The multiple alignment analysis similarity (*) and differences in 16S ribosomal RNA gene nucleotide sequences.

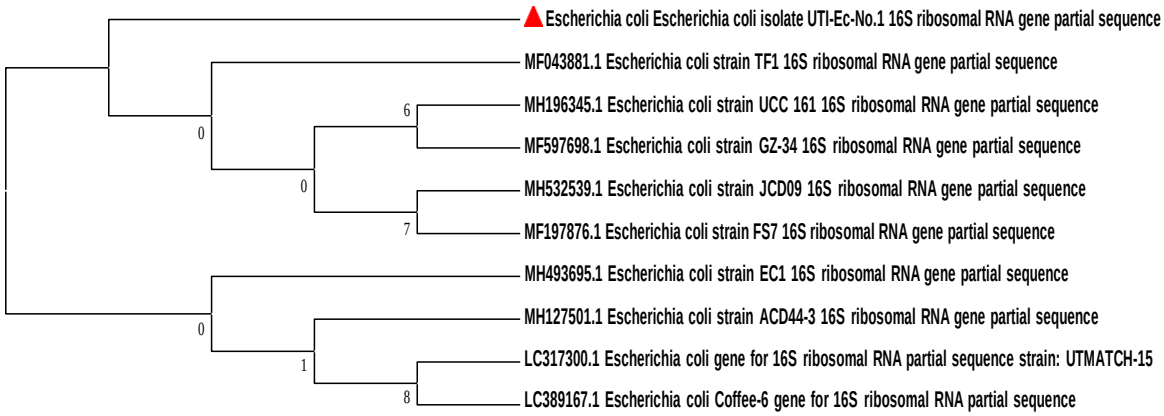

Figure 9: Phylogenetic tree analysis based on the 16S ribosomal RNA gene partial sequence used for confirmative detection study analysis. The phylogenetic tree was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0

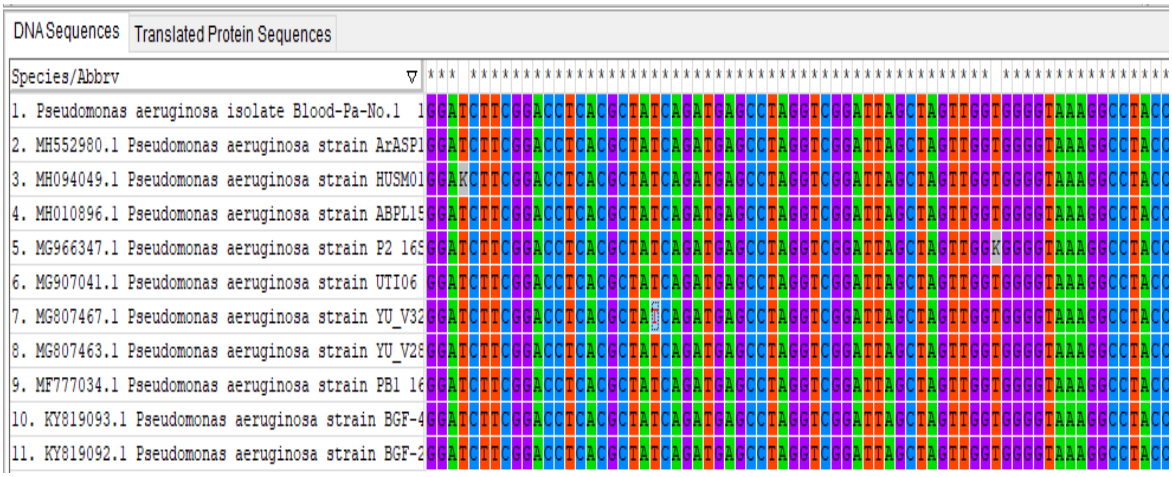

Figure 10: Multiple sequence alignment analysis of the partial 16S ribosomal RNA gene sequence for local Pseudomonas aeruginosaisolate (Blood-No.1) with NCBI-Blast Pseudomonas aeruginosa 16S ribosomal RNA gene by using (MEGA 6.0, multiple alignment analysis tools

