

CHAPTER ONE

1. Introduction

1.1. *Pseudomonas*:

The species that comprise the genus *Pseudomonas* are part of the wider family of bacteria that are classified as Pseudomonadaceae. There are more than 140 species in the genus. The species that are associated with opportunistic infections include *Pseudomonas aeruginosa*, *Pseudomonas maltophilia*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas cepacia*, *Pseudomonas stutzeri*, and *Pseudomonas putrefaciens*. *Pseudomonas aeruginosa* probably the most well known member of the genus (Brigham Narins, 2003).

P. aeruginosa is widely distributed in nature and is commonly present in moist environments in hospitals. It can colonize normal humans, in whom it is a saprophyte. It causes disease in humans with abnormal host defenses (Brooks *et al*, 2010).

The most common nosocomial infections occur in compromised hosts with severe burns, neoplastic disease, and premature birth. Cystic fibrosis patients in particular suffer from a lung condition in which mucus collects and creates a protective biofilm tenaciously colonized by *Pseudomonas* (Talaro & Chess, 2012).

Pseudomonas aeruginosa is a ubiquitous and versatile human opportunistic pathogen and has implications on morbidity, mortality and healthcare costs both in hospitals and in the community. The development of resistance to all available antibiotics in some organisms may preclude the effectiveness of any antibiotic regimen. Infections caused by *P.aeruginosa* are frequently life-threatening and difficult to treat as it exhibits intrinsically high

resistance to many antimicrobials and the development of increased particularly multi-drug resistance in health care settings (Anil& Shahid, 2013).

1.1.1 Objectives:

Main objective:

-*In vitro* evaluation of some groups of antibiotics on *Pseudomonas aeruginosa*.

Specific objectives:

- Isolation and identification of *Pseudomonas aeruginosa*.
- To determine frequency of *Pseudomonas aeruginosa*.
- To carry out sensitivity testing by using some of antibiotics.

CHAPTER TWO

2. Literature Review

2.1 *Pseudomonas aeruginosa*:

2.1.1 Definition:

Strictly aerobic gram negative bacteria, straight or slightly curved measured 0.5-1.0 x 1.5-5.0 none sporing non capsulated and is fimbriated, motile by presence of a polar flagellum (Subhash Parija, 2009).

2.1.2 Normal habitat:

Ps. aeruginosa is physiologically versatile and flourishes as a saprophyte in warm moist situation in the human environment including sinks, drains, respirators, humidifiers and disinfection solutions (Gerald *et al*, 1996).

2.1.3 Viability:

Ps. aeruginosa is heat labile bacteria readily killed at 55°C in 1 hr. It is highly susceptible to acid, silver salt, 2% alkaline of glutaraldehyde and disinfectant such as dettol and Cetrimide however it's very strongly resistant to common antiseptics and disinfectants such as chloroxylenol, hexachlorophene, and quaternary ammonium compounds (Subhash Parija, 2009).

The species has exceptional resistance to antibacterial agents and consequently an important potential contaminant of pharmaceutical and cosmetic preparations, contamination may be eliminated by heating to 70°C (Gerald *et al*, 1996).

2.1.4 Cell wall component:

- Pilli: they are important in mediating adhesion of the bacteria to the epithelial cells (Subhash Parija, 2009).

- Capsule: produces polysaccharide capsule also called as mucopolysaccharide ,alginate coat, or (glycocalyx) that has multiple functions .The polysaccharide layer anchors the Bacteria to epithelial cells and tracheobronchial mucin, also antibiotics such as aminoglycosides (Murray *et al* ,2002).

- Lipopolysaccharide: The adverse factor in gram-negative sepsis is the presence of an endotoxin in the outer membrane of the gram negative cell wall (Kathleen & Barry, 2012).

- Pyocyanin : is a pigment which catalyze production of superoxide and hydrogen peroxide and cause tissue damage also contribute to inflammation associated with a disease (Subhash Parija ,2009).

2.1.5 Antigenic structure:

O antigen: somatic or O antigens are the group specific Ag. *Ps.aeruginosa* possesses 19 distinct group specific OAg, so organism has been classified into 19 serogroups.

OAg are heat stable and can be extracted with acid or formamide.

H antigen: flagella or H Ag are found in the flagellum of *Ps.aeruginosa* are heat labile, two type of H Ag are demonstrated by slide agglutination (Subhash Parija ,2009).

2.1.6 Virulence factors:

- Toxins these include:

1. Exotoxin A: important virulence factor similar to diphtheria toxin, acts by preventing synthesis of protein in eukaryotic but it is different immunologically and structurally (Subhash Parija, 2009).

2. Exotoxin S and T: these toxins show adenosine diphosphate ribosyl transferase activity, it is facilitating spread of bacteria and invasion of tissues

followed by necrosis by causing damage in epithelial cells (Subhash Parija, 2009).

- Enzymes:

1. Elastase: two types serine protease (LasA) and zinc metalloprotease (LasB) these two enzymes act in combination to destroy elastin, caused damage in parenchyma tissue of lung and produce hemorrhagic lesion also facilitate spread of the infection by degrading several component of complement and inhibiting chemotaxis activities of neutrophil. In case of chronic infection antibodies are produced against LasA and LasB and form immunocomplex which deposited in the infected tissues (Subhash Parija, 2009).

2. Alkaline protease: is also responsible for destruction of tissue and dissemination of *Ps.aeruginosa* infection, also interfere with immune response of the host.

3. Phospholipase C is heat labile hemolysin contribute tissue destruction by break down lipids and lecithins.

4. Rhamenolipid: a heat stable hemolysin has the same action of phospholipase C.

2.1.7 Pathogenicity:

2.1.7.1 Respiratory tract infection:

Occurs almost exclusively in individuals with compromised respiratory system and most cases of *Pseudomonas* pneumonia occur in patient with malignancy and immunodeficient (Subhash Parija, 2009).

Primary non bacteremic *Pseudomonas* pneumonia usually occur in patient with cystic fibrosis, other chronic disease and in those with neutropenic, the condition that increase susceptibility of these patient to infection to *Pseudomonas aeruginosa* use of respiratory instrument during therapy and spectrum antibiotics (Subhash Parija, 2009).

2.1.7.2 Skin infection:

Pseudomonas aeruginosa can cause a variety of skin infection the most common are burn wound infection, Skin infection commonly seen in patient who exposure to moisture Pseudomonal wound infection is characterized by presence of dark brown eschar associated with edema and hemorrhagic necrosis (Subhash Parija, 2009).

