CHAPTER ONE

1. INTRODUCTION

Dialysis is a mechanical process that performs the work of healthy kidneys. Hemodialysis (HD) uses a man-made membrane (dialyzer) to remove wastes and extra fluid from the blood. It also restores the proper balance of certain minerals in the blood (electrolytes). The fluid used to filter or clean the blood is called dialysate. Hemodialysis is usually done in a hospital or dialysis center (Kumar and Clark, 2005; Rhodes, 2009).

Kidney failure (also called end-stage renal disease) occurs when kidney damage is so severe that a person needs dialysis or a kidney transplant to maintain life. The disease often occurs after kidney damage has been present for 10 years or more, which can be measured by how well the kidneys are able to filter wastes from the blood. This is called glomerular filtration rate (GFR). Kidney failure usually occurs when the GFR falls to below 15% of what is expected in a person with normal kidney function. It is treated with dialysis, which helps filter waste products from the blood when the kidneys are not working properly, or with kidney transplant (Kumar and Clark, 2005; Rhodes, 2009).

The spectrum of chronic kidney disease (CKD), also called chronic kidney failure (CKF) extends from the point at which there is slight kidney damage, but normal function, to the

point at which a patient may require either a renal transplant or renal replacement therapy to survive (Peter *et* al., 2003).

Hemodialysis continues to be an important treatment option for persons with end-stage renal disease. Infection is a serious complication of hemodialysis, arising from the percutaneous vascular access necessary to accomplish hemodialysis is the most common source of infection occurring in these patients (Taylor *et al.,* 2004).

Subcutaneously tunneled, cuffed, silicone, dual-lumen central venous catheters were introduced in 1984 and quickly gained acceptance for both temporary and permanent hemodialysis vascular access. Catheter-associated bacteremia (CAB) is a serious complication of permanent catheter use and a common cause of catheter failure (Saad, 1999).

In Sudan, there were about 5,967 patients with CKF on hemodialysis in 2012 most of them in about 2,919 patients were in Khartoum state and they are increasing annually. Hemodialysis-catheter related infections (HCRI) are one of the major causes of increasing mortality, morbidity and cost of therapy in HD patients in Sudan since about 50% of HD patients died from infections. So prevention of HCRI is one of the keys to success with HD (NCKDS, 2012).

The most serious problem of HD tunneled catheters is catheter-related blood stream infection (CRBI). The latter

condition, is typically a cause for removal of the catheter, and protracted systemic antibiotic therapy is prescribed for several weeks. Blood infection is often the reason for hospitalization and is the second highest cause of mortality across the globe (Quarello and Forneris, 2002).

It is also the second most common cause of mortality in HD patients, accounting for about one-quarter of all deaths. Moreover, infections are the leading cause of all hospital admissions (102 admissions/1,000 patient/year) in HD patients. Notably, infections are associated with excessive costs for both the patient and hospital (Fitzgibbons *et al.,* 2011).

Of the Gram-positive organisms, the causative agents for hemodialysis-associated infections are *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) which associated with most infections, but enterococcal infections are also in high increase. Management of Gram-positive blood stream infections (BSIs) in HD patients is frequently complicated by limited vascular access options. Additionally, emergence of decreased vancomycin susceptibility of these organisms is a further concern (Fitzgibbons *et al.,* 2011).

Patients undergoing HD are at high risk for infection because of impaired immune defenses, a high severity of illness, and the need for routine puncture of a vascular access site to remove blood for hemodialysis. Vascular access sites may

consist of fistulas (created from the patient's native vessels), grafts (created with synthetic materials), and cuffed (permanent) or noncuffed (temporary) catheters. Of these, the risk of infection is highest for catheters, intermediate for grafts, and lowest for fistulas. Infections in patients undergoing hemodialysis have adverse consequences for the individual patient, including increased morbidity and mortality, and for society, including increased costs, hospitalization rates, and need for antimicrobial agents. As a result of their frequent receipt of antimicrobials, particularly vancomycin, antimicrobial resistance has been common in patients undergoing dialysis. One of the first reports of vancomycin-resistant *enterococci* was from a renal unit in London, England. In the United States, patients undergoing dialysis have developed significant percentage of vancomycin-resistant *enterococci* cases in hospital-based studies (Tokars *et al*., 2002).

2.Rationale

Hemodialysis catheter related infections (HCRIs), in addition to an increase in morbidity and mortality contribute a tremendous financial burden to health care system, in which they often require the criteria of catheter removal and culture to make a definite diagnosis.

Previous studies in western countries have identified types of bacterial infection and antimicrobial therapy in patients on

dialysis, but in Sudan no previous studies in that issues, so catheter management decisions were not made according to a specific protocol, however, with a variety of treatment strategies used across units according to undefined criteria.

Therefore, this study was designed to determine the relative prevalence of bacterial infections among patients on hemodialysis and antibiotic regimen following sensitivity reports. This because the use of effective protocol lowers catheter blood stream infections rate in the dialysis population without inducing resistant pathogens.

3.Objectives:

3.1.General objective

To study phenotypic and genotypic characterization of bacterial pathogens in hemodialysis patients and their antibiotics susceptibility.

3.2.Specific objectives

1- To isolate the causative agent from dialysis patients suspected to have bacteremia.

2- To identify the isolates by VITEK 2 compact system.

3 - To confirm the identification by their 16S rRNA gene sequences.

4 - To perform antibiotic susceptibility and MICs of commonly used antibiotics by VITEK 2 compact system.

5- To amplify class 1 and 2 *intI* genes to determine whether integrons present in the isolates genomes.

CHAPTER TWO
2. LITERATURE REVIEW

2.1. Chronic Kidney Disease (CKD)

Chronic Kidney Disease is progressive, most often irreversible, and associated with multiple comorbidities and adverse outcomes. CKD defined as kidney damage of 3 or more months' duration caused by structural or functional abnormalities with or without a decreased glomerular filtration rate(GFR). Pathological markers, abnormalities in the blood or urine, or imaging tests, may reveal kidney

dysfunction. CKD may also be defined as a persistently low GFR of <60 mL/min/1.73m^2 for 3 or more months, with or without identifiable kidney damage (Carroll, 2006).

In the course of developing the Dialysis Outcome Quality Initiative (DOQI) guidelines, it became evident that in order to actually improve dialysis outcomes it was necessary to improve the health status of those who reach end-stage renal disease (ESRD), and that there in existed an even greater opportunity to improve outcomes for all individuals with chronic kidney disease (Kasiske and Cosio, 2003).

2.2 Dialysis

Dialysis is defined as the diffusion of molecules in solution across a semi permeable membrane along an electrochemical concentration gradient in which patients reached Stage 5 of Chronic Kidney Disease (CKD) or end stage renal disease (ESRD) and require dialysis or transplant for survival (Himmelfarb and Alp Ikizler, 2010).

2.2.1. Hemodialysis

The primary goal of hemodialysis is to restore the intracellular and extracellular fluid environment that is characteristic of normal kidney function. This is accomplished by the transport of solutes such as urea from the blood into the dialysate and by the transport

of solutes such as bicarbonate from the dialysate into the blood. Solute concentration and molecular weight are the primary determinants of diffusion rates. Small molecules, such as urea, diffuse quickly, whereas compartmentalized and larger molecules, such as phosphate, β2-microglobulin, and albumin, and protein bound solutes, such as p-cresol, diffuse much more slowly. In addition to diffusion, solutes may pass through pores in the membrane by means of a convective process driven by hydrostatic or osmotic pressure gradients a process called ultrafiltration. During ultrafiltration, there is no change in solute concentrations; its primary purpose is the removal of excess total body water (Himmelfarb and Alp Ikizler, 2010).

2.2.2. Vascular Access for Hemodialysis

Vascular access sites may consist of fistulas (created from the patient's native vessels), grafts (created with synthetic materials), and cuffed (permanent) or noncuffed (temporary) catheters (Tokars *et al*., 2002).

The use of tunneled central venous catheters (TCCs) for vascular access in chronic hemodialysis patients increased from 18% in 1998 to 27% in 2004. Moreover, the current use of TCC can be as high as 30 to 40% in prevalent patients and 74% in incident patients. Although providing life-saving therapy to those awaiting maturation or placement of an arteriovenous (AV) fistula or graft, there is a 2- to 3-fold increased risk of death and a 10-

to 20-fold higher risk of bacteremia in patients receiving hemodialysis through a TCC compared with a fistula (Landry *et al*., 2010).

Long-term HD in the pediatric age group is performed mainly through tunneled cuffed central venous catheters (CVCs) inserted angiographically or surgically, most often into the superior vena cava, arteriovenous fistulas (AVFs) or grafts (AVGs) (Eisenstein *et al*., 2011).

2.2.3. Hemodialysis Protocol and Catheter Care

Tunneled catheters were primarily either Quinton Permcaths or Tesio catheters, where as a few patients had the Life Site. All tunneled catheters were placed by one of four credentialed transplant surgeons under sterile conditions in an operating room setting. Usual TCC care in the outpatient dialysis setting is performed by a protocol described in the Fresenius Medical Care-North America (FMCNA) hemodialysis procedure manual. Using clean technique with handwashing, nonsterile gloves, masking of both nurse and patient, and a non sterile towel draped under the catheter, the nursing staff disinfected the connection ports using two gauze sponges soaked with aqueous-based povidine-iodine solution for 5 minutes. The gauze will be removed, and the solution will be allowed to dry before the catheter is opened.

TCCs are not used for any other purpose than for dialysis access. Exit site care should be performed during the intradialytic period and it consists of inspection of the catheter exit site, cleansing with povidine iodine, and placement of a sterile dry gauze dressing. Mupirocin ointment will be applied to the TCC exit site before placement of the dry gauze dressing for all patients during each dialysis treatment (Landry *et al*., 2010).

2.2.4. Complications

The two major complications of hemodialysis catheters are thrombosis and infection (Jain *et al*., 2009).

2.2.4.1. Catheter-Related Bacteramia (CRB)

2.2.4.1.1. Definition

Catheter-Related Bacteramia is defined as positive blood cultures in a patient with a HD catheter and no other source of this bacteremia (Haddad *et al*., 2012).

2.2.4.1.2. Risk Factors

Several factors increase the risk for catheter related bacteremia in hemodialysis patients with tunneled catheters. The likelihood of catheter related bacteremia is greater among patients with a previous catheter-related bacteremia and those with immunosuppressive therapy, but is not greater among patients with underlying human immunodeficiency virus infection. Hypoalbuminemia increases the likelihood of a recurrent episode of catheter-related bacteremia among patients treated for an initial catheter-related bacteremia. Other clinical factors, including age, sex, race, and diabetic state, have

not been shown to be direct causes of catheter-related bacteremia. The cumulative risk for infection is proportionate to the duration of catheter dependence. The presence of a catheter is a major risk factor for bacteremia among hemodialysis patients and can result in life-threatening complications, including septic shock, endocarditis, septic arthritis, osteomyelitis, or epidural abscess. The relative risk for infection-related hospitalization and infection-related death is increased 2- to 3-fold among catheter-dependent hemodialysis patients compared with those using fistulas or grafts (Allon, 2004).

Tunneled dialysis catheters (TDCs) are considered the vascular access of last resort when all other options for arteriovenous fistulas (AVFs) and grafts (AVGs) have been exhausted. Tunneled dialysis catheters are associated with an increased incidence of bacteremia there by leading to higher morbidity and mortality that results in significantly increased hospital costs. TDCs are also associated with less effective dialysis due to reduced blood flow rates, frequent malfunctions, and the development of central venous stenosis (Katzman *et al*., 2009).

