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Sudan University of Science and Technology

College of Graduate Studies

Determination of Antibiogram of *Pseudomonas aeruginosa* Isolated from Renal Failure Patients on Haemodialysis in Khartoum State

تحديد مرتسم المضادات الحيوية للزائفة الزنجارية المعزولة من مرضى الفشل الكلوي تحت الغسيل الدموي في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the Requirements of MSc in Medical Laboratory Science (Microbiology)

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الآية

قال تعالى:

بسم الله الرحمن الرحيم

(ٱقۡرَأۡ بِٱسۡمِ رَبِّكَ ٱلَّذِي خَلَقَ ۝١ خَلَقَ ٱلۡإِنسَٰنَ مِنۡ عَلَقٍ ۝٢ ٱقۡرَأۡ وَرَبُّكَ ٱلۡأَكۡرَمُ ۝٣

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صدق الله العظيم

سورة العلق، الآيات (1-5)

DEDICATION

To my parents,

husband,

sons,

brothers and sister

ACKNOWLEDGEMENT

First of all, thanks to ALMIGHTY ALLAH for giving me the power and willing to complete this study.

I heartily convey my sincere regards, respect and deepest gratitude to my supervisor **Prof. Humodi Ahmed Saeed** for encouragement, guidance and support from selection of the topic up to completion of this dissertation.

I would like to express my respect and thanks to all teaching staff of Microbiology Department for their kind help and support.

My thanks are extended to all friends for their co-operation and help during my work.

ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*) infection in renal failure patients undergoing haemodialysis constituted a true health problem. This study was essentially designed to determine antibiogram of *P. aeruginosa* isolated from haemodialysis patients. The study was carried out in the period from February to May, 2015.

The isolates *P. aeruginosa* were obtained from the Research Laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology (SUST).

The isolates were checked for purity by streaking on nutrient agar and examined microscopically. Re-identification was done by conventional microbiological methods, including colonial morphology, Gram's stain and biochemical tests. Antibiogram of the isolates was done by disk diffusion method, the antibiotics used were Ciprofloxacin, Imipinem, Gentamicin, Amikacin and Ceftriaxon.

Re-identification of the isolates confirmed that all isolates (n=12) were *P. aeruginosa*. Study on antibiogram of these isolates revealed 100% susceptibility to Ciprofloxacin and Imipenem, 83.3% to Gentamicin, 75% to Amikacin and 58.3% to Ceftriaxone.

The study concluded that *P. aeruginosa* in haemodialysis patients can be treated successfully using antipseudomonal drugs. Regular check up of antibiogram among large number of clinical isolates of *P. aeruginosa* recovered from hemodialysis patients is highly recommended.

المستخلص

الإصابة بالزائفة الزنجارية لدى مرضى الفشل الكلوي الذين تحت الغسيل الدموي تشكل مشكلة صحية حقيقية. هذه الدراسة صممت لتحديد حساسية ومقاومة الزائفة الزنجارية المعزولة من مرضى الغسيل الدموي للمضادات الحيوية. وقد أجريت الدراسة خلال الفترة من فبراير إلى مايو 2015 .

تم الحصول على عزلات الزائفة الزنجارية من مختبر البحوث بكلية علوم المختبرات الطبية بجامعة السودان للعلوم والتكنولوجيا. تم التحقق من نقاوتها بتزريعها في وسط الأجار المغذي وفحصها مجهرياً. إعادة تحديد الهوية أجريت بالطرق الميكروبيولوجية التقليدية وقد شملت شكل المستعمرة، صبغة جرام والاختبارات البيوكيميائية، وقد أجريت اختبارات التحسس للمضادات الحيوية للعزلات بطريقة الانتشار من القرص.

إعادة التعرف على العزلات أكدت أن كل العزلات وعددها (12) هي الزائفة الزنجارية. واختبار التحسس للمضادات الحيوية للعزلات أظهر أنها حساسة 100% للسيبروفلوكساسين والاميبنيم و83.3% للجنتاميسين و75%للاميكاسين و58.3% للسيفترايكزون.

وخلصت الدراسة أن الزائفة الزنجارية لدى مرضى الغسيل الدموي يمكن معالجتها بمضادات الزوائف، ويوصى بالمتابعة الدورية لاختبارات التحسس للعزل السريري للزائفة الزنجارية من مرضى الغسيل الدموي.

