"ISOLATION AND SCREENING OF MULTI-DRUG RESISTANCE MICRO-ORGANISM"

Manithe Shrutika Kishor

Assistant Professor – Microbiology Department

Indian Institute Of Food Science And Technology College

Aurangabad (MH), India

E-mail: shrutikamanithe55@gmail.com

I. ABSTRACT

Multiple Drug resistance occurs when Bacteria Are resistance to more than one antibiotic. Because of years of antibiotic over use, Multidrug resistance is now the rule rather than the exception among resistant bacteria. This situation as largely occurred through the sequential use of multiple different antibiotics. Multidrug- resistance organism infections are hard to treat because they do not respond to many common antibiotics, even the most powerful ones. The emergence of MDR phenotypes is a major Public health problem today in the bacterial infections.

MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories. To ensure correct application of these definitions, bacterial isolates should be tested against all or nearly all of the antimicrobial agents within the antimicrobial categories and selective reporting and suppression of results should be avoided.

For Antibiotic susceptibility testing 44 unknown Bacteria sample were isolated from hospital waste dumping area (source not to be expose) by expossing different agar media plates i.g., Actinomycete Isolation Agar, Brain Heart Infusion Agar, Manitol agar, antibiotic containing agar, Bismutase agar, MacConkey agar, Yeast extract peptone Dextrose agar and marked according to media on which the isolates were grown. For screening of the isolates Antibiotic susceptibility test were performed i.g. Disc Diffussion method using HIMedia ICOSA disc containing 20 set of antibiotics– Amikacin , Ampcillin , Cefadroxil , Amoxicilline , Co-Trimoxazole , Chloramphenicol, Ceftazidime, Ceftriaxdne , Ciprefloxacin , Cloxacillin , Cefoperazone , Erythromycin , Netillin, nitrofurantoin, Nalidixic acid, Norfloxacin, Gentamicin, Tobramycin, vancomycin, Penicillin.

When antibiotic resistance patterns of the Total 44 clinical isolates were studied for antibiotic susceptibility. Highest rates of antibiotic resistant were obtained for antibiotic ceftazidimine (68.18%) followed by Ampicillin(52.27%), Penicillin(47.72%), Cloxacillin(38.69%), Amoxicillin(36%), Nitrofuratonin(20.45%), Chloramphenical(20.45%), Co-Trimoxazole(15.90%), Netillin(13.63%), Erythromycin(11.36%), Ceftriaxomee(4.5%), Nalidixic acid(2.2%).

II. INTRODUCTION

A. Background

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria. Gram-positive and Gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance.In the strictest sense, multidrug-resistant organisms (MDROs) are labelled as such because of their in vitro resistance to more than one antimicrobial agent. Infections with MDROs can lead to inadequate or delayed antimicrobial therapy, and are associated with poorer patient outcomes. Of the MDROs, highly-resistant Gram-negative bacteria (e.g. multidrug-resistant carbapenemase-producing Klebsiella pneumoniae and Acinetobacter spp.) require special mention; these organisms can be resistant to all currently available antimicrobial agents or remain susceptible only to older, potentially more toxic agents such as the polymyxins, leaving limited and suboptimal options for treatment. The problem of increasing antimicrobial resistance is even more threatening when considering the very limited number of new antimicrobial agents that are in development.

B. Drug Resistance (D.R.)

Drug, toxin, or chemical resistance is a consequence of evolution and is a response to pressures imposed of any living organisms. Drug resistance is the reduction in effectiveness of a medication such as an anti-microbial or an antineoplastic in curing a disease or conditions. The development of antibiotic resistance in particular stems from the drugs targeting only specific bacterial molecules (almost always proteins). Because the drug is so specific, any mutation in these molecules will interfere with or negate its destructive effect, resulting in antibiotic resistance. Bacteria are capable of not only altering the enzyme targeted by antibiotics, but also by the use of enzymes to modify the antibiotic itself and thus neutralise it. Examples of target-altering pathogens are Staphylococcus aureus, vancomycin-resistant enterococci and macrolide-resistant Streptococcus, while examples of antibiotic-modifying microbes are Pseudomonas aeruginosa and aminoglycoside-resistant Acinetobacter baumannii The chances of drug resistance can sometimes be minimized by using multiple drugs simultaneously. This works because individual mutations can be independent and may tackle only one drug at a time; if the individuals are still killed by the other drugs, then the mutations cannot

persist. This was used successfully in tuberculosis. However, cross resistance where mutations confer resistance to two or more treatments can be problematic.

For antibiotic resistance, which represents a widespread problem nowadays, drugs designed to block the mechanisms of bacterial antibiotic resistance are used. For example, bacterial resistance against beta-lactam antibiotics (such as penicillins and cephalosporins) can be circumvented by using antibiotics such as nafcillin that are not susceptible to destruction by certain beta-lactamases (the group of enzymes responsible for breaking down beta-lactams). Beta-lactam bacterial resistance can also be dealt with by administering beta-lactam antibiotics with drugs that block beta-lactamases such as clavulanic acid so that the antibiotics can work without getting destroyed by the bacteria first. Recently, researchers have recognized the need for new drugs that inhibit bacterial efflux pumps, which cause resistance to multiple antibiotics such as beta-lactams, quinolones, chloramphenicol, and trimethoprim by sending molecules of those antibiotics out of the bacterial cell. Sometimes a combination of different classes of antibiotics may be used synergistically; that is, they work together to effectively fight bacteria that may be resistant to one of the antibiotics alone.

In the domestic environment, drug-resistant strains of organism may arise from seemingly safe activities such as the use of bleach, tooth-brushing and mouth washing, the use of antibiotics, disinfectants and detergents, shampoos, and soaps, particularly antibacterial soaps, hand-washing, surface sprays, application of deodorants, sun blocks and any cosmetic or health-care product, insecticides, and dips. The chemicals contained in these preparations, besides harming beneficial organisms, may intentionally or inadvertently target organisms that have the potential to develop resistance.

"Drug resistance develops naturally, but careless practices in drug supply and use are hastening it unnecessarily."