2.2.4.1.3. Etiology

Fourty five (52.3%) of infections were caused by Gram-positive cocci only, including *Staphylococcus aureus*, coagulase-negative *Staphylococcus* (CoNS), and

Enterococcus species. Twenty-three infections (26.7%) were caused by Gram-negative rods only, including a wide variety of enteric organisms (Saad, 1999). In which *Staphylococcus aureus* is a significant cause of morbidity and mortality in renal patients; indeed, it is the leading cause of haemodialysis related bacteraemia and coagulase-negative *staphylococci* are responsible for the largest proportion of central venous catheter-related bloodstream infections in inpatients. and many, in particular *Staphylococcus epidermidis* and *Staphylococcus hominis* (Casey *et al.,* 2007).

Enterobacter cloacae was repeatedly recovered from blood culture on several occasions after the fever spiked after dialysis (Cunha *et al*., 2000).

2.2.4.1.3.1 *Staphylococcus aureus*

Staphylococcus aureus is a pathogen isolated from haemodialysed patients most frequently and it has been reported that a large majority of infections in this clinical setting are of endogenous origin. Factors responsible for the increased risk of invasion by infectious agents among renal unit patients include decreased immunity, defective mucocutanous barriers, multiple needle punctures over the vascular access site required for dialysis, and the presence

of prosthetic devices that disrupt the normal host barriers and give direct access to normally sterile body sites, but also provide a site of colonization to which staphylococci are well adapted. The infections caused by *S. aureus* have been considered to originate from areas of impaired skin as a result of bacterial spread from anterior nares since colonization of this ecological niche appears to play a key role in the epidemiology and pathogenesis of invasive infections *S. aureus* may cause clinically significant bacteremia in certain groups of patients as a result of translocation from colonized mucosa directly to the bloodstream (Bogut *et al.,* 2007**).**

The incidence, spectrum of infecting organisms *Staphylococcus aureus,* was responsible for 1.92% of catheter-related bacteremia in hemodialysis (HD) patients in KSA (AlSaran *et al.*, 2013).

2.2.4.1.3.2 *Staphylococcus epidermidis*

Staphylococcus epidermidis, an organism routinely found on the skin and in the hospital environment, has become a primary pathogen in infections associated with prosthetic devices. Because these infections are indolent and often clinically silent, diagnosis and therapy are often difficult. Pathogens are often misidentified as contaminants. They are variable, often resistant to antibiotic susceptibility pattern and the uncertain correlation of in-vitro beta-lactam sensitivity testing with therapeutic efficacy make selection of

an effective antibiotic regimen difficult. Vancomycin combined with rifampin, gentamicin, or both, is recommended for empiric therapy of these infections. Usually, removal of the prosthetic device is also necessary and may contribute equally to a successful therapeutic outcome (Lowy and Hammer, 1983).

The incidence, spectrum of infecting organisms *Staphylococcus epidermidis,* was responsible for 50% of catheter-related bacteremia in hemodialysis (HD) patients in KSA (AlSaran *et al.*, 2013).

2.2.4.1.3.3 *Staphylococcus vitulinus*

Staphylococcus vitulinus is a member of the *Staphylococcus sciuri* group (*S. sciuri*, *S. lentus,* and *S. vitulinus*) are widespread in nature, and they can be isolated from a variety of farm animals, pets, and wild animals, as well as from various food products of animal origin. This group is made up of *Staphylococcus sciuri* subsp. *carnaticus*, *Staphylococcus sciuri* subsp. *rodentium*, *Staphylococcus sciuri* subsp. *sciuri, Staphylococcus lentus*, and *Staphylococcus vitulinus*. *Staphylococcus pulvereri* was a member of the *S. sciuri* group until recently, when it was shown that *S. pulvereri* is only a synonym of *S. vitulinus* (originally *S. vitulus*). Although they are principally associated with animals, members of the *S. sciuri* group may colonize humans, and it has been estimated that they may constitute 0.79 to 4.3% of the total number of coagulase-

negative *staphylococci* isolated from clinical samples. However, they have been associated with serious infections such as endocarditis, peritonitis, septic shock, urinary tract infection, endophthalmitis, pelvic inflammatory disease, and, most frequently, wound infections (Stepanovic *et al.,* 2005).

2.2.4.1.3.4 *Staphylococcus hominis*

Hemodialysis patient that presented a CRB episode caused by a coagulase negative (CoN) *Staphylococcus* rarely associated with human infections in dialysis patients. Macía-Heras *et al.* (2012) found the bacterial isolate recovered from catheter was identified as *Staphylococcus hominis* by 16S ribosomal gene sequencing. The *S. hominis* isolate was methicillin resistant, being *mec*A positive, and to daptomycin.

2.2.4.1.3.5 *Staphylococcus simulans*

Staphylococcus simulans is a common animal pathogen. It is usually isolated from cattle, sheep and their products that occasionally can colonize human skin. Unlike other coagulase-negative staphylococci, *S. simulans* tends to cause more severe infections that resemble those caused by *S. aureus* as stated by Vallianou *et al.* (2008). The incidence, spectrum of infecting organisms *Staphylococcus simulans,* was responsible for 3.84% of catheter-related bacteremia in hemodialysis (HD) patients in KSA (AlSaran *et al*., 2013).

2.2.4.1.3.6 *Streptococcus uberis*

Environmental Streptococci,

predominantly *Streptococcus uberis* are the leading cause of bovine mastitis. However, *S. uberis* is rarely associated with human infections, although, a limited number of human infections by *S.uberis* have been reported. Seven cases of urinary tract infections (UTIs), out of 148 culture positive urinary infections examined, were shown to be caused by *S. uberis* (Gülen *et al.*, 2013).

2.2.4.1.3.7 *Enterococcus faecalis and Enterococcus faecium*

Enterococci are Gram-positive, catalase-negative, non-spore-forming facultative anaerobic bacteria belonging to the group of so-called lactic acid bacteria. They can be seen as isolated cocci, pairs, short chains, and even long chains. Nearly 30 different species have been identified, but 2 namely, *E. faecalis* and *E. faecium* cause most of the human infections in which this genus is involved (Perez-Fontan *et al.,* 2011).

Recently, they have emerged as important pathogenic agents in nosocomial environments; they can cause severe infection with high mortality rates. From 1986 to 1989, they were the second-most commonly isolated agent of hospital-acquired infections in the United States. They can survive over long periods on inanimate objects such as thermometers and stethoscopes, and also on the hands of healthcare personnel; they often colonize the

gastrointestinal tract of patients and healthcare workers as well. *Enterococcus* spp. have markedly increased in frequency as etiological agents of urinary tract and bloodstream infections (BSI) (Strabelli *et al.*, 2006).

Vancomycin-resistant enterococci (VRE) infection has become a matter of great concern in patients with end-stage renal disease in America and Europe, and it has significantly contributed toward increasing the morbidity and mortality within this population. Fram *et al.* (2010) were able to identify VRE in dialysis and renal transplant patients in the year 2003, and the prevalence of colonization was 14.4% in dialysis patients, with type of dialysis treatment (hemodialysis *vs* peritoneal dialysis).

2.2.4.1.3.8 *Enterobacter cloacae*

Bacteria of the *Enterobacter cloacae* complex are Gram negative, chemo-organotrophic, facultatively anaerobic rods or coccobacilli. Recently. The importance of members of the *Enterobacter cloacae* complex as nosocomial pathogens has been high lighted, and strains belonging to this species are encountered among the most-often-isolated strains able to cause severe opportunistic infections in hospitalized and debilitated patients, especially in intensive care units. They cause pneumonia and urinary tract, wound, skin and soft tissue, ophthalmic and bloodstream infections (Mokracka *et al*., 2011).

The urinary and pulmonary systems are the organ systems most commonly colonized in these patients. *Enterobacter cloacae* bacteremia can also occur depending on the extent of immunocompromise. Outbreaks of coloacae infections are recorded in a number of hospital settings. Sporadic cases of *E. cloacae* infections have been linked to contaminated admixed intravenous fluids, total parenteral nutrition solutions, enteral feedings, infant formula, cardioplegic solution, and blood products. Another potential reservoir for nosocomial bacteremia is the heparin flush solution used to irrigate certain intravascular devices continually. This fluid had been implicated as a reservoir for outbreaks of device-associated bacteremia in several instances. Less commonly, outbreaks are linked to the colonization of devices such as long-term tunneled hemodialysis catheters (Musil *et al.*, 2010).

AlSaran *et al*. (2013) found the incidence, spectrum of infecting organisms *Enterobacter cloacae* was responsible for 7.69% of catheter-related bacteremia in hemodialysis patients in KSA.

2.2.4.1.3.9 *Serratia marcescens*

Serratia marcescens is a Gram-negative aerobic bacillus belonging to the family Enterobacteriaceae. *Serratia* species are widely distributed in nature and in hospitals and may even be found as commensals in the human gut microbiota. In recent years, *S. marcescens* has been increasingly

recognized as an important and frequent opportunistic pathogen. *Serratia* species rank among the 10 most common causes of bacteremia and skin and soft tissue infections, accounting for 1.4% and 2.0% of cases, respectively. Epidemiologic studies have shown an incidence rate of 1.3 cases of *Serratia* bacteremia per a population of 100,000. A substantial proportion (47%) of these events originates in the community (Rehman *et al.*, 2012).

2.2.4.1.3.10 *Escherichia coli*

Escherichia coli, originally called "*Bacterium coli* commune," was first isolated from the feces of a child in 1885 by Theodor Escherich and nowadays is the best-studied bacterium. *Escherichia coli* is a common inhabitant of the gastrointestinal tract of humans and animals. There are *E. coli* strains that are harmless commensals of the intestinal tract and others that are major pathogens of humans and animals. The pathogenic *E. coli* is divided into those strains causing disease inside the intestinal tract and others capable of infection at extra intestinal sites (Sousa, 2006).

Escherichia coli is the species that most frequently causes Gram-negative bacteremia (Maslow *et al*., 1993).The incidence, spectrum of infecting organisms *Escherichia coli* was responsible for 3.84% of catheter-related bacteremia in hemodialysis (HD) patients in KSA (AlSaran *et al.*, 2013).

2.2.4.1.3.11 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an important nosocomial pathogen, especially in individuals with neutropenia and those who are immunocompromised. During the 1960s, when *P. aeruginosa* first emerged as a common cause of Gram-negative bacteremia and effective antipseudomonal antibiotics were unavailable. However*, P. aeruginosa* continues to be a serious cause of infection, associated with a high rate of morbidity and a mortality rate ranging from 18% to 61% (Kang *et al*., 2003). .
Wagnild *et al.* (1977) found that ten of the 17 patients (59 percent) had a *Pseudomonas aeruginosa* from blood for culture was obtained over a six week period from 17 patients undergoing long-term hemodialysis, only one patient was symptomatic. The incidence, spectrum of infecting organisms *Pseudomonas aeruginosa* was responsible for 7.69% of catheter-related bacteremia in hemodialysis (HD) patients in KSA (AlSaran *et al.,* 2013).