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ABBREVIATIONS

ABGM	Antibiogram
BSI	Blood stream infection
CVD	Cardiovascular disease
CDC	Centra for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
ESRD	End stage renal disease
HD	Heamodialysis
MDR	Multidrug resistant
PQS	*Pseudomonas* quinolone signal
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

The name *Pseudomonas* referred to Professor Migula of the Karlsruhe Institute in Germany at the very end of the nineteenth century. His description of the new genus was short and inaccurate, It reads: 'Cells with polar organs of motility. Formation of spores occurs in some species, but it is rare (for instance: *Pseudomonas violacea).* That was all. Now it is well know that *Pseudomonas* strains do not produce spores. Shortly afterwards *Pseudomonas pyocyanea* was proposed, and later renamed *P. aeruginosa* (Palleroni, 2010).

Pseudomonas species are Gram-negative, motile, aerobic rods some of which produce water-soluble pigments. *Pseudomonas* occur widely in soil, water, plants, and animals. *P. aeruginosa* is frequently present in small numbers in the normal intestinal flora and on the skin of humans and is the major pathogen of the group. Other *Pseudomonas* infrequently cause disease. The classification of *Pseudomonas* is based on rRNA/DNA homology and common culture characteristics(Brooks *et al.,* 2007).

Occasionally, *P. aeruginosa* can colonise human body sites, with a preference for moist areas, such as the perineum, axilla, ear, nasal mucosa and throat; as well as stools. The prevalence of colonization by *P. aeruginosa* in healthy subjects is usually low, but higher colonization rates can be encountered

following hospitalization, especially amongst subjects treated with broad-spectrum antimicrobial agents. Colonization is common in the respiratory tract of mechanically ventilated patients, in the gastrointestinal tract of patients receiving anticancer chemotherapy, and on the skin of burn patients. Also, sinks, mops, disinfectant solutions, respiratory equipment, food mixers and other moist environments can act as reservoirs of *P. aeruginosa* in the hospital setting (Rossolini and Mantengoli., 2005).

P. aeruginosa is an important nosocomial pathogen, especially in individuals with neutronpenia and those who are immune compromised. During the 1960s, when *P. aeruginosa* first emerged as a common cause of gram-negative bacteremia and effective anti pseudomonal antibiotics were unavailable, the mortality rate was 90%. As anti pseudomonal antibiotics were introduced, treatment outcomes in cases of *P. aeruginosa* bacteremia improved. 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).

Pseudomonas infection is clinically indistinguishable from other forms of gram-negative bacterial infection. For this reason, patients with *Pseudomonas* infection might receive empirical antibiotics that are inactive against *Pseudomonas*, especially before antibiotic susceptibility results become available (Kang *et al.*, 2003).

The catheters infected with the greatest frequency are the central intravenous catheters. The microorganisms most frequently involved are gram positive (*Staphylococcus epidermidis* and *Staphylococcus aureus*), fungi (*Candida* sp), and gram negative (*Pseudomonas aeruginosa*) (Rello *et al*., 2000**)**.

The Clinical and Laboratory Standards Institute (CLSI) defines an antibiogram (ABGM) as an overall profile of antimicrobial susceptibility results of a microbial species to a battery of antimicrobial agents, which should reflect patient care needs along with the institution's formulary. When properly prepared and interpreted, ABGMs are an important resource for healthcare providers. While patient-specific cultures and susceptibility reports are pending, the ABGM may guide empirical therapy decisions based on likely pathogens and their probable susceptibilities to anti-infectives available at the institution (Zapantis *et al*., 2005).

Antibiotics are a group of medicines that are used to treat infections caused by germs (bacteria and certain parasites) (Jenny, 2014).

The risk of death in patients with end stage renal disease (ESRD) remains high, despite advances in dialysis care. The primary cause of death is typically cardiovascular disease (CVD), but infections and non-vascular sudden cardiac death also contribute significantly (Wagner *et al*., 2011).

Optimal survival and the quality of life of patients with (ESRD) on long term haemodialysis (HD) is largely dependent upon the adequacy of dialysis through

an appropriately placed and properly functioning permanent vascular access with minimal mechanical complications and infection rates. Vascular access-related blood-stream infections and related complications requiring hospitalization, account for nearly one third of the cost of ESRD management with reported mortality rates of 12–25.9% (Saxena and Panhotra, 2005).

