

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Haemodialysis (HD) acts wonders by improving the quality of life in patient of end stage renal disease. HD machine removes wastes from the blood stream and regulates the body's fluids and chemical balances (Gupta *et al.*, 2011).

Central venous catheters (CVCs) are commonly used for performance of HD. The ready availability of the CVCs as a vascular access (VA) for HD often makes them the access of choice, especially when urgent or emergent HD is required either at the time of initiation of renal replacement therapy or when a permanent access becomes dysfunctional (Haddad *et al.*, 2012).

The use of temporary haemodialysis catheters is often complicated by infectious or mechanical complications which are responsible for considerable morbidity and mortality in hemodialysis patients (Gupta *et al.*, 2011).

Catheter associated bacteremia is the most serious complication in hemodialysis patients and may be caused by both Gram-positive and Gram-negative bacteria and often results in serious systemic infections, including endocarditis, osteomyelitis, epidural abscess, septic arthritis, and even death (Krishnasami *et al.*, 2002). The incidence of catheter related blood stream infections (CR-BSI) varies considerably with the type of catheter, frequency of catheter manipulation, and patient-related factors such as underlying disease and acuity of illness. Peripheral venous catheters are the most frequently used venous access devices. While the incidence of local or bloodstream infections associated with

peripheral venous catheters is usually low, serious infectious complications are recognized by clinicians because of the large numbers of such catheters that are placed. Most serious catheter-related infections, however, are associated with central venous catheters (CVCs), especially those that are placed in patients in the ICU (Grady *et al.*, 2001).

The pathogenesis of CR-BSI can be attributed to two primary causes: bacterial colonization of the device and contamination of the fluid being administered (Abad & Safdar, 2011). Migration of skin organisms at the insertion site into the cutaneous catheter tract with colonization of the catheter tip is the most common route of infection for peripherally inserted, short-term catheters (Grady *et al.*, 2001).

Staphylococcus aureus (*S. aureus*) is a formidable pathogen that has the ability to colonize approximately half the dialysis population without any sign of disease but is also capable of causing wound and tissue infections; fulminant septicemia; and chronic difficult-to-eradicate and often foreign body-related infections. *S. aureus* is the main cause of infectious morbidity and mortality in haemodialysis patients (Vandecasteele *et al.*, 2009). The emergence of methicillin-resistant strains with high virulence potential in both hospital and community settings is contributing to a current public health crisis (Park *et al.*, 2008). The annual incidence of *S. aureus* bacteremia in patients on hemodialysis ranges from 6 to 27%. Complications of *S. aureus* bacteremia include meningitis, endocarditis, osteomyelitis, and metastatic abscesses. Patients with ESRD who acquire *S. aureus* bacteremia are at greater risk of death than patients in whom bacteremia is attributable to other organisms (Li *et al.*, 2009).

1.2. Rationale

The end stage renal disease patients treated with maintenance HD are at high risk of infection, because HD process requires frequent and prolonged vascular access. The success of maintenance HD process is measured by the survival of patients and their quality of life. Several thousand patients receive dialysis concurrently in Khartoum State. The patients received HD in units including Dr. Selma Center for Kidney Disease, Khartoum Teaching Hospital, Omdurman Teaching Hospital and Kidney Transplanting Association Hospital Dialysis Unit and others. The four major HD units have 97 HD machines and provide HD therapy to 781 patients with ESRD (Abdelwahab *et al*, 2013). This would increase the risk of transmitting the strains of infectious nosocomial opportunistic pathogens especially species of *Staphylococcus*, both coagulase negative and positive.

Furthermore, HD patients are immunosuppressed, which increases their susceptibility to infection, and they require frequent hospitalizations. Also infectious organisms can be transmitted from the dialysis centers through patients and healthcare workers to family members and into the community, which increase risk of developing infection by those infectious organisms.

1.3. Objectives

1.3.1. General objective

To isolate and identify the species of *Staphylococcus* from renal failure patients on haemodialysis.

1.3.2. Specific objectives

- a) To isolate *Staphylococcus* species from haemodialysis patients.
- b) To identify the common species of *Staphylococcus* isolated from haemodialysis patients.
- c) To calculate the percentage of the most common *Staphylococcus* species isolated.

