The most frequent bacteria in hemodialysis patients in Basra-Iraq

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Abstract

Kidney failure is one of the acute chronic diseases and it is the end of kidney disease, resulting in the body's inability to get rid of toxins, waste, and excess fluids. Here, either undergo a kidney transplant, or permanent dialysis, consequence the patient becomes vulnerable to bacterial and viral infections.75 samples were collected from patients undergoing hemodialysis (HD) in Al-Sadr Teaching Hospital in Basrah. with age ranged between 16 - 70 years old. The samples from water entering the dialysis machine and water leaving the patient, in addition to sample from HD patient's blood. Conventional microbiological and molecular approaches were applied. Bacteria were identification: 66 were Gram positive (72.5%) and 25 were Gram negative bacteria (27.4%). The 16S rDNA gene of 91 isolates was successfully amplified, sequenced, and proofreaded, the results showed that the bacterial species were belong to : *Bacillus* 46 (50.5%) included 9 species; *B. cereus* was the highly frequented (29) isolates , *B.paramycoides* 5, *B. safensis* 3,*B. lichenformis* 3, *B. subtilis* 2, whereas all the other *Bacillus* species frequency was 1 isolate for each as it will be demonstrated later, *Staphylococcus* 9, included *S. epidermidis* 4, while all the other *Staphylococcus* species frequency was 1 isolate for each, *Pseudomonas* 11,included *P. stutzeri* 9 , *P. putida* 1, and *P. aeruginosa* 1,*Enterobacter* 5, included *E.cloacae* 3, *E.xiangfangensis* 1,*E.asburiae* 1, *Enterococcus* 4included *E.casselifarus* 2, *E.faecium* 2. The present study was recorded the isolation and identify of three *Aerococcus* included *A. viridans* 2, *A. urinaeequi* 1 which it for our knowledge the first report of such bacteria in HD in Basrah-Iraq. In addition, *Lysinibacillus macrolides* 3, *Halomonas stevensii* 3, *Acinetobacter radiorsistens* 2, whereas *Stenotrophomonas rhizophila*, *Exiguobacterium aestuarii*, *Shigella sonnei*, *Klebsiella oxytoca* and *Margonella morganii* each of them 1. Out of 91 isolates fourteen new isolates were recorded as new strains in the National Center for Biotechnology Information (NCBI) and the Gene bank DNA sequences. The phylogenetic tree showed affinity between most of the studied isolates. Most of the 91 isolates were resistance to most of the antibiotics such as Ceftazidime and Cefepime more than Cefotaxime, Ceftriaxone, and Vancomycin. Among the ninety-one isolates that were tested for biofilm production on Congo red agar, (14) isolates gave positive result. However, the results of RAPD-PCR for 26 antibiogram closely related isolates, *B. cereus* were shown to be genetically close. As for the 4 isolates of *P. stutzeri* two of four were showed to be closely related, as well as two isolates of *S. epidermidis* showed genetically identical. The study has recommended that hemodialysis facilities' water supplies should be continuously monitored and that appropriate preventative measures be implemented to reduce the risk of these immunocompromised patients being exposed to polluted water sources.

Keywords: Renal failure, Hemodialysis, Bacterial infection, RAPD-PCR

1. Introduction

Renal failure occurs when the kidneys are damaged to the point that they are no longer able to do their functions. There are two types of renal failure, acute and chronic. Acute renal failure (ARF) occurs when the kidneys' ability to purify blood of harmful wastes and excess salt is impaired [1]. Chronic renal failure (CRF) is the last stage of kidney disease because it generally starts slowly, gradually, and catastrophically. In most cases, there are no underlying signs that signal kidney damage or illness until it has progressed to the point of kidney failure. Diabetes, high blood pressure, some immunological diseases, some genetic diseases, and other factors can all contribute to chronic kidney disease failure [2]. End-stage kidney disease (ESKD) is a huge healthcare burden across the world, necessitating expensive renal replacement therapy such as dialysis or transplantation. However, the availability of healthy donors' limits kidney transplantation as a therapy option [3].

