

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

The antibiogram is a periodic summary of antimicrobial susceptibilities of local bacterial isolates submitted to the microbiology laboratory. Consensus guidelines have been developed by the Clinical and Laboratory Standards Institute (CLSI) to standardize methods used in constructing antibiograms, with the goal of promoting the reporting of reliable and consistent antibiogram data. The antibiogram helps in monitoring antimicrobial resistance trends over different periods (Kaur *et al.*, 2013).

The performance of antimicrobial susceptibility testing is important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. So the susceptibility test of individual isolates is important with species that may possess acquired resistance mechanisms (James and Jane, 2009).

Staphylococci are members of the normal flora of the skin and mucous membranes of humans that cause suppuration, abscess formation, a variety of pyogenic infections and even fatal septicemia. The genus *Staphylococcus* has at least 35 species (Brooks *et al.*, 2007).

Staphylococci are differentiated by their ability to produce coagulase enzyme which divide them into coagulase-positive and coagulase-negative staphylococci (CoNS) groups. Although *Staphylococcus aureus* (*S. aureus*) is

the most common and important coagulase-positive staphylococcal species causing human disease, other staphylococci, including *S. intermedius*, *S. delphini*, *S. schleiferi* subsp. *coagulans*, *S. lutrae* and some strains of *S. hyicus* are also coagulase-positive and have been implicated in some human infections. CoNS are generally considered to have a low virulence potential some CoNS (e.g. *S. epidermidis*, *S. haemolyticus* and *S. lugdenensis*) have assumed increasing medical importance in hospital-acquired infections (Kwok and Chow, 2003).

S. aureus is an important animal and human pathogen that causes multiple types of serious infections with high morbidity rates. It possesses multiple virulence factors that affect host defenses, permit colonization and destruction of tissues, and induce sepsis syndromes (Moisan *et al.*, 2006).

Chronic kidney disease is a major public health burden. The prevalence of end-stage renal disease is increasing exponentially worldwide. For the vast majority of adult patients there will be haemodialysis (HD) treatment. Many patients still presented late HD access catheter either cuffed or noncuffed catheters. Infection is one of the most feared complications. Infection of the HD catheter was thought to cause an increase of >50% mortality in HD patients compared to patients on native fistulas and also cause significant morbidity in dialysis population. The cause of HD catheter-related bloodstream infections is multifactorial ranging from patient's factors (comorbidities and hygiene) to catheter's factors (types of catheter and sites of insertion) (Abdul-Gafor *et al.*, 2014).

Infection is the second most common cause of death in end-stage renal disease patients causing 12–22% of annual mortality. Patients receiving HD are at particular risk for the development of invasive infections caused by staphylococci. Infectious organisms most commonly come either from the patient's skin flora at the insertion site or from hands subsequently manipulating the catheter (Lew and Kaveh, 2000).

S. aureus and CoNS species are among the most prevalent pathogens of dialysis and kidney transplant patients. These individuals possess risk factors for colonization and infection with multidrug-resistant *S. aureus* because of their frequent and prolonged use of antimicrobials. Moreover, these patients undergo frequent admission to the hospital and invasive procedures (Giarola *et al.*, 2012).

The annual incidence of *S. aureus* bacteremia in patients on HD ranges from 6 to 27%. Complications of *S. aureus* bacteremia include meningitis, endocarditis, osteomyelitis, and metastatic abscesses (Li *et al.*, 2009).

Haemodialysis patients might also be prone to this mode of acquisition of staphylococcal infections because of their defective mucocutaneous barriers and immunodeficiency. Compounding the problem is striking ability of these microorganisms to develop resistance against a wide spectrum of antibiotics exemplified by the emergence and the worldwide spread of methicillin resistance (MR) and the increasing incidence of afflictions caused by CoNS other than *S. epidermidis* including *S. haemolyticus*, *S. lugdunensis*, *S. warneri* and *S. capitis*. Moreover, recent evidence indicates that the

epidemiology of methicillin resistant *S. aureus* (MRSA) may be undergoing a change through the appearance of community acquired MRSA (Koziol-Montewka *et al.*, 2004). In China Medical University Hospital, *S. aureus* was the most common species isolated from haemodialysis patients (27.53%) (Wang *et al.*, 2012).

In Turkey, *S. epidermidis* was the most prevalent species of CoNS isolated from nosocomial bacteremia followed by *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. capitis*, *S. xylosus*, *S. warneri*, *S. saprophyticus*, *S. lentus*, *S. simulans*, *S. chromogenes*, *S. cohnii*, *S. schleiferi* and *S. auricularis* (Koksall *et al.*, 2009).