CHAPTER TWO

LITERATURE REVIEW

2.1. The genus *Staphylococcus*

2.1.1. History

The name *Staphylococcus* (staphyle, bunch of grapes) was introduced by Ogston in 1883 for the group micrococci causing inflammation and suppuration. He was the first to differentiate two kinds of pyogenic cocci: one arranged in groups or masses was called “*Staphylococcus*” and another arranged in chains was named “Billroth’s *Streptococcus*.” A formal description of the genus *Staphylococcus* was provided by Rosenbach in 1884. He divided the genus into the two species *Staphylococcus aureus* (*S. aureus*) and *S. albus*. Zopf in 1885 placed the mass-forming staphylococci and tetrad-forming micrococci in the genus *Micrococcus*. In 1886, the genus *Staphylococcus* was separated from *Micrococcus* by Flügge in 1886 (Gotz *et al.*, 2006).

2.1.2. Definition

Members of *Staphylococcus* are Gram positive bacteria, with diameters of 0.5 – 1.5µm and characterized by individual cocci, which is divided in more than one plane to form grape-like clusters. To date, there are more than 30 species and eight sub-species in the genus *Staphylococcus*, many of which preferentially colonize the human body. However *S. aureus* and *S. epidermidis* are the two most characterized and studied strains (Harris *et al.*, 2002).

2.1.3. Description

The *Staphylococcus* species are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Most species have a relative complex nutritional requirement, however, in general they require an organic source of nitrogen, supplied by 5 to 12 essential amino acids, e.g. arginine, valine, and B vitamins, including thiamine and nicotinamide. Members of this genus are catalase positive and oxidase negative, distinguishing them from the genus *Streptococcus*, which are catalase- negative, and have a different cell wall composition to *Staphylococcus* (Harris *et al.*, 2002).

Staphylococci are tolerant to high concentrations of salt and show resistance to heat. Pathogenic *Staphylococcus* species are commonly identified by their ability to produce coagulase, and thus clot blood. This distinguishes the coagulase positive strains, *S. aureus* (a human pathogen), and *S. intermedius* and *S. hyicus* (two animal pathogens), from the other *Staphylococcus* species such as *S. epidermidis*, that are coagulase-negative (CoNS) (Harris *et al.*, 2002).

2.1.4. Normal habitat

The natural habitat of *Staphylococcus aureus* in humans is the moist squamous epithelium of the anterior nares. Several bacterial surface proteins are implicated in promoting adhesion to desquamated epithelial cells. Clumping factor B (ClfB) and iron-regulated surface determinant A both promote nasal colonization in rodent models, and in the case of ClfB, humans (foster, 2009).

Coagulase negative Staphylococci (CoNS) are normal inhabitants of human skin and mucous membranes (Piette & Verschraegen, 2009).

2.1.5. Mode of transmission

Staphylococcus bacteria can spread from one person to another through casual contact or contaminated objects and become a problem when they are a source for infection (Berg, 2007).

2.1.6. Pathogenecity

S. aureus causes a variety of suppurative infections and toxin in humans. It causes superficial skin lesions such as boils, styes and furuncles; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices (Abu-Rabei, 2009).

2.2. Haemodialysis

Chronic kidney disease (CKD) is a common public health problem, which occurs in many countries with an increasing prevalence. Over 50 million people throughout the world are known to have CKD, and of these, more than 1 million require renal replacement therapies such as dialysis and renal transplantation. In recent years, the rising incidence of diabetes and hypertension, the most common two causes of CKD, cause an increase in the prevalence of CKD (Özkan & Ulusoy, 2011).

Haemodialysis which is one of the renal replacement therapies, is a life-saving treatment. In the absence of this therapy, more than a million patients worldwide would have died within weeks. Haemodialysis was successfully performed for the first time in 1944 by

Willem Kollf in patients with renal failure. However, haemodialysis is accompanied by several complications (Özkan & Ulusoy, 2011).

The number of patients with end-stage renal disease treated by maintenance haemodialysis in the United States has increased sharply during the past 30 years. In 1999, more than 3,000 haemodialysis centers had >190,000 chronic haemodialysis patients and >60,000 staff members (CDC, 2001).

In June 2009, there were 2858 patients with end stage renal disease treated with maintenance HD in Sudan. The overall prevalence of the treated ESRD was 106 patients per million population (Elamin *et al.*, 2010).

