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Sudan University of Science and Technology

College of Graduate Studies

Isolation and Identification of *Pseudomonas* species from Renal Failure Patients in Khartoum State

العزل والتعرف على أنواع الزائفة من مرضى الفشل الكلوي في ولاية الخرطوم

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

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قرآن كريم

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صدق الله العظيم

سورة الرحمن:الآيات ١ ــ٤

DEDECATION

To my dear father.

To my lovely mother.

To my supervisor.

To my lovely brother and friends.

AKNOWLEDGEMENT

All thanks to ALMIGHTY**ALLAH,** for giving me health, strength and power to complete this study.

I would like to express my heartfelt gratitude to my supervisor, **Prof. Humodi Ahmed Saeed**for his endless guidance and for sacrificing his time to read my work. I am extremely grateful for his tireless support, advices and suggestions from the beginning to the end of this study.

My gratitude and thanks to the Administration of Sudan University of Science and Technology, College of Medical Laboratory Science for everythingprovided to me.

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ABSTRACT

*Pseudomonas*species are the most important pathogens for renal failure patients especially those undergoing hemodialysis. The objective of this study was to isolate and identify*Pseudomonas* species fromhemodialysis patients in Khartoum State. The study was conducted during period from March to June 2015.

Patientswith end-stage renal disease treated with maintenance hemodialysis from different centers (Ibn Sina Specialized Hospital, Ahmed Gasem Hospital, ALnaw Hospital,Medical Tropical Disease Hospital,ALwaldin Charity Hospital and Dr.Salma Center) were enrolled. The patients constituted both sexesmale (50) andfemale (59). Structured questionnaire was used for collection of data from each patient.Blood samples were collected and inoculated aseptically in brain heart infusion broth. Thentransported directly to the Research Laboratory of Sudan University of Science and Technology for processing.

Cultivation of 109 blood samples yielded bacterial growth. Isolated bacteria were identified by their colonial morphology, Gram stain and biochemical tests. The results revealed 12(11.0%)*Pseudomonas aeruginosa.*

This study concluded that considerable number of *Pseudomonas aeruginosa*isolated fromhemodialysis patients with bacteremia. Further studies using advanced technique are needed to confirm the results of the present study.

المستخلص

أنواع الزائفة من أهم مسببات العدوى لمرضى الفشل الكلوياللذين يخضعون للإستصفاء الدموي. كان الهدف من هذه الدراسة هو العزل والتعرف على أنواع الزائفةمن مرضى الإستصفاء الدموي في ولاية الخرطوم، أجريت هذه الدراسة في الفترة من مارس حتى يونيو٢٠١٥ .

شارك في الدراسة مرضى الفشل الكلوي في المراحل المتأخرة واللذين يخضعون للعلاج بالإستصفاء الدموي من مراكز مختلفة شملت مستشفى ابن سيناء التخصصي، مستشفى أحمد قاسم، مستشفى النو،مستشفى المناطق الحارة الطبي، مستشفى الوالدين الخيري ومركز الدكتورة سلمى. كان المرضى من كلا الجنسين الذكور(٥٠) والإناث (٥٩). تم إعداد استبيان استطلاعي على المرضى قبل إجراءالإختبار استخدمت لجمع البيانات من كل مريض.جمعت عينات الدم وحقنت مباشرة في مرق منقوع القلب والدماغ في ظروف معقمة، وبعد ذلك نقلت مباشرة إلى معمل الأبحاث في جامعة السودان للعلوم والتكنولوجيا للمعالجة.

تزريع ١٠٩ عينة دم أسفر عنه نموا" بكتيريا"، تم التعرف على البكتريا المعزولة بالشكل الظاهري للمستعمرات، وصبغة الجرام والإختبارات الحيوية الكيميائية. أظهرت النتائج أن الزائفة الزنجارية مثلت ١٢ (١١.٠%).

خلاصة هذه الدراسة ان الرقم الجدير بالإعتبار للزائفة الزنجارية تم عزله من مرضى الإستصفاء الدموي المتجرثم. عدة دراسات متطلبة بإستخدام وسائل تقنية متقدمة لتؤكد النتائج المقدمة في هذة الدراسة.

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Blood Stream Infection..... (BSI)

Catheter Related Bacteria..... (CRB)

Catheter Related Blood Stream Infection..... (CRBSI)

Central Catheter..... (CH)

Central Venous Catheters..... (CVCs)

Chronic Kidney Disease..... (CKD)

Cystic Fibrosis..... (CF)

Ecthyma Gangrenosum..... (EG)

End Stage Renal Disease..... (ESRD)

Gram Negative Bacteria ..... (GNB)

Health care Associated Infection..... (HAI)

Hemodialysis..... (HD)

Incidence rates ..... (IRs)

Peritoneal Dialysis ..... . (PD)

Renal Replacement Therapy..... (RRT)

Urinary tract infection..... (UTI)

Ventilator Associated pneumonia ..... (VAP)

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Renal failure describes a medical condition in which the kidneys fail to filter toxins and waste products from blood adequately; Hemodialysis (HD) is a procedure that is a substitute for many of the normal duties of the kidneys (Hassoon*et al.*, 2013).

Chronic kidney disease (CKD) has high prevalence and incidence worldwide. Difficulties such as lack of early diagnosis, inadequate treatment in the early stages, delayed specialized monitoring, and the complexity of the disease leads many people to need renal replacement therapy (RRT). RRT can be implemented through hemodialysis (HD), perit-oneal dialysis (PD), and renal transplantation. Each treatment has its own characteristics, advantages, disadvantages, and complications (Curty *et al.*, 2014).

Chronic kidney disease (CKD) is a common problem among males compared to females due to stress, alcoholism, hypertension and diabetes mellitus. Due to urinary stagnation, alkalization of urine and absence of flushing action, the presence of urinary tract infection (UTI) in CKD of males is higher compared to normal males (Jaiswal *et al.*, 2013).

Hemodialysis (HD) access-related infections are a majorcause of morbidity and mortality in HD patients; the responsiblemicroorganisms, prevention and treatment strategies,and outcome have been assessed in earlier studies (Yang *et al.*, 2012).

Infection of hemodialysis patients associated with frailty and disability are problems associated with the intravascular connection, white blood cell and complement dysfunction from contact with dialysis membranes, and exposure to bacteria and pyrogens from contaminated dialysis solutions or inadequately cleaned dialysis machines (Berman, 2010).

About 20% of the prevalent hemodialysis patients in the United States use a tunneled central vein catheter for vascular access; Infections are the most serious complication of tunneled dialysis catheters. Catheter-associated bacteremia occurs at a frequency of 2 to 5.5 per 1000 patients (Krishnasami *et al.*, 2002).

Patients with end-stage renal disease (ESRD) undergoes HD are especially prone to acquiring healthcare-associated infections (HAI). This is due to both the dialysis procedure and to the immune compromising effects of the underlying disease (Albuquerquea*et al.*,2014).

