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University of Babylon
College of Science for women
Biology department

# Evaluation of virulence factors bacteria from Dialysis patients and activity of Myrtus communis .L against some pathogenic bacteria.

**A thesis**
**Submitted to the Council of the College of science for women-University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology**

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**2021 A.D.** **1443 A.H.**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ رَبِّ اشْرَحْ لِي صَدْرِي ﴿٢٥﴾ وَيَسِّرْ لِي
أَمْرِي ﴿٢٦﴾ وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي
﴿٢٧﴾ يَفْقَهُوا قَوْلِي ﴿٢٨﴾

صدق الله العلي العظيم

سورة طه

الآية (٢٥ – ٢٨)

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To who reached the message and perform the Secretariat… and advised the nation … To the prophet of mercy and the light of the Worlds of our master **(Muhammad)**

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Finally, I would like to express my thanks to all my friends and to all lovely people who helped me , directly or indirectly to complete this work.

Dina

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# Dedication

To whom worried by thinking of my future… to hope candle whom shine my life… to whom bow every letters and pens … my mother (God prolong their ages).

To second Half in this life … To whom I see optimism in his eyes and my happiness in his laughter…To((my lovely husband)) thank you for supporting me and your encouragement god save you.
To my dear children Ali , Ahmed and Ayla
You are the light of my life.

To my loyal friends and loved ones.

I present my modest effort, deepest and sincere gratitude for their support.

**Dina**

# Summary

The current study included collection of 135 clinical samples of Dialysis(102) samples and burns (33) samples. The samples were collected from Imam Al-Sadiq Teaching Hospital and Margan medical city, during the period between October to December /2020. Bacterial species that belong to (*Klebsiella pneumonieae, Enterococcus faecalis, Escherichia coli, Staphylococcus aureus*) are frequently resistant to broad-spectrum antimicrobial agents. The main objective of our work was to molecular study of virulence factors in pathogenic bacteria among Dialysis patients and revealed the susceptibility of phytochemical compounds extracted from medicinal plants and compared with antibiotics.

The results of isolation and diagnosis showed that 161 isolates of bacteria were distributed as follows: *Klebsiella pneumoniae* 67(41.6%) , *Enterococcus faecalis* 48(29.8%), *Staphylococcus aureus* 24(14.9%), *Escherichia coli* 22(13.6%)

Molecular study involved detected virulence factors of bacterial pathogens by using polymerase chain reaction (PCR), started with Klebsiella pneumoniae genes (*fimH-1(38.7%),mrkD(58.1%),magA(3.22%), ,cnf*(0%)) and Enterococcus fecalis genes (*esp (25.14%),gelE(21.7%) ,hyl(53.53%),asa1*(27.43))

The study also included sensitivity testing for: *Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus aureus and Escherichai coli* pathogens towards 6 type's antibiotics by disk diffusion method , the results showed the most effective antibiotic against the different species of nosocomial pathogens was ATH15,AZM30 and AMB100 . Also, the current

study was conducted to investigate the effect of the crude Flavonoid, Alkaloid, and Terpenoid, compounds extract from the leaves of (*Myrtus communis L*.) against pathogenic bacteria isolated from hemodialysis fluid and burns. Antibacterial activity was achieved in vitro by using agar-well diffusion method against pathogenic bacteria isolated from hemodialysis fluid by preparing three concentrations for each crude compound (50, 100, and 200) mg/ml and compared with positive control represented by Azithromycin antibiotic and negative control represented by 10% dimethyl sulfoxide. the aimed of this study to investigate the antibacterial efficacy of the Secondary Metabolites Extracted from *Myrtus communis L*. leaves against some pathogenic bacteria isolated from Hemodialysis Fluid.

The data collected from the study revealed that, the crude Flavonoid and Alkaloid compounds extract from the leaves of (*Myrtus communis L*.) showed significant reduction at $P \leq 0.05$ in the growth of pathogenic bacteria isolated from Hemodialysis at 20 mg/ml compared with negative control. Finally, it can be concluded that Flavonoids and Alkaloid of *Myrtus communis L*. is most effective in controlling pathogenic bacteria isolated from hemodialysis fluid. While, terpenoid compounds was the least effective in controlling growth of pathogenic bacteria isolated from hemodialysis fluid and burns compared with flavonoid and alkaloid. Finally, it can be concluded that, Flavonoids of *Myrtus communis L*. is most effective in controlling pathogenic bacteria.

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## List of abbrivation

| Symbol | Definntion |
|---|---|
| **HD** | Hemodialysis |
| **RRT** | Renal replacement therapy |
| **ESRD** | End –Stage Renal Disease |
| **QOL** | Quality Of Life |

| | |
|---|---|
| **Kt/V** | measurement of the efficacy of a hemodialysis session. |
| **RKF** | Rate of Residual Kidney Function |
| **BMI** | Body mass index |
| **GV** | VGF |
| **OBI** | Occult hepatitis B virus infection |
| **CKD** | Chronic kidney disease |
| **CVC** | central venous catheter |
| **MRSA** | Methicillin-resistant Staphylococcus aureus |
| **HAI** | Health care-associated infections |

# *Chapter one*

# ***Introduction***

## 1.1. Introduction

Hemodialysis is a method of eliminating toxins from the bloodstream by diffusing them through a semipermeable membrane (Okunola and Olaitan, 2016). Each patient who employs HD machine is exposed to a huge volume of water (400 L per week) used to produce dialysate, which, if not adequately handled, might cause kidney failure. All low molecular weight contaminants in water, such as chemical, bacterial, and poisonous contaminants, have direct access to the HD patient's bloodstream through the dialyzer's semipermeable membrane. (Nikaido, 2009; Oumokhtar *et al*., 2013; Asserraji *et al*., 2014).

Care for people with chronic illnesses Due to the patients' impaired immune systems and the management of the bloodstream through catheters, patients undergoing hemodialysis for the treatment of end-stage renal failure have a greater rate of bloodstream-associated infection. (Shimohata *et al*., 2019). Water treatment systems are an important part of dialysis therapy, and precise bacteriological quality control of hemodialysis water is crucial for ensuring a higher quality of life for hemodialysis patients. The goal of this study was to determine the amount of bacteria contamination in hemodialysis water and dialysate. (Alipour *et al*., 2014). For hemodialysis, a comprehensive water purifying system is essential. Because dialysis patients are exposed to large amounts of water, which is mixed with dialysate concentrate to make dialysate, even trace mineral pollutants and bacterial endotoxins can enter the patient's bloodstream. \. Because the injured kidneys are unable to do their original job of eliminating pollutants, ions brought into the circulation through water can accumulate to dangerous levels, resulting in a variety of symptoms or death. (Tian *et al*., 2015). Furthermore, RRT patients with immunodeficiency may be malnourished, and the consequent imbalance in

bacteria, viruses, fungi, and other microorganisms in the body may increase the risk of nosocomial infections.( Zuo M *et al*., 2018). These infections not only have a negative impact on ESRD patients' quality of life (QOL), but they also increase to their financial burden. (Khan *et al*., 2015).

Antibiotics are produced on a global scale of around 100,000 tons per year, and their use had a significant impact on bacterial life on Earth. Antibiotic resistance has increased among pathogen strains, and some have developed resistance to multiple antibiotics and chemotherapeutic drugs, a condition known as multidrug resistance. Antibiotics were widely utilized for medical treatment, farm animals, and even aquaculture fish, resulting in the selection of hazardous bacteria resistant to a variety of medications. (De Lencastre *et al*., 2007).

One of two processes can cause multidrug resistance in bacteria. First, within a single cell, these bacteria may store many genes, each coding for drug resistance to a single treatment. On resistance (R) plasmids, this buildup is most common. Second, increased expression of genes that code for multidrug efflux pumps, which extrude a wide spectrum of medicines, can lead to multidrug resistance. (Nikaido, 2009). Natural medicines now not only cover the main health-care needs of the majority of the population in developing nations, but they are also gaining traction in wealthy countries as health-care expenses rise and everyone faces financial hardship. In the United States, about half of the population has tried the natural medicines for disease prevention and treatment. (Organization, 2013).

Myrtus communis L. (Myrtaceae) is a fragrant evergreen perennial shrub or small tree of the Myrtaceae family. (Satyavati *et al*., 1987). The

medicinal herb myrtle (*Myrtus communis L*) is utilized in traditional medicine all throughout the world. (Alipour *et al*., 2014). However, the aimed of this investigation was to see if secondary metabolites extracted harmful microorganisms obtained from haemodialysis fluid were tested using leaves.

**1.2.The Objective study:**

**1.** Isolation and Biochemical Identification of pathogenic bacteria from dialysis and burn patients.

**2.** Biological control to Pathogens by using *Myrtus communis L*. However,the aimed to determine the epidemiology, risk factors and complications of infections in patients receiving chronic hemodialysis, particularly bloodstream infections.

**3.**study to investigate the biological activity of phytochemical compounds extracted from *Myrtus communis L*. against some pathogenic bacteria isolated from Hemodialysis Fluid.

**1.3 The aim of study**

1.diagnosis of pathogenic bacteria isolated from Dialysis patient.
2.Identification their virulence factors .

3.test their susceptibility to phytochemical compounds extracted from medicinal plants and compound with antibiotics in Hillah city.

# Chapter Two

# literatures Review

## 2.1. Haemodialysis

Haemodialysis treatment of chronic renal failure was first used more than 50 years ago, and the technique has been widely developed ever since. New membrane materials have been introduced, and novel techniques for accessing the bloodstream have been developed. Haemodialysis is, however, still associated with a substantial number of complications (Tonelli *et al.*,2006) . In the majority of haemodialysis centres, cellulose dialysers are no onger used and dialysis-associated neutropaenia and dialyser-related severe reactions are rarely encountered by clinicians, but moderate neutropenia and thrombocytopenia do still occur (Rogacev *et al.*,2009 *;* School *et al.*,2011). Despite significant advances, The use of haemodialysis (HD) is still linked to a high rate of hospitalization and mortality. The results are still unsatisfactory, despite the fact that survival and quality of life have both improved since the previous study.. To address unmet clinical needs, new techniques and remedies are without any doubt a requirement (Vanholder *et al.*,2003).

Poor clinical outcomes, on the other hand, are caused not only by the population's increased comorbidity and age, but also by inherent 'current methods' limitations. These issues arise from current dialysis membranes' inability to remove the full spectrum of uraemic toxins accumulated in the body (Cianciolo *et al*.,2007). Complications in patients who have normal urea kinetics and Kt/V Dialysis-related amyloidosis, anemia, skeletal abnormalities, neuropathies are a few examples have been linked to uraemic toxins with molecular weights ranging from 5000 to 50 000 Da that Current dialysis techniques do not sufficiently clear them (Desjardins *et al*.,2013 ; Locatelli *et al*.,2008).

Classical and novel uraemia retention molecules have been discovered (Yu *et al*.,2017). Parathyroid hormone levels are particularly high. Other bone and

calcium–phosphate metabolism molecules, such as FGF23, osteoclastin, and osteoprotegerin, have all been identified to osteoodystrophy. Uraemic anaemia is caused by Proteins and hepcidin that bone marrow and erythropoiesis inhibition. Homocysteine and inflammatory mediators have been linked to accelerated atherosclerosis and cardiovascular complications. Increased leptin levels cause a significant decrease in appetite. j free light chains (22 000 Da) and k free light chains (Da (42 000)) in uraemic patients have recently been identified as toxic molecules (Desjardins *et al*.,2013). A number among these molecules have molecular weights (MW) that far exceed the ability to remove traditional membranes with high flux (HF) and molecular radii larger than dialysis membrane pores (Clark *et al*.,2017 ; Ronco *et al*.,2017).

Some small and middle molecules can be removed with traditional HF Membranes, but they leave behind others. We showed that the most important determinant of beta-2 microglobulin (b2M) removal in HF membranes is convection (flux) (Locatelli *et al*.,2008). These research paved the way for the widespread the application of the technique of haemodiafiltration (HDF) for maximizing the ability of HF membranes to remove contaminants (Ronco *et al.*,2015).

Despite procedures that have been simplified and automated machinery, HDF online necessitates a complicated assemble and specialized technology that is not in all cases accepted or readily available. Regardless of the results obtained HDF by onine, Dialysis clinical outcomes remain suboptimal and unsatisfactory. Mortality remains high, as do hospitalization and cardiovascular complications (Barreto *et al*.,2014).

### 2.1.1. The Hemodialysis mechanism

Wastes and excess water are removed during hemodialysis by using an external filter called a dialyzer, which contains a semipermeable membrane. The waste is separated by establishing a counter-current flow gradient in which blood flows in one direction and dialyzer fluid flows in the opposite direction. Peritoneal dialysis removes waste and water into the dialysate by using the peritoneum as a natural semipermeable membrane (the substance or fluid that passes through the dialysis membrane). The movement or diffusion of solute particles across a semipermeable membrane is the basic principle involved in dialysis (diffusion). Urea and creatinine, which are metabolic waste products, diffuse down the concentration gradient from the Dialysis fluid circulation (NaHCO3, NaCl, acid concentrate, and deionized water).

The size of the particles determines the rate of diffusion across the membrane during their diffusion into the dialysate.. The slower the rate of diffusion across the membrane, the larger the size of the solute particle. A vein connects the arteries that carry oxygenated blood from the heart. forming an arteriovenous shunt, which strengthens the vein(by forming muscles around it in the manner of an artery) sufficient to be punctured multiple times; its pressure is also monitored during the dialysis process (*Sabitha et al., 2017*). The diagrammatic representation of a dialyzer is shown in Figure 1.

Hemodialysis, as described in other reports However, there are a few potential limitations to this study that should be mentioned. To begin with, the authors There was a lack of information on patients who received incremental hemodialysis.

**2.2.**

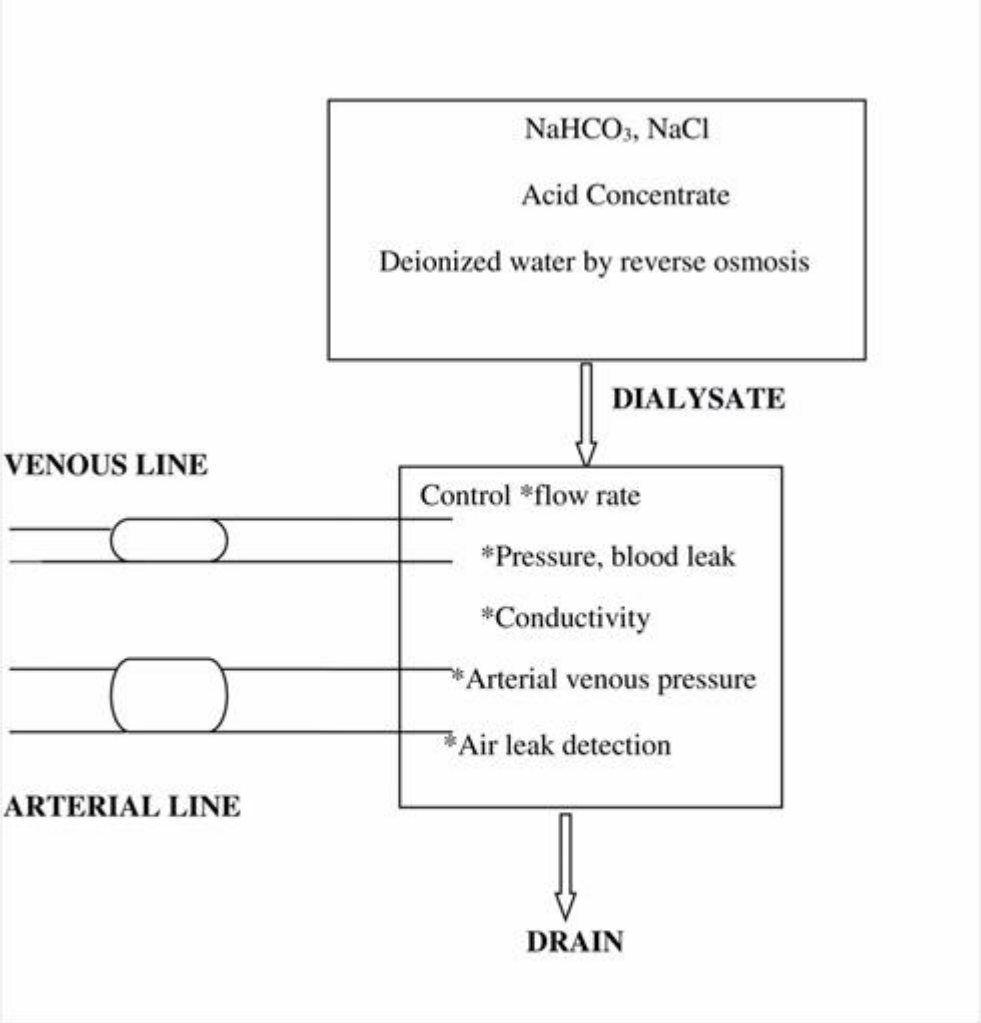



FIGURE 1: Diagrammatic Representation of a Dialyzer

$NaHCO_3$: sodium bicarbonate; NaCl: sodium chloride

## 2.2.Diseases associated with hemodialysis

### 2.2.1. Cardiovascular disease (CVD) :

In hemodialysis (HD) patients, cardiovascular disease (CVD) is the most common cause of death. Patients are at risk, and vascular calcification is an established risk factor (Verbeke *et al*.,2011). The possibility of vascular calcification increases with regard to renal function decline as well as greatest in renal failure at

the end of life (ESRD) (Temmar *et al*.,2010) . Amid mounting advances in Patients' mortality rate as a result of hemodialysis therapy on Dialysis on a regular basis remains unacceptable level This could happen be due to a disruption in uremic chronic inflammation, calcium-based therapies, or mineral metabolism . Furthermore, vascular smooth muscle cells are undergoing active osteogenesis. may have an impact on the risk of vascular calcification (Combe *et al*., 2001).

Normalized protein catabolic rate, serum albumin, serum creatinine, BMI, and normalized protein catabolic rate (nPCR)have all been suggested as ways to assess nutritional status in chronic kidney disease patients in the past (Pifer *et al*.,2002). Furthermore, several studies found that these factors were linked to an increased a patient's chance of dying while undergoing HD (Ishii *et al*.,2015; Rhee *et al*.,2015).

On the other hand, data on the impact of various patient characteristics on subsequent all-cause and cardiac mortality is scarce. The available data is limited and inconclusive. Hematologic indices have been found to influence the possibility of death in several studies. An increase in hemoglobin of 1 d/dL was associated with an increase in linked to a lower risk of death from any cause according to Ishii *et al*.,(2015), Park et al, on the other hand, found no link between hemoglobin and mortality risk (Park *et al*., 2015).