2.2.1. *Staphylococcus* blood stream infections in haemodialysis patients

This type of infection represents a main cause of morbidity, as well as a preventable cause of death, along with increased costs and hospitalization. Impaired immunity due to renal failure, comorbidities, malnourishment that increase the virulence and the adherence properties of hospital bacteria as well as the breakdown of the protective anatomical barriers due to repeated intravascular intervention required for haemodialysis, represent the main reasons for the high prevalence of blood stream infection in those patient (Fysaraki *et al.*, 2013).

Throughout the 1960s and 1970s, Gram negative organisms were most frequently isolated from patients with nosocomial blood stream infections. Since then, infections due to Gram-positive organisms have become increasingly frequent (Wisplinghoff *et al.*, 2004). *S. aureus* and coagulase negative *Staphylococcus* are the predominant pathogens (Lew & Kaveh, 2000).

Haemodialysis patients are immune suppressed, and this increases their susceptibility to infection. The nasal carriage of MRSA among dialysis patients is significant not only in terms of predisposing to subsequent infections, but also in playing an important role in transmission among dialysis unit staff and their family members (Abu-Rabei, 2009). Chronic haemodialysis patients are at high risk for infection because the process of haemodialysis requires vascular access for prolonged periods. In an environment where multiple patients receive dialysis concurrently, repeated opportunities exist for person-to-person transmission of infectious agents, directly or indirectly via contaminated devices, equipment and supplies, environmental surfaces, or hands of personnel (CDC, 2001).

2.2.1.1. Susceptibility to infection according to type of haemodialysis

Fistula or Graft haemodialysis: an arteriovenous (AV) fistula has the lowest infection rate at 1–4% per year. Polytetrafluoroethylene graft offers the second lowest infection rate at 10–20% per year (Lew & Kaveh, 2000).

Catheter haemodialysis: Central venous catheters with double lumens may provide temporary or permanent access. For both temporary or permanent access, tunneled cuffed catheters have lower infection rates than non tunneled, non cuffed catheters (Lew & Kaveh, 2000). Catheter related blood stream infections (CRBSIs) most of which associated with central venous catheters (CVCs) (Abad & Safdar, 2011).

Staphylococcus aureus is a frequent cause of catheter- related infections in dialysis patients. The worldwide emergence of methicillin-resistant *S. aureus* (MRSA) is of major concern, as its emergence has dramatically reduced the number of antibiotics available

for the prevention and treatment of infections in both hospitals and communities (Contreras *et al.*, 2012).

2.3. Previous studies

In a prospective cohort study performed in United State in period between 1998 – 2000, to determine the pathogenesis of central venous catheters (CVCs) related blood stream infections, revealed that, 35 (2.7%) caused CVC related BSI. Out of this 35 BSIs, 27 (77.1%) cases were caused by coagulase negative Staphylococci and the other 8 (22.9%) of cases were caused by other pathogens (Safdar & Maki, 2004).

Another study conducted in São Paulo, Brazil, to evaluate the incidence and risk factors of BSI among patients with double- lumen central venous catheter for haemodialysis, identify the microorganism isolated from blood stream. Of them 53 (41%) were *S. aureus* (Grothe *et al.*, 2010).

A group of researcher in Denmark performed a cohort study aimed to estimate the risk of blood stream infection among chronic HD patients during (1995-2010). The study subjects were 1792 HD patients. Out of them, only 461 with blood stream infection. Most common causative microorganisms isolated were *S. aureus* 43.8% and *E. coli* 12.6% (Dalgaard *et al.*, 2015).

A Prospective study in Mumbai conducted to estimate the incidence of infections associated with central venous catheter (CVC) access and incidence of secondary bacteremia in haemodialysis patients. Study subjects were hundred patients. Blood culture for catheter tip was used for processing. Catheter related bacteremia was diagnosed in 15 (15%) of patients. Gram positive bacteria account for majority of

infection (67%), *S. aureus* account for 5 (33.3%) and coagulase negative Staphylococci account also for 5 (33.3%) (Gupta *et al.*, 2011).