1.2. Rationale

Recently *Staphylococcus* species are increasingly becoming resistant to majority of the available antibiotics in use and subsequently have developed into a challenging public health problem (Aboud *et al.*, 2015). The monitoring of antimicrobial susceptibility patterns are important for clinicians in selecting empiric antimicrobial therapy and providing useful information on the surveillance of staphylococci (Akindele *et al.*, 2010). Hence, continuous surveillance on antimicrobial susceptibility of staphylococci is essential.

This study was conducted to determinate the antibiogram of *Staphylococcus* species which cause bacteremia in haemodialysis patients.

1.3. Objectives

1.3.1. General objective

To determine antibiogram of *Staphylococcus* species isolated from haemodialysis patients in Khartoum State.

1.3.2. Specific objectives

1. To re-identify species of *Staphylococcus* .
2. To perform susceptibility test against *Staphylococcus* species using different antimicrobial agents .

CHAPTER TWO

LITERATURE REVIEW

The introduction of penicillin in the early 1940s dramatically improved the prognosis of patients with staphylococcal infection. However, as early as 1942, penicillin-resistant staphylococci were recognized, first in hospitals and subsequently in the community. By the late 1960s, more than 80% of both community and hospital-acquired staphylococcal isolates were resistant to penicillin. More than 90% of staphylococcal isolates now produce penicillinase. Methicillin introduced in 1961 was the first of the semi synthetic penicillinase resistant penicillins. Its introduction was rapidly followed by reports of methicillin-resistant staphylococci (MRS). The *mecA* gene is part of a mobile genetic element responsible for methicillin resistant (MR) (Lowy, 2003).

Methicillin-resistant staphylococci are resistant to all penicillins, including semisynthetic penicillinase-resistant penems, carbapenems, and cephalosporins. The basis of this resistance is conferred by an additional penicillin-binding protein called PBP-2a which is absent in methicillin susceptible staphylococci (MSS) (Martineau *et al.*, 2000).

Because of increasing rates of MRS and the severity of infection it produces, vancomycin has become standard empirical therapy for Gram-positive bacteremia (Nicolson *et al.*, 2013).

In 1997, the first *Staphylococcus aureus* (*S. aureus*) isolate with reduced vancomycin susceptibility was reported in Japan. Since then, six confirmed

vancomycin-resistant *S. aureus* (VRSA) and at least 21 vancomycin-intermediate *S. aureus* (VISA) strains have been documented in the United States. A considerable prevalence of heterogeneous VISA in which only a subset of the bacterial population expresses the resistant phenotype has been reported among *S. aureus* isolates from various geographic and patient populations of the world. Although VRSA and VISA strains that demonstrate high level resistance to vancomycin are rare among *S. aureus* clinical isolates, there is great concern about the emergence of *S. aureus* with reduced susceptibility to vancomycin due to the high incidence of the organism in causing both health care and community-associated infections and its well known virulence and resistance to many other antimicrobial agents (Wang *et al.*, 2006).

The risk for invasive MRSA infections is 100-fold higher in dialysis patients than in the general population (45.2/1000 *versus* 0.2 to 0.4/1000). Dialysis patients currently account for up to 15.4% of all invasive MRSA infections. Twelve to 30% (2006 through 2007 European data), 33% (1994 through 2001 United States data), and up to 65% (most recent United States data) of patients on hemodialysis are colonized with MRSA (Vandecasteele *et al.*, 2009).

In another prospective study conducted in Eastern Province of Saudi Arabia, the prevalence of *S. aureus* nasal carriage on haemodialysis patients was 38.05%, including 27.3% for MSSA and 10.7% for MRSA. None of the isolated strains of *S. aureus* showed borderline oxacillin resistance (Saxena *et al.*, 2004).

A study in Sudan investigated the prevalence and antimicrobial susceptibility pattern of methicillin resistance *Staphylococcus. S. aureus* was 77% resistant to methicillin and 6% resistant to vancomycin (Kheder *et al.*, 2012).

In another descriptive study carried on haemodialysis patients, all isolated *S. aureus* were resistant to methicillin while 95% were resistant to cloxacillin, 6.81% to clindamycin, 6.81% to ciprofloxacin and 4.5% to rifampin. All isolates were sensitive to vancomycin (Aminzadeh *et al.*, 2006).

There is a significant increase in the MR in CoNS which was determined to be 67.5% isolated from blood samples of patients with true bacteremia in intensive care units (Koksal *et al.*, 2009).