2.1.7.3 Urinary tract infection:

Primary urinary tract infection acquired in the community because of *P. aeruginosa* is rare except in those patients with anatomical abnormalities and spinal cord injuries. The great majority of urinary tract infections is of nosocomial origin and is invariably the consequence of long-term catheterization. Rates of approximately 5% have been reported. Hamatogenous spread from a primary focus is uncommon. Paraplegic patients in institutional care are especially at risk, and reservoirs of contaminated urine such as drainage bottles and bed pans are probably the major sources for the organism. The infection is usually resolved by the removal of the catheter or other predisposing factor, but prostatic infections in the presence of calculi are exceedingly difficult to treat (Stephen & Peter, 2006).

2.1.7.4 Ear infection:

Frequently caused external otitis with swimming (swimmer ear), malignant external otitis is a virulence form of disease seen primarily in diabetics and elderly patients (Murray et al, 2002).

2.1.7.5 Eye infection:

The organism can cause conjunctivitis, Keratitis, or endophthalmitis when introduced into the eye by trauma or contaminated medication or contact

lens solution. Keratitis can progress rapidly and destroy the cornea within 24 to 48 hours (Kenneth *et al*, 2010).

2.1.7.6 Endocarditis:

This condition lead to distruction of heart valve and subsequent heart failure commonly occur in intravenous drug abusers of pentazocine and triphenylamine (Subhash Parija, 2009).

2.1.7.7 Nosocomial infection:

P. aeruginosa is among the top five causes of nosocomial bacteremia, and severe infection can lead to sepsis. Because of the multitude of virulence factors the organism elaborates, coupled with increasing antibiotic resistance, *P. aeruginosa* continues to be a problematic pathogen in this setting. When *P. aeruginosa* spreads from a tissue source, it probably does so by breaking down epithelial and endothelial barriers to gain access to the blood (Gerald L. Mandell *et al* 2010).

2.1.7.8 Cystic fibrosis (CF):

Pseudomonas aeruginosa is the most persistant infectious agent that complicates the course of CF, providing increased receptors for *Ps.aeruginosa* attachment defect in epithelia of CF patients may also retard their clearing by desquamation.

Once the bronchi are colonized, the organism remain, forming biofilm containing microcolonies of bacteria, which together are called a glycocalyx. Caused by mutant strain, their gene activated by high osmolarity of the thick CF secretions. The selective advantages of this biofilm include adhesion, inaccessibility of the immune system (complement, antibody, phagocytes) and interference with the access and action of antimicrobial agents (Subhash Parija, 2009).

2.1.8 Laboratory Diagnosis:

Specimen according to site of infection, e.g. urine, pus, ear swab, eye swab, sputum, effusion and blood for culture.

2.1.8.1 Microscopy:

Gram negative rod, motile by single polar flagellum, non spore forming and some strain are capsulated (Cheesbrough, 2006).

2.1.8.2 Culture:

Temperature 6-42°C with optimum 35- 37°C

Incubation time up 24 hours.

Aeration: obligated aerobe.

Nitrate can be used as an electron acceptor to permit an aerobic growth.

Optimum pH: 7.4-7.6 (Gerald *et al*, 1996).

2.1.8.3 Pigments:

i. Pyocyanin : non fluorescent bluish pigment

ii. Pyoverdin: Fluorescent greenish pigment

iii. Pyorubrin: dark red pigment.

iv. Pyomelanin: black pigment.

Note: *Pseudomonas aeruginosa* has a sweet grape or corn taco-like odor due to 2-aminoacetophenon (Brooks *et al*, 2004).

Blood Agar: large flat spreading colonies mostly β -hemolytic with green bluish pigment.

MacConkey Agar:

Pale yellow, non lactose fermenter colonies.

CLED: blue to green colonies (Cheesbrough, 2006).

PIA: (Pseudomonas Isolating Agar): selective medium contain pigment enhancing component and the selective agent irgasan (2.4.4 trichloro-2-hydroxyphenyl ether) (Gerald *et al*, 1996).

Acetamide media: blue color due to deamination of Acetamide.

Cetrimide agar: selective medium.

2.1.8.4 Set of sugars:

Pseudomonas aeruginosa attack or utilize carbohydrates by means of oxidation and produce a small amount of acid.

Lactose	Glucose	Maltose	Sucrose
Negative	Negative	Negative	Negative

2.1.8.5 Biochemical tests:

- 1 Oxidase: positive (key test).
- 2 Citrate: positive.
- 3 Catalase: positive.
- 4 Indole: negative.
- 5 Urease: different.
- 6 KIA:

Slope	Butt	Gas	H ₂ S
Red	Red	Negative	Negative

(Cheesbrough, 2006).

2.1.8.6 Epidemiological typing methods:

2.1.8.6.1 Serotyping:

Identification of group specific heat stable LPS Ag by agglutination test.

The major disadvantage of Serotyping is its limited discriminating power (Gerald *et al*, 1996).

2.1.8.6.2 Pyocin Typing:

Pyocin are bacteriocins produced by *P.aeruginosa*. Three types of pyocins are produced. They are R, F, and S pyocins. The capability to produce these pyocins is observed in more than 90% of strains of *P. aeruginosa*. Those strains which produce pyocins are resistant to their own pyocins but are sensitive to those produced by other strains. Typing of *Ps.aeruginosa* based on the production of Pyocin is the most commonly used method for typing *P.aeruginosa* strains. These are used in epidemiology studies (Subhash Parija, 2009).

2.1.8.6.3 Genotyping:

Include restriction enzyme analysis in combination with southern hyperidization with probes upstream from the exotoxin a gene (Ogle *et al* 1987) and chromosomal DNA analysis (Gerald *et al*, 1996).