In study investigated incidence, risk factors, clinical features and outcome of blood stream infections (BSIs) in haemodialysis patients, conducted in Nephrology of the University Hospital of Heraklion, Crete, Greece over a 7-year period (1999 to 2005). 102 blood samples were collected from study subjects, of whom there were 148 bacteremial isolates (14 samples were showed mixed infections). Gram positive organism were responsible for 96 episodes (65%), with *S. aureus* account for (55%) the most frequent, followed by *S. epidermidis* (26%) (Fysaraki *et al.*, 2013).

A cross sectional descriptive study conducted in Hashemi-nejad of Iran university to determine the frequency of hemodialysis catheter related infection risk factors. The study subjects were 116 patients with HDCRI. The pathogenic organisms isolated from blood cultures were: *S. aureus* (42%), coagulase negative Staphylococci (20%) (Sanavi *et al.*, 2007).

In study conducted in Turkey (in Istanbul University Cerrahpasa Medical Hospital), total of 200 CoNS strains were isolated from blood stream of hospitalized patients with true bacteremia. Among those 200 isolates, *S. epidermidis* was the most prevalent species 87(43.5), followed by *S. haemolyticus* 23(11.5%), *S. hominis* 19 (9.5%), *S. lugdunensis* 18(9%), *S. capitis* 15(7.5%), *S. xylosus* 10 (5%), *S. warneri* 8 (4%), *S. saprophyticus* 5(2.5%), *S. leuntus* 5(2.5%), *S. simulans* 4(2%), *S. chromogenes* 3(1.5%), *S. cohnii* 1(0.5%), *S. schleiferi* 1(0.5%) and *S. auricularis* 1(0.5%) (Koksal *et al.*, 2009).

In prospective cohort study undertaken to estimate the incidence of nosocomial blood stream infections in US hospitals, 179 cases of nosocomial BSI in 49 US hospitals over a 7-year period were enrolled. The study was concluded that, CoNS accounted for nearly one-third of all nosocomial BSIs (31%) and considered the common causative agents, followed by *S. aureus* (20%) (Wisplinghoff *et al.*, 2004).

A retrospective survey was performed among patients with ESRD treated with haemodialysis catheters in Nephrology unit at universities Academic Hospital, Bloemfontein. From 311 study subjects, only 25% (n=79) were episodes of suspected catheter related blood stream infections, 31(39%) of culture were positive. Pathogens which were isolated reported as: 15(48.4%) staphylococci were identified, *S. aureus* account for 7(22.6%) of these isolates and remaining eight were coagulase negative staphylococci (25.8%) (Bisiwe *et al.*, 2015).

A study was carried out in Chhatrapati Shahuji Maharaj Medical University, to assess the frequency of staphylococcal intravenous devices associated infections in a pediatric ward of a tertiary care hospital. OF 1141 venous blood, *S. aureus* was the most frequent blood isolate 99 (8.7%), and coagulase negative staphylococci (CoNS) were represent 30 (2.6%). *S. haemolyticus* 17 (56.7%), *S. epidermidis* 5 (16.7%), *S. xylosus* 4 (13.3%), *S. captis* 1 (3.3%), *S. cohnii* 2 (6.7%), *S. hominis*1 (3.3%) (Jain *et al.*, 2011).

Another study conducted at Nephrology Department of the University Hospital in Lublin, Poland, at 6-year intervals. Revealed that, 16 of 28 (57.1%) patients on haemodialysis were colonized with *S. aureus* in their anterior nares. The *S. aureus* isolates were cultured from specimens obtained from patients, who underwent haemodialysis. Twenty-six

isolates of CoNS were obtained from 24 (55.8%) patients: *S. epidermidis* 21 isolates(80%), *S. lugdunensis* 2 isolates (7.7%), *S. haemolyticus* 1 isolate (3.8%), *S. warneri* 1 isolate(3.8%), and *S. capitis* 1 isolate (3.8%) (Kozio-Montewka *et al.*, 2006).

Study was conducted to determine the epidemiology and significance of *S. aureus* and CoNS bacteremia, their resistance patterns and associated mortality in critically ill trauma patients. During 30 month study period, *S. aureus* and CoNS were isolated from 469 samples. Overall, 53% of isolates *S. aureus and* CoNS contributed for the remaining 47% (Tak *et al.*, 2013).

A Cohort study conducted during 2006-2008 to determine whether endometrial catheter colonization predict CRBSI, the common strain isolated from positive blood culture were *S. epidemadis* which accounted for 16 (76.2%) of the total (Rodriguez-Aranda *et al.*, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This was a cross-sectional study.