The exact risk factors for mortality in HD patients are still unknown. The goal of this meta-analysis was to conduct a comprehensive review of all available studies in order to identify risk factors for HD patient mortality. In patients with end-stage renal disease, cardiovascular disease (CVD) is the leading cause of morbidity and mortality (ESRD) (Olechnowicz-Tietz *et al*.,2013). that the rate of mortality in chronic hemodialysis (HD) is 7–8 times greater than general population, and 40%–

45% of all causes of mortality are related to CVD. (Collins *et al*.,2012; Charytan *et al*.,2007)

### 2.2.2.Diabetes and hemodialysis

Diabetes mellitus is becoming more common globally, as well as the quantity of diabetes mellitus patients undergoing hemodialysis on a regular basis (Lu *et al*.,2017; Kramer *et al*.,2018). Diabetes dialysis patients die at a higher rate than dialysis patients with other kidney diseases (National Kidney Foundation, 2012; Tien *et al*.,2013 ). Managing glucose in this population presents difficulties for both patients and clinicians. Previous research found an increase in GV in diabetics on hemodialysis ( Mirani *et al*.,2010). Furthermore, high GV has been observed linked to an increased danger of hypoglycemia. among others community (Williams *et al*., 2014). In this patient population, hypoglycemia is a risk factor for death (Zoungas *et al*.,2010; Investigators *et al*.,2013).

Despite the fact that there are numerous documents, no studies have been conducted to date. In diabetic patients on maintenance dialysis, there is a link between GV and mortality. In patients who do not have end-stage renal disease, HbA1c levels are typically used to evaluate glycemic control over time (ESRD).The methodology used to measure HbA1c in hemodialysis patients with ESRD determines its validity (Little RR *et al*.,2002) . Furthermore, a number of factors, including changes to the life span of red blood cells (RBCs) as well as mechanical and metabolic factors , may influence the measurement. One significant a restriction HbA1c in hemodialysis patients is that it does not provide information on glycemic control between dialysis days. In contrast, the GlucoDay device, which uses a biosensor to measure glucose every 3 minutes, is well suited to capturing the effect of hemodialysis on glucose levels over a 48-hour period. Diabetes mellitus (DM)

patients have a high risk of frailty. Espinoza and colleagues (2012) Furthermore, the average life expectancy for people over the age of 65 is The average duration of follow-up for DM patients with frailty was 23 months (Hubbard *et al*., 2010). As a result, it has been proposed that frailty and diabetes are linked. Diabetic nephropathy has been the leading cause of kidney disease since 1998. chronic hemodialysis patients in Japan. (DN.) After starting dialysis, the 5-year survival rate end-stage renal disease patients (ESRD) caused by DN is 60.3 percent, indicating a poor prognosis (data provided by The Japan Diabetes Society) ( Kohzuki *et al*.,2013). However, it is unknown whether DN is associated with frailty in HD patients.

### 2.2.3. Blood Pressure And Hemodialysis

In the general population, blood pressure (BP) is one of the most important modifiable risk factors for cardiovascular events and death (Vamos *et al*., 2012), Patients on maintenance hemodialysis with kidney failure have an extremely high rate of these events(Sarnak *et al*.,2003). However, BP management in hemodialysis patients is a conundrum because of paradoxical U-shaped associations between pre-hemodialysis systolic blood pressure (SBP) measured at the dialysis unit prior to the start of the hemodialysis session and cardiovascular disease and death reported in multiple observational studies (Bansal *et al*.,2015).

Hemodialysis patients with pre-dialysis SBPs less than 140 mm Hg are at a higher risk of death than those with SBPs greater than 140 mm Hg. Patients with pre-dialysis SBPs ranging from 150 to 179 mm Hg appear to have a risk of all-cause mortality that is comparable, if not lower. Those with pre-dialysis SBPs of 140 to 149 mm Hg had a lower risk of death even after controlling for case mix. These findings have caused concern among providers and the BP management guideline committees. Despite the fact that high blood pressure (BP) is a well-known risk

factor for heart disease in the general population, according to Bakris *et al*.,(2014) and Lim *et al*., (2012), the contribution of BP to cardiovascular disease is underestimated ,Among hemodialysis patients, hypertension contributes to cardiovascular and all-cause mortality (Georgianos *et al*.,2017). Outside of dialysis, however, elevated blood pressure is linked to death (Amar *et al*.,2000; Agarwal, 2010).

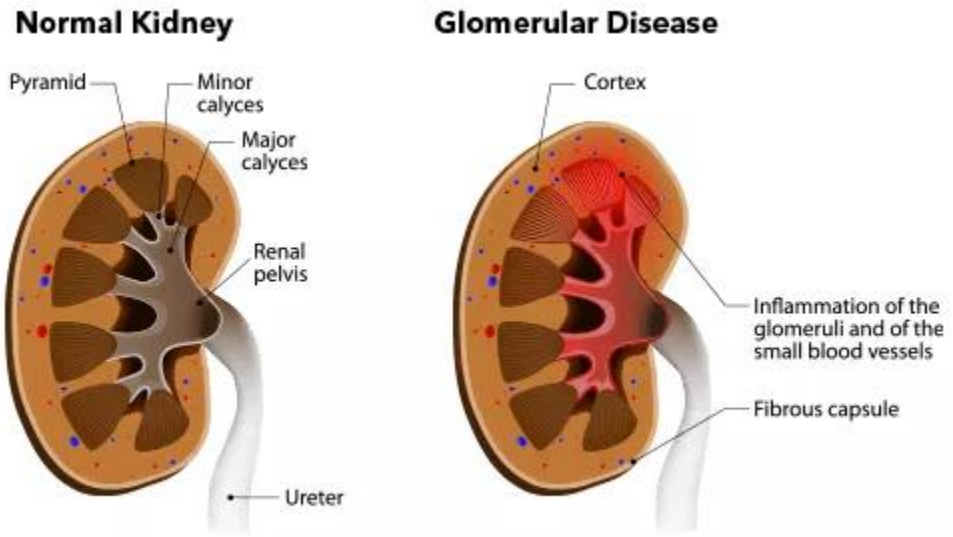
Furthermore, contrary to observational studies, intentional blood pressure reduction with antihypertensive medications improves clinical outcomes (Agarwal *et al*.,2009; Heerspink *et al*.,2009). To date, no trial has randomized patients in dialysis to two different BP targets, so optimal BP thresholds are unknown (Luther *et al*.,2008). The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative was founded more than a decade ago. The management of hypertension in hemodialysis was based on an arbitrarily chosen BP standard of 140/90 mmHg for predialysis BP and 130/80 mmHg for postdialysis BP, according to (KDOQI) guidelines(K/DOQI ,2004).

C

### 2.2.4. Glomerulonephritis and Hemodialysis

Glomerulonephritis causes inflammation in glomeruli and occurs alone or as part of diseases such as vasculitis, systemic lupus erythematosus, cancer, and infections End-stage renal failure is commonly caused by glomerulonephritis... Severe and prolonged inflammation can damage glomeruli and lead to kidney fibrosis. Post-streptococcal GN was first described in the nineteenth century, It's been known for a long time that it's a type of kidney damage (Brodsky *et al*.,2011). Although post-streptococcal disease has been the prototypical example of bacterial infection-related GN for the past two decades, the last two decades have seen an increase in the

prevalence GN caused by bacterial infection. Over the last few decades, For this group of diseases, there has been an epidemiological shift in the underlying cause, morphologic features, and therapeutic regimens (Nasr *et al*.,2008). In cases of bacterial infection-related GN, Staphylococcal species have emerged as a common etiologic agent (IRGN)in developed countries, and they are distinguished from classic post infectious GN by unique morphologic features and clinical implications . On immunofluorescence microscopy, GN associated with staphylococcal infection shows deposits that stain for IgA and the C3 component of complement in a dominant or co-dominant manner, and it occurs clinically in the setting of active infection..




## 2.3. Viral infection

In HD patients, infections are the leading cause of morbidity and death, second only to cardiovascular events. Unexpectedly, after an infection-related hospitalization, the risk of death from cardiovascular events increases significantly. The majority of severe infections in this population are caused by episodes of

bacteremia, with episodes of pneumonia following) Morbidity and Mortality. In HD patients, annual mortality from bacteremia is 100-300 times higher than in the general population. Even when age, race, gender, diabetes, and record errors are taken into account, mortality from bacteremia is 50 times higher ( Foley *et al*.,2004). In addition to bacterial infections, blood-borne viral infections are a common problem in HD units. Infections with the hepatitis B virus (HBV), hepatitis C virus (HCV), and the human immunodeficiency virus (HIV) are all very common (HIV). There are safety concerns about spreading the HD procedure among HD patients and the unit's staff due to the procedure's obvious nature. Furthermore, the natural histories of all of these infections, as well as the treatments and vaccine reactions available, differ from what is known about the general population. There are a number of notable reviews that examine each infectious agent in depth (Rao TK ,2003; Beathard and Urbanes, 2008).

### 2.3.1. The viral hepatitis

In haemodialysis patients, hepatitis B and C virus infections (HBV and HCV) cause morbidity and mortality. These patients are more likely to contract blood-borne infections as a result of prolonged vascular exposure and multiple blood transfusions..Devices, equipment, and supplies that have been tainted, Environmental surfaces, as well as attending personnel, may be important in the nosocomial transmission of these infections. Infections with hepatitis viruses are exacerbated in haemodialysis patients by significant immune status dysfunction caused by irreversible renal compromise (Abumwais *et al*., 2010; Reddy *et al*., 2010).

Furthermore, hepatitis viral infections in haemodialysis patients result in liver disease in patients on renal replacement therapy .Because patients with renal failure

are involved, they also pose serious problems in the management of these cases. Failure to do so will result in ineffective virus removal. Patients who are co-infected with these pathogens experience clinically serious manifestations and develop Interferon treatment resistance (Reddy *et al*.,2005).

## 2.4. Bacterial infections association with heamodialysis

Bacterial infections are incredibly common in patients with end-stage renal disease (Fram *et al*.,2015). In patients requiring HD, Infection is now the leading cause of death and morbidity, surpassing cancer. Dialysis patients die at a rate that is 6.5 to 7.9 times higher than the general public (Fram *et al*.,2015; Abou Dagher *et al*.,2015).

There are several risk factors for bloodstream infection in HD patients. CVC use, diabetes, hypoalbuminemia, anemia, and female gender are all risk factors. In this case, colonization with methicillin-resistant Staphylococcus aureus (MRSA) is also effective (Abou Dagher *et al*., 2015). Infection is now the most common cause of morbidity and death in HD patients (Abou Dagher *et al*., 2015). *Staphylococcus aureus* is currently the most common isolated pathogen infecting these patients (Abou Dagher *et al*.,2015). Because of their low immune activation, multiple needle punctures, and skin colonization, these patients are susceptible to staphylococcal infections (Abou Dagher *et al*.,2015). Gram-positive bacteria are the most commonly isolated bacteria in hemodialysis patients' blood cultures. The most common cause of infection is E. coli, with Staphylococcus coagulase negative infections coming in second (Abou Dagher *et al*.,2015 ; Chebrolu *et al*.,2015).

### 2.4.1. *Klebsiella pneumoniae*

A member of the Enterobacteriaceae family, which also includes *Salmonella* and E. coli (Adeolu *et al*., 2016.). *K. pneumoniae* has a long history as a pathogen (It

was identified as a cause of pneumonia by Carl Friedländer in 1882.) and is still one of the most common nosocomial pathogens in the world (Pendleton *et al*.,2013). It's also one of the leading causes of neonatal sepsis. accounting for one of the top three causative agents in the vast majority of cases (Zaidi *et al*.,2005; Okomo *et al*.,2019).

Carbapenem-resistant and extended-spectrum-lactam (ESBL)-producing *K. pneumoniae* (CRKp) is a serious public health threat, according to the World Health Organization (2017). Antibiotic-Resistant Bacteria Priority List to Guide Antibiotic Research, Exploration and development (WHO, 2017) Such strains are said to be responsible for more Multidrug-resistant (MDR) More than 90,000 infections, 7,000 deaths, and 25% of all infections have been caused by bacterial infections. disability-adjusted life years in Europe alone (Cassini *et al*.,2019).

Despite the lack of precise burden estimates in other regions, MDR rates are rising globally; for example, in Malawi, MDR infections now account for more than 75% of *K. pneumoniae* bloodstream infections (Musicha *et al*.,2017). The Enterobacteriaceae member *Klebsiella pneumoniae* is a remarkable human pathogen. *Klebsiella pneumoniae* infections have become more common in clinical settings in recent years (Decré *et al*., 2011; Vuotto *et al*., 2014). It has recently emerged as a multidrug-resistant hospital pathogen in hospitals all over the world, with few treatment options (Paczosa and Mecsas, 2016). Protection against phagocytosis, desiccation, and complement evasion have all been discovered through the study of its virulence factors, fimbriae, capsules, and lipopolysaccharide, which are all responsible for attachment to the host surface (Victor *et al*., 2007; El Fertas-Aissani *et al*., 2013; Piperaki *et al*., 2017).

Biofilms are a complex network of microbial exopolysaccharide matrixes that contain bacteria and are shaped by various host factors (Donlan and Costerton, 2002;

Soto, 2013). Biofilms have received a lot of attention in recent decades due to their role in nearly 80% of bacterial infections, with device-related infections, infections on body surfaces, and chronic infections being the most common. These biofilms are of particular concern because they protect both host defense mechanisms and traditional antimicrobial therapy, affecting antimicrobial treatment outcomes significantly ( Singh *et al*., 2017).

As part of its pathogenesis, *Klebsiella pneumoniae* forms biofilm (Wu *et al*., 2011). Imagine a biofilm on soft tissues and skin, lungs, urinary bladder, and so on (Piperaki *et al*., 2017). Klebsiella spp. infection of implants is a common occurrence. There have also been reports variants of small colony (Ronde-Oustau *et al*., 2017). As demonstrated in a rat model of bacterial prostatitis, *Klebsiella pneumonia* mediated urinary tract infections (UTI) are linked to biofilms. caused by bacterial adhesion to bladder mucosal tissue and the mucosal surface of the acini of prostate tissue, unlike Staphylococcus aureus/epidermidis (Murphy *et al*., 2013).

### 2.4.3. Siderophores obtained

Siderophore systems are made up of iron-chelating molecules that scavenge iron from host proteins or other sources, and surface receptors for internalization. Yersiniabactin, aerobactin, and salmochelin have been discovered in K. pneumoniae (core siderophore; encoded by ent) (Holt *et al*.,2015) . When compared to classical HAI or asymptomatic carriage, all three accessory systems have been shown to increase virulence in murine models (Fischbach *et al*.,2006; Bachman *et al*.,2011), and the presence of these loci is statistically linked to invasive CAIs in humans ( Bachman *et al*.,2011; Lam *et al*.,2018a; Lam *et al*.,2018b).

Despite some functional similarities, the siderophores differ in terms of iron binding affinities and host–immune interactions, such as macrophage survival

(Achard et al., 2013; Khater *et al*., 2015; Holden *et al*, 2015). Other conjugative elements, on the other hand, can transport them between bacterial cells; KpVP-1 regions fused with conjugative plasmid regions, as well as mosaic plasmids containing KpVP-1 regions fused with conjugative plasmid regions, can result in conjugative virulence plasmids (Lam *et al*.,2019 ; Yang *et al*.,2019). There have been several more plasmids discovered with distinct iuc or iro lineages (iuc3, iuc5, iro4, and/or iro5), many of which have genes predicted to encode conjugative machinery (iuc3, iuc5, iro4, and/or iro5) ( Lam *et al*.,2018b).

### 2.4.4. Colibactin

Colibactin, a genotoxic polyketide that causes DNA damage in eukaryotic cells and is encoded by the clb (pks) locus in ICEKp10, was discovered in *E. coli* first, However, it is found in 10% of *K. pneumoniae* strains (Nougayrède *et al*.,2006; Lam *et al*.,2018b). Colibactin is a protein that helps bacteria colonize mucosa and the gut, as well as spread to the blood and other organs, and it may play a role in the development of colorectal cancer (Nougayrède *et al*.,2006; Lu *et al*.,2017). This mutation is found only in the CG23-I sublineage, which is responsible for the vast majority of CG23 liver abscesses. Lam and his coworkers are a group of people who are passionate about what they do (Lam *et al*., 2018). The ICEKp10 gene has been acquired multiple times in CG258 and is common in the ST258-wzi154 (KL107) subclade, but the clb locus has been disrupted and is most likely inactive, indicating that it is negatively selected in this clon ( Russo *et al*.,2014).

### 2.4.5. Hypermucoidy

Although hypermucoidy is *K. pneumoniae's* most well-known virulence factor, little is known about its genetic basis or role in disease (Box 3). Due to the presence of one or both of the accessory regulator genes *rmpA* or *rmpA2* (which

share >80% homology), the phenotype is frequently linked to capsule overproduction (Walker *et al*.,2019 ). On virulence plasmids, *rmpA* and *rmpA2* are usually found near iro and iuc, and thus share the distribution of both KpVP-1 and KpVP-2; however, *rmpA* and iro are co-localized in ICEKp1, and *rmpA2* and iuc are co-localized in non-canonical virulence plasmids(Lam *et al*.,2018b; Lam *et al*.,2019).

## 2.5. *Enterococcus faecalis*

***Enterococcus faecalis (E. faecalis)***, a Gram-positive opportunistic bacterium that lives in the gastrointestinal tract of mammals and spreads through feces, is the leading cause of UTI, septicemia, endocarditis, and other infections, as well as meningitis, by colonizing wounds and the bloodstream (Arias *et al*.,2012; Agudelo Higuita *et al*.,2014; Lebreton *et al*., 2014). Vancomycin-resistant Enterococcus (VRE) has been designated as a pathogen with a second-tier high priority by the World Health Organization. MDR Enterococcus strains have also been reported to spread their resistance to other bacteria that cause disease, the majority notably *Staphylococcus aureus*, via a horizontal gene transfer mechanism (George *et al*.,1989; Mundy *et al*., 2000; Willems *et al*., 2011).

Tetracycline, erythromycin, chloramphenicol, amicillin, linezolid, and vancomycin resistance are the most notable and problematic MDRs expressed by *E. faecalis* V583, which was isolated first in the United States and is the most extensively studied clinical strain (Sahm *et al*., 1989; Palmer *et al*., 2010; Clewell *et al*., 2014; Raven *et al*., 2016). Exogenously acquired antimicrobial resistance (AMR) genes are plasmid-borne genes that also contribute to virulence and cytolytic toxin biosynthesis (Palmer *et al*., 2010). The bacterium has the highest proportion of exogenously acquired mobile DNA (> 25%), as well as 38 Insertion Sequence (IS) elements, according to the researchers. It possesses AMR genes and virulence

traits, allowing it to adapt to a wide range of conditions, and it actively contributes to and spreads its pathogenic potential.