2.2.4.1.4.Diagnosis

2.2.4.1.4.1.Clinical

Every patient with temporary, or true permanent access (catheters) (PCs) who had fever, chills, or other symptoms suggesting systemic infection (eg, nausea, vomiting, malaise, or back pain) was considered to have possible bacteremia (Saad, 1999). Allon (2004) mentioned that catheter-related bacteremia should be suspected clinically when a hemodialysis patient with a dialysis

catheter develops fever or chills. Three recent prospective studies observed positive blood culture results in 59% to 81% of symptomatic catheter-dependent patients.

2.2.4.1.4.2. Physical

Physical examination is usually performed, and other potential sources of infection were noted, including the catheter exit site or tunnel, peripheral venous catheter site, arteriovenous graft, skin ulcer, or wound. Further studies, including chest radiograph and urine analysis, will be performed as indicated for specific signs or symptoms. Patients presenting with severe symptoms, including hypotension, high fever, rigors, mental status changes, or vomiting, do not have HD initiated. Those who developed severe symptoms during HD had their treatment interrupted or discontinued. If the patient is clinically stable with mild symptoms, HD is initiated or continued as prescribed by Saad. (1999).

2.2.4.1.4.3. Bacteriology

2.2.4.1.4.3.1. Collection of Blood Samples

Using aseptic technique with hand washing, non sterile gloves, masking of both nurse and patient, and a non sterile towel draped under the catheter, the nursing staff disinfect the connection ports using two gauze sponges soaked with aqueous-based povidine-iodine solution for 5 minutes. The gauze is removed, and the

solution is allowed to dry before the catheter was opened as described by Landry *et al.* (2010).

2.2.4.1.4.3.2 Culture of Blood Samples

All patients with suspected bacteremia will have blood sample drawn, either directly through the catheter ports or from the ‘‘arterial’’ dialysis tubing port. Usually, at least two sets of aerobic and anaerobic cultures are obtained, although in a few cases, only one set is obtained. Peripheral blood cultures through separate venipuncture sites are not routinely performed (Saad, 1999).

2.2.4.1.4.3.3 VITEK 2 Compact System

The VITEK system originated in the 1970s as an automated system for identification and AST and has evolved today into the VITEK 2 system, which automatically performs all of the steps required for identification and AST after a primary inoculum has been prepared and standardized. This system allows kinetic analysis by reading each test every 15 min. The optical system combines multichannel fluorimeter and photometer readings to record fluorescence, turbidity, and colorimetric signals (Ligozzi *et al*., 2002).

The VITEK 2 as described by Pincus. (2006) is an automated microbial identification system that provides highly accurate and reproducible results as shown in multiple independent studies. With its colorimetric reagent cards, and associated hardware and software advances, the VITEK 2 offers a state-

of-the-art technology platform for phenotypic identification methods.

2.2.4.1.4.3.3 .1 Reagent Cards

2.2.4.1.4.3.3 .1.1 Identification Cards (ID)

The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. There are currently four reagent cards available for the identification of different organism classes include, GN - Gram-negative fermenting and non-fermenting bacilli, GP - Gram-positive cocci and non-spore-forming bacilli,YST - yeasts and yeast-like organisms and BCL-Gram-positive spore-forming bacilli (Pincus, 2006).

2.2.4.1.4.3.3 .1.2 Antimicrobial Susceptibility Testing (AST) Cards

VITEK® 2 and Etest™ meet a laboratory's antimicrobial susceptibility testing (AST) needs and deliver minimum inhibitory concentrations (MICs) for most organisms include Gram negative antibiotic susceptibility testing (AST) cards, Gram positive antimicrobial susceptibility testing (AST) cards and fluconazole antimicrobial susceptibility testing (AST) card for yeast susceptibility testing(Pincus, 2006).

2.2.4.1.4.4. Molecular Study

2.2.4.1.4.4.1. Integrons

Integrons were defined by Fluit and Schmitz. (2004) as elements which contain the genetic determinants of the components of a site-specific recombination system that recognizes and captures mobile gene cassettes. An integron includes the gene for an integrase (int) and for an adjacent recombination site (attI). Gene cassettes are not necessarily part of the integron, but they become part of the integron when integrated. Nearly all known gene cassettes from resistance integrons encode resistance to antibiotics or disinfectants. These integrons are found on transposons, plasmids and the bacterial chromosome. Integrons can now be divided into two major groups: the resistant integrons (RI) and the super integrons (SI). RI carry mostly gene cassettes that encode resistance against antibiotics and disinfectants, and can be located either on the chromosome or on plasmids. The large chromosomally-located integrons,

which contain gene cassettes with avariety of functions, belong to the SI group. Three classes of RI are known. SI are not given a specific name. The integron originally designated as class 4 is now named *Vibrio cholera* SI. Most RI belong to class 1, and class 1 integrons have been reported in many Gram negative genera including *Acinetobacter, Aeromonas, Alcaligenes, Burkholderia, Campylobacter, Citrobacter, Enterobacter, Escherichia, Klebsiella, Pseudomonas, Salmonella, Serratia, Shigella* and *Vibrio.* They have also been found in other bacteria such as *Corynebacterium glutamicum* and *Mycobacterium fortuitum*, and a gene cassette has been discovered in *Enterococcus faecalis*. Class 2 integrons are embedded in the Tn7 family of transposons and consist of an integrase gene followed by gene cassettes. Class 2 integrons have been found in *Acinetobacter, Shigella* and *Salmonella.* Class 3 integrons have been described in *Pseudomonas aeruginosa*, *Serratia marcescens*, *Alcaligenes xylosoxidans, Pseudomonas putida* and *Klebsiella pneumonia* isolates from Japan.

Gene cassettes include an open reading frame and a recombination site, 59-bp element or attC. Integration and excision of cassettes occurs by a site-specific recombination mechanism catalysed by the integrase. Based on the intI sequences, at least six classes of integrons have been described to date. Class 1 integrons are the most studied

and are largely implicated in the dissemination of antibiotic resistance among clinical isolates. Although the mobilisation of gene cassettes has been demonstrated on various occasions, the stability of integron structures has been indicated by the finding of conserved gene cassette combinations among isolates of different origins, or their maintenance in bacteria under antibiotic pressure over extended periods of time (Severino and Magalha~es, 2004).

All class 1 integrons possess the following key components: an integrase gene (*intI1*), a recombination site (*attI1*), and a promoter (Pc) for the transcription of cassette-associated genes.These components encompass the basic functions needed for the acquisition and expression of a second type of mobile genetic element known as a gene cassette. Gene cassettes typically consist of an open reading frame (ORF) associated with a recombination site known as a 59-base element (59-be). IntI1 most commonly mediates recombination between 59-be sites and *attI1*, resulting in insertion of gene cassettes into *attI1.* Although recombination between two 59-be sites is also possible. As a result of multiple insertion and excision events (Holmes *et al*., 2003).

2.2.4.1.4.4.2. 16S rRNA gene

Identification of bacterial species from culture material by 16S rRNA gene sequencing is now widely used in many clinical microbiology laboratories (Hartmeyer and Justesen, 2010).

The use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy has been by far the most common housekeeping genetic marker used for a number of reasons. These reasons include (i) its presence in almost all bacteria, often existing as a multigene family, or operons; (ii) the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); and (iii) the 16S rRNA gene (1,500 bp) is large enough for informatics purposes **(**Janda and Abbott, 2007)**.**

Although 16S rRNA gene sequencing has been widely used in reference laboratories, one of the major limitations to its wider use in routine clinical microbiology laboratories is the difficulty associated with interpretation of 16S rRNA gene sequence results. The use of 16S rRNA gene sequencing for bacterial identification depends on significant inter-species differences and small intra-species differences in 16S rRNA gene sequences. As a result, one of the major limitations is that when two bacterial species share a similar 16S rRNA gene sequence, this technology alone

would not be useful for distinguishing them confidently (Teng *et al*., 2011).

2.2.4.1.5. Treatment

All patients are treated with intravenous antibiotics pending culture diagnosis. Initial coverage in most cases consisted of vancomycin combined with either gentamicin or ceftazidime. Some patients with only mild signs and symptoms of infection or a history of allergy to vancomycin received only a cephalosporin initially. All episodes confirmed by positive blood cultures are diagnosed as CAB regardless of other possible sources. Antibiotics are adjusted based on culture and sensitivity results and continued for 21 days unless other indications warranted a longer course. If initial culture results are negative, the patient is not diagnosed with CAB and antibiotics are discontinued. Management of the PC is determined by the treating nephrologist. In cases with severe or uncontrolled clinical sepsis, the PC was usually removed without an attempt to salvage the catheter (Saad, 1999).

2.2.4.1.6. Epidemiology

Japan and Taiwan are the countries with the highest prevalence of end-stage renal disease (ESRD) patients. In 2003, the prevalence was <1800 patients per million population (pmp) in Japan and 1600 pmp in Taiwan. Prevalence is a little lower in the United States (1500

pmp) and Spain (1000 pmp). Prevalences in developing countries are lower than those from developed countries, perhaps reflecting the lower quality of the public health systems. However, the number of ESRD patients is increasing worldwide. In Malaysia, dialysis is the main modality of renal replacement therapy. There were about 26,000 patients on dialysis in 2011 with the prevalence of 900 per million populations (Lugon, 2009 and Abdul Gafor *et al.,* 2014).

Approximately, 25% of the approximately 300,000 US hemodialysis patients, or 75,000 patients, use catheters as their vascular access (Allon, 2004).

The incidence of catheter-related blood stream infections (CRIs) in hemodialysis patients ranges from 2.5 to 5.5 cases per 1000 catheter-days (Landry *et al.,* 2010).

Several studies have reported a rate of bacterial infection in CVCs used for HD in children with ESRD in the range of 1.5 to 4.8 episodes/1000 catheter days (Eisenstein *et al*., 2011).

The projected annual number of catheter-related bacteremia episodes is 67,500 to 150,000. Of those, approximately 10% of patients would be hospitalized with either severe sepsis (hemodynamic instability or high fever with shaking chills) or a metastatic infection. This translates to approximately 7,000 to 15,000 serious complications of

catheter related bacteremia annually in the United States (Allon, 2004).

2.2.4.1.7. Prevention

2.2.4.1.7.1. Gentamicin and Heparin Lock (GHL) Protocol

A standard protocol was implemented where gentamicin and heparin were mixed by a specified dialysis nurse to a final concentration consisting of gentamicin (4 mg/ml) and heparin (5000 units/ml). The choice of final gentamicin dose was based on *in vitro* evidence indicating that supraphysiologic drug concentrations (100- to 1000-fold higher than therapeutic plasma drug levels) are needed to eradicate biofilm organisms, whereas higher concentrations can be associated with systemic toxicity (Landry *et al.,* 2010).

2.2.4.1.7.2. Core Interventions for CDC's Dialysis Bloodstream Infection BSI Prevention Collaborative (CDC, 2011)

1. **Surveillance and feedback using NHSN** should be conducted monthly surveillance for BSIs and other dialysis events and enter events into CDC's National Healthcare

Safety Network (NHSN). The facility rates should be Calculated and compared to rates across other facilities using NHSN. Facilities should actively share results with front-line clinical staff.

2. **Chlorhexidine for skin antisepsis** An alcohol-based chlorhexidine (>0.5%) solution should be used as the first line agent for skin antisepsis, particularly for central line insertion and during dressing changes. Povidone-iodine, preferably with alcohol, or 70% alcohol are alternatives.

3. **Hand hygiene audits** Hand hygiene audits with feedback of results to clinical staff should be Performed monthly.

