# Training Report


**Internship Training Report**

**Submitted to**

**Lovely Professional University, Punjab**

**in partial fulfillment of the requirements**

**For the degree of Master of Science in Clinical Microbiology**

**Submitted by**
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**SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES**
**LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA, MAY, 2016**

# DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Guide **Mr. Gurinder Singh (**Assistant Professor ), COD Paramedical Sciences (LSPPS)(Internal supervisor), Lovely Professional University and **Dr. Mamta Kumari** Chief Microbiologist (MD), SRL Diagnostics (External Supervisor). This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Saurabh
Date:
Place: Lovely professional University, Punjab

# *CERTIFICATE*

This is to certify that Mr./Ms. *Saurabh* bearing Registration Number 11400990 has completed his/her Master of Science in Clinical Microbiology internship under our guidance and supervision. This report is record of the candidate own work carried out by him/her under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

.....

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***Date :*** 06/05/16

.....

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# ABSTRACT

**Context:** Bacteria isolated from the pus samples were resistant to multiple drugs. Multiple drug resistance have become an important clinical problem. Antimicrobial resistance showed by different pathogens might hinder a successful treatment.

**Aim:** The present prospective study was done to isolate and identify the bacteria from pus samples with their antimicrobial susceptibility pattern.

**Materials and Methods:** The present study was carried out for a period of 4 months (1st January 2016-15th April 2016) in the microbiology department of SRL diagnostics, Gurgaon, Haryana, to isolates various bacterial pathogens present in the pus samples and to determine their Antibiotic Susceptibility Pattern. Total 583 samples were received. Out of 583 samples (357, 61.23%) were collected from male patients and (226, 38.76%) were collected from female patients. All the pus samples were processed as according to the Standard operating procedure. For the identification and antibiotic susceptibility pattern Vitek 2 Compact ID/AST Instrument was used. bacteria were identified with their antibiotic susceptibility pattern. *Staphylococcus aureus* showed the resistance to most antibiotics. Erythromycin, Ciprofloxacin, Oxacillin. Gram positive bacteria *Staphylococcus aureus* showed the highest resistance to ciprofloxacin.

**Results:** Total (328, 56.26%) cases were found as positive with (193, 58.84%) male and (135, 41.15%) female patients and 255 (43.73%) were negative. Total 13 bacterial genus were identified with (6, 46.15%) Gram positive and (7, 53.84%) Gram negative bacteria. In Gram positive bacteria *Staphylococcus aureus* (160, 32.62%) was the most frequent grown organism followed by the Gram negative bacterium *E. coli*(39, 11.89%). Others Gram positive and negative bacteria were Identified as *S. epidermidis*(35, 18.81**%),** *S. haemolyticus*(22, 11.82**%),** *Enterococcus spp*(18, 09.67**%),** *S. pyogenes*(04,02.15**%),** *Pseudomonas aeruginosa* (35,24.64**%),** *Klebsiella pneumonia* (29,20.42**%),** *Proteus mirabilis*(15,10.56**%**), *Enterobacter Species*(11,7.74**%),** *Acinetobacter baumanni*(09,6.33**%),** *Morganella morganii* (04, 2.81**%).**

**Conclusion:** Our study confirms that the most pyogenic Gram positive bacterium was *Staphylococcus aureus* and in there were variations in Gram negative bacteria which are responsible for the formation of pus.


**KEYWORDS:** Pathogens, Antibiotics, *Staphylococcus aureus*, Multiple Drug Resistance.

# ACKNOWLEDGEMENT

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# CHAPTER 1

# INTRODUCTION

Pus is a protein rich fluid that is usually whitish-yellow, yellow-brown in color. Pus exudates due to the necrosis of the tissue. Pus is a fluid that had filtered from the circulatory system. The presence of pus is the result of our body's natural immune system responding to an infection caused by different groups of Micro-organisms Including bacteria, Fungi, and Protozoa. Bacterial infection is the most common cause of drainage the pus. [1]

The whitish-yellow, yellow-brown, greenish color of pusis result of an accumulation of dead Neutrophils. Pus can sometime be green because some WBC's produce a green Anti-Bacterial protein called Myeloperoxidase. *Pseudomonas aeruginosa* produces Pyocyanin (green pigment). Infection caused by *Pseudomonas aeruginosa* is particularly foul smelling.

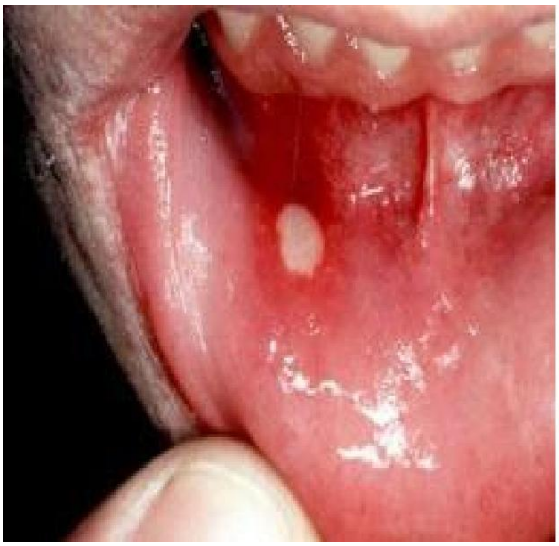
The development of wound infection depends on the integrity and prospective function of Skin. The potential for infection depends on a number of patient variables such as the state of hydration nutrition and existing medical conditions as well as extrinsic factors such as pre, intra and postoperative care if the patient has undergone surgery. Thus it is difficult to predict which wound will become infected. [2]

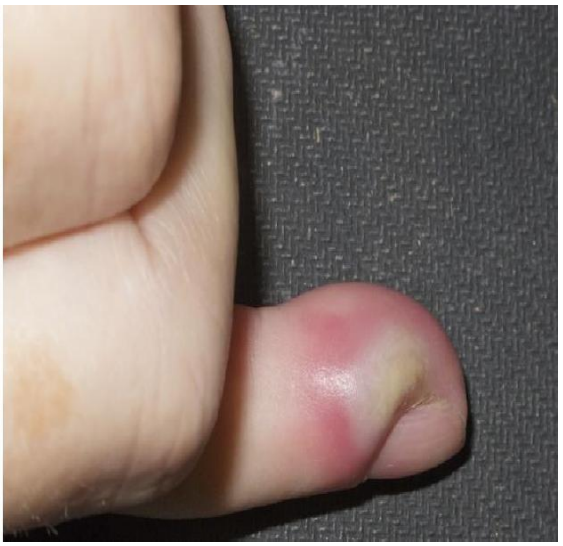
Wound infection is very common infection throughout the world it creates great fears in both developed and developing countries. India being a developed country, the socioeconomic condition of the people is not so good and their knowledge regarding sanitation and hygiene is poor. People in India are generally prone to agricultural wounds, traffic accident and domestic. People are not aware of prevention from the Injuries minor or major wounds can be prevented with the help of proper care and by using disinfectant and treatment protocols, clean surgical procedures, proper care of wound and hygiene Environment.

The overall incidence of wound sepsis in India is from 10-33%. Relative resistance to Antibiotics relatively more virulent strains and capacity to adapt quickly to changing environment make the pathogens acquired in hospitals a matter of concern. Wound infection is one of the most common hospital acquired infections and important cause of morbidity and accounts for 70-80%. [3]

Skin, the largest organ in the human body, plays a crucial role in the sustenance of life through the regulation of water and electrolyte balance, thermoregulation, and by acting as a barrier to external noxious agents including microorganisms, however, when the epithelial integrity of skin is disrupted, a wound results.[4]

The common Portal of entry of pathogenic Bacteria into the body are the sites where Mucus membrane meets with the Skin such as Lower and Upper Respiratory airways, Gastrointestinal Tract, Skin(Cuts, Burns, other Injuries).





Pus formation in the mouth (Figure 1)  Pus formation in the skin (Figure 2)

According to survey there are 3-7% Risk of post operative wounds infection. A study on anaerobic Bacterial profile and Anti-Bacterial susceptibility pattern of pus isolates in a south Indian tertiary hospital revealed *S. aureus* (24.29%) was the most commonest isolates followed by *Pseudomonas aeruginosa* (21.49%), *E.coli* (14.02%),*Klebsiella pneumonia* (12.15%), *S. pyogenes* (11.23%), *S. epidermidis* (9.31%) and *Proteus species* (7.47%).

## 1.1WOUNDS

Wounds are injuries that break the skin or other body tissues. They include cut, scraps, scratches punctured skin. They often happen because of an accident, surgery, sutures. It is very important to clean the minor as well as major wounds. At the site of the wound there is dead and living WBC, Micro-organisms. They all suspend in inflammatory exudates. The formation of Pus is a common sequel of Acute Inflammation. [5]

## 1.2PRINCIPLE OF WOUND

Some wounds are superficial that require local first aid including cleansing and dressing but some wound are deeper that need medical attention to prevent infection and loss of function. Skin is the largest organ of the body. Skin having epidermis and dermis layers, the cells present in the epidermis layer provides a moist warm

and Nutritive Environment for conductive Microbial growth and colonization. Pus formation is only due to the killing of WBC and Micro-organisms. Some common Bacteria are said to be Pyogenic (pus forming) and include *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E.coli*, etc.

## 1.3 CLASSIFICATION OF WOUND

### 1.3.1 Acute wound

Acute Wound is an injury to the skin that occurs suddenly rather than over time. It heals at a predictable and expected rate according to the normal wound healing process. Acute wound can happen anywhere on the body. Acute wound includes bites, Burns, cut, surgical wounds and other Injuries. Acute wound are expected to heal within expected time period and treatment required for healing depends upon the severity of the wound. [6]

### 1.3.2 Chronic wound

Chronic wound develops when any acute wound fails to heal in the expected time frame for that type of wound, which might be a couple of weeks or up to six weeks in some cases. Failure of any wound to heal can be due to a lack of one or more of the main requirements of healing, including a good supply of blood, oxygen and nutrients, and a clean and infection-free environment. An important aspect in caring for wounds is to remove the causing agent, as in cases of wounds caused by weight-bearing or wounds that are under constant pressure. When wounds do not get relief from constant pressure, there can be a cumulative breakdown of the tissue. [7]

## 1.4 CLASSIFICATION OF WOND INFECTION

### 1.4.1 BURN WOUND INFECTION

Burn wound infection is problematic because it delays healing, encourages scarring and may result in Bacteremia. Bacteria and fungi are the most common pathogens of burn wounds. These microbes form multi-species Biofilms on burn wounds within 48 – 72 hours of injury.

In a two year period Study done on Bacterial profile of burn wounds infections at a burn unit Nishtar hospital Multan, the frequency of Gram negative organisms was found to be high with *Pseudomonas aeruginosa* (54.4%) being the most common isolate, followed by *Staphylococcus aureus* (22%), *Klebsiella species* (8.88%), Staphylococcus epidermidis (5.79%), *Acinetobacter species* (4.63%), *Proteus species* (2.70%) and *Escherichia coli* (1.54%). [8]

## 1.4.2 SURGICAL WOUND INFECTION

Surgery that involves a cut (incision) in the skin can lead to a wound infection after surgery. most Surgical wound infections show up within the first 30 days after surgery. Surgical wound infections may have pus draining from them and can be red, painful or hot to touch.

Pre-antibiotic 963 treatment specimens from 676 individuals were examined. One-thousand sixty Bacterial strains were isolated from 614 individuals. Particularly, a single agent was identified in 271 patients, multiple agents were found in 343 patients, and no agent was identified in 62 patients. A high preponderance of aerobic Bacteria was observed. Among the common pathogens were *Staphylococcus aureus* (191, 28.2%), *Pseudomonas aeruginosa* (170 patients, 25.2%), *Escherichia coli* (53 patients, 7.8%), *Staphylococcus epidermidis* (48 patients, 7.1%), and *Enterococcus faecalis* (38 patients, 5.6%). pure cultures most commonly yielded S. *aureus* (98 strains), P. *aeruginosa* (82 strains), and *Enterobacteriaceae* (102 strains). [9]

## 1.4.3 NOSOCOMIAL WOUND INFECTION

An infection that is contracted from the environment or staff of a healthcare facility. It can be spread in the Hospital environment, nursing home environment, rehabilitation facility, clinic or other clinical settings. Different types of Bacteria are Responsible for the Nosocomial Wound Infection.