The bacterium has the highest proportion of exogenously acquired mobile DNA (> 25%) and 38 Insertion Sequence (IS) elements, both of which aid in the disease's spread. Pathogenicity, antimicrobial resistance genes, and virulence traits are all considerations, allowing it to adapt to a variety of situations. (Moscoso *et al*.,2011). In the United States, the first vancomycin-resistant *E. faecalis* clinical strain was discovered in 1989. *E. faecalis* is a leading cause of hospital-acquired infection and multidrug resistance (Lebreton et al., 2014). Because of these factors, *Enterococcus faecalis* is not generally considered safe (GRAS) (Ogier *et al*.,2008).

*E. faecalis* has been isolated from a wide range of environments and can be found in the wild. Human and animal gastrointestinal tracts are widely regarded as E. faecalis' primary habitat, where it lives as a commensal (Lukasova *et al*.,2003; Lebreton *et al*.,2014 ; Barretto *et al*.,2015). Furthermore, human and animal blood and urine specimens are major sources of *E. faecalis* (Lebreton *et al*.,2014).

*E. faecalis* is a type of lactose acid bacteria that is commonly used to make fermented foods, especially fermented dairy products. Several *E. faecalis* Traditional dairy products have yielded strains that have been recovered in recent years (Chen *et al*.,2015 ; Terzic-Vidojevic *et al*.,2015). Furthermore, *E. faecalis* can be found in water and soil (Weigand *et al*.,2014; Veljović *et al*.,2015). Given *E. faecalis'* primary habitat is the gastrointestinal tract, but its widespread distribution in a variety of niches, studying the relationship between enteric and extra-enteric strains of *E. faecalis* is fascinating. The central question is whether extra-enteric *E. faecalis* strains are related to faecal pollution or belong to different lineages. Another

intriguing feature is *E. faecalis'* ability to adapt to and survive in a variety of environments.

### 2. 5.1. Virulence factors *of Enterococcus faecalis*

Virulence factors such as enterococcal surface protein (Esp), hyaluronidase (Hyl), gelatinase (GelE), aggregation substance (AS) proteins (Asa1), collagen-binding protein (Ace), and cytolysin (CylA) play a role in infection adherence, colonization, evasion of the host immune response, extracellular enzyme production, and pathogenicity (Barbosa-Ribeiro *et al*.,2016 ; Strateva *et al*.,2016). Treatment of enterococcal infections has also become more difficult , Antimicrobial resistance has increased to -lactams, macrolides, fluroquinolones, glycopeptides, and aminoglycosides, among other antimicrobials ( Heidari *et al*.,2016; Singh *et al*.,2016). Enterococci have sophisticated genetic exchange systems. These systems have the ability to transfer virulence determinant genes to resistant strains (Heidari *et al*.,2016; Singh *et al*.,2015).

The emergence of antimicrobial-resistant virulent enterococci is a major concern in the treatment and control of nosocomial infections (Niu *et al*., 2016). Some virulence factors have been linked to the formation of E. faecalis biofilms, and others have been linked to pathogenicity in E. faecalis.. Esp is a large surface protein found in E. faecalis cells that helps them adhere, colonize, and persist in the urinary tract, as well as evade the immune system and form biofilms (Toledo-Arana *et al*., 2001; Paganelli *et al*., 2012). Gelatin, collagen, and hemoglobin are hydrolyzed by GelE, an extracellular metalloprotease. GelE controls autolysis and the release of high-molecular-weight eDNA, which is needed for *E. faecalis* biofilm formation. It's also linked to the formation of *E. faecalis* biofilms and bacterial adhesion (Kayaoglu and rstavik, 2004; Park *et al*., 2007; Thomas *et al*., 2008). However, some studies

have found no correlation between Esp or GelE and biofilm formation in *E. faecalis* (Kristich *et al*., 2004; Mohamed and Murray, 2005; Anderson *et al*., 2016).

### 2.5.2. Enterococcal surface protein(*Esp*) gene

Esp, the enterococcal surface protein, is a Bap-like protein (Toledo-Arana *et al*.,2001; Tendolkar *et al*.,2005). Esp is an 1873-amino-acid protein with a large molecular weight. It has well-defined modules such as the Nterminal domain, which shares 26 percent The central core region has the same sequence as Bap., The C-terminal domain, which has two domains (A and B) and a series of tandem repeats that share 23% sequence identity with Bap's C-repeats, and the C-terminal domain, which has two domains (A and B) and a series of tandem repeats that share 23% sequence identity with Bap's C-repeats, This protein has an LPXTG-like motif similar to those found in surface-associated proteins.. Biofilm formation is aided by *E. faecalis* via Esp. The N-terminal domain was discovered to be the most important region in Esp-mediated biofilm enhancement (Tendolkar *et al*.,2005). However, the molecular mechanisms by which Esp's N-terminal region accomplishes this function are unknown. We hypothesize that these proteins could mediate intercellular communication via a common molecular mechanism of amyloid-like conformation, based on previous findings with S. aureus Bap. We demonstrated this when the pH of the media turned acidic. Biophysical in vitro assays, cell-based and microscopic approaches, and dyebinding analyses revealed that Esp's N-terminal domain formed aggregates with amyloid-like propertiesEsp caused multicellular behavior in bacteria lacking the esp gene by forming amyloid-like material when it was added exogenously to the culture or expressed from a plasmid, demonstrating that Esp's

Nterminal domain is functional... Our findings point to amyloid-like aggregation as a common cellular mechanism. BAP-like proteins are found in the biofilm matrix.

### 2.5.3. Gelatinase

Gelatinase is a secreting factor that is important in the pathogenesis of Enterococci (Todokoro *et al*., 2017). This enzyme's proteolytic activity contributes to the production of peptides for the bacteria, which directly and indirectly harms host tissue (Ali *et al*.,2017).Gelatinase is a zinc endo peptidase/metallo endo peptidase that is encoded by a chromosomal gene (gelE). Gelatin, collagen, hemoglobin, and other biologically active peptides can all be hydrolyzed by this enzyme (Vidana *et al*., 2011). Gelatinase is an extracellular zinc-endopeptidase/protease from E. faecalis that can hydrolyze gelatin, collagen, casein, hemoglobin, and other peptides (Su *et al*.,1991). In both animals and humans, *E. faecalis* produces gelatinase, which contributes to virulence. Gelatinase damages host tissue, allowing bacteria to migrate, spread, colonize, and persist via bioflm formation (Franz *et al*., 2011).

### 2.5.4. Hyaluronidase

HyaluronidaseAs a hyaluronidase is a degradative enzyme cancause damage to the tissue Hyaluronidase works by depolymerizing connective tissue mucopolysaccharides. Its purpose is to facilitate the spread of bacteria and their toxins through host tissue. The chromosomal hyl gene encodes a hyaluronidase that is specific for *E. faecium* (Rice *et al*.,2003; Vankerckhoven *et al*.,2004) and is related to the hyaluronidases of other Gram-positive cocci. Hyaluronidase promotes the migration of other bacteria from the root canal to the periapical lesions, exacerbating the situation. Hyaluronidase causes other bacteria to produce the toxins that they have, which increases the damage (Sunde *et al*.,2002). *E. faecalis* contains

hyaluronidase, which aids in the spread of bacteria and toxins to host tissue. Other bacteria will continue to migrate from the root canal to the periapical lesions as a result of hyaluronidase. Furthermore, hyaluronidase stimulates the production of toxins by other bacteria, which increases damage and inflammation (Abou-Rass *et al*.,1998). This condition is extremely beneficial to the growth of *E. faecalis*.

### 2.5.5. Aggregation substance

Asa1 encodes an aggregation substance that aids conjugation transfer of plasmids containing sex pheromone genes (Galli *et al*., 1990) and increases virulence (adherence to renal tubular cells (Kreft *et al*.,1992), endocardial cells in the heart and intestinal epithelial cells). Asa1 is a virulence factor that encodes a surface protein involved in adherence and is dependent on the pheromone inducible conjugative plasmid (Sava *et al*., 2010) Aggregation substance is encoded on a sex pheromone plasmid and mediates bacterial aggregation, allowing plasmid transfer (Süßmuth *et al*., 2000).

### 2.5.6. Characteristics of *E. faecalis*

*E. faecalis'* ability to survive in high concentrations of sodium hypochlorite (NaOCl), sodium dodecylsulfate, hydrogen peroxide, heat, hyperosmolarity, ethanol, as well as acidity and alkalinity Del (Fabbro *et al*.,2014). In addition to such traits, *E. faecalis* can cause extra-radicular infection either directly or indirectly by secreting toxins or inducing inflammation. It can also acquire and transfer extrachromosomal elements, as well as encode virulence traits, which aid in colonization and competition with bacteria from other sources, Pathological changes and resistance to host defense mechanisms *E. faecalis* can also form a biofilm that

is well-organized that is resistant to recuperation. It can cause hydroxyapatite precipitation in a mature biofilm, leading to calcification ( Fabbro *et al*.,2014).

The aggregation compounds Plasmids encode adhesive substances. that help the donor and recipient bacteria exchange plasmids during the conjugation process.They also help *E. faecalis* attach to various eukaryotic cells, strengthen *E. faecalis'* adhesion to types I and IV collagens, and protect *E. faecalis* from host neutrophils. Microorganisms that produce these aggregation factors, such as E. faecalis, can cause macrophages to release tumor necrosis factors (TNF-), as well as interferon (INF-) and tumor necrosis factors (TNF-), as a result of bacteria-induced T-cell proliferation (Kayaoglu *et al*.2004). As a result, TNF release leads to bone resorption, whereas INF- release stimulates The amount of hydrogen peroxide and superoxide anions secreted increases., causing cell and tissue damage. Surface adhesins, on the other hand, contribute to *E. faecalis* virulence bacteria adhering to variety of essential substances, Abiotic surfaces (for biofilm formation), other bacteria species (for gene and nutrient exchange), The materials used include collagen fibers, human serum, and dentinal tissues (Kayaoglu *et al*.,2004).

## 2.6. *Staphylococcus aureus*

*S. aureus* is a dangerous pathogen that causes infections such as *Klepsilla pneumonia,* soft tissue infections, wounds, arthritis, and skin infections. Increased morbidity and mortality, as well as significant economic loss, have resulted from the spread of multidrug-resistant and highly virulent S. aureus strains around the world. Antibiotic resistance develops as a result of recurrent infections and antibiotic overuse, which aids in the spread of *S. aureus* (Sampedro *et al*., 2014). In the meantime, in stressful environments, this pathogen can form biofilms that protect active cells from antibiotics and host defense mechanisms. As a result, S. aureus

infection is becoming more common, but treatment options are limited .Staphylococcus aureus colonizes the nares without causing any symptoms containing approximately 30%of people permanently colonized (Wertheim *et al*.,2005). It's also the leading cause of skin and soft tissue infections, particularly among colonized people (Dryden,2009).

Although most local skin infections are self-limiting, they can serve as a portal for this pathogen to enter underlying tissue and the bloodstream on rare occasions. (In fact, S. aureus bacteremia is most commonly caused by skin infections) (Wilson *et al*.,2011; Yarovoy *et al*.,2019) . The mechanisms of *S. aureus* systemic spread from skin lesions are unknown, but the main risk factors for developing S. aureus sepsis are age (highest risk in infants and the elderly), additional comorbidities (heart disease, diabetes, renal disease, HIV infection), the presence of indwelling medical devices, intravenous drug use, and low socioeconomic status (Asgeirsson *et al.*,2018). Amidst the fact that the majority of people colonized with S. aureus will not develop an invasive infection, *S. aureus* is one of the most common pathogens causing bloodstream infections due to the sheer number of people infected ( Kern and Rieg ,2019).

These infections are marked by high mortality rates despite proper treatment (ranging from 20% to 50%, depending on infection severity), frequent recurrences (5–10%), and long-term impairments in more than one-third of survivors (Jacobsson *et al*.,2008; Asgeirsson *et al*.,2018). In developed countries, the prevalence of Infections with S. aureus have been found in the bloodstream increased in the recent past (Asgeirsson *et al*.,2018; Kern and Rieg ,2019 ). In developing countries, severe *S. aureus* infections are another underappreciated but significant problem (Nickerson *et al*.,2009).

In general, there is a pressing It is necessary to gain a better understanding of these invasive infections and develop new therapies to improve patient care. *S. aureus* is a spherical bacterium with grape-like clusters that is Gram-positive and coagulase-positive. It belongs to the Staphylococcaceae family. *S. aureus* is a commensal bacteria that can be found asymptomatically on the skin, skin glands, and mucous membranes, including healthy people's noses and guts (Gould and Chamberlaine,1995). According to studies, approximately a quarter of people are persistent S. aureus nasal carriers, 30% are intermittent carriers, and the remaining 50% are noncarriers (Wertheim *et al*.,2005). As a result, by acting as a reservoir for the pathogen, this colonization greatly increases the likelihood of infection. In most cases, the affected individuals *S. aureus*, which they normally carry as a commensal, infects them.

### 2.6.1. Virulence factors *of Staphylococcus aureus*

Infants and children are the most susceptible to the severe consequences of *S. aureus* infections, as they may be exposed in the community or in the hospital to this bacteria (Kaplan,2016). Pathogenic *S. aureus* strains colonize and cause infection by expressing a variety of virulence factors that play an important role in host–pathogen interactions. These virulence factors help the pathogen not only enter but also survive in the host tissues. They evade immune responses and adhere to host cells, but they also cause tissue damage by secreting exoenzymes and toxins that cause tissue damage (Tong *et al*.,2015; Thomer *et al*.,2016).

Within the slime layer, *S. aureus* strains produce a multi-layered biofilm, which contains a protein that is heterogeneous. The innate resistance of biofilms to host

immune defenses and antimicrobial agents is a key feature, as it leads to chronic and destructive infections (Lister and Horswill ,2014). *S. aureus* causes infectious endocarditis and osteomyelitis when it attaches to and persists on native host tissues like heart valves and bone. It can also adhere to and stay on medical devices such as catheters, prosthetic joints, artificial heart valves, and orthopedic implants, resulting in severe chronic infections in hospitalized patients (Barrett *et al*.,2014; Hogan *et al*.,2015). Adhesion to various surfaces and colonization of host tissues are the first steps in *S. aureus's* formation of a biofilm. Microbial surface components such as fibronectin-binding proteins A and B (finbA and finbB), clumping factors A and B (clfA and clfB), collagen-binding proteins A and B (collagen-binding proteins A and B), and collagen-binding proteins C and D (collagen-binding protein (cna), bone sialoprotein bind) are expressed by the bacterium and recognize adhesive matrix molecules (MSCRAMMs) (Khoramian *et al*.,2015; Kot *et al*.,2018).

## 2.7. *Escherichia coli*

*E. coli* is a type of bacteria. Theodor Escherich, a German bacteriologist, discovered a Gram-negative, facultative anaerobic bacterium in the human colon in 1885 (Feng *et al*.,2002). As a result of considerable research and development, *E. coli* has become the best-characterized organism on the globe, the workhorse in molecular biology laboratories, and one of the most important organisms employed in industry. Due to its rapid expansion, simplicity of culture, metabolic adaptability, and abundance of biochemical and physiological knowledge, it is a popular model organism. In the past, non-pathogenic strains were widely used in the pharmaceutical, food, chemical, and fuel industries. For industrial use, *E. coli* is not the most adaptable organism. On the other hand, rapid development in *E. coli* metabolic engineering and synthetic biology has been aided by a substantial body of

knowledge in *E. coli* biochemistry, physiology, and genetics. As a result, a number of previously believed hurdles have been overcome, and new E. coli phenotypes have been developed to outperform typical native producers. *E. coli*, for example, has been genetically modified to produce industrial quantities of amino acids that were previously produced by the natural producer Coryne bacterium glutamicum (Shen *et al*.,2011; Gusyatiner *et al*.,2017 ;Ohtake *et al*.,2017). *E. coli* is a frequent alternative for metabolic engineering when no natural producers are available. *E. coli* has long been utilized as a proof-of-concept model organism as well as an industrial producer. Lysine can be made in a number of ways (Kojima *et al*.,2000), 1,3-propanediol (PDO) (Sabra *et al*.,2017), Two well-known and effective examples are Burgard et al.,2016; Sanford et al.,2016. As a result, *E. coli* is the most common prokaryote found in both laboratory and industrial environments. E. coli strains are easy to grow, have a quick doubling time, and may survive in a range of environments. Above all, *E. coli* is a simple genetically manipulable organism that allows us to better understand its physiology and produce new phenotypes. *E. coli* has been used to develop a variety of molecular cloning techniques and genetic tools. These techniques allow for quick strain growth, which can save industrial development costs dramatically (Meyer *et al*.,2011).

### 2.7.1. Toxicity

Metabolic engineering involves the modification of native pathways or the introduction of non-native enzymes to produce compounds that are desired. In these processes, intermediates that are potentially harmful, poisonous products, or sludge of toxic side reactions may accumulate (Nicolaou *et al*.,2010; Jarboe *et al*.,2011). Metabolite toxicity is a complex phenomenon that can be caused by a number of reasons, such as a general stress response, essential enzyme inhibition or deactivation, or cell membrane damage. The majority of the time, toxicity has a non-

specific, condition-dependent effect. Toxicology can stifle growth, product development, or both. Because growth and production toxicity are uncoupled, It is usual for the cell to accumulate products to a titer that above the toxicity threshold, preventing the cell from growing (Atsumi *et al*.,2008; Atsumi *et al*.,2010).

### 2.7.2. Virulence factors of *E.coli*

*E .coli* Bacteria have many virulence factors that increase their susceptibility to disease The most important of them are urinary tract injuries disease, the most significant of which is urinary tract infections, one of these factors is the ability to analyze red blood cells and the possession of these cells. The hemolysin enzyme is divided into several types relying on how it degrades (alpha, beta, kama). The first type, vahimolysin-, supplies bacteria only with ability to reproduce Beta-hemolysin, the second type, allows bacteria to break down blood cells. The red blood decomposes entirely, while hemolysin, the third type, allows bacteria to degrade red blood cells. Other mammals' red blood cells, not human red blood cells. The bacteria also possess the cytotoxic factor factor necrotizing that analyzes red blood cells(Jaweetz *et al*.,2016).

Adhesins, toxins (e.g., alpha-hemolysin, cytotoxic necrotizing factor 1, autotransporter toxins), iron/heme acquisition pathways, and iron ion transport have all been found in uropathogenic *E. coli*. P, S, and type 1 fmbriae are implicated in colon, kidney, and lower urinary tract epithelial cell adhesion, as well as cytokine production in T cells.. Furthermore, they play an important role in the colonization of extraintestinal infections. The ability of UPEC to multiply intracellularly is a distinguishing feature (Ewers *et al*.,2007; Baldy-Chudzik *et al*.,2015). The deletion of a region of the genome involved in the formation of type 1 fmbriae and the silence of genes expressing P fmbriae have resulted in the generation of strains that generate

asymptomatic bacteriuria (ABU).These strains can colonize the urinary tract without causing infection (Terlizzi *et al*.,2017).

