Drug Resistant Pathogens Isolated from Wound Infection

By

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A Thesis Submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of B.Sc. in Microbiology

Department of Mathematics and Natural Sciences

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The report does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The report does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Ethics statement

The departmental review board of BRAC University in Dhaka, Bangladesh, provided ethical clearance. IBN SINA Diagnostic & Imaging Center gave permission to complete my thesis in their microbiology laboratory. The respondents’ privacy and the data's confidentiality were strictly protected during data collection.

Abstract

For several decades antimicrobial resistance is growing rapidly and limiting the efficacy of antibiotics. Resistance to antibiotics can be accelerated by a variety of factors including human practices, drug tolerance, drug destruction and drug impermeability.

In this study, the prevalence, types and antibiotic susceptibility of microorganisms isolated from pus samples were investigated. A total 200 pus samples of both sex between age group 0-90 years were analyzed in this study. These specimens were analyzed to observe the antibiotic susceptibility having significant growth of pyogenic bacteria. Gram-positive organisms accounted for 35% and Gram-negative organisms accounted for 64% which is almost double of total Gram-positive isolates comparatively. Most of the Gram-positive bacteria were resistant against Cephalosporin group and sensitive towards Carbapenem group's Imipenem but resistant against Meropenem. Such as Cefixime (92)% and Ceftazidime (94)% were highly resistant in *Staphylococcus aureus*. In *Enterococcus* Imipenem was (20)% and Meropenem was (80)%. On the other hand, in Gram-negative bacteria *Escherichia coli, Acinetobacter* and *Pseudomonas spp* showed most resistance against the antibiotics.

This investigation intended to decide the prevalence of various bacterial microbes and their antibiotic susceptibility in different sorts of wound contaminations.

Key words: Antibiotic resistance, Susceptibility, Wound contamination

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Introduction

1.1 Introduction

Wound infection is seemingly the most common, and simultaneously the most devastating complication of the wound healing process. Bacteria can get easy access to a wound infection site therefore the site is more susceptible to bacterial infection. A wound infection can occur during accident, trauma, burn, surgical procedures and also as a result of chronic disease condition such as Diabetes mellitus and leprosy (Mekonnen Sissey et, al., 2019). If the procedure is imperfectly managed, a wound infection can cause secondary complications which may include cutting of limbs or loss of limbs for life. Pus a yellowish-white, thick and non-transparent fluid gets formed in the site of wound infection are composed with various pathogenic components such as bacteria, fungi, etc. All wounds can be contaminated from surrounding skin/immediate environment, endogenous (gastrointestinal tract, tumor, birth defect, nasopharyngeal) source of the patient. Skin provides a first line defense system in the attack against pathogens. A patient's infection can severely modified based on the local environment he is put into. Due to the distinctive biological, non-sterile wound environment and the extremely intricate system of wound healing can also burdens the patient. Other factors including the virulence characteristics of microbes, selection pressures, the host immune system, age and comorbid conditions of a patient play a critical role. Wound infections have resulted in acceptable morbidity, mortality, prolonged hospitalization and escalation of direct and indirect healthcare costs (Siddiqui AR et, al., 2010). A few common agents responsible for causing infections are *Staphylococcus aureus (S.aureus), Streptococcus pyogenes, Escherichia coli (E.coli), Klebsiella spp, Proteus spp, Acinetobacter spp, Pseudomonas spp, Candida albicans etc* (Basista et,al., 2017). Though it varies based on the wound source, the most commonly isolated gram-positive cocci are *S.aureus* and *Coagulase Negative Staphylococci* (CoNS). Besides, gram-negative aerobic bacilli such as *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis* are the most prevailing clinically relevant isolates (Rice LB, 2006).

High infection rates occur when the surgical site is complicated by the implantation of medical devices, such as osteosynthesis plate or soft tissue alloplastic augmentation. *S.aureus* and *S. epidermidis*, among other Gram-positive microorganisms, are

commonly found in the skin and are frequent causes of infections associated with surgical implants. Indeed, staphylococcal wound infections can cause severe local and systemic complications. Wound dehiscence, failure of the operation, hernia formation, septic thrombophlebitis, pain, and scars can occur at the incision site. Systemic complications include bacteremia, metastatic infection, hypotension, organ failure, and death. Chronic wounds and burn wounds are prone to infection by staphylococci as well as the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa.* This bacteria's wound infections are also associated with ulcer enlargement or healing delay.

To inhibit these pathogen's growth on the site of infection, there are immense usage of antibiotics for years. Antibiotics are substances derived from a microorganism or produced synthetically, that destroys or limits the growth of living pathogenic organism. The pharmacology of antibiotics consists of the destruction of a bacterial cell and the interchange of important cellular functions and mechanisms within the cell. Antimicrobial agents are classified into two groups established on in vitro effect on bacteria:

- Bactericidal
- Bacteriostatic

Bactericidal antibiotics kill bacteria and bacteriostatic antibiotics prevent the growth of pathogen. Antibiotics are also chemotherapeutic agents (use of drugs with selective toxicity against infection), which have been an integral asset in the clinical administration of bacterial diseases since the 1940s (Saswati Sengupta et al., 2013). However now that bacteria has developed resistance against antibiotics, they have also developed some mechanisms to survive and promote the resistance. A high MIC of bacterial isolate above the susceptibility threshold are known as resistant bacteria. Certain antibiotics can work against certain bacteria. As an example, vancomycin an antibiotic known to target to work against gram-positive bacteria, is not able to cross the cell wall of gram-negative bacteria (Chara Calhoun et al., 2020). Also, β-lactam will not be effective against some bacteria because the function of it's work procedure requires a cell wall. As an example, it won't be effective against

bacteria as Mycoplasma species which lack this cellular component (Chara Calhoun et al., 2020). The emergence of antibiotic-resistant strains has come from inappropriate and continued use of systemic and topical antimicrobial agents which provided selective pressure. In any case, in the resulting days the benefits have reduced for the far-reaching development and spread of anti-microbial resistant strains (Saswati Sengupta et al., 2013). Microorganisms can show their resistance in multiple ways. For instance, they can release enzymes (like penicillinase) to inactivate the antibiotic before it kills the microorganism. Also they can stop producing the drug-sensitive structure or modify the structure so that it is no longer sensitive to the drug.

Hence the wide number of antibiotics and antifungal antibiotics used in health care industry to treat against the bacteria are given below-

Antibiotics used against Gram positive bacteria-

Penicillin: Gram positive bacteria functions by forming cell wall and that's their major component. Penicillin works as their destruction of peptidoglycan layer. Without peptidoglycan layer bacteria is forced to burst from internal pressure. Penicillin along is not a antibiotic but with other members and they are ampicillin, amoxicillin, amoxyclav, cloxacillin, nafcillin, and ticarcillin. Penicillin at first was derived from the green mold *Penicillium*, but most penicillins are now produced by synthetic means. A few are used against Gram-negative bacteria.

Cephalosporin: These antibiotics were first produced from by a mold *Cephalosporium.* As a substitute of penicillin resistant bacteria cephalosporin is used. They prevent synthesis of bacterial cell walls. The new version of cephalosporin are used against gram negative bacteria too (RL Thompson et al., 1983). Cephalexin, Cefuroxime, Ceftazidime, Cefixime,Ceftriaxone, Cefotaxime, Cefepime are included under cephalosporin.

Azithromycin: A broad spectrum macrolide antibiotic mostly active against gram positive bacteria. Also, some are active against gram negative as well such as Bordetella pertussis and Legionella species

Vancomycin: Vancomycin is a very expensive antibiotic with numerous side effects, and it is used only in life-threatening situations. It interferes with cell wall formation in bacteria as well. Current use of vancomycin is mainly against bacteria displaying resistance to penicillin, cephalosporin, and other antibiotics.

Antibiotics used against Gram negative bacteria -

Tigecycline: A broad spectrum drugs that inhibit the growth of Gram-negative bacteria, rickettsiae, chlamydiae, and certain Gram-positive bacteria (D.Y. Aksoy et al., 2008). Tigecylcine is a class of tetracycline antibiotic medication. Their function of destruction is by inhibiting protein synthesis. Unlike other antibiotics, tigecyclines have relatively mild side effects, but they are known to kill useful bacteria in the body. Very young children are prohibited from using this.

Aminoglycoside: These antibiotics prohibits protein synthesis in gram- negative bacteria. Aminoglycoside are now synthetically produced and they are originally originated from bacterial genus *Streptomyces*. Members include gentamicin, netilmicin, amikacin, streptomycin.

Some antifungal antibiotics are now used to treat infectious diseases caused by fungus. A very common form of fungal infection in women of Bangladesh is vaginal infection caused by *Candida albicans*. To fight this infection cotrimoxazole, ketoconazole, and miconazole are widely used.

All medication have the tendency of complication and antibiotic is no different from others. With an accurate figure firstly the Mode of Action and then Mode of Resistance of antibiotic is explained further –

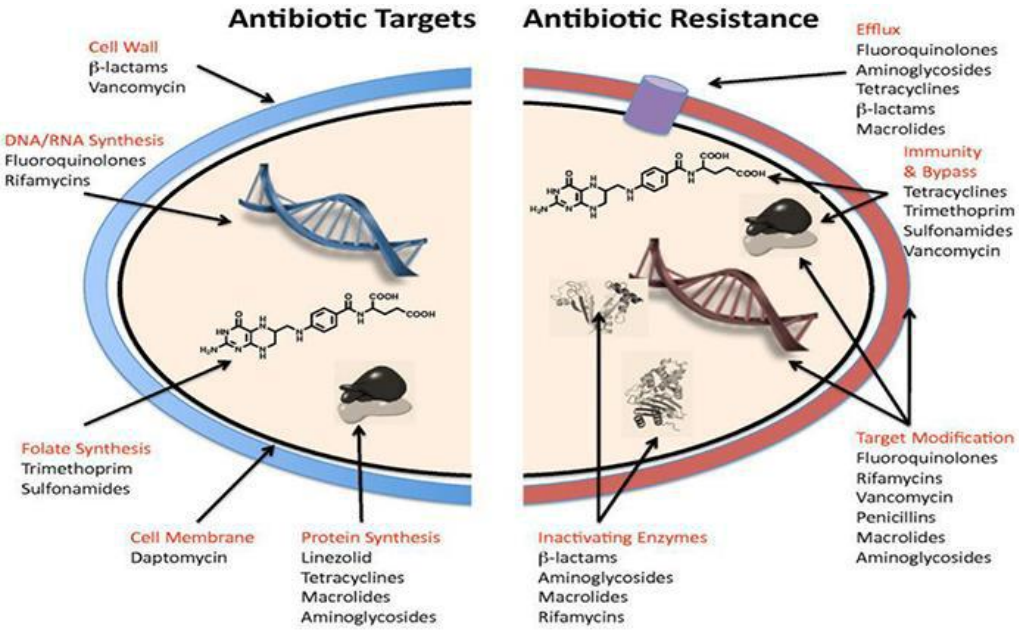


Figure 1: Mode of Action & Resistance

Mode of Action:

Basis of molecular mechanism of action against bacterial cells antibiotics are divided into 4 classes-

- β- Lactam inhibition -

Peptidoglycan layer is the prior form of structure in a bacterial cell wall. Without this a bacteria can't survive in the adverse environmental condition such as, changes in osmotic pressure. Glycopeptides and β-lactams are specific types of antibiotics that interfere with critical steps in homeostasis of cell wall biosynthesis. Important members of the β-lactam family include penicillin, cephalosporin, monobactam, and carbapenem. Specific inhibitors which can inhibit cell wall synthesis can result in alteration of cell shape and size. Also a cellular stress results in cell death (Srinivasa and Moreshwar, 1980).