2.1.9 Treatment:

Of the pathogenic bacteria, *P.aeruginosa* is the organism most consistently resistant to many antimicrobials. This is primarily due to the porins that restrict their entry to the periplasmic space. *P.aeruginosa* strains are regularly resistant to penicillin, ampicillin, cephalothin, tetracycline, chloramphenicol, sulfonamides, and the earlier aminoglycosides (streptomycin, kanamycin). Much effort has been directed toward the development of antimicrobials with anti-*Pseudomonas* activity. The newer aminoglycosides gentamicin, tobramycin, and amikacin all are active against most strains despite the presence of mutational and plasmid-mediated resistance. Carbenicillin and ticarcillin are active and can be given in high doses, but permeability mutations occur more frequently than with the aminoglycosides. The most prized feature of some of the third-generation cephalosporins (ceftazidime, cefepime, cefoperazone), carbapenems

(Imipenem, meropenem), and monobactams (aztreonam) is their activity against *Pseudomonas*. In general, urinary infections may be treated with a single drug, but more serious systemic *P.aeruginosa* infections are usually treated with a combination of an anti-*Pseudomonas* lactam and an aminoglycoside, particularly in neutropenic patients. Ciprofloxacin is also used in treatment of such cases. In all instances, susceptibility must be confirmed by in vitro tests (Kenneth *et al*, 2010).

The treatment for *P.aeruginosa* infection in CF presents special problems because most of the effective antimicrobics are only given intravenously. There is a reluctance to hospitalize in many patients, and oral agents are used instead. There is less experience with their efficacy under these conditions, and the chronic nature of CF is a setup for development of resistance during therapy. This has already been seen with ciprofloxacin and aztreonam. Aerosolized tobramycin has also been used in some CF patients, with some evidence of clinical improvement (Kenneth *et al*, 2010).

2.2 Antimicrobial agent

2.2.1 Definition:

Antimicrobial chemotherapy involves the use of chemicals to prevent and treat infectious diseases. These chemicals include antibiotics, which are derived from the natural metabolic processes of bacteria and fungi, as well as synthetic drugs. To be effective, antimicrobial therapy must disrupt a necessary component of a microbe's structure or metabolism to the extent that microbes are killed or their growth is inhibited. At the same time, the drug must be safe for humans, while not being overly toxic, causing allergies or disrupting normal biota (Crown & Talaro, 2009).

Antibiotics are metabolites produced by certain microorganisms, which inhibit the growth of certain other microorganisms (Hogg, 2005).

2.2.2 Cephalosporins:

2.2.2.1 Chemistry:

This important group of semi synthetic β -lactam antibacterial agents is derived from 6-aminopenicillanic acid; however, the thiazolidine ring is replaced by dihydrothiazins (Gabriel virella et al, 1996).

2.2.2.2 Classification:

The cephalosporins are classified into three generations that roughly parallel their chronologic development.

(a) First generation cephalosporins are available in oral and injectable forms. Preparations include cefazolin, cephalexin, and cephalothin.

(b) Second generation cephalosporins are mostly injectable. Preparations include cefamandole, cefaclor, cefoxitin, and cefuroxime

(Gabriel virella et al, 1996).

(c) Third generation cephalosporins are also mostly injectable preparations include cefotaxime and ceftriaxone.

(d) Fourth generation cephalosporins: cefepime (Gabriel virella *et al*, 1996).

2.2.2.3 Spectrum of activity:

First-generation cephalosporins are variably absorbed from the intestines and are useful against gram-positive cocci, and certain gram negative rods. Second-generation drugs, have an expanded activity against gram-positive cocci, as well as numerous gram negative rods (e.g., *Haemophilus influenza*), third-generation cephalosporins, including cefotaxime and ceftriaxone, are used primarily against gram-negative rods (e.g., *Pseudomonas aeruginosa*) and for treating diseases of the central nervous system. The fourth generation cephalosporins (e.g. cefepime) have improved activity against the gram negative bacteria involved with urinary tract infections (Pommerville, 2011).

2.2.2.4 Toxicity:

(a) Because the structures of cephalosporin and penicillin are similar, 10%-15% of patients who are allergic to penicillin might also be allergic to cephalosporin. (b) The cephalosporins are more toxic than penicillin, and most are mildly nephrotoxic. Later generation cephalosporins, however, are less nephrotoxic than early generation cephalosporins (Gabriel virella *et al*, 1996).

2.2.3 Aminoglycosides:

2.2.3.1 Mechanism of action:

Aminoglycosides inhibit protein synthesis by binding to the bacterial ribosomes either directly or by involving other proteins. This binding destabilizes the ribosomes, blocks initiation complexes, and thus prevents elongation of polypeptide chains. The agents may also cause distortion of

the site of attachment of mRNA, mistranslation of codons, and failure to produce the correct amino acid sequence in proteins (Kenneth *et al*, 2010).

2.2.3.2 Spectrum of activity:

The aminoglycoside are bactericidal and their spectrum of activity is broad, especially against Gram negative organism's .All aminoglycoside are virtually non absorbed by the gastrointestinal tract, so they must be administered parentally to achieve therapeutic blood levels.

(a)Preparations and therapeutic uses

(i) Streptomycin: the first to be developed ,has been relegated to use as a second line antimycobacterial agent ,because of its marked ototoxicity Streptomycin is only used when multidrug regimens are necessary and the bacteria have become resistant to less toxic antibacterial agents.

(ii)Neomycin is mostly used as a topical antibiotic in oral preparations when given orally (e.g.to treat neonatal *Escherichia coli* diarrhea) neomycin is not absorbed; therefore, oral administration is also considered a form of local treatment.

(iii)Kanamycin is primarily used to treat *M.tuberculosis* infections (Gabriel *et al* 1996).

(iv)Gentamicin is less toxic and is widely administered for infections caused by gram-negative rods (*Escherichia*, *Pseudomonas*, *Salmonella*, and *Shigella*). Two relatively new aminoglycosides, tobramycin and amikacin, are also used for gram-negative infections, with tobramycin especially useful for treating *Pseudomonas* infections in cystic fibrosis patients (Talaro & Barry, 2010).

(b)Aminoglycoside and β -lactam are synergistic when used in combination because both groups are bactericidal, but have independent mechanisms of action (Gabriel *et al*, 1996).

2.2.3.3 Toxicity:

They have a toxic effect both on the kidneys and on the auditory and vestibular portions of the eighth cranial nerve. To avoid toxicity, serum levels of the drug, blood urea nitrogen, and creatinine should be measured, they are poorly absorbed from the gastrointestinal tract and cannot be given orally, they penetrate the spinal fluid poorly and must be given intrathecally in the treatment of meningitis and they are ineffective against anaerobes, because their transport into the bacterial cell requires oxygen (Levinson, 2010).

2.2.4 Polymyxin E (Colistin):

2.2.4.1 Mechanism of action:

Bind to negatively charged phospholipids and disrupt the membrane integrity with subsequent osmotic perturbation (leakage of intracellular contents). Also bind to LPS but this binding will not result in disruption of the cytoplasmic membrane (Hardy, 2002).