A study conducted in which 109 wound swabs were collected from patients who had developed postoperative wound infection. Conventional technique for isolation of Bacteria was applied with analytical profile index (API system) for identification to confirm primary and secondary isolates. Antibiotics susceptibility was applied for all isolated bacteria. Aerobic bacterial isolates were *S.aureus* (55.0%), *P. mirabilis* (35.0%), *Ecoli* (5.0%),*Pseudomonas aeruginosa* (3.0%) and *Proteus vulgaris* (2.0%). The prevalence rate of hospital acquired infection was 25.23%. [10]

## 1.4.4 BITE WOUND INFECTION

Between 5% and 60% of all bite wounds are complicated by infection. Approximately 20% to 50% of cat bites, 10% to 50% of human bites, and 3% to 20% of dog bites will become infected. With the exception of monkey bites, which have a 25% infection rate, infection developing after mammalian bites is uncommon. The most common aerobic organisms isolated at a research laboratory were *Pasteurella*(50%),

*Streptococcus*(46%), *Staphylococcus*(46%), *Neisseria*(32%) *and Corynebacterium* (12%). *Other aerobic organisms, in order to decreasing frequency, including Moraxella species* (10%), *Enterococcus species*(10%),*Bacillus species*(8%), *Pseudomonas species*(6%),*Actinomyces species*(6%), *Lactobacillus species*(4%), *Stenotrophomonas maltophilia*(4%), *Pediococcus species*(2%),*Stomatococcus species*(2%)[11]

## 1.5 BACTERIAL INFECTION OF SKIN

### 1.5.1 BOILS

A boil is a skin infection that commonly caused by *Staphylococcus aureus* boils starts in a hair follicle or oil gland. At first, the skin turns red in the area of the infection, and a tender lump develops. After four to seven days, the lump starts turning white as pus collects under the skin. If the infection spreads to the deeper tissues of the skin, then it becomes an abscess or furuncle. Boils usually resolve by themselves but severe or recurring cases require medical treatment. [12]

### 1.5.2 CELLULITIS

Cellulitis is a Bacterial infection of the skin and tissues beneath the skin. Unlike impetigo, which is a very superficial skin infection, Cellulitis is an infection that also involves the skin's deeper layers: the dermis and Subcutaneous tissue. *Streptococcus* and *Staphylococcus* are the Bacteria commonly responsible for Cellulitis the same Bacteria that can cause impetigo. MRSA (Methicillin-Resistant *Staph. aureus*) can also cause Cellulitis. Sometimes, other Bacteria (for example, *Haemophilus Influenzae*, *Pneumococcus*, and *Clostridium* species) may cause Cellulitis. [13]

### 1.5.3 FOLLICULITIS

This common type is marked by itchy, white, Pus-filled bumps. When bacterial Folliculitis affects a man's Beard area, it’s called Barber's itch. It occurs when hair follicles become infected with Bacteria, usually *Staphylococcus aureus*. *Staphylococcus* lives on the skin all the time. But they generally cause problems only when they enter your body through a cut or other wound. [14]

### 1.5.4 IMPETIGO

Impetigo is the most common Bacterial infection in children. This acute, highly contagious infection of the superficial layers of the epidermis is primarily caused by *Streptococcus pyogenes* or *Staphylococcus aureus*. Secondary skin infections of existing skin lesions (Eg. cuts, Abrasions, Insect bites, chickenpox) can also occur. Methicillin-resistant *S aureus* (MRSA) and Gentamicin-Resistant *S aureus* strains have also been reported to cause impetigo. Impetigo is classified as either Non-bullous (about 70% of cases).

### 1.5.5ABSCESS

An abscess is a tender mass generally surrounded by a colored area from pink to deep red, painful and warm to touch, abscesses are often easy to feel by touching. The middle of an abscess is full of pus and debris. abscesses can show up any place on your body. The middle of the abscess liquefies and contains dead and living cells, Bacteria. the most common sites are in armpits (Axillae), areas

around anus and vagina(Bartholin gland abscess), the base around a tooth (dental abscess).S. aureus was the most common Pathogenic organism isolated in breast abscesses at Al-Amiri Hospital, Kuwait, of which 23% were MRSA. [15]

# CHAPTER 2

# LITERETURE REVIEW

A study of pus samples was conducted in a JLN Hospital, Ajmer for a period of 6 months. Total 100 samples were received in a bacteriology section. Out of 100 culture, 48 (48%) were Gram Negative and 25 (25%) were gram positive. Mixed growths were seen in 20 (20%) samples and no growth in (7%) cases. Biochemical tests applied were standard Catalase test, citrate utilization, Coagulase, Oxidase, Methyl red, Voges-Proskauer, Indole Production, motility, Carbohydrate fermentation test using glucose, sucrose, maltose and lactose. Antimicrobial susceptibility testing of isolates was performed by standard Kirby Bauer disc diffusion methods according to CLSI protocol. *Klebsiella pneumoniae* is 100% sensitive to Imipenem, 71.42% of Amikacin and Cefotaxime, 67.5%,Ceftazidime, 60.71% Ciprofloxacin, 53.57 %, Tetracycline and Amoxyclave. *Pseudomonas aeruginosa* is 100% sensitive to Imipenem, and Amikacin followed by Piperacillin (75%), Ceftazidime (65%), Amoxyclave, Ciprofloxacin and Cefotaxime (60%) and Tetracycline (50%). *Staphylococcus aureus* 100 % sensitive to Vancomycin, 87.5% to Linezolid, 62.5% to Gentamicin, 62.5% to Amoxyclave, 25% to Oxacillin, 75% to Ciprofloxacin 75% to Erythromycin and 25%to Cotrimoxazole.[16]

A study conducted from August, 2013 to January, 2014, in GSL Medical college central lab. Pus samples received for diagnostic microbiology was processed and identification by standard protocols. Out of 114 pus samples received for culture and sensitivity in the microbiology central laboratory, 102 (89.47%) cases yielded positive culture while 12 (10.53%) cases had no aerobic Growth. A total number of 107 organisms were isolated out of 102 Pus samples. Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method. The Antibiogram of Gram Positive cocci revealed that the Vancomycin (100%) was the most susceptible drug followed by Levofloxacin (76.92%) and Oxacillin (73.07%). Gram Negative Bacilli are susceptible to Imipenem (80%), Aztreonam (80%), Piperacillin + Tazobactum (80%), Levofloxacin (80%). [17]

A study was conducted in Tertiary care hospitals attached to Dr. S.N.Medical College (Jodhpur) Western Rajasthan. A total number of 70 samples were collected for aerobic culture and sensitivity from both inpatients and outpatients of various departments of Hospitals. Identification and antimicrobial susceptibility testing of Grown bacteria was done by Vitek-2 Compact System. The Antibiogram of Gram Positive cocci obtained by Vitek-2 compact revealed that Tigecycline (58.33 %) was the most susceptible drug followed by Nitrofurantoin (45.69%) and Vancomycin (40.36%). *Staphylococcus aureus*was most susceptible to Nitrofurantoin (57.14%) followed by Tigecycline (50.00%) and Linezolid (39.28%). MRSA was detected with the help of Cefoxitin screen and 16 (57.14%) were found to be MRSA. Enterococcus was most susceptible to Vancomycin (57.69 %) followed by Linezolid (53.84 %) and Tigecycline (50%). CONS was most susceptible to Tigecycline (75.00 %) followed by Nitrofurantoin (50.00 %). [18]

This prospective study was carried out from Tertiary Care Institute Haryana, Indian Microbiology department from January 2015 to July 2015. A total of 438 pus samples were obtained for aerobic culture and sensitivity from different IPDs & OPDs of hospital. A total of 364 organisms were isolated. The commonest organism were family *Enterobacteriaceae* (38.5%) followed by *Staphylococcus spp* (26.9%), *Pseudomonas* (21.4%), *Enterococcus* (6.6%), *Diphtheroids* (3.8%), *Streptococcus pyogens*(2.2%) and Acinetobacter (0.5%). The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines. Sensitive to all the drugs tested in our set up. Gram Negative bacteria of Enterobacteriaceae were most susceptible to Imipenem (sensitivity 90-100%), only two isolates of *Klebsiella spp* showed resistance. Enterobacteriaceae showed very high resistance to Ampicilline, Amoxyclav and Ciprofloxacin (sensitivity ranging from 5-30%). Resistance to Cephalosporin ranged from no resistance to resistance in 50% isolates, where in *E.coli* and *Klebsiella spp* were the main contributors followed by *Proteus, Citrobacter* and no resistance in *Morganella spp*..Sensitivity to Polymixin B and Colistin was 100% as all Enterobacteriaceae was sensitive. Sensitivity to Tigecycline ranged from 68-100%, *Klebsiella* spp. showing highest resistance as seven isolates were resistant. No resistance against Imipenem, Polymixin B and Colistin was reported in *Pseudomonas* and *Acinetobacter spp* isolated from wounds (Table 3). Piperacillin and Netilmicin are no more effective against Pseudomonas as sensitivity is only 1-6%, Piperacillin Tazobactum combination being slightly better (sensitivity being 33.3%). Sensitivity to Amikacin and Gentamicin is still there in *Pseudomonas* being 52.6 and 65.4% respectively. Sensitivity to Ceftazidime is also good in our set up being 78%. [19]

In the study of pus samples collected from 3 different centers. The Doctor's X-ray and pathology Institute Pvt. Ltd. Civil lines, Kanpur, Akash Ganga centre, Shuklaganj, this centre is located outside main Kanpur city and caters to semi-urban population, Akash ganga centre, Unna. Indole Production, Urea Hydrolysis, Citrate Utilization etc. Antibiotic susceptibility testing was done by using Vitek 2-Compact. *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the major microbial pathogen associated with wound infection. On studying the antibiotic sensitivity pattern of the isolated bacteria, it was seen that commonly used Oral antibiotics on patient with for pus like Amoxicillin/Clavulanic Acid, Cefuroxime, Cefexime were not effective on bacteria in >50% cases, however fortunately the resistance to Quinolones (Ciprofloxacin, Ofloxacin, Prulifloxacin, Levofloxacin) is relatively less. Data on inject able antibiotics demonstrate that Gentamicin has less efficiency in Kanpur region but other inject able like Amikacin, Piperacillin/Tazobactum,Cefoperazone/Sulbactum, Carbapenem etc can be safely used. Low resistance to 3rd generation Cephalosporin also Demonstrate absence of E.S.B.L producing strains in pus sample in Kanpur region. Amikacin are the most effective agents against whole gram negative organism. Gram negative bacteria isolated were sensitive to Gentamicin, Ceftazidime and Ciprofloxacin. However, most of the gram negative bacteria isolated were resistance to Ampicilline, Chloramphenicol and ciprofloxacin are third generation Cephalosporin. [20]

One hundred pus samples were screened from different orthopedic hospitals of Nagpur (central India) for *S. aureus*, by growth on Mannitol salt agar (MSA), Baird-Parker agar (BPA), Deoxyribonuclease test, tube Coagulase test, and latex agglutination test. Fifty-one *S. aureus* isolates were obtained which were further subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method (DDM). Minimal inhibitory concentrations (MICs) were determined by an automated system, the VITEK 2 system. Emergence of Linezolid resistance in orthopedic patients has important implications for the use of Linezolid as a therapeutic agent. Twelve (23.52%) LRSA isolates and seven (58.33%) LR-MRSA was recorded in our study in contrast to studies where higher susceptibility rates to Linezolid were observed among MRSA infections. The significant observation of this study was that all the 12 LRSA were multidrug-resistant strains. [21]

# CHAPTER 3

# AIM AND OBJECTIVE

1. To isolate and identify the bacteria from pus samples.

2. To study the antimicrobial susceptibility pattern in various Gram positive and Gram negative bacterial isolates.

# CHAPTER 4

# MATERIAL AND METHODS

In this study a total 583 pus samples were received within a period of 4 months (1st January 2016-15th April 2016) In the Microbiology Department of SRL diagnostics, Gurgaon, Haryana. Received pus samples were processed on blood agar, MacConkey agar media and incubated at 37 degree C under aerobic condition in incubator and the organisms were identified by Vitek 2 Compact ID/AST Instrument.

## 4.1 MATERIALS

Materials Used during the study are Mention in Appendix No. III

## 4.2 METHOD

In order to Identify the bacteria from pus samples along with their antibiotic susceptibility pattern. A total 583 pus samples were received. All the samples are processed as according to the Standard operating procedure of microbial techniques.

## 4.2.1 COLLECTION OF THE SAMPLES

Pus samples were receivedin a sterile cotton swab, syringes, sterile container or tube. After the collection of the pus samples, transported by using different types of transport media (Amies swab with or without Charcoal).

## 4.3 PROCESSING OF SAMPLES

## 4.3.1 MACROSCOPIC EXAMINATION

The Physical characteristics of the samples were observed, Physical characteristics including of the quantity, color, thickness, etc.