- Quinolone -

Bacterial DNA synthesis is severely important for it's reproduction system. Two major enzyme which play the foremost role of various nucleic acid processes are

gyrase and topoisomerase IV. Quinolone, a type of antibiotic which targets bacterial DNA synthesis, mRNA transcription and cell division and breaks the DNA- topoisomerase complex. Thus by breaking the chemical structure this antibiotic kills the bacteria. Several studies have shown that topoisomerase IV is the prior target of quinolones in Gram-positive bacteria like *Streptococcus pneumoniae*, where gyrase is the primary target and topoisomerase IV the second major target in Gram-negative bacteria, as an example, *E.coli* and *Neisseria gonorrhea* (Domagala, 1994)

- Inhibition of protein biosynthesis -

  The aminocyclitol and tetracycline families of antibiotics are antibiotics which inhibit 30S ribosome. The aminocyclitol class of antibiotics (spectinomycin) and the aminoglycoside family of antibiotics (streptomycin, kanamycin, tobramycin, framycetin, gentamicin, etc.) bind to the 30 S ribosome subunit at its 16 S rRNA component. Spectinomycin acts on the stability of peptidyl-tRNA. Spectinomycin ties at the 30 S ribosome subunit and represses the extension factor-catalyzed movement without causing protein mistranslation (Ian and Marilyn, 2001). Conversely, aminoglycosides and the 16 S rRNA interaction cause conformational change in the complex framed between the mRNA codon and its charged aminoacyl tRNA at the ribosome, further causing tRNA crisscross which at that point brings about protein mistranslation (ref et al., 1986; Epe, 1984; Leach et al., 2007).
- Sulfa drugs -

  Sulfonamides are chemical compounds that are interrelated to Para- amino benzoic Acid and this acid is also intermediate compound of bacterial synthesis of Folic acid. Folic acid is a major component for the synthesis of nitrogen base. Sulfonamides not only block the formation of folic acid but their incorporation into the precursors causes the formation of a pseudo metabolite which is reactive and antibacterial. The advantage of using this drug is that mammalian cells have no side-effect of sulfonamide because they absorb and use preformed folic acid.

The fusion of sulfonamides with trimethoprim or other diaminopyrimidines increases the strength of these antibiotics (Choquet-Kastylevsky et al., 2002).

Table 1:

Resistance Mechanism	Specific Examples
Diminished intracellular drug concentration	
Decreased outer membrane permeability	β -Lactams (eg, OmpF, OprD)
Decreased cytoplasmic membrane transport	Aminoglycosides (decreased energy) Quinolones (eg, OmpF)
Increased efflux	Tetracyclines (eg, tetA) Quinolones (eg, norA) Macrolides (eg, mefA) Multiple drugs (eg, mexAB-OprF)
Drug inactivation (reversible or irreversible)	β -Lactams (b-lactamases) Carbapenemases (carbapenems) Aminoglycosides (modifying enzymes) Chloramphenicol (inactivating enzymes)
	Quinolones (gyrase modifications) Rifampin (DNA polymerase binding) β -Lactams (PBP changes) Macrolides (rRNA methylation)
Target bypass Glycopeptides (vanA, vanB)	Trimethoprim (thymidine-deficient strains)

1.2 Objectives

- Isolation and identification of pyogenic bacteria from clinical specimens of various wound infection.
- To do biochemical tests to identify and differentiate the microorganisms.
- To determine the prevalence of pyogenic infection among different ages and sex.
- Determination of the drug resistance pattern of the isolates.

Chapter 2

Methods & Materials

2.1 Study Design

All samples were collected from patients of IBN SINA Diagnostic & Imaging Center having clinical symptoms of microbial infection.

Samples were collected from both sexes and different age groups.

Study Period

January 5th – February 15th , 2021

Study Site

IBN SINA Diagnostic & Imaging Center, Dhanmondi, Dhaka 1209

Types of specimen

The specimen type that included in this study was Pus (from wound infection).

Quantity of specimen

A total of 200 clinical isolates were tested from patients.

Used Media, Biochemical test & Reagents

Media

- MacConkey agar media
- Blood agar media
- 2
- TSI slants
- MIU media
- Simmons citrate agar slants
- Muller-Hinton broth

Biochemical tests & Reagents

- TSI
- MIU
- Citrate
- Bile esculin
- Coagulase
- Catalase
- Oxidase

2.2 Methods and working procedure

Table 2:

Flow chart of working procedure:

Collection of pus sample

Inoculation on MacConkey ager, Blood agar & Chocolate agar plates

Incubation for 24 hours

Visual observation of bacterial colony

Microscopic Observation of bacteria

Identification of identified bacteria by using biochemical tests from positive plates

Antibiotic sensitivity test for identified bacteria

Sterilization:

All the media were sterilized (15 lbs for 15 minutes) by using autoclave. Glass materials sterilized at 180 degree Celsius for 1 hour in a hot air oven prior to use. All solutions were sterilized under the same condition.

Collection of sample:

Pus samples were collected from patient having wound infection from the diagnostic center and also hospitalized patients. Pus samples were taken from the patients by cotton swab and then put into a sterilized test tube.

Inoculation of specimens:

All the samples were directly inoculated on MacConkey agar, Blood agar and chocolate agar media by streaking technique as soon as possible. Then the plates were incubated at 37°C for 24-48 hours and observed the plates on the next day.

MacConkey agar-

- A selective and differential media used for the isolation and differentiation of non-fastidious gram negative- rods.
- Differentiation of lactose fermenting and non-lactose
- fermenting gram negative bacteria.
- The pink color is due to production of acid from lactose, absorption of neutral red.
- E.g. *Shigella, Salmonella* (non-lactose fermenting)
  Esherichia coli, Klebsiella(lactose fermenting)

Figure 2: MacConkey agar

Wound samples were directly inoculated on the media with the inoculating loop by streaking technique and then incubated for 24-48 hours at 37°C.

Blood agar-

- It is an enriched and differential (based on hemolytic reaction) media.
- It contains defibrinated mammalian blood (usually 5% sheep blood)
- Different types of hemolysis (alpha, beta, gamma) on blood agar.
- E.g. *Streptococcus pyogenes, Staphylococcus aureus*

Figure 3: Blood agar

Wound samples were directly inoculated on the media with the inoculating loop by streaking technique and then incubated for 24-48 hours at 37°C.

Chocolate agar-

- When blood agar is heated, the red blood cells are lysed and medium becomes chocolate brown in color. Then it's referred as chocolate agar.
- It's used to culture nutritionally demanded or fastidious organisms.
- E.g., *Haemophilus influenza, Neisseria meningitidis*

Wound samples were directly inoculated on the media with the inoculating loop by streaking technique and then incubated for 24-48 hours at 37°C.

Figure 4: Chocolate agar

2.3 Characteristics of different pyogenic bacteria on different media

Pseudomonas spp -

Pseudomonas aeruginosa is a species known as Gram negative, rod- shaped or bacilli and motile (polar flagella). Diagnosis is done on general purpose media and also get identified in biochemical tests. It develops well on most lab media and normally is separated on blood agar plates or eosin- methylthionine blue agar. It is distinguished based on its Gram morphology, a positive oxidase response and β hemolysis with an irregular shape (S.Baron 1996). Mostly found in blue-green, red-brown and yellow- green.

Staphylococcus aureus -

Staphylococcus aureus is a Gram positive, golden yellow bacteria which is responsible for a multiple kind of clinical diseases. It doesn't cause disease on healthy skin however if by any means it can enter the blood stream, the bacteria can create multiple potential diseases (T.A.Taylor et al., 2020). General culture media are easy to grow this bacteria such as blood agar and chocolate agar media.

Escherichia coli-

Escherichia coli is a significant Gram-negative bacteria that causes mostly waterborne and foodborne diseases. Consumption of contaminated food, uncooked food, liquid are responsible and transmission through fecal-oral route (M.A.Ameer et al., 2021). All general culture plates can grow this bacteria within just (24-48) hours incubation period. It's opaque, off-white, big and moist on culture plates.

Enterococcus-

A Gram positive opportunistic pathogen which causes multiple infections. By entering the food chain they can easily contaminate the food and environment. (Carmen Torres et al.,2018). The enterococci are strong and versatile species which are able to survive under any conditions, making them adapted to the hospital environment. Two prior species which cause the majority of enterococcal infections they are *Enterococcus faecalis* and *Enterococcus faecium* (Mónica García-Solache, Louis B Rice).

Klebsiella spp-

Klebsiella is a Gram negative, rod-shaped, lactose fermenting bacteria. The bacteria once entering into human body can show high antibiotic resistance and virulence (John V. Ashurst et al., 2021). *K. pneumonia* a type of *Klebsiella spp* complicates in pleural abnormalities, pulmonary gangrene or lung abscess (W K Moon et al., 1995). The eosin methylene blue (EMB) agar medium is specifically good for the bacteria's growth as it's a selective media. Also on MacConkey agar plates it gives mucoid texture, regular shape and opaque white color.