Thenumberof patients with ESRD is increasing by 9% per year. Among those undergoing HD, the majority will require at least one hospitalization every year; these patients have an intrinsic susceptibility to developing aninfection as a result of numerous co-morbid conditions, uremic toxicity and anemia of chronic renal failure, all of which contribute to an immune compromised state. In addition, chronic hemodialysispatients depend on vascular access, which increases the risk for developing bacteremia, the nosocomial infection with the greatestmortality rate(Krishnasami*et al.*, 2002).

Gram-negative bacteria (GNB) have been reported in up to 33% of cases with catheter-related bacteremia (CRB) indicating that empiric antibiotic therapy should target both Gram-positive and Gram-negative organism (Yang*et al.*,2012).

Bacterial infections caused by Gram-negative bacilli, especially those involving vascular access, are considered as frequent infectious complication of haemodialysis and a major cause of morbidity and mortality among haemodialysis patients. The most common isolates, in order of frequency were *Pseudomonas aeruginosa* (22.7%), *Chryseobacterium meningosepticum* (14.9%), *Stenotrophomonasmaltophilia*(13.5%), *Escherichia coli* (12.8%) and *Enterobacter cloacae* (7.8%), representing 71.6% of all isolates(Arvanitidou *et al.,* 2003).

1.2. Rationale

Pseudomonas species have great implication on human health. It causes multiple infections arrange of mild and severe infections to more virulent damaging internal forms as in case of bacteriamia and septicemia and high rate of morbidty and mortality among hemodialysis patients.

This research is expected to highlight the problem of infection caused by *Pseudomonas* species in hemodialysis patients in Khartoum State.

1.3. Objectives

1.3.1. General objective

To isolate and identify *Pseudomonas* species in hmodialysis patients in Khartoum State.

1.3 .2. Specific objectives

1- To isolate *Pseudomonas* species from hemodialysis patients.

2- To identify *Pseudomonas* species among hemodialysis patients.

3-To determine the ratio of *Pseudomonas* species which infect hemodialysis patients.

CHAPTER TWO

LITERATURE REVIEW

2.1. Dialysis

Is the process of removing waste productsand excess fluids from the body, there are two types of dialysis: hemodialysis and peritoneal dialysis. Inhemodialysis (HD), blood is removed from the bodyand pumped by a machine outside the body into dialyzer (artificial kidney). Doctors decide to place a person on dialysis when the person's kidney failure is causing certain conditions such as uremic, encephalopathy, pericarditis, acid-osis, heart failure, pulmonary edema and hyperkalemia (Manhal*et al.*, 2012).

Patients with end-stage renal disease (ERSD) requiring dialysis are at increased risk for getting bloodstream infection (BSI). This type of infection represents a main cause of morbidity, as well as a preventable cause of death, along with increased costs and hospitalization (Fysaraki *et al.*, 2013).

Hemodialysis can be performed through a central catheter (CH) inserted in the internal jugular or subclavian vein or through an arterio-venous fistula (AVF) preferably in the upper limbs whose optimal functionality delay varies from one to three months. Noso-comial infection is one of the most serious complications and the second cause of death in dialysis patients. The risk factors that predispose to nosocomial infection in RRT may be influenced by patient characteristics, site of dialysis access and disorders of the skin and mucous membranes and co-morbidities such as diabetes mellitus, anemia, cardio vascular disease, immune-suppression and metabolic imbalances (Curty *et al.*,

2014).Catheter-related bacteremia(CRB) was defined as the occurrence of a positive blood culture from the catheter with or without a positive peripheral blood culture, in the presence of systemic symptoms of infection with no other source of infection identified. Bacteremia was classified as primary in the absence of an identified source growing the same organism(s) as that recovered from blood. When the organism isolated from blood was the same as the organism causing an infection at another site, the Blood Stream Infection was classified as secondary. Exceptions to this were intravascular device-associated BSIs, all of which were classified as primary even if localized signs of infection were present at the access site (Fysaraki *et al.*, 2013).

Infection is the second most common cause of death and hospitalization among hemodialysis patients. Previous prospective studies have focused on the subset of infections causing death or requiring hospitalization without including infections treated in the outpatient setting. Moreover, many publications have included primarily patients with fistulas or grafts. Catheters are used in approximately 80% of patients initiating hemodialysis and 25% of all prevalent patients as a bridge to a permanent vascular access or because the patient has exhausted all options for a permanent access. Catheter-dependent patients are at increased risk for all-cause infection .When catheter-dependent hemodialysis patients present with symptoms suggesting infection; the assumed diagnosis is catheter-related bacteremia (AL Solaiman *et al.*, 2011).

Septicemia is the most frequent complication among patients on HD, especially when conducted through a central venous catheter. The kind of vascular access for HD has

significant influence on patient survival. Catheters are associated with substantially greater risk of septicemia, hospitalization, and mortality compared to arteriove-nous fistula (Curty *et al.*, 2014).

Central venous catheters(CVCs) refer to prolonged vascular access devices indicated for the administration of intravenous medication treatments, fluids, or total parenteral nutrition, repeated blood sampling and for hemodialysis, (CVC) - and HD-catheter usage are associated with complications that occur during catheter insertion, throughout the catheter dwell period and at the time of removal (Napalkov *et al.*, 2013).

Identification and prevention of catheter-related complications is critical to improving patient care. Common complications include catheter misplacement or breakage, catheter occlusion due to local or systemic infection and thrombosis (Napalkov *et al.*, 2013).

The reported incidence rates (IRs) of catheter-related complications vary widely depending on the terminology and definition of complications, patient population, units of measurement, duration of catheterization and follow-up, catheter location, placement and care procedures and diagnostic methods. The most common type of complication is catheterrelated bloodstream infection (CRBSI), with an incidence rate of 0.46 to 30 per 1000 catheter-days, or in 4.3% to 26% of placed catheters (Napalkov *et al.*, 2013).

Catheter-related bacteraemia may arise via two paths: (a) direct spread of microorganisms from the skin along the outside of the catheter leading to contamination of the bloodstream; or, (b) colonisation of the inner lumen of the catheter leading to the formation of biofilm and direct migration of organisms into the bloodstream. A biofilm is

A multi-layered cell cluster with a strong propensity to adhere to polymer surfaces and provides a protected niche environment for microorganisms with physical barrier protection against antibiotics. Within the biofilm, bacteria exhibit increased growth rates, a higher cell density and more active gene transcription. This further contributes to the heightened resistance of bacteria to antibiotics. Even in the absence of overt infection, microbial colonisation of catheters may engender a chronic inflammatory state, which in turn increases the risk of erythropoietin-resistant anemia, malnutrition and cardiovascular disease (Gunatillake *et al.*, 2011).