"The overuse of antibacterial cleaning products in the home may be producing strains of multi-antibiotic-resistant bacteria"

"The use and misuse of antimicrobials in human medicine and animal husbandry over the past 70 years has led to a relentless rise in the number and types of microorganisms resistant to these medicines - leading to death, increased suffering and disability, and higher healthcare costs."

"Deaths from acute respiratory infections, diarrhoeal diseases, measles, AIDS, malaria, and tuberculosis account for more than 85% of the mortality from infection worldwide. Resistance to first-line drugs in most of the pathogens causing these diseases ranges from zero to almost 100%. In some instances resistance to second- and thirdline agents is seriously compromising treatment outcome. Added to this is the significant global burden of resistant, hospital-acquired infections, the emerging problems of antiviral resistance and the increasing problems of drug resistance in the neglected parasitic diseases of poor and marginalized populations."

Mechanisms of action - The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are: Drug inactivation or modification: e.g., enzymatic deactivation of Penicillin G in some penicillin-resistant bacteria through the production of β-lactamases.

Alteration of target site: e.g., alteration of PBP — the binding target site of penicillins — in MRSA and other penicillin-resistant bacteria.

Alteration of metabolic pathway: e.g., some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid.

Reduced drug accumulation: by decreasing drug permeability and/or increasing active efflux (pumping out) of the drugs across the cell surface.

C. Mechanisms of action – The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are:

- Drug inactivation or modification: e.g., enzymatic deactivation of *Penicillin* G in some penicillin-resistant bacteria through the production of β-lactamases.
- Alteration of target site: e.g., alteration of PBP — the binding target site of penicillins — in MRSA and other penicillin-resistant bacteria.
- Alteration of metabolic pathway: e.g., some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid.
- Reduced drug accumulation: by decreasing drug permeability and/or increasing active efflux (pumping out) of the drugs across the cell surface.

D. Type of Multi Drug Resistance –

MULTIDRUG RESISTANCE (M.D.R.) - MDR was defined as acquired non-suseptibility to at least one agent in three or more antimicrobial categories. Multiple drug resistance (MDR), multidrug resistance or multi resistance is antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drugs. The types most threatening to public health are MDR bacteria that resist multiple antibiotics; other types include MDR viruses, fungi, and parasites (resistant to multiple antifungal, antiviral, and antiparasitic drugs of a wide chemical variety). Recognizing different degrees of MDR, the terms extensively drug resistant (XDR) and pandrug-resistant. PDR) have been introduced. Many different definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria are being used in the medical literature to characterize the different patterns of resistance found in healthcare-associated, antimicrobial resistant bacteria. Sometime bacteria find way to fight the antibiotic you are

taking and your infection won't go away. When antibiotic resistance develop, Your doctor must prescribe a different antibiotic in order to fight the infection.

Common multidrug-resistant organisms are usually bacteria:

- Vancomycin-Resistant Enterococci (VRE)
- Methicillin-Resistant *Staphylococcusaureus* (MRSA)
- Extended-spectrum β-lactamase (ESBLs) producing Gram-negative bacteria
- *Klebsiellapneumoniae* carbapenemase (KPC) producing Gram-negatives
- Multidrug-Resistant gram negative rods (MDR GNR) MDRGN bacteria such as *Enterobacter species*, *E.coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*

A group of gram-positive and gram-negative bacteria of particular recent importance have been dubbed as the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and Enterobacter species).

Bacterial resistance to antibiotics Various microorganisms have survived for thousands of years by their ability to adapt to antimicrobial agents. They do so via spontaneous mutation or by DNA transfer. This process enables some bacteria to oppose the action of certain antibiotics, rendering the antibiotics ineffective. These microorganisms employ several mechanisms in attaining multi-drug resistance:

- No longer relying on a glycoprotein cell wall
- Enzymatic deactivation of antibiotics
- Decreased cell wall permeability to antibiotics
- Altered target sites of antibiotic
- Efflux mechanisms to remove antibiotics
- Increased mutation rate as a stress response

Many different bacteria now exhibit multi-drug resistance, including staphylococci, enterococci, gonococci, streptococci, salmonella, as well as numerous other gram-negative bacteria and *Mycobacterium tuberculosis*. Antibiotic resistant bacteria are able to transfer copies of DNA that code for a mechanism of resistance to other bacteria even distantly related to them, which then are also able to pass on the resistance genes and so generations of antibiotics resistant bacteria are produced.This process is called horizontal gene transfer.

EXTENSIVELY DRUG RESISTANCE (X.D.R.)- Bacteria that are classified as XDR are epidemiologically significant due not only to their resistance to multiple antimicrobial agents, but also to their ominous likelihood of being resistant to all, or almost all, approved antimicrobial agents. In the medical literature XDR has been used as an acronym for several different terms such as 'extreme drug resistance', 'extensive drug resistance', 'extremely drug resistant' and 'extensively drug resistant'. Initially, the term XDR was created to describe extensively drug-resistant Mycobacterium tuberculosis (XDR MTB) and was defined as 'resistance to the first-line agents isoniazid and rifampicin, to a fluoroquinolone and to at least one of the three-second-line parenteral drugs (i.e. amikacin, kanamycin or capreomycin)'. Subsequent to this, definitions for strains of non-mycobacterial bacteria that were XDR were constructed according to the principle underlying this definition for XDR MTB (i.e. describing a resistance profile that compromised most standard antimicrobial regimens). Two sets of criteria have mainly been used to characterize bacteria as XDR. The first is based on the number of antimicrobials or classes or subclasses to which a bacterium is resistant, and the second on whether they are 'resistant to one or more key antimicrobial agents'. And XDR was defined as non-susceptibility to at least one agent in all but two or fewerantimicrobial categories.

PANDRUG RESISTANCE (PDR**)** - From the Greek prefix 'pan', meaning 'all', pandrug resistant (PDR) means 'resistant to all antimicrobial agents'. Definitions in the literature for PDR vary even though this term is etymologically exact and means that, in order for a particular species and a bacterial isolate of this species to be characterized as PDR, it must be tested and found to be resistant to all approved and useful agents. Examples of current definitions are: 'resistant to almost all commercially available antimicrobials', 'resistant to all antimicrobials routinely tested 'and 'resistant to all antibiotic classes available for empirical treatment'.