Hemodialysis HD is a life-sustaining therapy for those whose kidneys aren't functioning correctly. A catheter is inserted into a big vein and the individual is attached to a dialysis machine, which cleans the blood and returns it to the person [4]. As a direct result of uremia and its metabolic ramifications, patients on hemodialysis have immune system abnormalities, rendering them more susceptible to infections. Poor antigen processing, antibody production, and cell-mediated immune response are all affected by defects in neutrophils, lymphocytes B and T, and monocytes, for example, resulting in an increase in the incidence of microbial infections [5]. Patients are exposed to quantities of water ranging from 15000 to 20000 L of dialysis fluid annually and non-selectively during treatment sessions that last between 4 and 6 hours, three times a week. As a result, all low-molecular-weight compounds in water have direct access to the patient's bloodstream. Dialysis patients are at an increased risk of infections [6]. Most of the bacteremia, sepsis, and pyrogenic responses acquiring

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during hemodialysis are caused by the failure of water treatment systems, such as filtration and reverse osmosis, as well as loop and machine disinfection procedures [7]. Bloodstream infection is one of the most serious consequences in HD patients. HD patients are 26 times more likely than the general population to develop bacteremia, which increases the risk of systemic infections, hospitalization, and patient death [8]. The Centers for Disease Control and Prevention (CDC) and the American Society of Nephrology (Transforming Nephrology Dialysis Safety Initiative) have focused on developing methodologies for preventing bloodstream infections in HD patients, implying that prophylactic measures to reduce infections in these patients are needed [9].

Bacterial development in hemodialysis water is dependent on the kind of water treatment system, dialysate distribution systems, dialysis machine type, and disinfection method utilized [10]. To ensure patient safety, the water used for HD must be of high quality. One of the most serious problems faced the dialysis patients is bacterial infection, so the aim of the study; is to investigate the microbiological quality of the dialysis water used at hemodialysis center by isolating and identifying the most frequent gram-negative and gram –positive bacteria from in and out washing machine in addition to isolate bacteria from hemodialysis patient blood.

2. Material and Methods

2.1. Isolation and Identification

A total of (75) samples were collected from patients undergoing hemodialysis in Al-Sadr Teaching Hospital at the period from November 2020 to February 2021. The age of patients ranged between 16 - 70 years old. Water samples were collected in sterilized containers, where one liter of water entering the dialysis machine and one liter of water leaving the patient. The water samples were transferred to the laboratory; the water was filtered using a filter paper with 0.45µ m pores. Then the filter paper was placed in brain heart infusion broth (BHIB) and incubated at 37ºC for 24h or until the appearing of turbidity. In addition, one ml of blood was taken from each patient after obtaining consent from the patient who were either with or without symptoms such as fever, chills, vomiting and abdominal pain. Blood was taken before the patient has given Anti-coagulation substance or antibiotic

Samples were inoculated directly into 10 ml of brain heart infusion broth in sterile condition, after that, they were transferred to the biological department's laboratory and incubated at 37 °C for 7 days or until the appearing of turbidity. Then BHIB for all samples were cultured on Blood agar, Mannitol salt agar and MacConkey agar. The cultured plates were incubated at 37ºC for 24 h, subculture was performed each 24 h. The grown sub cultured colonies were stained with Gram's stain then Oxidase and catalase assays were performed [11].

2.2.DNA extraction and 16S rDNA gene amplification

One hundred and eighteen bacterial DNA was successfully extracted from gram-positive and gram-negative bacteria using DNA extraction kit (Geneaid Presto™ Mini gDNA bacteria kit) and then bacterial isolates were identified by amplifying 16 S rDNA gene using universal primers 27F5´-AGAGTTTGATCCTGGCTCAG3´,1492R5´GGTTACCTTGTT ACGACTT-3´ [12]. Reaction was done in 50 µl,25 µl of Master mix, 19 µl of nuclease-free water, 2 µl of each DNA template, forward and reverse primers. The mixture was run as follow: Initial denaturation 1 cycle of 95°C for 5 min, (95°C for 30 sec,55°C for 30 sec,72°C for 1min, each of them for 35 cycles), followed by final extension at 72°C for 5 minuts. The PCR products were visualized by agarose gel electrophoresis. Twenty µl of 16S rDNA PCR product of each sample was labeled with a number identical to the number of excel sheet and sent to Microgen company " http://dna.macrogen.com . for sequencing.