1.2. Rationale

End-stage renal disease is a significant social and economic burden on the Sudan. And hemodialysis patients are immune suppressed, which increases their susceptibility to infection, which is a frequent cause of rehospitalization and the second leading cause of death in chronic renal patients on hemodialysis. The central venous catheter is largely responsible in the majority of cases. *P. aeruginosa* is an important nosocomial pathogen with its ability to propagate on medical devices, hospital environment and even in disinfectants, Infections due to this virulent organism are difficult to both control and treat because it is clinically indistinguishable from other forms of gram-negative bacteria. For this reason, patients with *Pseudomonas* infection might receive empirical antibiotics that are inactive against *Pseudomonas,*

1.3. Objectives

1.3.1. General objective

To determine antibiogram of *P. aeruginosa* isolated from haemodialysis patient in Khartoum State.

1.3.2. Specific objectives

1. To re identify the isolates of *P. aeruginosa*.

2. To perform susceptibility test using different antimicrobial agents against *P. aeruginosa* isolates.

CHAPTER TWO

LITERATURE REVIEW

CAPTER TWO

LITERATURE REVIEW

The total number of people with chronic kidney disease in Sudan is estimated to be about 7% of the adult population (unpublished data from our university), while end-stage renal disease is estimated to affect 5400 new cases per year (estimated incidence is150 new cases per million population per year (Elhassan *et al*., 2007). Infection is the leading cause of morbidity and the second most frequent cause of mortality among patients on renal replacement therapy. North American data show that rates of bloodstream infection (BSI) in hemodialysis patients range from 0.5 to 27.1/100 patients/month, depending on the type of access used. More recent studies demonstrated a reduction in the incidence of BSI in this population from 1.09 to 0.89/100 patients/month and from 2.04 to 0.75/100 patients/month after implementation of specific control measures (Fram *et al*., 2014).

Bacterial infections caused by Gram-negative bacilli, are considered as frequent infectious complication of haemodialysis and a major cause of morbidity and mortality among haemodialysis patients. However, the prevalence of antibiotic resistance among non-fermentative Gram-negative bacteria increases rapidly in healthcare facilities, including haemodialysis

units. Numerous outbreaks of Gram-negative bacterial infections in the haemodialysis setting have been reported (Arvanitidou *et al*., 2003) .

Antibiotics Natural or synthetic compounds that either kill (bactericidal) or inhibit growth (bacteriostatic) of bacteria (or other microorganisms) Antibiotics may be classified in several ways. Most common classification schemes are based on chemical structure of the antibiotic (Ware, 2011). Antimicrobial drugs act in one of several ways: by selective toxicity, by inhibition of cell membrane synthesis and function, by inhibition of protein synthesis, or by inhibitionof nucleic acid synthesis (Brook *et al*., 2007).

The original method of determining susceptibility to antimicrobials was based on broth dilution methods, which although still the gold standard today, are time consuming to perform. This prompted the development of a disk diffusion procedure for the determination of susceptibility of bacteria to antimicrobials (Hudzicki, 2013).

By the early 1950s, most clinical microbiology laboratories in the United States had adopted the disk diffusion method for determining susceptibility of bacteria to antimicrobials. Each lab modified the procedure to suit its own needs, which included using different types of media, inoculums concentration, incubation time, incubation temperature, and concentration of the antimicrobial compound. Interpretation of susceptibility and resistance

was based only on the presence or absence of a zone of inhibition surrounding the disk, and two or three different concentrations of the same antimicrobial were routinely tested against the pathogen. Many researchers published variations for the procedure resulting in multiple protocols that resulted in widespread confusion. In1956, Kirby and his colleagues at the University of Washington School of Medicine and the King County Hospital proposed a single disk method for antimicrobial susceptibility testing (Hudzicki, 2013).

The proportion of healthcare-associated infections caused by multidrug-resistant pathogens is increasing. This epidemiological and microbiological phenomenon poses an infection-control challenge and is a significant threat to patient safety compared with infections caused by susceptible strains of the same organism, infections caused by several antibiotic-resistant bacteria have been associated with worse outcomes, including longer hospitalizations, higher mortality rates, and greater healthcare expenditures. these poor outcomes are likely multi factorial in etiology, including greater severity of underlying illness, delays in initiation of effective therapy, and, in some cases, a lack of effective antimicrobial therapy (Patel *et al.*, 2008).