2.2.4.2 Spectrum:

They act against *Ps.aeruginosa* and other Gram negative rods. Although these antimicrobics were used for systemic treatment in the past, their used is now limited to topical applications resistance to them rarely develops.

2.2.4.3 Toxicity:

Nephrotoxicity and neurotoxicity (Kenneth *et al*, 1996).

2.2.5 Carbapenems:

2.2.5.1 Mechanism of action:

Are classes of β -lactam antibiotics that substitute a carbon atom for a sulfur atom and add a double bond to the penicillin nucleus these antibiotics, which inhibit cell wall synthesis (Tortora *et al*, 2010).

2.2.5.2 Spectrum:

Imipenem and meropenem have the broadest spectrum of all β -lactam antibiotics. This fact appears to be due to the combination of easy penetration of G-ve and G+ve bacterial cells and high level of resistance to lactamases. Imipenem is rapidly hydrolyzed by a renal tubular dehydropeptidase-1; therefore, it is administered together with an inhibitor of this enzyme (cilastatin), which greatly improves its urine levels and other pharmacokinetic characteristics. Meropenem is not significantly degraded by dehydropeptidase-1 and does not require co-administration of cilastatin; it has largely replaced Imipenem in clinical use (Kennth *et al*, 2010).

Complicated by intra-abdominal infections where other beta-lactam agents are not appropriate due to intolerance or resistance and also Necrotizing pancreatitis (Amyarmbrust *et al*, 2006).

CHAPTER THREE

3. Materials and Methods

3.1 Study area:

Clinical isolates were collected from Khartoum Teaching, Soba, Omdurman Military & Royal Care hospitals.

3.2 Study population:

This study done on clinical isolates collected from patients having different infections (urinary tract infection , wound infections , bed sore , cystic fibrosis and blood infection).

3.3 Study location:

The study was performed in Sudan University of medical laboratory science.

3.4 Study duration:

The study was conducted during the period from February till April 2014.

3.5 Sample size:

The total sample size was 60 samples.

3.6 Data collection:

Isolates of *Pseudomonas aeruginosa* from different specimens.

3.7 Materials

3.7.1 Media:

1. Blood agar
2. MacConkey agar
3. Oxidase disc
4. KIA medium
5. Urea agar
6. Peptone water
7. Simmon's citrate
8. Ceftrimide media

9. Muller Hinton agar

3.7.2 Glass wares:

1. Petri dishes
2. Tubes
3. Slides

3.7.3 Others:

1. Bensen burner
2. Straight loop, wire loops and forceps
3. Cotton wool swabs
4. Antibiotics discs

3.8 Methods:

3.8.1 Sub culturing:

Under aseptic condition, clinical isolates were subcultured on MacConkey, blood, Cetrimide and nutrient agar media and incubated for 18-24 hrs at 37°C aerobically. At the end of incubation period, the colonial morphology was studied.

3.8.2 Identification:

3.8.2.1 Colonial morphology

The cultures were morphologically examined for size, color, fermentation of lactose on MacConkey agar, hemolysis on blood agar and growth on Cetrimide.

3.8.2.2 Gram reaction:

From single colony smears were done from fifty culture media by using normal saline by air and fixed by flame. The fixed smears were covered by crystal violet for 30-60 seconds then washed by clean tap water and then covered by logal iodine for 30 – 60 seconds, washed off by clean tap water, were decolorized by alcohol for few seconds followed by washing by clean tap

water. Finally smears covered by Saffranin for 2 minutes, washing by clean water examine the slides by using of oil immersion lens (100X).

Result:

Gram negative: red color

Gram positive: violet color

3.8.3 Biochemical Tests:

3.8.3.1 Oxidase test:

This test is used to detect the ability of the organism to produce Oxidase enzyme, Oxidase disk was placed inside sterile petridish, small inoculums was taken by using wooden stick, and then smeared on the disk by opening the petridish partially.

Result:

Positive: deep blue purple color within 10-30 seconds.

Negative: absence of coloration

3.8.3.2 Citrate utilization test:

In this organism has the ability to use citrate as sole source carbon (c).

By using of the loop a part of tested organism (colony) was emulsified in koser citrate media and incubated for 18 – 24 hours in 37°C.

Result:

Positive: blue color

Negative: no change =green color

3.8.3.3 Indole test:

In this test the tested organism produced tryptophanase enzyme which brake down the tryptophan and produce indole. When the organism is incubated at 37°C for overnight, the kovac's reagent was added.

Result:

Positive: pink- red ring

Negative: yellow brown ring

3.8.3.4 Urease test:

In this test the organism produce urease enzyme which break down the urea and produce ammonia and carbon dioxide, which change the pH into alkaline PH, in the presence of phenol red indicator the color of the media changed into pink in case of positive result and no change in the media in case of negative result.

3. 8.3.5 Kligler iron agar:

The tested organism inoculated on the media by using sterile straight loop, by stabbing on the butt, then streaked the slope of the media then incubated for 24 hours in 37°C. The result depends on the ability of the organism to ferment the glucose in the butt and lactose in the slope, gas production appears as gap and cracking of the media H₂S production as blacking on the media.

3.8.4 Antimicrobial sensitivity testing

3.8.4.1 Preparation of inoculums:

Suspension from all growth culture media is prepared by using normal saline; 2–3 colonies are emulsified from each isolate in separate tube and compared with McFarland stander for adjustment until match occurred.

3. 8.4.2 Seeding of media:

Under septic condition by using sterile swabs, swabs are immersed in suspension in the surface of the tubes to remove the excess Muller Hinton surface is inoculated by swabbing.

3.8.4.3 Application of antimicrobial discs:

By using sterile forceps applicate the chosen antibiotics on the surface of the media according to the international measurement. The discs are pressed to

confirm that it is fixed well on the surface of the media incubate at 37 c for 18–24 hours. At the end of the incubation period read the zone of inhibition.

3.8.4.4 Interpretation of the zone size:

The results were interpreted according to the following criteria.

	Resistant	intermediate	sensitive
Amikacin	14	15 – 16	17
Colistin	14	15 – 18	19
Cefotaxime	14	15 – 17	18
Meropenem	13	14 – 15	16
Imipenem	13	14 – 15	16
Streptomycin	11	12 – 14	15

3.8.5 Quality control:

Standard strain of *Pseudomonas aeruginosa* with NCCLS No(8.2) were brought from National Health laboratories .sensitivity testing were performed on Mueller Hinton agar in similar way (disc diffusion method) and condition to our isolates to determine the validity of the selected antibiotics.