3.1.2. Study area

Five hospitals in Khartoum State were enrolled in this study, which were including (Omdurman Military Hospital, Hospital of Tropical Diseases, Alnaw Hospital, Alwalidain Charity Hospital and Ibn Sina Hospital). The experimental work was carried out in the Research laboratory, Sudan university of Science and Technology, Sudan.

3.1.3. Study duration

This study was conducted in the period from February to May, 2015.

3.1.4. Study population

Adult (males and females) all of them with renal failure and undergoing hemodialysis were enrolled in this study.

3.2. Inclusion criteria

Participants of this study included haemodialysis patients with symptoms of septicemia such as fever.

3.3. Exclusion criteria

This study excluded all haemodialysis patients without symptoms of bacteremia or on antibiotics.

3.4. Sample size

One hundred and fifteen (n=115) renal failure patients under-going haemodialysis were enrolled.

3.5. Sampling technique

This study was based on non-probability convenience sampling technique.

3.6. Method of data collection

Data were collected according to structured questionnaire (appendix 1).

3.7. Ethical considerations

Participants in the study were informed and had got all the information about the research study, and all ethical roles were followed during sampling and data collection. Permission was issued by College Ethical Committee, Sudan University of Science and Technology.

3.8. Collection of specimens

Blood samples were collected from haemodialysis patients by vein puncture technique under complete aseptic condition. This was achieved by sterilization of the site of collection using 70% alcohol and iodine. 5 ml were collected from each patient using sterile syringes.

3.9. Laboratory work

3.9.1. Inoculation

The blood culture bottles were sterilized using 70% alcohol and iodine and the needle of syringe which used in sample collection was replaced by sterile one, then the 5ml of samples were inoculated immediately into 70 ml of Brain Heart Infusion broths blood culture bottles aseptically and incubated aerobically at 37⁰C for up to 7 days.

3.9.2. Subculture

Before the subculture was done all blood culture bottles were examined for the signs of bacterial growth such as (turbidity, haemolysis, presence of clot). Then subculture was done by using sterile syringes and small amount of inoculated broth were subcultured into mannitol salt agar by streaking out on the upper edge of the agar until 1/3 of the plate was covered while the plate was rotated at 60 degrees. The sample was spread from the end of the first streak into second area using regular streaking until zigzag shape was finally formed to obtain pure culture. Then the blood culture bottles were re-incubated at 37°C. Subcultured procedure was repeated two times after each 24 hr incubation.

3.9.3. Isolation

Growth which was obtained after 1st, 2nd or 3rd subculture, were examined for colonial morphology and ability to ferment mannitol. Sample which was showing no growth for up to one week was discarded and reported as sterile or negative blood culture.

3.10. Identification

3.10.1. Colonial features

Colonial feature used as first identification step, depending on size, color, density, edges, side view and fermentation of mannitol sugar.

3.10.2. purification

The isolated organisms were purified in sterile nutrient agar slop which incubated at 37°C overnight, after overnight incubation the purified organisms were used for purposes of identification

3.10.4. Storage

The purified organisms were stored in sterile crayon tubes at -20⁰C using sterile nutrient glycerol broth medium.

3.10.4. Gram stain

The Gram stain was used for the identification of pathogens in cultures by determining their Gram reactions, cell shape and arrangement according to Gram procedure. The method was performed as follows;

The smear was prepared on clean slide and fixed by rapidly passing the slide over the Bunsen flame. The smear was covered with crystal violet and left for 1min, then rinsed carefully with water, then the smear float with mordant (Lugol's iodine) and left for 2 minutes. Then the smear was decolorized with alcohol and rinsed again carefully for few seconds. Finally the smears were covered with safranin and left for 2min, rinsed as before and dried by blotting on a filter paper (Washington *et al.*, 2006).

3.10.5. Biochemical tests

Several biochemical tests were carried out to identify the isolated strains of microorganisms for examples: (catalase, coagulase, DNase, sensitivity to novobiocine and polymixin B and sugar fermentation tests)." According to Chessbrough".