Bacterial species were identified by using the coming back sequences after trimming and proofreading and use of Basic Local Alignment search tool (BLAST) in the National Center for Biotechnology Information (NCBI) " http://www.ncbi.nlm.nih.gov [13]. After concatenation, by adding all the corrected nucleotide sequences of the bacterial species together multiple alignment was performed. Then using UPWGA choice the tree was viewed in mega X as rooted tree The Phylogenetic tree of bacterial species was constructed and drawing by Mega X program.

2.3. Biofilm production and Antibiotic sensitivity

All the identified strains were tested for biofilm formation using Congo red agar method and disc diffusion method for detection antibiotic susceptibility. Nine antibiotics were used (Vancomycin 30 µg, Ceftazidime 30 µg, Ceftriaxone 30 µg, Cefepime 30 µg, Cefotaxime 30 µg, Erythromycin 15µg, Ampicillin 30µg, Gentamicin10µg, Methicillin 5µg).

2.4. Random amplified polymorphic DNA (RAPD) PCR

The 26 isolates that have the same results of the antibiotic susceptibility tests were subjected to RAPD-PCR test to determine the identical strains.

RAPD-PCR procedure and primers for *Bacillus cereus*, were based on [14]. The OPR13 (5′- GGA CGA CAA G–3′) primer was used. The RAPDPCR experiment was carried out in a 25- µl reaction tube containing 12.5 µl of Master mix, 11 µl of nuclease-free water, 0.5 µl of primer, and 1 µl of DNA template. An initial denaturation step at 94°C for 4 minutes was followed by 40 cycles of DNA

denaturation at 94°C for 1 minute, primer annealing at 35°C for 1 minute, and DNA extension at 72°C for 2 minutes, followed by a final extension step at 72°C for 5 minutes

RAPD-PCR. for *P. stutzeri* was according to Sikorski et al. [15], experiment was carried out with 25 µl volumes containing 1 µl of DNA following primers (0.5 µl): (i) (5´-CGAGCTTCGCGTACCACCCC-3´), (ii) (5´-GTTTCGCTCGATGCGCTACC-3´)12 µl of Master mix, 11 µl of nuclease-free water the program was 4 cycles for 5 min each at 94, 40, and 70 °C were run, followed by 30 cycles for one minute each at 94 and 55 °C and two min at 70 °C, with a final primer extension cycle for five min at 70 °C.

RAPD-PCR for *S. epidermidis* was done based on Olorunfemi et al. [16]. primers (5'-TCGCCAGCCA-3') and (5'-GACACGGACC-3') in a 20- µl reaction mixture using 5 µl of genomic DNA and 5 µl of master mix 1.5 µl of each primer was employed in a reaction with 7 µl of free water. 1 cycle at 94°C for 3 minutes, 45 cycles of 94°C for 1 minute (denaturation), 36°C for 1 minute (annealing), and 72°C for 2 minutes (extension), and a final extension at 72°C for 7 minutes. Electrophoresis in a 2 % agarose gel at 60V for 1.30 h. resolved the reaction products.

RAPD-PCR of *E. casselifavus* was run according to Cocconcelli et al. [17] in a total volume of 25 µl, reactions were carried out with 2 µl template DNA, 12.5 µl Master mix, and 1.5 µl random primers CC1(5'-AGCAGCGTGG-3'), 9 µl of free water. The amplification protocol consisted of 5 minutes at 94 % and 40 cycles of denaturation at 94 % for 1 minute, annealing at 33 % for 1 minute, and extension at 72 %. 2 min, then 72 °C for the last elongation 1 minute.

RAPD-PCR Profile Analysis each gel was inspected, with the presence or absence of polymorphic bands in specific lanes being assigned a score of 1 or 0. The Numerical Taxonomy System software (NTSYSPC.V2-10e) was applied to the scored bands. The dispersion in the scored bands can help reveal a pattern grouping. Then the phylogenetic tree for RAPD-PCR was draw to show the resembling or differences.