4. **Catheter care/ vascular access observations** It is an important to perform monthly or quarterly audits of catheter care and accessing practices to ensure adherence to facility protocols. This includes following recommended procedures and maintaining aseptic technique while connecting and disconnecting catheters and during dressing changes. The results with front-line clinical staff should be showed.

5. **Patient education/engagement** It is an important to provide standardized, basic education to all patients on topics including care of vascular access, hand hygiene, risks related to catheter use, recognizing signs of infection, and instructions for access management when away from the dialysis unit.

6. **Staff education and competency** Regular training of staff on infection control topics, including care of access and aseptic technique has to be provided. Evaluation of competency for skills such as catheter care and accessing at least every 6-12 months and upon hire should be performed.

7. **Catheter reduction** Efforts within the facility (e.g., through patient education, vascular access coordinator) has to be incorporated in order to reduce catheters by identifying barriers to permanent vascular access placement and catheter removal.

CHAPTER THREE

2. MATERIALS AND METHODS

3.1. Study Design

3.1.1. Type of Study

The study is a descriptive and cross-sectional study of 201 patients on dialysis with symptoms of catheter-related blood stream infections.

3.1.2. Study Area

Blood collection and patients' data were done in 17 Dialysis Units. These were Khartoum Teaching Hospital, Gaffer Iben Auff Specialized Hospital for Children, Omdurman Teaching Hospital, Mohamed Elamin Hospital for Children, Alacademy Teaching Hospital, Al waledain dialysis centers, Giad hospital, El gamea Hospital for renal disease, Ahmed Gasim Hospital, El Safia Dialysis Center, Military Medical Hospital, Ribat University Hospital, Ibn Seina Hospital, Alamal Alwatani Hospital, Ompada Hospital, Sharg Elneel Hospital and Elban Gadead Hospital in Khartoum State.

3.1.3. Inclusion Criteria

The study was done in Sudanese patients at different age group, Every patient with temporary, or true permanent access (catheters) (PCs) who had fever, chills, or other symptoms suggesting systemic infection (eg, nausea, vomiting, malaise, or back pain) was considered to have possible bacteremia were included in this study.

3.1.4. Exclusion Criteria

Patients with clinical or laboratory evidence of another infected site were excluded from study.

3.1.5. Data Collection

Data was collected using structural interviewing Questionnaire. Demographic information, including age, sex, race, cause and duration of end stage renal disease (ESRD), signs and symptoms during dialysis (fever, chills, weakness), and indication for permanent catheters (PCs) access, was collected at the time of PC placement. All study patients were prospectively monitored for the total number of days, the PC was in place from the date of insertion to the date of catheter removal or exchange. Unassisted catheter survival was defined as the number of days from initial catheter placement to the first catheter intervention (removal or exchange for any purpose).

3.2. Collection of Specimens

Blood samples were drawn, either directly through the catheter ports or from the ‘‘arterial’’ dialysis tubing port. Usually, one set was obtained by using aseptic

technique with hand washing, non sterile gloves, masking of both nurse and patient, and a non sterile towel draped under the catheter, the nursing staff were disinfected the connection ports using two gauze sponges soaked with aqueous-based povidine-iodine solution for 5 minutes. The gauze was removed, and the solution was allowed to dry before the catheter was opened as mentioned by Landry *et al*. (2010).

3.3. Inoculation of Specimens

Media were selected for the culture of blood were capable of providing the fastest growth and isolation of as wide range of pathogens as possible.

3.3.1. Culture Media

Thioglycollate broth is a medium of choice for culture of blood samples as mentioned by Collee *et al.* (1996) who found the use of a single good all-purpose medium can give nearly as many positive results as the use of the range of different media, with the advantages of economy in costs and labour and the avoidance of confusion, so if a single media is used as a routine, it must be richly nutritive and suitable for aerobes and anaerobes so thioglycollate broth supports the growth of anaerobes and aerobes and has been used in modified form for blood culture purposes,

3.3.2. Dilution of the Sample

The blood was diluted between 1 in 5 and 1 in 10 in the culture medium to reduce the concentration of natural antimicrobial constituents to a sub-effective level (Collee *et al*., 1996).

3.3. 3. Procedure of Inoculation of Blood Samples

Thioglycollate broth (50 ml) for blood culture was generally dispensed in a special round blood culture bottle of about 100-120 ml capacity and fitted with a screw cap with a central hole giving access to a rubber washer seal, the bottle cap and the exposed part of the washer were protected by foil which was removed immediately before the 5 ml of blood was inoculated by inserting the syringe needle through the washer and the inoculated bottles were incubated as soon as possible at 35-37˚C (Collee *et al.*, 1996 and Cheesbrough, 2000).

3.4. Examination of Bacterial Growth

Growth was observed as generalized turbidity or there may be discrete colonies on the surface of the sedimented red cells, then subculture was done from all bottles to solid media which was done at least twice during the first 2-3 days. It was usual to continue incubation and inspection for up to 5-7 days with a final subculture, then Gram-stained smears were made from any broth that showed visible signs of growth as this can allow an early presumptive report (Collee *et al*., 1996). .

3.5 Subculturing of Blood Culture Broth

A strict aseptic technique was used to avoid contamination of the culture, by cleaning the top of the bottle by using an ethanol-ether swab. A sterile needle and small syringe was inserted through the rubber liner in the cap, and about 1 ml of broth culture was withdrawn and inoculated on blood agar, chocolate agar and MacConkey's agar. Blood agar plate was incubated anaerobically for up to 48 hours, the chocolate agar plate was incubated in a carbon dioxide atmosphere for up to 48 hours and the MacConkey agar plate was incubated aerobically for an overnight, then the top of the bottle was swabbed and reincubated (Cheesbrough, 2000).

3.5 Identification and Susceptibility Testing of the Isolated Bacteria by Using VITEK 2 Compact System (BioMérieux, Marcy I'Etoile, France)

VITEK 2 compact system (BioMérieux, Marcy I'Etoile, France) is a semi-automated bacterial identification and susceptibility testing system enabling rapid determination of MICs by analysis of bacterial growth kinetics with antimicrobials in sealed test cards and resistant mechanisms. The procedures recommended by the manufacturer were strictly followed.

3.5.1. Primary Identification

Appropriate culture and inoculum preparation was done, which required the acceptable ranges for isolation media, temperature, atmosphere, age of culture, incubation, and organism suspension turbidity which has a significant importance for successful identification of microorganism. In automated systems it is even more important to get a huge amount of fresh and pure isolated samples. These bacteria are needed for making high concentration of active exponential phase bacterial suspensions which prevents environmental contaminations. Since sterile working condition couldn't reach properly during inoculation of cards, old colonies of bacteria or low concentrations of inoculants could be corrupted by contaminations.

3.5.1.1 Colonial Morphology

The primary culture on agar media that showed significant growth was examined and the morphological character, size, shape, color and haemolysis were observed and recorded. A single well isolated colony was removed by using sterile wire loop and replated, and incubated again for 16 to 24 h at 37°C just before testing.

3.5.1.2 Gram's Stain

Tested Colonies were stained by Gram's stain as follow: A well prepared, dried and fixed smear was covered with crystal violet stain for two minutes. The stain was washed off rapidly with clean tap water. All the water was tipped off and the smear was covered with lugol's

iodine for 30-60 seconds. The iodine was washed off with clean tap water and decolorized rapidly (few seconds) with acetone-alcohol and washed immediately with clean water. Then the smear was covered with neutral red stain for two minutes and washed off with clean tap water. The dried smear was examined microscopically, first with the 40x objective to check the staining and then with the oil immersion objective to report the bacteria (Cheesbrough, 2000).

3.5.2. Suspension Preparation

3.5.2.1 Identification Cards (ID)

For both identification cards, bacterial suspensions were prepared by transfering a sufficient number of colonies of a pure culture and to suspend the microorganism in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12x75 mm clear plastic (polystyrene) test tube. The turbidity was adjusted according to 0.50-0.63 McFarland's turbidity standard and was measured using a turbidity meter VITEK 2 instrument called DensiChek. (BioMe´rieux) (software version 4.01).

Vitek2® Gram negative (GN) and Gram positive (GP) cards were set up according to instructions given by BioMérieux®. All reagents and equipment needed for processing supplied by Manufacturer Company. All isolates introduced to the computer before processing and inoculated cards were processed in the instrument within 30 min of inoculation.

3.5.2.1.1 The GP identification card

The GP identification card is based on established biochemical methods and newly developed substrates which includes colorimetric tests for the following reactions: phosphatidylinositol phospholipase C, arginine dihydrolase (two tests), galactosidase,glucosidase, alanine-phenylalanine- proline arylamidase, L-aspartic acid arylamidase, galactosidase, mannosidase, alkaline phosphatase, L-leucine arylamidase, proline arylamidase-glucuronidase (two tests), galactosidase, L-pyroglutamic acid arylamidase, alanine arylamidase, tyrosine arylamidase, and urease. Also tests acid production from the following substrates: amygdalin, xylose,cyclodextrin, sorbitol, galactose, ribose, lactate, lactose, *N*-acetylglucosamine, maltose, mannitol, mannose, methyl-D-glucopyranoside, pullulan, raffinose, salicin, sucrose, and trehalose. Finally, growth in 6.5% NaCl and tests for resistance to polymyxin B, bacitracin, novobiocin, O129, and optochin were also included in the ID-GP identification card.

3.5.2.1.2 The GN identification card

The GN card was based on established biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic activities, and resistance, there are 47 biochemical tests and one negative control well. Final identification results are available in approximately 10 hours

or less, test substrates including 18 enzymatic tests for aminopeptidases and -osidases. Substrates used for detection of aminopeptidases are usually coupled with 7-amino-methylcoumarin (7AMC); substrates for detection of -osidases were usually coupled with 4-methylumbelliferone (4MU). The 18 test substrates were as follows: 4MU-a-arabinopyranoside, 4MU-a-D-galactoside, a-Lglutamic acid-7AMC, 4MU-b-D-cellobiopyranoside, 4MU-b-D-galactoside, 4MU-b- D-glucoside, 4MU-b-D-glucuronide, 4MU-b-D-mannopyranoside, 4MU-*N*-acetylb-D-glucosaminide, 4MU-*N*-acetyl-b-D-galactosaminide, 4MU-b-D-xyloside, glutaryl-glycyl-arginine-7AMC, g-L-glutamic acid-7AMC, 4MU-phosphate, L-proline-7AMC, L-pyroglutamic acid-7AMC, L-lysine-7AMC, and *Z*-arginine-7AMC. Furthermore, the ID-GNB card includes 18 fermentation tests (adonitol, L-arabinose, D-cellobiose, D-galacturonate, D-glucose, glucose-1-phosphate, D-glucuronate, inositol, 5-keto-gluconate, D-maltose, D-mannitol, D-melibiose, palatinose, D-raffinose, L-rhamnose, sucrose, D-sorbitol, and D-trehalose), 2 decarboxylase tests (ornithine and lysine), and 3 miscellaneous tests (urease, utilization of malonate, and tryptophane deaminase).

3.5.2.2 Antimicrobial Susceptibility Testing (AST) Cards

A volume of 145µl and 280µl of the inoculum from the ID test tube for Gram negative and Gram positive organisms respectively, were pipetted into the antibiotic susceptibility

testing (AST) test tube of 3.0 mL of sterile saline and mixed thoroughly.