2.4 Isolation and Identification of Microorganisms

Identification of bacterial isolate was carried out by observation of colony characteristics and different bio-chemical test such as Triple sugar Iron (TSI) test, Motility Indole Urease (MIU) test, Citrate utilization test, Catalase test, Coagulase test and Oxidase test.

Triple Sugar Iron(TSI) test-

- It contains 3 sugars glucose, lactose, sucrose.
- It is used to study different properties of bacterium–sugar fermentation, gas production, H2S production.
- An orange red medium with slant and butt.
- Reaction – Yellow – Acid
  Pink/Red - Alkaline
- Yellow slant/ Yellow butt – Lactose fermenters
  Pink slant / Yellow butt - Non lactose fermenters
  Pink slant /No color change – Non fermenters
- Black precipitate formation – H2S production
- Gas/bubble formation - Gas production

Note- LF – *Klebsiella spp.*
NLF – *Salmonella, Shigella*

Motility Indole Urease (MIU Test) –

- Three tests in a single tube determines the differentiation of organisms on motility, indole, urease.

- Identification of gram-negative bacilli

Motility Test-

- It determines if bacteria is able to move in the agar media.
- Thinner agar is used to allow mobile bacteria to move through agar.
- Any color change in insertion point is considered positive if it the media color is changed.

Indole Test –

- Bacteria that possess the enzyme "tryptophanase" are capable of hydrolyzing and de-amminate tryptophan by producing indole, pyruvic acid & ammonia.
- Indole paper is used to determine positive result if the paper changes the color.

Urease Test –

Development of ammonia will increase the pH of media and converts colorless phenolphthalein to pink color.

Simmons Citrate Test-

- A test which determines the usage of carbon source of certain bacteria for their growth.
- The medium is alkaline.
- Dark blue determines positive result and dark green determines negative result.

Figure 5: (Left) Simmons Citrate, (Middle) TSI, (Right) MIU

Bile esculin agar-

- Used for isolation and identification of group D streptococci.
- Esculin in the medium is hydrolyzed to esculitin and dextrose.
- Later esculitin gives the dark brown/black complex by reacting to ferric citrate.

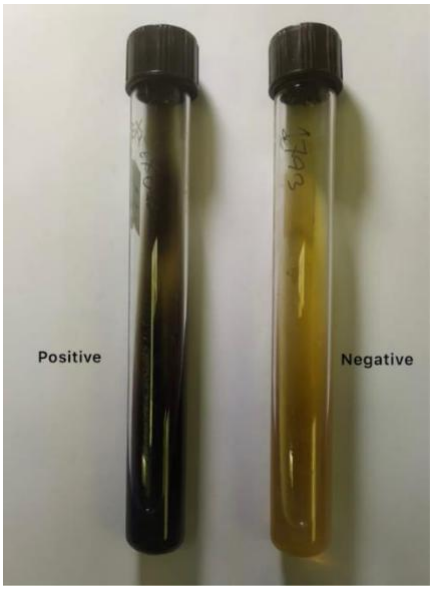


Figure 6: Bile esculin agar

Coagulase test –

- An enzyme produced by Staphylococcus aureus
- Converts fibrinogen to fibrin in plasma
- Absence of clumping is negative, presence of clumping is positive.

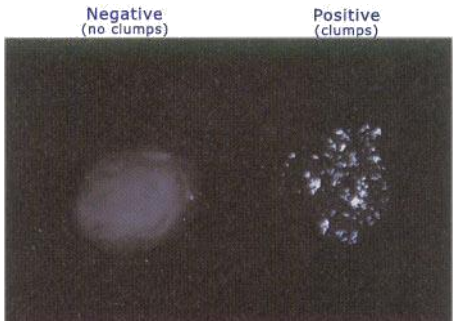


Figure 7: Coagulase test

Oxidase test-

- An enzyme of bacterial electron transport chain
- Enzyme's presence gives purple color
- Enzyme's absence gives no color

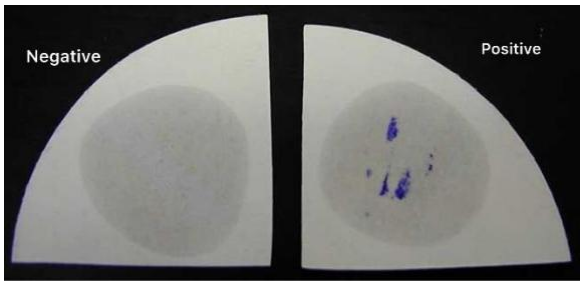


Figure 8: Oxidase test

2.5 Determination of antibiotic Susceptibility of different antimicrobial agents isolates

Antibiotic Susceptibility Test:

A laboratory test which determines how effective antibiotic therapy is against a bacteria. The goal of antimicrobial susceptibility testing is to predict the in vivo success or failure of antimicrobial therapy. Tests are performed in vitro, and measure the growth response of an isolated organism to a particular drug.

Among all the tests for susceptibility in the microbiology laboratory, Disc diffusion method (Kirby- Bauer) is used for the antibiotic susceptibility testing.

Disc Diffusion Method-

- The most commonly used methods in a laboratory to determine susceptibility of bacteria isolation to antibiotics.
- In this method, discs impregnated with known concentrations of antibiotics are placed on agar plate that has been inoculated with a culture of the bacterium to be tested.
- The plate is incubated at 37°C for 24 hours.
- The susceptibility of drug is determined from the zones of inhibition of bacterial growth surrounding the antibiotic discs.
- The diameters of the zone of inhibition are calculated with thin transparent millimeter scale to the nearest millimeter.

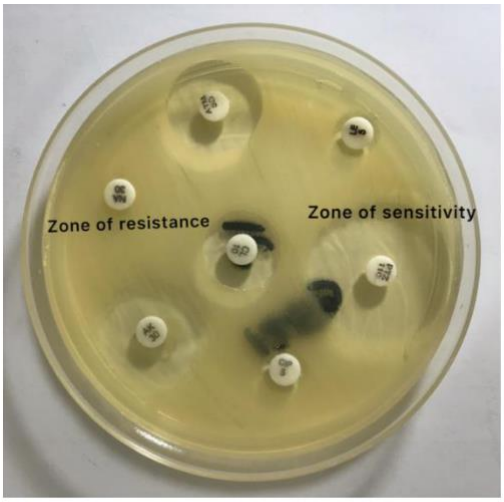


Figure 9: Antibiotic susceptibility test

Selection for antimicrobial disk:

- Antibiotic discs are obtained commercially.
- The discs are applied with sterile forceps, onto the surface of the medium, streaked with test strains, and the reading is reported after incubating the plate for 24 hours at 37°C aerobically

2.5.1 Antibiotic discs used for Gram Negative organisms

- **Inhibitors of Cell Wall synthesis**

  β **- Lactam antibiotics:** Amoxycillin, Cephalexin, Cephradine, Cefuroxime, Ceftaxidime, Cefixime, Ceftriaxone, Cefotaxime, Imipenem, Meropenem.

  β **- Lactamase inhibirors** : Tazobactam

- **Protein synthesis inhibitors:** Amikacin, Gentamycin
- **Inhibitors of metabolism:** Cotrimoxazole
- **Inhibitors of nucleic acid synthesis:** Ciorofloxacin, Amoxyclav

2.5.2 Antibiotic discs used for Gram Positive organisms

- **Inhibitors of Cell Wall synthesis**

  β **-Lactam antibiotics:** Amoxycillin, Cloxacillin, Cephalexin, Cephradine, Cefuroxime, Ceftaxidime, Cefixime, Ceftriaxone, Cefotaxime

  β **- Lactamase inhibirors :** Tazobactam

  Other: Vancomycin

- **Protein synthesis inhibitors:** Azithromycin, Gentamycin, Linezolid, Fusidic Acid
- **Inhibitors of metabolism:** Cotrimoxazole
- **Inhibitors of nucleic acid synthesis:** Ciprofloxacin, Amoxyclav

2.5.3 Used antibiotic discs with their resistance & sensitivity zone

Table 3:

NAME of Antibiotics	Code	Zone of resistance	Zone of Sensitive
Amoxicillin	AML	≤ 13	≥ 18
Gentamycin	CN/GN	≤ 12	≥ 15
Cotrimoxazole	SXT	≤ 10	≥ 16
Amoxyclav	AMC	≤ 13	≥ 18
Cephalexin	CL	≤ 14	≥ 18
Cephradine	CE	≤ 14	≥ 18
Cefuroxime	CXM	≤ 14	≥ 18
Ceftazidiame	CAZ	≤ 14	≥ 18
Cefixime	CFM	≤ 15	≥ 19
Ceftriaxone	CRO	≤ 13	≥ 21
Ciprofloxacin	CIP	≤ 15	≥ 21
Cefotaxime	CTX	≤ 14	≥ 23
Colistin	CT	≤ 08	≥ 11
Chloramphenicol	C	≤ 12	≥ 18
Amikacin	AK	≤ 14	≥ 17
Imipenem	IM	≤ 13	≥ 16
Meropenem	MEM	≤ 13	≥ 16
Azithromycin	AZM	≤ 13	≥ 18
Cloxacillin	OB	≤ 14	≥ 19
Fusidic Acid	FD	≤13	≥ 17
Vancomycin	VC	≤ 14	≥ 15
Linezolid	LD	≤ 17	≥ 21

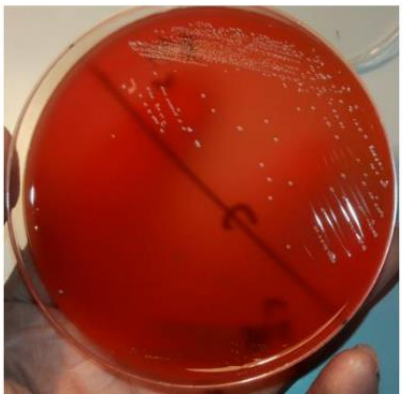
Chapter 3

Result

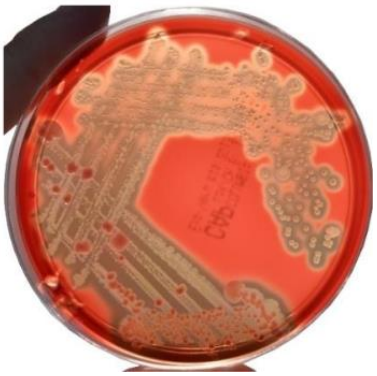
3.1 Pyogenic bacteria isolated from pus specimen