E. POSSIBLE MECHANISM BY WHICH ORGANISM BECOME DRUG RESISTANC

The three fundamental mechanisms of antimicrobial resistance are (1) enzymatic degradation of antibacterial drugs, (2) alteration of bacterial proteins that are antimicrobial targets, and (3) changes in membrane permeability to antibiotics. Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome. The most important mechanism of resistance to the penicillins and cephalosporins is antibiotic hydrolysis mediated by the bacterial enzyme beta-lactamase. The expression of chromosomal beta-lactamase can either be induced or stably depressed by exposure to beta-lactam drugs. Methods to overcome resistance to beta-lactam antibiotics include the development of new antibiotics that are stable to beta-lactamase attack and the coadministration of beta-lactamase inhibitors with beta-lactam drugs. Resistance to methicillin, which is stable to gram-positive beta-lactamase, occurs through the alteration of an antibiotic target protein, penicillin-binding protein 2. Production of antibiotic-modifying enzymes and synthesis of antibiotic-insensitive bacterial targets are the primary resistance mechanisms for the other classes of antibiotics, including trimethoprim, the sulfonamides, the aminoglycosides, chloramphenicol, and the quinolone drugs. Reduced

antibiotic penetration is also a resistance mechanism for several classes of antibiotics, including the beta-lactam drugs, the aminoglycosides, chloramphenicol, and the quinolones.

F. Resistance mechanisms to antibiotics

Resistance to antibiotics may result from innate (intrinsic) or acquired mechanisms. Intrinsic resistance is a trait of a bacterial species. For example, the target of the antimicrobial agent may be absent in that species, the cell envelope (cell membranes and peptidoglycan) may have poor permeability for certain types of molecules or the bacterial species may produce enzymes that destroy the antimicrobial agent. These bacteria are clinically resistant, but should more accurately be referred to as “unsusceptible”, as it is often merely a matter of increasing the concentrations of the antimicrobial agent to levels that may never be reached during therapy, or only at certain sites.

A bacterial strain can acquire resistance either by mutation or by the uptake of exogenous genes by horizontal transfer from other bacterial strains. Genes encoding enzymes that can modify the structure of an antimicrobial are commonly transferable (penicillinases and cephalosporinases (bla-genes), acetyl transferases modifying e.g. aminoglycosides (aac-genes), as are genes leading to target modification (erm-genes), methicillin-resistance (mecA-genes) and glycopeptide-resistance (van-genes). There are several mechanisms for horizontal gene transfer, mainly based on mobile genetic elements, which often function in concert (Dobrindt 2004). Large plasmids with many different genes can be transferred from bacterium to bacterium by conjugation. Transposons can carry several resistance genes. They cannot replicate by themselves, but can move within the genome, e.g. from plasmid to plasmid or from chromosome to plasmid. Integrons can also encode several resistance genes. They cannot move by themselves, but encode mechanisms both to capture new genes and to excise and move cassettes with genes within and from the integron. Integrons are commonly carried on plasmids (EFSA, 2005), but may also be chromosomally-integrated such as in Salmonella Typhimurium DT 104.

G. Antibiotics, targets and activities

The diverse antibioticmolecules used during antibiotherapy of bacterial infections may be classified according to their mechanism of action on bacterial cell. There are 4 major mechanisms: (1)alteration of cell envelope, (2) inhibition of protein synthesis, (3) inhibition with nucleic acid synthesis, and (4) inhibition of a metabolic pathway.

The ß-lactams (penicillins, cephalosporins, carbapenems, etc), polymyxins, CAMPs and glycopeptides (vancomycin and teicoplanin) work by perturbing the bacterialcell wall synthesis or the membrane stability/integrity. ß-lactam molecules block synthesis of the bacterial cell wall by interfering with the enzyme activity involved in the final step of peptidoglycan synthesis. Polymyxins and cationic antimicrobialpeptides exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial contents (ions, ATP etc.). The cyclic lipopeptide daptomycin induces depolarisation of the outer membrane and subsequent cell death by inserting its lipid part into bacterial membrane. Vancomycin and teicoplanin interfere with the final cross-linking steps of pentapeptide units during cell wall synthesis preventing stable cell wall synthesis. Macrolides, aminoglycosides, tetracyclines, chloramphenicol, streptogramins and oxazolidinones inhibit various steps involved in protein synthesis: macrolides, aminoglycosides, and tetracyclines bind to the subunits of the ribosome or to rRNA (e.g. S12 protein, 23S rRNA etc.), whereas chloramphenicol binds to the 50S subunit interfering with the translation process. Fluoroquinolones exert their antibacterial effects by disrupting DNA synthesis and causing lethal double-strand DNA breaks during DNA replication (inhibition of gyrase and topoisomerase activities) whereas sulfonamides and trimethoprim block the pathway for folic acid synthesis, which ultimately inhibits DNA synthesis. The drug combination of TMP, a folic acid analogue, plus sulfamethoxazole (a sulfonamide) inhibits steps in the enzymatic pathway for bacterial folate synthesis.

1. Table : Mechanisms of action of a Bacteria

Action	Alteration of bacterial envelope	Inhibition of protein synthesis	Inhibition of nucleic acid synthesis	Inhibition of metabolic pathway
Antibiotic family	ß-lactam	MLS	Quinolone	Sulfamide
	Glycopeptide	Phenicol,	Rifamycine, Ansamycine	Folic acid
	Polymyxin, daptomycin	Oxazolidinone		Nitro-imidazole
	CAMP	Aminoglycoside		
		Cycline (tetracycline)		

H. Main bacterial mechanisms of antibioticresistance

Bacteria may resist antibiotic action by using several mechanisms. Some bacterialspecies are innately resistant to one class of antibiotics, e.g. bacteria are resistant due to their intrinsic envelope that limits the antibiotic penetration or to the presence of a low level of efflux systems that decrease intracellular antibiotic concentration. In such cases, all strains of that bacterial species are likewise resistant to all the members of those antibacterial classes An ongoing resistant: e.g. initially susceptible bacteria become resistant to antibiotics and bacterial genera. A simple technical definition of the various resistance mechanisms may be proposed for classification: mechanical barrier (altering the required intracellular dose of antibiotic); enzymatic barrier (expression of a detoxifying

enzyme that modify modifies the antibiotic); target protection barrier (mutation or expression of a molecule impairing the antibiotic recognition and activity).