3. Result

3.1. Isolation and identification of bacteria

From75 samples collected from male and female patients undergoing dialysis during the current study, 91 bacterial isolates were obtained: 66 were identified as Gram positive (72.5%) and 25 as Gram negative bacteria (27.4%) There were few samples from their no growth of bacteria were obtained. The extracted DNA for all samples were subjected to amplify the 16S rDNA gene using PCR. The amplified bands of all 91 bacteria isolates were visualized under UV transilluminated which showed a single band for each isolate with size roughly 1500bp when compared to a typical ladder.

The 16S rDNA gene of 91 isolates was successfully sequenced, and the bacterial species were identified after trimming and treating using (BLAST) in (NCBI) as bellow : *Bacillus* 46 (50.5%) included 9 species; since *B. cereus* was the highly frequented (29 isolates) , *B.paramycoides* 5, *B. safensis* 3 ,*B. lichenformis* 3, *B. subtilis* 2, whereas all the other *Bacillus* species frequency was 1 isolate for each, *Staphylococcus* 9 (9.8%), included *S. epidermidis* 4, whereas all the other *Staphyiococcus* species frequency was 1 isolate for each, *Pseudomonas* 11(12%) included *P. stutzeri* 9 , *P. putida* 1, *P. aeruginosa* 1,*Enterobacter* 5, included *E.cloacae* 3, *E.xiangfangensis* 1,*E.asburiae* 1, *Enterococcus* 4 included *E.casselifarus* 2, *E.faecium* 2, *Aerococcus* 3 included *A.viridans* 2, *A.urinaeequi* 1, *Lysinibacillus macrolides* 3, *Halomonas stevensii* 3, *Acinetobacter radiorsistens* 2, *Stenotrophomonas rhizophila* 1, *Exiguobacterium aestuarii* 1, *Shigella sonnei* 1, *Klebsiella oxytoca* 1 and *Margonella morganii* 1.

3.2. 16S rDNA gene phylogenetic tree of bacterial species

For the 16SrDNA gene sequences of the isolated bacterial species, a rooted phylogenetic tree was generated Fig1. The tree depicting the evolutionary connection and distribution of (60) distinct bacterial species recovered from hemodialysis unit compared to reference strains. The tree was drawn using Mega x program and after concatenated of the sequences for all subjected isolates. The phylogenetic tree showed similarity between 25 isolates of *Bacillus*, 4 *S. epidermides* isolates, 8 *P. stutzeri,* 3 *Halomonas stevensii* ,2 *E. Cloacas,* 2 *Acinetobacter radiorsistens*, 2 *Enterococcus faecium* and 2 *Enterococcus casselifavus.*