The host's own intestinal flora are a potential source of UPEC, However, The virus can be feces is a type of communication that can be passed on orally or sexually (Terlizzi *et al.*,2017). The presence and amount of capsular antigen are directly proportionate to the severity of neonatal invasive infections such meningitis and bacteremia/sepsis, which are caused by *E. coli* K1 strains. Hosts could spread *E. coli* K1 infection (Alkeskas *et al*.,2015). The urogenital or digestive tract may be the source of early E. coli infection, especially in the case of perinatal UTI.

## 2.8. Role of Plant extraction Anti-infective Drugs

The belief has existed since ancient times that plants contain biologically active compounds with therapeutic properties that can be used to treat a variety of aliments, such as asthma, gastro-intestinal problems, skin disorders, respiratory and urinary complications, hepatic and cardiovascular disease, and so on. Because of the chemical compounds in these plants that have a favorable physiological effect on the human body, they have a significant potential for the discovery and development of new medications (A Review. Dilfuza Egamberdieva). PlantsMedicinal plants benefit from the accumulation of bioactive phytochemicals in plant tissue, known as primary and secondary metabolites. Primary metabolites are organic molecules that contribute in the formation and development of the human body. Examples include glucose, starch, polysaccharide, protein, lipids, and nucleic acid. Secondary metabolites produced by plants include alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, and others( A Review. Dilfuza Egamberdieva; Arvind Kumar Shakya.,2016).

### 2.8.1. *Myrtus communis L*. (Myrtaceae)

Linnaeus initially described Myrtus, popularly known as myrtle( Bouzabata *et al*.,2016) in 1753. It is a genus of flowering plants in the Myrtaceae family. The common myrtle (Myrtaceae) is an evergreen plant with antibacterial, anti-inflammatory, ant hyperglycemic, and antioxidant effects. It's also used as a sedative and hypnotic herb in traditional Iranian medicine. Myrtus communis, often known as the common or true myrtle, is found across the northern Mediterranean region (particularly on the islands of Sardinia and Corsica, where it is known as murta)( Ogur *et al*.,2014).

### 2.8.2. Distribution

Myrtle (M. communis L.) is a common Mediterranean plant. The plant can be found growing in abundance from the northwest to the east of the Mediterranean, encompassing bordering countries and western Asia, as well as the Aegean (Baytop 1997). Myrtleis is a plant that grows in southern Europe, North Africa, and western Asia. It can also be found in South America, the northeastern Himalaya, and Fig. Myrtle is grown in gardens, especially in the Northwest Indian region, because of its aromatic blooms (Nadkarni 1989). With a wide range over the Mediterranean, this species is one of the most important evergreen shrubs in the Mediterranean maquis. It thrives on the beaches and in the inner hillsides of Italy, and it is especially common on the islands, where it is one of the most common plants.

### 2.8.3. Characterization

The plant grows to a height of 2.4–3 meters, with branches producing a complete head that is heavily covered in leaves (Sumbul *et al*., 2011). Fruits are small and

black, with small green leaves (Asif *et al*., 2011). The evergreen leaves are 2–5 cm long and fragrant when crushed, similar to myrrh or eucalyptus. The taste of it is bitter and intensive (Gortzi *et al*., 2008; Özkan and Güray, 2009) which is mainly due to its astringency. Flowers are star-shaped, white or pinkish in color, and quite fragrant (Charles, 2013). Several seeds are contained in the spherical blue-black berry fruit. Pollination is carried by by bees. The seeds are disseminated by insects, and the seeds are dispersed by birds that consume the insects.

### 2.8.4 Chemical compound

Geraniol (1.6 percent), -Humulene (1.5 percent), eugenol (1.3 percent), isobutyl-isobutyrate (0.8 percent), and methyl chavicol (0.5 percent) were the main chemicals in M. communis. Among all of the medications examined, chlorhexidine had the lowest MIC. M. communis oil had lower MIC values against both bacteria than NaOCl, although it had a higher MIC against Candida albicans(Nora *et al*.,2015; Nabavizade *et al*.,2017).

### 2.8.5. Pharmacological effects

#### 2.8.5.1. Antimicrobial

Alem et colleagues investigated the antibacterial activity of the crude preparation of Myrtle against *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Pseudomonas vulgaris, Pseudomonas mirabilis, Klebsiella aerogenes, Salmonella typhi, and Salmonella* and *shigiella* (Alem *et al*.,2008). Mansouri et altested the antibacterial activity of M. communis methanol crude extract against ten laboratory strains of bacteria, including six Gram positive (*Staphylococcus aureus,*

*Micrococcus luteus*, *Streptococcus pneumoniae*, *S. pyogenes*, *S. agalactiae*, and *Listeria monocytogenes*) and four Gram negative bacteria (*Escherichia coli*, *Proteus vulgar vulgaris*, *Pseudomonas aeruginosa* and *Campylobacter jejuni*). The crude extract inhibited the growth of all tested bacteria except *C. jejuni* (Mansouri *et al* .,2001).

# Chapter Three

# Materials and Methods

## 3. Materials and Method

### 3.2. Materials

#### 3.2.1. Equipments:

The equipments which used with their sources are shown in table (3-1)

**Table (3-1):**Equipment's used in this project.

| No. | Gadgets | Company | Country |
|---|---|---|---|
| 1 | Autoclave | Hirayama | USA |
| 2 | Biological safety cabinet | Labogene | Korea Denmark |
| 3 | Distillator | FineTech | Korea |
| 4 | EDTA vacuum tube | Xinle | Germany |
| 5 | E-graph –UV (Gel documentation) | ATTO | Korea |
| 6 | Electrophoresis power | supply Pelex | France |
| 7 | flasks andbeakers | Hirschman | Germany |
| 8 | Gas burner | GFL | Germany |
| 9 | Gel electrophoresis | Cleaver | USA |
| 10 | Glass EDTA tubes 10 ml | AFCO | Jordan |
| 11 | Hood | Bio Lab | Korea |
| 12 | Incubator | Memmert | Germany |
| 13 | Loop, wood sticks | Roche | China |
| 14 | Medical injection Syringes | MEDECO | UAE |
| 15 | Micro centrifuge | Himedia Beckman | India Germany |
| 16 | Micropipettes | Slamid | USA |
| 17 | 5-50 ml, 100-1000 ml , 0.5 – 10 ml | BIO BASIC | Canada |
| 18 | Microcentrifuge tubes 1.5ml | Kardelen | Turkey |
| 19 | Medical cotton | Memmert | Germany |
| 20 | Oven | Ravi scientific industries | India |
| 21 | Paraffin film | Cleaver | USA |
| 22 | PCR system/Conventional | Hirschman | Germany |
| 23 | Quantum vilber lourmat | | France |
| 24 | Refrigerator | Concord | Lebanon |
| 25 | Screw capped tubes 10 ml | | |
| 26 | Spectrophotometer | Optima sp3000 | Japan |
| 27 | Sensitive electron balance | A & D | Japan |

| | | | |
|---|---|---|---|
| **28** | Vortex | Gemmy | Taiwan |
| **29** | Water bath | GFL | Germany |
| **30** | 100-100µl , 10-50 µl tips | Dolphin | Syria |
| **31** | 0.1-10µl with filter tips | Promega | USA |
| **32** | 1.5 µl Micro-centrifuge tube | Sterile | S. Korea |

### 3.2.2. Chemical and biological Material

Chemical and biological materials are listed in table(3-2)

**Table (3-2):**The chemicals and biological materials and their sources.

| NO. | Chemical material (molecular) | Company/country |
|---|---|---|
| **1.** | 99%,95% and 70% alcohol (Ethanol) | Flukachemika Switzerland |
| **2.** | Agarose | Froggabio, Canada |
| **3.** | Nuclease-Free-Water | Bio basic, Canada |
| **4.** | DNA Ladder Marker 100bp | Promega, USA |
| **5.** | Primers | Macrogen, Korea |
| **6.** | Tris borate TBE buffer (loading buffer) | Promega ,USA |
| **7.** | PCR pre mix (master mix) | Bioneer, Korea |
| **8.** | Master mix ARMS PCR – Kit | Promega, USA |
| **9.** | DNA loading dye | Promega/USA |
| **10.** | Redsafe nucleic acid staining solution 1ml | BDH,England |
| **11.** | Distilled water | |
| **12.** | Isopropanol 99% | BDH/England |

**Table (3-3):** Culture media used in this study

| Culture Media | Company /Country |
|---|---|
| Urinary Tract Infection Chromogenic Agar (UTIC) | Condalab(spain) |
| Brain heart infusion broth (BHI) | Prondisa (spain) |
| Nutrient Agar media | Oxoid (England) |

## Samples Collection

The practical aspect of this study was extended for the period between October to December /2020, which included(102) Dialysis samples collected from Imam Al-Sadiq Teaching Hospital (Hemodialysis unit) and Margan Medical City hospital in Babylon City.

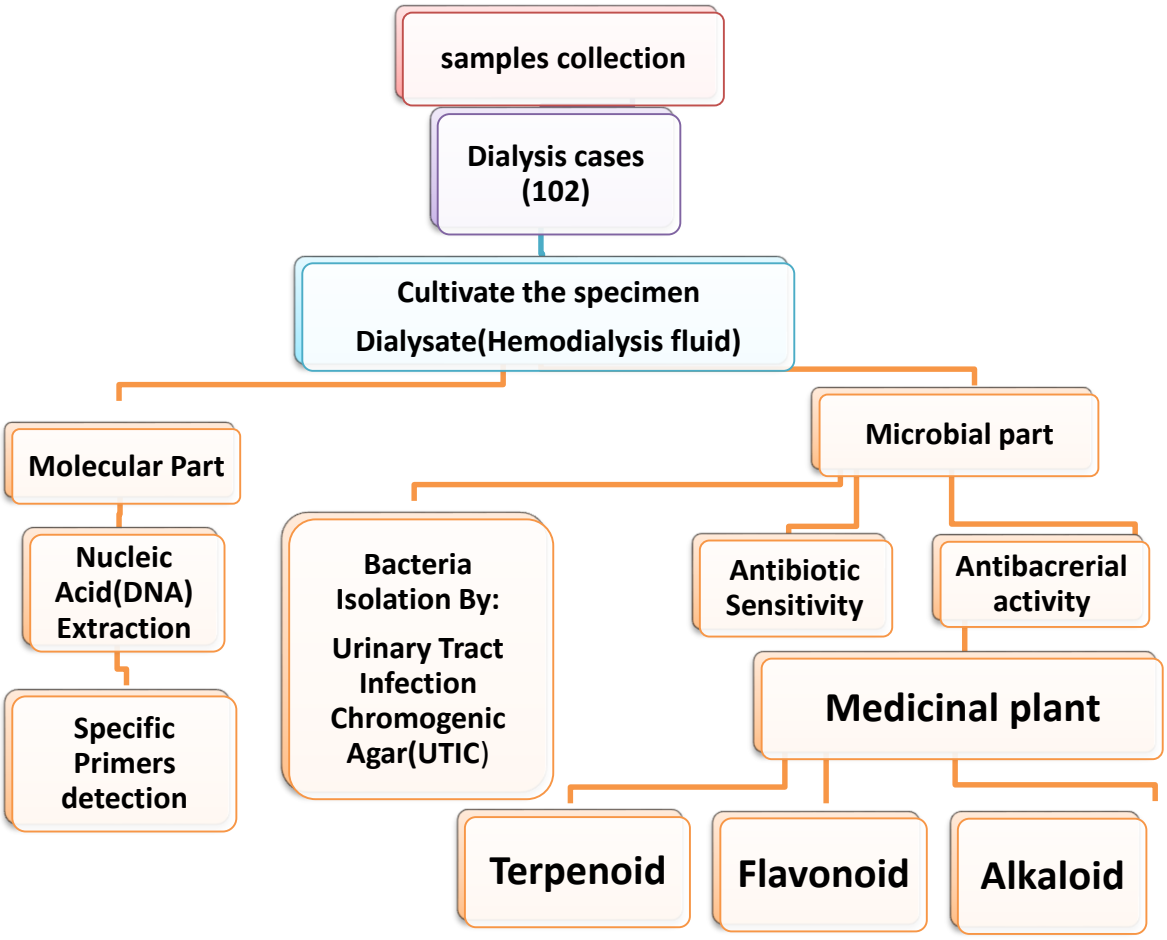



**Fig.(3-1):**Study design (plan of study).

## 3.3. Methods

### 3.3.1 Dialysis Sampling:

One hundred and two of Dialysis fluid samples were collected from patients suffering from Dialysis Specimens were aseptically obtained, 5-10 ml of dialysis fluid was transported to the laboratory in a sterile container and then cultivating them on culture media for the purpose of isolating and diagnosing bacterial species .

### 3.3.3 Isolation of Bacterial species

In this study the method of isolation of bacteria species .Included using the following medium:

#### 3.3.3.1 Nutrient Agar Medium

To isolate, cultivate and to save bacterial strains.

#### 3.3.3.2 MacConkey Agar Medium

To isolate Gram-negative bacilli and to differentiate lactose fermented from non-lactose fermented bacteria.

#### 3.3.3.3 Mannitol salt agar(MSA)

This medium was used to detect the growth of Staphylococcus species that have the ability to fermentation Mannitol.

#### 3.3.3.4 Muller Hinton Agar

Muller Hinton agar medium was prepared according to manufacturing company, it was autoclaved at (121°C \ for 15 minutes), then cooled to 50°C, This medium was used to detect anti-bacterial activity of plant Extracted.

### 3.3.3.5 Brain Heart Infusion Agar (BHIA)

is a solid medium rich in nutrients, suitable for the cultivation of several fastidious strains of bacteria

### 3.3.3.6 Urinary Tract Infections Chromogenic Agar (UTIC)

Chromogenic UTI Medium is a differential agar which provides presumptive identification of the bacteria which cause infection of the urinary tract.

## 3.3.5. Isolation of Genomic DNA (Promega Genomic DNA Purification Kit )

Genomic DNA that **used for molecular study obtained by Wizard Genomic DNA Extraction .**

**3.3.5.1.** Components of an Isolation Kit

| Components | Amount | Components | Amount |
|---|---|---|---|
| **Cell lysis Solution** | 100 ml | **Protein Precipitation Solution** | 200 µl |
| **Nuclei Lysis Solution** | 600 µl | **DNA Rehydration Solution** | 100 µl |
| **RNase A Solution** | 3 µl | | |

### 3.3.5.2. The Protocol for DNA extraction from Gram negative bacteria

Procedure which Promega Kit recommend for DNA separation as reveled in the following :

**Step 1 –Preparing Pellet Cells**

**1.**First of all , add 1 ml of a bacterial culture grown for 24 hours in Brain Heart infusion broth to a 1.5 ml microcentrifuge tube.

**2.**Centrifuge at 13,000 - 16,000 × g for 2 minutes to pellet the cells the supernatant completely removed .

**Step 2 –Lyse Cells**

**3.**Six hundred µl of nuclei lysis solution was added. Gently pipet until the cells is suspended.

**4.**Incubation at 80 °C for 5 minutes to lyse the cells; then cool to room temperature.

**5.**Three µl of RNase Solution then added to the cell lysate. Invert the tube 2-5 times to mix.

**6.**Incubation at 37°C for 15-60 minutes. Cool the sample to room temperature .

**Step 3 – Protein Precipitation**

The RNase-treated cell lysate was combined with 7.200µl of protein precipitation solution.

To mix the Protein Precipitation Solution with the cell lysate, vortex vigorously at high speed for 20 seconds.

**8.**Then 5 minute ice incubation.

**9.** For 3 minutes, centrifuge at 13,000-16000 g.

**Step 4 – DNA Precipitation and Rehydration**

**10.** The DNA supernatant was transferred to a clean 1.5ml micro centrifuge tube containing 600µl of room temperature isopropanol.

**11.** Gently invert the strands of DNA until they form a visible mass.

**12.** Centrifuge for 2 minutes at 13,000-16,000 g.

**13.**it Drain the tube on clean absorbent paper after carefully pouring out the supernatant. Add 600 l of 70 percent ethanol at room temperature and gently invert the tube several times to wash the DNA pellet.

**14.** For 2 minutes, centrifuge at 13,000-16,000 g. Aspirate the ethanol with caution.

**15.** Allow the pellet to air-dry for 10-15 minutes after draining the tube on clean absorbent paper.

**16.** In the tube, add 100µl of DNA Rehydration Solution and rehydrate the DNA by incubating at 65°C for 1 hour.

**17** By gently tapping the tube, mix the solution on a regular basis. Alternatively, you can hydrate the DNA by incubating the Solution at room temperature or at 4 °C overnight..

**Final Step –Pure DNA**

**18.**At last ,the DNA fragment stored at 4°C or -20 °C as the final step.

## 3.3.6. Estimation of DNA Purity and Concentration

The Nano drop was used to assess the DNA concentration of samples by putting 3µl by the the extracted DNA machine to detect concentration in mg/l and purity by observing the ratio of optical density (OD) 260/280 nm to detect the purity concentration of samples containing protein For purifying DNA, the standard 260/280 ratio was 1.7-1.9. (Sambrook and Rusell,2001).

## 3.3.7. An agarose gel electrophoresis

After genomic DNA extraction, agarose gel electrophoresis was used to confirm the closeness and uprightness of the separated DNA. (Sambrook and Maniatis,1989).

### 3.3.8. Primers

In this study ,sets of primers were used to detect of virulence genes as shown in the following Table (3-4).The primers were supplied by Ligo /USA.

**Table (3-4)** Sequence of Primers

| Genes | bp. | Primer | |
|---|---|---|---|
| ***Klebsiella pneumoniae*** | | | |
| *fimH-1* | **688** | Forward | ATGAACGCCTGGTCCTTTGC |
| | | Reverse | GCTGAACGCCTATCCCCTGC |
| *mrkD* | **240** | Forward | CCACCAACTATTCCCTCGAA |
| | | Reverse | ATGGAACCCACATCGACATT |
| *magA* | **1,282** | Forward | GTGCTCTTTACATCATTGC |
| | | Reverse | GCAATGGCCATTTGCGTTAG |
| *cnf* | **498** | Forward | AAGATGGAGTTTCCTATGCAGGAG |
| | | Reverse | CATTCAGAGTCCTGCCCTCATTATT |
| ***Enterococcus feacalis*** | | | |
| *esp* | **510** | Forward | AATTGATTCTTTAGCATCTGG |
| | | Reverse | AGATTCATCTTTGATTCTTGG |
| *gelE* | **213** | Forward | TATGACAATGCTTTTTGGGAT |
| | | Reverse | AGATGCACCCGAAATAATATA |
| *hyl* | **276** | Forward | GACTGACGTCCAAGTTTCCAA |
| | | Reverse | ACAGAAGAGCTGCAGGAAATG |
| *asal* | **375** | Forward | GCACGCTATTACGAACTATGA |
| | | Reverse | TAAGAAAGAACATCACCACGA |

### 3.3.9. Master Mix

Bioneer's master premix was used, with the components listed in the table. (3-5).