CVCs get colonised either through extraluminal (skin-related) or intraluminal (hub or perfusate related) routes. In the first case, organisms migrate from the skin insertion site along the catheter up to the catheter tip, finally reaching the blood stream. In the second case the catheter hubs are contaminated during catheter manipulation by dialysis personnel. The colonised bacteria then spread through the lumen of the catheter. For long-term catheters particularly those that are cuffed and/or surgically planted, the hub is a major source of colonisation of catheters (Saxena and Panhotra, 2005).

The development of CR-BSI is facilitated in the presence of certain potential risk factors such as the presence of an under lying disease, poor patient hygiene and poor hygiene of the medical staff, inexperienced medical attendant inserting the catheter, insertion site and method of catheter insertion, duration of catheterization, cumulative number of catheter manipulation and high number of hemodialysis runs. Moreover, CR-BSI often result in serious systemic infections, including endocarditis, osteomyelitis, epidural abscess, septic arthritis, and even death (Caylan *et al.*, 2010).

Gram positive organisms are the most common etiologic agents of bacteremia in this setting; however, infections may also be caused by gram-negative pathogens. The latter are of particular concern due to the absence of new drug development against them (Fysaraki *et al.*, 2013).

Pseudomonas is regarded as a particularly lethal bacterial isolate. High mortality rates have been reported in episodes of *Pseudomonas* sepsis when associated with visceral infections as seen in immune-suppressed, hospitalized patients (Golestaneh*et al.*, 2006).

Pseudomonas infections are an important cause of morbidity and mortality in immune-compromised patients, with *Pseudomonas aeruginosa* (*P. aeruginosa*) being the most common species isolated from clinical specimens (Wong *et al.*, 2011).The organism is a less frequent pathogen associated with catheter infection, accounting for 4–16% of isolates. Nevertheless, this pathogen should always be considered as one potential causative agent of CVC related infections, especially in immune-compromised hosts. Metastatic infectious foci are important determinants of the morbidity and mortality of CVC related infections. Endocarditis, septic embolism, and visceral abscesses are rare but serious complications whose mere suspicion demands careful clinical and radiological search (Caravaca *et al.*, 2014).

2.2. *Pseudomonas*

2.2.1. History

The name *Pseudomonas* comes from the greek and latin and means "false unit"(pseudo = false, greek; monas = single unit, latin). "Monas" was used in the early history of microbiology to describe single-celled organisms. In 1786 Otto Friedrich Muller, from

Copenhagen, classified the bacteria and named the *Pseudomonads*, they came into the group of vibriones (which was defined as group of shaking bacteria (Siegrist, 2010*)*.

Many years later it was detected that *Pseudomonas* are motile. They therefore were given the name "Pseudo", because they appeared to be shaking but in reality they were motile. Because of their widespread occurrence in water, the *Pseudomonads* were characterized and named as one of the first microorganism groups. The organism was defined in rather vague terms in 1894 as a genus of Gram-negative, rod-shaped and polar-flagella bacteria (Siegrist, 2010*)*.

2.2.2. Definition and general characteristics

Pseudomonas are motile (one or more polar flagella), rod shaped and aerobic, Gram-negative, non-fermentative bacteria. The typical bacterial size is 0.5 – 1.0 x 1.5 – 5.0 µm. The catalase test gives a positive result, but in some rare cases species show a negative reaction in the oxidase test, e.g. *P. syringae* (Siegrist 2010*)*.

Another known feature associated with *Pseudomonas* species (e.g. *P. aeruginosa,P. fluorescens, P. putida)* is the secretion of pyoverdin (fluorescein, a siderophore), a fluorescent yellow-green pigment under iron-limiting conditions (Siegrist 2010).

Pseudomonas needs siderophores to build a complexwith iron (III) and to be able to take upiron. Certain *Pseudomonas* species may also produce additional pigments, such aspyocyanin (blue pigment, a siderophore) by *P. aeruginosa* , quinolobactin (yellow, darkgreen in presence of iron, a siderophore) by *P. fluorescens* or/and a reddish pigmentcalled pyorubrin and pyomelanin (brown pigment) by *P. aeruginosa* . On blood agar ahemolytic reaction can be observed (Siegrist, 2010).

They grow well on standard broth and solid media such as blood agar, chocolate agar, and MacConkey agar, which are recommended to isolate *Pseudomonas* species from clinical specimens. Selective agar containing inhibitors such as cetrimide can also be used for isolation and presumptive identification. *Pseudomonas* colonies may be nearly colorless, but white, off-white, cream, and yellow colony pigmentation is common. Fluorescent colonies can be readily observed under ultraviolet light (PHE, 2015).

Pseudomonas comprises a genus of species capable of utilizing a wide range of organic and inorganic compounds and of living under diverse environmental conditions (Moore*et al.*, 2006).

Consequently, they are ubiquitous in soil and water ecosystems and are important as plant, animal and human pathogens. The genus *Pseudomonas* is well known for its metabolic versatility and genetic plasticity. The species of *Pseudomonas*, in general, grow rapidly and are particularly renowned for their ability to metabolize an extensive number of substrates, including toxic organic chemicals, such as aliphatic and aromatic hydrocarbons. Strains of *Pseudomonas* species are often resistant to antibiotics, disinfectants, detergents, heavy metals, and organic solvents. Some strains have been confirmed to produce metabolites that stimulate plant growth or inhibit plant pests (Moore *et al.*, 2006).

Pseudomonas spp. are highly adaptable bacteria that can colonize various environmental niches, including soil and marine habitats, plants and animals. *Pseudomonas* spp. are also opportunistic human pathogens, causing infection of the eyes, ears, skin, urethra and

respiratory tract in cystic fibrosis (CF) in burned patients, as well as other immune compromised individuals (Maschio*et al.*, 2015).

2.2.3. Species

The genus *Pseudomonas* once comprised over 100 species but over the period of a decade many of these have been reclassified into different genera. The main groups of *Pseudomonads* of medical interest are:

The fluorescent or 'true' *Pseudomonas*: *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and*Pseudomonas putida*(PHE, 2014).

Burkholderia spp: Within this genus, there are at least 30 species in the genus but the medically important species are*Burkholderia cepacia*, *Burkholderia pseudomallei* and *Burkholderia mallei*, which are associated with human and animal infection (PHE,2014).

Burkholderia cepacia is an important pathogen of pulmonary infections in people with cystic fibrosis (PHE, 2014).

Burkholderia pseudomallei is the causal agent of melioidosis, a life-threatening septic infection prevalent in Southeast Asia and Northern Australia.*Burkholderia mallei* causes glanders, a rare disease in horses and other species (PHE,2014).

Delftia acidovorans: Occasionally found in clinical specimens and the hospital environment (PHE, 2014).

Brevundimonas spp.: *Brevundimonas diminuta* and *Brevundimonas vesicularis* are rare in clinical specimens and of doubtful clinical significance.