- **Mechanical barrier mechanism -** Bacteria may modify membrane permeability, such as a decrease of porin content or an alteration of the LPS structure, two responses that prevent the antibiotic access to the target at required concentrations (minimal inhibitory concentration).Alternatively or conjointly, bacteria may produce efflux pumps that extrude the antibacterial agent from the cell before it can reach its target site and exert its effect.
- **Enzymatic barrier mechanism -** Bacteria may acquire plasmid genes or over-expressed chromosomal genes encoding enzymes that cleave the antibacterial agent before it can have an effect, such as ß-lactamases, cephalosporinases etc.Bacteria may acquire several genes for other modifications of the antibiotic such as acetyltransferase, phosphotransferase etc.
- **Target protection barrier mechanism-** Bacteria may protect the antibiotic target by acquiring mutations that strongly decrease the affinity of the antibiotic for the target, by producing mimicked targets that lure antibiotics.Bacteria may synthesise a protective molecule masking the target access to antibiotics.Consequently, susceptible bacteria may exhibit an efficient level of resistance to antibiotics via mutation and selection, by expressing special resistance mechanisms (down-regulation of porins, overproduction of efflux pumps etc.) in response to external stimuli, or by acquiring from other bacteria the genetic information that provides resistance mechanism (e.g. gene for enzyme, efflux transporter). The last event may occur by several genetic mechanisms including transformation, conjugation or transduction.

2. Table

Mechanism	**Mechanical barrier**		**Enzymatic barrier**		**Target protection barrier**	
Type of activity	Influx	Efflux, active expel	Cleavage	Alteration	Target mutation	Protective molecule, new molecules
Susceptible antibiotic	ß-lactam, Quinolone etc.	ß-lactam, Aminoside etc.	ß-lactam	Phenicol, aminoside etc.	Quinolone, MLS etc.	ß-lactams, quinolone

Multi-drug resistant bacteria

Many bacteria have become resistant to multiple classes of antibiotics (at least three unrelated antibiotic classes) and deploy multiple strategies to overcome the stress of antibiotic chemotherapy. Resistance is not necessarily limited to a single class of antibiotics. It can apply, simultaneously, to many chemically unrelated compounds to which the cell has never been exposed: this is termed « multi-drug resistance » (MDR).

Today, these MDR bacteria are a cause for serious concern in hospitals and other health care institutions where they are commonly detected. The major mechanism of MDR is the active transport of drugs from the cell to the environment by pumps which expel a broad spectrum of compounds that are noxious to the bacterium (including antibiotics, biocides etc.). In addition, the poly-specificity of efflux transporters confers a general resistance phenotype that can reinforce the effect, and/or drive the acquisition of additional mechanisms of resistance such as mutation of antibiotic targets or synthesis of enzymes that alter the drugs.

There is strong evidence for the role of AcrAB-TolC efflux in Enterobacteriaceae: the expression of this efflux pump is an important prerequisite for the selection of fluoroquinolone resistant mutants that exhibit mutated targets (mutation in gyrase and topoisomerase) in various Gram-negative bacteria such as Salmonella or Campylobacter, two major food-borne pathogens (Piddock 2006). These two mechanisms, conjointly expressed, confer a high resistance level against quinolones. Similar synergies have been recently reported for macrolides in Campylobacter and other examples may be mentioned with ß-lactams, CAMPs, polymyxins, and Enterobactericeae (Davin-Regli et al. 2008, Piddock 2006).

In all of these cases, strains of bacteria carrying resistance factors are selected by the use of antimicrobialmolecules which kill the susceptible strains but allow the newly resistant strains to survive and grow. Acquired resistance due to chromosomal mutation and selection is termed vertical evolution since the advantage will be conferred to a bacterial line. Bacteria also develop resistance through the acquisition of new genetic material from other resistant organisms. This is termed horizontal transfer, and may occur between strains of the same species or between different bacterial species or genera sharing a same ecological niche. Mechanisms of genetic exchange include conjugation, transduction, and transformation. For each of these processes, transposons facilitate the transfer and incorporation of the new resistance genes into the genome of the bacterial host or into plasmids.

III. CLINICAL WASTE/ H0SPITAL WASTE

Disposal is one of the biggest day-to-day challenges faced by healthcare providers. Medical waste is any kind of waste that contains infectious material (or material that potentially infectious). This includes waste generated by healthcare facilities like physician offices, hospitals, dental practices, laboratories, medical research facilities like physician office, and veterinary clinics. Hospitals wastewater is dangerous for Health and environment because it likely contains many kinds of pollutants such as radioactive, chemical and pharmaceutical wastes and pathogenic microorganisms . Medical waste can contain bodily fluid like blood or other contaminants like wholly or partly of human or animal tissue, excretions, drugs, or other products, swabs or dressings, or syringes, needles or other instruments which unless renders safe may prove hazardous to any person who come in contact with it . The 1988 Medical Waste Tracking Act defines is as Waste Generated during Medical Research, Testing, Diagnosis, Immunization or treatment of Human Being

or Animal. Waste water Discharge into the Hospitals surrounding can be an important factor to pathogens proliferation such as *Enterobacteriaceae* family like *Escherichia coli* strains in the environment. Although E.coli bacteria in general are not pathogenic, pathogenic strain of these bacteria have been observed. For example, urinary tract infection *E.coli* can be named pollution of drinking or pool water with some phenotype of E.coli is found in environment, can indicate the presence of other intestinal bacteria because environmentally adapted strains of E.coil can stay a long time in the environmental water. Hospitals are important sites for the generation of hazardous waste according to its location. Disposal of this waste is an environmental concern, as many medical wastes are classified as infectious or biohazard and could potentially lead to the spread of infection disease.