Clinically significant infections with *P. aeruginosa* should not be treated with single-drug therapy, because the success rate is low with such therapy and because the bacteria can rapidly develop resistance when single drugs are employed (Brooks *et al.,* 2007).

A penicillin active against *P. aeruginosa,* ticarcillin or piperacillin is used in combination with an amino glycoside, usually tobramycin. Other drugs active against *P. aeruginosa* include aztreonam, imipenem, and the newer quinolones, including ciprofloxacin. Of the newer cephalosporins, ceftazidime and cefoperazone are active against *P. aeruginosa,* ceftazidime is used in primary therapy of *P. aeruginosa* infections. The susceptibility patterns of *P aeruginosa* vary geographically, and susceptibility tests should be done as an adjunct to selection of antimicrobial therapy (Brooks *et al., 2007*).

The first case of Multidrug-resistant *P. aeruginosa* strain was isolated in the hematologic unit in 1992. After that, the hospital prevalence of MDR strains increased significantly from 8% in 1993 to 17% in 1999 (Tacconelli *et al.*, 2002).

Pseudomonas aeruginosa is inherently resistant to many antimicrobial agents owing to impermeability, multi-drug efflux and a chromosomal Amp C *β*-lactamase. Useful activity is seen only among alpha carboxy and amino-penicillins, third and fourth generation cephalosporins, monobactams,

carbapenems, amino glycosides and fluoro quinolones. Resistance to each of these drug classes can arise by various mutations causing up regulation of efflux or down regulation of permeability or, in the case of amino penicillins and cephalosporins, via hyper production of the chromosomal Amp C *β*-lactamase. Resistance to *β*-lactams and aminoglycosides can also arise by the acquisition of plasmids, transposons or integrons encoding *β*-lactamases, or amino glycoside-modifying enzymes. Recent interest has also centred on the emergence of carbapenemases in *P. aeruginosa* (Henwood *et al.,* 2001). A retrospective study of clinical records was conducted for cases between January 2005 and January 2009 where all episodes of catheter related bacteremia in the preceding 4 years were a subject of this study. The study included 93 chronic hemodialysis patients from Prince Salman Center for Kidney Diseases (PSCKD), confounded fifty-two episodes of catheter-related bacteremia occurred in 93 patients during the study period, and single species of Gram-negative rod was isolated in 17 of 52 episodes (32.7%), 4 (7.69%) of them was *P. aeroginosa* (Al saran *et al.,* 2013). The retrospective nested case–control study conducted in São Paulo, Brazil. To investigated the risk factors for morbidity and mortality related to BSI in patients on hemodialysis, from January 2010 to June 2013. The study subject were 221 patients in the hemodialysis program during the study period, and 93 (42.1%) had BSI. The study was conducted that Gram negative bacteria

accounted for15.7% (n=5) with 80% showing multiple resistance (Fram *et al*., 2014).

A prospective cohort study performed in Rome, Italy in period between 1990-1999 undertaken to determine the prevalence and epidemiology of multidrug-resistant *P. aeruginosa* bloodstream infections. Among 358 patients with *P. aeruginosa* bacteremia confined that 14% of *P. aeruginosa* bloodstream infections are multidrug resistant (Tacconelli *et al*., 2002). In a cross sectional descriptive study conducted in Hashemi-nejad of Iran university, to determine the frequency of Hemodialysis catheter related infections, studied 116 patients with HCRI between 2003-2004. Pathogenic organisms isolated from blood cultures were *P. aeruginosa* 4% (Sanavi *et al*., 2007).

In study conducted in India to assess the prevelance and resistance pattern of *P. aeruginosa* in tertiary care hospital from Odisha. Total of 319 *P. aeruginosa* isolated from 3783 culture positive clinical samples. 47% were multidrug resistant strains of *P. aeruginosa* isolated from positive blood culture. All MDR stains were sensitive to Imipenem , polymixin B, Colistin (Pathi *et al*., 2013).

Study was conducted over 6 month in university Kebangsaan Malaysia Medical Center. It was across-sectional study which include ESRD patient with the diagnosis HD CRBSIs. Out of 28 cases with suspected HD CRBSIs, 18 patient were confirmed to HD CRBSIs. 11(44.4%) patient had Gram

negative infection 2 (9.52%) of them were *P. aeruginosa*, and Cefepime was the most effective antibiotic with 100% sensitivity to *P. aeruginosa*. This was followed by 50% to amikacin, 50% to ceftazidime, 50% to Gentamicin (Abdul Gafor *et al*., 2014).