In a study in Lublin, Poland, the prevalence of *S. aureus* and CoNS nasal carriage among patients undergoing haemodialysis, thirty-five (81.3%) out of 43 patients examined in 2004 were positive for staphylococci in their anterior nares. *S. aureus* was isolated from 12 (27.9%) patients. Twenty-six isolates of CoNS were isolated from 24 (55.8%) patients: *S. epidermidis* (21 isolates), *S. lugdunensis* (2 isolates), *S. haemolyticus* (1 isolate), *S. warneri* (1 isolate), and *S. capitis* (1 isolate). Antibiotic susceptibility testing identified 1 (8.3%) *S. aureus* strain and 10 (38.4%) CoNS isolates (9 *S. epidermidis* isolates and 1 *S. haemolyticus* isolate) demonstrating resistance to methicillin. The MRSA strain identified in the study was resistant only to β -lactam antibiotics (oxacillin, ampicillin, ampicillin/sulbactam and cefazolin) and tetracycline. Among MR CoNS, high rates of resistance to tetracycline (70%) and trimethoprim/sulfamethoxazole (40%) were observed. In total 50% of the

isolates demonstrated constitutive macrolides-lincosamides- streptogramins B resistance mechanism by the disc diffusion method. One isolate (10%) was resistant to ciprofloxacin and demonstrated low-level resistance to mupirocin. MSSA isolates demonstrated high rates of resistance to penicillin (81.8%) and tetracycline (63.6%). Among methicillin- susceptible (MS) CoNS the following resistance rates were observed: penicillin (68.7%), tetracycline (31.2%), trimethoprim-sulfamethoxazole (25%), and erythromycin (18.7%). It is noteworthy that non-*S. epidermidis* CoNS isolates (except for one methicillin-resistant *S. haemolyticus* isolate), two *S. lugdunensis* isolates and single isolates of *S. warneri* and *S. capitis* were fully sensitive to all antibiotics used in the susceptibility testing (Kozioł-Montewka *et al.*, 2006).

A large cohort study conducted for *S. aureus* screening involved 578 HD patients. Of them 288 (49%) were positive, 10% of isolates were resistant to methicillin (Price *et al.* , 2015). In another study in the North of Iran, 74.2% of isolated *S. aureus* were resistant to methicillin. Of the MR *S. aureus* isolates, 3 (13%) were resistant to vancomycin. Resistance frequencies to clindamycin, ciprofloxacin, and trimethoprim-sulfamethoxazole were 12.9%, 9.7%, and 19.3%, respectively (Ghasemian *et al.*, 2010).

A study in the University Hospital of Larissa, Greece, was carried out to determine the clonal relatedness of MR CoNS on haemodialysis patients. In total, 42 CoNS isolates were recovered; 36 *S. epidermidis*, 4 *S. hominis* and 2 *S. haemolyticus*. Among them, MR was identified in 37 isolates (88.1%) (32 *S. epidermidis*, 3 *S. hominis* and 2 *S. haemolyticus*). Apart from β -lactams, MR

CoNS were also resistant to other antibiotics: erythromycin 33 (89.1%), ofloxacin 21 (56.7%), fusidic acid 30 (81%), trimethoprim-sulphamethoxazole 22 (59.4%), gentamicin 17 (45.9%) and tetracycline 12 (32.4%). None of the isolates showed reduced susceptibility to vancomycin, teicoplanin and linezolid (Liakopoulos *et al.* , 2008).

A study conducted in Turkey to determine antibiotic resistance of CoNS, resulted in detection of 67.5% of CoNS isolates to be resistant to methicillin. MR CoNS strains were determined to be more resistant to antibiotics than MS CoNS strains. Resistance rates of MR CoNS and MS CoNS strains to the antibacterial agents, respectively, were as follows: gentamicin 90% and 17%, erythromycin 80% and 37%, clindamycin 72% and 18%, trimethoprim-sulfamethoxazole 68% and 38%, ciprofloxacin 67% and 23%, tetracycline 60% and 45%, chloramphenicol 56% and 13% and fusidic acid 25% and 15%. None of the strains were resistant to vancomycin and teicoplanin (Koksal *et al.*, 2009).

A study in Malaysia was conducted to determine the antibiogram for haemodialysis catheter-related bloodstream infections. The CoNS was 100% susceptible to gentamicin, vancomycin and 33.3% susceptible to ciprofloxacin, trimethoprim-sulfamethoxazole (Abdul-Gafor *et al.*, 2014).

In United States a study was conducted to determine antimicrobial susceptibility of isolated *Staphylococcus* species. Trimethoprim-sulfamethoxazole resistance was about 17% in MS CoNS compared with

nearly 57% in MR CoNS. Similar trends were seen for gentamicin, ciprofloxacin, clindamycin and erythromycin (Diekema *et al.*, 2001).

Cross resistance was most common in the MR CoNS. The resistant rates of MR CoNS to trimethoprim-sulphamethoxazole, ciprofloxacin and gentamicin were 64%, 50.5% and 72.3% respectively. MR *S. haemolyticus* showed higher frequencies of cross resistance. For ciprofloxacin resistance was 65.0%–66.7% across four European countries (France, Germany, Italy, and Spain) (John and Harvin, 2007).