Gram Positive: *S. aureus, Enterococci spp., S. pyogenes.*

S. aureus



Enterococci spp

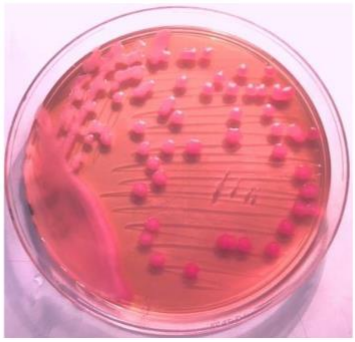


S. pyogenes

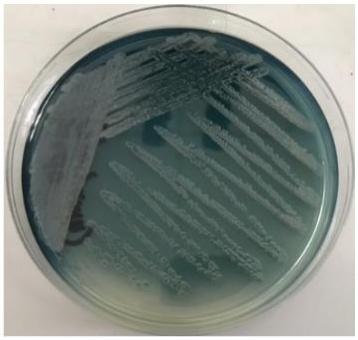
Figure 10: Gram-positive bacteria

Gram Negative: *E. coli, Klebsiella spp., Pseudomonas spp., Acinetobacter spp., Enterobacter spp.*

E.coli



Klebsiella spp.



Pseudomonas spp.

Enterobacter spp

Acinetobacter spp

Proteus spp.

Figure 11: Gram-negative bacteria

3.2 Results of biochemical tests for isolated organisms

Table 4:

Organisms	Citrate test	Oxidase test	MIU Medium			TSI		
			Motility	Indole	Urea	Slant	Butt	H_2S
E .coli	-	-	+	+	-	A	A	+
Klebsiella spp	+	-	-	-	+	A	A	+
Pseudomonas spp.	+	+	+	-	-	A	A	+
Staphylococcus aureus	-	-	-	-	-	K	K	-
Acinetobacter spp.	+	-	-	+	+	A	A	-

*A= Acidic; K= Alkaline

3.3 Antibiotic susceptibility test of isolated organisms

After identification of the infection causing bacteria disc diffusion antibiotic sensitivity testing (Kirby–Bauer antibiotic testing) were done to determine Antibiotic susceptibility patterns of isolated organisms.

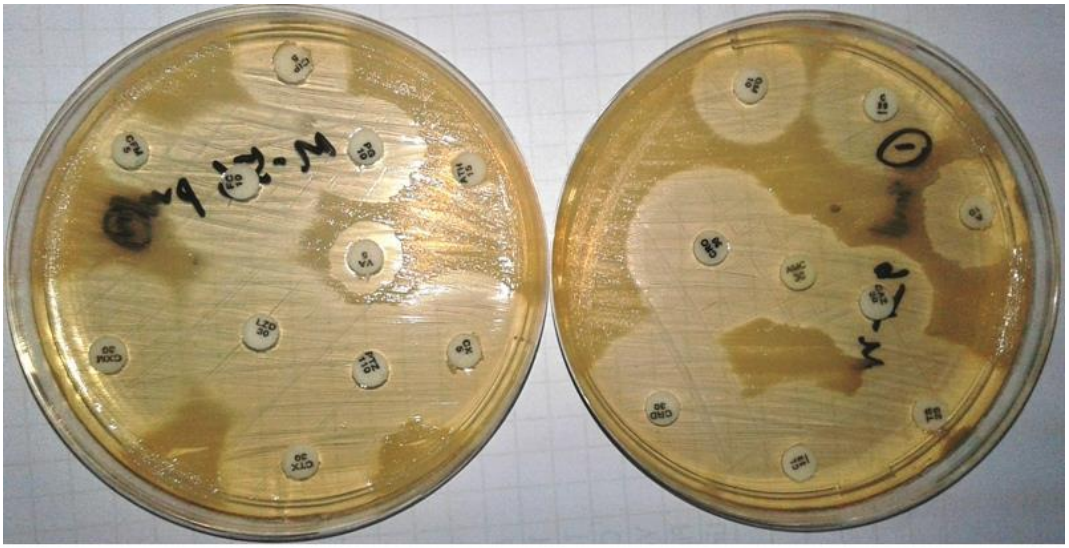


Figure 12: Antibiotic susceptibility test of Gram-positive bacteria

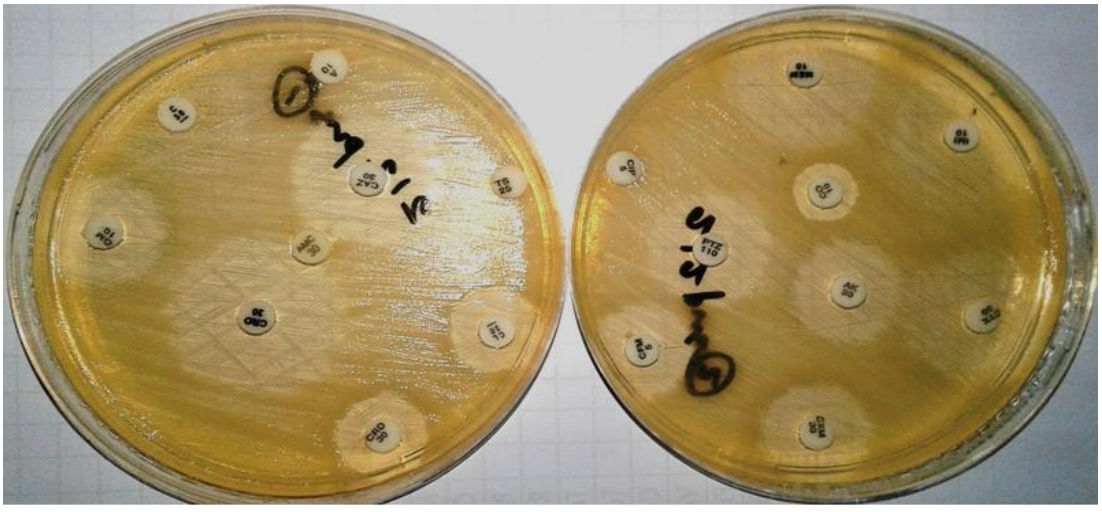


Figure 13: Antibiotic susceptibility test of Gram-negative bacteria

3.4 Percentage of Gram positive & negative organisms of total positive samples

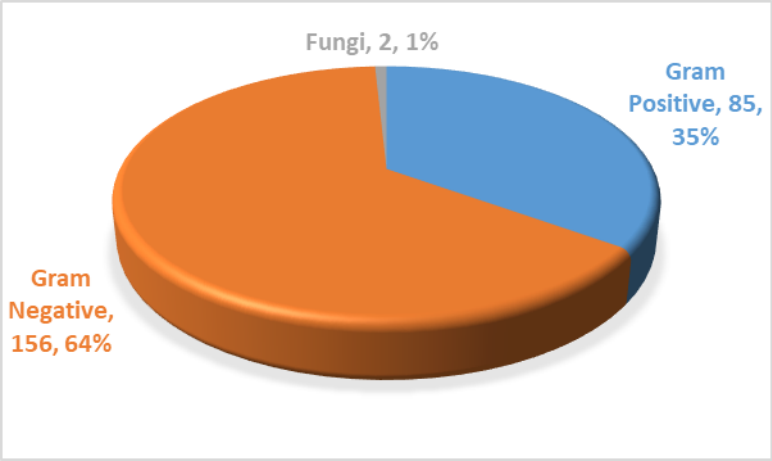


Figure 14: Percentage of all pathogens

Table 5:

Category	**Pathogen**	**(n)%**
Positive	*Streptococcus*	8 (3.32)%
	S.aureus	72 (29.88)%
	Enterococcus	5 (2.07)%
Total		85 (35.27)%

Among all the Gram-positive pathogens *S.aureus* is likely to have the most advantage on showing positive growth results from the wound samples.

Table 6:

Category	Pathogen	(n)%
Negative	*Klebsiella*	24(9.96)%
	E.coli	57(23.35)%
	Proteus	8(3.32)%
	Acinetobacter	8(3.32)%
	Pseudomonas	57(23.65)%
Total		156(64.73)%

Among all the Gram-negative pathogens *E.coli* and *Pseudomonas* are advanced in number which is more than 20% and other pathogens *Klebsiella*, *Proteus*, *Acientobacter* are mostly in 2-10% in number which is very minimal.

Table 7:

Pathogen	Isolated type	(n)%
Fungi	Candida	2 (0.83)%
Total		2 (0.83)%

Apart from finding of Gram-positive and Gram-negative pathogens, there are some minimal finding of *Candida* species (0.83) %. Invasive contamination because of *Candida* species is generally a condition related with clinical advancement, and is broadly known as a significant reason for morbidity and mortality in the health care sector (Peter G. Pappas et al., 2016).

3.5 Percentages of positive patients according to sex

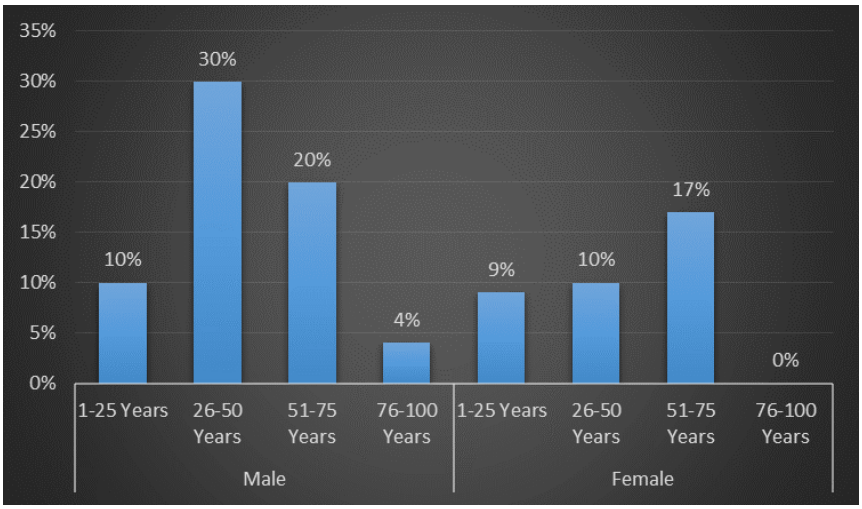


Figure 15: Percentage of positive patients according to sex

Among (26-50) years of male is showing predominance in positive patients which is likely to be 30% and gradually (51-75) years is 20% and (76-100) years is 4%. On the other hand, female patients are showing predominance in the age group of (51-75) years and gradually decreases to (26-50) years in 10% and (1-25) years in 9%.