3.5.2.2.1 AST-GP 67 Card

AST-GP 67 card were used for Gram-positive cocci including *Staphylococcus spp*, *Enterococcus spp*. and *S. agalactiae* contained benzylpenicillin, erythromycin, gentamicin, nitrofurantoin, oxacillin, rifampin, tetracycline, trimethoprim-sulfamethoxazole, tigecycline, linezolid, moxifloxacin, levofloxacin, clindamycin, ciprofloxacin, and vancomycin.

3.5.2.2.2 AST-GN 75 Card

AST-GN 75 card was used for Gram-negative bacilli contained ampicillin, gentamicin, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, nitrofurantoin, ciprofloxacin, cefoxitin, levofloxacin, ceftriaxone, amikacin, cefazolin cefepime, ceftazidime, meropenem, pipracillin and tobracillin.

3.5.3 Inoculation

Identification cards and AST test cards were inoculated with microorganism suspensions using an integrated vacuum apparatus. Test tubes containing the microorganism suspension were placed into a special rack (cassette) and the cards were placed in the neighboring slot while inserting the transfer tubes into the corresponding suspension tubes. The cassette can accommodate up to 10 tests. The filled cassette was placed into a vacuum chamber station. After

the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells.

3.5.4 Card Sealing and Incubation

Inoculated cards were passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards. All card types were incubated on-line at 35.5 ± 1.0ºC. Each card was removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next reading time. Data were collected at 15-minute intervals during the entire incubation period.

3.5.5 Optical System

A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum was used. During incubation, each test reaction was read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm was used to eliminate false readings due to small bubbles that may be present.

3.5.6 Test Reactions

Calculations were performed on raw data and compared to thresholds to determine reactions for each test and the test reaction results were appear as “+”,” –“, “(–)” or “(+)”.

Reactions that appear in parentheses were indicative of weak reactions that were too close to the test threshold.

3.5.7 Database Development

The databases of the VITEK 2 identification products were constructed with large strain sets of well-characterized microorganisms tested under various culture conditions. These strains were derived from a variety of clinical and industrial sources as well as from public (e.g., ATCC) and university culture collections.

3.5.8 Analytical Techniques

Test data from an unknown organism are compared to the respective database to determine a quantitative value for proximity to each of the database taxa. Each of the composite values is compared to the others to determine if the data are sufficiently unique or close to one or more of the other database taxa. If a unique identification pattern is not recognized, a list of possible organisms is given, or the strain is determined to be outside the scope of the database.

3.5.9 Identification Levels

An unknown biopattern is compared to the database of reactions for each tax on, and a numerical probability calculation is performed. Various qualitative levels of identification are assigned based on the numerical probability calculation: excellent identification, very good identification, good identification, acceptable identification (each of these four categories shows only one identification

result), low discrimination (more than one identification result is given, where upon the software suggests performing additional tests such as oxidase, hemolysis, pigmentation, indole, and motility tests in order to obtain the correct identification), inconclusive identification, and unidentified.

3.5.10 Interpretation of Results

(i) Agreement. The VITEK 2 system and the reference method were considered to be in agreement when the species identification of the VITEK 2 system agreed exactly with the species identification of the reference method.

(ii) Essential agreement. MICs obtained with the VITEK 2 system and by the reference methods were considered to be in essential agreement when the MIC obtained with the VITEK 2 system was within 1 twofold dilution of the reference MIC obtained by either the microdilution method or the agar dilution method. In the case of high-level resistance to aminoglycosides, “category agreement” occurred when the categorization of high-level resistance with the VITEK 2 system coincided with the results obtained by the reference methods.

(iii) MIC discrepancies. MIC discrepancies were considered “very major” (the VITEK 2 system indicated susceptible and the reference method indicated resistant), “major” (the VITEK 2 system indicated resistant and the reference method indicated susceptible), and “minor” (the VITEK 2 system indicated intermediate and the reference method indicated

susceptible or resistant, or the VITEK 2 system indicated susceptible or resistant and the reference method indicated intermediate).

3.6 Molecullar Diagnosis

3.6.1 Extraction of Genomic DNA

Genomic DNA was extracted using Vivantis nucleic acid extraction kit (GF-1 bacterial DNA extraction kit, Vivantis Technologies, Malaysia). According to the manufacturer's suggestions 1-3 ml of bacterial culture grown over night on nutrient broth at 37°C, were pelleted by centrifugation at 6.000×g for 2 min, at room temperature and the supernatant were decanted completely then the pellet was resuspended completely by adding 100 µl buffer R1 and pipetting up and down. Then lysozyme treatment were done by adding 10µl lysozyme (50 mg/ml) into the cell suspention of Gram negative and 20 µl lysozyme (50 mg/ml) into the cell suspention of Gram positive mixed and incubated at 37 ˚C for 20 min. They were digested by centrifugation at 10.000×g for 3 min and supernatants were decanted completely. Protein denaturation was done by resuspention of the pellet in 180 µl of buffer R2 and addition of 20 µl of proteinase K, which was mixed thoroughly and incubated at 65˚C for 20 min with occasionally mixing every 5 min, then 2 volumes (400 µl) of buffer BG were added and mixed

thoroughly by inverting tube several times until homogeneous solution was obtained then incubated for 10 min at 65 °C. Absolute ethanol was added and mixed immediately and thoroughly. A sample of 650 µl was transferred into a column assembled in a clean collection tube and centrifuged at 10.000×g for 1 min and flow discarded through, column was washed with 650 µl of wash buffer and centrifuged at 10.000×g for 1 min and discarded flow through. Then column was centrifuged at 10.000×g for 1 min to remove residual ethanol, then column was placed into a clean microcenterifuge tube, and then 50-100 µl of sterile water was added directly onto column membrane and stood for 2 min. DNA was eluted by centrifugation at 10.000×g for 1 min, and finally DNA were stored at -20°C for further identification.

3.6.2 Application of Polymerase Chain Reaction

Polymerase Chain Reaction amplification for all primers used in this study was performed in 20 µl volumes of Maxime PCR PreMix kit (i-Taq) was mixed every component 2.5U i-Taq DNA Polymerase, dNTPs mixure 2.5 Mm each, reaction buffer 10x and gel loading buffer in one tube for 1 rxn PCR (iNtRON Biotechnology-Korea) and 5 µl bacterial template DNA, 2 µl of Forward and Reverse primer in concentration of 10 Pmol, in a Thermal Cycler (Primus 96 Advanced, Biotechnologie, GmbH, Germany).

The presence of class 1 and 2 integrons was tested by PCR using primers specific for the integron integrase genes intI 1 and intI 2 (Table 1) (Chang *et al.,* 2011; Mobarak-Qamsari *et al.,* 2013). The PCR conditions were as follows:- Initial denaturation at 95 °C for 1 min, 30 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 65 °C and extension for 1 min at 72 °C, and a final extension for 7 min at 72 °C for intI1 and initial denaturation at 95 °C for 5 min followed by 35 cycles of 1 min at 94 °C, 1 min at 65 °C and 1 min at 72 °C with a final extension at 72 °C for 10 min for intI 2.

A species–specific PCR assay with primers targeted to the 16S rRNA gene was done for *Staphylococcus epidermidis, E. faecalis, E. faecium*, *Staphylococcus aureus* and *P. aeruginosa* to confirm the VITEK 2 compact system species identification (Table 1). Using the primers Forward: 5-TACATGCAAGTCGAGCGAAC3, Reverse: 5AATCATTTGTCCCACC TTCG-3 targeted to the *Staphylococcus epidermidis* 16SrRNA, Forward: 5'-GTTTATGCCGCATGGCATAAGAG-3', Reverse: 5'-CCGTCAGG GGACGTTCAG-3' targeted to the *E.faecalis* 16SrRNA, Forward: 5'ATCGCAAGATTGTTCGAAC3, Reverse: 5'CTTAGAAAGGAGGTG ATCCAG-3 targeted to the *E.faecium* 16SrRNA, Forward: 5-CGTCACACCACGAGAGTTTGTAA3, Reverse: 5'ACCTTTCGACGG CTAGCTCC-3 targeted to the *Staphylococcus aureus* 16SrRNA and Forward:

5’GACGGGTGAGTAATGCCTA3, Reverse: 5’CACTGGTGT TCCTTCCTATA-3 targeted to the *P. aeruginosa* 16SrRNA, which designed on previously published studies (Vandecasteele *et al*., 2001, Dumani *et al.*, 2012, Galloway-Pen˜a *et al.*, 2012, Zhang *et al.*, 2000 and Hassan *et al*., 2012 respectively). PCR reaction used to assess the occurrence of all target bacteria was performed as mentioned above for each specific primer. the amplification program of *Staphylococcus epidermidis, Staphylococcus aureus and P. aeruginosa* were run as follow: initial denaturation One pre cycle of 95°C for 2 minutes, 30 cycles of denaturation at 92°C for 1 minute, annealing for 1 minute at 55°C for *Staphylococcus epidermidis* and *P. aeruginosa* and at 60°C for *Staphylococcus aureus* and extension at 72°C for 1 minute and One final extension cycle at 72°C for 10 minutes. The temperature profile for *E. faecalis* and *E. faecium* included an initial denaturation step of 95°C for 15 min and followed by 35 cycles of a denaturation step at 94°C for 30 s and primer annealing step for 1 min at 60°C for *E. faecalis* and at 53°C for *E. faecium* an extension step at 72°C for 1 min, and a final step at 72°C for 2 min, for negative control ultra pure water was used. PCR products were analyzed by 1.5% agarose gel (Agarose, Vivantis Technologies, Sdn. Bhd, Malaysia), electrophoresis performed at 120 V in Tris-Borate EDTA buffer. The gels were stained with 1.5 µl of ethidium bromide and visualized under

ultraviolet light, and photographed with the (BioRad Gel doc 2000, USA) Imaging System. 50-bp and 100-bp DNA ladder digest (Vivantis Technologies, Malaysia) were used as a molecular weight marker.

3.7 Statistical Analysis

The data collected were statistically analyzed using SPSS (ver. 19.0; SPSS). Catagorical variables between the groups were analyzed using the Chisquare test and two-tailed Mann-Whitney U test. Results are presented as *n* (%). *P* values <0.05 was considered significant.

Table (1): Primers used in this study

Gene	Primer	Amplicon size (bp)	Reference
16S rRNA of *Staphylococcus epidermidis*	Forward: 5-TACATGCAAGTCGAGCGAAC-3 Reverse: 5-AATCATTTGTCCCACCTTCG-3	1443	Vandecasteele *et al.*(2001)
16S rRNA of E. faecium		1569	Pena *et al.* (2012)
16S rRNA of *E.faecalis*	Forward: 5-ATCGCAAGATTGTTCGAAC-3 Reverse: 5-CTTAGAAAGGAGGTGATCCAG-3	310	Dumani *et al.*(2012)
16S rRNA of *P. aeruginosa*		956	Hassan *et al* .(2012)
16S rRNA of *Staphylococcus aureus*	Forward: 5’-GTT TAT GCC GCATGG CAT AAG AG-3’ Reverse: : 5’-CCG TCA GGG GACGTT CAG-3’	70	Zhang *et al.* (2000)
Class 1 intI	Forward: 5-GACGGGTGAGTAATGCCTA-3 Reverse: 5-CACTGGTGTTCCTTCCTATA-	280	Zhao *et al.* (2011)
		233	Mobarak-Qamsari *et al.* (2013)

Class 2 intI	3	
	Forward: 5-CGTCACACCACGAGAGTTTGTAA-3 Reverse: 5-ACCTTTCGACGGCTAGCTCC-3	
	Forward: 5-CCTCCCGCACGATGATC-3 Reverse: 5-TCCACGCATCGTCAGGC-3	
	Forward: 5-TTATTGCTGGGATTAGGC-3 Reverse: 5-ACGGCTACCCTCTGTTATC-3	

CHAPTER FOUR

4. RESULTS

4.1. Status of Patients in Dialysis

During the study period, 201 cases with suspected hemodialysis (HD) catheter-related blood streaminfections (HD CRBSIs) were identified. The patients were divided into 115 (57.2%) males and 86 (42.8) females (Table 2). The patients attended different dialysis centers in Khartoum State, 85 (42.3%) were in Khartoum north, 69 (34.3%) were in Khartoum, and 47 (23.4%) were in Omdurman (Table 2).