Cross sectional analytical study was conducted in tertiary care hospital in UP at 2014. Aimed to comparing the antibiogram of hospital and community acquired *P. aeruginosa* infection and determination of ESBL production on the same isolate. Total of 90 isolate of *pseudomonas* from various sample. All isolates (100%) were sensitive to imipenim, 40% to Ceftaxidime, 61.54% to Gentamicin (Sarwat *et al*., 2014).

In a cross-sectional study conducted in the São Paulo, Brazil in 2013. Aimed to identify the microorganisms isolated on the pericatheter skin, catheter tip and blood stream of patients on hemodialysis by central venous catheter. 128 microorganisms were isolated in the bloodstream in the 94 patients studied. Among the Gram-negative microorganisms, *P. aeruginosa* were prevalent (40%), and most frequently isolated in the blood (43%), and *P. aeruginosa* was 100% sensitive only to clavulanic acid and tazobactam (Esmanhoto *et al*., 2013).

1n a prospective case-control study was performed at a tertiary care hospital in Ankara, in 2004 to determine the risk factors for nosocomial infections of imipenem-resistant *P. aeruginosa*. During the study period, *P. aeruginosa*

infections were detected in 170 patients, of the 170 patients, 75 (44.1%) had imipenem-resistant *P. aeruginosa* (Onguru *et al.,* 2008).

A study in United Kingdom conducted to establish the prevalence of antibiotic resistance among clinical isolates of *P. aeruginosa*. The *P. aeruginosa* susceptibility to amikacin, gentamicin, ceftazidime, imipenem and ciprofloxacin was 94.4%, 88.9%, 97.7%, 91.1% and 91.1% respectively (Henwood *et al.,* 2001).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE
MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a laboratory-based experimental study conducted to determine antibiogram of *P. aeruginosa* isolated from renal failure patients undergoing haemodialysis in Khartoum State hospitals.

3.1.2. Study area

The study was done 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 in the period from February to May, 2015.

3.1.4. Source of *Pseudomonas* isolates

The isolates were obtained from the Research Laboratory, SUST. The isolates were previously recovered from renal failure patients undergoing heamodialysis in different hospitals in Khartoum State.

3.2. Sample size

A total of twelve (n=12) bacterial isolates were used in this study.

3.3. Methods

3.3.1. Checking purity of the isolates

The isolates (n=12) were streaked on nutrient agar plates and examined microscopically for purification then a discrete colony was picked up and sub-cultured on nutrient agar (Appendix 1) slope for performing Gram stain and biochemical tests.

3.3.2. Re-identification of the isolates

3.3.2.1. Gram's stain (Appendix 2)

Bacterial smear was prepared by transferring portion of discrete colony to a drop of normal saline. The smear was covered with crystal violet stain for 30-60 seconds, rapidly washed off the stain with clean water, then the smear was covered with lugol's iodine for 30-60 seconds, washed off the iodine with clean water, decolorized rapidly (few seconds) with acetone-alcohol, washed immediately with clean water, then the smear was covered with safranine for 2 minutes, washed off the stain with clean water, wiped back of the slide clean and placed it in draining rack for the smear to air dry, the smear was examined microscopically with the oil immersion objective to report bacterial Gram reaction and cells shape. Gram positive bacteria; stain dark purple, Gram negative bacteria; stain red (Cheesbrough, 2006).

3.3.2. 2. Biochemical tests

3.3.2.2.1. Oxidase test

This test is used to determine bacteria that produce oxidase enzyme which oxidized the oxidase reagent (tetramethyl-ρ-phenylenediaminedihydrochloride) to give a dark-blue color. The test was performed by commercial discs impregnated with the oxidase reagent; a pure colony was smeared on the disc by sterile wooden stick. A positive reaction was indicated by developing deep blue color within 10 seconds (Cheesbrough, 2006).

3.3.2.2.2. Citrate utilization test

The test is based on the ability of an organism to use citrate as its only source of carbon**.** The test was performed by prepared Simmons's citrate agar (Appendix 3) according manufacture and inoculated with the organism under test by using straight wire then incubated at 37^{o}C for 24 hours and checked, bluing of the media was observed (Cheesbrough, 2006).

