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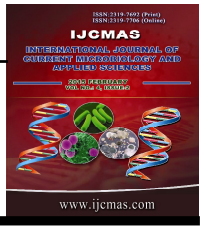

Original Research Article

Biofilm Quantification and Comparative Analysis of MIC (Minimum Inhibitory Concentration) & MBIC (Minimum Biofilm Inhibitory Concentration) Value for Different Antibiotics against *E. coli*

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Keywords

Chryseobacterium indologenes,
UTI,
Resistant

A B S T R A C T

Biofilm is an aggregate of microorganisms in which cells are adhere to each other to a surface. The adherent cells are embedded within a self-produced matrix of extracellular polymeric substance. Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface initially through weak, reversible Van der waals forces, after this cell use adhesion molecules such as pili for permanently binding. The work demonstrates that polymer surfaces are not only differentially susceptible to microbial colonization but also they show differential response against the antibiotics. The study also clearly demonstrates that the Minimum Biofilm Inhibitory Concentration (MBIC) values are independent of pattern of biofilm development on polymeric surfaces. Material properties are directly related to microbial adhesion and colonization is followed by biofilm formation process. The comparative analysis is done on the basis of different antibiotics against *E. coli* using different catheters and obtained the results for the material used for the catheters making and antibiotic action. Earlier researchers have showed that when the microorganism reached the proximity of a surface, attachment is determined by physical and chemical interaction which may be attractive and repulsive, depending upon the complex interplay of the chemistries of the microorganism and substratum surfaces and the aqueous phase. It has also been reported that surface materials with different porosity, groove and braid are with higher infection rate than flat ones, probably due to increase surface area. Microbes preferentially adhere to irregularities that confirm to their size since this maximizes microbial surface area. Grooves or scratches that are an order of microbial size increase the contact area and hence the binding potential and thus the extent of biofilm formation *in vitro* studies to be perform by laboratories will determine that microbial adherence to biomaterial occur in the following order: latex >silicon

Introduction

Biofilm is an aggregate of microorganisms in which cells are adhere to each other to a surface (living/nonliving). The adherent cells are embedded within a self-produced matrix of extracellular polymeric substance.

Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface initially through weak, reversible Van der waals forces, after this cell use adhesion molecules such as pili for

permanently binding. Biofilms have been found in variety of microbial infections in the body like urinary tract catheter, dental plaque, endocarditis, infections in cystic fibrosis and infections prostheses and heart valves. There are four stages of biofilm development:

1. Initial attachment
2. Irreversible attachment
3. Maturation and proliferation
4. Detachment

The predominant form of life for the majority of microorganisms in any hydrated biologic system is a cooperative community termed a "biofilm". A biofilm on an indwelling urinary catheter consists of adherent microorganisms, their extracellular products, and host components deposited on the catheter.

The longer the urinary catheter remains in place, the greater the tendency of these organisms to develop biofilms and result in urinary tract infections. Urinary catheters are silicone devices, which readily acquire biofilms on the inner or outer surfaces. The organisms commonly contaminating these devices and developing biofilms are *S. epidermidis, Enterococcus faecalis, E. coli*, Proteus mirabilis, *P. aeruginosa*, *K. pneumoniae*, and other gram-negative organisms.

The biofilm mode of life conveys a survival advantage to the microorganisms associated with it and, thus, biofilm on urinary catheters results in persistent infections that are resistant to antimicrobial therapy.

Externally Implanted Devices (EID) are commonly used in care of patient in routine hospital practices. The infections related to these devices are matter of serious concern which leads to patient discomfort and serious consequences in terms of blood streams infections.

The increasing use of EID has lead to a concomitant increase in incidences of biofilm associated infections, with the most common colonizer *E. coli*. *E. coli* addition and colonization depends on many factors including both biotic and abiotic. Material surface properties and chemistry are among the important abiotic factor affecting microbial colonization.

Market available devices are more prone to infections and quality of EID is generally compromised by manufacturers. To evaluate quality of EID available in the market and their susceptibility to infections, *E. coli* biofilms was developed over the above mention device surfaces and MIC, MBIC to plank tonic sessile cell was measured for Oxytetracycline.