3.10.5.1. Catalase test

Catalase act as catalyst in the breakdown of hydrogen peroxide to oxygen and water. Three milliliter of hydrogen peroxide were poured into sterile test tubes, using sterile wooden sticks several colonies of test organism were removed and immersed in the

hydrogen peroxide solution and looking for immediate bubbling. Active bubbling indicates positive catalase test, no bubbles indicates negative test (Cheesbrough, 2006).

3.10.5.2. Coagulase test

The test was used to differentiate *S. aureus* which produce enzyme coagulase from other Staphylococci. Coagulase cause plasma to clot by converting fibrinogen to fibrin. There are two types of coagulase; bound and free coagulase. The free coagulase which converting fibrinogen to fibrin was done by adding 0.2 ml of plasma to 0.8 ml of test organism in test tube then the mixture was incubated at 37⁰C for 4 hours and positive result was determined by appearance of fibrin clot, the absence of clot was indicates negative result (Cheesbrough, 2006).

3.10.5.3. DNase test

Test was done by inoculation the test organism in DNA agar by spotting and then all plates were incubated overnight at 37⁰C. After incubation period the plates were poured by hydrochloric acid solution and left for 5 min. looking for clear zone around the organism spot which indicate hydrolysis of DNA. No clearing indicates negative test result (Cheesbrough, 2006).

3.10.5.4. Sensitivity to 5µg novobiocin

Test organism was inoculated in sterile normal saline and turbidity of organisms suspensions were compared with 0.5 McFarland standard then the organism was inoculated into blood agar using sterile swab and disc of 5µg novobiocin were applied, then the blood agar plate was incubated overnight at 37⁰C. After overnight incubation inhibition zone around novobiocin disc was observed. The presence of inhibition zone

(16mm) or more indicates that the organism is susceptible. Zone about (6-12 mm) or less indicates that the organism is resistant (Washington *et al.*, 2006).

3.10.5.5. Sensitivity to 30µg polymixin B

Test organism was inoculated into sterile normal saline and turbidity were compared with 0.5 McFarland standard then the test organism was inoculated into blood agar and disc of 30µg polymixin B was applied, then the blood agar plate was incubated overnight at 37°C. After overnight incubation inhibition zones about (10mm or more) around polymixin B discs indicates sensitivity to disc. Zone of less than (10mm) indicates that the organism is resistance (Washington *et al.*, 2006).

3.10.5.6. Sugar fermentation test

This test was done after preparation of broth media containing phenol red indicator and sugar was to be tested. The test organism inoculated in broth media with several sugars under aseptic condition, then all test tubes were incubated overnight at 37°C. After overnight incubation all tubes of different sugars (glucose, maltose, sucrose, mannose, Arabinose, Trehalose, lactose, Xylose, and Raffinose) were examined for yellow color produced by acid PH. Pink color indicateS negative result (Macki & MacCarteney, 1996)

CHAPTER FOUR

RESULTS

This study was conducted to isolate and identify the common *Staphylococcus* species in haemodialysis patients. One hundred and fifteen (n=115) patients under-going haemodialysis in different centers were enrolled (Table 1 and 2).

The study was done in Khartoum State in period from February to May 2015. The samples were inoculated directly into Brain Heart Infusion broths media and incubated up to 7 days aerobically at 37°C. Out of 115 blood samples investigated, only 33 samples showed positive bacterial growth (Table 3).

The isolated organisms were differentiated by Gram stain technique as Gram-positive and Gram-negative. The former were further investigated. Different biochemical tests were carried out to identify *Staphylococcus* species. The biochemical test results revealed that, the coagulase-negative *Staphylococci* were *S. leugdunensis*, *S. hemolyticus*, *S. hominis*, *S. epidermatis*, *S. saprophyticus*, *S. lutrea*, *S. schlerferi*, *S. arlettea*, *S. vituleneus*, *S. picifermentans*, *S. capitis*, *S. caprea* and *S. pasteuri*. They were represented 87.9% of the total isolates. While coagulase-positive *Staphylococci* were *S. aureuse* and *S. intermedius*. The percentage was 12.1% (Table.4).