Stenotrophomonas maltophilia: May be clinically significant in severely immune-compromised patients and is increasingly isolated from sputum of patients with cystic

fibrosis. The overall incidence in 2011 for *S. maltophilia* bacteraemia was 0.8 Cases/100,000 population in England, Wales and Northern Ireland (PHE, 2014).

Sphingomonas paucimobilis: *S. paucimobilis* has been found in clinical material and recovered from hospital equipment (PHE, 2014).

2.3. *Pseudomonas aeruginosa* (*P.aeruginosa*)

2.3.1. General characteristics

P.aeruginosa is a Gram-negative bacterium that is widespread in the environment and engages in a wide variety of interactions with eukaryotic host organisms. It is a common opportunistic pathogen in humans, causing a broad range of infections in community and healthcare settings (Snyder *et al.*, 2013).

P. aeruginosa produce pyocin, which has characteristic property and can be used for typing of strains. The worldwide emergence of multidrug resistant bacterial strains is of growing concern, especially in nosocomial infections caused by *P. aeruginosa.* These infections are difficult to eradicate due to resistance to many antimicrobials, thus major cause of morbidity and mortality, leading directly and indirectly to an enormous increase in cost of hospital stay for the patients and also emergence of new health hazards for the community (Pal *et al.*, 2010).

The most serious manifestations of infection include bacteraemia (particularly in neutropenic patients), pneumonia (particularly in cystic fibrosis patients and critically ill patients), urinary tract infections and wound infections (especially in patients with burn injuries) (Snyder *et al.*, 2013).

Despite the wide distribution of *P. aeruginosa* in the environment, this microorganism rarely colonizes humans. However, the chance of colonization increases significantly in hospitalized patients.More than 70% of *Pseudomonas* infections occur as nosocomial or healthcare-associated infections.In some hospitals, *P. aeruginosa* can be the first agent of infection, mainly in respiratory and urinary tract infections. Bloodstream infections are mainly caused by Gram-positive cocci, although this rule cannot be expanded to developing countries, where environmental conditions favor Gram-negative bacilli infections.Some risk factors for *Pseudomonas* bacteremia have been described as increased age, hemodialysis, solid organ transplant, neoplasms, heart disease, diabetes mellitus, and chronic obstructive airwaydisease.However, these factors are unalterable, and efforts should focus on appropriate antibiotic therapy and prevention (Tuon *et al.*, 2012).

In the United States, *P. aeruginosa* is among the most common hospital pathogens and is the second most common pathogen isolated from patients with ventilator-associated pneumonia VAP, Given the severity of *P. aeruginosa* infections and the limited antimicrobial arsenal with which to treat them, finding alternative prevention and treatment strategies is an urgent priority (Gellatly and Hancock, 2013).

2.3.2. Habitat

Pseudomonas aeruginosa is an opportunistic pathogen that normally inhabits the soil andsurface in aqueous environments. Its adaptability and high intrinsic antibiotic resistance enable it to survive in a wide range of other natural and artificial settings, including surfaces in medical facilities (Gellatly and Hancock, 2013).

2.3.3. Pathogenesis

The pathogenesis of this organism is multi-factorial and involves various toxins and proteases (exotoxin A, lecithinase) and the glycocalyx "slime." *P. aeruginosa* is both invasive and toxigenic. The 3 stages of *Pseudomonas* infections are (1) bacterial attachment and colonization, (2) local infection, and (3) bloodstream dissemination and systemic disease (Selina *et al.*, 2015).

In healthy children disease is primarily limited to the first 2 stages (as in diseases such as otitis externa, urinary tract infections (UTIs), dermatitis, cellulitis, and osteomyelitis), although recent case reports describe bacteremia and sepsis in previously healthy children.In immunocompromised hosts, including neonates, infection can progress rapidly through the 3 stages and cause pneumonia, endocarditis, peritonitis, meningitis, ecthyma gangrenosum (EG), bacteremia, and overwhelming septicemia (Selina *et al.*, 2015).

2.4. Previous studies

Caylan *et al.*, (2010) conducted study aimed to evaluate the incidence and risk factor of catheter related blood stream infection, prospective data were collected on all temporary hemodialysis catheters inserted in Turkey, between October 2003 and October 2006. Sixty-seven microorganisms were isolated as etiologic agents for the catheter related-blood stream infections CR-BSI, 18 (26.9%) were Gram negative bacilli, and*P. aeruginosa* the most frequently isolated microorganism that was(9.0%).

Another study carried out byTokars *et al.*, (2002) in Atlanta and Georgia in October 1999 to May 2001, from the Dialysis Surveillance Network, Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, 109 outpatient dialysis centers that voluntarily report data to this system. Among 1747 positive blood cultures, 1919 isolates were reported. Of the 1919 isolates, 1244 (66%) represented access-related bacteremias in patients with catheters; 232 (12%) access-related bacteremias in patients with fistulas or grafts; 363 (19%) secondary bacteremias; and 80 (4%) contaminants. Among isolates from access-related bacteremia in patients with catheters, 27(2.2%) were *Psudomonas* spp,and among isolates from access-related bacteremias in patients with fistulas or grafts, 5(2.2) were *Psudomonas* spp and 14(3.9%) represented secondary bacteremia.

In a case–control study, six hundred forty-four patients undergoing renal replacement therapy were selected from Hospital Santa Casa de Misericórdia de Ponta Grossa in

Brazil. The study conducted to compare the prevalence of infection and related deaths, as well as the sensitivity profile of the putative bacteria in patients treated with peritoneal dialysis, arteriovenous fistula hemodialysis and catheter hemodialysis. One hundred sixteen patients (18.01%) developed infection, *P. aeruginosa* was one of the etiology of infections from patients on different types of dialysis, represented 3 (4.76%) from central catheter hemodialysis and 3(5.08%) from peritoneal dialysis (Curty *et al.*, 2014).

Another study in southeastern Brazil aimed to provide a wide overview of Health care-associated infections epidemiology in a hemodialysis unit. Collected data from prospective surveillance carried out from March 2010 through May 2012. This study demonstrated the most frequent agents of blood stream infection recovered from blood cultures. *P. aeruginosa* was represented(15.0%) (Albuquerque *et al.*, 2014).

A group of researchersperformed a study investigated incidence, risk factors, clinical features and outcome of bloodstream infections (BSIs) among 239 patients undergoing hemodialysis at the Department of Nephrology of theUniversity Hospital of Heraklion, Crete, Greece over a 7-year period (1999 to 2005). The predominant strains isolated from polymicrobial events were *S. aureus* (7 isolates; 50%), *Enterobacter* spp. (3; 21%), *E. coli* (3;21%), *Enterococcus faecalis* (3; 21%) and *P. aeruginosa* (3; 21%) (Fysaraki *et al.*, 2013).