The patients were ranged in age between 10 and 90 years. Most patients ages were between 51-70 years ie 72(35.8%) patients (Table 3).

The results revealed that out of 201 patients, 62 (30.8%) patients gave positive bacterial growth on blood cultures 14 (7.0%) though they were under antibiotic treatment and 48 (23.9%) did not take antibiotics, while 139 patients were negative for growth in blood culture and 31(15.4%) were under antibiotic treatment, while 108 (53.7%) were not taking antibiotics (Table 4).

Of the 201 patients, 147(73.1%) received hemodialysis through a permanent catheter and 54(26.9%) received hemodialysis through a temporary catheter. Permanent and temporary catheters showed 40 (19.9%) and 22 (10.9) bacterial growth respectively (Table 5).

The majority of the infections 107 (53.2%) occurred in the first 6 months insertion period of the catheter (Table 6).

4.2. Phenotypic results

Out of 201 cases, 62 cases gave positive growth blood cultures. Out of them, 56 (90.3%) patients showed Gram-positive bacteria and 6 (9.7%) patients showed Gram-negative bacteria. *Staphylococcus epidermidis* was the most common microorganism associated with HD CRBSIs, it involved in 35 out of the 62 (56.5%) cases. Other isolated

bacteria include *Enterococcus faecalis, Enterococcus faecium*, 6 (9.7%) each, *Staphylococcus aureus* 4(6.5%), *Pseudomonas aeruginosa* 3 (4.8%), *Staphylococcus vitulinus, Staphylococcus hominis, Staphylococcus simulans, Streptococcus uberis*, *Enterobacter cloacae, Serratia marcescens,* and *Escherichia coli,* each once (1.6%) (Table 7).

4.3. Antibiotic Sensitivity

The antibiotic susceptibility results showed that only vancomycin, linezolid, tigecycline and nitrofuranation were fully efficacious against Gram-positive isolates (0%), and highly resistant to benzylpenicillin (92.9%) and oxacillin (83.9%). Resistance rate to other antibiotics were: erythromycin (51.8%), trimethoprim/Sulfamethoxazole (37.5%), tetracycline (35.7%), clindamycin (28.6%), ciprofloxacin (16.1%), gentamicin (10.7%), levofloxacin (7.2%), rifampicin (5.4%) and moxifloxacin (3.6%). Susceptibility results of Gram-negative isolates showed fully resistance (100%) to ampicillin, ampicillin/sulbactam, cefazolin and cefoxitin. Resistant towards piperacillin, nitrofuranation, trimethoprim/sulfamethoxazole all (50%), cefepime (33.3%) and meropenem, gentamicin, tobramycin, ciprofloxacin and levofloxacin all (16.7%) each. Were found all isolates were susceptible to amikacin, (Appendix 4) and (Appendix 5) the MICs and resistance rate of Gram-positive and Gram-negative bacterial sensitivity testing shown.

4.4 Genomic DNA Isolation

The result showed that the full amount of DNA obtained using this protocol was very efficient method for DNA extraction from isolates, since good yields of genomic DNA were obtained .

PCR amplifications for classes 1 and 2 integrase Genes showed that 54 (87.1%) of the 62 isolates harbored class 1 (Fig 1) and the class 2 intI gene was not found in this study. Classes 1 integrase Gene present in all Gram negative isolate and in 48 of 56 Gram positive islates (Table 8).

The integron harboring isolates were significantly more resistant to erythromycin (54.2%), clindamycin (31.3%), ciprofloxacin (16.7%) and rifampicin (6.3%) (Table 9). On the other hand, resistance to levofloxacin, moxifloxacin, benzylpenicillin, oxacillin, gentamicin, tetracycline and Trimethoprim/Sulfamethoxazole were significantly higher in integron negative isolates (Table 9).

The association between integron carriage and antibiotic resistance was significant for oxacillin (p = 0.002) (Table 9).

Two primer pairs were used for each species in this study, These primers targeted the variable regions in the 16S rRNA gene. PCR assays results (Table 10), employing this primer pair produced DNA products of the predicted size of 34 of 35 *Staphylococcus epidermidis* (1443 bp) (Fig 2), also 5 of 6 *E. faecium* (1569bp) (Fig 3), and all *E. faecalis* (310 bp) (Fig 4), *P. aeruginosa* (956bp) (Fig 4) and *Staphylococcus aureus* (70

bp) (Fig 5). 16S rRNA gene sequence offered a useful method for the identification of bacteria.

Table (2): Distribution of patients in dialysis centers according to state

State	Male (%)	Female (%)	Total (%)
Khartoum	42 (20.9)	27 (13,4)	69 (34.3)
Khartoum north	45 (22.4)	40 (19.9)	85 (42.3)
Omdurman	28 (13.9)	19 (9.5)	47 (23.4)
Total	115 (57.2)	86 (42.8)	201 (100)

Table (3): Prevalence of pathogens according to patient's age group

Age groups/years	Male (%)	Female (%)	Total (%)
10- 30	41 (20.4)	18 (9.0)	59 (29.4)
31- 50	25 (12.4)	33 (16.4)	58 (28.9)
51- 70	41 (20.4)	31 (15.4)	72 (35.8)
71- 90	8 (4.0)	4 (2.0)	12 (6.0)
Total	115 (57.2)	86 (42.8)	201 (100)

Table (4): Association between patients antibiotic status and growth pattern

Antibiotic status	Growth (%)	No growth (%)	Total (%)	Level of significance
Patients take antibiotic	14 (7.0)	31 (15.4)	45 (22.4)	.965 non-significant
Patients not take antibiotic	48 (23.9)	108 (53.7)	156 (77.6)	
Total	62 (30.8)	139 (69.2)	201	

(100)

Table (5) : Association between type of catheter and growth pattern

Type of catheter	Growth (%)	No growth (%)	Total (%)	Level of significance
Permanent catheter	40 (19.9)	107 (53.2)	147 (73.1)	.o66 non-significant
Temporary catheter	22 (10.9)	32 (15.9)	54 (26.9)	
Total	62 (30.8)	139 (69.2)	201 (100)	

Table (6): Association between period of catheter and growth pattern

Days of catheter/month	Growth (%)	No growth (%)	Total (%)	Level of significance
< 1 Month	6 (3.0)	11 (5.5)	17 (8.5)	0.887 non-significant
1- 6 Month	30 (14.9)	77 (38.3)	107 (53.2)	
7- 12 Month	18 (9.0)	38 (18.9)	56 (27.9)	
13- 18 Month	4 (2.0)	7 (3.5)	11 (5.5)	
>19 Month	4 (2.0)	6 (3.0)	10 (5.0)	
Total	62 (30.8)	139 (69.2)	201 (100)	

Table (7): Bacterial isolates from 62 blood cultures.

Gram-positive organisms	Prevalence (%)	Gram-negative organisms	Prevalence (%)

Staphylococcus epidermidis	35	(56.5)	*Pseudomonas aeruginosa*	3	(4.8)
Enterococcus faecalis	6	(9.7)	*Enterobacter cloacae*	1	(1.6)
Enterococcus faecium	6	(9.7)	*Serratia marcescens*	1	(1.6)
Staphylococcus aureus	4	(6.5)	*Escherichia coli*	1	(1.6)
Staphylococcus vitulinus	1	(1.6)			
Staphylococcus hominis	1	(1.6)			
Staphylococcus simulans	1	(1.6)			
Streptococcus uberis	1	(1.6)			
Total	56	(90.3)		6	(9.7)

Table (8): The presence of integrons in 62 bacterial isolates

Type of organisms	N0	Integron positive isolates	Integron negative isolates
Gram-positive organisms	56	48 (85.7%)	8 (14.3%)
Gram-negative organisms	6	6 (100%)	0
Total	62	54 (87.1%)	8 (12.9%)

Table (9): Association between antimicrobial susceptibility and integron carriage in 56 Gram positive clinical isolates

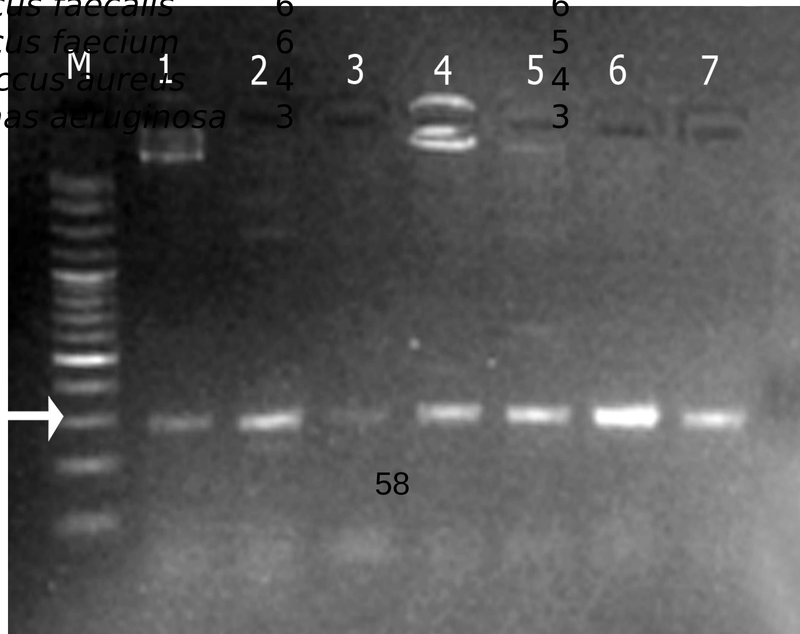
Antibiotic	Integron positive isolates (n= 48)		Integron negative isolates (n=8)		Level of significance*
	% R	% S	% R	% S	

P	91.7	8.3	100	0	NS
OX	81.3	18.7	100	0	**
GM	10.4	89.6	12.5	87.5	NS
CIP	16.7	83.3	12.5	87.5	NS
LEV	6.3	93.7	12.5	87.5	NS
MXF	2.1	97.9	12.5	87.5	NS
E	54.2	45.8	37.5	62.5	NS
CM	31.3	68.7	12.5	87.5	NS
LNZ	0	100	0	100	NS
VA	0	100	0	100	NS
TE	35.4	64.6	37.5	62.5	NS
TGC	0	100	0	100	NS
FT	0	100	0	100	NS
RA	6.3	93.7	0	100	NS
SXT	35.4	64.6	50	50	NS

The significance level was determined using the two-tailed Mann-Whitney U test. **, 1%, NS, non-significant; R, resistant; S, sensitive. P Benzylpenicillin OX Oxacillin GM Gentamicin CIP Ciprofloxacin LEV Levofloxacin MXF Moxifloxacin E Erythromycin CM Clindamycin LNZ Linozolid VA Vancomycin TE Tetracycline TGC Tigecycline FT Nitrofuranation RA Rifampicin SXT Trimethoprim/Sulfamethoxazole

Table (10): PCR assay with primers targeted to the 16S rRNA gene for isolates

Isolates	**Total**	**16srRNA gene**
Staphylococcus epidermidis	35	34
Enterococcus faecalis	6	6
Enterococcus faecium	6	5
Staphylococcus aureus	4	4
Pseudomonas aeruginosa	3	3



280 bp

Fig 1: PCR amplification product of class 1 integrone integrase genes in 54 of 62 clinical isolates. (1-7) 280 bp: positive isolates, M: Ladder of DNA molecular weight marker (100 bp,) .