## 4.3.2 MICROSCOPIC EXAMINATION

Each pus sample was spread on clean sterile glass slide for the smear preparation. The smear was than dried in the air and fixed by heating. Bacteria were identified by Gram staining.

## 4.3.2.1 COMPOSITION AND PREPARATION OF GRAM STAINING

Composition and Preparation of Gram staining is mentioned in Appendix No. IV

## 4.3.3 CULTURE MEDIA USED FOR THE PUS SAMPLES

After receiving of the samples, samples were streaked on blood agar and MacConkey Agar plates. After 24 hour incubation in aerobic incubator blood agar and MacConkey agar plates were observed. Blood agar is a type of Growth medium (Trypticase soya agar enriched with 5% Sheep blood) that encourages the growth of bacteria. Mostly Gram positive bacteria grown on the blood Agar which can be identified on the basis of Haemolysis. MacConkey agar is selective for Gram negative organisms and helps to differentiate lactose fermenting Gram negative rods from non lactose fermenting Gram negative rods. It is primarily used for detection and Isolation of members of *Enterobacteriaceae* family and *Pseudomonas* spp., other Gram negative bacteria.

*Pseudomonas aeruginosa*(Figure.1)

*Streptococcus pyogenes* (Beta haemolysis)(Figure.2)

## 4.3.4 IDENTIFICATION OF THE ORGANISM

After the overnight incubation, the culture plates were examined for the growth of micro-organism. In the case of Positive sample, colonies were appeared in the culture plate. For the identification of the micro-organism Standard operating procedures of microbial techniques were followed. Bacteria confirmed with the help of Gram staining and then Vitek 2 compact ID/AST Instrument was used for the identification and antibiotic susceptibility tests. Bacteria as well as other Micro-organisms can be identified with Vitek 2 compact ID/AST Instrument.

### 4.3.4.1 Suspension preparation

A sterile swab or applicator stick is used to transfer a sufficient number of colonies of a pure culture and to suspend the microorganism in 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube. The turbidity is adjusted accordingly and measured using a turbidity meter.

### 4.3.4.2 Inoculation

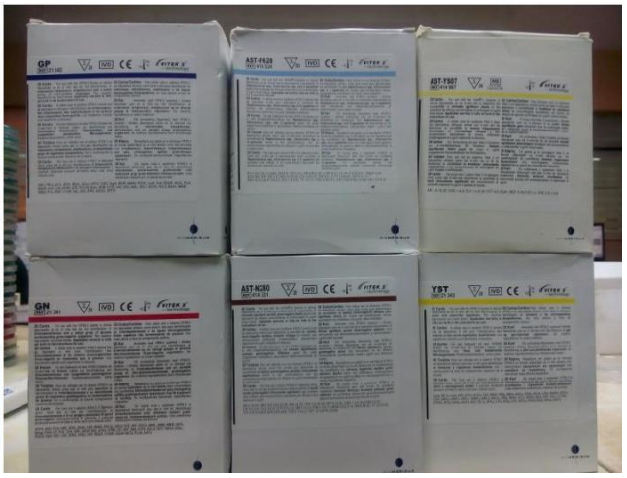
The suspension tubes were placed in cassettes and GN, GP cards were inserted into the first tube that has marked with ID and the AST-281, AST-280,P-628 cards were inserted into the second tube that has marked with AST. These filled cassettes then load manually into the vacuum chamber station of the instrument. After the vacuum is applied and the air is reintroduced into the station, the organism suspension is forced through the transfer tube into micro-channels that fill all the test wells.

### 4.3.4.3Incubation

After loading of suspension into the wells, the transfer tubes were cut and the cards were sealed by the machine itself. The cards then loaded into the carousel incubator where online incubation was given (35.5 +/- 1.0˚C). Each card was removed from the incubator once every 15 min. and transported to an optical system for reaction readings and then returned to the incubator until the next read time.[22]

Vitek2 Compact ID/AST instrument.

(Figure 3)




Cards Used For the ID/AST.

(Figure 4)

# CHAPTER 5

# RESULTS

The study was carried out for a period of 4 months (1st January 2016-15th April 2016) In the Microbiology Department of SRL Diagnostics, Gurgaon, Haryana, to isolates various Bacterial pathogens present in the pus samples collected from different wounds including vagina, mouth, skin, etc. and to determine their antibiotic susceptibility pattern. Pus samples were processed and analyzed by standard operating procedure of microbial identification.

## 5.1 Growth pattern of total samples

In the study of pus samples a total 583 samples were received, out of which 328 (56.26%) were positive and 255 (43.73%) were negative. The positive samples were identified as a single growth.

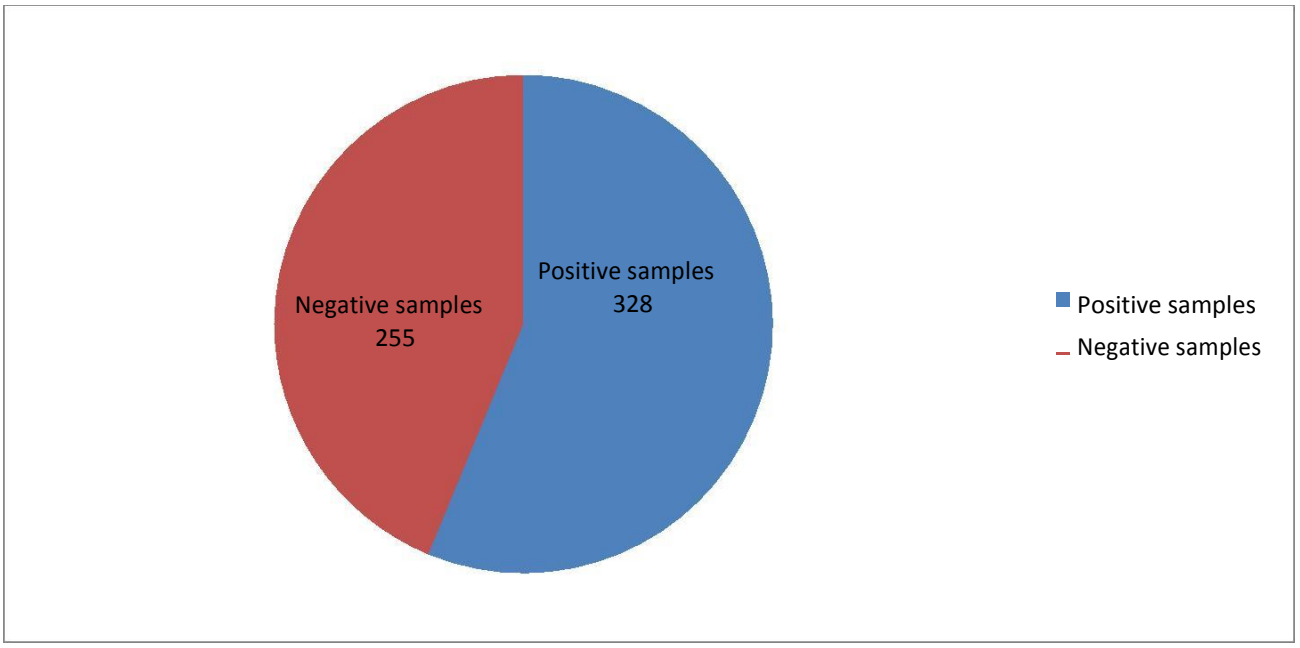



**Chart 1 Growth Pattern of total samples.**

## 5.2 DITRIBUTION OF ORGANISMS ISOLATED

### Table 1. Distribution of Gram positive isolates

| Organism isolated | No. of isolates | Percentage (%) |
|---|---|---|
| *S. aureus* | 107 | 57.52**%** |
| *S. epidermidis* | 35 | 18.81**%** |
| *S. haemolyticus* | 22 | 11.82**%** |
| *Enterococcus spp.* | 18 | 09.67**%** |
| *S. pyogenes* | 04 | 02.15**%** |

[**Note- Percentage is according to the only Gram positive organisms**]

Gram Positive cocci *S. aureus* was most frequently isolated organism 107 times contributes 57.52% of Gram Positive organisms followed by *S. epidermidis* (35, 18.81%), *S. haemolyticus*(22, 11.82%), *Enterococcus spp.*(18, 9.67**%**), *S. pyogenes* (04, 2.15%).

### Table 2.Distribution of Gram negative isolates

| Organism isolated | No. of isolates | Percentage (%) |
|---|---|---|
| *E. coli* | 39 | 27.46**%** |
| *Pseudomonas aeruginosa* | 35 | 24.64**%** |
| *Klebsiella pneumoniae* | 29 | 20.42**%** |
| *Proteus mirabilis* | 15 | 10.56**%** |
| *Enterobacter Species* | 11 | 7.74**%** |
| *Acinetobacter baumanni* | 09 | 6.33**%** |
| *Morganella morganii* | 04 | 2.81**%** |

[**Note- Percentage is according to the only Gram positive organisms**]

Gram Negative bacilli *E. coli* was most frequently isolated in Gram negative organisms contributes 27.46% of Gram Negative organisms followed by *Pseudomonas aeruginosa*(35,24.64**%),** *Klebsiella pneumonia*(29,20.42**%),** *Proteus mirabilis*(15,10.56**%**), *Enterobacter Species*(11,7.74**%),** *Acinetobacter baumanni*(09,6.33**%),** *Morganella morganii* (04,2.81**%).**

## 5.3 GENDER WISE DITRIBUTION OF PATIENTS WITH POSITIVE CASES

### Table 3.Gender Wise Distribution

| GENDER | TOTAL SAMPLES | POSITIVE SAMPLES | NEGATIVE SAMPLES | PERCENTAGE OF POSITIVE SAMPLES | PERCENTAGE OF NEGATIVE SAMPLES |
|---|---|---|---|---|---|
| **MALE** | 357 | 193 | 164 | 54.06% | 45.93% |
| **FEMALE** | 226 | 135 | 91 | 59.73% | 40.26% |

Out of 583 samples 357 (61.23%) were collected from male patients with 193 (58.84%) positive cases, 164 (64.31%) with negative cases and 226 (38.76%) were collected from female patients with 135 (41.15%) Positive cases 91(35.68%), negative cases. *Staphylococcus aureus* is the bacterium which was most frequently isolated in both male and female.

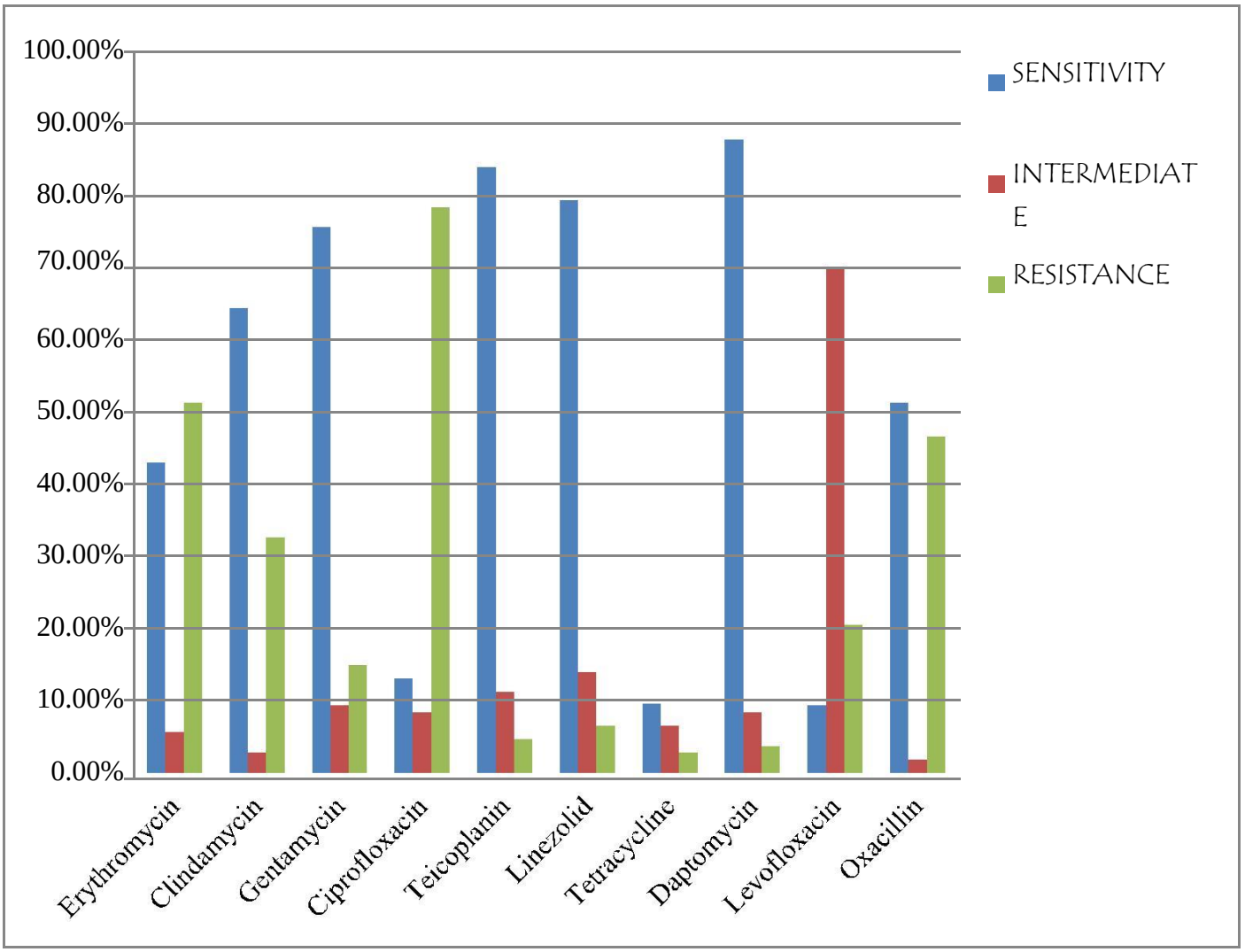
## 5.4 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES

### Table4.Antibiotic Susceptibility pattern for *S.aureus*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Erythromycin | 42.99% | 5.60% | 51.40% |
| Clindamycin | 64.48% | 2.80% | 32.71% |
| Gentamicin | 75.70% | 9.34% | 14.95% |
| Ciprofloxacin | 13.08% | 8.41% | 78.50% |
| Teicoplanin | 84.11% | 11.21% | 4.63% |
| Linezolid | 79.43% | 14.01% | 6.54% |
| Tetracycline | 9.65% | 6.54% | 2.80% |
| Daptomycin | 87.85% | 8.41% | 3.73% |
| Levofloxacin | 9.34% | 70.09% | 20.56% |
| Oxacillin | 51.40% | 1.86% | 46.72% |

[Note: Percentage of antibiotics against total no. of *S.aureus*]

## Chart 2.Antibiotic Susceptibility Pattern for *S.aureus*



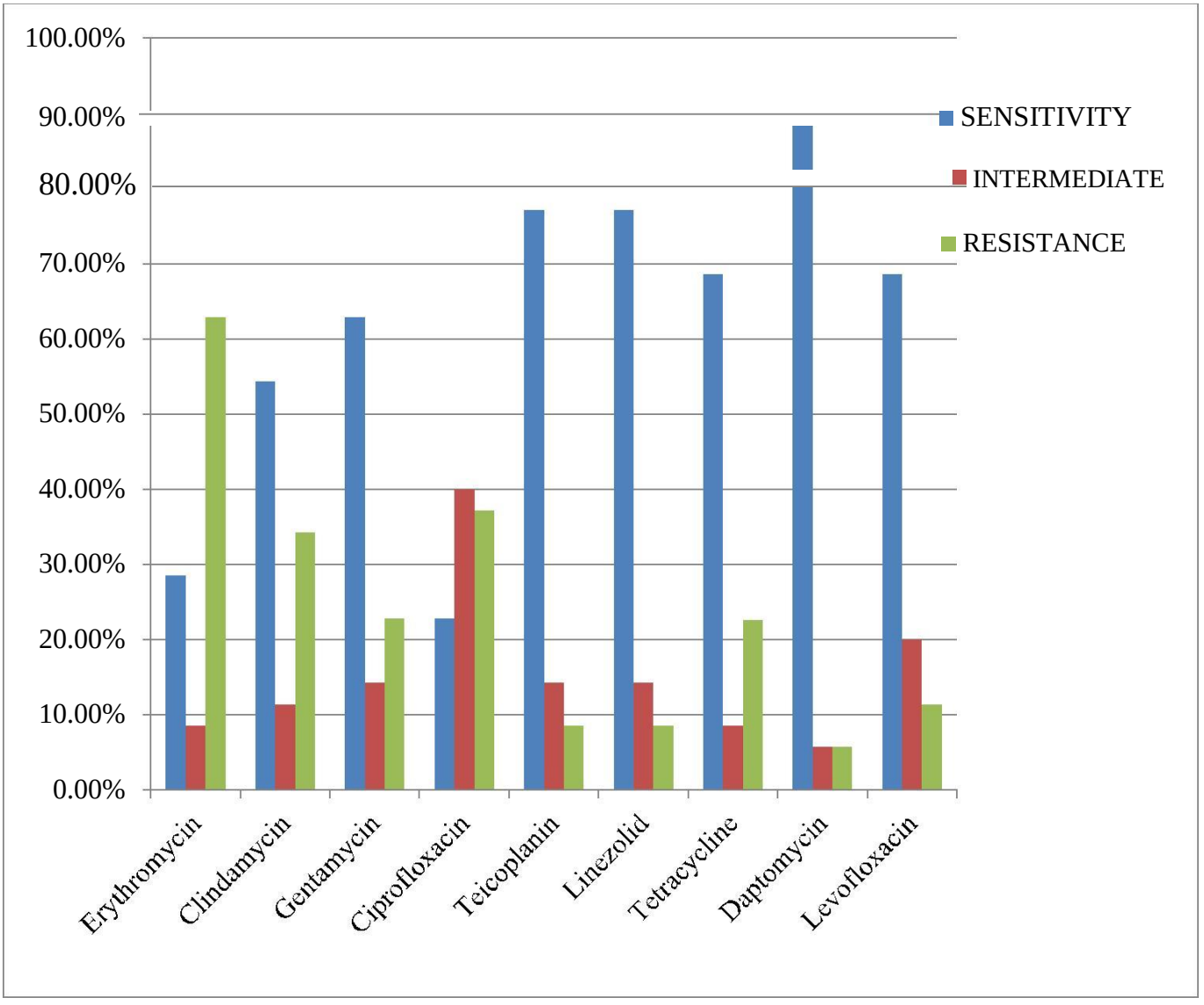

[Note: Percentage of antibiotics against total no. of *S.aureus*]

## Table 5.Antibiotic Susceptibility Pattern for *S.epidermidis*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Erythromycin | 28.57% | 8.57% | 62.85% |
| Clindamycin | 54.28% | 11.42% | 34.28% |
| Gentamicin | 62.85% | 14.28% | 22.85% |
| Ciprofloxacin | 22.85% | 40% | 37.14% |
| Teicoplanin | 77.14% | 14.28% | 8.57% |
| Linezolid | 77.14% | 14.28% | 8.57% |
| Tetracycline | 68.57% | 8.57% | 22.65% |
| Daptomycin | 88.57% | 5.71% | 5.71% |
| Levofloxacin | 68.57% | 20% | 11.42% |
| Oxacillin | 17.14% | 5.71% | 77.41% |

[Note: Percentage of antibiotics against total no. of *S. epidermidis]*

## Chart 3.Antibiotic Susceptibility Pattern for *S.epidermidis*



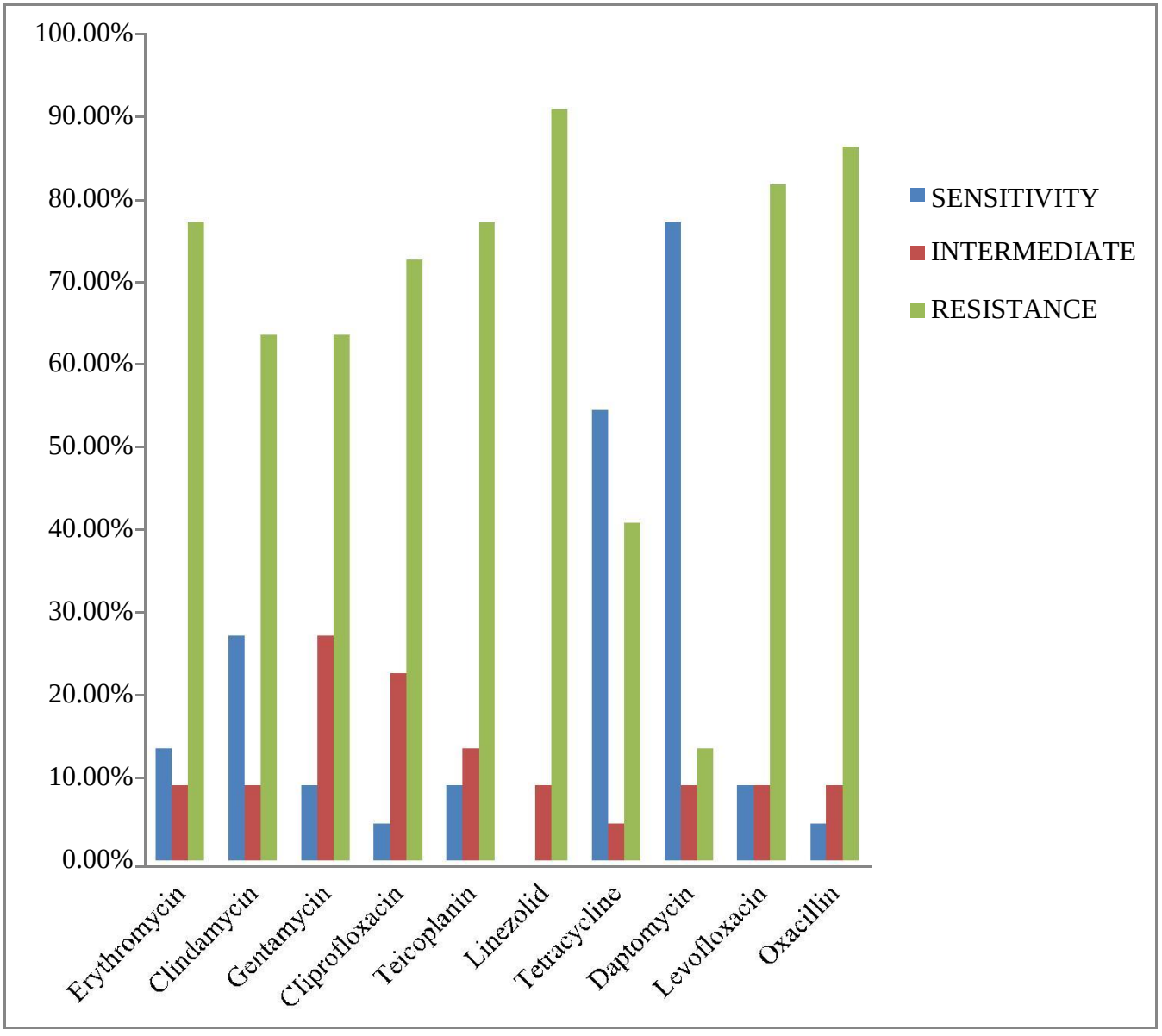

[Note: Percentage of antibiotics against total no. of *S.epidermidis]*

## Table 6.Antibiotic Susceptibility Pattern for *S.haemolyticus*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Erythromycin | 13.63% | 9.09% | 77.27% |
| Clindamycin | 27.27% | 9.09% | 63.63% |
| Gentamicin | 9.09% | 27.27% | 63.63% |
| Ciprofloxacin | 4.5% | 22.72% | 72.72% |
| Teicoplanin | 9.09% | 13.63% | 77.27% |
| Linezolid | 00% | 9.09% | 90.90% |
| Tetracycline | 54.54% | 4.52% | 40.90% |
| Daptomycin | 77.27% | 9.09% | 13.63% |
| Levofloxacin | 9.09% | 9.09% | 81.81% |
| Oxacillin | 4.5% | 9.09% | 86.36% |