A cross-sectional descriptive study was conducted during 2003-2004 in hemodialysis patients with temporary catheter infections who were referred to Hashemi-nejad Hospital of Iran University of Medical Science. The pathogenic organisms isolated from blood cultures were: *S.aureus* (42%), Coagulase-negative staphylococci(20%), *E. coli* (19%),

Enterococci(7%),StreptococciD(7%), *P. aeroginosa* (4%), *Klebsiella pneumoniae* (1%)(Sanavi *et al.*, 2007).

In another study which performed to evaluate the occurrence, identity and antimicrobial resistance of Gram-negative bacteria isolated from municipal water supplies, treated water, and dialysate of all 85 Greek haemodialysis centres. The most common isolates, in order of frequency were *P. aeruginosa* (22.7%), *Chryseobacterium meningosepticum* (14.9%), *Stenotrophomonas maltophilia* (13.5%), *Escherichia coli* (12.8%) and *Enterobacter cloacae* (7.8%), representing 71.6% of all isolates. *P. aeruginosa* was the most prevalent isolate in all types of water samples followed by *C. meningosepticum* in tap and treated water and by *E. coli* in dialysate (Arvanitidou *et al.*, 2003).

In a cohort study all intensive care unit patients with acute renal failure who were treated with renal replacement therapy were screened to determine whether there was an increased incidence of nosocomial blood stream infections and whether this resulted in a worse outcome between 1995 and2000 in Gent, Belgium. The incidence of nosocomial blood stream infections was 8.8%;*P. aeruginosa* was represented 2(2.9%) (Hoste*et al.*, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a cross-sectional descriptive study.

3.1.2. Study area

This study was conducted in Khartoum State hospitals (Ahmed Gasim Hospital, Ibn Sina Specialized Hospital, Medical Tropical Disease Hospital, ALnaw Hospital, ALwaldin Charity Hospital and Dr.Salma Center). The practical part was done in the Research Laboratory, SUST.

3.1.3. Study duration

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

3.1.4. Study population

Renal failure patientssufferingfrom symptoms and signs of bacteremia were enrolled in this study.

3.2. Inclusion criteria

Hemodialysis patients suffering from symptoms and signs of bacteremia were included.

3.3. Exclusion criteria

Hemodialysis patients on antibiotics who did not have symptoms of bacteremia were excluded.

3.4. Ethical considerations

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

3.5. Sample size

One hundred and nine (n=109)hemodialysis patients were participated in this study.

3.6. Data collection

Data were collected from patients according to the structured questionnaire (Appendix 1).

3.7. Experimental work

3.7.1. Collection of blood and inoculation

Most patients were anaemic thus five ml blood instead of the normal volume 10-20 ml were collectedfrom eachhemodyalysis patient. The collection of blood was carried outunder aseptic condition using 70% ethanol to clean the area around the puncture. Sterile single use syringeswere used to collect the blood. Before inoculating the samples into the bottle of blood culture(brain heart infusion broth),the top of each bottle were

disinfected by ethanol alcohol.The bottles were labeled by patients name, number,date and time of collection. As soon as possible the inoculated media was incubated at 35-37°C for up to 7 days. The culture media was checked every day for bacterial growth.

3.7.2. Sub culture of blood

A strict aseptic technique was used by using ethanol-alcohol swab to clean the top of bottles, sterile needles and syringes were used and inserted through the rubber liner in the cap and about 1 ml withdraw from the broth culture, then inoculated on MacConkey agar and incubated aerobically overnight at 37°C.

3.7.3. Examination of the growth

After incubation period, the growth was examined macroscopically. Colonial morphology (shape, size, edge, and odor) was reported.

3.7.4. Purification

All isolated organisms were purified by streaking on nutrient agar plate then incubated at 37°C overnight. At the end of incubation period, a discrete colony was picked up and streaked on the surface of slope of nutrient agar for further investigations.

3.7.5. Storage

All purified organisms were stored in sterile crayo tubes at -20°C using sterile nutrient glycerol broth medium.

3.8. Identification of the isolates

3.8.1. Gram stain

The smears wereprepared from isolated bacteria, and then allowed todry. Then they covered with crystal violet for 30 seconds, rinsed water and then covered by Lugol's iodine for 30 seconds, rinsed water. The decolorization was done by acetone -alcohol; rinsed water and then the smear was stained by safranin as counter stain, rinse in water and allow to dry then examined microscopically using oil immersion lenses (Cheesbrough, 2000).

3.8.2. Biochemical tests

3.8.2.1. Oxidase test

A filter paper impregnated with the reagent was allowed to dry completely. A loopful bacteria from nonselective plate was smeared onto the paper and examined for development of a violet or purple color within 10 seconds (positive reaction). No color change indicated negative result (Charles, 2006).

3.8.2.2. Citrate utilization test

A well isolated colonies were selected and streaked on the surface of the citrate slant in test tube using an inculcating loop, The tube was then incubated at 35ºC in non -CO_2 incubator for 24 hours. Growth with intense blue color on the agar slant indicated a

positive reaction when no growth and no color change (green) indicated a negative reaction (Charles, 2006).

3.8.2.3.Urease test

The surface of the urea agar slant was streaked with heavy inoculums of pure culture. The tube was then incubated at 35°C in non- CO_2 for 18-24hours. Production of intense pink-red color on the slant, which might penetrate into the butt, was considered a positive reaction. No color change indicated a negative reaction (Charles, 2006).

3.8.2.4. Indole test

Approximately 2-3 colonies of the bacteria were inoculated in the indole nitrate broth medium and incubated at 37°C for 24-48 hours, then the tube was examined for growth, when the broth was visibly turbid, 0.5 ML Kovacs reagent was added. Production of a pink to red color at the interface of the reagent and the broth within seconds indicated a positive reaction.No color change indicated a negative reaction(Charles, 2006).

3.8.2.5.Fermentation of sugar and production of Gas and H_2S

The butt of the KIA tube was stabbed using sterile inoculating needles and then streaked back and forth along the surface of the agar with the organism. The tube was incubated at 35°C in non-CO_2 incubator for 18-24hours. If acid slant –acid butt gave yellow –yellow color it was an indication that both glucose and lactose were fermented. If alkaline slant-acid butt gave red –yellow color it was an indication that only glucose was fermented. If alkaline slant-alkaline butt gave red-red color it was an indication that glucose was not

fermented,the presence of black precipitate (butt) indicated H_2S production, and presence of splits or cracks with air bubbles indicated gas production(Charles, 2006).

3.8.2.6. Motility test

The organism was taken by straight wire and stabbed into the medium making a single stab about 1-2 cm down into the medium. The tubes were then incubated overnight at 37°C. The motility was indicated by the presence of diffuse growth (appearing as the clouding of the medium away from the line of inoculation). Non motile organisms did not show growth out from the line of inoculation(Charles, 2006).

3.8.2.7. Growth at 42°C and 4°C

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

3.8.2.8.Oxidative-fermentationtest

Two tubes were required for the test,each inoculated with the organism under test using straight needle. The medium was stabbed three to four times half way to the bottom of the tube. One tube of each pair was covered with a 1cm layer of sterile mineral oil. The other tube was left open to the air. The two tubes incubated at 35°Cand examined daily for several days. Acid production was detected in the medium by the appearance of a yellow color in covered tube(Washington *etal.*,2006).