3.3.2.2.3. Sugar fermentation, gas and H_2S production

A tube of Kligler Iron Agar (Appendix 4) was inoculated using a sterile straight wire, first the butt was stabbed then the slope was streaked and incubated at 35–37°C overnight. Lactose fermenting bacteria was appeared as yellow butt and yellow slope, glucose fermenting bacteria was appeared as yellow butt and red slope, non-lactose and non- glucose fermenting bacteria was appeared as red butt and red slope, blackening in the media indicated hydrogen sulphide

production and cracks in the medium was due to gas production (Cheesbrough, 2006).

3.3.2.2.4. Urease test

The organism under test was inoculated in a medium which contained urea and the indicater phenol red (Appendix 5). When the strain was urease producing, the enzyme will hydrolyzed the urea to give ammonia and carbon dioxide. With the release of ammonia, the medium became alkaline as shown by a chang in color of the indicator to pink-red. The organism under test was stabbed by straight wire in urea slop medium and incubated overnight, chang in color to pink indicated a positive test (Cheesbrough, 2000).

3.3.2.2.5. Indole production

The test organism was cultured in a medium which contained tryptophan. Indole production was detected by kovac's reagent (Appendix 6), which contained *P*-dimethyl aminobenzaldehyde which reacted with indole to produce a red colored compound. The organism under test was inoculated in peptone water, then incubated at 35-37°C overnight. The detection of indole was done by addition of kovac's reagent which gave red ring with 10 minutes in a positive test while it gave a yellow to green in the negative test (Cheesbrough, 2000).

3.3.2.3. Motility test

The organism were taken by straight wire loop and stabbed into the medium making a single stab about 1-2 cm down into the medium. The tubes were incubated over night at 37c.The motility was indicated by the presence diffuse growth (appearing as the clouding of the medium away from the line of inoculation) .

3.3.2.4. Growth at 42^0C and 4^0C

Under a septic condition nutrient agar was inoculated with the organism under test, then incubated at 42^oC and 4^oC for 24 hours and checked.

3.3.2.5. Oxidative –fermentation test

The acids formed in oxidative degradation of glucose extremely weak, and the more sensitive oxidation fermentation medium (Appendix 7) is required for their detection, OF medium poured into Tow tubes, each inoculated with the organism under test using straight needle, the medium were stabbed three to four times half way to the bottom of the tube, one tube of each pair were covered with a 1cm layer of sterile mineral oil, leaved the other tub open to the air. Incubated each tube at 35^oC and examined daily for several days. Acid production was detected in the medium by the appearance of a yellow color in covered tube (Washington *et al*., 2006) .

3.3.3. Susceptibility testing

This was done by NCCLS Kirby Bauer disc diffusion technique according to WHO (1996) and NCCLS (1997)

3.3.3.1. Culture media

Sterilized Muller-Hinton agar(Appendix 8) (PH 7.4±2) was prepared, cooled to 45-50^{o}C, and poured in sterile, dry Petri plates on a level surface, to depth of 4mm. The presence of any excess surface moister on the medium was removed by keeping the plates inverted in an incubator at (35-37^{o}C).

3.3.3.2.Antibiotics

Discs containing the following antibiotics were obtained from (Bioanalyse-turkey). Ciprofloxacin (CIP) 5µg, Gentamicin (CN) 10µg, Ceftriaxon (CRO) 30µg, Amikacin (AMK) µg30, Impenem(IMP) 10µg.

3. 3.3.3. Preparation of inoculums

The inoculums was prepared by direct colony suspension method. Five well isolated colony(of similar appearance) of the organism to be tested were touched with sterile loop and the growth was transferred to sterile saline ,then the inoculums turbidity was adjusted by using McFarland standard turbidity (Cheesbrough., 1989).

3.3.3.4. Inoculation of Muller Hinton agar

The plate were inoculated by dipping a sterile cotton swabs into the inoculums. The excess inoculums was removed by pressing rotating the swab firmly against the side of the tube above the level of the liquid. The swab was seeded evenly over the surface of the medium in three direction ,the inoculums was left to dry, with lid closed for a few minutes at room temperature.

3.3.3.5. Antimicrobial disc application

By using a pair of sterile forceps, antibiotic disc were applied, and evenly distributed on the inoculated plate(9cm plate). The plate was inverted and incubated aerobically at 37^{o}C for 18-24 hours.

