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% bacteraemia. The main bacterial isolates from urine samples represented Escherichia coli (40%), and Proteus mirabilis (11.1), Staphylococcus aureus (12.5%), Enterocloacae (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 multocida (19.44%), Enterocloacae (11.11%), and, Streptococcus spp (2.55%). Antimicrobial sensitivity for Pseudomonas aeruginosa appeared sensitivity for Amikacin, Ciprofloxacin, Ceftazidime, and imipenem while Pasteurella multocida 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.

Keywords:
Bacterial Infection, Hemodialysis Patients, Wasit, Iraq

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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 electrolyte 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 Providence 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 H2S, 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 Simon’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 pastuereellaceae 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 (Genaid – 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

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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)

RESULT

The results of urine culture showed that 60 urine 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%], *Saureus* 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, 64 samples [64%] shows no growth, but 36

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**Table 1: list of sequence primer gene**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'-3')</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S-rRNA gene <em>E.coli</em></td>
<td>F: GGAACCTGAGCACGGGTCCAG</td>
<td>589bp</td>
</tr>
<tr>
<td></td>
<td>R: TTTAACCTTGCGGGCCGTACT</td>
<td></td>
</tr>
<tr>
<td><em>E.coli</em> Pap gene</td>
<td>F: CGCAGTGAGGGCATCCTATT</td>
<td>416bp</td>
</tr>
<tr>
<td></td>
<td>R: ACGGAGTGAAGCAGAAATGTT</td>
<td></td>
</tr>
<tr>
<td>16S-rRNA gene <em>P.mutocida pneumoniae</em></td>
<td>F: TGATAAAATGACGGCCTGGG</td>
<td>454bp</td>
</tr>
<tr>
<td></td>
<td>R: AGTTCGAGACTCAATCCGGG</td>
<td></td>
</tr>
<tr>
<td><em>P.mutocida pneumoniae</em> toxA gene</td>
<td>F: GCTTTTCGGGATATGCTG</td>
<td>300bp</td>
</tr>
<tr>
<td></td>
<td>R: TCAATAGGGCTCTCAACGCT</td>
<td></td>
</tr>
<tr>
<td>16S-rRNA gene <em>P. mirabilis</em></td>
<td>F: CTTCTTTGAGTCCGGCAT</td>
<td>366bp</td>
</tr>
<tr>
<td></td>
<td>R: ATACCCCTGTAGTCACGCT</td>
<td></td>
</tr>
<tr>
<td>UreC <em>P. mirabilis</em></td>
<td>F: CAAGCCCAAGAAGTCTCGT</td>
<td>453bp</td>
</tr>
<tr>
<td></td>
<td>R: ATTTGGCTCTCTCAGCATC</td>
<td></td>
</tr>
<tr>
<td>16S-rRNA gene <em>P. aeruginosa</em></td>
<td>F: TCAACCTGGGAACTCGATCC</td>
<td>698bp</td>
</tr>
<tr>
<td></td>
<td>R: CAGACTGCGATCGGACTAC</td>
<td></td>
</tr>
<tr>
<td>lasB gene <em>P. aeruginosa</em></td>
<td>F: ACTACAAGCAGTGACCGGA</td>
<td>524bp</td>
</tr>
</tbody>
</table>

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**Table 2: Results of blood culture**

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>Number &amp; percentage</th>
<th>Type of bacteria</th>
<th>Number &amp; percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em></td>
<td>12(33.33)</td>
<td><em>E.coli</em></td>
<td>16(40)</td>
</tr>
<tr>
<td><em>Paste. multo</em></td>
<td>7(19.44)</td>
<td><em>P. mirabilis</em></td>
<td>12(30)</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>11(30.55)</td>
<td><em>staphylococcus</em></td>
<td>5(12.5)</td>
</tr>
<tr>
<td><em>Ent. clo</em></td>
<td>4(11.11)</td>
<td><em>E. cloa</em></td>
<td>5(12.5)</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>2(5.55)</td>
<td>*Pant.</td>
<td>1(2.5)</td>
</tr>
<tr>
<td><strong>------</strong></td>
<td><strong>------</strong></td>
<td><strong>Kleb.</strong></td>
<td><strong>1(2.5)</strong></td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>total</td>
<td>40</td>
</tr>
<tr>
<td><em>X²</em></td>
<td>12.924*</td>
<td>33.360*</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 3: Antibiotic sensitivity testing for isolated bacteria

<table>
<thead>
<tr>
<th>Type of antibiotics</th>
<th>Sensitivity No &amp;%</th>
<th>Type of isolated bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.coli (N=16)</td>
<td>Proteus (n=12)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>16(100)</td>
<td>10(83.33)</td>
</tr>
<tr>
<td>Nitrofurantion</td>
<td>5(31.25)</td>
<td>10(83.33)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>6(37.5)</td>
<td>9(75)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>5(31.25)</td>
<td>6(50)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8(50)</td>
<td>8(66.66)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>10(62.5)</td>
<td>12(100)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5(31.25)</td>
<td>11(91.66)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>6(37.5)</td>
<td>3(25)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>7(43.75)</td>
<td>10(83.33)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>12(75)</td>
<td>9(75)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2(12.5)</td>
<td>1(8.33)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>6(37.5)</td>
<td>6(50)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>X²</td>
<td>38.937*</td>
<td>44.308*</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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.

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 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.
**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.
with multiple copies of 16s rRNA genes that we can find in single bacterial genomes. We compare the 16s rRNA 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. (Meiharwal, et al., 2002). Antibiotic resistance is a main.
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%], Ciprofloxacine [31%], Nalidixic acid [38%], Gentamicin[50%], Amoxicillin, and clavulanic acid [37%], Aztreonam [31%], Chlorampheni-
These results were agreement with the previous studies (Mahmood, 2011) in Alnahrain -Iraq, (Al-Mayahie &Al-Kuriashy, 2016) in Wasit -Iraq, and (Al-Mjalll, 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 Kirkuk -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 (Vanhata, 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

![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).](image_url)
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 Imipen. Ciprofloxaxin, 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 (Domann et al., 2003) in Germany, (Rahmani et al., 2003) 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.

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