CHAPTER FOUR

Results

Sixty clinical isolates of *Pseudomonas aeruginosa* were recovered from wound swab (29), urine (22), bed sore (4), blood (4), sputum (1) and meningeal swab (1), with the following percentage 48.3%, 36.7%, 6.7%, 5%, 1.7% and 1.7% respectively (**Table 1**).

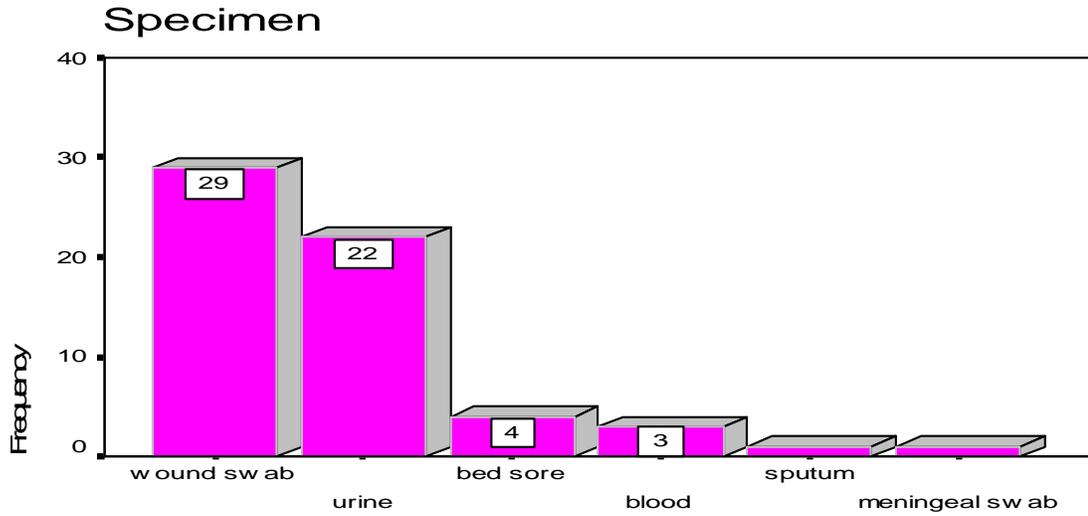
Susceptibility testing were performed on above isolates and it was Highly resistance to Cefotaxime (95%) then Streptomycin (41.7%), Meropenem (26.7%), Colistin (10%), Amikacin (6.7%) but no resistance was detected with Imipenem (0%) (**Table 2**).

Table (1) Distribution of *Pseudomonas aeruginosa* among clinical isolates

Specimen	Frequency	Percent
Wound swab	29	48.3%
Urine	22	36.6%
Bed sore	4	6.7%
Blood	3	5.0%
Sputum	1	1.7%
Meningeal swab	1	1.7%

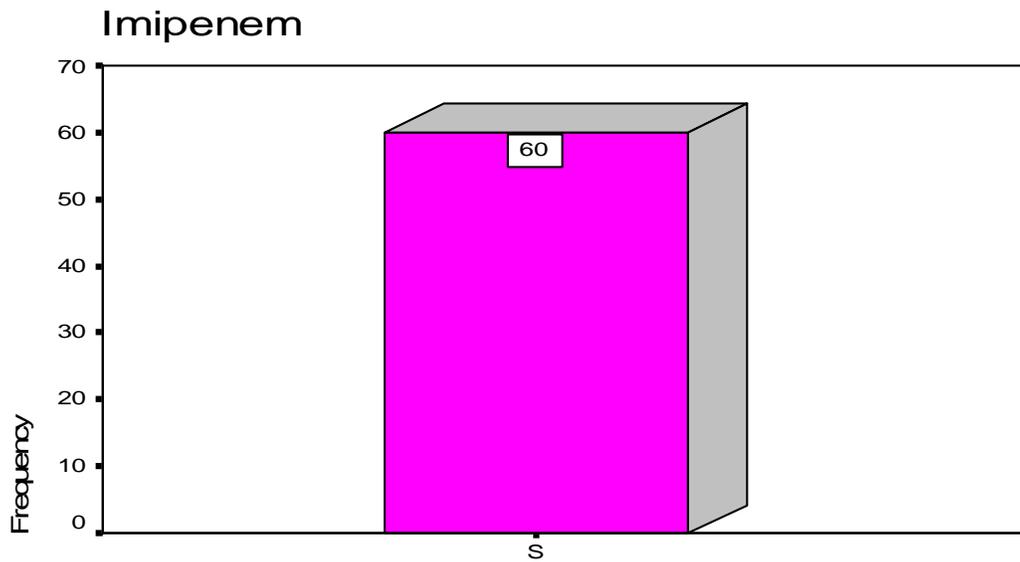
Table (2) Show susceptibility of *Pseudomonas aeruginosa* to different antibiotics

Antibiotic	Percentage	
	Sensitive	Resistant
Imipenem	100%	0%
Colistin	90%	10%
Cefotaxime	5%	95%
Streptomycin	58.3%	41.7%
Meropenem	73.3%	26.7%
Amikacin	93.3%	6.7%