**Table (3-5):** Components of the Master Mix

| Item | Concentration |
|---|---|
| DNA Taq polymerase | 1U/µl |
| Each :dNTP (dATP ,dCTP ,dGTP,d TTP) | 250Mm |
| Tris-HCL(pH9.0) | 10mM |

| | |
|---|---|
| KCL | 30Mme |
| MgC12 | 1.5mM |

### 3.3.10. Polymerase chain Reaction Technique

In this study were used conventional PCR was used to detect genes by using eight primers as shown in the table (3-4).

Lyophilized preliminary was disintegrated in a free DNase/RNase water to give a final concentration of 100 pmol / µl kept as a stock in -20°C.

### 3.3.11. Polymerase Chain Reaction Protocol

With primer sequences, standard amplification conditions were used in PCR. In PCR programming, the annealing temperature at which primers hybridize to complimentary sequences on template DNA is likely the most important. Melting temperature (Tm) calculations are used to determine the annealing temperature for PCR primers. Tm is the temperature at which half of the material melts DNA stands have been denatured The guanine and cytosine (G+C) content of PCR primers is used to calculate Tm. In 2-12 °C, the annealing temperature is frequently below the Tm .

### 3.3.12. Detection of *fimH-1*

The *fimH-1* genes were amplification by conventional PCR the primer sets manufactured by Ligo, USA .Sequences *fimH-1* primer was:

5 -ATGAACGCCTGGTCCTTTGC-3'

5' -GCTGAACGCCTATCCCCTGC-3'

The gradient condition for *fimH-1* are similar as shown in the following table (3-6). The PCR reaction mixture for gradient consisted of 5µl

template DNA , 5µl master mix ,5µl of each forward and reverse primer in 20 µl of total reaction volume

**Table (3-6):** Gradient condition for *fimH-1*

| ***fimH-1*** **Cycles** | **Temperature C°** | **Time/min** | |
|---|---|---|---|
| Initial denaturation | **94** | **5 min** | **1** |
| Denaturation | **94** | **1min** | **30** |
| Annealing Zones | **51-53-55-57-59-61** | **1min** | |
| Extension | **72** | **1min** | |
| Final extension | **72** | **5min** | **1** |
| Storage | **4** | ∞ | |

After determining the best annealing temperature for *fimH-1* by select the most visible band, which is 55C°, The PCR mixture consisted of 5 µl of DNA, 5 µl of master mix, 5 µl of forward and reverse primers, and PCR conditions were as listed in the table below. (3.7). PCR items were dissected on 2% agarose gel recolored with 5 µg/ml Redsafe nucleic acid staining solution ,the *fimH-1* produce 688 bp.

**Table (3.7):** condition for *fimH-1*

| **PCR Steps** **Cycles** | **Temperature C°** | **Time/min.** | |
|---|---|---|---|
| Initial denaturation | **94** | **5min** | **1** |
| Denaturation | **94** | **1min** | **30** |
| Annealing | **55** | **1min** | |
| Extension | **72** | **1min** | |
| Final extension | **72** | **5min** | **1** |
| Storage | **4** | ∞ | |

### 3.3.13. Detection of *mrkD*

The *mrkD* genes were amplification by conventional polymerase chain reaction (PCR). The primer sets manufactured by Ligo ,USA . Sequences *mrkD* primer was :

5'- CCACCAACTATTCCCTCGAA -3'
5'-ATGGAACCCACATCGACATT -3'

The gradient condition for *mrkD* are similar as shown in the following table (3.8). The PCR reaction mixture for gradient consisted of 5µl template DNA, 5µl master mix , 5µl of each forward and reverse primer in 20 µl of total reaction volume

**Table (3.8)**Gradient condition for *mrkD*

| PCR Steps | Temperature C° | Time/min. | Cycles |
|---|---|---|---|
| Initial denaturation | **94** | **5 min** | **1** |
| Denaturation | **94** | **1 min** | **30** |
| Annealing Zones | **49-51-53-55-57-59** | **1 min** | |
| Extension | **72** | **1 min** | |
| Final extension | **72** | **5 min** | **1** |
| Storage | **4** | ∞ | |

After picking the optimal annealing temperature for mrkD by determining the clearest band, which is 52C° , The PCR mixture consisted of 5 µl of DNA and 5 µl of master mix. ,5µl forward and reverse primer , PCR condition performed as illustrated in the following table (3.9).

PCR items were dissected on 2% agarose gel recolored with 5 µg /ml Redsafe nucleic acid staining solution . The *mrkD* produce 240 bp.

**Table (3.9)** Condition for PCR for *mrkD*

| PCR Steps | Temperature C° | Time/min. | Cycles |
|---|---|---|---|
| Initial denaturation | **94** | **5 min** | **1** |
| Denaturation | **94** | **1 min** | **30** |
| Annealing | **55** | **1 min** | |
| Extension | **72** | **1 min** | |
| Final extension | **72** | **5 min** | **1** |
| Storage | **4** | ∞ | |

## 3.3.14. Determination of *magA*

The *magA* genes were amplification by conventional polymerase chain reaction (PCR). The primer sets manufactured by Ligo ,USA .Sequences *magA* primer was:

5' - GTGCTCTTTACATCATTGC - 3'

5' – GCAATGGCCATTTGCGTTAG-3'

The gradient condition for *magA* are similar as shown in the following table (3.10). The PCR reaction mixture for gradient consisted of the 5µl template DNA ,5µl master mix,5µl of each forward and reverse primer in 20 µl of total reaction volume

**Table (3.10) Gradient condition for *magA***

| PCR Steps | Temperature C° | Time/min. | Cycles |
|---|---|---|---|
| Initial denaturation | **94** | **5 min** | **1** |
| Denaturation | **94** | **1 min** | **30** |
| Annealing Zones | **51-53-55-57-59-61** | **1 min** | |
| Extension | **72** | **1 min** | |
| Final extension | **72** | **5 min** | **1** |
| Storage | **4** | ∞ | |

After determining the ideal annealing temperature, for *magA* by select the apparent band, which is 59C°, The PCR mix was 5 µl DNA, 5µl master

mix ,5µl forward and reverse primer,PCR condition were carried out as illustrated as follows (3.11).

PCR items were dissected on 2% agarose gel recolored with 5 µg/ml Redsafe nucleic acid staining solution . The *magA* produce 1,282 bp.

**Table (3.11)**PCR condition for *magA*

| PCR Steps | Temperature C° | Time/min. | Cycles |
|---|---|---|---|
| Initial denaturation | **94** | **5 min** | **1** |
| Denaturation | **94** | **1 min** | **30** |
| Annealing | **55** | **1 min** | |
| Extension | **72** | **1 min** | |
| Final extension | **72** | **5 min** | **1** |
| Storage | **4** | ∞ | |

### 3.3.15. Detection of *cnf*

The *cnf* genes were amplification by conventional polymerase chain reaction (PCR). The primer sets manufactured by Ligo ,USA .Sequences *cnf* primer was:

5' - AAGATGGAGTTTCCTATGCAGGAG -3'

5' – CATTCAGAGTCCTGCCCTCATTATT-3'

The gradient condition for *cnf* are similar as shown in the following table (3.12). The PCR reaction mixture for gradient consisted of the 5µl template DNA ,5µl master mix,5µl of each forward and reverse primer in 20 µl of total reaction volume

**Table (3.12)** Gradient condition for *cnf*

| PCR Steps | Temperature C° | Time/min. | Cycles |
|---|---|---|---|
| Initial denaturation | **95** | **5** | **1** |
| Denaturation | **94** | **1** | **30** |
| Annealing Zones | **52-54-56-58-60-62** | **1** | |
| Extension | **72** | **1** | |
| Final extension | **72** | **10** | **1** |
| Storage | **4** | ∞ | |

for *cnf* by selecting the clearst band After determining the ideal annealing temperature,which is 58C°, PCR mixture was 5 µl DNA, 5µl master mix 5µl forward and reverse primer,PCR condition were performed as illustrated in the following (3.13).

PCR items were dissected on 2% agarose gel recolored with 5 µg/ml Redsafe nucleic acid staining solution . The *cnf* produce 498 bp.

**Table (3.13):** PCR condition for *cnf*

| PCR Steps | Temperature C° | Time/min. | Cycles |
|---|---|---|---|
| **Initial denaturation** | **95** | **5** | **1** |
| **Denaturation** | **94** | **1** | **30** |
| **Annealing** | **58** | **1** | |
| **Extension** | **72** | **1** | |
| **Final extension** | **72** | **10** | **1** |
| **storage** | **4** | ∞ | |

## 3.3.16. Detection of *Enterococcus feacalis* genes

*Enterococcus feacalis* genes were amplification by conventional PCR.As shown in the table(3.14)

**Table (3.14):**PCR condition for *Enterococcus feacalis* Genes

| ***esp*** | | | |
|---|---|---|---|
| **PCR Steps** | **Temperature C°** | **Time/min.** | **Cycles** |
| **Initial denaturation** | **95** | **3** | **1** |
| **Denaturation** | **95** | **30s** | **30** |
| **Annealing** | **54** | **30s** | |
| **Extension** | **72** | **60s** | |
| **Final extension** | **72** | **10min** | **1** |
| **storage** | **4** | ∞ | |
| ***gelE*** | | | |
| **PCR Steps** | **Temperature C°** | **Time/min.** | **Cycles** |
| **Initial denaturation** | **95** | **3s** | **1** |
| **Denaturation** | **95** | **30s** | |

| | | | |
|---|---|---|---|
| **Annealing** | **56** | **30s** | **30** |
| **Extension** | **72** | **60s** | |
| **Final extension** | **72** | **10min** | **1** |
| **storage** | **4** | ∞ | |
| ***hyl*** | | | |
| **PCR Steps** | **Temperature C°** | **Time/min.** | **Cycles** |
| **Initial denaturation** | **95** | **3s** | **1** |
| **Denaturation** | **95** | **30s** | **30** |
| **Annealing** | **56** | **30s** | |
| **Extension** | **72** | **60s** | |
| **Final extension** | **72** | **10min** | **1** |
| **storage** | **4** | ∞ | |
| ***asa1*** | | | |
| **PCR Steps** | **Temperature C°** | **Time/min.** | **Cycles** |
| **Initial denaturation** | **95** | **3s** | **1** |
| **Denaturation** | **95** | **30s** | **30** |
| **Annealing** | **56** | **30s** | |
| **Extension** | **72** | **60s** | |
| **Final extension** | **72** | **10min** | **1** |
| **storage** | **4** | ∞ | |

After the determination of optimum annealing temperature of *Enterococcus fecalis* genes for *Esp, asa1, gelE, hyl* by selecting the clearest band, which is (54 C°, 56 C°, 56 C°, 56 C°) respectively, PCR mixture was 5µl DNA, 5µl master mix, 5µl forward and reverse primer, PCR items were dissected on 2% agarose gel recolored with 5 µg/ml Red safe nucleic acid staining solution.

**Table (3.15):** Gradient condition for *Enterococcus feacalis* Genes

| ***esp*** | | | |
|---|---|---|---|
| **PCR Steps** | **Temperature C°** | **Time/min.** | **Cycles** |
| **Initial denaturation** | **95** | **3** | **1** |
| **Denaturation** | **95** | **30s** | |

| | | | |
|---|---|---|---|
| **Annealing Zones** | **50-52-54-56-58-60** | **30s** | **30** |
| **Extension** | **72** | **60s** | |
| **Final extension** | **72** | **10min** | **1** |
| **storage** | **4** | ∞ | |
| ***gelE*** | | | |
| **PCR Steps** | **Temperature C °** | **Time/min.** | **Cycles** |
| **Initial denaturation** | **95** | **3s** | **1** |
| **Denaturation** | **95** | **30s** | **30** |
| **Annealing Zones** | **52-54-56-58-60-62** | **30s** | |
| **Extension** | **72** | **60s** | |
| **Final extension** | **72** | **10min** | **1** |
| **storage** | **4** | ∞ | |
| ***hyl*** | | | |
| **PCR Steps** | **Temperature C°** | **Time/min.** | **Cycles** |
| **Initial denaturation** | **95** | **3s** | **1** |
| **Denaturation** | **95** | **30s** | **30** |
| **Annealing Zones** | **50-52-54-56-58-60** | **30s** | |
| **Extension** | **72** | **60s** | |
| **Final extension** | **72** | **10min** | **1** |
| **storage** | **4** | ∞ | |
| ***asa1*** | | | |
| **PCR Steps** | **Temperature C°** | **Time/min.** | **Cycles** |
| **Initial denaturation** | **95** | **3s** | **1** |
| **Denaturation** | **95** | **30s** | **30** |
| **Annealing Zones** | **50-52-54-56-58-60** | **30s** | |
| **Extension** | **72** | **60s** | |
| **Final extension** | **72** | **10min** | **1** |

| | | |
|---|---|---|
| **storage** | **4** | ∞ |

## 3.3.17. Materials and Methods of Medicinal plant

## 3.3.17.1. Plant extraction:

Myrtle (*Myrtus communis L*.), leaves were collected from the gardens at University of Babylon, during October 2020, identified based on the taxonomic features in Iraqi Flora(Townsend et al,1974)Table (3-15). Leaves of these plants were cleaned, dried, and kept according to(Harorne et al,1975)

**Table(3-15) :** Scientific, Local, English name, Family, and active parts

| Scientific name | Local name | English name | Family | Active part used |
|---|---|---|---|---|
| ***Myrtus communis L.*** | **Yas** | **Myrtle** | **Myrtaceae** | **Leaves** |

### 3.3.17.2. Alkaloid determination using Harborne (1973) method:

Five g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide (NH4OH) was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute 1% ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

### 3.3.17.3. Extraction of the Crude Flavonoid and Crude Flavonoid (Boham et al ,1974):

Ten g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous Methanol at room temperature. The whole solution was

filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight

### 3.3.17.4. Extraction of the Crude Terpenoid Compounds (Harborne et al,1984):

Stock solution of 200 mg/ml for Flavonoid, Terpenoid, and Alkaloid, were prepared in 10% Dimethyl Sulfoxide (DMSO) then sterilized by Millipore filter (0.22µm) and stored at (-20C˚) until use (Al-Jassani et al,2017)

### 3.3.17.5. Antibacterial Efficacy:

The anti-bacterial activity of the secondary metabolite's compounds extracted from the leaves of (*Myrtus communis L*.) was tested against the isolated bacteria by using agar-well diffusion method (Perez L et al , 1990)Wells were made by using cork porer (6mm) in diameter. Dimethyl sulfoxide 10% (DMSO) was used as a negative control and Azithromycin (30 µg/Disc) antibiotic as a positive control Table(3-16).

**Table:(3-16)** Types of antibiotic, Abbreviation, and concentration per disc

| Antibiotic | Abbreviation | Concertation | Bacteria |
|---|---|---|---|
| **Azithromycin** | **AZM** | **(Disc/µg)** | ***Staphylococcus aureus*** |
| | | | ***Enterococcus faecalis*** |
| | | | ***Escherichia coli*** |
| | | | ***Klebsiella pneumonieae*** |

**Bacterial Isolates:** All isolates used in this study was isolated from hospitals located at Hillah city, Iraq Table(3-17).

**Table:(3-17) .** Types of Bacterial Isolates and their sources

| N0 | Bacteria isolate | Source of specimen |
|---|---|---|

| | | |
|---|---|---|
| **1** | ***Staphylococcus aureus*** | **Hemodialysis Fluid(102)** |
| **2** | ***Escherichia coli*** | |
| **3** | ***Enterococcus faecalis*** | |
| **4** | ***Klebsiella pneumonieae*** | |

## 3.4. Statistical analysis:

All data of treatments were dictated by three replicates. Data were subjected to an analysis of variance by using SPSS 16.0 program, a completely randomized design was used and least significant difference (L.S.D) was performed at $P \leq 0.05$.

## 3.5. Ethical Approval:

A valid consist was achieved from hospitals administration and from each patients and control before their inclusion in the study. For every patients or their followers, the procedure had been informed before the samples were collected, making absolutely sure that they understood the procedure that was to be carried out. The subjects were sentient that they had the right to reject to be included in the study without any detrimental effects.

# *Chapter Four*

# *Results and Discussion*

## 4. Results and Discussion:

### 4.1. Characteristics of the study Subjects:

The study was performed in Hilla city's and all samples collected from Imam AL-Sadiq teaching hospital (Hemodialysis unit and burns unit) and Margan medical city hospital. The study conducted in period from October to December / 2020, which included 102 samples of Dialysis. All cases were prognosticated by physicians, and samples were taken based on clinical evaluation for the patients, and all specimens were sent to the center's official.

In this study, 102 Hemodialysis fluid specimens were collected and divided into two groups: male (56) and females (46) patients, cultures were used to grow Hemodialysis fluid samples on urinary tract infection chromogenic (UTIC) media. The VITEC2 system was used to confirm the diagnosis of bacterial species.

The demographic distribution shows that the majority of Hemodialysis fluid specimens' patients were 56 males (54.9%) versus 46 females (45.1%), with statistically significant discrepancies between the two classes (males and females) at (P=0.05).

The primary goal of hemodialysis is to restore the intracellular and extracellular fluid environment that is characteristic of normal kidney function (Himmelfarb and Ikizler, 2010). In the United Kingdom, approximately 7,000 people start a form of renal treatment each year (Hole *et al*., 2018). The primary reason for renal failure is diabetes (28%), with the average age being 64 years. More than 60% of patients starting dialysis are male (Hole *et al*., 2018), with research indicating females are the predominant care partners (Low *et al.*, 2008). For more than 80% of ESRD patients, dialysis will be their

first form of treatment (Gilg *et al*., 2017).this study showed that percentage of male (54.9%), that continued with dialysis more than female percentage (45.1%), as show in table (4-1).