1443 bp

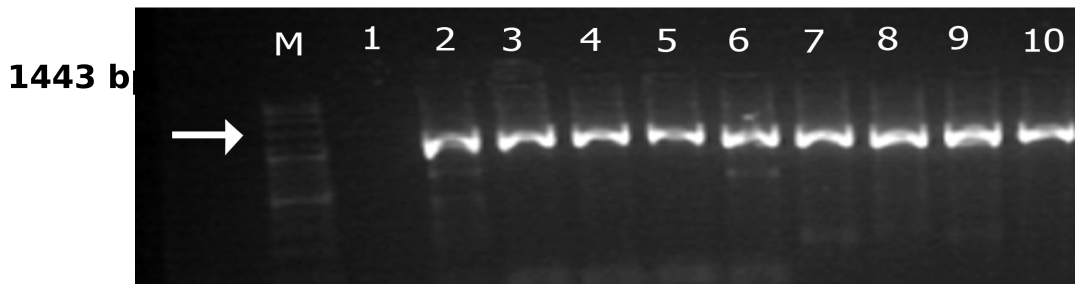


Fig 2: PCR amplification product of 16S rDNA which was specific for *Staphylococcus epidermidis* were identified in 34 of 35 of isolates in 1.5% agarose gel electrophoresis. (2-10) 1443 bp: *Staphylococcus epidermidis* isolates, M: (100 bp) DNA ladder,1:negative control.

1569 bp

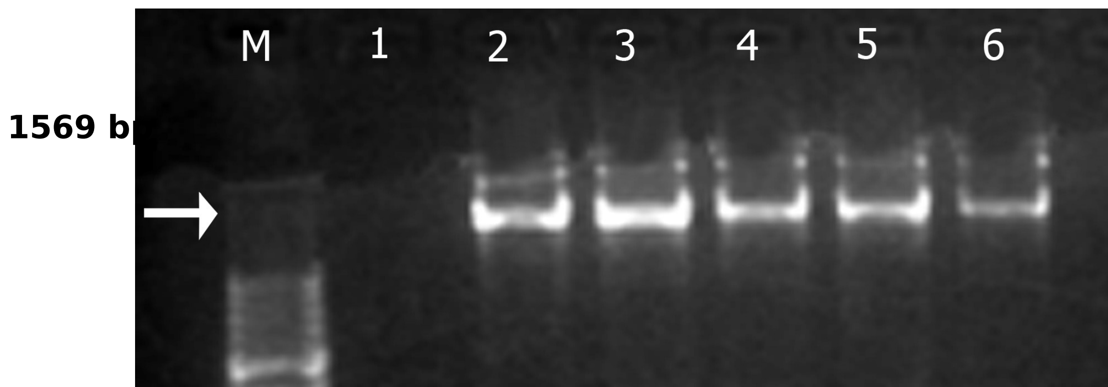


Fig 3: PCR amplification product of 16S rDNA which was specific for *E. faecium* were identified in 5 of 6 of *E. faecium* isolates in 1.5% agarose gel electrophoresis. M: (100 bp)

DNA ladder. (2-6) 1569 pb: *E. faecium* isolates, 1: isolate negative.

956 bp
310 bp

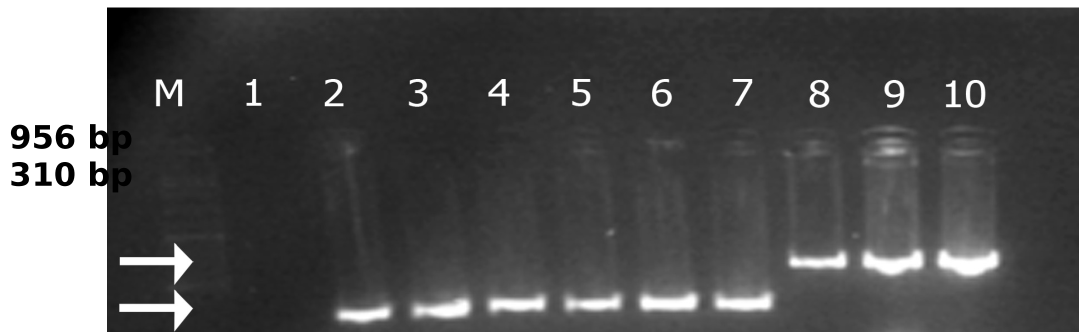


Fig 4: PCR amplification product of 16S rDNA which was specific for *E. faecalis and P. aeruginosa* were identified in all *E. faecalis* and *P. aeruginosa* isolates, lane (2-7) 310 bp: *E. faecalis* . and lane 8,9,10 (956 bp): *P. aeruginosa,* M: (100 pb) DNA ladder.1: negative control in 1.5% agarose gel electrophoresis.

70 bp

Fig 5: PCR amplification product of 16S rDNA which was specific for *Staphylococcus aureus* were identified in all *Staphylococcus aureus* isolates, in 1.5% agarose gel electrophoresis, (1-4) 70 bp: *Staphylococcus aureus* isolates, M: (100 bp) DNA ladder.

CHAPTER FIVE

5. DISCUSSION

This study confirms that bacteremia have frequent occurrence in the chronic HD patients with long-term, permanent and temporary venous catheter access. In this study most infections (73.1%) occur in permenent catheters.

This result is similar to the result shown by Taylor *et al*. (2004) who found that most infections (57%) were for permenent catheters. Our rate of median catheter duration of use for 1-6 months was (53.5%) and this is within the range obtained by Abdul Gafor *et al.* (2014) who reported that the median catheter duration was three months.

This results showed that the average patient age was from 51-70 years. This result is in line with the results obtained by Abdul Gafor *et al.* (2014) who reported that the average patient age was 61 years.

This study diagnosed CAB based on blood cultures drawn directly from the PC port or from the dialysis blood tubing coming from the PC. This technique is similar to that of Saad. (1999). Results also reported a predominance of Gram-positive organisms (90.3%) in contrasts with Saad (1999). The wide variety of both Gram-positive and Gram-negative infections seen, is similar to the spectrum of organisms reported by Landry *et al*. (2010) who found that a predominance of Gram-positive organisms (87.7%). Also this study is reported a predominance of Gram-positive coagulase-negative *staphylococci* (59.7%). This result substantiated the result reported by Taylor *et al*. (2004) who found the most microbial etiology of CAB in hemodialysis patients was coagulase-negative *staphylococci* (45%).

In this study, both VITEK 2 compact system and PCR assay with primers targeted to the 16S rRNA gene identification to

the species level, reliably distinguished one species from each other. With VITEK 2 compact system it could identify 100% of *P. aeruginosa, Staphylococcus aureus and Enterococcus faecalis* but 97.2% and 85.7% of *Staphylococcus epidermidis* and *Enterococcus faecium* respectively, compared to 16S rRNA based PCR which was used as a reference method in this study.

Foundation Dialysis Outcomes Quality Initiative recommend initial coverage for CAB with antibiotics effective against *Staphylococcus* and *Streptococcus* organisms, but did not include a recommendation for treatment to include Gram-negative rods or *Enterococcus* organisms. Because the result showed that incidence of Gram-negative infections, including *Pseudomonas aeruginosa, Enterobacter cloacae, Serratia marcescens* and *Escherichia coli* and *Enterococcus spp.* This result is similar to the result obtained by Saad. (1999).

Antibiogram is a list of laboratory testing for the sensitivity of an isolated bacterial strain to different antibiotics. In an era of bacterial resistance, a careful and correct selection of antibiotics is important to increase the chance of successful treatment and to reduce the rate of bacterial resistance. Prior to this study, the empirical antibiotics for HD CRBSIs in our centres were intravenous vancomycin and gentamicin. Based on this study, we noted that all Gram-negative bacteria were sensitive to amikacin. Unfortunately,

gentamicin resistance was noted among Gram-negative organisms. This study also realized that coagulase-negative *Staphylococcus* were the most common Gram-positive organisms and they were sensitive to vancomycin. Thus, following this study results, empirical antibiotics for HD CRBSIs in the Sudanese centres might switch to intravenous vancomycin and amikacin.

Bacterial resistance has become a worldwide problem as a result of the abuse of antibiotic drugs and the transfer of resistance genes between bacteria. The present result revealed that the class 1 intI gene was detected in 87.1% (54/62) of isolates and class 2 intI gene was not found in this study. This result is in agreement to the result obtained by Zhao *et al*. (2011).

Researchers thought that the occurrence rate of integrons in Gram-negative bacteria was higher than in Gram-positive bacteria (Zhao *et al*. 2011). This results are simulate to their results in which the occurrence rate of integrons in Gram-negative bacteria was 100%, while the occurrence rate of integrons in Gram-positive bacteria was 85.7%.

The present study found a significant association between integron carriage and higher rates of resistance to oxacillin. This result is similar to the result obtained by Zhao *et al*. (2011) who reported that Class 1 integrons including genes that encode aminoglycoside resistance and β-lactam resistance.

In this study, there was an inverse association between resistance to levofloxacin, moxifloxacin, benzylpenicillin, oxacillin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole with integron carriage, where integron negative isolates were more resistant to these antibiotics.

While this is so, more research to investigate antimicrobial susceptibility of bacterial pathogens isolated from dialysis patients with more bacterial isolates and their susceptibility to various antibiotics as well as their integrons and the resistance to specific antibiotics should be warranted.

CONCLUSION

The study concluded that:

1. Coagulase-negative *Staphylococcus* was the most common microorganism associated with HD CRBSIs, they were sensitive to vancomycin, and most their infections (73.1%) occured in permanent catheters.
2. Gram-positive isolates were highly resistant to benzylpenicillin and oxacillin while Gram-negative isolates showed fully resistance to ampicillin, ampicillin/sulbactam, cefazolin and cefoxitin and all isolates were susceptible to amikacin.
3. PCR assay with primers targeted to the 16S rRNA gene identification to the species level, reliably distinguished one species from each other.

4. Class 1 intI gene was detected in 87.1% (54/62) of isolates with occurrence rate 100% in Gram-negative bacteria and 85.7% in Gram-positive bacteria, while class 2 intI gene was not found in this study.

RECOMMENDATIONS

1. Following this study results, we recommended empirical antibiotics for HD CRBSIs in Sudanese centres must include Gram-negative rods and might switch to intravenous vancomycin and amikacin.
2. It is strongly recommended to establish effective protocol for catheter management decisions in Sudan.
3. Further in depth studies including large sample size and other location are recommended.