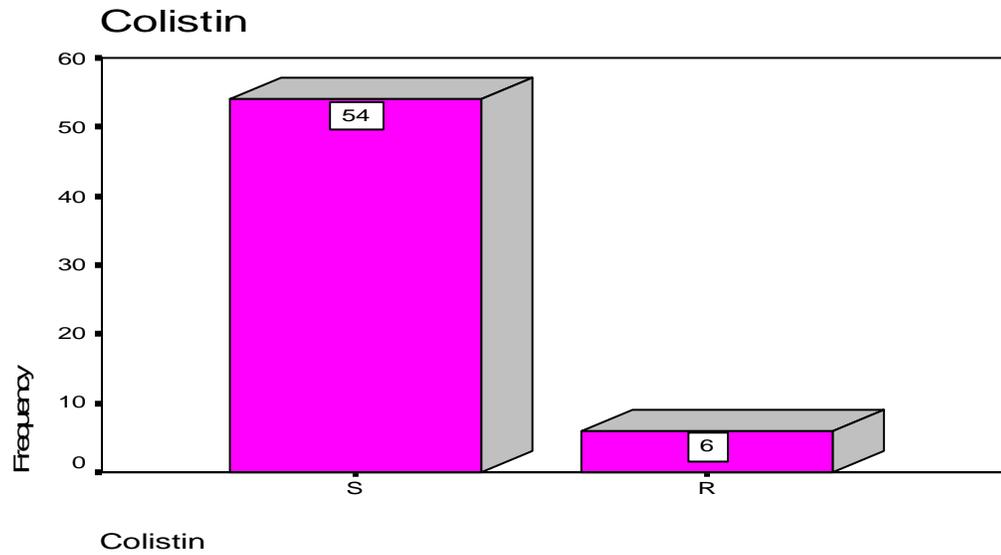
Specimen

Graph (1) Distribution of *Pseudomonas aeruginosa* among clinical isolates

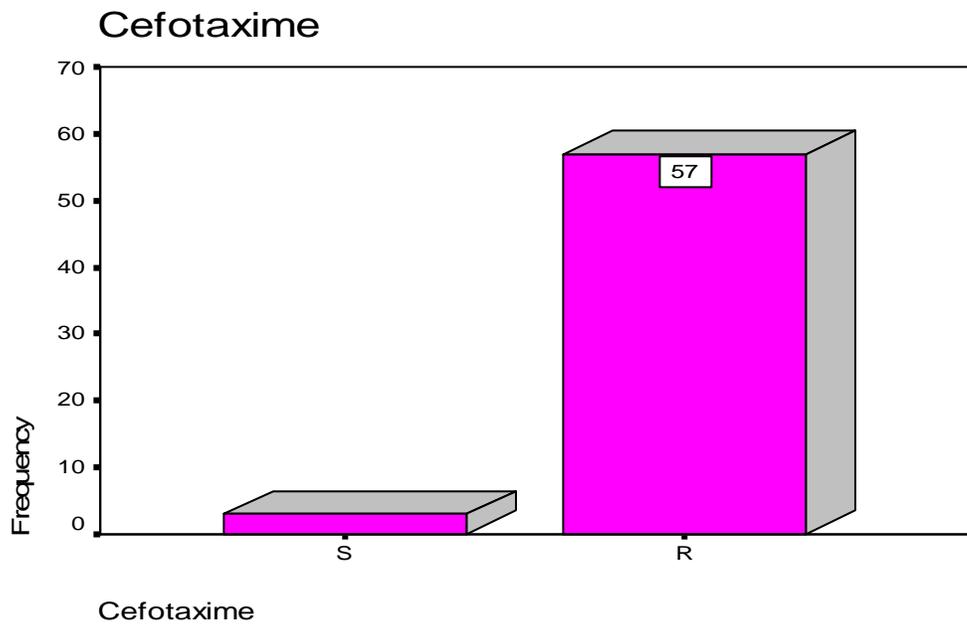


Imipenem

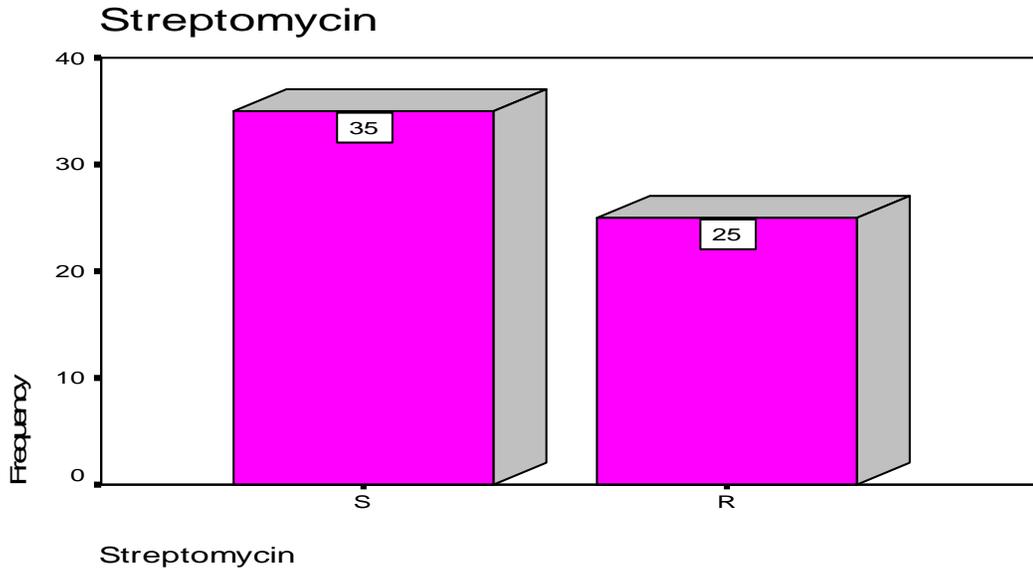
Graph (2) Percentage of imipenem resistant *Pseudomonas aeruginosa*



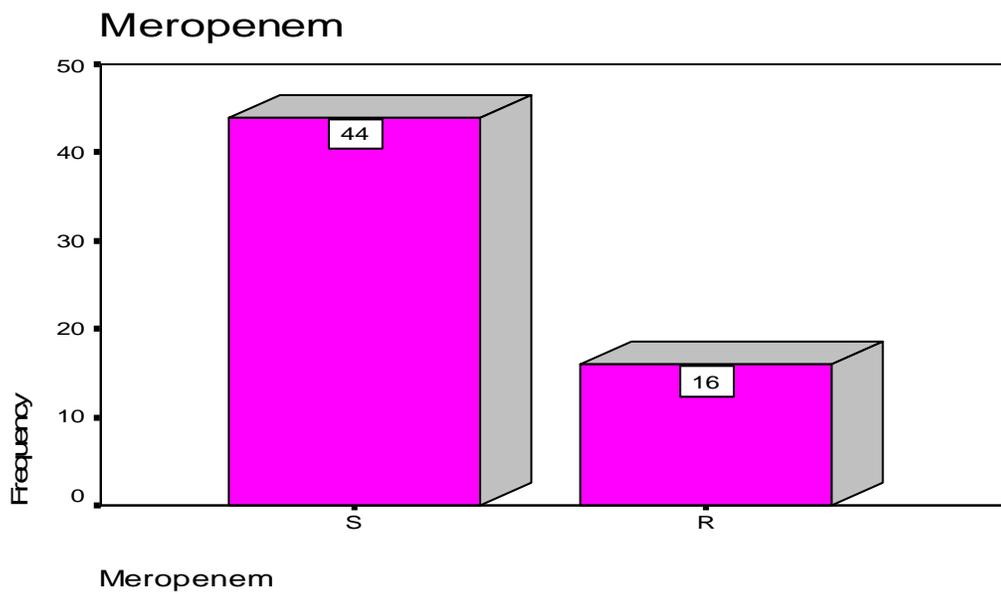
Graph (3) Percentage of Colistin resistant *Pseudomonas aeruginosa*