3.6 Percentages of etiological agents of bacterial infection

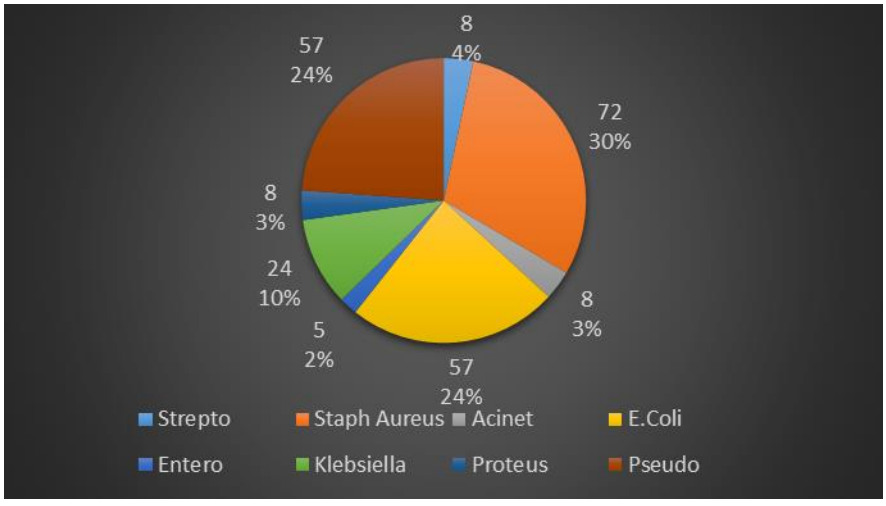


Figure 16: Percentages of etiological agents of Positive

After categorizing the pathogens in a specific order from Gram-positive to Gram-negative, this chart shows the pathogens all together in their specific percentage.

3.7 Antibiotic resistance pattern of different organisms

Klebsiella spp:

In this study Vancomycin, Linezolid, Cloxacillin, Fusidic Acid, Azithromycin gradually show 100% sensitivity against *Klebsiella spp.* And with this result these antibiotics can be suggested as some right medicines to treat infections with *Klebsiella spp.* There are couple of other antibiotics which are moderately resistant and mostly sensitive starting with Amoxyclav (96)%, Imipenem (79)%, Colistin (83)%, Amoxycillin (79)%, Tigecycline (87)%. Antibiotics which are mostly resistant against this pathogen are Cefotaxime (75)%, Cotrimoxazole (88)%, Ceftazidime (88)%, Cephalexin (83)% and Cefuroxime (75)%.

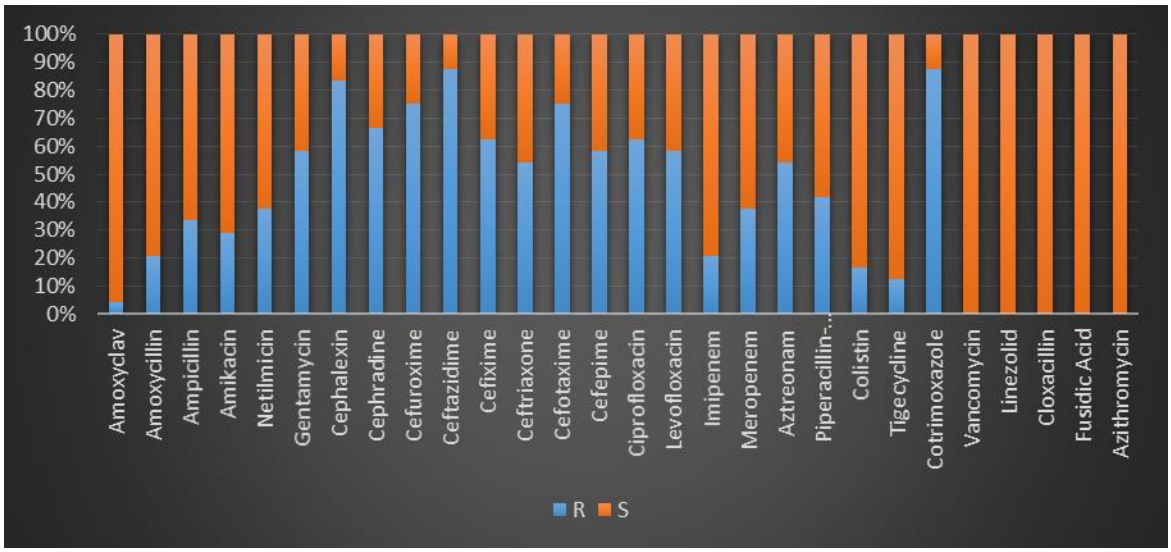


Figure 17: Antibiotic resistance pattern of *Klebsiella spp*.

Pseudomonas spp:

According to this chart, Cephalexin (95)%, Cefuroxime (93)%, Ceftazidime (81)%, Cefotaxime (88)%, Cotrimoxazole (89)% are showing the most resistance against *Pseudomonas spp.* So antibiotics mostly under Cephalosporin group will not be effective in treating diseases under this pathogen. Antibiotics which are moderately resistant are Amoxycillin (84)%, Ampicillin (75)%,

Amikacin (68)%, Imipenem (68)%, Meropenem (41)%. 100% sensitive towards this pathogen are Vancomycin, Linezolid, Cloxacillin, Fusidic Acid, Azithromycin, Amoxyclav.

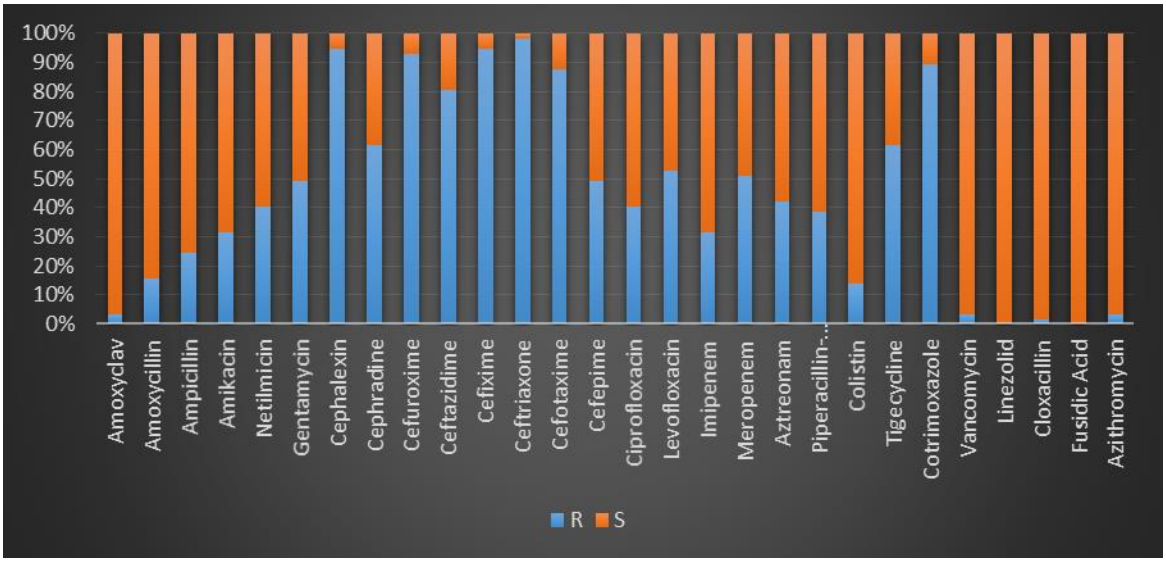


Figure 18: Antibiotic resistance pattern of *Pseudomonas spp*.

Proteus spp:

In this chart Amoxyclav, Vancomycin, Linezolid, Cloxacillin, Fusidic acid, Azithromycin show 100% sensitivity, that determines the possibility of proper medication against this bacteria. Cephalexin (88)%, Cefuroxime (63)%, Ceftazidime (75)%, Collistin (88)%, Cotrimoxazole (63)% are respectively showing more resistance towards *Proteus.* Antibiotics which are moderately resistant are Amikacin (25)%, Netilmicin (25)%, Cephradine (38)%, Cefixime (25)%, Ceftriaxone (25)% and Imipenem, Azteronam, Tigecycline are showing minimal resistance and mostly sensitivity.

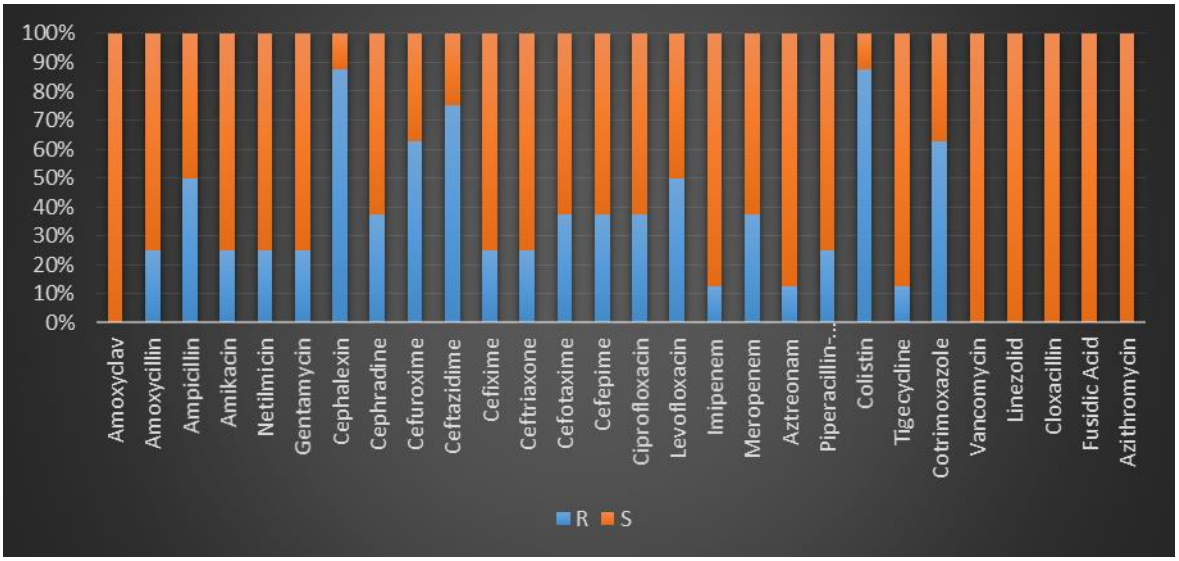


Figure 19: Antibiotic resistance pattern of *Proteus spp*.