Escherichia coli strains are the most frequent cause of urinary tract infections. Biofilm formation allows the strains to persist a long time in the genitourinary tract and interfere with bacterial eradication.

We determined the possible relationships between the different urinary tract infections and in vitro biofilm formation.

Biofilm

A biofilm is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS, which is also referred to as slime (although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on

living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. The microbial cells growing in a biofilm are physiologically distinct from plank tonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium.

Microbes form a biofilm in response to many factors, which may include cellular recognition of specific or non-specific attachment sites on a surface, nutritional cues, or in some cases, by exposure of plank tonic cells to sub-inhibitory concentrations of antibiotics. When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated.

Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible adhesion via Van der Waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili. Hydrophobicity also plays an important role in determining the ability of bacteria to form biofilms, as those with increased hydrophobicity have reduced repulsion between the extracellular matrix and the bacterium. Some species are not able to attach to a surface on their own but are sometimes able to anchor themselves to the matrix or directly to earlier colonists. It is during this colonization that the cells are able to communicate via quorum sensing using products such as AHL.

Some bacteria are unable to form biofilms as successfully due to their limited motility. Non motile bacteria cannot recognize the surface or aggregate together as easily as motile bacteria. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. Polysaccharide matrices typically enclose bacterial biofilms. In addition to the polysaccharides, these matrices may also contain material from the surrounding environment, including but not limited to minerals, soil particles, and blood components, such as erythrocytes and fibrin. The final stage of biofilm formation is known as dispersion, and is the stage in which the biofilm is established and may only change in shape and size. The development of a biofilm may allow for an aggregate cell colony (or colonies) to be increasingly antibiotic resistant.

Stages of biofilm formation

There are five stages of biofilms formation:

1. Initial attachment of microorganisms
2. Irreversible attachment
3. Maturation I
4. Maturation II
5. Dispersion

Initial attachment of microorganisms

The primary adhesion stage constitutes the beneficial contact between a conditioned surface and plank tonic microorganisms. During the process of attachment, the organism must be brought into close proximity of the surface, propelled either randomly or in a directed fashion via chemotaxis or mortality (Prakash *et al.,* 2003). Once microorganism reaches critical proximity to the surface the final determination of the adhesion depends on the net sum of the attractive and repulsive forces generated between the two surfaces. These forces include eletrostatic and hydrophobic interactions (Melo *et al.,* 1997; Kumar and Prasad, 2006) and Van der

wall's attraction (Denyer *et al.,* 1993). The attachment is unstable and reversible and if the environment is unfavorable for microbial attachment, cells can detach from the surface (Ghannoum and O'Tolle, 2004).The solid and liquid interface between a surface and aqueous medium provides the ideal environment for the attachment and the growth of the micro organism (Spiers *et al.,* 2003). Attachment will mostly occur in a rougher, more hydrophobic and coated by conditioning films (Liu *et al.,* 2004).

The primary stage is reversible and is characterized by a no. of physiochemical variables that define the interaction between the bacterial cell surface and conditioned surface of interest (Liu *et al.,* 2004). When a biofilm is composed of heterogeneous species, the metabolic byproduct of the organisms might support the growth of another, while adhesion of one species might provides the ligand which allow the attachment of others. Conversely the depletion of nutrients and accumulation of toxin byproduct generated by primary colonizers may limit the species diversity within a biofilm.

Irreversible attachment

The secondary stage involves the anchoring of bacteria to the surface by molecular mediated binding between the specific adhesions and the surface (Kumar and Prasad, 2006) in this process the loosely bounded organisms gather together and produce exo-polysaccharides that complex with the surface materials (An *et al.,* 2000). Once the bacteria attaches irreversibly to the surface, they undergo a genotypic and phenotypic changes to ensure the development and the maturation of the biofilm. All bacteria produced the multiple adhesion some of which are regulated by at the transcriptional level depending on the genes encoded, permitting organisms to switch from sessile to plank tonic forms under different environmental influences the changes described above result in the production of increased amount of EPS, increased resistance to antibiotics, increased UV resistance, gene exchange events that occur more frequently and higher amount of secondary metabolites that are produced.