[Note: Percentage of antibiotics against total no. of *S.haemolyticus]*

## Chart 4.Antibiotic Susceptibility Pattern for *S.haemolyticus*



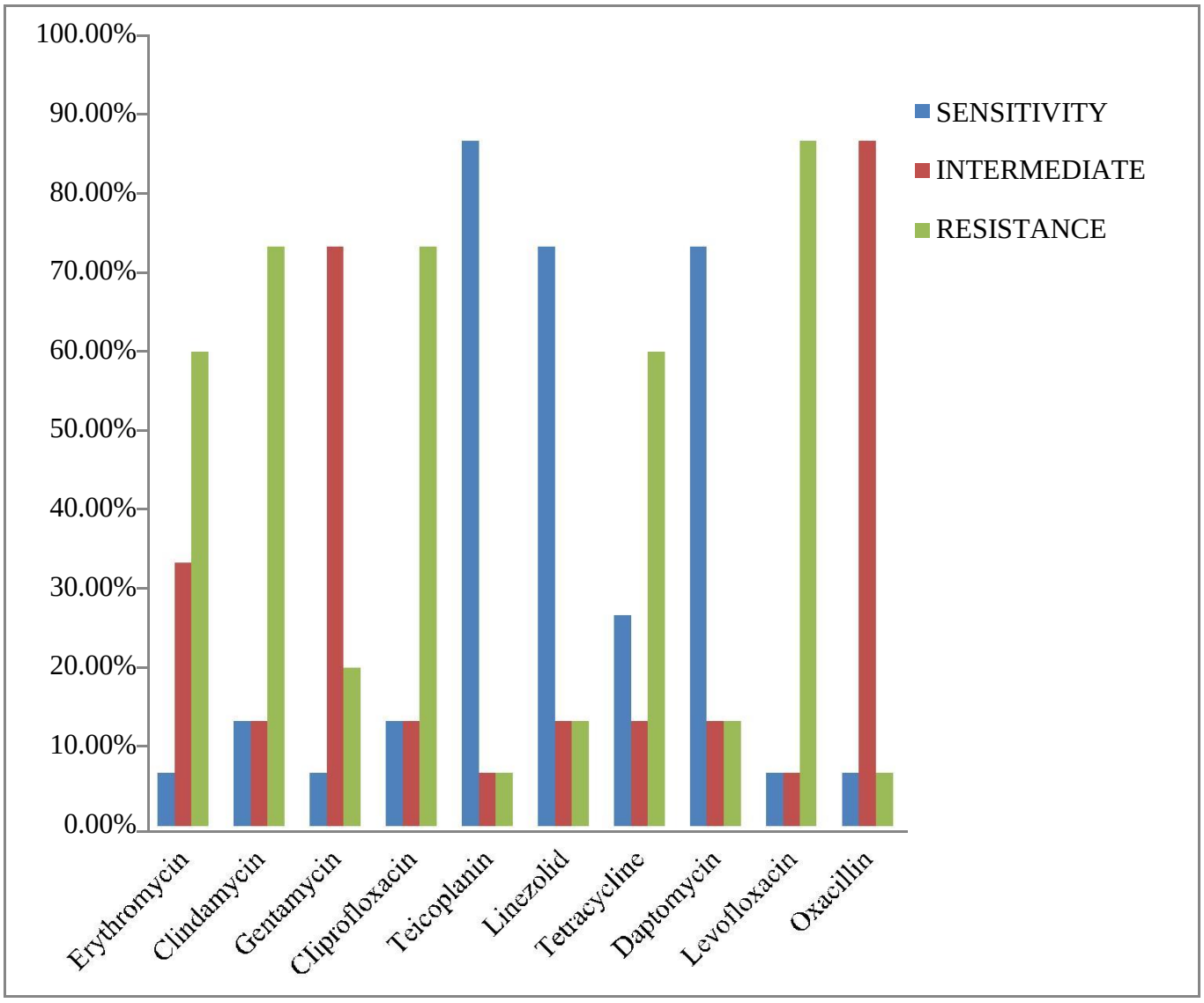

[Note: Percentage of antibiotics against total no. of *S.haemolyticus]*

## Table 7. Antibiotic Susceptibility Pattern for *Enterococcus species*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Erythromycin | 6.66% | 33.33% | 60% |
| Clindamycin | 13.33% | 13.33% | 73.33% |
| Gentamicin | 6.66% | 73.33% | 20% |
| Ciprofloxacin | 13.33% | 13.33% | 73.33% |
| Teicoplanin | 86.66% | 6.66% | 6.66% |
| Linezolid | 73.33% | 13.33% | 13.33% |
| Tetracycline | 26.66% | 13.33% | 60% |
| Daptomycin | 73.33% | 13.33% | 13.33% |
| Levofloxacin | 6.66% | 6.66% | 86.66% |
| Oxacillin | 6.66% | 86.66% | 6.66% |

[Note: Percentage of antibiotics against total no. of *Enterococcus* Species*]*

## Chart 5.Antibiotic Susceptibility Pattern for *Enterococcus* species



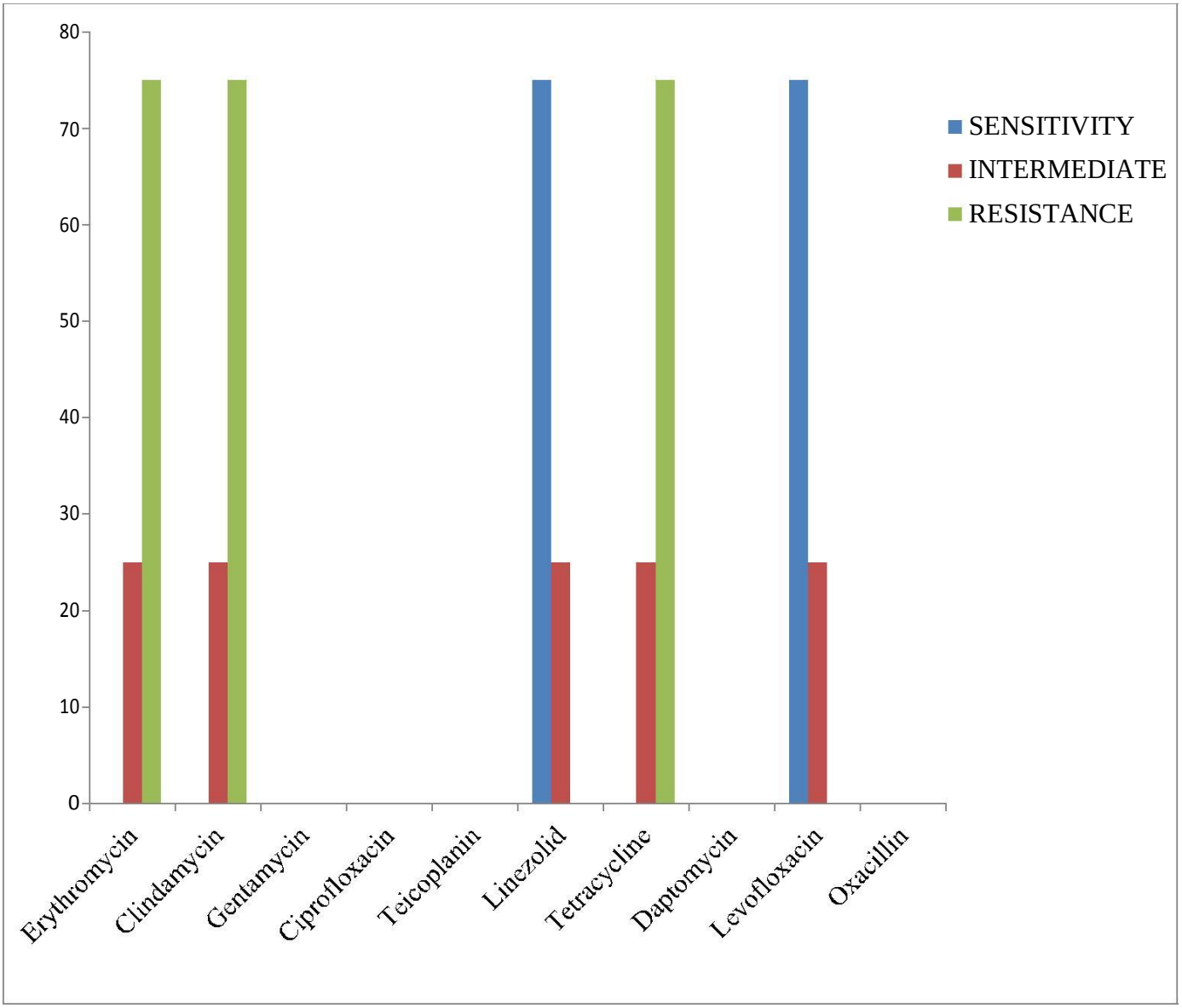

[Note: Percentage of antibiotics against total no. of *Enterococcus Species]*

## Table 8.Antibiotic Susceptibility Pattern for *Streptococcus pyogenes*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Erythromycin | --- | 25 | 75 |
| Clindamycin | --- | 25 | 75 |
| Gentamicin | --- | --- | --- |
| Ciprofloxacin | --- | --- | --- |
| Teicoplanin | --- | --- | --- |
| Linezolid | 75 | 25 | --- |
| Tetracycline | --- | 25 | 75 |
| Daptomycin | --- | --- | --- |
| Levofloxacin | 75 | 25 | --- |
| Oxacillin | --- | --- | --- |

[Note: Percentage of antibiotics against total no. of *Streptococcus pyogenes]*

## Chart 6.Antibiotic Susceptibility Pattern for *Streptococcus pyogenes*



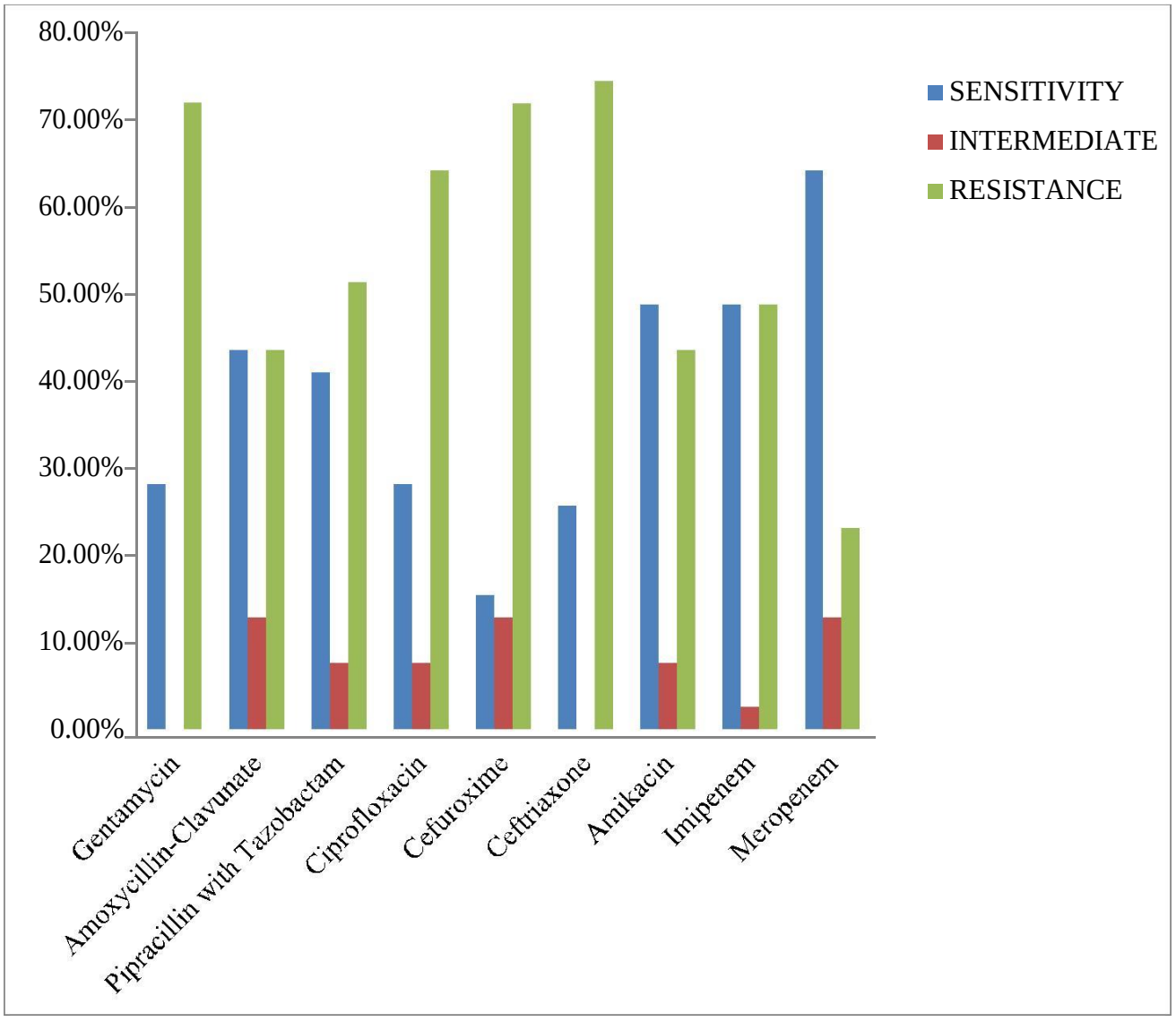

[Note: Percentage of antibiotics against total no. of *Streptococcus pyogenes]*

## Table 9.Antibiotic Susceptibility Pattern for *E.coli*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Gentamicin | 28.20% | 00% | 71.92% |
| Amoxycillin-Clavunate | 43.58% | 12.82% | 43.58% |
| Piperacillin/Tazobactum | 41.02% | 7.69% | 51.28% |
| Ciprofloxacin | 28.20% | 7.69% | 64.10% |
| Cefuroxime | 15.38% | 12.82% | 71.79% |
| Ceftriaxone | 25.64% | 00% | 74.35% |
| Amikacin | 48.71% | 7.69% | 43.58% |
| Imipenem | 48.71% | 2.56% | 48.71% |
| Meropenem | 64.10% | 12.82% | 23.07% |