Enterococcus spp:

In this study, Amoxyclav (60)%, Ampicillin (80)%, Gentamycin (80)%, Cephalexin (80)%, Cefuroxime(80)%, Ceftazidime (80)%, Cefixime (80)%, Cefotaxime(80)%,Meropenem(80)%, Azithromycin(80)% are respectively showing most resistance against *Enterococcus* .None of these antibiotics would be a good suggestion as medication against this bacteria then. However Levofloxain, Azteronam, Piperacillin-tazobactam, Colistin, Tigecycline, Vancomycin, Linezolid, Fusidic acid are showing 100% sensitivity towards this bacteria.

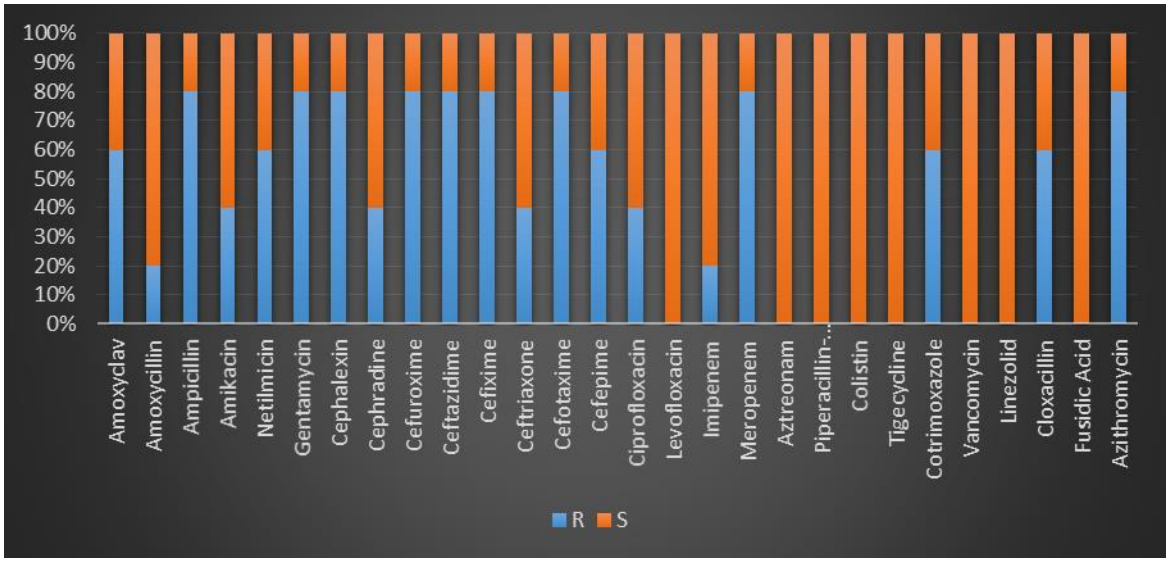


Figure 20: Antibiotic resistance pattern of *Enterococcus spp*.

E.coli:

Here Amoxyclav, Imipenem, Meropenem, Piperacillin-tazobactam, Colistin, Tigecycline, Vancomycin, Linezolid, Cloxacillin, Fusidic acid, Azithromycin are sequentially showing more than 80% of sensitivity. Some of the antibiotics which are mostly resistant against this bacteria are Cephalexin (93)%, Cefuroxime (82)%, Ceftazidime(79)%, Cefixime(79)%, Cotrimoxazole(75)%.

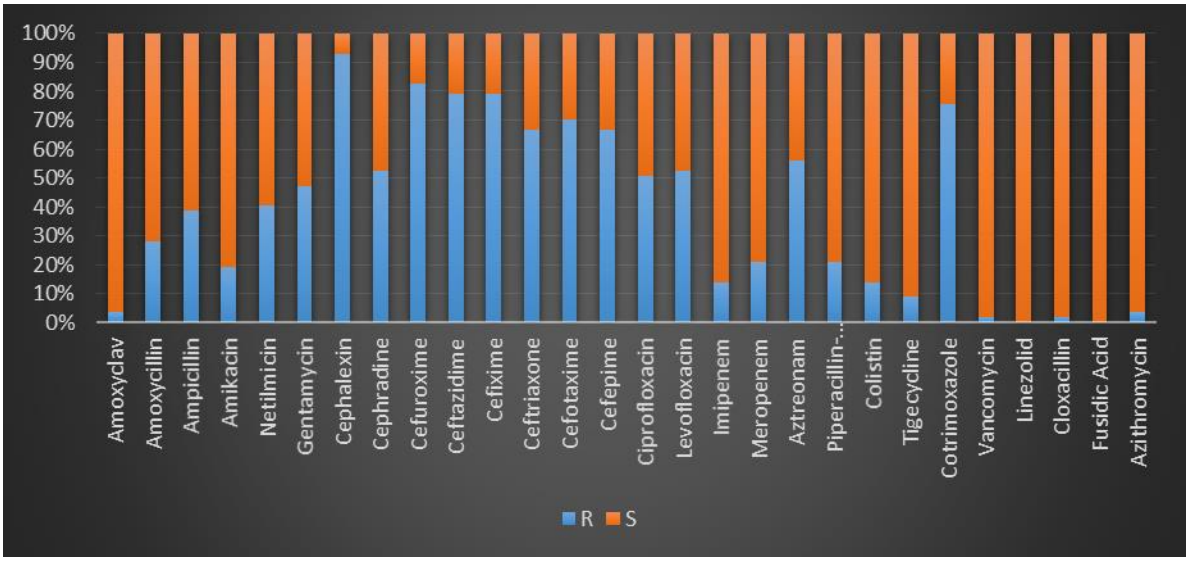


Figure 21: Antibiotic resistance pattern of *E.coli*

Acinetobacter:

According to this chart *Acinetobacter* shows high resistance to a potential antibiotic group Cephalosporin, which are Ceftazidime(100)%, Cefixime (100)%, Ceftriaxone(100)%, Cefotaxime(100)%, Cefepime (88)% accordingly. Also Meropenem, Azteronam, Piperacillin-tazobactam are not behind in terms of showing resistance. On the other hand, Amoxyclav (100)%, Vancomycin (100)%, Linezolid (100)%, Cloxacillin (100)%, Fusidic acid (100)%, Azithromycin (100)% are highly sensitive towards this bacteria.

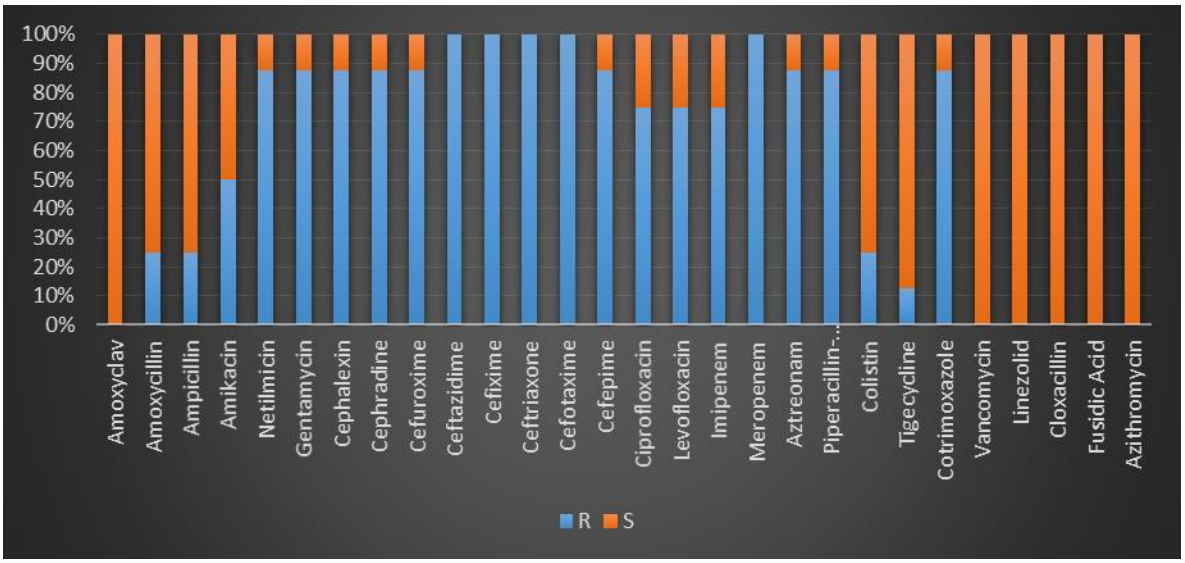


Figure 22 : Antibiotic resistance pattern of *Acinetobacter*

Staphylococcus aureus:

S.aureus seems hugely sensitive towards Levofloxacin(99)%, Imipenem (87)%, Azteronam (99)%, Piperacillin-tazobactam(100)%, Colistin (100)%, Tigecycline (100)%, Vancomycin (100)%, Linezolid (93)%, Fusidic acid (94)%. Antibiotics which are showing most resistance against this bacteria are Ampicillin (93)%, Ceftazidime(94)%, Cefixime(92)%. It was moderately sensitive Amoxycillin (86)% and Netilmicin (88)%.