Table 1. Distribution of hemodialysis patients according to hospitals

Hospital	Patients	
	No	%
Omdurman Military Hospital	36	29
Alnaw Hospital	19	19
Al Walidain Charity Hospital	16	16
Hospital of Tropical Diseases	29	29
Ibn Sina Hospital	15	7
Total	115	100

Table 2. Distribution of hemodialysis patients according to gender

Gender	Patients	
	No	%
Males	55	48
Females	60	52
Total	115	100

Table 3. Bacterial growth on Brain Heart Infusion Broth media

Growth	No	%
Positive growth	33	29
Negative growth	82	71
Total	115	100

Table 4. Frequency and percentage of isolated *Staphylococcus* species

Staphylococcus species	Frequency	%
Coagulase – positive staphylococci		
<i>S. aureus</i> subsp. <i>aureus</i>	1	3
<i>S. intermedius</i>	2	6.1
<i>S. lutrae</i>	1	3
Coagulase – negative staphylococci		
<i>S. epidermatis</i>	1	3
<i>S. schlerferi</i> subsp. <i>schlerferi</i>	1	3
<i>S. arlettae</i>	1	3
<i>S. vitulenuse</i>	1	3
<i>S. piscifermentans</i>	1	3
<i>S. capitis</i> subsp. <i>urealyticus</i>	1	3
<i>S. saprophyticus</i> subsp. <i>Saprophyticus</i>	2	6.1
<i>S. caprea</i>	2	6.1
<i>S. hominis</i> subsp. <i>hominis</i>	2	6.1
<i>S. pasteurii</i>	2	6.1
<i>S. hominis</i> subsp. <i>novobiosepticus</i>	3	9.1
<i>S. hemolyticus</i>	5	15.2
<i>S. lugdunensis</i>	7	21.2
Total	33	100

Table 5. Number and percentage of positive and negative blood culture yielding

***Staphylococcus* spp. for both males and females according to hospital**

Hospital	Patients		Blood culture results								% of Staph
	No										
	M	F	M				F				
+ve			%	-ve	%	+ve	%	-ve	%		
Omdurman military Hospital	21	15	5	15.2	16	19.5	0	0	15	18.3	15.1
Alnaw Hospital	9	10	3	9.1	6	7.3	4	12.1	6	7.3	21.2
Al Walidain Charity Hospital	4	12	1	3.0	3	3.6	4	12.1	8	9.8	15.1
Hospital of Tropical Diseases	16	13	7	21.2	9	11	4	12.1	9	11	33.3
Ibn Sina Hospital	5	10	4	12.1	1	1.2	1	3.0	9	11	15.1
Total	55	60	20		35		13		47		100
	115		55				60				

Key: +ve ; positive growth in Brain Heart Infusion broth , -ve ; negative growth in Brain

Heart Infusion broth, M; male , F; female, Staph; *Staphylococcus* isolates.

CHAPTER FIVE

DISCUSSION

5.1. Discussion

In hospital setting, infection are commonly caused by *Staphylococcus* species which are important nosocomial and opportunistic pathogens especially for immune compromised patients such as those with end stage renal disease treated with maintenance haemodialysis. The objective of this study was to isolate and identify the *Staphylococcus* species among renal failure patients treated with haemodialysis in Khartoum State. One hundred and fifteen blood samples (n=115) were collected from haemodialysis patients in five different hospitals. Most of those patients use the temporary catheter as type of vascular accesses which may increase risk for developing bactremia. Out of 115 blood samples examined, only 33(29%) showed positive bacterial growth. Of those, (12.1%) were coagulase-positive staphylococci including 1(3%) *S. aureus* subsp. *aureus*, 1(3%) *S. lutrea* and 2(6.1%) *S. intermedius*. The remaining 28(87.9%) isolates were coagulase-negative and were reported as *S. lugdunensis* (21.2%), *S. hemolyticus* (15.2%), *S. hominis* subsp. *novobiosepticus* (9.1%), *S. pasteurii* (6.1%), *S. hominis* subsp. *hominis* (6.1%), *S. caprea* (6.1%), *S. saprophyticus* subsp. *saprophyticus* (6.1%), *S. capitis* subsp. *urealyticus* (3%), *S. picifermentas* (3%), *S. vitulenus* (3%), *S. arlettae* (3%), *S. schlerferi* subsp. *schlerferi* (3%) and *S. epidermatis* (3%). The results were similar to those obtained by Safdar & Maki, in US (2004), Wisplinghoff *et al.*, (2004) in US and Bisiwe *et al.*, (2015) in Universites academic hospital, Bloemfontein, which were reported the CoNS as common causative agent of bacteremia. This may attributed to presence of

prosthetic material and frequent breaches of the skin associated with vein puncture provide chance for infection by CoNS which are common bacterial species surrounded the environment of immune compromise HD patients (either patients skin normal flora, hand of personnel, contaminated medical devices or water), therefore infections by these bacterial species were more frequent.