A prospective study was carried out to determine the prevalent bacterial agents of neonatal sepsis and their antimicrobial susceptibility in Urmia, Iran. The CoNS isolates were highly resistant to commonly used antibiotics; ampicillin (100%), ceftriaxon (65%), cefotaxim (67%) and gentamicin (51%), but comparatively low resistance to vancomycin (10%), imipenem (19%), and ciprofloxacin (23%) (Gheibi *et al.*, 2008).

In a study in USA, 53% of *S. lugdunensis* were resistant to penicillin G, 36% resistant to methicillin, 37% resistant to trimethoprim-sulfamethoxazole, and 36% resistant to clindamycin. All *S. lugdunensis* were sensitive to vancomycin (Byrnes *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

The present work is a laboratory based study.

3.1.2. Study area

The experimental work of the present study was carried out in the Research Laboratory, College of Medical Laboratory science, Sudan University of Science and Technology (SUST).

3.1.3. Study duration

The study was carried out during the period from March to June, 2015.

3.1.4. Source of *Staphylococcus* isolates

Staphylococcus isolates were obtained from the Research Laboratory, (SUST).

The samples used in this study were previously collected from end stage renal failure patients under heamodialysis by inoculation the blood samples in brain heart infusion broth. The isolates were stored in 16% glycerol broth at -20⁰ C.

3.2. Sample size

A total of thirty-three (n=33) *Staphylococcus* isolates were used in this study.

3.3. Microbiological methods

3.3.1. Purification of isolates

The isolate were streaked on nutrient agar (Appendix 1) and incubated overnight at 37⁰C, at the end of incubation period, a discrete colony was picked

up and checked for purity under microscope and then stored in Bijou bottle containing glycerol for further investigations.

3.3.2. Re-identification of isolates

3.3.2.1. Gram's stain (Appendix 2)

A smear from the young culture (16-18 hrs) was prepared by emulsifying a small portion of the bacterial colony in a drop of normal saline and spread evenly on clean glass slide. The smear was allowed to air dry and fixed by passing the slide three times through Bunsen burner flame. Crystal violet stain was added to fixed smear for 30-60 seconds then washed by tap water followed by 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 safranin for 2 minutes, and washed off with clean water. The slide was placed in a draining rack for the smear to air dry and then examined microscopically by oil immersion objective 100x (Cheesbrough, 2006).

3.3.2.2. Biochemical tests

3.3.2.2.1. Catalase test

Test was carried by adding of 2 ml of 3% hydrogen peroxide in small test tube. Growth organism was immersed in the test tube solution by using a sterile wooden stick. Positive results were shown by formation of air bubbles (Cheesbrough, 2006).

3.3.2.2.2. Coagulase test

The tube coagulase test use to detect organism that produce free coagulase which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Test was carried by adding of 0.2 ml of diluted plasma (1:10) to 0.8ml of test broth organism. After mixing gently, incubate at 37° C on water bath. Examined for clotting after 1-3 hours. If the test is negative, the tube was leaved at room temperature overnight and examined again (Cheesbrough, 2006).

3.3.2.2.3. DNase test

The organism was cultured on a medium containing deoxyribonucleic acid (DNA) (Appendix 3). After overnight incubation the colonies were tested by flooding the plate with a weak hydrochloric acid (Appendix 4). The acid precipitate the unhydrolyzed DNA. DNase producing colonies should be formed were surrounded by clear area (Cheesbrough, 2006).

3.3.2.2.4. Carbohydrates fermentation

Peptone water sugar containing phenol red as pH indicator was prepared at pH 7.4 (Appendix 5). The carbohydrates that were used included lactose, Glucose, Maltose, Sucrose, Mannose, Mannitol, Arabinose, Trehalose, Xylose and Raffinose. The broth was a light red color, supports the growth of most organisms whether they are able to ferment the sugar or not. The test organism was inoculated into a broth containing the test sugar and incubated overnight at 37⁰C. A bright yellow color indicates the production of enough acid products

from fermentation of the sugar to drop the pH to 6.9 or less (Washington, 2006).

3.3.2.3. Sensitivity to Novobiocin and Polymixin B

Suspension was prepared from isolated colonies in normal saline. The suspension was mixed well and matched against 0.5 MacFarland standard (Appendix 6). By using sterile swab the plate of blood agar (Appendix 7) inoculated with suspension. The disc of 5 µg Novobiocin and 30 µg Polymixin B was applied. Then the plate was incubated overnight at 35°C. The zones of inhibition were measured and interpreted (Washington, 2006).