IV. ANTIBIOTICS USE FOR SUCCEBTIBILITY TESTING

NAME OF ANTIBIOTICS	
a. AMIKACIN (AK30)	b. CEFOPERAZONE (CPZ 75)
c. AMPICILLIN (AMP 10)	d. ERYTHROMYCIN (e)
e. AMOXICILLINE	f. NITROFURANTOIN (NIT 300)
g. CO-TRIMOXAZOLE (COT 25)	h. NALIDIX (NX 10)
i. CEFADROXIL (CFR 30)	j. NORFLOXACIN (NX 10)
k. CHLORAMPHENICOL (C 30)	l. NETILLIN (NET 30)
m. CEFTAZIDIME (caz 30)	n. GENTAMICIN (GEN 10)
o. CEFTRAXONE (CTR 30)	p. TOBRAMYCIN (TOB10)
q. CIPROFLOXACIN (CIP5)	r. VANOMYCIN (VA 30)
s. CLOXACILLIN (COX 1)	t. PENICILLIN (P 10)

AGAR MEDIA USE FOR PROCDURE

Actomycetes Isolation Agar, Brain Heart Infusion Agar, Manitol agar, Antibiotic containing agar, Bismuth sulphite agar, MacConkey agar, Yeast extract peptone Dextrose agar.

V. ISOLATION OF ORGANISMS FROM CLINICAL SOLID WASTE

A total of 20 waste solid sampe were collected from the clinical waste disposal area. Sample (soil mixed with waste) was collected in sterile zip lock plastic maintaining aseptic conditions, stored at 4^0c and mark according to there source and location. The collected sample was brought to the Laboratory for isolation of soil bacteria.

Serial dilution technique were used for the isolation of bacteria. In this technique sample suspension was prepared by adding soil mixed with waste (1g) was added to 10 ml of sterile water (the stock) and shaken vigorously for at least 1 minute. The dilute was then sediment for a short period. Sterile dilution blanks were marked sequentially starting from stock and10-1 to 10-4. One ml from the stock was transferred to the 10-1 dilution blank using a fresh sterile pipette. One ml from the 10-1 dilution was transferred to the 10-2 tube for each succeeding step then from the 10-2 to the 10-3, then from the 10-3 to the 10-4. From each dilution tube 0.1 ml of dilution fluid was transferred into Nutrient Agar culture media and incubated at 37 °C for 24 hours. Nutrient Agar (NA) culture media contained 0.5% peptone, 0.3% yeast extract, 0.5% Nacl, 0.25% glucose, 1.5% agar, distilled water and pH was adjusted to 7 at room temperature. After successful growth of microorganisms the pure cultures of bacteria were sub-cultured in NA slants; incubated at 37 °C to achieve vigor. Different agar media also use i.g., ., Actomycetes Isolation Agar, Brain Heart Infusion Agar, Manitol agar, antibiotic containing agar, Bismuth sulphite agar, MacConkey agar, Yeast extract peptone Dextrose agar.

Collection of Bacterial culture - Bacterial culture were grown isolate on different agar media and bacterial slants were prepared. These bacterial slant were studied in the present work. The culture of bacteria were maintained in theirappropriate agar slants at 4^0C throughout te study and used as a stock cultures.

According to appropriate agar media and source of isolates name were given as follow:AFG action -1 , AFG action-3 , AFL action-4 , TP4 mani 1, AFL action 1, AFL Mac 2, TP3 bis-1, Afg mani-2,AFG ypd-1,AFL bhi-1,SFL actino-1,AFG YPD-2,AFL mani-4,AFG bis-1,AFL actino-3,SFL CET-2,AFL BHI-1,TP-3 MAC-1,AFL ACTINO-2,AFL BHI-2,AFG YPD-3,TP-4 MANI-3,AFG MANI-1,TP-3 MANI-1,AFG BHI-1,AFL ACTINO-2,AFL MANI-3,AFL ANTI-1,TP-1 MANI-2,AFL ANTI-3,AFL BHI-3,AFL YPD-2,AFL EMB-2,AFL MAC-1,AFG MANI-3,AFG MANI-4,AFG MANI-6,AFG BIS-2,AFG MANI-5,AFG ACTINO-2,AFG BHI-1,AFG EMB-1,AFL YPD-3.

- **GLYCEROL STOCK PREPARATION**

Bacteria on an LB agar plate can be stored at 4^0c for a weeks. However, if you want to store bacteria for a longer time, you will need to establish glycerol stocks. The adiition of glycerol stabilizes the frozen bacteria, preventing damage to the cell membranes and keeping the cells alive. A glycerol stock of bacteria can be stored stably at -80^0C for many years.

PROTOCOL

1. Followed the steps for inoculating an overnight liquid culture.
2. After bacterial growth were obtained, in 1micro liter sterile LB broth 200microlitter sterile glycerol and 100microlitter grown culture were added.
3. Mixture were mixed welled,labelled and packed.
4. Stored at -20^0c.

- **ANTIBIOTIC SUSCEPTIBILITY TEST**

By the early 1950s, most clinical microbiology laboratories in the united state had adopted the Disc Diffusion Method for determining susceptibility test of a bacteria to antimicrobials. Antibiotic susceptibility pattern of test cultures were detected by Kirby-Bauer disc diffusion method. Following set of 20 Antibiotic was used-

From isolates slants Bacterial inoculums

was prepared by suspending in 5ml sterile nutrient broth and incubated overnight.

From Overnight freshly grown bacterial culture 0.5ml was mixed in 50ml sterile luria burtani agar.

Immediately mixture of Bacterial culture and agar was aseptically poured in Petridish.

HiMedia ECOSA Disk of antibiotic containing twenty antibiotics were placed on Agar surface and plates were incubated at 37^0 C for 24 hours.