CHAPTER FOUR

RESULTS

This study was conducted to isolate and identify*Pseudomonas* species from hemodialysis patients. One hundred and nine (n=109) participants; 50(45.9%) males and 59(54.1%) females were enrolled. Blood samples were collected from each patient in different centers of hemodialysis in Khartoum State. These were10(9.2%)samples from Ahmed Gasim Hospital,23(21.1%)from Ibn Sina Specialized Hospital,37(33.9%) from Medical Tropical Disease Hospital,20(18.3%) from ALnaw Hospital,3(2.8%) from Dr. Salma Center and 16(14.7%)from ALwaldin Charity Hospital (Tables 1&2).

Cultivation of blood samples yielded bacterial growth. Suspected *Pseudomonas* spp. when identified by biochemical tests(Table 3). The results revealed 12(11.0%)*P. aeruginosa.* Among 50 males were investigated 7 (58.3%) infected by *P.aeruginosa,* all of them with catheter for hemodialysis. While among 59 females wereinvestigated 5 (41.7%) infected by *P.aeruginosa.* Fourof them with catheter and one female with fistulafor hemodialysis(Table 4).

Table 1. Distribution of participants according to hemodialysis centers

Centers	Participants	
	Number	%
Ahmed Gasim Hospital	10	9.2
Ibn Sina Specialized Hospital	23	21.1
Medical Tropical Disease Hospital	37	33.9
ALnaw hospital	20	18.3
Dr. Salma Center	3	2.8
ALwaldin Charity Hospital	16	14.7
Total	109	100

Table 2. Distribution of participants according to gender

Gender	Participants	
	Number	%
Male	50	45.9
Female	59	54.1
Total	109	100

Table 3. Tests adopted for identification of *Pseudomonas* spp

Isolate code	Biochemical tests							Growth at 42°C	Growth at 4°C	Motility	OF test	Identified organism
	Indole	Urease	Citrate	KIA								
				Slope	Butt	Gas	H_2S					
P1	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P2	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P3	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P4	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P5	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P6	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P7	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P8	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P9	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P10	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P11	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*
P12	–	–	+	R	R	–	–	G	NO	M	O	*P. aeruginosa*

Key

- = Negative reaction G= Growth OF = Oxidation Fermentation
+ =Positive reaction NO =no growth O = Oxidative
R =Red M = Motile

Table 4. Distribution of pathogens according to hemodialysis device among gender

Gender	Number	%	Pathogen	
			device of hemodialysis	
			Catheter	Fistula
Male	7	58.3	7	-
Female	5	41.7	4	1
Total	12	100	11(91.7%)	1(8.3%)

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Hemodialysis in end stage renal failure patients is a life saving procedure. However, patients undergoing hemodialysis potentially have an increased risk of exposure to infections.Blood stream infection is one of the main causes of morbidity and mortality among hemodialysis patients. Infections in patients receiving hemodialysis are often caused by resistant pathogens; due to frequent hospital admissions and frequent need for antimicrobial therapy. *Pseudomonas* species is an important pathogen which has been responsible for many infections in renal failure patients.

This study was designed to isolate and identify *Pseudomonas* species in renal failure patients in Khartoum State as well as to determine the ratio of *Pseudomonas* infection in hemodialysis patients. In this study only 12(11.0%) *P. aeruginosa*were isolated.

This result is in line with result obtained by Caylan *et al.*, (2010) in Turkey which reported the percentage of infection by *P. areuginosa* as (9.0%), but less than that reported by Albuquerque *et al.*, (2014) in Brazil (15.0%), and Arvanitidou*et al.*, (2003) inGreek (22.7%).

The device used for hemodialysis may play a crucial role in infections according to study conducted by Tokars *et al.*, (2002) was performed in Atlanta and Georgia, among isolates from access-related bacteremia in patients with catheters, 27(2.2%) were *Psudomonas* sppand among isolates from access-related bacteremias in patients with fistulas or grafts, 5(2.2) were *Psudomonas* sppand 14(3.9%) represented secondary bacteremia.In the

present study the patients used catheter 11(91.7%) and 1(8.3) with fistula for hemodialysis were *P. areuginosa.*

Other results reported by Hoste *et al.*, (2004) in Belgium, Fysarki *et al.*, (2013) in Greece, Sanvani *et al.*,(2007) in Iran and Curty *et al.*,(2014) in Brazil whom reported the percentage of isolated*P. aeruginosa* as 2.9%, 3.21%,4%, 4.76 respectively .

Most of previous studies reported that the common causative agents of blood stream infections were caused by*P. aeruginosa.* The organism is nosocomial pathogen that normally inhabits in hospital environments. Therapeutic options are increasingly limited due tothe continued emergence and spread of antimicrobialresistant strains enable it to survive in a widerange of other natural and artificial settings, includingsurfaces in medical facilities. *P. aeruginosa* infections are associated with compromised host defenses such as in hemodialysis patients. Hence, careful clinical evaluation, limitation of the catheter use, improvement of nutritional status, finding alternative prevention andtreatment strategies is an urgent priority management of infections risk factors considerably reduces the incidence of BSIs. Finally, surveillance of local microbiology is of utmost importance for appropriate empirical antimicrobial treatment.

5.2. Conclusion

1-This study concluded that*Pseudomonas aeruginosa* was isolated from hemodialysis patients represented 11.0 %.

2-The kind of vascular access for hemodialysis has significant influence on patient's survival. The arterio-venous fistula (AVF) for hemodialysis is the safest access.

Recommendations

1-Good hygiene of patients and medical staff should be management when manipulation of vascular accesses: Proper choice of insertion site, local antisepsis, personnel appropriate attire, care and maintenance of the catheter.

2- Preferable use of arterio-venous fistula for hemodialysis.

3-Since the *Pseudomonas aeruginosa* is highly resistant to antimicrobials; sensitivity testing to these bacteria should be carried out routinely.

4- Increase sample size in the future studies.

5- More studies are needed using molecular technique to confirm this result.

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Appendix 1

Questionnaire

-Hospital name……. **-Date / /2O15**

-Patient name……–Patient NO……....

- Age: …….. Years

- Gender: Male ☐ Female ☐

-Type of hemodialysis: Catheter ☐ Fistula ☐

- Starting of dialysis: ……

-Antibiotic received: Yes ☐ No ☐

-Co-morbid conditions: Yes ☐ No ☐

-If yes :

Diabetes ☐ Malnutrition ☐ Hypertention ☐

-Laboratory processing……

……

……

……

.