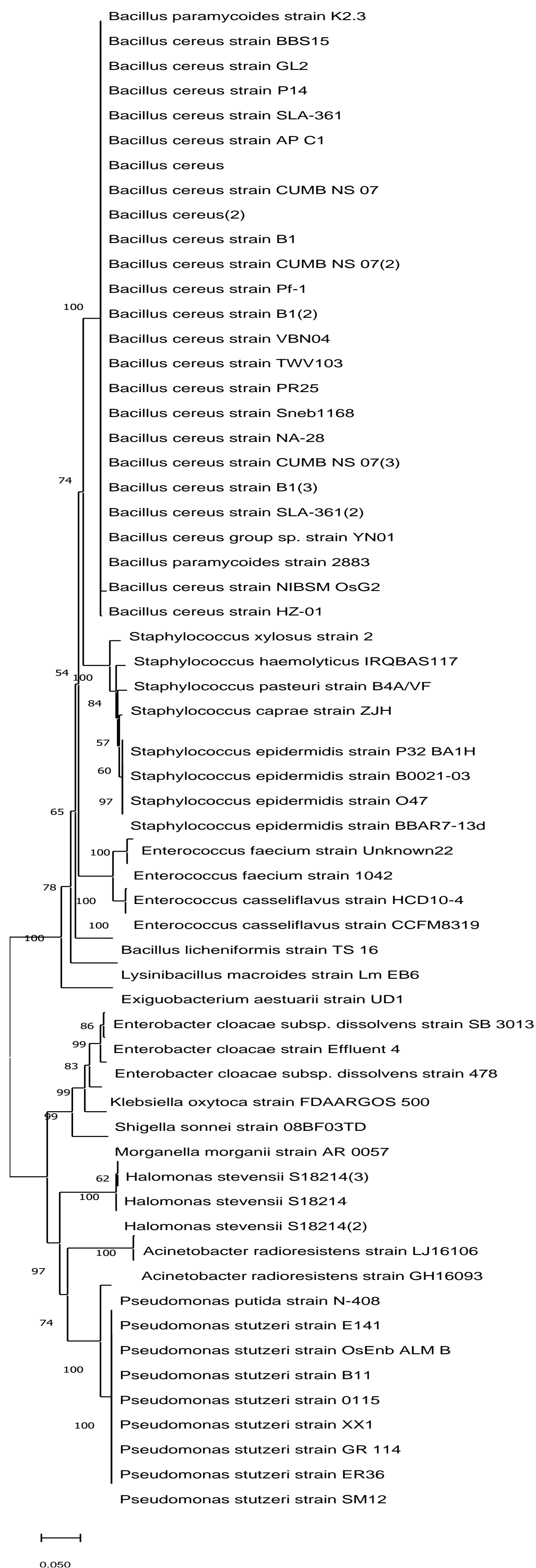

Figure 1. Rooted Neighbor Joining phylogenetic tree constructed using concatenated sequences of 16SrDNA gene for isolated bacteria during the present study This Neighbor Joining tree showing the distribution and Phylogenetic relationships of 12 different bacterial species isolated from hemodialysis patients with their reference strains (ATCC). All horizontal branch lengths were drawn to scale.

3.3. Recording new bacterial strains

After identification and comparing nucleotides with their type strains in NCBI, fourteen novel worldwide strains were recorded. The new strains databases were deposited in the DNA Data Bank of Japan (DDBJ), and they are available in the National Center for Biotechnology Information (NCBI) and the Gene bank. The new strains including: *Bacillus cereus* strain yasmun8 , *Pseudomonas stutzeri* strain yasmun20, *Bacillus cereus* strain yasmun27 , *Bacillus cereus* strain yasmun29, *Bacillus cereus* strain yasmun30, *Lysinibacillus macroides* strain yasmun35, *Bacillus cereus* strain yasmun41, *Bacillus cereus* strain yasmun54 , *Staphylococcus haemolyticus* strain yasmun69, *Enterobacter cloacae* strain yasmun89 , *Halomonas stevensii* strain yasmun95, *Bacillus cereus* strain yasmun70b, *Bacillus paramycoides* strain yasmun80b,in addition to isolation and recording *Aerococcus viridans* strain yasmun83b for the first time to our knowledge in Iraq from HD water.

3.4. Congo red agar test and antibiotic susceptibility

The ability of identified bacteria to form biofilm was investigated during the present study. Among the ninety-one tested isolates, there were (14) isolates gave positive result, of which (3) intermediate whereas the rest (77) were negative. The antimicrobial susceptibility test results for 46 isolates of *Bacillus* species were as bellow; most of isolates (91.3%) resistant to Ceftazidime CAZ and Cefepime FEP, whereas less were resistant to Vancomycin VA, Ceftriaxone CRO, and Cefotaxime CTX (52.1%, 58.6 %, and 63 %) respectively was less, with significant differences at P<0.01. The results of antimicrobial susceptibility test for nine isolates of *Staphylococcus* species, 100% resistant to CAZ and FEP, whereas less the resistant to VA was less CRO, and CTX (77.7%, 66.6 %, 33.3 %) respectively with significant differences at P<0.01. Among the eleven isolates of *Pseudomonas* species, 90.9% were resistant to CAZ, 72.7% were resistant to FEP, 54% were resistant to VA and less were resistant to CRO and CTX 27.2% with significant differences at P<0.01. About the rest of the bacterial species, there was a difference in antibiotic susceptibility, concerns of *Enterococcus*, 100% were resistant to CAR, FEP, 50% were resistant to CRO, VA, and 25% were resistant to CTX. In terms of *Enterobacter* isolates, they were 100% resistant to CAZ, FEP, 60% to CRO, VA and 20 to CTX. *Aerococcus* isolates, were 100% sensitive to CTX, CRO and 66.6% to CAZ, VA, FEP. Whereas *Klebsiella* were 100% sensitive to CTX, CAZ, VA while it was resistant to CRO and intermediate to FEP. For *Lysinibacillus macrolides*, all isolates were sensitive to CTX. resistant to CAZ, FEP, and an intermediate effect toward VA. However, 66.6% were sensitive to CRO. All *Acinetobacter radiorsistens* isolates showed resistance to CTX, CAZ and intermediate effect to CRO, FEP and sensitive to VA. *Shigella sonnei* and *Margonella morganii* isolates showed resistance to all antibiotics. While *Exiguobacter aestuarii* isolate was sensitive to CTX, CRO and resistant to CAZ, VA, FEP with significant differences at P<0.01. The results demonstrated that 26 isolates from different sources (in,