[Note: Percentage of antibiotics against total no. of *E.coli]*

## Chart 7.Antibiotic Susceptibility Pattern for *E.coli*



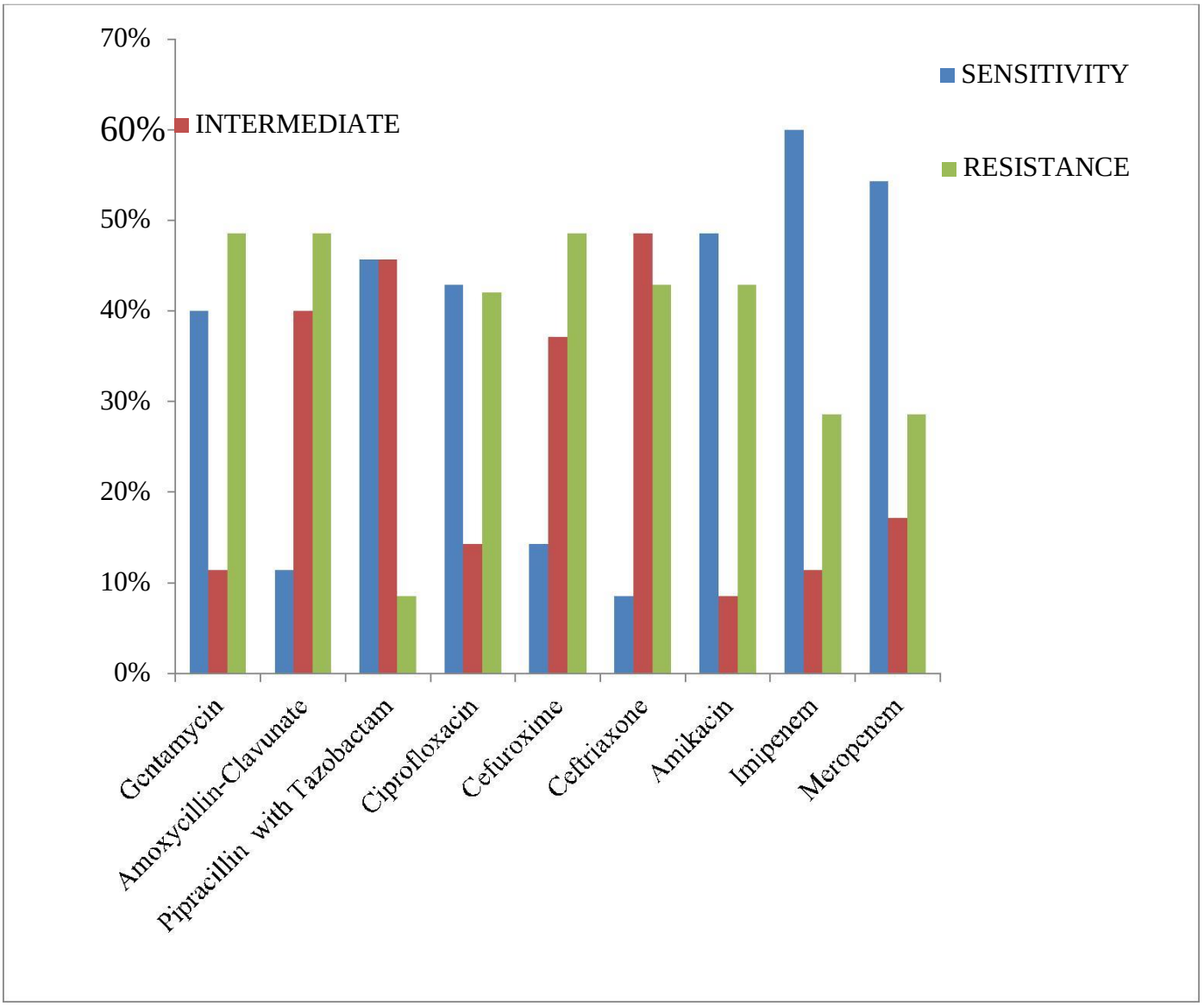

[Note: Percentage of antibiotics against total no. of *E.coli]*

## Table 10.Antibiotic Susceptibility Pattern for *Pseudomonas aeruginosa*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Gentamicin | 40% | 11.42% | 48.57% |
| Amoxycillin-Clavunate | 11.42% | 40% | 48.57% |
| Piperacillin/Tazobactum | 45.71% | 45.71% | 8.57% |
| Ciprofloxacin | 42.85% | 14.28% | 42.05% |
| Cefuroxime | 14.28% | 37.14% | 48.57% |
| Ceftriaxone | 8.57% | 48.57% | 42.85% |
| Amikacin | 48.57% | 8.57% | 42.85% |
| Imipenem | 60% | 11.42% | 28.57% |
| Meropenem | 54.28% | 17.14% | 28.57% |

[Note: Percentage of antibiotics against total no. of *Pseudomonas aeruginosa]*

## Chart 8.Antibiotic Susceptibility Pattern for *Pseudomonas aeruginosa*



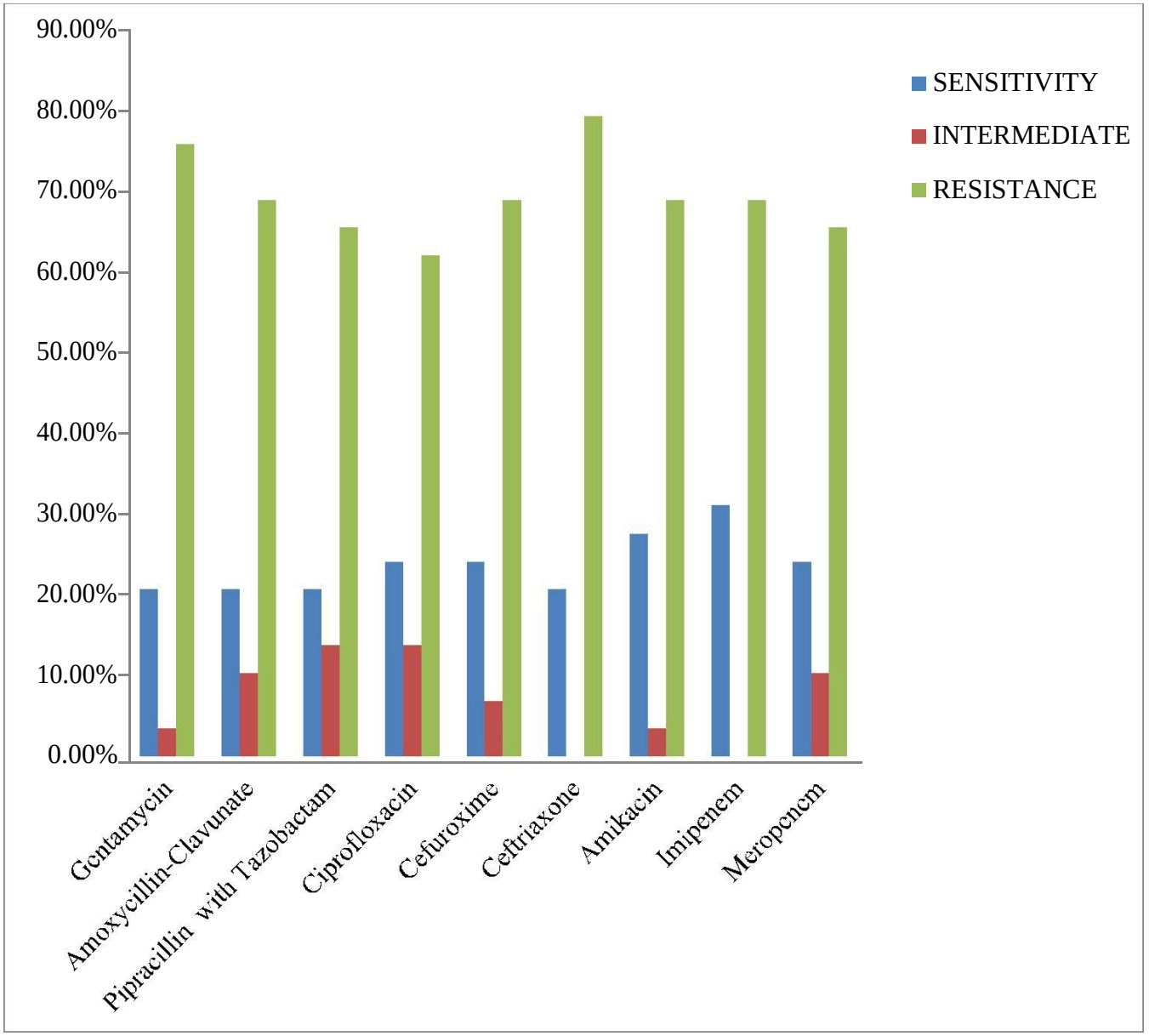

[Note: Percentage of antibiotics against total no. of *Pseudomonas aeruginosa]*

## Table 11.Antibiotic Susceptibility Pattern for *Klebsiella pneumoniae*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Gentamicin | 20.68% | 3.44% | 75.86% |
| Amoxycillin-Clavunate | 20.68% | 10.34% | 68.96% |
| Piperacillin/Tazobactum | 20.68% | 13.79% | 65.51% |
| Ciprofloxacin | 24.13% | 13.79% | 62.06% |
| Cefuroxime | 24.13% | 6.89% | 68.96% |
| Ceftriaxone | 20.68% | 00% | 79.31% |
| Amikacin | 27.58% | 3.44% | 68.96% |
| Imipenem | 31.09% | 00% | 68.96% |
| Meropenem | 24.13% | 10.34% | 65.51% |