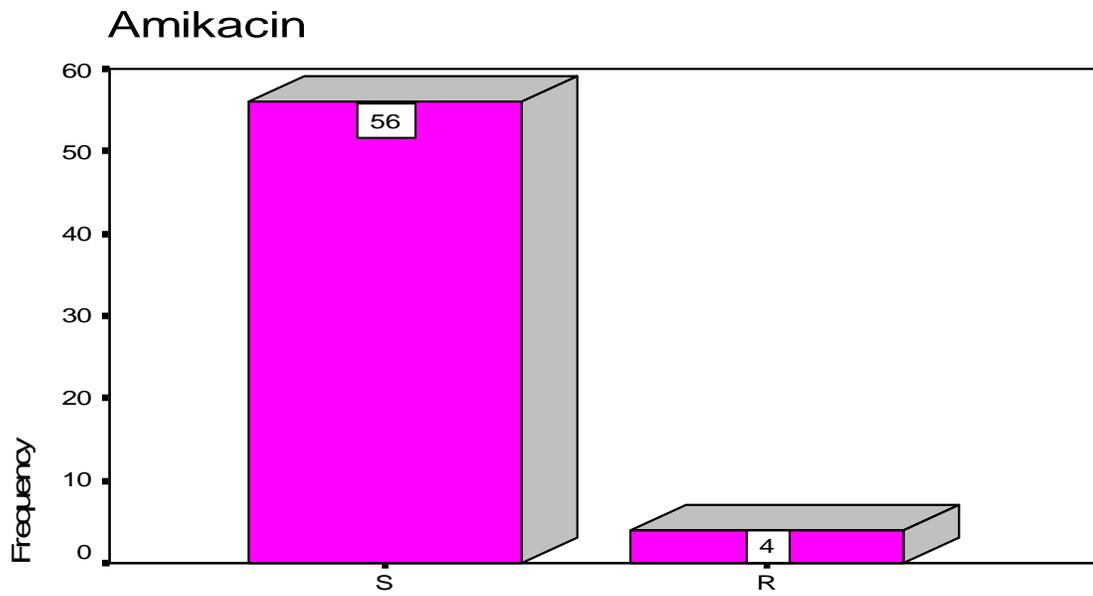
Graph (4) Percentage of Cefotaxime resistant *Pseudomonas aeruginosa*



Graph (5) Percentage of Streptomycin resistant *Pseudomonas aeruginosa*



Graph (6) Percentage of Meropenem resistant *Pseudomonas aeruginosa*



Amikacin

Graph (7) Percentage of Amikacin resistant *Pseudomonas aeruginosa*



Plate (1) *Pseudomonas aeruginosa* on blood agar.



Plate (2) *Pseudomonas aeruginosa* on MacConkey agar

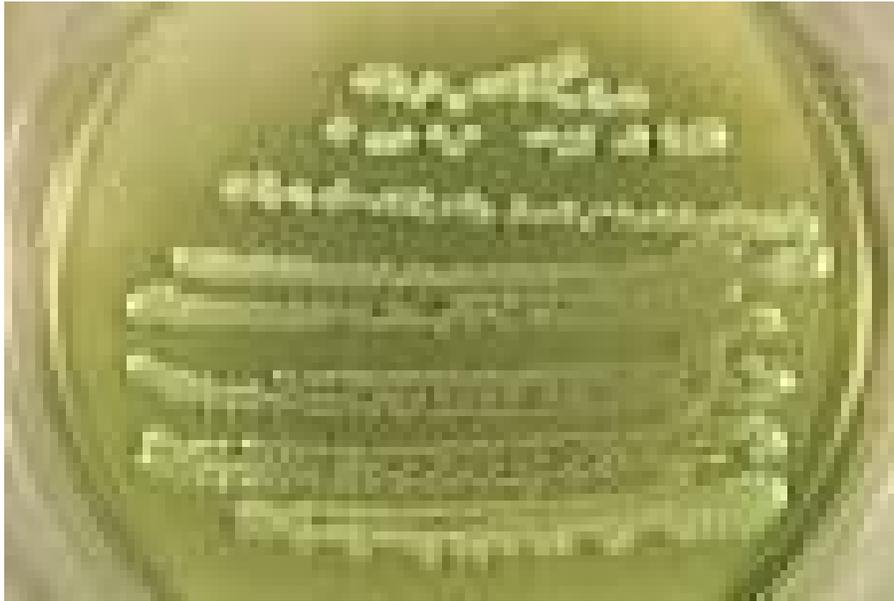


Plate (3) *Pseudomonas aeruginosa* on Cetrimide agar



Plate (4) *Pseudomonas aeruginosa* Oxidase test



Plate (5) Set of biochemical tests



Plate (6) *Pseudomonas aeruginosa* on Muller's Hinton agar.

CHAPTER FIVE

5. Discussion, Conclusion and Recommendations

5.1 Discussion

This study had been conducted to *in vitro* evaluation of some groups of antibiotics on *Pseudomonas aeruginosa* isolates.

According to table (2) percentage of Imipenem resistant *Pseudomonas aeruginosa* was (0%) and this result were agreement with those obtained by (Anil.C and Shahid R.M, 2013) and (Rashid .H *et al*, 2014) that found the percentage of Imipenem resistant *Pseudomonas aeruginosa* (0%).

According to table (2) percentage of Amikacin resistant *Pseudomonas aeruginosa* was (6.5%) and this result were disagreement with those obtained by (Rezvan *et al*,2005),that found the percentage of Amikacin resistant *Pseudomonas aeruginosa* (23.2%).

According to table (2) percentage of Cefotaxime resistant *Pseudomonas aeruginosa* was (95%) and this result were agreement with those obtained by (Saeed A.H and Awad A.A,2009). that found the percentage of Cefotaxime resistant *Pseudomonas aeruginosa*(97%).

According to table (2) percentage Meropenem resistant *Pseudomonas aeruginosa* was (26.7%) and this result were agreement with those obtained by (Pathmanathan S.G, 2009).that found the percentage of Meropenem resistant *Pseudomonas aeruginosa* (22.7%).