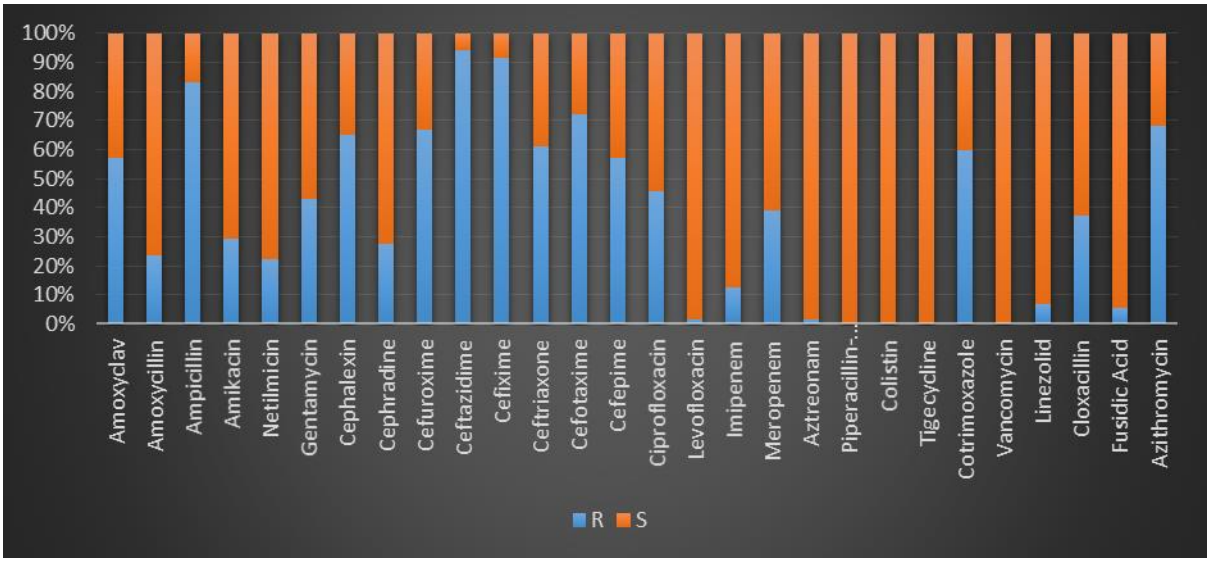


Figure 23: Antibiotic resistance pattern of *S.aureus*

Streptococcus:

This bacteria is showing maximum sensitivity (100)% towards various antibiotics which are accordingly Amoxyclav, Cephradine, Ciprofloxacin, Levofloxacin, Imipenem, Meropenem, Azteronam, Piperacillin-tazobactam, Colistin, Tigecycline, Vancomycin, Linezolid, Fusidic acid. So in order to treat infection of this species there are multiple options in hands. It is moderately sensitive to Amoxycillin, Ampicillin, Cephalexin, Cefepime, Cloxacillin accordingly which is around (87)%.

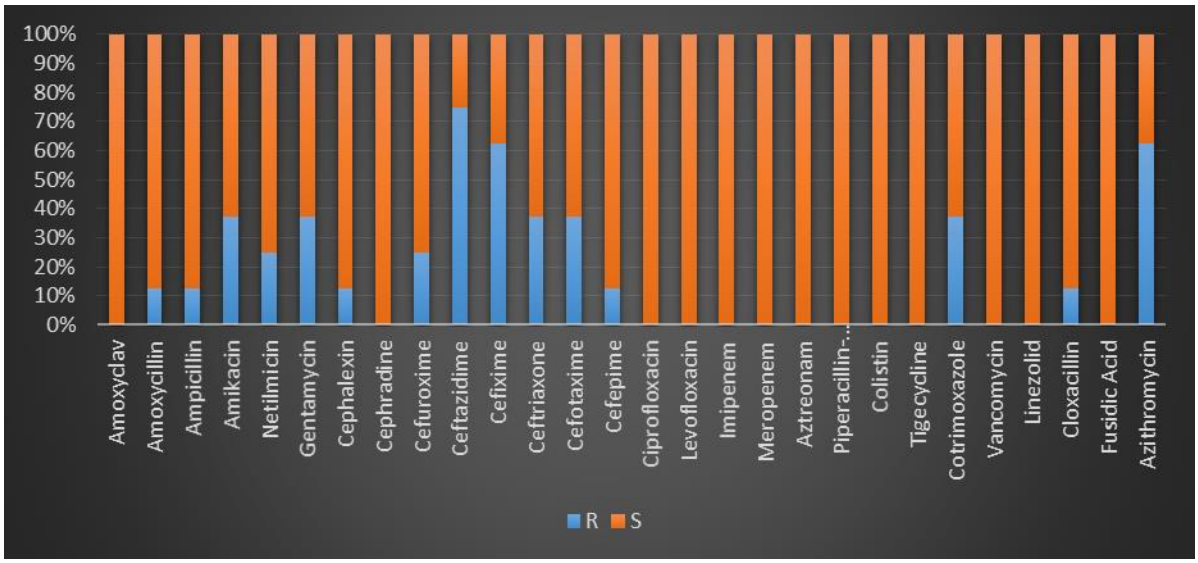


Figure 24: Antibiotic resistance pattern of *Streptococcus*

Sensitivity pattern of Gram-positive and Gram-negative bacteria-

Most of the Gram-positive bacteria were resistant against Cephalosporin group and sensitive towards Carbapenem group's Imipenem but resistant against Meropenem. Such as Cefixime (92)% and Ceftazidime (94)% were highly resistant in *Staph.aureus.* In *Enterococcus* Imipenem was (20)% and Meropenem was (80)%. On the other hand, in Gram-negative bacteria *E.coli*, *Acinetobacter* and *Pseudomonas spp* showed most resistance against the antibiotics.

Table 8:

Antibiotic resistance pattern (All)

Group Name	Drug Name	Gram Positive			Gram Negative				
		Strepto	Staph Aureus	Entero	Klebsiella	E.Coli	Acenit obacter	Pseudom onas	Proteus
Penicillin	Amoxycillin	13%	24%	20%	21%	28%	25%	16%	25%
	Ampicillin	13%	83%	80%	33%	39%	25%	25%	50%
	Cloxacillin	13%	38%	60%	0%	2%	0%	2%	0%
	Amoxyclav	0%	57%	60%	4%	4%	0%	4%	0%
Aminoglycoside	Amikacin	38%	29%	40%	29%	19%	50%	32%	25%
	Gentamycin	38%	43%	80%	58%	47%	88%	49%	25%
	Netilmicin	25%	22%	60%	38%	40%	88%	40%	25%
Cephalosporin	Cefepime	13%	57%	60%	58%	67%	88%	49%	38%
	Cefixime	63%	92%	80%	63%	79%	100%	95%	25%
	Cefotaxime	38%	72%	80%	75%	70%	100%	88%	38%
	Ceftazidime	75%	94%	80%	88%	79%	100%	81%	75%
	Cefuroxime	25%	67%	80%	75%	82%	88%	93%	63%
	Ceftriaxone	38%	61%	40%	54%	67%	100%	98%	25%
	Cephalexin	13%	65%	80%	83%	93%	88%	95%	88%
	Cephradine	0%	28%	40%	67%	53%	88%	61%	38%
Fluroquinolones	Ciprofloxacin	0%	46%	40%	63%	51%	75%	40%	38%
	Levofloxacin	0%	1%	0%	58%	53%	75%	53%	50%
Carbapenem	Imipenem	0%	13%	20%	21%	14%	75%	32%	13%
	Meropenem	0%	39%	80%	38%	21%	100%	51%	38%
Peptide	Colistin	0%	0%	0%	17%	14%	25%	14%	88%
Sulfonamide	Cotrimoxazole	38%	60%	60%	88%	75%	88%	89%	63%
Oxazolidnone	Linezolid	0%	7%	0%	0%	0%	0%	0%	0%
Glycopeptide	Vancomycin	0%	0%	0%	0%	2%	0%	4%	0%
Tigecycline	Tigecycline	0%	0%	0%	13%	9%	13%	61%	13%
Fusidane	Fusidic Acid	0%	6%	0%	0%	0%	0%	0%	0%
Monobactem	Aztreonam	0%	1%	0%	54%	56%	88%	42%	13%
Piperacillin-tazobactam	Piperacillin-tazobactam	0%	0%	0%	42%	21%	88%	39%	25%
Microlide	Azithromycin	63%	68%	80%	0%	4%	0%	4%	0%

Chapter 4

Discussion

A total 200 pus samples of both sex between age group 0-90 years were analyzed in this study. These specimens were analyzed to observe the antibiotic susceptibility having significant growth of pyogenic bacteria. Gram-positive organisms accounted for 35% and Gram-negative organisms accounted for 64% which is almost double of total Gram-positive isolates comparatively.

The samples were inoculated on MacConkey agar media, Blood agar and Chocolate agar media for primary identification of pyogenic bacteria such as *S.aureus, Pseudomonas spp., E.coli., Klebsiella spp., Enterococcus spp*. etc. Depending on colony formation, pigmentation, elevation and margins, colonies were presumably identified. Then the presumed isolates were further tested for a more confirmation. All isolates were examined through biochemical tests- TSI (Triple Sugar Iron) test, MIU (Motility Indole Urea) test, Citrate utilization test, Coagulase test and Oxidase test.

This study also showed that male 64% are more infected than female 36%. Among the causative agents *S.aureus* (30%) was the most prevalent .The second prevalent bacteria were accordingly *E. coli* **(**24%) and *Pseudomonas* (24%) and others are *Klebsiella spp*. (10%), *Streptococcus* (4%), & *Proteus spp* (3%), *Acinetobacter spp.* (3%) and *Enterococcus* (2%),

In this study, *Streptococcus* has shown the most sensitivity towards the antibiotics among all the isolated pathogens. On the other hand another Gram-negative bacteria *Staph.aureus* was highly resistant against to some of the most prescribed antibiotics. Cephalosporin group was mostly active in this resistance pattern of this bacteria. It's clearly shown that about 30% of infection rate is only from *Staph.aureus* itself. Gram- negative bacteria were mostly resistant against Penicillin and Cephalosporin. *E.coli* (24)%, *Pseudomonas spp* (24)%, *Klebsiella spp* (10)% accordingly were most found pathogens in the Gram-negative group. In a research, it is also found that *E.coli* is one of the major pathogen in the wound infection, after that comes *Staph.aureus* but in a different platform (Sushmita Roy et al., 2017). In addition Linezolid, Vancomycine, Tigecycline, Fusidic acid were some most active antibiotics as they were susceptible to most of the organisms. Also the pattern observed in this study has shown that there were pathogens which were multi-drug resistant. However Fungus species were not much available in the findings which was only 0.83%.