out and patients' blood) have antibiogram similarity to each other (for the same sample) so they were subjected to other four antibiotic, that included Erythromycin (E), Ampicillin (AM), Gentamicin (CN) and Methicillin (MET). The results of the similar isolates were almost identical, most isolates were 96% resistant to AM, whereas less were resistant to MET, E, and CN (76%, 32%, 4%) respectively with significant differences at P<0.01. Among the ninety-one isolates that were cultured on Congo red agar test, there were (14) isolates gave the positive result, of which (3) intermediate whereas the rest (77) was negative as shown in Figure 2.

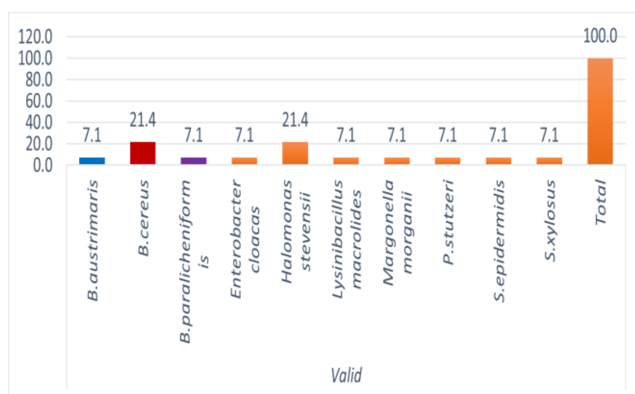

Figure 2. The percentage of positive isolates in the Congo red agar

3.5. Detection the identical bacterial strain by DNA RAPD-PCR

The RAPD-PCR genotyping were performed for the 24 similar isolates using different primers from various references. The visualized bands of RAPD-PCR in agarose for 24 strains (identical in antibiotic susceptibility) (16 *B. cereus* were shown in Figure 3. As well as four *P. stutzeri*, two *S. epidermides, two E. casselifavus*). Since the results of *B. cereus* showed relation between isolate (56 & 57) and (72 & 73) as shown in figure 3. As for the isolates of *P. stutzeri* the four showed, two of them related (47 & 48), as well as the two isolate of *S. epidermidis* showed closely related.

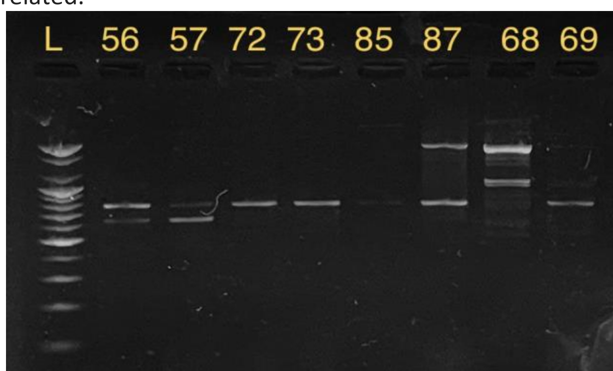

Figure 3. Agarose gel electrophoresis showing RAPD pattern of B. cereus, it shows identity between the isolates (56&57) from in and outward water respectively and (72&73) from in and outward water respectively.