According to table (2) percentage Colistin resistant *Pseudomonas aeruginosa* was (10%) and this result were disagreement with those obtained by (Rashid .H *et al*, 2014) that found the percentage of Colistin resistant *Pseudomonas aeruginosa* (0%).

According to table (2) percentage Streptomycin resistant *Pseudomonas aeruginosa* was (41.7%) and this result were disagreement with those obtained by (Olayinka,B.O *et al* ,2004) that found the percentage of Streptomycin resistant *Pseudomonas aeruginosa* (70%).

5.2 Conclusion and recommendations

5.2.1 Conclusion:

This study concluded to that:

-Percentage of *Pseudomonas aeruginosa* isolated from wound swab was (48.3%).

-Percentage of *Pseudomonas aeruginosa* isolated from urine was (36.7%).

-Percentage of *Pseudomonas aeruginosa* isolated from bed sore swab was (6.7%).

-Percentage of *Pseudomonas aeruginosa* isolated from blood was (5%).

-Percentage of *Pseudomonas aeruginosa* isolated from sputum was (1.7%).

-Percentage of *Pseudomonas aeruginosa* isolated from meningeal swab was (1.7%)

-No Imipenem resistant *Pseudomonas aeruginosa* was isolated.

- Percentage of Cefotaxime resistant *Pseudomonas aeruginosa* was (95%).

-Percentage of Streptomycin resistant *Pseudomonas aeruginosa* was (41.7%).

-Percentage of Meropenem resistant *Pseudomonas aeruginosa* was (26.7%).

- Percentage of Colistin resistant *Pseudomonas aeruginosa* was (10%).

- Percentage of Amikacin resistant *Pseudomonas aeruginosa* was (6.7%).

5.2.2 Recommendation:-

-Since the *Pseudomonas aeruginosa* are highly resistant to antimicrobial agents, susceptibility testing to these bacteria should be carried out routinely before prescribed the treatment.

- All laboratories should be facilitated with microbiology equipments
- For more accurate evaluation further well designated studies are needed with increased number of samples.

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Appendices

Appendix 1

MacConkey agar (Himedia laboratories Pvt.Ltp.Mumbai, India).

Ingredients:	gms/litter
Peptic digest of animal tissue	17.00
Protease peptone	3.00
Lactose	10.00
Bile salts	1.50
Sodium chloride	5.00
Neutral red	0.03
Agar	15.00

Appendix 2

Blood agar (Himedia laboratories Pvt.Ltp.Mumbai, India).

Ingredient

Nutrient agar	500 ml
Sterile defibrinated blood	25 ml

Preparation

Prepare the agar medium, sterilized by autoclave at 121C for 15 minutes, when agar has cooled to 50C add aseptically the sterile blood and mix gently but well. To avoid forming air bubbles.

Appendix 3

Cetrimide agar: (Himedia laboratories Pvt.Ltp.Mumbai, India).

Enzymatic Digest of Gelatin	20 g ml
Magnesium Chloride	1.4 g
Potassium Chloride	10 g
Cetrimide (Cetyltrimethylammonium Bromide)	0.3 g
Agar	13.6 g

Glycerol 10ml

Final pH: 7.2 ± 0.2 at 25°C

Preparation

1. Suspend 45.3 g of the medium and 10 ml of glycerol in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Appendix 4

Gram's stain:

1. Crystal violate:-

Crystal violate	20 gm
Ammonium oxalate	99gm
Ethanol	95gm
Distil water	1L

2. Logal's iodine:-

Potassium iodine	20gm
Iodine	10gm
Distilled water	1L

3. Acetone –alcohol decolorizer:-

Acetone	500ml
Ethanol or methanol absolute	465ml
Distilled water	25ml

4. Saffranin 0.54g

Distilled water	100ml
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Appendix 5

Peptone water (Himedia laboratories Pvt.Ltp.Mumbai, India).

Ingredient

Peptic digest of animal tissue	10g
Sodium chloride	5g

Preparation

15g of powder dissolve in 1 liter of D.W then sterilize by autoclaving at 15 lbs pressure at 121°C for 15 minute.

Appendix 6

Urea agar base (Christensen) (Himedia laboratories Pvt.Ltp.Mumbai, India).

Ingredient

Peptides digest of animal tissue	1g
Dextrose	1g
Sodium chloride	5g
Monopotassium phosphate	0.80g
Phenol red	0.012g
Agar	15

Preparation

24g of powder dissolve in 1L of D.W then sterilize by autoclaving at 15 lbs pressure at 121°C for 15 minutes then cool and add aseptically 50 ml of 40% urea, mix and pour in tube in vertical position.

Appendix 7

Kosser's citrate medium (Himedia laboratories Pvt.Ltp.Mumbai, India).

Ingredient

Magnesium sulfate	0.2g
Potassium dihydrogen sulfate	1g
Sodium ammonium sulfate	1.5g
Trisodium citrate	2.5g
Bromothymole blue	0.016g

Preparation

5.2 g of powder dissolve in 1l of D.W, sterilize by autoclaving at 15 Ibs pressure at 121°C for 15 minutes and pour in tube.

Appendix 8

Kligler iron agar (KIA) (Himedia laboratories Pvt.Ltp.Mumbai, India).

Ingredient

Peptic digest of animal tissue	15g
Beef extract	3g
Yeast extracts	3g
Protease peptone	10g
Lactose	10g
Dextrose	1g
Ferrous sulfate	0.20g
Sodium chloride	5g
Sodium thiosulfate	0.3g
Phenol red	0.024g
Agar	15g

Preparation

57.5 of powder dissolve in 1L of D.W and sterilize by autoclave at 15 Ibs pressure at 121°C for 15 minutes then cool and pour in tube in slop slant position.

Appendix 9:

Muller's Hinton agar (Himedia laboratories Pvt.Ltp.Mumbai, India).

Ingredients	Gms / Litre
Beef, infusion from	300.000
Casein acid hydrolysate	17.500
Starch	1.500

Agar 17.000

Final pH (at 25°C) 7.3±0.1

Preparation:

Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 1 minute. Mix well before pouring.

Antimicrobials:

Amikacin (gm) (30mcg)

Colistin (gm) (10mcg)

Cefotaxime (gm) (30mcg)

Meropenem (gm) (10mcg)

Imipenem (gm) (10mcg)

Streptomycin (gm) (10mcg)