[Note: Percentage of antibiotics against total no. of *Klebsiella pneumoniae]*

## Chart 9.Antibiotic Susceptibility Pattern for *Klebsiella pneumonia*



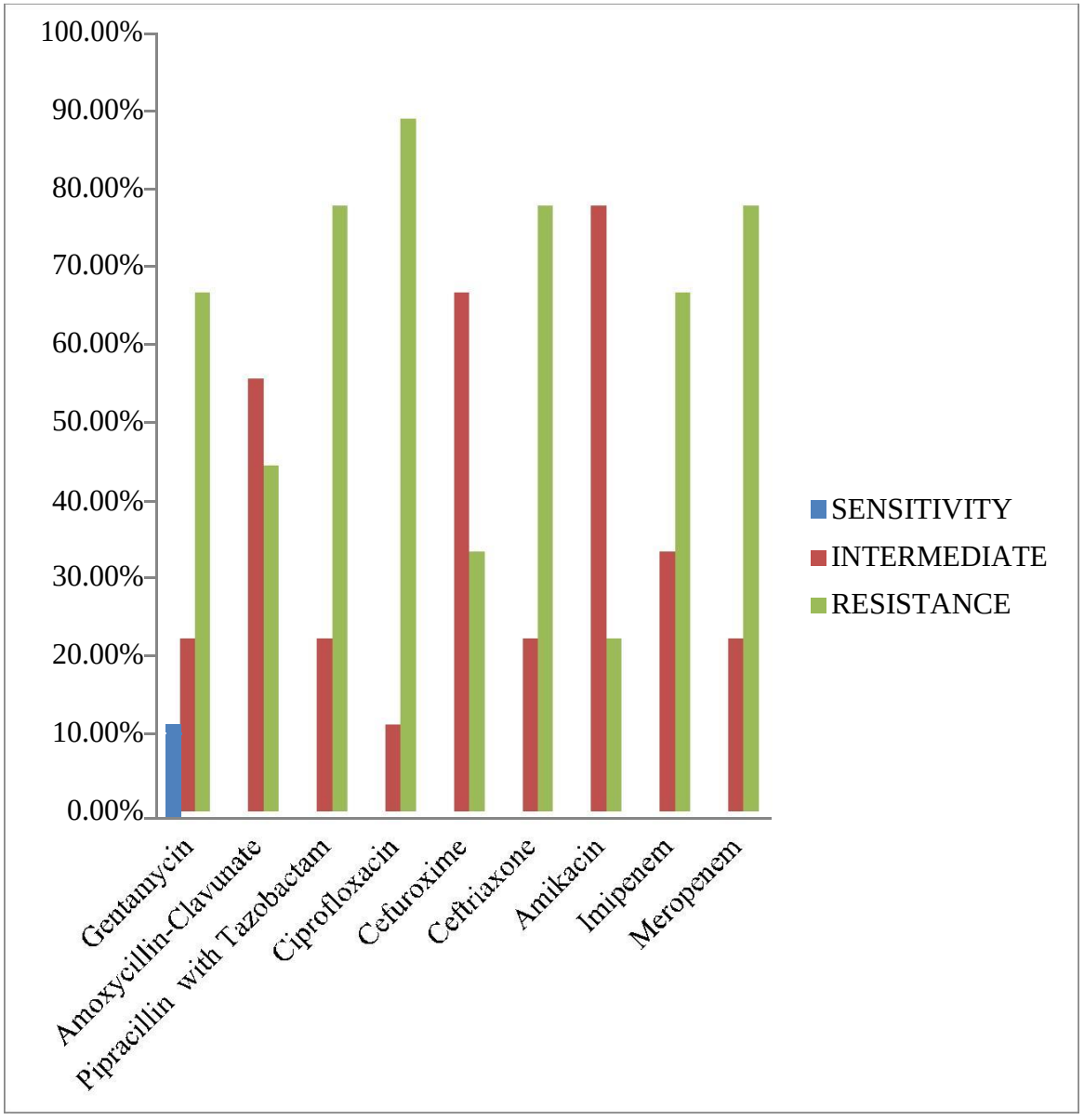

[Note: Percentage of antibiotics against total no. of *Klebsiella pneumoniae*

## Table 12.Antibiotic Susceptibility Pattern for *Acinetobacter baumannii*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Gentamicin | 11.11% | 22.22% | 66.66% |
| Amoxycillin-Clavunate | 00% | 55.55% | 44.44% |
| Piperacillin/Tazobactum | 00% | 22.22% | 77.77% |
| Ciprofloxacin | 00% | 11.11% | 88.88% |
| Cefuroxime | 00% | 66.66% | 33.33% |
| Ceftriaxone | 00% | 22.22% | 77.77% |
| Amikacin | 00% | 77.77% | 22.22% |
| Imipenem | 00% | 33.33% | 66.66% |
| Meropenem | 00% | 22.22% | 77.77% |

[Note: Percentage of antibiotics against total no. of *Acinetobacter baumannii]*

## Chart 10.Antibiotic Susceptibility Pattern for *Acinetobacter baumannii*



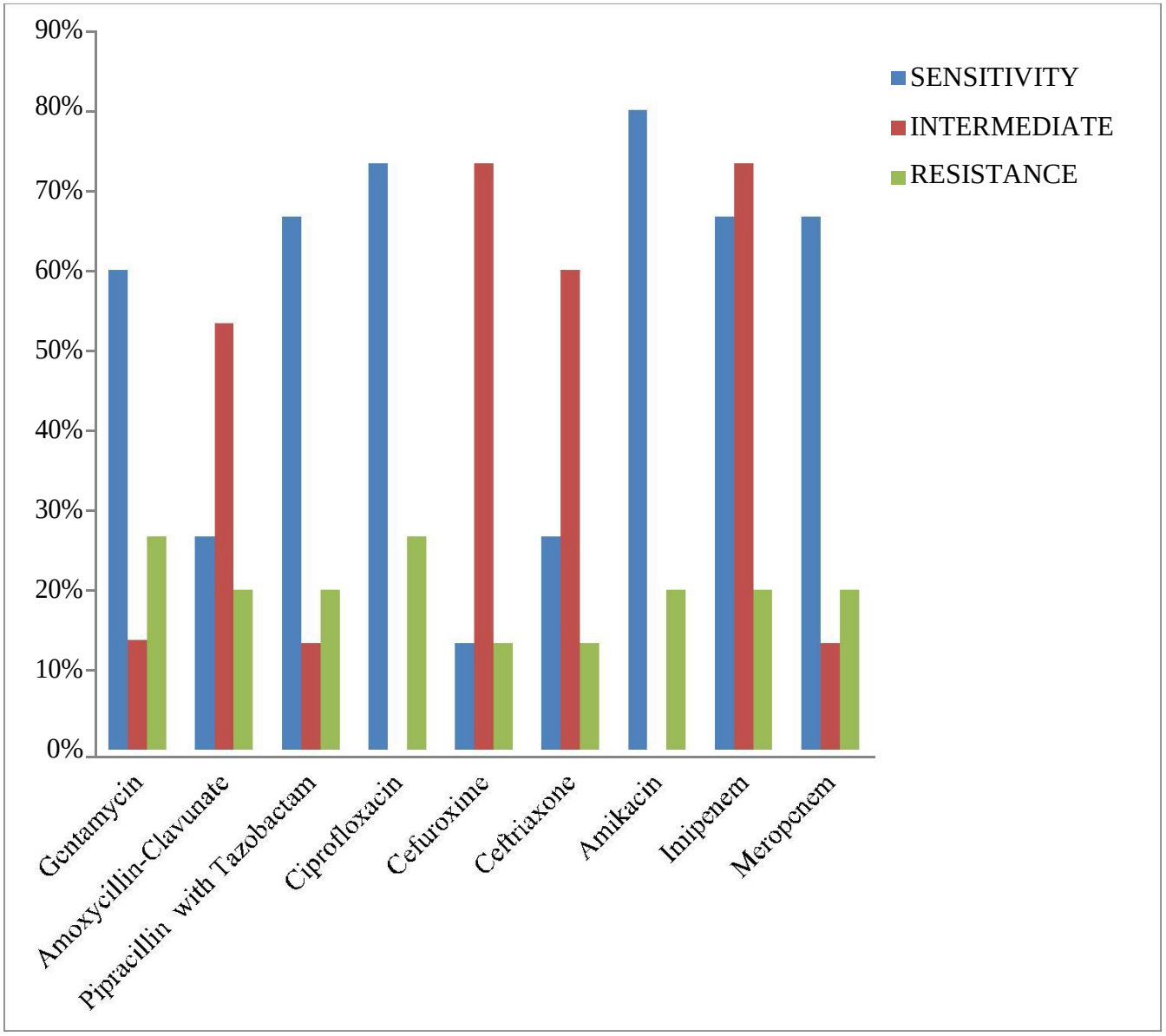

[Note: Percentage of antibiotics against total no. of *Acinetobacter baumannii]*

## Table 13.Antibiotic Susceptibility Pattern for *Proteus mirabilis*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Gentamicin | 60% | 13.73% | 26.66% |
| Amoxycillin-Clavunate | 26.66% | 53.33% | 20% |
| Piperacillin/Tazobactum | 66.66% | 13.33% | 20% |
| Ciprofloxacin | 73.33% | 00% | 26.66% |
| Cefuroxime | 13.33% | 73.33% | 13.33% |
| Ceftriaxone | 26.66% | 60% | 13.33% |
| Amikacin | 80% | 00% | 20% |
| Imipenem | 66.66% | 73.33% | 20% |
| Meropenem | 66.66% | 13.33% | 20% |

[Note: Percentage of antibiotics against total no. of *Proteus mirabilis]*

## Chart 11. Antibiotic Susceptibility Pattern for *Proteus mirabilis*



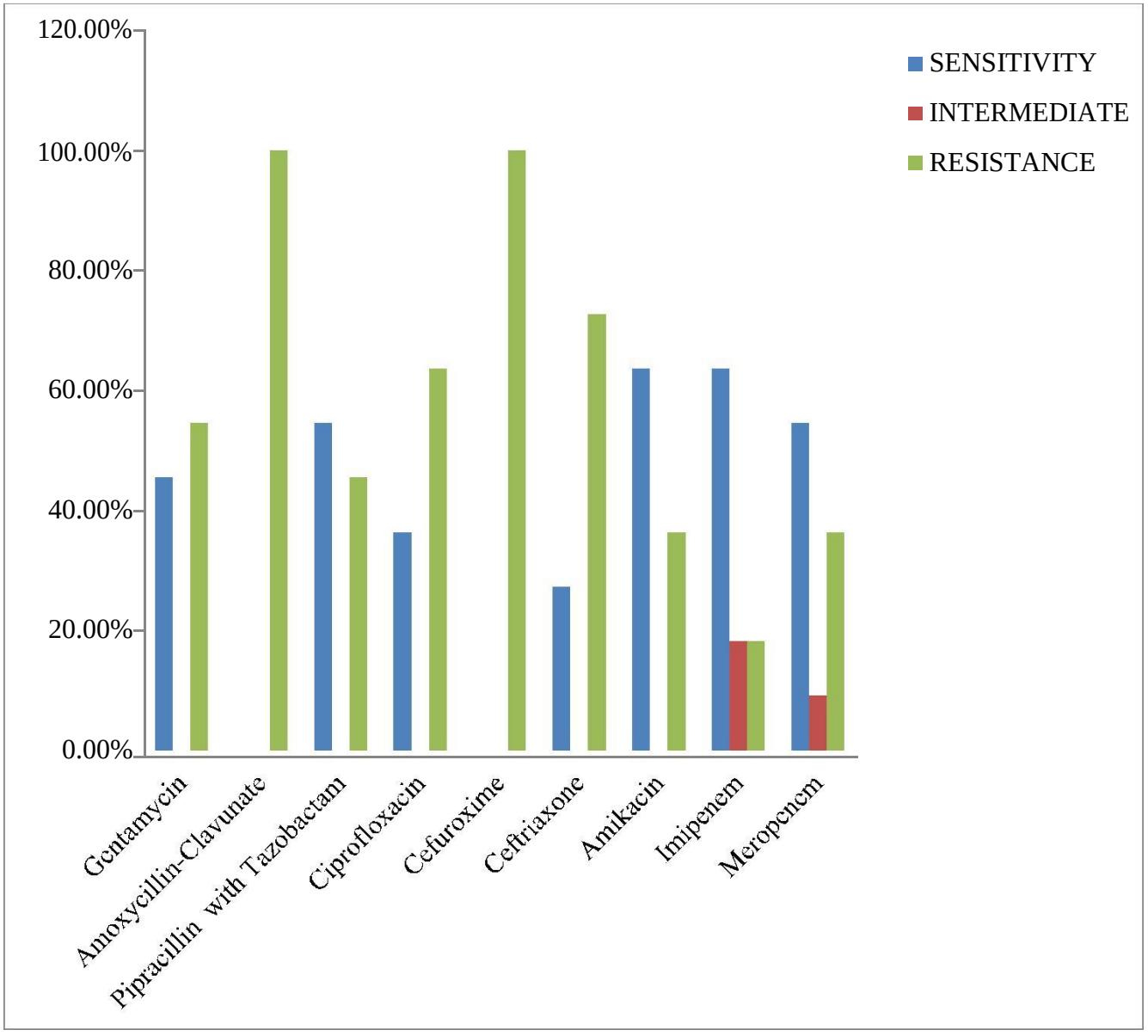

[Note: Percentage of antibiotics against total no. of *Proteus mirabilis]*

## Table 14.Antibiotic Susceptibility Pattern for *Enterobacter species*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Gentamicin | 45.45% | 00% | 54.54% |
| Amoxycillin-Clavunate | 00% | 00% | 100% |
| Piperacillin/Tazobactum | 54.54% | 00% | 45.45% |
| Ciprofloxacin | 36.36% | 00% | 63.63% |
| Cefuroxime | 00% | 00% | 100% |
| Ceftriaxone | 27.27% | 00% | 72.72% |
| Amikacin | 63.63% | 00% | 36.36% |
| Imipenem | 63.63% | 18.18% | 18.18% |
| Meropenem | 54.54% | 9.09% | 36.36% |