3.3.3. Antimicrobial susceptibility testing

Susceptibility test was performed using Kirby-Bauer disc diffusion method according to NCCLS (2012) as follows :

3.3.3.1 Culture medium

Sterilized molten Muller-Hinton agar (Appendix 8) (PH 7.4±2) was prepared, cooled to 45-50°C and poured in sterile dry Petri plates on a level surface, to a depth of 4mm.

3.3.3.2. Antibiotics

The following antibiotics were obtained from Himedia laboratories PVT. Ltd INDIA: Methicillin (MET) 5 µg, Vancomycin (VA) 30 µg, Ciprofloxacin (CIP) 5 µg, Ceftriaxone (CRO) 30 µg , Trimethoprim-sulphamethazole (SXT) 25 µg and Gentamicin (GEN) 10 µg.

3.3.3.3. Quality control

Quality control was performed to measure the effectiveness of antimicrobial agents by using a control *S. aureus* ATCC 25923 obtained from the Central Public Health Laboratory.

3.3.3.4. Preparation of inoculums

The inoculum was prepared by transfer of 3-5 well isolated colonies of same appearance with sterile wire loop to 2 ml of sterile physiological saline. The turbidity of this suspension was adjusted to a 0.5 MacFarland standard. This suspension was used within 15 minutes of preparation.

3.3.3.5. Seeding of plates

A sterile non toxic cotton swab was dipped into the inoculums tube and then the swab was rotated against the side of the tube above the level of the suspension to remove excess fluid. The plate of Muller Hinton agar was inoculated by streaking the swab evenly over the surface of the medium in three directions. The surface of agar was allowed 3-5 minutes to dry.

3.3.3.6. Antibiotic disc application

The selected antibiotics were applied on the surface of agar by using sterile forceps which evenly distributed in the inculcated plate. Each disc was pressed down to ensure its contact with the agar.

3.3.3.7. Incubation

The inverted plates were incubated aerobically at 35°C for 16-18 hours.

3.3.3.8. Reading of zones of inhibition

Following overnight incubation, by using a ruler on the underside of the plate, the diameter of each zone of the inhibition was measured in millimeters.

3.3.3.9. Interpretation of the results

The zone of each antibiotic was compared to their standard inhibition zone on the chart provided by manufacture's (Appendix 9). The results were interpreted as sensitive (S) or resistance (R) (Appendix 10).

CHAPTER FOUR

RESULTS

A total of thirty three (n=33) *Staphylococcus* isolates were obtained from the Research Laboratory (SUST). These were 4 coagulase-positive staphylococci and 29 coagulase-negative staphylococci (Table 1).

Study on antibiogram of *Staphylococcus* species to traditionally used antibiotics revealed that the susceptibility to Methicillin (MET), Vancomycin (VA), Ciprofloxacin(CIP), Ceftriaxone (CRO), Trimethoprim-sulphamethazole (SXT) and Gentamicin (GEN) were 66.7%, 84.8%, 84.8%, 6%, 36.3% and 94% respectively. The 11 (33.3%) MET resistant *Staphylococcus* isolates were: 2, *S. hemolyticus*; 2, *S. caprae*; 1, *S. hominis* subsp. *novobiosepticus*; 1, *S. intermedius*; 1, *S. pasteurii*; 1, *S. schleiferi* subsp. *schleiferi*; 1, *S. piscifermentans*; 1, *S. arlettae* and 1, *S. lutrae*. The 5(15.1%) VA resistant *Staphylococcus* isolates were: 1, *S. hemolyticus*; 1, *S. intermedius*; 1, *S. caprae*; 1, *S. schleiferi* subsp. *schleiferi* and 1, *S. arlettae* (Table 2).

Table (3) demonstrates the antibiogram of CoNS as follows; MET 69%, VA 86.2%, CIP 82.8%, CRO 3.5%, SXT 31% and GEN 93%.

Table 1. Types, number and percentage of re-identified *Staphylococcus* isolates

<i>Staphylococcus</i> species	No	%
Coagulase-positive staphylococci		
<i>S. aureus</i> subsp. <i>aureus</i>	1	3
<i>S. lutrae</i>	1	3
<i>S. intermedius</i>	2	6.1
Coagulase-negative staphylococci		
<i>S. lugdunensis</i>	7	21.2
<i>S. haemolyticus</i>	5	15.2
<i>S. hominis</i> subsp. <i>novobiosepticus</i>	3	9.1
<i>S. hominis</i> subsp. <i>hominis</i>	2	6.1
<i>S. saprophyticus</i>	2	6.1
<i>S. pasteuri</i>	2	6.1
<i>S. caprae</i>	2	6.1
<i>S. epidermidis</i>	1	3
<i>S. capitis</i> subsp. <i>urealyticus</i>	1	3
<i>S. schleiferi</i> subsp. <i>schleiferi</i>	1	3
<i>S. piscifermentans</i>	1	3
<i>S. arlettae</i>	1	3
<i>S. vitulinus</i>	1	3
Total	33	100

Table 2. Susceptibility patterns of *Staphylococcus* isolates to antibiotics.