**Table (4-1):** The Study Population's Socio-demographic Characteristics.

| Variable's | Patients (No= 102) | | Statistical test | P-Value |
|---|---|---|---|---|
| | No | Percentage % | | |
| Age | 10-20 year (6 ) | 5.8 % | *X2= 8.65* *df= 2.4* | 0.041 |
| | 21-30 year( 13) | 12.7 % | | |
| | 31-40 year(12 ) | 11.7 % | | |
| | 41-50 year( 15) | 14.7 % | | |
| | 51-60 year(26 ) | 25.4% | | |
| | 61 to 70 year(25) | 24.5 % | | |
| | 71-80 year(4 ) | 3.9 % | | |
| | 81-90 year(1 ) | 0.9 % | | |
| Gender | Male (56 ) | 54.9% | *X2 = 3.71* *df=1.56* | 0.049 |
| | Female (46 ) | 45.1 % | | |

### 4.2.1. Isolates and identification bacteria:

Classic microbiological methods were used to identify bacteria that are most common in hemodialysis fluid samples. Figure (4-1), show the number of isolates for each bacterium from hemodialysis samples, where the *Klebsiella pneumoniae* was the high prevalence among the study samples. *K. pneumoniae* is the etiological agent of pneumonia and UTI. Although the pathogenesis and virulence factors of *K. pneumoniae* have been widely studied, the differences between infectious cases and colonization/asymptomatic cases are not well understood (Ikeda *et al*., 2018). This study determined the prevalence of hypermucoviscosity, specific serotypes, and virulence genes in *K. pneumoniae* isolated from the hemodialysis fluid obtained from selected patients., CKD has been recognized as a risk factor for poor outcome in patients with community-acquired pneumonia (CAP) (Carratalà, 2007). Despite these findings, however, no study has comprehensively evaluated pneumonia in patients with CKD requiring hospitalization through the emergency department. The main findings were that the CKD patients had more severe pneumonia at admission compared with non-CKD patients; Hospitalization for pneumonia is a relatively common event for CKD patients (Viasus *et al*., 2011). The *Enterococcus faecalis* are also represent high value after *K. pneumonia* 48(47% ) of isolates in this study, *Staphylococcus aureus* 24(23.5 %), *E.Coli* 22(21.5% ) respectively as shown in figure (4-2).

Zacharioudakis *et al*., (2015), reported that *Enterococcal* is associated with an increased risk of catheter removal, permanent transfer and death and this in agree with our results which refer to this bacteria more prevalence among haemodialysis patients.

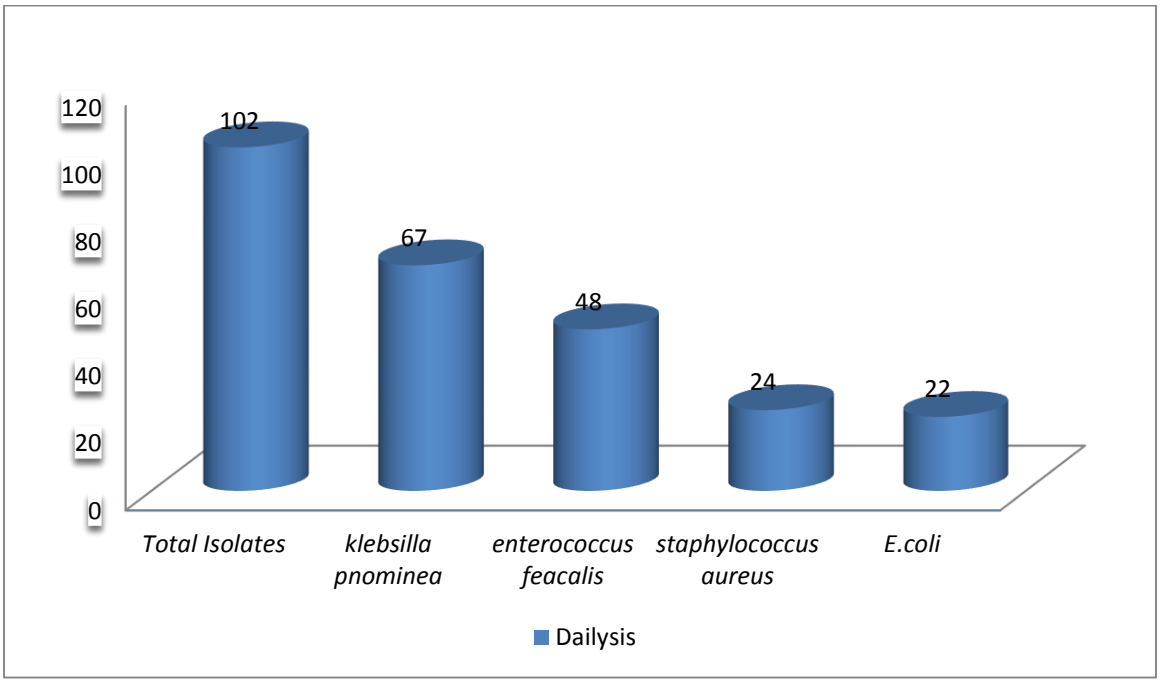



**Figure (4-1):** Isolates of bacteria from hemodialysis fluid.

### 4.3. Dialysis and other complications:

various complications are associated with vascular access in patients who are on hemodialysis and are associated with abdominal catheters in patients using continuous ambulatory peritoneal dialysis (CAPD). In present results there is (13) male and (15) female from total number of patients (102) were with Diabetes. Diabetes appears to be an independent risk factor for developing ESRD due to non-diabetic causes, possibly due to accelerated Reno vascular disease. In addition, patients who are non-diabetic on initiation of dialysis are more likely to develop diabetes; so-called new onset diabetes after dialysis (Rhee *et al.*, 2014). One study showed an incidence rate of new diabetes of 20/1000 patient years compared to 6.5 in the non-dialysis population (Salifu *et al.*, 2010). The incidence and prevalence of new-onset diabetes after initiation of hemodialysis (NODAD) is largely unknown.

Diabetic patients with *K. pneumoniae* bacteremia tend to be older than the general population. Tsay *et al, (*2002), reported that the common underlying diseases in patients with *K. pneumoniae* bacteremia were DM (34.2%).

Study demonstrate that the dialysis modality differentially affected the risk of hepatitis infection in ESRF patients, On the one hand, HD was associated with higher HCV prevalence's. The prevalence's of HCV infection observed in dialysis patients in the present study were considerably higher than those in the corresponding general populations of many countries (Johnson *et al*., 2009).

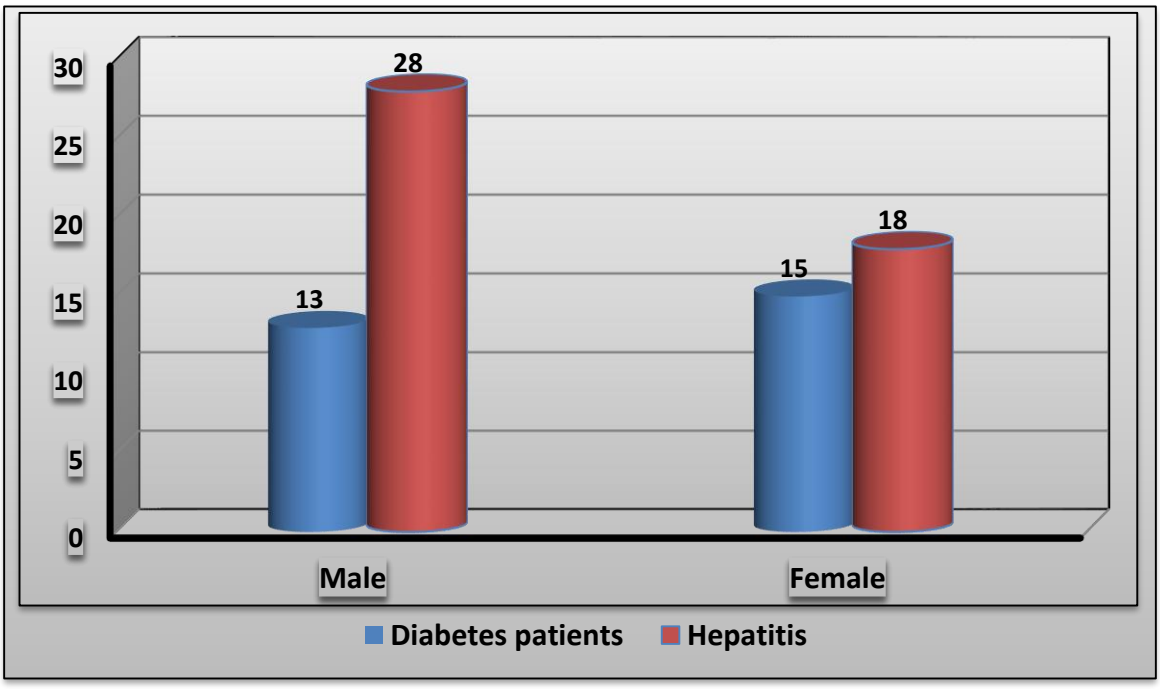



**Figure (4-3):** The numbers of patient samples with confirmed Hepatitis and Diabetes.

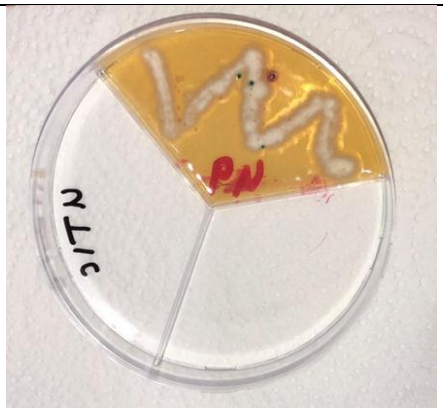
### 4.4. Isolation and identification of Bacteria:

### 4.4.1. Culture of Bacteria:

Colonial Morphology Accurate and definitive microorganism identification, including bacterial identification and pathogen detection, is essential for correct disease diagnosis, treatment of infection and outbreaks associated with microbial infections. According to the colonial morphology, bacteria can be identified as: **Form** – It is the shape of the colony, e.g., circular, filamentous, irregular or radiate, etc. **Elevation** – It is the cross sectional shape of the colony, such as flat, elevated, low convex, convex, and umbonate. **Surface** – It is the surface of the colony appeared, such as smooth, glistening, rough, dull (opposite of glistening), rugose (wrinkled), etc. **Opacity** – For example, transparent (clear), opaque, translucent (almost clear, but distorted vision, like looking through frosted glass), iridescent (changing colors in reflected light), etc. **Consistency** – Mucoid, firm frable, membranous, butyrous, etc. Chromogenesis (pigmentation) – For example, white, buff, red, purple, etc. Edges – Entire, ciliated, crenated, lobate, etc.

*K. pneumonia* (A-1)

*K. pneumonia* (A-2)




*Staphylococcus (B)*

*Enterococcus faecalis (C)*

Mix growth (D)

**Figure (4-4):** isolation bacteria on urinary tract infection chromogenic agar agar(UTIC).(A-1)*K. pneumonia*, *(B) Staphylococcus aureus (C) Enterococcus faecalis* (D)Mix growth).at 37c°for 24 hrs.

### 4.4.2.Microscopic Diagnosis:

They were mostly were negative. After staining with a Gram stain, bacterial isolates are examined microscopically.

### 4.4.3. Vitek2 and Biochemical Diagnosis:

The biochemical tests for diagnosing isolates are shown in Table 4-2. The Vitek-2 was used to diagnose bacterial isolates since it has 46 different tests. To diagnose isolates, bacterial tests as well as biochemical tests are needed.,

It was then diagnosed using biochemical tests and diagnostic confirmation by the VITEC2 method, as it was done after the culture characteristics of the colonies and the microstructure of the bacterial colonies were determined. 102 bacterial isolates were identified and diagnosed. In the samples taken from dialysis patients, *Klebsiella pneumoniae* bacteria were found to be the most common. It findings are consistent with those of Grishin *et al*., (2013), who found that the bacterium *K. pneumoniae* is associated with a 31.60 percent increase in the incidence of dialysis . *Klebsiella pneumoniae* is a gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe, catalase positive, oxidase negative belonging to the Enterobacteriaceae family.

### 4.5. Molecular study:

DNA was extracted from clinical *K. pneumonia* and *Enterococcus feacalis* isolates taken from hemodialysis and burn patients using a DNA extraction kit provided by Promega, and then electrophoresis was performed for the Molecular detection of *K. pneumonia* genes. Using the Polymerase chain reaction (PCR) method, the researchers discovered the *MrkD*, *FimH-1*,*magA* and *Cnf* genes in the bacterial isolates under investigation. Analysis of gel electrophoresis employing a 100-bp DNA markers(KAPA Universal Ladder)" reported the results of the active binding of isolated DNA to certain primers for the *MrkD*, *FimH-1* and *Cnf* genes promoter site.

### 4.5.1. Identification of virulence genes of *K. pneumonia* by PCR.

**Table (4-4):** *Klebsiella pneumoniae* genes with each cases and their percentage.

| Gene | *mrkD* | | *fimH-1* | | *Cnf* | | Total | |
|---|---|---|---|---|---|---|---|---|
| **Cases** | ***No*** | ***%*** | ***No*** | ***%*** | ***No*** | ***%*** | ***No*** | ***%*** |
| **Dialysis (67)** | ***36*** | ***53.7*** | ***24*** | ***35.8*** | ***---*** | ***---*** | ***60*** | ***100%*** |
| **Total** | ***36*** | ***53.7*** | ***24*** | ***35.8*** | ***---*** | ***---*** | ***60*** | ***100%*** |

### 4.5.1.1.*mrkD* Gene *of Klebsiella pneumoniae :*

*MrkD* protein is an important factor in binding of the microorganism to collagen molecules, It was found that not all *Klebsiella pneumonia* 67 sample (100%) isolated from hemodialysis are positive for *mrkD* gene, only 36 were positive. The burn isolates were not detected in PCR. The prevalence of *mrkD* gene was well studied by many researcher in different countries (Bellifa *et al*., 2013).

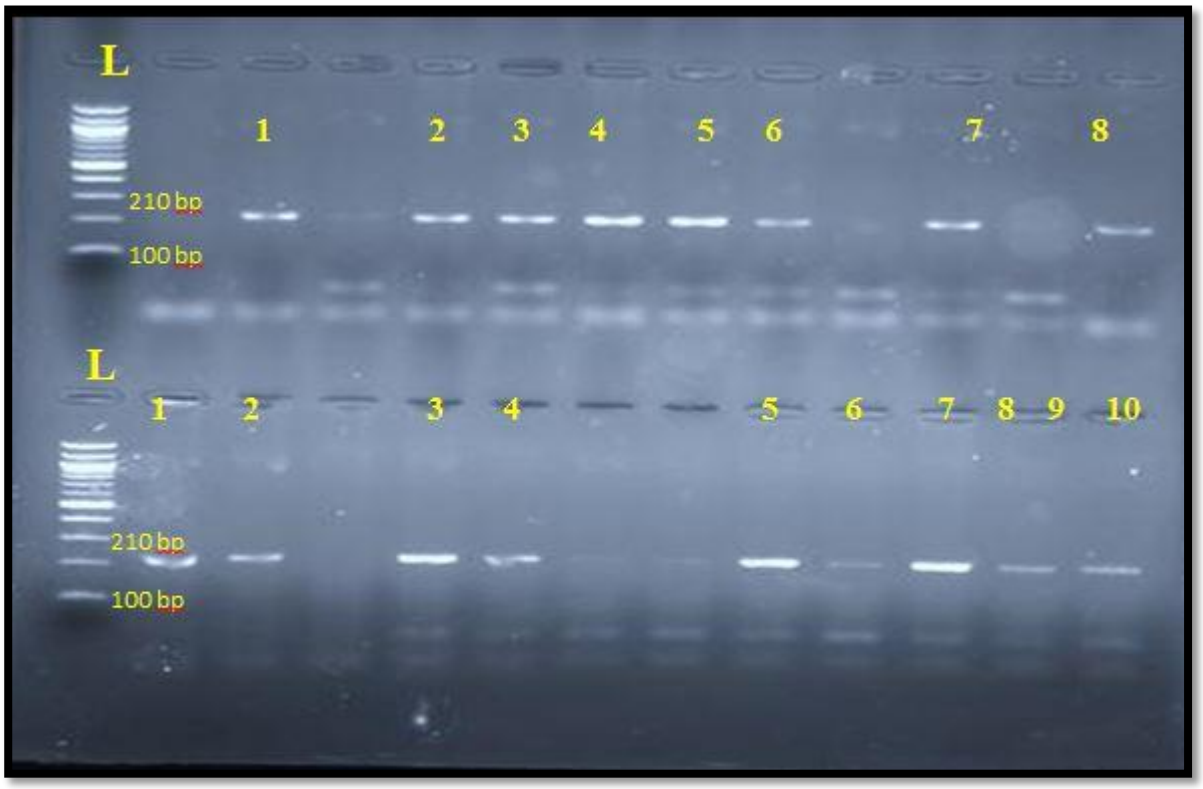



**Figure (4-5):** electrophoresis of *mrkD* Gene products *of Klebsiella pneumoniae*. L lane contain the 100 bp DNA Ladder, 5 % NuSieve®:2 agarose gel in 1X TBE buffer containing 5µl redsafe. (1-8) and (1-10) Lanes positive, other well negative results for *mrkD* gene with 210 bp.

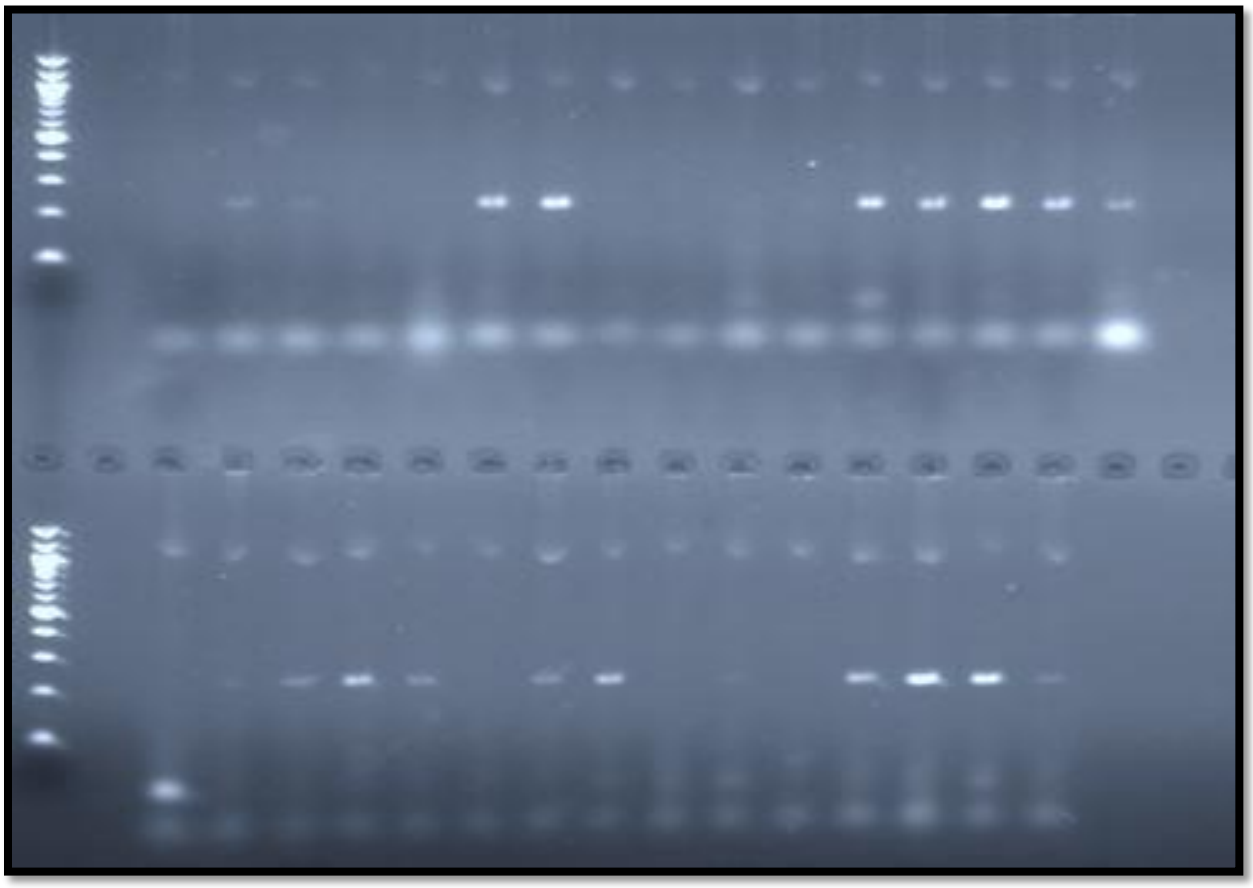


**Figure (4-6):** Electrophoresis of *mrkD Gene* products *of Klebsiella pneumoniae*. L lane contain the 100 bp DNA Ladder, 5 % NuSieve®:2 agarose gel in 1X TBE buffer containing 5µl redsafe. (1-8) and (1-9) Lanes positive, other well negative results for *mrkD* gene with 210 bp.