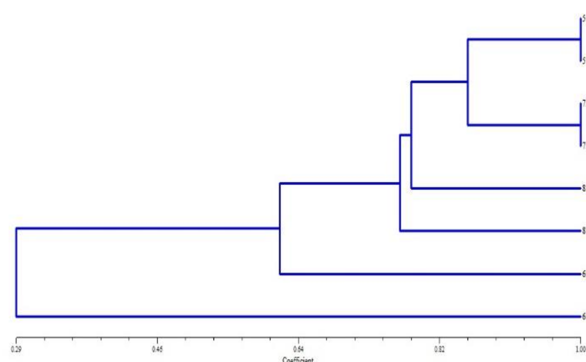

Figure 4. Dendrogram of 8 B. cereus strains 56 and 57, 72 and 73, 85 and 87, 69 and 68 constructed by a set of variables RAPD The Numerical Taxonomy System was applied to the scored bands. Software for statistics (NTSYSPC.V2-10e).

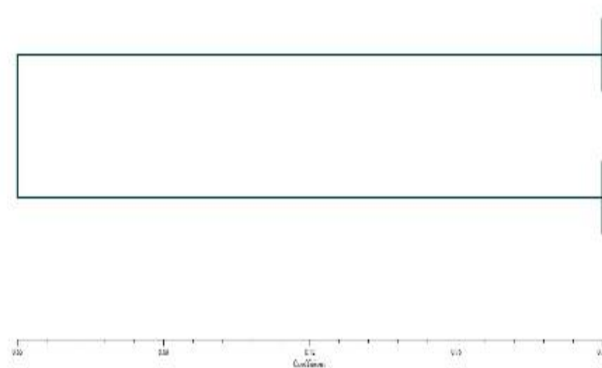

Figure 5. Dendrogram of 4 P. stutzeri strains 47 and 32, 50 and 83, constructed by a set of variables RAPD The Numerical Taxonomy System was applied to the scored bands. Software for statistics (NTSYSPC.V2-10e).

4. Discussion

Re-hospitalization is a common cause of infection, and infection is the second highest cause of death among chronic renal patients on hemodialysis. Infections are caused by both Gram positive and Gram-negative bacteria, with Gram positive bacteria, such as Staphylococcus aureus, being the most prevalent causal pathogen [18]. For the efficacy and safety of HD using high permeability dialysis membranes, it is critical to provide pure dialysis fluid. Many techniques, including reverse osmosis (RO), can be used to treat water. Metal ions, aqueous salts, and polymers can all be removed from treated water using RO [19]. The quality of municipal water in Iraq is regulated by a local standard no.417–2009, which is quite comparable to the World Health Organization's drinking water guideline in numerous aspects. A minimum of 0.3 mg/L free chlorine should be maintained at the furthest point from the plant, according to Iraqi standards. In Iraq, there is no national regulation for dialysis water quality [20]. The results in the current study showed the predominance of gram-positive bacteria. Out of 91 samples isolate, 66 were Gram positive (72.5%) and (25) as Gram negative bacteria (27.4%) some of them were found only in the incoming water or outward water, whereas the other was isolated from the incoming, outward and the patients' blood. However, the

less bacterial isolates were obtained from the patients' blood samples. A study conducted in treatment system of the hemodialysis unit in Tlemcen, Algeria was showed the predominance of gram-positive bacteria as well as study conducted in Cameroon in 2016 showed same result [7]. The Gram-positive bacteria were found in 28 of the 45 isolates was isolated from hemodialysis fluid at a hospital in Tehran, [21]. The types of bacteria obtained in the current study, indicate that *Bacillus* the most frequent type of bacteria 46, followed by , *Pseudomonas* 11, *Staphylococcus* 9, *Enterobacter* 5 , *Enterococcus* 4, *Aerococcus* 3, *Lysinibacillus macrolides* 3, *Halomonas stevensii* 3, *Acinetobacter radiorsistens* 2, *Stenotrophomonas rhizophila* 1, *Exiguobacterium aestuarii* 1, *Shigella sonnei* 1, *Klebsiella oxytoca* 1, and *Margonella morganii* 1. In the current study the presence of such types of bacteria is caused by the source of the water, as a study conducted at the University of Basrah proved that such types are present in drinking water as well as in tap water [22]. The presence of Gram-positive bacteria in current study such as the *Bacillus,* due to their ability to form spores and *Bacillus cereus* has a distinctive thermal property due to the temperature in which it grows, and it is well known that *Staphylococcus* resistance to difficult conditions. In current study, 14 isolates from both in coming and outward water were identified as novel strains. The similarity of strains to their reference strains was more than 99%. (1 % difference in 16S rDNA sequence). These novel strains might be the consequence of mutations induced by unrepaired DNA or RNA strand damage, which is commonly caused by radiation or chemical mutagens like antibiotics or sterile chemicals used in hemodialysis units. Since the frequency of the new isolates in the outward water was 64.3%, which is higher than their frequency in the incoming water 35.7%, and this may be a result of their exposure to chemicals such as citric acid at a high temperature ranging between 80-90, its exposure to such substances as well as its exposure to ultraviolet rays in the filter plant may be the reason for these mutations.