Appendix 2

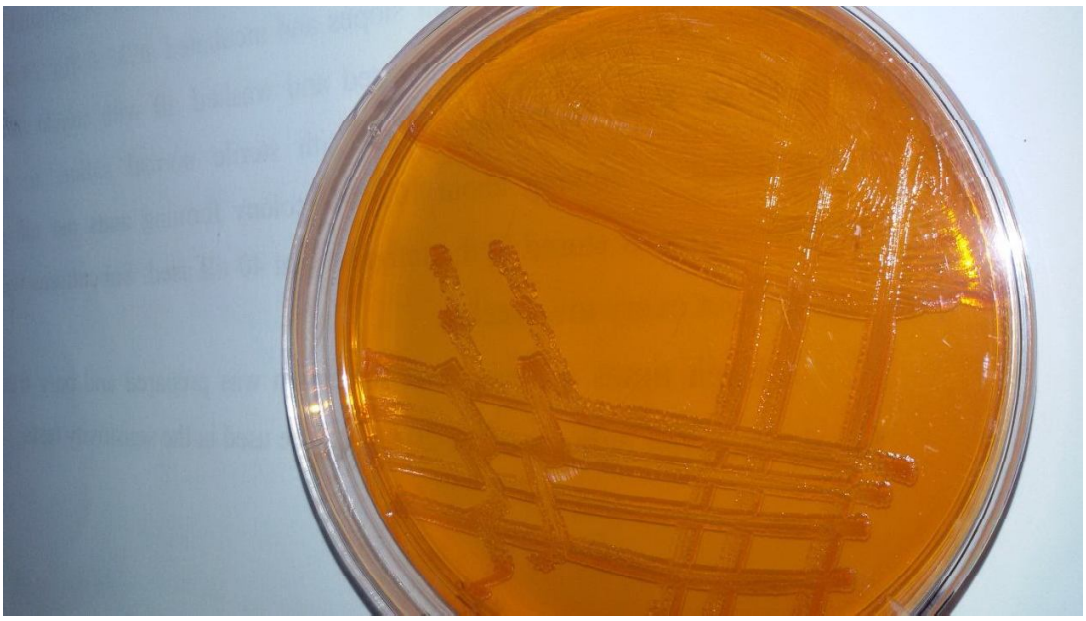
Original work

Oxidative Fermentation test

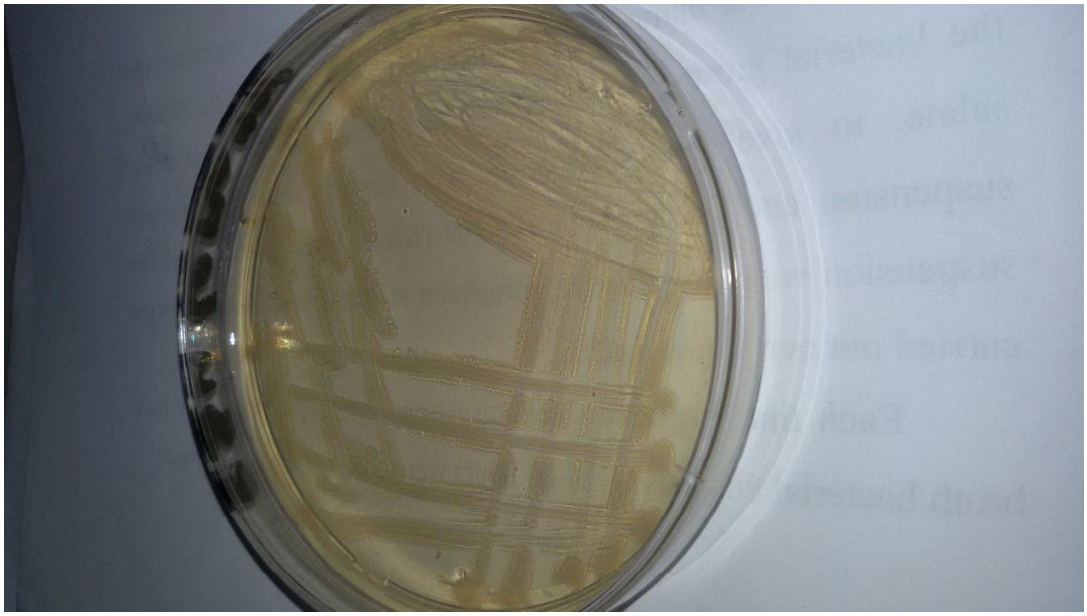
Isolate NO.	Sugar								Inter-pretation
	Glucose		Maltose		Lactose		Mannitol		
***P.aeruginosa* (1)**	Open tube	Close tube	Open tube	Close Tube	Open tube	Close tube	Open tube	Close tube	
***P.aeruginosa* (2)**	Pink	Yellow	Pink	Yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (3)**	Pink	Yellow	pink	Yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (4)**	Pink	Yellow	pink	Yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (5)**	Pink	Yellow	pink	Yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (6)**	Pink	Yellow	pink	Yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (7)**	Pink	Yellow	pink	Yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (8)**	Pink	Yellow	pink	Yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (9)**	Pink	Yellow	pink	Yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (10)**	Pink	Yellow	pink	yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (11)**	Pink	Yellow	pink	yellow	pink	pink	Pink	yellow	oxidative
***P.aeruginosa* (12)**	Pink	Yellow	pink	yellow	pink	pink	Pink	yellow	oxidative