### 4.5.1.2. *fimH-1 Gene of Klebsiella pneumoniae :*

The *fimH* gene was amplified using specific primers and appeared as a band of (530 bp), on agarose gel as shown in (Figures : 4-7).

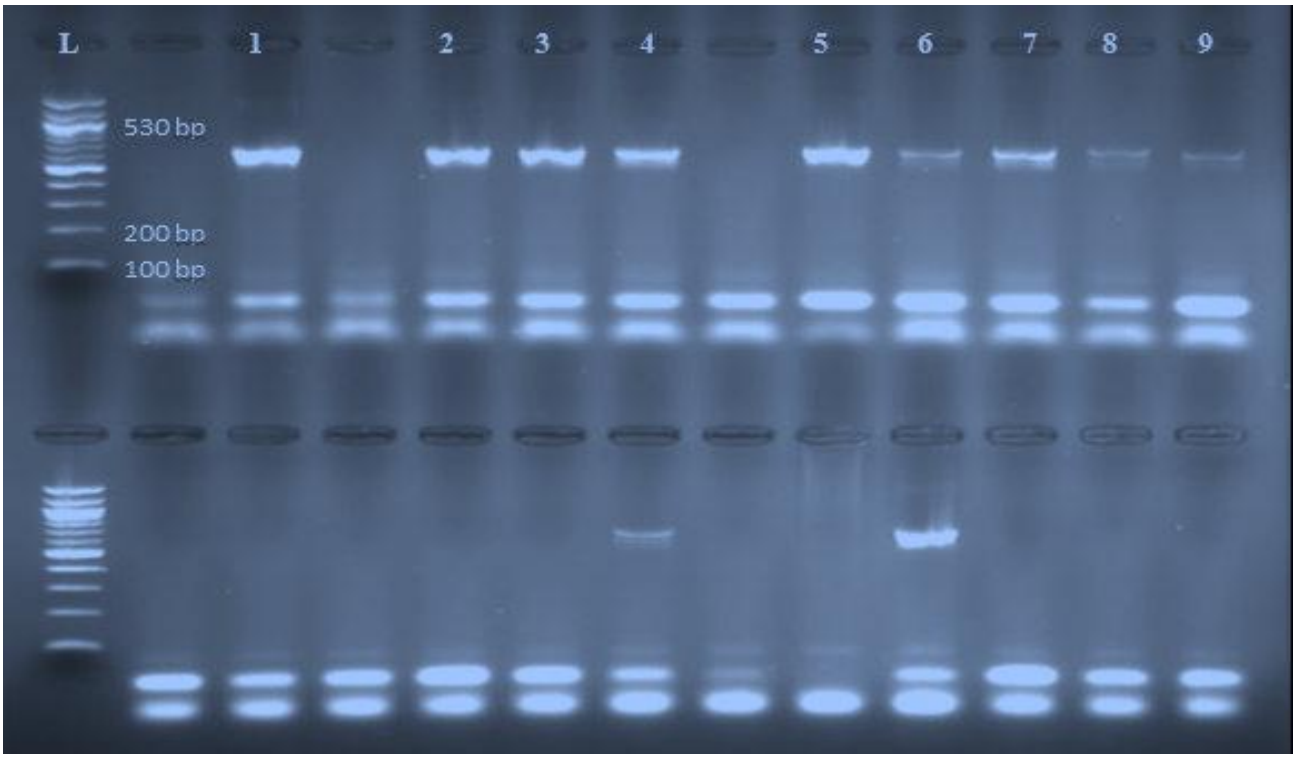



**Figure (4-7):** electrophoresis of *fimH-1* Gene products *of Klebsiella pneumoniae*. L lane contain the 100 bp DNA Ladder, 5 % NuSieve®:2 agarose gel in 1X TBE buffer containing 5µl redsafe. (1-8) and (1-10) Lanes positive, other well negative results for *fimH-1* gene with 210 bp.

### 4.5. 2. Identification of virulence genes of *Enterococcus faecalis* by PCR.

**Table (4-5): *Enterococcus faecalis* genes with each cases and their percentage.**

| Gene | *gelE* | | *Esp* | | *asa1* | | *hyl* | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cases | No | % | No | % | No | % | No | % | No | % |
| Dialysis (48) | 30 | 62.5 | 32 | 66.6 | 42 | 87.5 | 38 | 79.16 | 142 | 81.14 |
| Burn (22) | 8 | 36.7 | 12 | 45.5 | 6 | 27.3 | 7 | 31.8 | 33 | 18.9 |
| Total | 38 | | 44 | | 48 | | 45 | | 175 | 100.0 |

### 4.5. 2.1. *gelE Enterococcus faecalis* gene:

Gelatinase, encoded by the chromosomal *gelE*, is an extracellular zinc endopeptidase that hydrolyzes collagen, gelatin, and small peptides (39) and that has been shown to exacerbate endocarditis in an animal model (Didem and Kuştimur, 2019). It can hydrolyze gelatine, collagen, fibrinogen, casein, hemoglobin, insulin, certain *E. faecalis* sex-pheromone-related peptides, and some other bioactive peptides (Yordanova and Stanilova, 2020). Fig (4-8) that show *gelE* gene in *E.fecalis* isolates.

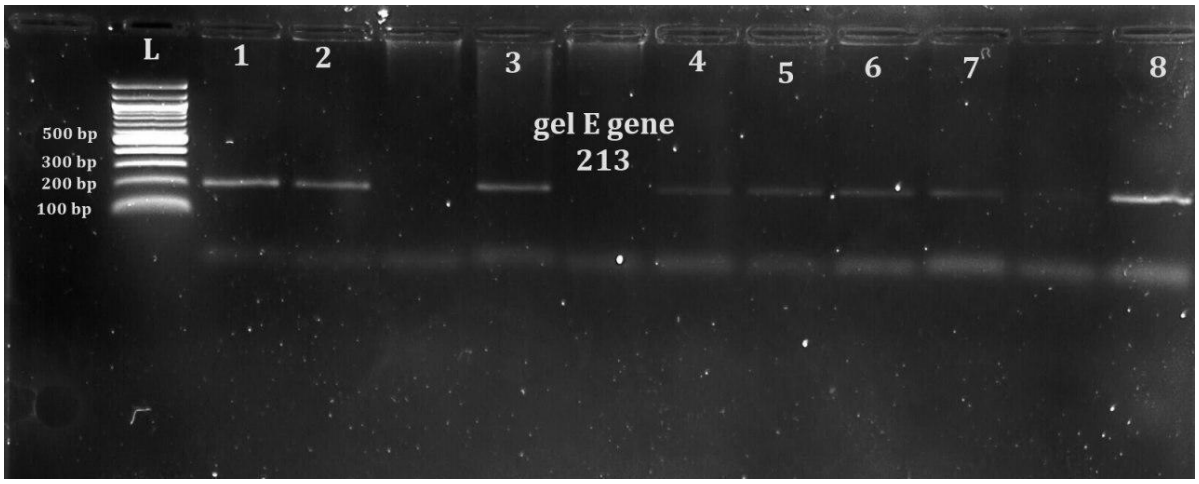



**Figure (4-8): Electrophoresis of *gel E* gene products of *E.fecalis*.** L lane contain the 100 bp DNA Ladder, 5 % NuSieve®:2 agarose gel in 1X TBE buffer containing 5µl redsafe. (1,2,3, 4, 5,6, 7, 8) Lanes positive , other well negative results with 213 bp.

### 4.5. 2.2. *Esp Enterococcus faecalis* gene*:*

Enterococcal surface protein (ESP) with a molecular weight of about 200 kDa is the largest identified enterococcal protein. The *ESP* gene encoding this protein is located on the pathogenicity island (PAI), which also contains proteins responsible for the active outflow of antibiotics (Heikens *et al*.,2007). This location is probably a result of horizontal gene transfer between *E. faecalis* and *E. faecium*. The ESP protein shows some structural similarity to other proteins present in Gram-positive bacteria: C-a in Beta -haemolytic *Streptococcus agalactiaceae* encoded by the bca gene, R28 in *Streptococcus pyogenes* and Bap in *Staphylococcus aureus* - a protein associated with formation of biofilm (Elhadidy and Elsayyad ,2013). The contribution of the surface protein ESP to colonization and persistence of *E. faecalis* in urinary tract infections has been shown in an animal model (Tendolkar *et al*., 2005).

*ESP* is also associated with promotion of primary attachment and biofilm formation of *E. faecalis* on abiotic surfaces.

L lane contain the 100bp DNA Ladder, 5 % NuSieve®:2 agarose gel in 1X TBE buffer containing 5µl redsafe. (1, 2, 3, 4, 5) Lanes positive . other well negative results with 510 bp.

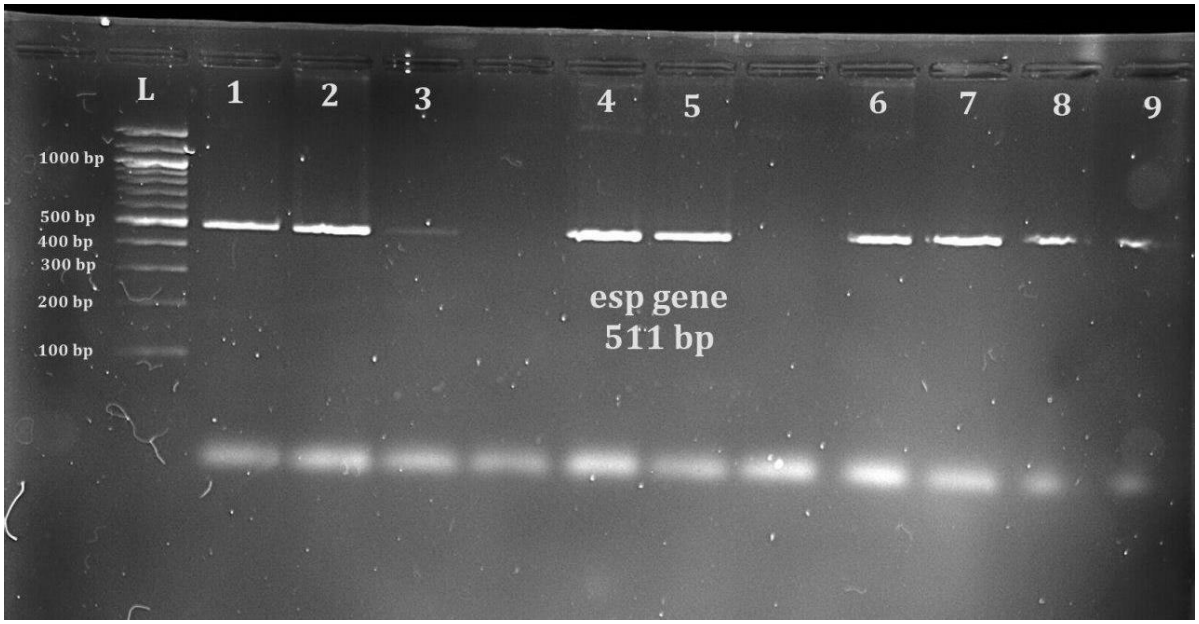



**Figure (4-10): electrophoresis of *ESP* gene products of *E. fecalis*.** L lane contain the 100bp DNA Ladder, 5 % NuSieve®:2 agarose gel in 1X TBE buffer containing 5µl redsafe. (1,2,3,4,5,6,7,8,9) Lanes positive, other well negative results with 510 bp .

### 4.5.2.3. *Asa1 Enterococcus faecalis* gene

Aggregation substance, which is expressed by *asa1* and carried on a plasmid, is a pheromone-inducible protein that allows the conjugative transfer of plasmids encoding sex pheromone genes via clumping of one *Enterococcus* to another (Kiruthiga *et al.*, 2020 ). Serum can cause it to be expressed on the cell surface. Aggregation substance has been shown to increase valvular

vegetation mass in an animal model of endocarditis by increasing bacterial adherence to renal tubular cells and heart endocardial cells, augmenting internalization by intestinal epithelial cells, and increasing bacterial adherence to renal tubular cells and heart endocardial cells as a virulence factor. (Fahmy *et al.,* 2021).

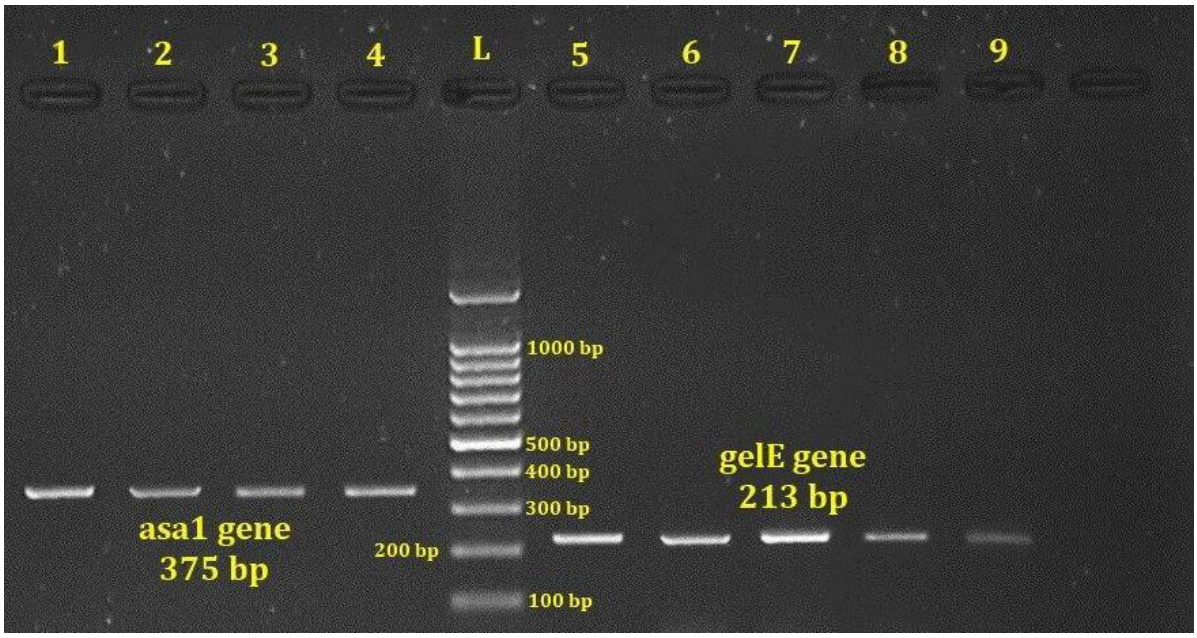



**Figure (4-11): electrophoresis of *asa1, gelE gene*s products of *E.faecium*.** L lane contain the 100 bp DNA Ladder, 5 % NuSieve®:2 agarose gel in 1X TBE buffer containing 5µl redsafe. asa1 (1, 2, 3, 4) Lanes positive , other well negative results with 375 bp, *gelE* (5, 6, 7,8,9) Lanes positive results with 213 bp

### 4.5.2.4. *hyl Enterococcus faecalis gene:*

Hyaluronidase is a degradative enzyme that degrades hyaluronic acid and is linked to tissue injury as a result of its action (Khalkhali and Mojgani, 2017). Hyaluronidase promotes bacterial invasiveness by depolymerizing the mucopolysaccharides moiety of connective tissues. Because the breakdown

products of its target substrates are disaccharides, which may be carried and processed intracellularly by bacteria, hyaluronidase may also serve as a source of nutrition for the bacteria (Maasjost *et al.*, 2019). Fig (4-12) show *hyl* gene in  and *E.fecalis* isolates.