Antibiotic resistance is accelerated because of the misuse and overuse of antibiotics. Adequate cleaning, dressing and bandaging can prevent most infections. Only after prescribed by a health professional antibiotics should be taken also reducing the number of prescribing antibiotics and increasing the need for improved water, sanitation and immunization is important. Incentives should be focused on antibiotic stewardship rather than focusing on antibiotic overuse or misuse. Patients also need to be educated about the proper usage of antibiotics. Also sustainable antibiotic usage needs to be taught among health professionals, policy makers etc. Periodic studies are also needed as evaluation measure of the level of infection control practices. Food industry/animal farm industry need to take proper measurements to reduce health risks. Ensuring political commitment to meet the threat of antibiotic resistance is another important needs.

References

1. Basista Prasad Rijal, Deepa Satyal, and Narayan Prasad Parajuli, “High Burden of Antimicrobial Resistance among Bacteria Causing Pyogenic Wound Infections at a Tertiary Care Hospital in Kathmandu, Nepal”,2017.
   [file:///E:/Important/THESIS%20ARTICLES/9458218.pdf]
2. Carmen Torres, Carla Andrea Alonso, Laura Ruiz-Ripa, Ricardo León-Sampedro, Rosa Del Campo, Teresa M Coque, “Antimicrobial Resistance in Enterococcus spp. of animal origin”,2018.
   [https://pubmed.ncbi.nlm.nih.gov/30051804/]
3. Chara Calhoun, Harrison R. Wermuth, Gregory A. Hall, “Antibiotics”, Medical University of South Carolina, Michigan State University College of Human Medicine, MUSC, 2021. [https://www.ncbi.nlm.nih.gov/books/NBK535443/]
4. Christopher J. Kristich , Louis B. Rice, Cesar A. Arias, “Enterococcal Infection—Treatment and Antibiotic Resistance”, 2014.
   [https://pubmed.ncbi.nlm.nih.gov/24649502/]
5. Choquet-Kastylevsky MD, PhD, Thierry Vial MD, Jacques Descotes MD, PhD, “Allergic adverse reactions to sulfonamides”, Current Allergy and Asthma Reports volume 2, pages16– 25 (2002).
   [https://link.springer.com/article/10.1007%2Fs11882-002-0033-y]
6. Davis, L L Chen, and P C Tai, “Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides”, 1986. [https://www.pnas.org/content/83/16/6164/tab-article-info]
7. Derek. W. Moore, “Antibiotic Classification & Mechanism” , 2020.
   [https://www.orthobullets.com/basic-science/9059/antibiotic-classification-and-mechanism]
8. D Y Aksoy, S Unal, “New antimicrobial agents for the treatment of Gram-positive bacterial infections”,2008.
   [https://pubmed.ncbi.nlm.nih.gov/18261129/]
9. Irina Negut, Valentina Grumezescu, Alexandru Mihai Grumezescu “Treatment Strategies for Infected Wounds”,2018.
   [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6225154/]

10. John V. Ashurst, A, “dam Dawson, “Klebsiella Pneumonia”, Kingman Regional Medical Center, 2021. [https://www.ncbi.nlm.nih.gov/books/NBK519004/]
11. John M. Domagala, “Structure-activity and structure-side-effect relationships for the quinolone antibacterials”,1994. [https://academic.oup.com/jac/article-abstract/33/4/685/672331?redirectedFrom=fulltext]
12. Jean-Christophe Lagier, Sophie Edouard, Isabelle Pagnier, Oleg Mediannikov, Michel Drancourt, Didier Raoult, “Current and Past Strategies for Bacterial Culture in Clinical Microbiology”, 2015. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4284306/]
13. Luke F. Chen, MBBS (Hons), CIC, FRACPa ,Teena Chopra, MD , Keith S. Kaye, MD, MPH, “Pathogens Resistant to Antibacterial Agents”, 2009. [https://sci-hub.do/https://pubmed.ncbi.nlm.nih.gov/19909886/]
14. Muhammad Atif Ameer, Abdul Wasey, Philip Salen “Escherichia Coli (E Coli 0157 H7)”, University of Nebraska Medical Center, St. Luke's University Health Network St, Luke's University Hospital, 2021. [https://www.ncbi.nlm.nih.gov/books/NBK507845/]
15. Mónica García-Solache , Louis B Rice, “The Enterococcus: a Model of Adaptability to Its Environment” 2019. [https://pubmed.ncbi.nlm.nih.gov/30700430/]
16. Neha Upmanyu, Viveka Nand Malviya, “Antibiotics: mechanisms of action and modern challenges” , INSTITUTE FOR VETERINARY-PHYSIOLOGY AND BIOCHEMISTRY, SCHOOL OF VETERINARY MEDICINE, JUSTUS-LIEBIG-UNIVERSITY, GIEßEN, GERMANY 2DEPARTMENT OF NEUROBIOLOGY, MAX PLANCK INSTITUTE FOR BIOPHYSICAL CHEMISTRY, GÖTTINGEN, GERMANY, 2020. [https://scihub.do/https://www.sciencedirect.com/science/article/pii/B9780128190012000188]
17. Peter Davey, Charis A Marwick, Claire L Scott, Esmita Charani, Kirsty McNeil, Erwin Brown, Ian M Gould, Craig R Ramsay, and Susan Michie, “Interventions to improve antibiotic prescribing practices for hospital inpatients” , Cochrane Database Syst Rev. 2017. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6464541/]

18. Peter G. Pappas,Carol A. Kauffman, David R. Andes, Cornelius J. Clancy, Kieren A. Marr, Luis Ostrosky-Zeichner, Annette C. Reboli, Mindy G. Schuster, Jose A. Vazquez, Thomas J. Walsh,Theoklis E. Zaoutis, and Jack D. Sobel, "Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America" , Clinical Infectious Diseases, 2016. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4725385/]
19. Rima Moghnieh,Georges F. Araj, Lyn Awad, Ziad Daoud, Jacques E. Mokhbat,Tamima Jisr, Dania Abdallah,Nadim Azar, Noha Irani-Hakimeh,Maher M. Balkis,Mona Youssef, Gilbert Karayakoupoglou,Monzer Hamze, Madonna Matar, Roula Atoui, Edmond Abboud, Rita Feghali, Nadine Yared, and Rola Husn, "A compilation of antimicrobial susceptibility data from a network of 13 Lebanese hospitals reflecting the national situation during 2015–2016", Antimicrobial Resistance Infection Control,2019. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6381724/]
20. R L Thompson, A J Wright, "Cephalosporin antibiotics",1983. [https://pubmed.ncbi.nlm.nih.gov/6401833/]
21. Saswati Sengupta, Madhab K. Chattopadhyay and Hans-Peter Grossart, "The multifaceted roles of antibiotics and antibiotic resistance in nature", Independent, Hyderabad, India,Centre for Cellular and Molecular Biology (Council of Scientific and Industrial Research), Hyderabad, India, Limnology of Stratified Lakes, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany, Institute for Biochemistry and Biology, Potsdam University, Potsdam, Germany, 2013. [https://www.frontiersin.org/articles/10.3389/fmicb.2013.00047/full]
22. Samuel Baron, "Medical Microbiology, 4th edition" 1996. [https://www.ncbi.nlm.nih.gov/books/NBK7627/]
23. Shehab, N., Patel, P.R., Srinivasan, A., Budnitz, D.S., n.d. Emergency department visits for antibiotic associated adverse events. Clin. Infect. Dis., 47(6), 735-743
24. Tracey A. Taylor; Chandrashekhar G. Unakal, "Staphylococcus Aureus", Oakland University,The University of the West Indies, 2020 [https://www.ncbi.nlm.nih.gov/books/NBK441868/]

25. [https://www.cliffsnotes.com/study-guides/biology/microbiology/control-of-microbial-growth/antibiotics]

Appendix I

Instruments & Reagent kits

The important equipment used through the study are listed below:

1. Autoclave
2. Sterilizer
3. Disposable micro plate tips
4. Petri dishes
5. Incubator
6. Glass slide
7. Platinum Loop
8. Water bath
9. Refrigerator
10. Hot air oven
11. Microscope
12. Cover slip
13. Disposable syringe
14. Glass cylinder
15. Beaker
16. Bunsen burner

17. Incinerator

Appendix II

Media Composition

Media used were prepared by standard methods using appropriate compositions. The compositions used for different media given below:

1. Muller-Hinton agar

Ingredients	**Amount (g/l)**
a) Beef, infusion	300
b) Casamino acids	17.5
c) Strach	1.5
d) Agar	17

2. MacConkey Agar Medium

Ingredients	Amounts (g/l)
a) Peptone	20.0
b) Lactose	10.0
c) Bile salts	5.0
d) Sodium Chloride	5.0
e) Neutral red	0.075
f) Crystal violet	0.001
g) Agar	12.0
h) pH	7.4

3. Blood agar

Ingredients	Amount (%)
a) Beef extract	0.3
b) Peptone	0.5%
c) NaCl	1.5
d) Agar	1.5%
e) Sheep Blood	5%

4. Triple sugar-iron agar slant

Ingredients	**Amount (g/l)**
a) Beef extract	3
b) Yeast extract	3
c) Peptone	15
d) Proteose peptone	5
e) Lactose	10
f) Saccharose	10
g) Dextrose	1
h) Ferrous sulfate	0.2
e) Sodium chloride	5
f) Sodium thiosulfate	0.3
g) Phenol red	0.024
h) Agar	12

5. MIU agar media

Ingredients	Amount (g/l)
a) Peptone	10
b) Dextrose	1
c) Sodium chloride	5
d) Phenol red	0.002
e) Agar	2
f) Urea	20

6. Simmons citrate agar

Ingredients	Amount (g/l)
a) Ammonium dihydrogen phosphate	1
b) Dipotassium phosphate	1
c) Sodium chloride	5
d) Magnesium sulfate	0.2
e) Agar	15
f) Bromothymol blue	0.08
g) Sodium citrate	2