Key : Pink =alkaline ph = No CHO utilization

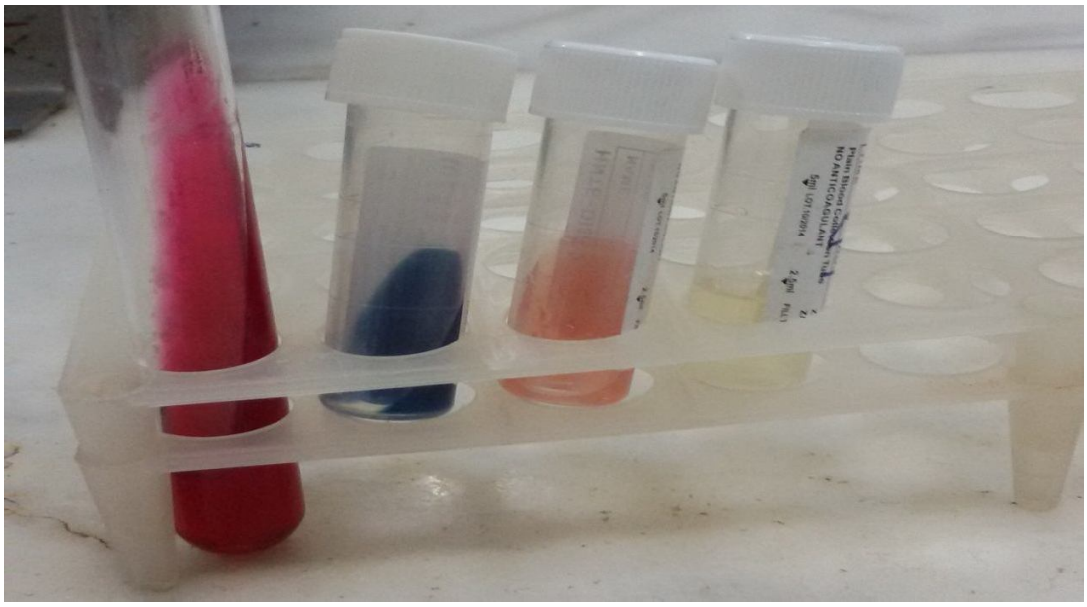
Yellow=acid ph= CHO utilization



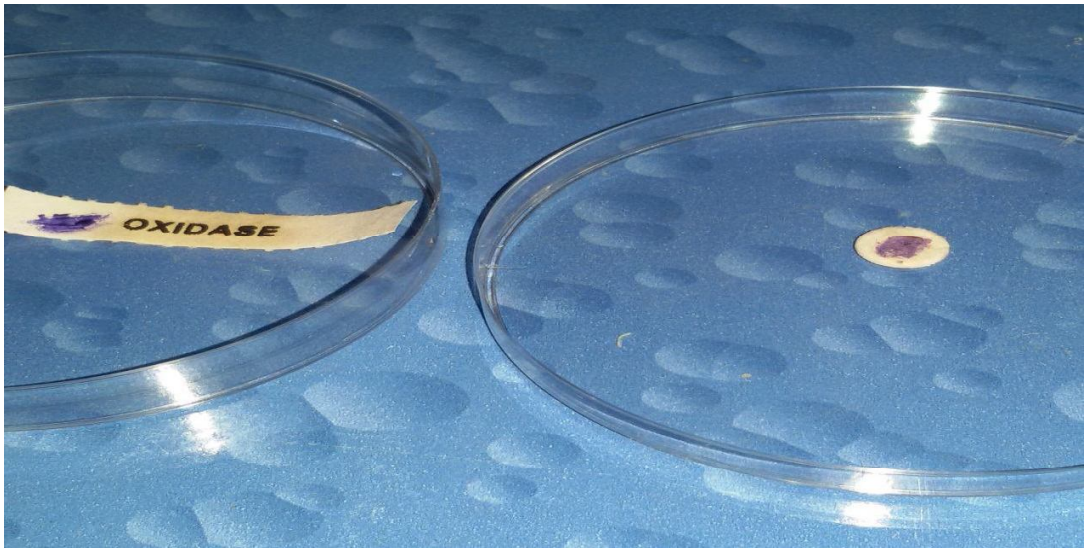
(1) Growth on MacConkey agar.



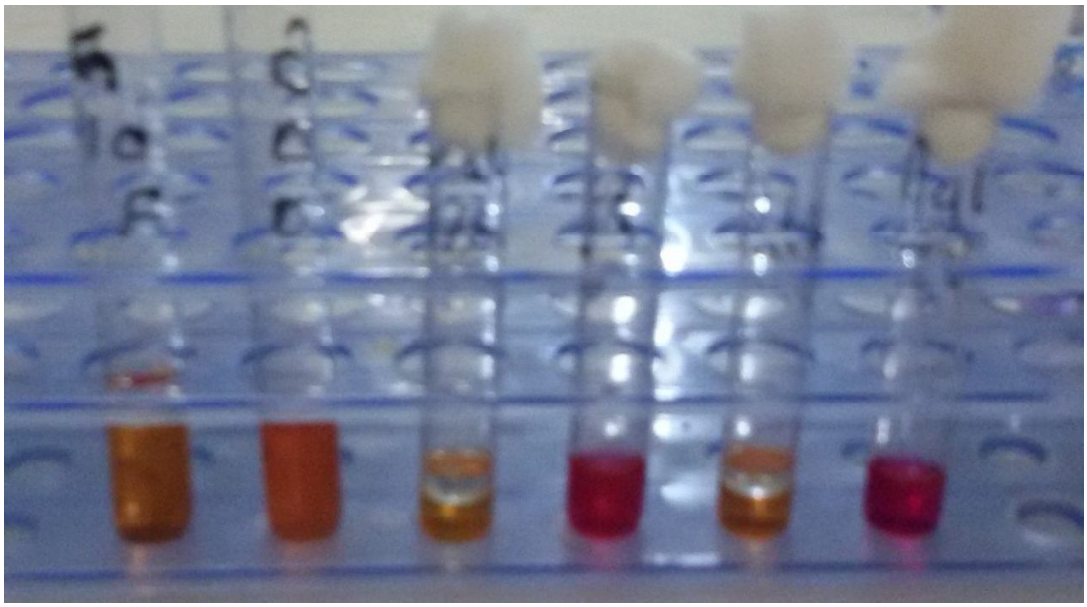
(2) Growth on nutrient agar.

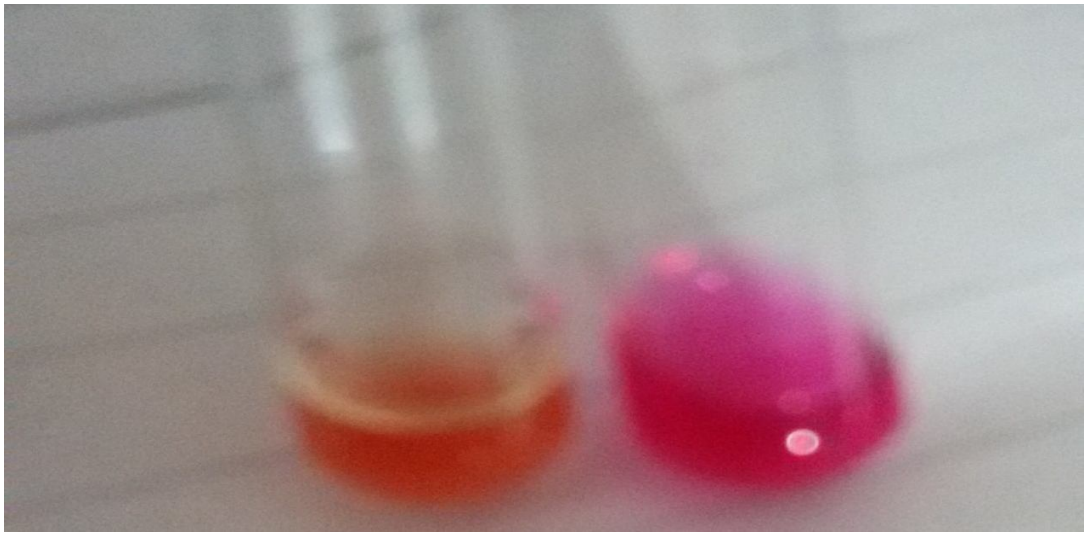


(3)Biochemical tests.



(4)Oxidase test





.(5)Oxidation fermentation test

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER TWO

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CHAPTER THREE

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APPENDICES

CHAPTER FIVE

DISCUSSION