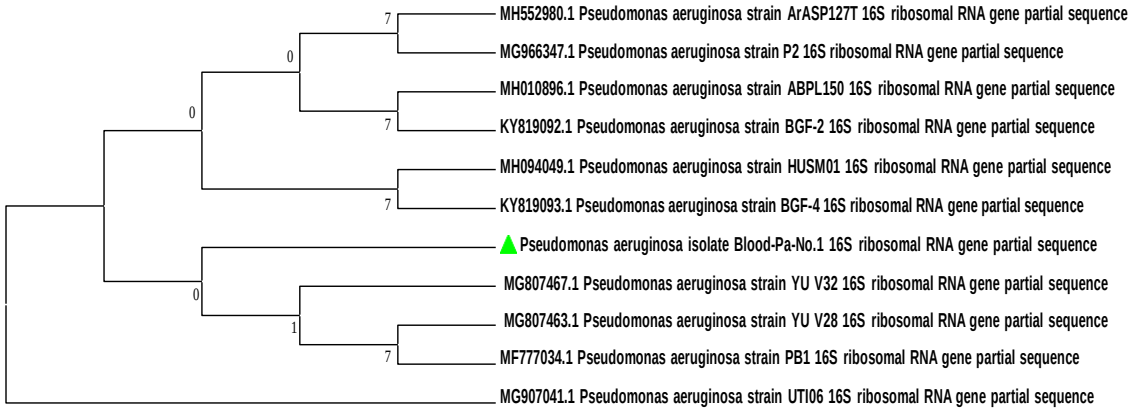

Figure 11: Phylogenetic tree analysis based on the 16S ribosomal RNA gene partial sequence used for confirmative detection study analysis. The phylogenetic tree was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0).

with multiple copies of 16srRNA genes that we can find in single bacterial genomes. We compare the 16srRNA gene from multiple species of bacteria to see how much these genes differ between a variety of bacteria; these results appear as follows;

DISCUSSION

Antimicrobial sensitivity test for isolates from blood and, urine hemodialysis patients form blood, and urine samples. These organisms due to a variety of infections include bacteremia and UTI. (Meharwal, et al., 2002). Antibiotic resistance is a main

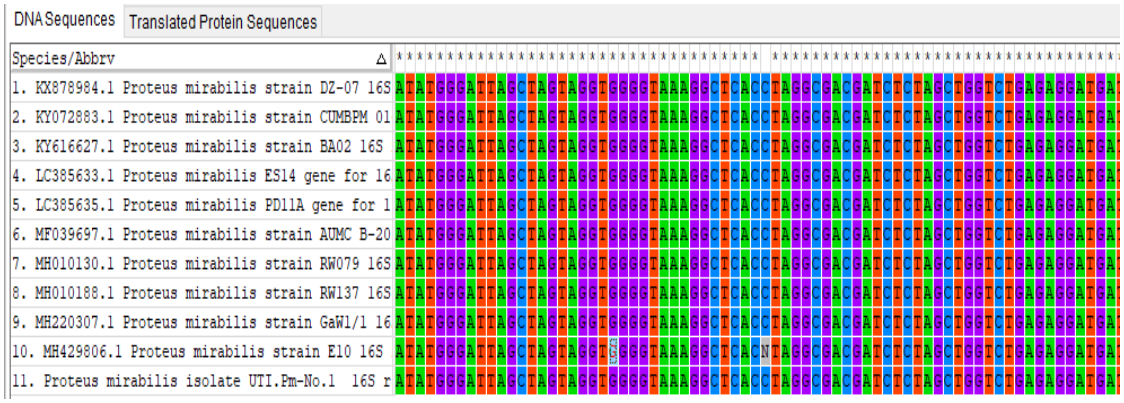

Figure 12: Multiple sequence alignment analysis of the partial 16S ribosomal RNA gene sequence for local Proteus mirabilis isolate (UTI-No.1) with NCBI-Blast Proteus mirabilis 16S ribosomal RNA gene by using (MEGA 6.0, multiple alignment analysis tools). The multiple alignment analysis similarity (*) and differences in 16S ribosomal RNA gene nucleotide sequences.