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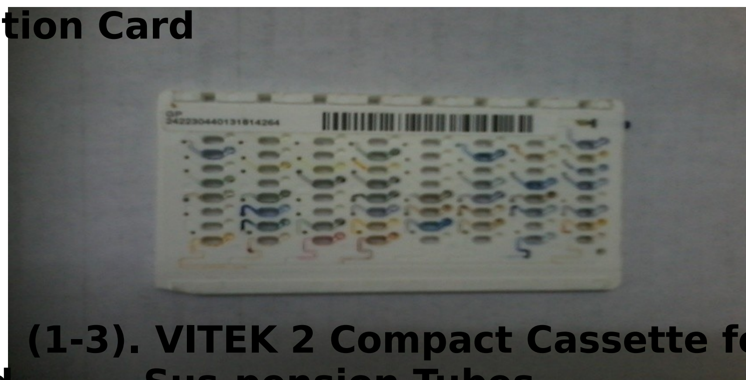
APPENDIX (1)

VITEK 2 Compact System

Appendix (1-1). VITEK 2 Compact System Instrument

Appendix (1-2). VITEK 2 Compact System Identification Card



Appendix (1-3). VITEK 2 Compact Cassette for 10 Cards and Sus-pension Tubes.

Appendix (1-4). VITEK 2 Compact Densichek

Appendix (1-5). VITEK 2 Compact Vortex

APPENDIX (2)

Polymerase Chain Reaction Instruments

Appendix (2-1). Polymerase Chain Reaction Thermal Cycler

Appendix (2-2). Polymerase Chain Reaction Electrophoresis

Appendix (2-3). Polymerase Chain Reaction Gel Documentation System

APPENDIX (3)

Questionnaire

Date:………………
Hospital: ………………………………… Sample type: …………………
Patients name: …………………………Sample No: ………………………
Age……………
Gender: ⌅ male ⌅ female
Residence:
State:………………………… Locality: ………………………………
City/ Village: ………………………………
Has the patient been treated with antibiotic during the last 10 days?

⌅ Yes ⌅ NO

If yes: Specify: …………… Days.

Type of antibiotic…………………………………

Clinical presentation: fever ⌅ …C ⌅ chills ⌅ weakness

Type of vascular access:………………………………………………………

No of catheter-days: ………………………………………………………….

Complication:……………………………………………………………

Outcome:………………………………………………………………

Appendix (4): MICs of the 56 Gram positive isolates

Strain no.*	MIC (μg/ ml)												
	P	OX	GM	CIP	LEV	MXF	E	CM	LNZ	VA	TE	TGC	
	FT	RA	SXT										
1	0.5	4	8	0.5	0.12	1	8	0.25	1	1	1	0.12	16
2	0.5	80											
3	0.5	0.25	0.5	0.5	0.12	0 .25	0 .25	0.25	2	1	16	0.12	16
4	0.5	10											
5	0.5	4	0.5	0.5	0.12	0 .25	0 .25	0.25	1	1	1	0.12	16
6	0.5	10											
7	0.25	0.25	0.5	0.5	0.5	0 .25	0.5	0.25	2	0.5	1	0.12	16
8	0.5	10											
9	2	0.25	0.5	8	8	1	8	8	2	1	16	0.12	128
10	0.5	80											
11	0.5	4	0.5	4	0.12	1	8	0.25	2	1	2	0.12	16
12	0.5	10											
13	0.5	4	8	0.5	0.12	1	8	0.25	1	2	16	0 .25	16
14	32	320											
15	0.5	4	0.5	0.5	0.12	0 .25	0 .25	0.25	1	1	1	0.12	16
16	0.5	20											
17	0.5	0.25	4	8	4	2	0 .25	0.25	1	1	1	0.12	16
18	32	80											
19	2	4	0.5	1	1	0.5	0.5	8	2	1	16	0.12	16
20	0.5	10											
21	32	4	8	8	8	8	0 .25	0.25	2	0.5	16	0.12	16
22	0.5	80											
23	2	4	0.5	1	0.5	0.25	0 .25	8	2	1	1	0.12	16
24	0.5	10											
25	0.5	4	16	4	4	1	8	0.25	1	1	1	0.12	16
26	0.5	160											
27	0.5	4	16	8	4	1	0.25	8	1	0.5	1	0.12	16
28	0.5	20											
29	0.5	0.5	1	8	8	2	8	0.25	2	0.5	1	0.12	16
30	0.5	80											
31	0.5	0.25	0.5	0.5	0.25	0.25	0.25	0.25	2	1	16	0.12	16
32	0.5	10											
33	0.5	4	16	1	0.5	0.25	8	8	1	2	16	0.5	16
34	0.5	20											
35	0.25	4	0.5	4	4	1	0.25	0.25	1	2	2	0.25	16
36	0.5	10											
37	0.5	4	0.5	0.5	0.25	0.25	0.25	0.25	2	1	16	0.12	16
38	0.5	10											
39	0.5	4	0.5	0.5	0.5	0.25	8	0.25	1	1	1	0.12	16
40	0.5	10											
41	4	4	0.5	0.5	1	0.25	0.5	8	2	1	16	0.12	16
42	0.5	10											
43	0.5	4	0.5	0.5	0.5	0.25	8	0.25	1	1	1	0.12	16

44 0.5 80
45 0.5 4 16 0.5 0.12 0.25 8 0.25 1 1 16 0.5 16
46 0.5 160
47 0.5 4 0.5 0.5 0.5 0.25 0.25 0.25 2 0.5 16 0.12 16
48 0.5 320
49 0.12 4 4 0.5 0.12 0.25 8 0.25 1 2 2 0.12 16
50 0.5 10
51 0.5 4 0.5 0.5 0.12 0.25 0.25 0.25 1 1 16 0.12 16
52 0.5 10
53 2 4 0.5 1 1 0.25 0. 25 8 2 1 1 0.12 16
54 0.5 10
55 4 4 0.5 8 4 2 8 8 1 2 16 0.12 16
56 0.5 10
1 4 0.5 1 2 0.5 0.25 8 2 0.5 1 0.12 32
BP 0.5 10 0.5 4 0.5 0.5 0.12 0.25 8 0.25 1
2 1 0.12 16 0.5 10
RS 1 4 0.5 1 2 1 0.25 0.25 2 0.5 1 0.12 16
0.5 10
% 0.5 4 0.5 0.5 0.12 0.25 8 0.25 1 1 16 0.12 16
0.5 160
0.5 4 16 1 4 1 0.25 0.25 1 2 16 0.5 16
0.5 80
0.5 4 0.5 8 8 2 8 0.25 1 2 2 0.25 16
0.5 10
0.5 4 0.5 0.5 0.12 0.25 0.25 0.25 1 1 1 0.12 16
0.5 160
0.5 4 16 0.5 0.12 0.25 8 0.25 1 1 1 0.12 16
0.5 160
1 4 0.5 0.5 2 0.25 0.25 8 2 0.5 1 0.12 64
0.5 10
0.5 4 0.5 8 4 2 8 0.25 1 2 16 0.5 16
0.5 10
0.5 4 0.5 4 4 1 8 0.25 1 2 16 0.5 16
0.5 10
0.5 4 8 1 0.5 0.25 8 0.25 1 1 2 0.12 16
0.5 10
0.5 4 0.5 4 4 8 8 0.25 1 2 2 0.12 16
0.5 160 0.25 0.25 0.5 0.5 0.12 0.25 0.25 0.25 1
1 1 0.12 16 0.5 160 0.5 0.25 0.5 0.5 0.12 0.25
8 0.25 1 0.5 16 0.12 16 0.5 10
0.5 4 0.5 0.5 0.12 0.25 0.25 0.25 1 2 1 0.12 16
0.5 10
0.5 4 0.5 0.5 0.12 0.25 0.25 0.25 1 2 1 0.12 16
0.5 80
0.5 4 0.5 4 4 1 8 0.25 1 1 1 0.12 16
0.5 10
0.5 4 0.5 1 0.5 0.25 8 0.25 1 1 2 0.12 16
32 160
0.5 0.25 0.5 0.5 0.12 0.25 8 8 1 1 1 0.12 16
0.5 10
0.5 4 0.5 0.5 0.12 0.25 8 8 1 2 1 0.12 16
0.5 10
0.5 4 0.5 0.5 0.12 0.25 8 8 1 2 1 0.12 16
0.5 10
0.5 4 0.5 0.5 0.12 0.25 8 8 2 1 16 0.12 16
0.5 10
0.5 4 0.5 0.5 0.12 0.25 8 0.25 1 1 1 0.12 16
0.5 160
0.5 4 0.5 0.5 0.12 0.25 0.25 0.25 1 1 1 0.12 16
0.5 160
0.5 4 0.5 0.5 0.12 0.25 0.25 0.25 1 1 16 0.25 16
0.5 10
0.5 4 0.5 8 0.12 0.25 8 8 1 2 1 0.12 16
0.5 160
0.5 4 0.5 0.5 0.12 0.25 0.25 8 1 2 1 0.12 16
0.5 10

≥0.5 ≥4 ≥ 16 ≥ 8 ≥ 8 ≥ 8 ≥ 8 ≥ 8 ≥ 8 ≥ 32 ≥ 16 ≥ 2 ≥ 512
≥ 32 ≥ 32

52/56	47/56	6/56	9/56	4/56	2/56	29/56	16/56	0/56	0/56	20/56	0/56	0/56	3/56	21/56
92.9	83.9	10.7	16.1	7.2	3.6	51.8	28.6	0	0	35.7	0	0	5.4	37.5

P Benzylpenicillin OX Oxacillin GM Gentamicin CIP Ciprofloxacin
LEV Levofloxacin MXF Moxifloxacin E Erythromycin CM Clindamycin
LNZLinozolid VA Vancomycin TE Tetracycline TGC Tigecycline
FT Nitrofuranation RA Rifampicin SXT Trimethoprim/Sulfamethoxazole

Appendix (5): MICs of the 6 Gram negative isolates

Strain no.*	MIC (μg/ ml)															
	AM	SAM	PIP	CZ	FOX	CAZ	CRO	FEP	MEM	AN	GM	TM	CIP	LEV	FT	SXT
1	32	32	4	64	64	2	64	1	0.25	2	1	1	0.25	0.25	512	320
2	32	32	128	64	64	2	64	1	0.25	2	16	16	4	8	16	320
3	32	32	4	64	64	1	1	1	0.25	2	1	1	0.25	0.12	16	20
4	32	32	4	64	64	64	6	64	16	2	1	1	0.25	0.25	512	20
5	32	32	128	64	64	2	1	64	0.25	2	1	1	0.25	0.25	512	20
6	32	32	128	64	64	2	64	1	0.25	2	1	1	0.25	0.25	16	320
BP	≥32	≥32	≥128	≥64	≥64	≥64	≥64	≥64	≥16	≥64	≥16	≥16	≥4	≥8	≥512	≥320
RS	6/6	6/6	3/6	6/6	6/6	1/6	3/6	2/6	1/6	0/6	1/6	1/6	1/6	1/6	3/6	3/6
%	100	100	50	100	100	16.7	50	33.3	16.7	0	16.7	16.7	16.7	16.7	50	50

AM Ampicillin SAM Ampicillin/Sulbactam PIP Piperacillin CZ Cefazolin FOX Cefoxitin CAZ Ceftazidime
CRO Ceftriaxone FEP Cefepime MEM Meropenem AN Amikacin
GM Gentamicin TM Tobramycin CIP Ciprofloxacin LEV Levofloxacin FT Nitrofuranation SXT Trimethoprim/Sulfamethoxazole