[Note: Percentage of antibiotics against total no. of *Enterobacter Species]*

## Chart 12.Antibiotic Susceptibility Pattern for *Enterobacter species*



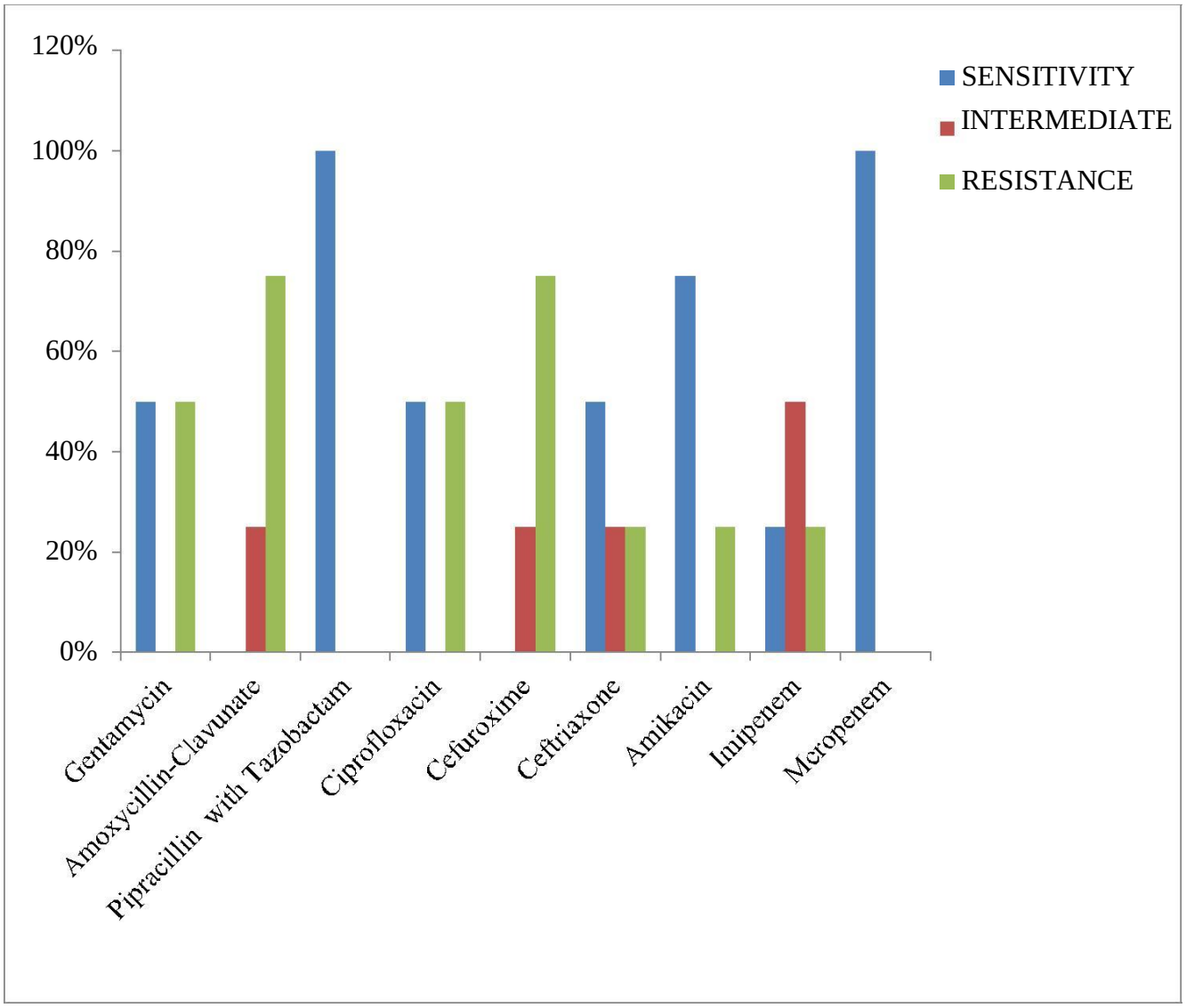

[Note: Percentage of antibiotics against total no. of *Enterobacter Species]*

## Table 15.Antibiotic Susceptibility Pattern for *Morganella morganii*

| ANTIBIOTICS | SENSITIVITY | INTERMEDIATE | RESISTANCE |
|---|---|---|---|
| Gentamicin | 50% | 00% | 50% |
| Amoxycillin-Clavunate | 00% | 25% | 75% |
| Piperacillin/Tazobactum | 100% | 00% | 00% |
| Ciprofloxacin | 50% | 00% | 50% |
| Cefuroxime | 00% | 25% | 75% |
| Ceftriaxone | 50% | 25% | 25% |
| Amikacin | 75% | 00% | 25% |
| Imipenem | 25% | 50% | 25% |
| Meropenem | 100% | 00% | 00% |

[Note: Percentage of antibiotics against total no. of *Morganella morganii]*

## Chart 13.Antibiotic Susceptibility Pattern for *Morganella morganii*




[Note: Percentage of antibiotics against total no. of *Morganella morganii]*

# CHAPTER 6

# DISCUSSION

The present study was carried out for a period of 4 months (1st January 2016-15th April 2016) In the Microbiology department of SRL diagnostics, Gurgaon, Haryana, to isolates various bacterial pathogens present in the pus samples and to determine their antibiotic susceptibility pattern.

Out of 583 samples 357(61.23%) were from male patients with 193 (58.84%) positive and 164(64.31%) negative cases, 226(38.76%) were from female patients with 135 (41.15%), positive and 91 (35.68%) negative cases. Bacterial genus was identified with 5(41.66%) Gram positive and 7(53.84%) Gram negative bacteria. In Gram positive bacteria *Staph.aureus* 107(32.62%) was the most frequent grown organism. followed by the Gram negative bacteria *E. coli* 39 (11.89).Our study is well comparable with the study conducted by Jyoti Sangwanl. Pooja Singla, Pratibha Mane1, Sumit Lathwal and A. K. Malik, who have reported that a total of 364 organisms were isolated. The commonest organism were family *Enterobacteriaceae* (38.5%) followed by *Staphylococcus spp* (26.9%), *Pseudomonas* (21.4%), *Enterococcus* (6.6%), *Diphtheroids*(3.8%), *Streptococcus pyogens*(2.2%) and Acinetobacter (0.5%).The most predominant gram positive bacteria (n=144) isolated was *Staphylococcus aureus* (61.1%) followed by *Enterococcus* (16.7 %),CONS (6.9%), *Streptococcus pyogens*(5.6%) and Diphtheroids (1.4%). Though *Enterobacteriaceae* was the most predominant family isolated, *Pseudomonas* (35.5%) was the most predominant single Gram negative organism (n=220). Enterobacteriaceae (n=140) mainly comprised of *E.coli* (38.6%) followed by Proteus (22.8%), *Citrobacter* (21.4%), *Klebsiella* (15.7%) and *Morganella* (1.4%). [23]

*Staphylococcus aureus* showed the resistance to most antibiotics. Erythromycin, Ciprofloxacin, Oxacillin. Gram positive bacterium *Staphylococcus aureus s*howed the highest resistance to Ciprofloxacin. Gram Positive cocci *Staph.aureus* was sensitivity to Teicoplanin 84.11% and Linezolid 79.43%, were concluded that most sensitive and resistance to the Erythromycin 51.40% and Ciprofloxacin 78.50%, were considered as the most resistant antibiotic. Most of the Gram positive organisms were sensitive To Teicoplanin, Linezolid.Most of the Gram negative organisms Sensitivity to the Imipenem and Meropenem. *Enterobacter* species were 100% Resistant to the Cefuroxime, was the most resistant antibiotic to the Gram negative bacteria. Some Gram negative bacteria showed variable pattern, Amikacin, Imipenem, Meropenem showed different pattern to Gram negative bacteria. Our study is well comparable with the study conducted by Shivani Khullar, Laxmi Rathore, P. K. Khatri, R. S. Parihar, Saroj Meena, Archana Bora, Vinod Maurya and Niranjan Sharma who have reported The Antibiogram of gram positive cocci obtained by Vitek-2 compact revealed that Tigecycline (58.33 %) was the most susceptible drug followed by Nitrofurantoin (45.69%) and Vancomycin (40.36%). *Staphylococcus aureus* was most susceptible to Nitrofurantoin (57.14%) followed by Tigecycline (50.00%) and Linezolid (39.28%). MRSA was detected with the help of Cefoxitin screen and 16 (57.14%) were found to be MRSA. *Enterococcus* was most susceptible to Vancomycin (57.69 %) followed by Linezolid (53.84 %) and Tigecycline (50%). CONS was most susceptible to Tigecycline (75.00 %) followed by Nitrofurantoin (50.00 %). [24]

# CHAPTER 7

# CONCLUSIONS

In conclusion, pyogenic infection has been the major cause of mortality and morbidity since long. Emerging of multidrug resistant strains is of major concern to treat these conditions. Even though Gram negative bacteria are being increased significantly but still *Staphylococcus aureus* is being continued as a major etiological agent of pyogenic infections. Changing antimicrobial resistance pattern poses challenge in treating these conditions bacterial contaminations of wounds is a serious problem. This study shows that pyogenic infections are important cause of morbidity in patients with Gram negative bacteria (*E.coli*) as well as Gram Positive organisms (*Staphylococcus aureus*).The Antibiogram of Gram positive cocci revealed that the Daptomycin (75%-85%) was the most sensitive drug followed by Teicoplanin(75%-80%), Linezolid (70%-85%). Some Gram negative bacilli were sensitive to Carbapenem Group and some Gram negative bacteria showed resistance to Carbapenem group. Some Gram negative bacteria resist to Ceftriaxone and Cefuroxime. Our study there by will guide the clinician in choosing appropriate Antibiotics which not only contributes to better treatment but there judicious use will also help in preventing emergence of resistance to the drug which are still sensitive and There is a need for a central database in India where various laboratories can upload their Antibiogram regularly and this data can be very useful in formulating guidelines for treatment of various infectious diseases.

# CHAPTER 8

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# APPENDICES

## Appendix 1

## LIST OF ABBREVATIONS

| | |
|---|---|
| AST | Antimicrobial Sensitivity Testing |
| CFU | Colony Forming Unit |
| GPC | Gram positive cocci |
| GNB | Gram negative Bacilli |
| *S.aureus* | *Staphylococcus aureus* |
| *S. epidermidis* | *Staphylococcus epidermidis* |
| *A. baumanni* | *Acinetobacter baumanni* |
| *E. coli* | *Escherichia coli* |
| *P mirabilis* | *Proteus mirabilis* |
| *S. pyogenes* | *Streptococcus pyogenes* |
| BA | Blood Agar |
| MA | MacConkey Agar |
| W.H.O | World Health Organization |

# Appendix 2

## ANTIBIOTICS

| | |
|---|---|
| **ERY** | Erythromycin |
| **CLIN** | Clindamycin |
| **GEN** | Gentamicin |
| **CTX** | Ceftriaxone |
| **CFU** | Cefuroxime |
| **AK** | Amikacin |
| **CIP** | Ciprofloxacin |
| **PIP** | Piperacillin |
| **TET** | Tetracycline |
| **TEI** | Teicoplamin |
| **LEV** | Levofloxacin |
| **OXA** | Oxacillin |

# Appendix 3

## MATERIALS

### 4.1.1 EQUIPMENTS

- Weighing machine
- Autoclave
- Biosafety cabenet
- Bunsen burner
- VITEK 2 compact ID/AST instrument
- Incubator
- Refrigerator

### 4.1.2 GLASS WARES

- Petri plates
- Slides
- Glass tubes

### 4.1.3OTHERS

- Cotton
- Assorted Nichrome loop
- Forceps
- Staining rack
- Gloves
- Face mask

# Appendix 4

## Composition and Preparation of Gram staining

## 1. CRYSTAL VIOLET STAIN

### Solution A

| Composition | Gm/Liter |
|---|---|
| Crystal violet | 2gm |
| Ethanol | 10ml |

### Solution B

| Composition | Gm/Liter |
|---|---|
| Ammonium oxalates | 4 gm |
| Distilled water | 400 ml |

### Preparation

1. Mix well Solution A and Solution B and store for 24 hour to dissolve the stain completely.
2. Filter the solution through filter paper after 24 hour.
3. store in a tightly stopper bottle.

## 2. GRAM IODINE SOLUTION

| Composition | Gm/Liter |
|---|---|
| Potassium iodide | 2gm |
| Iodine | 1gm |
| Distilled water | 100ml |

### Preparation

1. Dissolve Potassium iodide in distilled water and then iodine.
2. Store in a tightly stopper bottle.

## 3. ACETONE – ALCOHOL DECOLORIZER

| Composition | Gm/Liter |
|---|---|
| Acetone | 250 |
| Ethanol | 250 |

## Preparation

1. Mix 250ml acetone in 250ml ethanol
2. Store in a tightly stopper bottle.

## 4. COUNTER STAIN SAFRANIN-O

| Composition | Gm/Liter |
|---|---|
| Safranin | 0.34gm |
| Absolute alcohol | 10ml |
| Distilled water | 90ml |

## Preparation

1. Dissolve Safranin in Absolute alcohol and then add distilled water.
2. Filter the solution through filter paper.
3. Store in a tightly stopper bottle.