Various structures such are flagella, fimbrie and outer membrane protein, curli and extra cellular polymers structures are involved in biofilm formation (Watnick *et al.,* 1999). They have distinct role in different species and under different environmental (Giaouris and Nychas, 2006) condition. Flagella motility is importance to overcome the forces that repel the bacteria from reaching the abiotic material. Once it reaches the surface, appendages such as pili, OMPS and curli are required to achieve stable cell to cell and cell to surface attachment. Flagella play an important role in the early stages of bacterial attachment by overcoming the repulsive forces associated with the substratum (Giaouris and Nychas, 2006).

Micro colony formation

After the adherence of the bacteria to the inert surface, the association becomes stable for micro colony formation (Palmer *et al.,* 1997; O'Toole *et al.,* 2000). The bacteria begin to multiply while sending out chemical signals that intercommunicate among the bacterial cell.

Once the signal intensity exceeds certain threshold level, the genetic mechanisms underlying the exopolysaccharides production are activated in this way the bacteria multiply within the embedded exopolysaccharides matrix, thus giving rise to the formation of micro colony formation (Prakash *et al.,* 2003).

Micro colonies further develop into macro colonies which are divided into fluid-filled channel and enclosed in an extra polysaccharides matrix (Allison, 2000). Macrocolonies, compared to microcolonies, are composed of more bacterial cell and are enclosed in extracellular matrix and have higher metabolic and physiological heterogeneity (Ghannoum and O'Toole, 2004).

Maturation I

Du+ the transcription of specific genes takes place. These are required for the synthesis of EPS (Prakash *et al.,* 2003) attachment itself initiate the synthesis of the extracellular matrix in which the sessile bacteria are embedded followed by water filled channels in the circulatory system that helps in delivering the nutrients and removing the waste product from the cell communities in the micro colonies (Prakash *et al.,* 2003)**.**

Maturation II

Once bacteria have irreversibly attached to a surface, the process of biofilm maturation begins. The overall density and complexity of the biofilm increases as surface-bound organisms begin to actively replicate and extra cellular components generated by attached bacteria interact with organic and inorganic molecules in the immediate environment to create the glycocalyx (Carpentier and Cerf, 1993).The availability of nutrients in the immediate environment within the biofilm and the removal of waste, limits the growth potential of any bacterial biofilm (O'Toole *et al.,* 2000).

In addition, there is an existence of an optimum hydrodynamic flow across the biofilm that determines the maximum growth (Carpentier and Cerf, 1993). Other factors that control biofilm maturation include the internal pH, oxygen, carbon source, osmolarity, temperature, electrolyte concentration and the flux of materials and surface types. The surface types can be either:

- High surface energy materials that are negatively charged ; hydrophilic materials such as glass, metals or minerals

- Low surface energy materials that are either low positively or low negatively charged; hydrophobic materials such as plastic made up of organic polymers (O'Toole *et al.,* 1998).

At some point, the biofilm reaches a critical mass and a dynamic equilibrium is reached at which the outermost layers of growth begin to generate plank tonic organisms. These organisms are free to escape the biofilm and colonies other surfaces. Cells nearest the surface become inactive or die due to a lack of nutrients, decrease in pH, pO2 or an accumulation of toxic metabolic byproducts (Dunne, 2002)

Dispersion

As the biofilm gets older, cells detach and disperse and colonize a new niche. This detachment can be due to various factors including, fluid dynamics and shear effects of the bulk fluid (Brugnoni *et al.,* 2007). Some bacteria are shed from the colony and some stop producing EPS and are released into the surrounding environment (Herrera *et al.,* 2007). Biofilm cells may be dispersed either by shedding of daughter cells from actively growing cells or detachment as a result of nutrient levels (Spiers *et al.,* 2003).

The released microorganisms may be transported to new locations and restart the

biofilm process (Prakash *et al.*, 2003).As the thickness of the EPS increases, anaerobic conditions develop within the biofilm (Spiers *et al.*, 2003).