After 24-48 hrs of incubation at 37 plates were observed for zones of inhibition and zone diameters were recorded taking zone edge as the point of inhibition. Using zone diameter dada bacterial strains were categorise as sensitive, resistant .The introduction of various antimicrobials for treating variety of infections showed the necessity of performing antimicrobial susceptibility testing as a routine procedure in all microbiology laboratories. In laboratories it can be made available by using antibiotic disk which will diffuse slowly into the medium where the suspected organism is grown. The basic principle of the antibiotic susceptibility testing has been used in microbiology laboratories over 80 years. Various chemical agents such as antiseptics, disinfectants, and antibiotics are employed to combat with the microbial growth. Among these, antibiotics are generally defined as the substances produced by the microorganism such as Penicillium, which has the ability to kill or inhibit the growth of other microorganisms, mainly bacteria. Antimicrobial susceptibility tests (ASTs) basically measures the ability of an antibiotic or other antimicrobial agent to inhibit the invitro microbial growth.

There are many different procedures that microbiologists use to study the effects of various antimicrobial agents in treating an infection caused by different microorganisms. Mueller Hinton Agar is considered as best for the routine susceptibility testing since it is has batch-to-batch reproducibility, low concentration of inhibitors of sulphonamide, trimethoprim and tetracyclines and produce satisfactory results for most of the non-fastidious pathogens. Fastidious organisms which require specific growth supplements need different media to grow for studying the susceptibility patterns.

Factors Affecting antibiotic susceptibility testing

Many conditions can affect the accuracy of the AST results, which is described in detail below.

s (ICOSA DISC) **pH** - pH of the medium is an important factor which influences the accuracy of the antibiotic susceptibility testing. If the pH of the medium is too low than the desired pH, certain drugs such as amino glycosides, quinolones and macrolides lose their potency, on the other hand, antibiotic classes such as tetracyclines appear to have excess activity a lower Ph and the vice versa happens in the case of the higher pH.

Moisture - The presence of moisture content on the medium can counter act with accuracy of the susceptibility testing. It is important to remove the excess moisture present in the agar surface, by keeping it in the laminar flow hood for few minutes.

Effects of medium components - If the media selected for the antibiotic susceptibility contains excessive amounts of thymine or thymidine compounds, they will reversibly inhibit the action of certain antimicrobial agents such as trimethoprim groups. This reversible inhibition yields smaller or less distinct or even no zones and will be misinterpreted as resistant antibiotics. MHA is low in thymine and thymidine content and it can be used successfully to study the susceptibility of antibiotics. Also the medium containing excessive cation reduces the zone size, while low cation content results in unacceptably large inhibition zones.

Amount of organism - The amount of the organism used for the susceptibility testing is standardized using a turbidity standard. This is obtained by a visual approximation using McFarland standard of 0.5 or else it can be determined by using a spectrophotometer with Optical density of 1 at 600 nm wavelength

Materials Required

1. Different Agar slants containing microbial culture (For example, Unkown Isolate culture)
2. Inoculation loop (wire loop)
3. Bunsen burner
4. MicroPippete, sterile tips
5. Nutrient broth
6. Antibiotic disc

VI. Procedure

Select a pure culture plate of one of the organisms to be tested.

1. Aseptically emulsify a colony from the slant in the sterile 5ml Nutrient Broth solution. Mix it thoroughly to ensure that no solid material from the colony is visible in the broth and incubated for overnight at 37^0c in incubator.
2. After 24hours freshly grown culture were taken.
3. Nutrient agar were sterilized by autoclaving at 121^0c for 15min.
4. In a50ml sterilized 1.5% nutrient agar (NA) prepared in 100ml conical flask (Media must be prepared using distilled water), 0.5ml freshly grown culture were added with the help of sterile micropipette
5. The NA containing organism were mixed well and poured the mixture in a sterile petriplate
6. Then allowed the nutrient agar plate to solidify for 5min.
7. After the solidification is completed, HiMidia ICOSA Disc Antibiotic discs can be placed on the surface of the agar using sterilized forceps.
8. Gently press the discs onto the surface of the agar using flame sterilized forceps or inoculation loop.
9. Carefully invert the inoculated plates and incubate for 24 hours at 37° C.
10. After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used.
11. Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone.
12. Compare the measurement obtained from the individual antibiotics to the standard table to determine whether the tested bacterial species is sensitive or resistant to the tested antibiotic.

VII. RESULT

Susceptibility of Isolates Antibiotic susceptibility pattern for different beta-lactams antibiotic was performed on isolates obtained from clinical waste collected during August 2017 to September 2017 and Susceptibility was tested for different antibiotic which include - Amikacin , Ampcillin, Amoxicilline , Co-Trimoxazole , Chloramphenicol, Ceftazidime, Ceftriaxone, Cefadroxil, Ciprofloxacin , Cloxacillin , Cefoperazone , Erythromycin , Netillin, nitrofurantoin, Nalidixic acid, Norfloxacin, Gentamicin, Tobramycin, vancomycin, Penicillin and partially broad spectrum and third generation Blactam Ceftazidime, Cefotaxime, and fourth generation Cefepine. Majority of the isolates were obtained from hospital waste