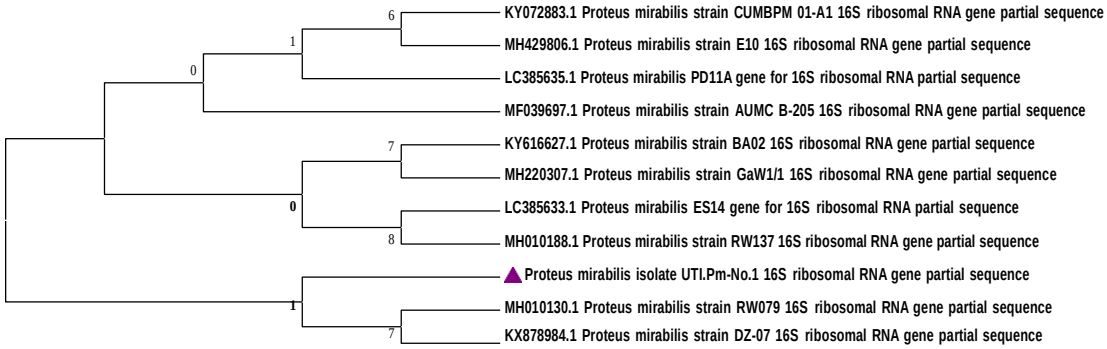

Figure 13: Phylogenetic tree analysis based on the 16S ribosomal RNA gene partial sequence used for confirmative detection study analysis. The phylogenetic tree was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local Proteus mirabilis isolates (UTI-No.1) showed closed related to NCBI-Blast Proteus mirabilis (MH010130.1).

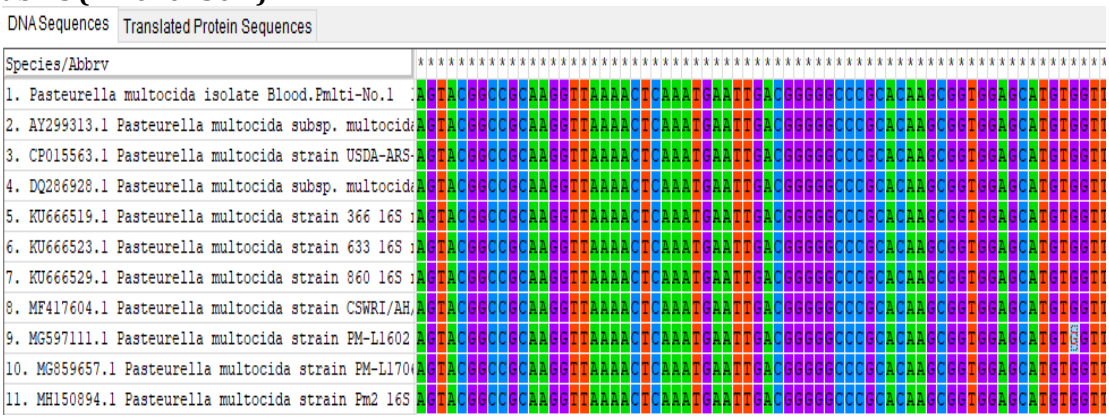

Figure 14: Multiple sequence alignment analysis of the partial 16S ribosomal RNA gene sequence for local Pasteurella multocida isolate (Blood-No.1) with NCBI-Blast Pasteurella multocida 16S ribosomal RNA gene by using (MEGA 6.0, multiple alignment analysis tools). The multiple alignment analysis similarity (*) and differences in 16S ribosomal RNA gene nucleotide sequences.

The clinical problem in treating infections due to these microorganisms. The resistance to the antimicrobials was increased over the years. Resistance rates vary from country to country due to the different strains of these microorganisms.

In table [3-5]. *E. coli* appears sensitivity [100%] for Imipenem, but sensitive to Cefepime [75%], Amikacin [63%], Nitrofurantoin for urine isolate [32%], Ciprofloxacin [31%], Nalidixic acid[38%], Gentamicin[50%], , Amoxicillin, and clavialunic acid [37%], Aztreonam [31%], Chloramphenі-