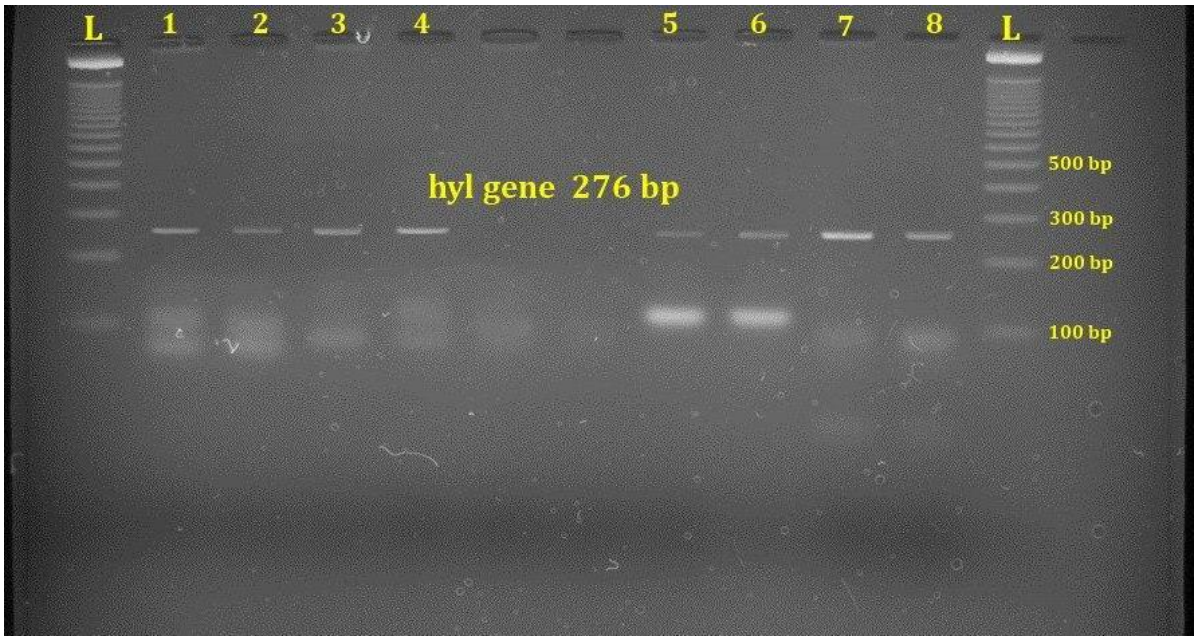



**Figure (4-12):**Electrophoresis of *hyI* gene products of (E.faecium, E.fecalis). L lane contain the 100 bp DNA Ladder, 5 % NuSieve®:2 agarose gel in 1X TBE buffer containing 5µl red safe. (1,2,3,4,5,6,7,8) Lanes positive , other well negative results with 276 bp

## 4.6. Antibacterial efficacy of the Secondary Metabolites Extracted from Myrtus communis L.

The antibacterial activity of secondary metabolites extracted from leaves of (*Myrtus communis* L.), such as (Flavonoid, Alkaloid, and Terpenoid) against pathogenic bacteria isolated from Hemodialysis Fluid .Activity of the secondary metabolites was screened by agar well diffusion methods .The results revealed that, the extracts of Flavonoid, Alkaloid, and Terpenoid of

(*Myrtus communis* L.) leaves showed significant reduction at P≤ 0.05 in the growth of pathogenic bacteria isolated from Hemodialysis Fluid. Antibacterial activity was applied at (50, 100, and 200 mg/ml), and then, compared with 10% dimethyl sulfoxide (DMSO) as a negative control and with Azithromycin antibiotic (30 µg/Disc) as a positive control. Inhibitory zone diameter increases significantly at (P≤ 0.05) by increasing concentration from 50 to 200 mg/ ml. The results also revealed that, flavonoid compounds extracted at (50, 100, and 200 mg/ml) showed significant effect at (P≤ 0.05) compared with negative control, and showed a similar effect (There in no significant difference at P≤ 0.05) between flavonoid compounds and the Azithromycin antibiotic as the inhibition diameter reached (30± 1) in the flavonoid compounds compared with (30± 0) in the antibiotic when applied to S. aureus pathogenic bacteria. In the same context, the results also showed a similar effect (There in no significant difference at P≤ 0.05) between flavonoid compounds and the Azithromycin antibiotic when applied to E. coli (30.66± 1.15) and K. pneumonieae (29.66± 0.57). On the other hand, the results of flavonoid compounds at (200 mg/ml) showed significant superiority at (P≤ 0.05) over the Azithromycin antibiotic as the inhibition diameter reached to (32± 1) in the flavonoid compared with (27± 0) in the Azithromycin antibiotic

**Table (4-6):** Antimicrobial activity of Myrtus communis L. extraction against bacteria isolated by (WDA)method.

| Concentration | Pathogenic bacteria | | | |
|---|---|---|---|---|
| | ***S. aureus*** | ***E. coli*** | ***E. faecalis*** | ***K.pneumonieae*** |
| | **Inhibition zone/mm** | | | |
| **Control negative** | **0± 0** | **0± 0** | **0± 0** | **0± 0** |
| **50 mg/ml** | **21± 1** | **26± 1** | **22± 2** | **23.66±0.57** |
| **100 mg/ml** | **24.66± 0.57** | **28± 1** | **29± 1** | **27± 2** |
| **200 mg/ml** | **30± 1** | **30.66± 1.15** | **32± 1** | **29.66± 0.57** |
| **Control positive(Azithromycin)** | **30± 0** | **30± 0** | **27± 0** | **30± 0** |
| **LSD** | **1.24** | **1.48** | **1.99** | **1.75** |
| ***Mean± standard deviation** | | | | |

The present study also revealed that, there are significant decrease in the growth of pathogenic bacteria with the increasing of concentration of alkaloid compounds extracted from (*Myrtus communis* L.) compared with the negative control DMSO 10% (Table 4-6). In the same context, the results also revealed that, alkaloid compounds at (200 mg/ml) showed significant superiority at ($P \leq 0.05$) over the Azithromycin antibiotic as the inhibition diameter reached to (32± 1) in the alkaloid extract compared with (27± 0) in the antibiotic when applied to *E. faecalis* pathogenic bacteria. In addition to that, there is no significant difference between alkaloid compounds extracted from (*Myrtus communis* L.) at (200 mg/ml) and Azithromycin antibiotic at $P \leq 0.05$ when applied to *E. coli* and *K. pneumonieae* pathogenic bacteria. In contrast, the

Azithromycin antibiotic showed significant superiority at (P≤ 0.05) over alkaloid compounds when applied to *S. aureus* (Table 4- 7).

**Table(4-7):** Antibacterial efficacy the Alkaloid compounds Extracted from *Myrtus communis* L. against some pathogenic bacteria isolated from Hemodialysis Fluid.

| **Concentration** | **Pathogenic bacteria** | | | |
|---|---|---|---|---|
| | ***S. aureus*** | ***E. coli*** | ***E. faecalis*** | ***K.pneumonieae*** |
| | **Inhibition zone/mm** | | | |
| **Control negative** | **0± 0** | **0± 0** | **0± 0** | **0± 0** |
| **50 mg/ml** | **16± 1** | **18.33± 1.52** | **19± 1** | **21.33± 1.15** |
| **100 mg/ml** | **18± 1** | **25± 1** | **24.33± 0.57** | **25± 1** |
| **200 mg/ml** | **24± 1** | **30± 1** | **29± 1** | **30± 1** |
| **Control positive** | **30± 0** | **30± 0** | **27± 0** | **30± 0** |
| **LSD** | **1.47** | **1.69** | **1.24** | **1.48** |
| ***Mean± standard deviation** | | | | |

The current study also uncovers that, terpenoid compounds at (50, 100, and 200 mg/ml) showed significant superiority at (P≤ 0.05) compared with negative control. But, in contrast, Azithromycin antibiotic showed significant superiority at (P≤ 0.05) over terpenoid compounds in all concentrations when applied to *S. aureus, E. coli, E. faecalis, and K. pneumonieae* (Table 4-8) Thus, terpenoid compounds extracted from leaves of (*Myrtus communis* L.)

was the least effective in controlling growth of pathogenic bacteria isolated from hemodialysis fluid compared with flavonoid and alkaloid.

**Table(4-8):** Antibacterial efficacy the Terpenoid compounds Extracted from *Myrtus communis* L. against some pathogenic bacteria isolated from Hemodialysis Fluid.

| Concentration | Pathogenic bacteria | | | |
|---|---|---|---|---|
| | ***S. aureus*** | ***E. coli*** | ***E. faecalis*** | ***K.pneumonieae*** |
| | **Inhibition zone/mm** | | | |
| **Control negative** | **0± 0** | **0± 0** | **0± 0** | **0± 0** |
| **50 mg/ml** | **15± 1** | **10.66± 0.57** | **14.66± 1.52** | **12.33± 0.57** |
| **100 mg/ml** | **17.33± 0.57** | **12± 2** | **20± 1** | **15± 1** |
| **200 mg/ml** | **20± 1** | **15± 1** | **21.66± 0.57** | **20.66± 0.57** |
| **Control positive** | **30± 0** | **30± 0** | **27± 0** | **30± 0** |
| **LSD** | **1.24** | **1.87** | **1.55** | **1.04** |
| **Mean± standard deviation** | | | | |

Figure: 1. Antibacterial activity of the crude flavonoid compounds extracted (M. communis L.) leaves at (50, 100, and 200 mg/ml) against *K. pneumaniese*

Figure: 2. Antibacterial activity of the crude alkaloid compounds extracted (M. communis L.) leaves at (50, 100, and 200 mg/ml) against *E. coli*

Figure: 3. Antibacterial activity of the crude flavonoid compounds extracted (M. communis L.) leaves at (50, 100, and 200 mg/ml) against *S. aureus*

Figure: 2. Antibacterial activity of the crude alkaloid compounds extracted (M. communis L.) leaves at (50, 100, and 200 mg/ml) against *E. faecalis*

The present study was proved that, the secondary metabolites include Flavonoids, Alkaloid, and Terpenoids, extracted from the leaves of (*Myrtus Communis L.*) have antibacterial activity against pathogenic bacteria isolated

from hemodialysis fluid especially Flavonoid and Alkaloid compounds. The plant kingdom provided and is still providing endless sources of medicinal plants of various uses for example, Bioactive compounds such as phenolic, terpenoids, and alkaloids extracted from several medicinal plants like (*Lactuca serriola L., Lepidium sativum L., Myrtus Communis L., Cassia senna L., Ricinus communis L., Cassia didymobotrya* (Fresenius) Irwin & *Barneby, Melia azedarach L., Dianthus caryophyllus L., and Salvia hispanica L.*) have antibacterial efficacy against different pathogenic microorganisms (Al-Marzoqi *et al*.,2015;Al-Marzoqi *et al*.,2016; Hussein *et al*., 2017; Hussein *et al*., 2018; Hussein *et al*., 2019; Hussein *et al*., 2020; Kamil *et al*.,2020). Hussein *et al*.,(2018) were used primitive plant like Chlorella vulgaris as antibacterial. Kamal AS *et al*.,(2019) was used Hibiscus sabdarifa extracts against member of *Enterobacteriaceae* microorganisms. Kamal SA *et al*.,(2020) was used leaves of Ficus carica Linn against pathogenic bacteria. AL-Masoodi *et al*., (2020) was used *Curcuma longa L.* and Boswellia carteri Birdwood against Fusarium species isolated from maize seeds.

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts of biologically active compounds isolated from plant species used in herbal medicines (Saxena,1997). Antimicrobial properties of medicinal plants therefore, may have a significant clinical value in treatment of resistant microbial strains(Hammer and Carson, 1999) .Mert *et al*.,(2008) was reported that, The antibacterial activity of the *(Myrtus Communis L.*) extracts against

*Staphylococcus aureus* ATCC 6538P and *Staphylococcus aureus* ATCC 29213 was more than Ceftazidime antibiotic. *M communis* leaves extracts showed greatest antibacterial effect against *S. aureus* and *V. cholerae* cerotype Ogawa (Taheri *et al*.,2013). Myrtle essential oil has a moderate inhibitory activity against *Staphylococcus aureus* and Acinetobacter baumannii (El Hartiti *et al*.,2020).

Biologically active compounds such as tannins, flavonoids, coumarins, essential oil, fixed oil, fibres, sugars, citric acid, malic acid and antioxidants are present in the plant (Sumbul *et al*., 2011). In contrast, natural bioactive compounds extracted from medicinal plants make their effects by many mechanisms, for example polyphenols binding with polysaccharides and proteins (Macromolecules), thus inhibiting their roles in biochemical metabolites. Terpenoids and flavonoids make their effects by disruption of microbial membranes and Polypeptide's embarrassment of linkage of bacterial proteins to host polysaccharide receptors and alkaloids complexes make their effect by inhibiting of efflux pump (Okusa *et al*.,2009).

Finally, bacterial activity of *Myrtus Communis L*. might be belonging to secondary metabolites like Flavonoids, Alkaloids, and Terpenoids, and their effect in proteins, RNA, and DNA synthesis and disruption in membranes permeability or disturbance in metabolic activity. Secondary metabolites compounds extracted from *Myrtus Communis L*. especially Flavonoids, Alkaloids regard a good source for controlling pathogenic bacteria isolated from hemodialysis fluid .

# Conclusions and Recommendations

**Conclusions:**

1. In this study, the detection of a variety of bacteria in hemodialysis water indicates the necessity of regular appropriate monitoring by the local health authority, to ensure the best possible control over the water treatment system.
2. Conventionally, water treatment is a major determinant of morbidity and mortality in HD units, and the microbial quality is a major factor involved. There is evidence of bacterial contamination in the dialysis units sampled in this study. There is compelling need for periodic microbiological monitoring of water after each treatment step.
3. Catheter-related bacteremia is a significant cause of poor health outcomes in infected HD patients.
4. The result of our study also emphasizes the need for continuous evaluation of local antibiotic sensitivity patterns of pathogen for the formulation of a rational antibiotic policy. Studies such as this should provide a useful information base to guide practice and policies on rational use of antibiotics.
5. In this study, the plants extract produced some effects against bacterial pathogens so, they may help in controlling microbial infections and contamination.

**Recommendations:**

1. Infections caused by contaminated water and equipment can be prevented by a well-designed water-treatment system, routine cleaning and disinfection of system components, and routine bacteriologic monitoring of dialysis water and dialysis fluid.
2. Standard precautions with additional measures recommended specifically for dialysis centers will prevent transmission of bacteria and viruses from

patient to patient. These precautions include routine use of gloves, hand washing, and cleaning and disinfection of the external surface of the dialysis machine and other environmental surfaces. In addition, preventing transmission of hepatitis C virus infection requires vaccination of susceptible patients and staff, avoiding dialyzer reuse, and use of a dedicated room, dialysis machine, and staff members when treating patients chronically infected with this virus.

3. It is necessary to conduct more studies about the other etiological agents like (bacteria, viruses, fungi) associated with HD patients.
4. Antibiogram profile is most critical subject, so its recommended to focused on resistance and sensitivity for bacteria associated with HD.
5. It can be useful to use the different types of plants extract like (Alkaloids, terpenoids, and others) especially with MDR bacteria and measure the activity of them as bacteriostatic and bactericidal.

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# الخلاصة

تضمنت الدراسة الحالية جمع ١٣٥ عينة سريرية من (102) عينة من غسيل الكلى وحروق (33) عينة. جمعت العينات من مستشفى الإمام الصادق التعليمي ومدينة مرجان الطبية ، خلال الفترة من أكتوبر إلى ديسمبر / ٢٠٢٠. الأنواع البكتيرية التي تنتمي إلى (الكليبسيلا الرئوية ، المكورات المعوية البرازية ، العصيات القولونية، المكورات العنقودية الذهبية) غالبًا ما تكون مقاومة للعوامل المضادة للميكروبات واسعة الطيف. كان الهدف الرئيسي من عملنا هو الدراسة الجزيئية لعوامل الفوعة في البكتيريا المسببة للأمراض بين مرضى غسيل الكلى وكشف مدى حساسية المركبات الكيميائية النباتية المستخلصة من النباتات الطبية ومقارنتها بالمضادات الحيوية.

أظهرت نتائج العزل والتشخيص أن 161 عزلة من البكتيريا توزعت على النحو التالي: الكليبسيلا الرئوية ٦٧ (٤١,٦%) ، المكورات المعوية البرازية٤٨ (٢٩,٨٪ ) ، المكورات العنقودية الذهبية ٢٤ (١٤,٩٪) ، العصيات القولونية ٢٢ (١٣,٦٪) .

اشتملت الدراسة الجزيئية على عوامل الفوعة المكتشفة لمسببات الأمراض البكتيرية باستخدام تفاعل البلمرة المتسلسل (PCR) ، والتي بدأت بجينات الكليبسيلا الرئوية fimH-1 (٣٨,٧%) ، mrkD (٥٨,١٪) ، magA (٣,٢٢٪) ، cnf (٠٪) والجينات المعوية البرازية esp (٢٥,١٤٪) ، gelE (٢١,٧ ٪) ، hyl (٥٣,٥٣٪) (، ) asa1 (٢٧,٤٣% ) .

كما اشتملت الدراسة على اختبار الحساسية لكل من: الكليبسيلا الرئوية و المكورات المعوية البرازية و المكورات العنقودية الذهبية و العصيات القولونية تجاه المضادات الحيوية من النوع ٦ بطريقة الانتشار القرصي ، وأظهرت النتائج أن المضاد الحيوي الأكثر فاعلية ضد الأنواع المختلفة من مسببات الأمراض المستشفوية كان ATH15 و AZM30 و AMB100. كما أجريت الدراسة الحالية لمعرفة تأثير مركبات الفلافونويد الخام والقلويد والتربينويد المستخلص من أوراق نبات الآس (*Myrtus communis L*.) ضد البكتيريا المسببة للأمراض المعزولة من سائل غسيل الكلى والحروق. تم تحقيق الفعالية المضادة للبكتيريا في المختبر باستخدام طريقة انتشار آجار جيدا ضد البكتيريا المسببة للأمراض المعزولة من سائل غسيل الكلى عن طريق تحضير ثلاثة تراكيز لكل مركب خام (٥٠ ، ١٠٠ ، و ٢٠٠) مجم / مل ومقارنة بالتحكم

الإيجابي المتمثل بالمضاد الحيوي أزيثروميسين والسالب. يمثل التحكم بنسبة ١٠٪ ثنائي ميثيل سلفوكسيد. هدفت هذه الدراسة إلى معرفة الفعالية المضادة للبكتيريا للأيضات الثانوية المستخرجة من أوراق نبات الآس *Myrtus communis L*. ضد بعض البكتيريا المسببة للأمراض المعزولة من سائل غسيل الكلى.

أظهرت البيانات التي تم جمعها من الدراسة أن مستخلص مركبات الفلافونويد والقلويد الخام من أوراق نبات الآس (*Myrtus communis L*.) أظهر انخفاضًا معنويًا عند $P \leq 0.05$ في نمو البكتيريا المسببة للأمراض المعزولة من غسيل الكلى عند ٢٠ مجم / مل مقارنة بالسالب. مراقبة. أخيرًا ، يمكن استنتاج أن مركبات الفلافونويد والقلويد من نبات الآس *Myrtus communis* L. هي الأكثر فعالية في السيطرة على البكتيريا المسببة للأمراض المعزولة من سائل غسيل الكلى. بينما كانت مركبات التربينويد الأقل فاعلية في التحكم في نمو البكتيريا الممرضة المعزولة من سائل غسيل الكلى والحروق مقارنة بالفلافونويد والقلويد. أخيرًا ، يمكن استنتاج أن مركبات الفلافونويد من نبات الآس *Myrtus communis L*. هي الأكثر فعالية في السيطرة على البكتيريا المسببة للأمراض.

**جمهورية العراق**

**وزارة التعليم العالي والبحث العلمي**

**جامعة بابل / كلية العلوم للبنات**

**قسم علوم الحياة**

# تقييم عوامل ضراوة البكتريا المعزولة من مرضى. الديلزة وفعالية نبات Myrtus communis L ضد بعض انواع البكتريا المرضية .

رسالة ماجستير

مقدمة الى مجلس كلية العلوم للبنات، جامعة بابل وهي جزء من

متطلبات نيل درجة ماجستير علوم في علوم الحياة

من قبل

دينا طارق مزهر

**إشراف**

**الأستاذ الدكتور**

علي حسين المرزوكي

**الأستاذ الدكتور**

حسين جبر حسين

**١٤٤٣ هـ**

**٢٠٢١م**