Jain *et al.*, (2011) in India, Grothe *et al.*, (2010) in Brazil, Sanavi *et al.*, (2007) in Iran University, Dalgaard *et al.*, (2015) in Denmark, Tak *et al.*, (2013) in India and Fysaraki *et al.*, (2013) in Greece, were all reported that, *S. aureus* as the common species isolated from DH patients with percentages 8.7%, 41%, 42%, 43.8%, 53% and 55% respectively. These findings disagree with results of this study in which *S. aureus* represented about only 3% of the total isolates. These difference may be contributed to the fact that, haemodialysis patients have an increased *S. aureus* nasal carriage rate varying between 32% and 82% and the probable predisposing factors include immunodeficiency (Kozio-Montewka *et al.*, 2006). Therefore, this is considered to play a key role in the development and pathogenesis of life-threatening staphylococcal BSI infections in haemodialysis patients.

Koksal *et al.*, (2009), Rodriguez-Aranda *et al.*, (2011) and Kozio-Montewka *et al.*, (2006) reported that, the common species isolated were *S. epidermidis* and reported as 43.5%, 76.2% and 80% respectively, these findings disagree with result of this study in which the common species isolated were *S. lugdunensis* which represented about (21.2%) of the total isolates, and *S. epidermidis* were isolated at only (3%). In most situations, *S. lugdunensis* has been regarded as more pathogenic than most members of the genus

Staphylococcus because of the aggressive nature of infections observed in many cases. Also it has ability to express a clumping factor and/or produce a thermo-stable DNase and produce virulence determinants. It also has also been much less commonly than other CoNS species considered to be contaminant or colonizing microorganism and is much more likely to be considered a significant isolate (Kozio-Montewka, 2006).

CoNS isolated in this study, were similar to those obtained by Jain *et al.*,(2011) and Koksai *et al.*,(2009) but they differ in the representative ratios of such organisms which include *S. hominis*, *S. epidermidis*, *S. hemolyticus*, which were reported by Jain as 3.3%, 16.7%, 56.7% respectively and were reported by Koksai *et al* as 9.5%, 43.5% and 11.5% respectively. These organisms in presented study were 15.2%, 3% and 15.2% respectively. Ratio of *S. capitis* obtained in the result of this study was similar to those obtained by Jain *et al* (2011) as 3%. In addition to the above species obtained by Koksai *et al.*,(2009) also they were isolated *S. lugdunensis*, *S. saprophyticus*, and *S. schleiferi* which were reported as 9%, 2.5% and 0.5% respectively and these species were also isolated during this study but in different percentages as follows 21.2%, 6.1% and 3% respectively. The differences between these findings may attributed to different in sample size, behavior and diversity in skin microbial flora within different ethnic groups in those population.

5.2. Conclusion

This study concluded that, the rate of *Staphylococcus* species isolated from hemodialysis patients was moderate. Although all HD patients enrolled in this study were suffering from symptoms of bacteremia, most of patients revealed negative growth on blood culture, this may due to pathogen other than staphylococci or anaerobic pathogen.

5.3. Recommendations

1. Surveillances must be conducted and large number of sample size should be enrolled to validate the results of this study.
2. Undertaking of further studies to isolate and identify different causative agents of BSI in HD patients.
3. When the investigation of BSI in HD is requested, its recommended to use both aerobic and anaerobic blood culture media to provide chance for isolation of large number of different microorganisms.

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APPENDICS

1.Questionnaire

Date / / 2015

Hospital Name

1. Gender: Male () female ()

2. Age years .

3. Type of hemodialysis: Catheter () Fistula ()

4. Duration:

5. Symptomes

Fever () Hypotension ()

6. History of bactremia Yes () No ()

7. Antibiotic receive Yes () No ()

8. Co-morbid conditions

Diabetes Yes () No ()

Malnutrition Yes () No ()

9. Laboratory processing

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2. Biochemical tests results for different Staphylococcus species isolates

3.Color plates :