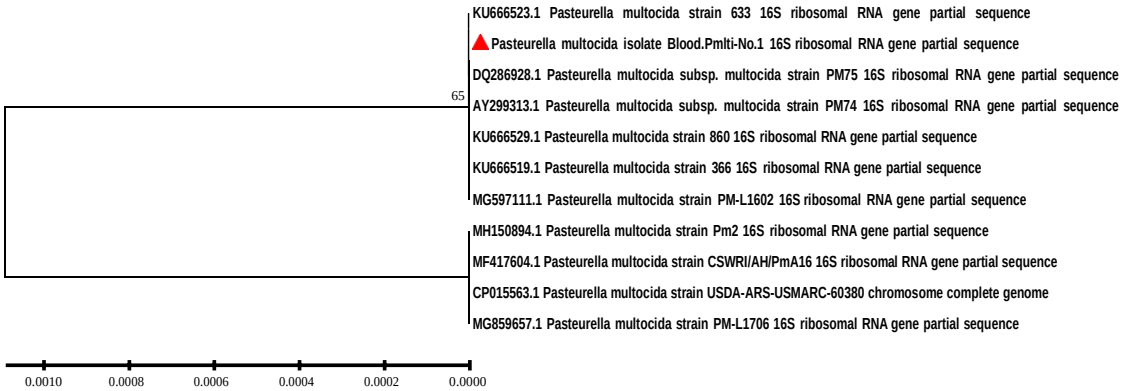

Figure 15: Phylogenetic tree analysis based on the 16S ribosomal RNA gene partial sequence used for confirmative detection study analysis. The phylogenetic tree was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local Pasteurella multocida (Blood-No.1) showed closed related to NCBI-Blast Pasteurella multocida (KU666523.1).

Table 4: NCBI BLAST Homology sequence identity for 16rRNA gene between local bacterial isolates and NCBI-Blast isolates

Local bacterial isolates	Genbank accession numbers	NCBI BLAST Homology sequence identity	
		NCBI-BLAST identical isolates	Identity (%)
Escherichia coli UTI-No.1 isolate	MH654987	MF043881.1	100%
Pseudomonas aeruginosa Blood Isolate No.1	MH654988	MG807467.1	100%
Proteus mirabilis UTI-No.1 isolate	MH654989	MH010130.1	100%
Pasteurella multocida Blood Isolate No.1	MH654990	KU666523.1	100%

col[44%] and Sulphamethoxazole Trimethoprim[37%]. While resistance [88%]to Tetracycline only.

These results were agreement with the previous studies (Mahmood, 2011) in Alnahrain -Iraq, (Al-Mayahie &Al-Kuriashy, 2016) in Wasit -Iraq, and (Al-Mijalli, 2017)in Saudi Arabia, but disagreement with (Cunha, et al., 2016) in Brazil, and(Osman, et al., 2017) in Lebanon, because of the sensitivity to Nitrofurantoin [93-98%], Azethronem [62%], Amikacin [94-99%], Amoxicillin-clavulanic acid [79-82%], Trimethoprim-Sulphamethoxazole [49-55%], and Azethronem [62%], while in this study the results sensitivity average between [22.2 - 55.5%], these results were different from other countries may be due to the difference in strain of bacteria.

In table [3-5] *P. mirabilis* revealed (100%) sensitive for Amikacin, Cefepime 75%, Ciprofloxacin 92%, Chloramphenicol 83%, Imipenem (83%), Aztreonam 50%, and Trimethoprim 50%, to Tetracycline 50%, Nalidixic acid 75%, Nitrofurantoin 83.3%, and Gentamicin 67%. while resistance to Amoxicillin 75%. These results were agreement with (Al-Samarai &Ali, 2016) in Kirkuk - Iraq, and (Tawfeeq, 2014) in Krikuk -Iraq, but disagreement with (Mashouf, et al., 2009) in Iran, (Khan, et al., 2014) in Pakistan, and (Osman, et al., 2017) in Lebanon because of the sensitivity to Imipenem [80-100%], Amikacin [40 -93%], Gentamicin [40-100%], ciprofloxacin [0 -86%], and Amoxicillin-clavulanic acid [60-83%], while in this study sensitivity to Imipenem [50%], Amikacin [100%], ciprofloxacin [100%], and Amoxicillin-clavulanic acid [100%] while gentamicin resistance [100%], these results were difference of results from other countries may be due to the difference in the strain of bacteria.