3.3.3.6. Reading and interpretation

The diameter of each zone of inhibition (including the diameter of the disc) was measured to nearest millimeter by using ruler. The susceptibility of isolates was reported according to the manufactures standard zone size interpretative manual. Sensitive organisms were when the zone of inhibition was equal to or greater than the standard.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of twelve (n=12) bacterial isolates were obtained from the Research Laboratory (SUST). These were previously recovered from renal failure patients undergoing heamodialysis in different hospitals in Khartoum State. Re-identification of these isolates revealed that all isolates belonging to *P. areuginosa*. Biochemical tests, growth at 42^0C, oxidative -fermentation test, and motility test were adopted for re-identification. The results were tabulated in table (1).

All isolates were processed for susceptibility testing using disk diffusion method. The results showed that susceptibility of *P. areuginosa* to different antibiotics as follows; Imipinem and Ciprofloxacin 100%, Gentamicin 83.3%, Amikacin 75%, Ceftriaxon 58.3% (Table2).

Table 1. Biochemical tests, growth at 42^0C and motility test adopted for re-identification

Isolate code	Biochemical tests							GrowTh at 42^0C	OF test	Motility test	Suggested organism
	Indole	Urease	Citrate	KIA							
				Slope	Butt	H_2S	Gas				
P1	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P3	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P4	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P5	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P6	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P7	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P8	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P9	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P10	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P11	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*
P12	–	–	+	R	R	–	–	Growth	Oxidative	Motile	*P. aeruginosa*

Key

– =Negative reaction

\+ =Positive reaction

R =Red

Table 3. Antibiogram of *P. areuginosa*

Antibiotics	Susceptibility	Intermediate	Resistance
CIP 5µ	100%	0%	0%
IMP 10µ	100%	0%	0%
CN 10µ	83.3%	16.7%	0%
AMK 30µ	75%	0%	25%
CRO 30µ	58.3%	0%	41.7%

Key: **CIP**=Ciprofloxacin, **CN**=Gentamicin, **CRO**=Ceftriaxon, **AMK**=Amikacin, **IMP**=Imipineme

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Pseudomonas aeruginosa (*P. aeruginosa*) is an important pathogen for heamodialysis patient. The occurrence of infection caused by resistent microorganism constitutes a public health problem. Resistant bacteria such as *P. aeruginosa* have become increasingly common in health care institutions. The present study was done to determine the antibiogram of *P. aeruginosa* isolated from renal failure patients undergoing heamodialysis in different hospitals in Khartoum State.

The result revealed that *P. aeruginosa* was sensitive 100% to Imipinem. This result is in a agreement with that obtained by Pathi *et al*., (2013) in India and Sarwat *et al*., (2014) in India. But disagrees with that obtained by Abdul-Gafor *et al*., (2014) in Malaysia and Onguru *et al*., (2008) in Turkey and Henwood *et al.,* (2001) in United Kingdom who reported susceptiblity to Imipinem as 50%, 55.9% and 91.9% respectively.

In this study the susceptibility of *P. aeruginosa* to Ciprofloxacin was 100%. This result is completely agrees with that obtained by Abdul Gafor *et al*., (2014). The finding of the present study disagree with that of Henwood *et al*., (2001) who reported susceptibility to Ciprofloxacin as 91.1%.

On other hand, the susceptibility of *P. aeruginosa* to Gentamicin in the present study was 83.3%. This result is higher than that obtained by Abdul Gafor *et al*., (2014) and Sarwat *et al*., (2014) who reported the susceptibility of *P. aeruginosa* to Gentamicin as 50% and 61.54% respectively, but less than that obtained by Henwood *et al.,* (2001) who reported the susceptibility to Gentamicin as 88.9%

The susceptibility of *P. aeruginosa* to Ceftriaxon was 58.3%. This result in line with that obtained by Abdul-Gafor *et al*., (2014) who reported susceptibility of *P. aeruginosa* to Ceftazidim as 50%. But the finding of the present study was less than that obtained by Henwood *et al.,* (2001) who reported the susceptibility to Ceftazidim as 97.7%.

Moreover, 25% of *P. aeruginosa* were resistant to Amikacin. This result disagrees with that reported by Onguru *et al*., (2008), Abdul Gafor *et al*., (2014) and Henwood *et al.,* (2001) who reported that 70%, 50% and 5.6% of *P. aeruginosa* isolates were resistant to Amikacin respectively.