solid sample. Result shown in figure 1 depict antibiotic susceptibility profile for all 44 isolates obtained from clinical/hospital waste area. It is evident from figure 1 that resistance to Ceftazidime was differential and found to be high ranging from 68.18 to 70% among the clinical isolates. Resistance to broad spectrum antibiotic Norflaxacin, nalidixic acid, Amakacin was comparitively low accounting for 2.2% for this three antibiotic. Out of these isolates 2 isolates e.g., AFG actino-3 and AFL mac-2 were found to exhibit resistance to Ceftazidime. Isolate resistant for Nalidixic acid was TP1 mani-2. Cloxacillin resistant strains was AFG actino-1, AFL actino-4, TP 4 actino-2, TP4 mani-1, AFL mac-2, TP3 bis-1, AFG mani-2, AFG ypd-1, AFL actino-1, AFG ypd-2, AFL Bhi-1, AFL bhi-2, AFG bhi-1, TP1 mani-2, AFG mani6, AFG bhi-1, AFG EMB-1. Cefoperazone resistant strains was AFL actino-4, AFG mani-2 and AFG EMB-1. Nitrofurantonin resistant 9 isolates were found AFL actino-3, AFL actino-2, AFG mani-1, TP1 mani-2, AFL anti3, AFL EMB-2, AFG mani-6, AFG EMB-1, AFL ypd-3. 6.Penicillin resistant 21 isolates were AFG actino-1, AFL actino-4, AFL actino-1, AFL mac-2, TP3 bis-1, AFL actino-1, AFG ypd-2, AFL actino-3, AFL Bhi-1, AFL actino-2, AFG ypd-3, AFG mani-1, AFL anti-3, AFL bhi-3, AFL EMB-2, AFG mani-3, AFG mani-4, AFG mani-5, AFG bhi-1, AFG EMB-1, AFL ypd-3. Chloramphenicol resistant 9 isolates were found AFG actino-1, AFL actino-4, TP4 mani-1, AFL actino-1, AFG mani-2, AFL bhi-1, AFG bis-2, AFG EMB-1. Single Norfloxacin resistant isolate was found e.g., AFL bhi-1. Co-trimoxazole resistant 7 isolates were found AFL mac-2, TP3 mac-1, AFL bhi-2 AFL anti -1, AFL ypd-2, AFG actino-2, AFG bhi-1. 48 Amoxicillin resistant 16 isolates were found AG actino-1, AFL actino-4, TP4 actino-2, TP4 mai-1, AFL actino-1, AFL mac-2, TP3 bis-1, AFG ypd-2, AFL mani-4, AFL actino-3, AFL bhi-2, AFG mani-1, AFL bhi-3, AFG mani-6, AFG EMB-1. Amikacin resistant 1 isolate was found – AFL bhi-1. Ceftazidimine resistant 29 isolates were found – AFG action-1, TP4 actino-4 , AFG actino-1, TP4 mani-1, AFG ypd-1, AFL actino-3, AFL-bhi-1, AFL bhi-2, TP4 mni-3, AFG mani-1, TP3 mani-1, AFG bhi-1, AFL actino-2, AFL mani-3, AFL anti-1, TP1 mani-2, AFL anti-3, AFL bhi-3, AFL ypd-2, AFL mac-1, AFG mani-4, AFG mani-6, AFG mani-5, AFG actino-2, AFG bhi-1, AFG EMB-1. 13. Netillin resistant 6 isolates were found AFL bhi-2, AFG ypd-3, TP4 mani-3, AFL actino-2, AFL mani-2, AFG mani6. Ampicillin resistant 22 isolates were found – AFG actino-1, AFG actino-3, AFL actino-4, TP4 actino-2, TP4 mani1, AFL actino-1, AFL actino-3, AFL bhi-1, TP3 mac-1, AFL actino-2, AFG mani-1, AFG bhi-1, AFL anti-1, AFL anti-3, AFG bis-2, AFL bhi-3, AFG bhi-3, AFG bis-2, AFG EMB-1, AFL ypd-2, AFG mani-5, AFL ypd-3, AFGmani-4, AFG bhi-1. Erythromycin resistant 5isolates were found- AFL actino-4, AFL bhi-1, TP1 mani-2, AFG bis-2, AFG mani-5. MDR bacteria was found in isolates. When the antibiotic resistance pattern of the isolates were studied, following result in percentages was calculated. Among 44 isolates highest rates of antibiotic resistance were obtainedTotal 44 clinical isolates were studied for antibiotic susceptibility and result of the test are as shown in table given above.

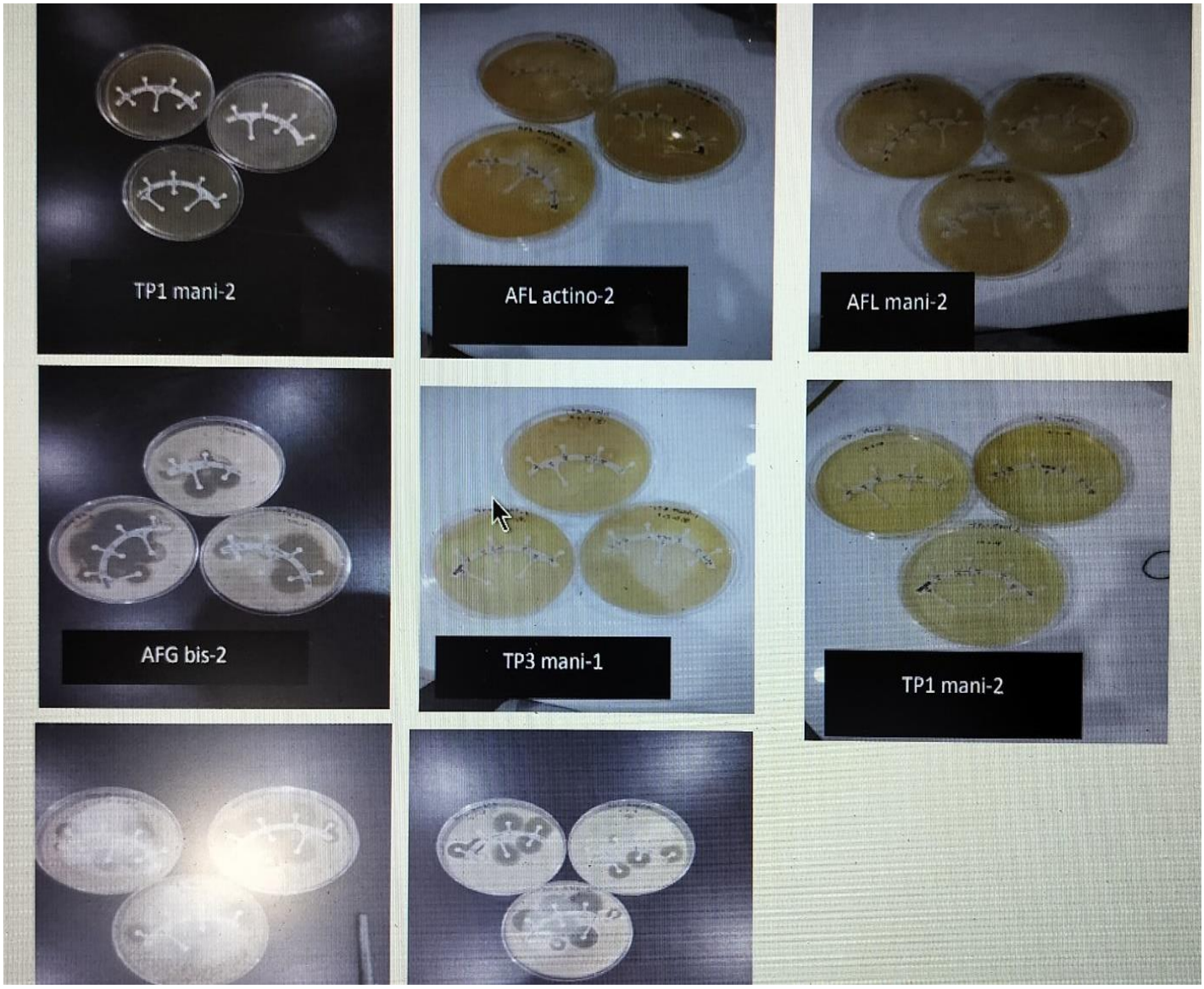
Highest rates of antibiotic resistant were obtained for antibiotic ceftazidimine (68.18%) followed by Ampicillin(52.27%),Penicillin(47.72%),Cloxacillin(38.69%),Amoxicillin(36%),Nitrofuratonin(20.45%),Chloramphenical(20.45%),Co-Trimoxazole(15.90%),Netillin(13.63%),Erythromycin(11.36%),Ceftriaxomee(4.5%),Nalidixic acid(2.2%).Amikacin (2.2%), Norfloxacin (2.2%). Whereas total 44 isolates were observed completely sensitive for 4 antibiotics Vancomycin, Cefadroxil, Gentamicin, Ciprofloxacin & Tobramycin.