In table [3- 5]. *Pseudomonas aeruginosa* shows sensitivity [100%] of isolates to Nitrofurantoin and Imipenem. Ciprofloxacin, and Amikacin but, sensitive to Aztreonam [50%]and Cefepime 71%, While resistance [100%]to Chloramphenicol, Gentamicin, Tetracycline, amoxicillin, and Trimethoprim. These results were agreement with the previous studies [Al-Marzoqi et al., 2008] in Hillah -Iraq, (Hussein et al., 2015) in Baghdad-Iraq, and (Vijayalakshmi, 2016) in India, (Dickson, 2016) in Ghana, but disagreement from results with(Ghadiri, et al., 2012) in Iran, and (Vanitha, et al., 2012) in Chennai because resistance to Imipenem [60-80%], ceftazidime[40-60%], Amikacine [20-40%], and ciprofloxacin [13-40%] while in this study the resistance average between [0-25%]. The misuse of

antibiotics and, isolated strains and, other environmental factors cause their differences.

In table [3- 5]. *Pasteurella multocida* shows sensitivity [100%] of isolates to Nalidixic acid and Imipenem. Ciprofloxacin, Aztreonam while Cefepime [71%]. But sensitive to Amikacin [57%]. However, resistance to amoxicillin Nitrofurantoin, Gentamicin, Trimethoprim and Tetracycline 42%, 28%, 14%, respectively. While resistance [100%]to Chloramphenicol.

The results of this study were agreement with (Doman et al., 2003) in Germany, (Rahmani et al., 2006) in Iran, (ElGamal, et al., 2013) in Egypt, (Shbib and Soukkarieh, 2015) in Syria and (Pourzare, et al., 2016) in Iran, all *E. coli* identified using sequencing 16S rRNA gene confirmed this species.

REFERENCES

Al-Marzoqi, A.H.; Shemmran, A.R.; Hindi, Z.Sh.; Al Taee, Z.M.; &Al-Nafee.M.K. Bacterial& Viral infection associated with thalassemia patients in Hillah city, Babylon University /College of Science for Woman.2008.

Al-Mayahie, S.M.G.; & Al-Kuriashy, J.J.H. Distribution of ESBLs among Escherichia coliisolates from outpatients with recurrent UTIs & their antimicrobial resistance. Wasit –Iraq.J Infe Dev Ctries.2016; 10[6]:575-583.

Al-Mijali, S.H.S. Bacterial Uropathogens in Urinary Tract Infection of Antibiotic Susceptibility Pattern Riyadh Hospital, Saudi Arabia.IMedpub Journals.Cellular &Molecular Medicine .2017; Vol 3, No.1:

Al-Samarai, A.; & Ali, S. Urinary tract infection in a female in Kirkuk city, Iraq: Causative Agent &Antibiogram. World Journal of the College of Pharmacy & Pharmaceutical Science .2016; Vol.5, Issue ^, 261-273.

Cunha, M.A.; Assuncao, G.L.M.; Medeiros, I.M.; &Feritas, M.R. Antibiotic resistance patterns of urinary tract infections in a northeastern Brazilian capital.Rev Inst Med Trop Sao Paulo [2016]; 58.2.

Dickson, O.W.K. Characterization of *Salmonella* & other Gram-negative Bacterial Pathogens obtained form stool &blood at cape coast teaching Hospital. MSc. Kwame Nkrumah University of Science &technology, Ghana 2016.

Domann, E.; Hong, G.; Imirzalioglu, C.; Turchner, S.; Kuhle, J.; Watzel, C.; Hain, T.; Hossian, H.; & Chakraborty, T. Culture-Independent Identification of pathogenic bacteria &Polymicrobial Infections in the Genitourinary Tract of Renal Transplant Recipients.Germany. Journal of Clinical Microbiology .2003; Vol.41.no.12.

Elgaml, A.; Hassan, R.; Shokralla, S.; & El-Naggar, W. Analysis of 16s rRNA ribosomal RNA gene seg- ments for diagnosis of Gram-negative pathogenic isolated from urinary tract infections.African Journal of Microbiology research. Egypt. 2013; Vol.27[33]. pp.2862-2869.

Ghadiri, H.; vaez, H.; Khosravi, S.; & Soleymani, E. The Antibiotic Resistance Profiles of Bacteria Stain from Patients with Hospital-Acquired Blood Stream &Urinary Tract Infection.Iran. Critical care research & practice. 2012; Articale ID890797. 6 pages.