5.2. Conclusion

The study concluded that:

1. Members of antiseudomonal remain with high potency
2. *P. aeruginosa* were completely susceptible to Ciprofloxacin and Imipinem (100%).

5.3. Recommendations

1. Screen a large number of antibiotics.
2. Further study with large number of isolates is recommended to validate the result of this study.

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REFERENCES

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APPENDICES

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 ^{o}C.

2. Gram's stain

Crystal violet

Approximate formula * per liter

Crystal violet…….…….20.0g

Ammonium oxalate…….…….9.0g

Ethanol, obsolute……95ml

Distilled water……..……...to 1 liter

Lugol s iodine

Approximate formula * per liter

Potassium iodine……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 liter

Saffranin……2.5g

95% ethanol…….……10ml

Distilled water……to 100ml

3. **Simmons's citrate medium**

Koser's medium……..1 litre

Agar…….....20.0g

Bromothymol blue,0.2%.....40ml

4. **Kliglar iron agar(KIA)**

Approximate formula * per liter

Lab-Lemco powder…….....3.0g

Yeast extract…….....3.0g

Peptone…….....20.0 g

Sodium chloride…….....5.0 g

Lactose…….....10.0 g

Dextrose(gluocose)……..1.0 g

Ferric citrate…….....0.3 g

Sodium thiosulphate…….....0.3 g

Phenol red……..0.05 g

Agar……12.0 g

5. Christensen's urea agar

Glucose……....5.0g

Sodium chloride……..……5.0g

Potassium dihydrogen phosphate…….....2.0g

Peptone…….....1.0g

Agar……..20.0g

Distilled water…….1.0litre

6. kovac s reagent

Approximate formula * per liter

Amyle or isoamyl alcohol……15ml

P-Dimethyl-aminobenzaldehyde……..…..10g

Hydrochloric acid concentrated……..……...50ml

7. Oxidative fermentation media

Peptone……..……...10g

Sodium chloride……..…….5.0g

D. glucose……..……...5.0g

Phenol…….0.01g

Agar……15g

Distilled water…….....1L

Final PH: 7.0±0.1at 25C^0

8. Muller Hinton Agar

Approximate formula * per liter

Beef Extract……...…..……2g

Acid Hydrolysate of Casein…….……..…17.5g

Starch……..……..……..……1.5g

Agar…….17g

Final PH: 7.3 ±0.1at 25C^0

Appendix 9: Bioanalyse zone size interpretation chart

Antimicrobial Agent	Symbol	Disc content	Interpretative Criteria		
			Sensitive (mm or more)	Intermediate (mm)	Resistant (mm or less)
Ciprofloxacin	CIP	5 mcg	21	16-20	15
Gentamicin	CN	10 mcg	15	13-14	12
Ceftriaxon	CRO	30 mcg	21	14-20	13
Amikacin	AMK	30 mcg	18	14-17	13
Impenem	IMP	10 mcg	19	16-18	15

Appendix 10: Original work

Diameter of inhibition zone of various antimicrobial discs against *pseudomonas areuginosa*

antibiotics / *pseudomonas*	Ciprofloxacin (5µ)	Gentamicin (10µ)	Ceftriaxon (30µ)	Amikacin (30µ)	Impenem (10µ)
P.aeru Co1	40	25	35	40	35
P.aeru Co2	32	14	6	8	25
P.aeru Co 3	33	27	5	20	38
P.aeru Co4	38	26	32	34	45
P.aeru Co5	37	20	38	30	40
P.aeru Co6	36	21	31	28	38
P.aeru Co7	32	14	7	0	25
P.aeru Co8	31	20	12	8	33
P.aeru Co9	32	21	29	38	40
P.aeru Co10	35	23	28	31	38
P.aeru Co11	32	29	9	22	38
P.aeru Co12	32	25	31	31	34

Appendix 13: original work

Oxidative Fermentation test

Sugar NO	Glucose		Maltose		Lactose		Mannitol		Interpretation
	Open tube	Closed tube	Open tube	Closed tube	Open tube	Closed tube	Open tube	Closed tube	
P.aeru Co1	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co2	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co3	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co4	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co5	Pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co6	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co7	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co8	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co9	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co10	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co11	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative
P.aeru Co12	pink	yellow	pink	yellow	pink	pink	pink	yellow	Oxidative

Key: Pink=alkaline ph=no CHO utilization Yellow=acid ph=CHO utilization

P.areu =pseudomonas areuginosa Co=Code