VIII. Conclusion

Antibiotic susceptibility pattern of 44 clinical isolates against 20 antibodies were studied by Disc diffusion method. All the isolates were found to be resistant to 16 different antibiotics and sensitive for 4 antibiotics. MDR of commonly used antibiotics is observed to be high in the hospital waste area. The contamination of different environmental factors by antibiotics lead to the rise of resistance. The incidence of antibiotic resistance organism in this clinical waste should not overlooked; since these organism may be vital to the safety and wellbeing of patients who are

hospitalized as well as individuals who are susceptible to infection as are harmful to the society. These organism may leads to variety of diseases. Therefore proper clinical waste treatment plant should be established and improved sanitary measure should be practiced.

IX. PHOTO



X. FUTURE ASPECTS

Bacterial infections are raising serious concern across the globe. The effectiveness of conventional antibiotics is decreasing due to global emergence of multi-drug –resistance bacterial pathogen. This process seems to be primarily caused by an indiscriminate and inappropriate use of antibiotics in non-infected patients and in the food industry. New classes of antibiotics with different actions against MDR pathogens need to be developed urgently. -Concerns over the treat of antibiotic resistance have fuelled and regulatory efforts to promote development of new treatments for serious infections .fears arises that the world could face serious, multidrug resistant bacterial diseases that lack effective treatments. An optimal approach would be to develop drugs that improve on current effective therapies for today's

patients. -Drug resistance in a cancer cell is a serious complication that is always continuously evolving. Rather than just one or two factors, drug resistance is a combination of a handful of elusive mechanism. Many of these mechanisms and factors have been studied in the past, however new methods of analysis and treatment are being developed and tested rigorously. Along with the new progress and breakthrough, the pharmaceutical industry must also recognize the increasing expensive cost factor and its burden on cancer patients of the future. Ultimately, new treatment methods accompanied by cost-efficient analysis will provide patients with the best cancer treatment possible

XI REFERENCES

1. Sharpe M: High on pollution: drugs as environmental contaminants. J Environ Monit 2003, 5:43–46

2. IversenA, KühnI, FranklinA, MöllbyR: High prevalence of vancomycinresistant Enterococci in Swedish wastewater. Apply Environ Microbial 2002, 68:2838–2842.

3. Yang CM, Lin MF, Liao PC, Yeh HW, Chang BV, Tang TK, Cheng C, Sung CH, Liou ML: Comparison of antimicrobial resistance patterns between clinical and wastewater strains in a regional hospital in Taiwan. Lett Appl Microbial 2009, 48:560–565.

4. Colomer-Lluch M, Imamovic L, Jofre J, Muniesa M: Bacteriophages carrying antibiotic resistance genes in fecal waste from cattle, pigs, and poultry. Antimicrob Agents Chemother 2011,

5. Pathak SP, Gaur A, Bhattacherjee JW: Distribution and antibiotic resistance among aerobic heterotrophic bacteria from rivers in relation to pollution. J Environ Sci Health A 1993, 28:73–87.

6. Kruse H: Indirect transfer of antibiotic resistance genes to man. Acta Vet Scand 1999, 92:59–65.

7. Wegener H, Aarestrup F, Gerner-Smidt P, Bager F: Transfer of resistant bacteria from animals to man. Acta Vet Scand 1999, 92:51–58. 8. Kümmerer K, Henninger A: Promoting resistance by the emission of antibiotics from hospitals and household into effluent. Clinical Microb Infection 2003, 12:1203.

8. Kümmerer K, Henninger A: Promoting resistance by the emission of antibiotics from hospitals and household into effluent. Clinical Microb Infection 2003, 12:1203.

9. Grabow W, Prozesky O: Drug resistance of coliform bacteria in hospital and city sewage. Antimicrob Agents Chemother 1973, 3:175–180.

10. Linton KB, Richmond MH, Bevan R, Gillespie WA: Antibiotic resistance and R factors in coliform bacilli isolated from hospital and domestic sewage. J Med Microbial 1974, 7:91–103.

11. Al-Ahmad A, Daschner FD, Kümmerer K: Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of wastewater bacteria. Arch Environ Contam Toxicol 1999, 37:158–163.

12. Gelaw A: Isolation of Bacterial Pathogens from Patients with Postoperative Surgical Site Infections and Possible Sources of Infections at University of Gondar Hospital, Northwest Ethiopia. (MSc. Thesis). Ethiopia: Addis Ababa University; May 2011