Hussein, V.H.; Taha, B.M.; Rassol, K.H.; & Hussein, J.D. Study the Prevalence & Antimicrobial Susceptibility Pattern of Bacteria Isolated from Blood Culture of Children in Baghdad. Paripex – Indian Journal of Research.2015; Vol.4, Issue: 6.

Khan, I.U.; Mirza, I.A.; Afzal, A.; Ali, S.; Hussain, F.M.; & Ghafoor, T. Antimicrobial Susceptibility Pattern of Bacteria Isolated from Patients with Urinary Tract Infection Journal of the College of Physicians & Surgeons Pakistan.2014; Vol.14[11]:840-844.

Lysaght, M.J.Maintenance dialysis population dynamic: current trends and long –term implications.J.of the Amer. Soci.of nephrol., 13, 37-40.2002.

Mahmood. M.A Prevalence and antimicrobial of pathogens in Urinary tract infections.Journal of Al-nahrain University.Vol. 14 [4] pp.146-152.2011.

Mashouf, R.Y.; Babalhavaeii, H.; & Yousif, J. Urinary tract infections: Bacteriology &Antibiotic Resistance Pattern, Iran.2009; Vol.46.

Mehrawal, S.K., Taneja, N. Sharma, S.K, and Shama, M. Complication nosocomial UTI caused by non-fermenters. Indian J Urol: 18:123-128, 2002.

Narula, C.A.;Hooda, C.A. Conservative management of chronic renal failure M.J.A.F.I. 2007. 63(2) 56-61.

O'Shaughnessy, D. An introduction to hemodialysis.Fresenius Medical, Care, 1st ed (2005), pp P 5-7.

Osman, M.; Mallate, H.; Hamze, M; &Raad, E.B. Prevalence & antibiotic susceptibility pattern of bac- teria causing urinary tract infections in Youssif Hospital centre. The first report from AHKar gov- ernorate, North Lebanon. The Internation Arabic Journal of Antimicrobial Agents.iMedpub Journals .2017; Vol.7, No.1:2. ISSN 2174-9094.

Pourzare, M.; Derakhshan, S.; & Roshani, D. Distribution of uropathogenic virulence genes in Escherichia coli isolated from children with urinary tract infection in sanadaj, Iran.2016.

Rahmani, S.; Forozandeh, M.; Mosavi, M.; & Rezee, A.Detection of bacteria by amplifying the 16s rRNA gene with universal primers & RFLP, Medical Journal of the Islamic Republic of Iran.2006. ; Vo.19, No.4.

Rubin, N.T., Treatment of irreversible renal failure in Arend, Armitage, Clemmons, Drazen, Griggs, Larusso, Cecil Textbook of Medicine 23rd ed, Chapter 133 Saunders.pp:936-4.2007.

Salih, B.J.; Salim, A.A.; Rahi, K and Hasan.F.O. Acute Renal Failure in children below 12 years causes clinical presentation and management. Iraqi medic.J.1 (1), 2009, 33-39.

Stevens, A and Lowe, J. Pathology, 2nd ed, mosby, Edingborough pp. 350-375, 2000.

Tawfeeq, A.A. Prevalence & Antimicrobial Sensitiv- ity of Uropathologenic Bacteria in a group of Patients in Kirkuk city. Al-Taqani.2014; vol.27, No.2.

Tortora, G and Grabowski, S.R. Principles of Anatomy and Physiology, 9th ed, JohnWiley and Sons, NewYork, 2000, pp 914-954.

Vanitha, R.N.; Kannan, G.; Vankata, N.M.; Vishwakanth, D.; Nagech, V.R.D.; Yogitha, M., Venkata, S.M.; & Thennarasu, P. A Retrospective study on bloodstream infections & Antibiotic Susceptibility in a Tertiary care Teaching Hospital. Chennai. International Journal of Pharmacy & Pharmaceutical science.2012; Vol.4, Issue 1.

Vijuyalakshmi, P. A study on rare cases & bacterial infection in children. Journal of Medical Science &Clinical Research .2016; Vol.[4]. Issue [08]. page 12196-2300.

Zilva, F.; Pannall., and Mayne, P.H. Clinical chemistry in diagnosis and treatment, 6th ed London, Arnold, 1994. Pp;133-398.