<i>Staphylococcus</i> species	Susceptibility (%) of bacterial isolates to					
	MET	VA	CIP	CRO	SXT	GEN
Coagulase-positive staphylococci						
<i>S. aureus</i> subsp. <i>aureus</i>	1/1	1/1	1/1	0/1	1/1	1/1
<i>S. lutrae</i>	0/1	1/1	1/1	0/1	0/1	1/1
<i>S. intermedius</i>	1/2	1/2	2/2	1/2	2/2	2/2
Coagulase-negative staphylococci						
<i>S. lugdunensis</i>	7/7	7/7	6/7	0/7	4/7	7/7
<i>S. haemolyticus</i>	3/5	4/5	3/5	0/5	1/5	4/5
<i>S. hominis</i> subsp. <i>novo</i> .	2/3	3/3	2/3	0/3	2/3	3/3
<i>S. hominis</i> subsp. <i>hominis</i>	2/2	2/2	2/2	0/2	0/2	2/2
<i>S. saprophyticus</i>	2/2	2/2	2/2	0/2	0/2	2/2
<i>S. pasteurii</i>	1/2	2/2	2/2	0/2	0/2	2/2
<i>S. caprae</i>	0/2	1/2	2/2	0/2	0/2	1/2
<i>S. epidermidis</i>	1/1	1/1	0/1	1/1	1/1	1/1
<i>S. capitis</i> subsp. <i>ure</i> .	1/1	1/1	1/1	0/1	0/1	1/1
<i>S. schleiferi</i> sub. <i>schleiferi</i>	0/1	0/1	1/1	0/1	0/1	1/1
<i>S. piscifermentans</i>	0/1	1/1	1/1	0/1	1/1	1/1
<i>S. arlettae</i>	0/1	0/1	1/1	0/1	0/1	1/1
<i>S. vitulinus</i>	1/1	1/1	1/1	0/1	0/1	1/1
Percentage	66.7%	84.8%	84.8%	6%	36.3%	94%

Key: MET=Methicillin, VA=Vancomycin, CIP=Ciprofloxacin, CRO=Ceftriaxone, SXT=Trimethoprim-sulphamethazole, GEN= Gentamicin.

Table 3. Susceptibility patterns of Coagulase-negative staphylococci (n=29)

Result Antibiotic	Sensitive	Resistant
MET 5 µg	20 (69%)	9 (31%)
VA 30 µg	25 (86.2%)	4 (13.8%)
CIP 5 µg	24 (82.8%)	5 (17.2%)
CRO 30 µg	1 (3.5%)	28 (96.5%)
SXT 25 µg	9 (31%)	20 (69%)
GEN 10 µg	27 (93%)	2 (7%)

Key: **MET**=Methicillin, **VA**=Vancomycin, **CIP**=Ciprofloxacin,
CRO=Ceftriaxone, **SXT**=Trimethoprim-sulphamethazole, **GEN**= Gentamicin.

CHAPTER FIVE

5. DISCUSSION

5.1. Discussion

This study determined antibiogram of *Staphylococcus* species isolated from end stage renal failure patients undergoing haemodialysis. The main pathogenic species of staphylococci was *S. aureus*. Study on antibiogram of this organism revealed high degree of susceptibility to Methicillin (100%), Vancomycin (100%), Ciprofloxacin (100%), Trimethoprim-sulphamethazole (100%), Gentamicin (100%). Similar study carried out in Khartoum State, Sudan, by Kheder *et al.*, (2012) reported low degree of susceptibility to Methicillin (23%) and Vancomycin (94%). This variation may be attributed to number of *S. aureus* tested in the two studies, although the same organism revealed high degree of resistance to Ceftriaxone (100%). Furthermore, the findings of the present study was different from that of Ghasemian *et al.*, (2010) in Iran who reported resistant to Methicillin, Vancomycin, Ciprofloxacin and Trimethoprim-sulphamethazole as 74.2%, 13%, 9.7% and 19.3% respectively. On the other hand, the susceptibility of *S. lugdunensis* to vancomycin was (100%) and to Trimethoprim-sulphamethazole was (57.1%). This results is in line with that reported by Byrnes *et al.*, (2014) in USA who reported susceptibility of *S. lugdunensis* to Vancomycin and Trimethoprim-sulphamethazole as 100% and 63% respectively. Moreover, 31% of coagulase-negative staphylococci (CoNS) investigated in this study were resistant to Methicillin. These results are almost similar to that reported by Koziol-Montewka *et al.*, (2006) in Poland who reported that 38.4%

of CoNS were resistant to Methicillin. High prevalence of Methicillin-resistant CoNS (MR CoNS) was reported by Koksai *et al.*, (2009) in Turkey and Liakopoulos *et al.*, (2008) in Greece as 67.5% and 88.1% respectively.