Antimicrobial susceptibility test in the current study findings were for 46 isolates of *Bacillus* species, most isolates were (91.3%) resistant to CAZ and FEP, whereas less were resistant to VA, CRO, and CTX (52.1%, 58.6 %, and 63 %) respectively as well as (28.5%) from *B. cereus* resistant to all antibiotic above. Since it has shown high percentage of resistant to both MET and AM. This is consistent with the results of Zhao who found that Antibiotic-resistant of *B. cereus* strains have been discovered because of improper antibiotic usage over time or the establishment of resistance genes [23]. The resistance shown in the current study against the β-lactam antibiotics (ampicillin) was comparable to that seen in numerous other studies, in which more than 90% of *B. cereus* isolates were found to be resistant. Furthermore, chloramphenicol, gentamicin and vancomycin [23]. The current study suggests that the resistance of *Staphylococcus* and *Pseudomonas* isolates to antibiotics may be due to their ability to form biofilm. Due to the increase in bacterial resistance, the current study suggests the use of antibiotic susceptibility testing to aid in the selection of antibiotics and treatment modalities. However, the percentage of antibiotic resistance was not correlated strongly with biofilm production, since only 14 isolates were recorded as biofilm producing bacteria. Biofilm in reverse osmosis (RO) membranes is a common issue in hemodialysis water purification. Germs attach to and multiply on RO membranes, generating biofilms that clog and damage the membranes, allowing bacteria and/or biological components to be transferred and possibly detrimental to the health of hemodialysis patients [9]. It has been suggested that although dialysis machines are sterilized before each use using citric acid and a certain temperature, it is not sufficient to eliminate all contaminants.

The applying antibiogram was showed the similarity of sixteen *B. cereus,* four *P. stutzeri*, two *S. epidermides*, and two *E. casselifavus* isolated from same samples (in, out and patients' blood). Because of their relevance in epidemiology and ecology, bacterial species and strains must be precisely documented. Biochemical approaches are difficult to distinguish between closely related isolates since they are so identical. As well as phenotypes are too varied to distinguish between closely related strains, genotyping, can distinguishes between bacterial strains based on their genetic content, has become commonly employed for bacterial strain typing due to its high resolution. The use of this technique in the current study is necessary to know that the species that appeared in the patient's inward water are the same as those that appeared in the water leaving him or in blood samples. Since the results of *B. cereus* shown related between isolate (56 from water in & 57 from water out) and (72 from water in & 73from water out) as shown in figure (2). As for the isolates of *P. stutzeri* out of the four, two of them were related (47 from water in & 48 from water out), as well as the two isolate of *S. epidermidis* were genetically relatedness. To distinguish epidemiologically important strains, genotyping methods have been employed extensively. For the separation of strains belonging to the same species, random amplified polymorphic DNA (RAPD-PCR) analysis has been used. This technique has been successfully used to a wide range of microorganisms. It is a PCR-based approach of genetic typing that relies on genomic polymorphisms and is quick and sensitive for epidemiological investigations [24].

However, the presence of such bacterial species poses a risk to such immunocompromised patients such as *Bacillus cereus* is an opportunistic bacterium that frequently causes gastrointestinal infections and is rapidly becoming recognized as a cause of severe local or systemic illnesses. *B. cereus'* pathogenicity is based on the release of a variety of poisons and enzymes, as well as the capacity to undergo swarming differentiation in response to surface-sensing signals [25]. *Pseudomonas aeruginosa* is a common nosocomial Gram-negative bacteria linked to increased morbidity and death during hospitalization [26]. As such the presence of most of the isolates in the precent study itself or their products (toxin, enzyme, etc.) may be consider as dangerous pointer in HD.

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