In this study the susceptibility of CoNS to Vancomycin was 86.2%. This result was less than that obtained by Abdul-Gafor *et al.*, (2014) in Malaysia, Liakopoulos *et al.*, (2008) in Greece and Koksai *et al.*, (2009) in Turkey who reported that CoNS were 100% sensitive to Vancomycin. But the susceptibility of CoNS to Gentamicin and Trimethoprim-sulphamethazole were similar to that obtained by Abdul-Gafor *et al.*, (2014) in Malaysia who reported susceptibility of CoNS to Gentamycin and Trimethoprim-sulphamethazole as 100%, 33.3% respectively. Moreover, susceptibility of CoNS to Ciprofloxacin was higher than result of Abdul-Gafor *et al.*, (2014) who reported susceptibility of CoNS to Ciprofloxacin as 33.3%.

Generally, 94% of *Staphylococcus* isolates tested in this study were resistant to Ceftriaxon. This result confirms the findings of Gheibi *et al.*, (2008) in Iran who reported 65% resistant *Staphylococcus* isolates.

5.2. Conclusion

The study concluded that the *Staphylococcus* species isolated from hemodialysis patients are:

1. Highly susceptible to Gentamicin (94%), Vancomycin (84.8%) and Ciprofloxacin (84.8%).
2. low susceptibility to Ceftriaxone (6%).

5.3. Recommendation

1. Further study with large number of *Staphylococcus* isolates from hemodialysis patients are highly recommended to validate these findings.
2. Screen another antibiotics to determine antibiogram of *Staphylococcus* species.
3. Further study recommended to validate these findings.

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APPENDICES

1. Nutrient agar

Approximate formula * per Liter

Beef Extract.....3.0g

Peptone.....5.0g

Agar.....15.0g

Final pH 7.3 ± 0.2 at 25°C.

2. Gram's stain

Crystal violet

Approximate formula * per Liter

Crystal violet.....20.0g

Ammonium oxalate.....9.0g

Ethanol, absolute.....95ml

Distilled water.....to 1 liter

Lugol's iodine

Approximate formula * per Liter

Potassium iodide.....20.0g

Iodine.....10.0g

Distilled water.....to 1 liter

Acetone-alcohol decolorizer

Approximate formula * per Liter

Acetone.....500ml

Ethanol, absolute.....475ml

Distilled water.....25ml

Saffranin

Approximate formula * per 100ml

Saffranin.....	2.5g
95% ethanol.....	10ml
Distilled water.....	to 100ml

3. DNase agar

Approximate formula * per Liter

Peptone	20.0g
Deoxyribonucleic acid.....	2.0g
Sodium chloride.....	5.0g
Agar.....	12.0g

Final pH 7.3 ± 0.2 at 25°C.

4. Hydrochloric acid, 1mol/l

Approximate formula * per 100ml

Hydrochloric acid, concentrated.....	8.6ml
Distilled water.....	to 100 ml

5. Peptone water sugars

- **Peptone water with indicator**

Approximate formula * per Liter

Peptone.....	10.0g
Sodium chloride.....	5.0g
Phenol red, 2g/l(0.2%).....	12.5ml
Distilled water.....	to 1 liter

- **Peptone water sugars**

Peptone water with indicator.....100ml

Sterile 10% sugar solution.....5ml

Final pH 7.3 ± 0.1 at 25°C .

6. 0.5 McFarland standard

Approximate formula * per 100ml

1% w/v barium chloride.....0.6ml

1% v/v Sulphuric acid.....99.4ml

7. Blood agar

- Blood agar base

Approximate formula * per Liter

Meat extract.....10.0g

Tryptone.....10.0g

Sodium chloride5.0g

Agar.....15.0g

- 5% Sterile defibrinated blood.

- Final pH 7.3 ± 0.2 at 25°C .

8. Muller-Hinton Agar

Approximate formula * per Liter

Beef extract.....2.0g

Acid Hydrolysate of Casein.....17.5g

Starch.....1.5g

Agar.....17.0g

Appendix 9: Himedia zone size Interpretation chart

Antimicrobial Agent	Symbol	Disc Content	Interpretative Criteria		
			Sensitive (mm or more)	Intermediate (mm)	Resistant (mm or less)
Ceftriaxone	CRO	30mcg	21	14-20	13
Ciprofloxacin	CIP	5mcg	21	16-20	15
Trimethoprim-sulphamethazole	SXT	5mcg	16	11-15	10
Gentamicin	GEN	10mcg	15	13-14	12
Methicillin	MET	5mcg	14	10-13	9
Vancomycin	VA	30mcg	17	15-16	14

Appendix 10: Original work

Diameter of inhibition zones of various discs of antibiotics against *Staphylococcus* isolates

Antibiotic isolates	Met	VA	CIP	CRO	SXT	GEN
<i>S. lugdunensis</i> ¹	20(S)	24(S)	43(S)	20(I)	39(S)	26(S)
<i>S. lugdunensis</i> ²	20(S)	25(S)	31(S)	14(I)	0(R)	15(S)
<i>S. lugdunensis</i> ³	18(S)	18(S)	34(S)	9(R)	23(S)	20(S)
<i>S. lugdunensis</i> ⁴	20(S)	18(S)	16(I)	11(R)	17(S)	21(S)
<i>S. lugdunensis</i> ⁵	20(S)	20(S)	27(S)	9(R)	9(R)	21(S)
<i>S. lugdunensis</i> ⁶	18(S)	23(S)	40(S)	10(R)	23(S)	25(S)
<i>S. lugdunensis</i> ⁷	19(S)	18(S)	25(S)	10(R)	0(R)	19(S)
<i>S. haemolyticus</i> ¹	20(S)	21(S)	27(S)	10(R)	0(R)	23(S)
<i>S. haemolyticus</i> ²	18(S)	18(S)	30(S)	13(R)	0(R)	17(S)
<i>S. haemolyticus</i> ³	0(R)	15(I)	19(I)	0(R)	15(I)	9(R)
<i>S. haemolyticus</i> ⁴	17(S)	19(S)	26(S)	12(R)	0(R)	19(S)
<i>S. haemolyticus</i> ⁵	0(R)	19(S)	10(R)	10(R)	21(S)	20(S)
<i>S. hominis novo.</i> ¹	12(I)	25(S)	33(S)	10(R)	31(S)	18(S)
<i>S. hominis novo.</i> ²	18(S)	27(S)	34(S)	18(I)	0(R)	32(S)
<i>S. hominis novo.</i> ³	16(S)	25(S)	14(R)	12(R)	31(S)	31(S)
<i>S. hominis hom.</i> ¹	21(S)	24(S)	37(S)	18(I)	7(R)	28(S)
<i>S. hominis hom.</i> ²	21(S)	26(S)	35(S)	20(I)	0(R)	29(S)
<i>S. intermedius</i> ¹	0(R)	0(R)	31(S)	22(S)	23(S)	16(S)
<i>S. intermedius</i> ²	19(S)	17(S)	25(S)	10(R)	22(S)	15(S)

<i>S. saprophyticus</i> ¹	14(S)	23(S)	39(S)	14(R)	0(R)	15(S)
<i>S. saprophyticus</i> ²	16(S)	18(S)	26(S)	15(I)	0(R)	22(S)
<i>S. pateuri</i> ¹	0(R)	22(S)	30(S)	0(R)	15(I)	28(S)
<i>S. pateuri</i> ²	19(S)	19(S)	39(S)	11(R)	0(R)	21(S)
<i>S. caprae</i> ¹	11(R)	25(S)	30(S)	10(R)	0(R)	10(R)
<i>S. caprae</i> ²	0(R)	8(R)	26(S)	0(R)	10(R)	16(S)
<i>S. aureus aureus</i>	21(S)	19(S)	32(S)	16(I)	24(S)	22(S)
<i>S. epidermidis</i>	24(S)	23(S)	20(I)	27(S)	28(S)	30(S)
<i>S. capitis urea.</i>	14(S)	20(S)	23(S)	10(R)	0(R)	24(S)
<i>S. schleiferi</i>	0(R)	9(R)	28(S)	0(R)	11(I)	15(S)
<i>S. piscifermentans</i>	0(R)	18(S)	38(S)	12(R)	18(S)	29(S)
<i>S. arlettae</i>	0(R)	11(R)	25(S)	18(I)	0(R)	18(S)
<i>S. vitulins</i>	19(S)	20(S)	26(S)	8(R)	0(R)	24(S)
<i>S. lutrae</i>	0(R)	21(S)	35(S)	9(R)	0(R)	23(S)

Key

MET=Methicillin **VA**=Vancomycin **CIP**=Ciprofloxacin **CRO**=Ceftriaxone

SXT= Trimethoprim-sulphamethazole **GEN**= Gentamicin. **S**= Sensitive

R= Resistant **I**= Intermediate