Because of film thickness and the activity of anaerobic species, the film detaches and sloughs off from the surface of substrate. Polysaccharide enzymes specific for EPS degradation from different organisms may be produced during different phases of biofilm growth and contribute to detachment. It has been suggested that the escape of *P. aeruginosa* cells from the biofilm matrix involved the action of an enzyme that digests the alginate (Prakash *et al.*, 2003).

There are primarily three types of catheters

- Indwelling or Foley catheters
- Intermittent catheters
- External catheters

Indwelling or Foley catheters are flexible tubes placed into the bladder to drain urine. Patients who require long-term catheterization use them. These are inserted into the bladder and might remain inside for a relatively longer period of time. One use could be after a Prostate Procedure; the catheter helps keep the urethra open during healing. The catheter is anchored in place by a soft, water filled balloon at the tip (Fig. 3)

Intermittent catheters are the most commonly used catheters. These catheters are best suited for those patients who need to empty their bladder once in a while.

External catheters are male-specific and are worn on the penis like a condom. External catheters are safer than intermittent or Foley catheters because they are not placed inside the body. One reason for using a male external catheter is to assist in control of incontinence or for patients that have restricted mobility.

Intravenous catheter

It is a catheter that is inserted into a vein for supplying medications or nutrients directly into the bloodstream or for diagnostic purposes such as studying blood pressure (Fig. 1 and 2).

In medicine, Intravenous catheter is a catheter (small, flexible tube) placed into a peripheral vein in order to administer medication or fluids. Upon insertion, the line can be used to draw blood.

The catheter is introduced into the vein by a needle (similar to blood drawing), which is subsequently removed while the small tube of the cannula remains in place. The catheter is then fixed by taping it to the patient's skin (unless there is allergy to adhesives). Newer catheters have been equipped with additional safety features to avoid needle stick injuries. Modern catheters consist of synthetic polymers such as teflon (hence the often used term 'Venflon' or 'Cathlon' for these venous catheters).

Intravenous catheter is the most commonly used vascular access in medicine. It is given to most emergency room and surgical patients, and before some radiological imaging techniques using radiocontrast, for example. In the United States, more than 25 million patients get a peripheral venous line each year.

An Intravenous catheter is usually placed in a vein on the hand or arm. It should be distinguished from a central venous catheter which is inserted in a central vein (usually in the internal jugular vein of the neck or the subclavian vein of the chest), or

an arterial catheter which can be placed in a peripheral as well as a central artery. In children, a local anaesthetic gel (such as lidocaine) is applied to the insertion site to facilitate placement.

Urinary catheter

In **urinary catheterization** ("**cathing**" for short), a latex, polyurethane, or silicone tube known as a urinary catheter is inserted into a patient's bladdervia the urethra. Catheterization allows the patient's urine to drain freely from the bladder for collection. It may be used to inject liquids used for treatment or diagnosis of bladder conditions. A clinician, often a nurse, usually performs the procedure, but self-catheterization is also possible. The catheter may be a permanent one (indwelling catheter), or an intermittent catheter removed after each catheterization.

A **Foley catheter** (indwelling urinary catheter) is retained by means of a balloon at the tip that is inflated with sterile water. The balloons typically come in two different sizes: 5 cm^3 and 30 cm^3. They are commonly made in silicone rubber or natural rubber.

Endotracheal catheter: A catheter that is inserted into the trachea through the mouth or nose in order to maintain an open air passage or to deliver oxygen or to permit the suctioning of mucus or to prevent aspiration of the stomach contents (Fig. 4)

A **tracheal tube** is a catheter that is inserted into the trachea in order for the primary purpose of establishing and maintaining a patent airway and to ensure the adequate exchange of oxygen and carbon dioxide. Many different types of tracheal tubes are available, suited for different specific applications. An *endotracheal tube* is a specific type of tracheal tube that is nearly always inserted through the mouth (orotracheal) or nose (nasotracheal).

A *tracheostomy tube* is another type of tracheal tube; this 2–3 inch-long (51–76 mm) curved metal or plastic tube may be inserted into a tracheostomy stoma to maintain a patent lumen. A *tracheal button* is a rigid plastic cannula about 1 inch in length that can be placed into the tracheostomy after removal of a tracheostomy tube to maintain patency of the lumen.

Most endotracheal tubes today are constructed of polyvinyl chloride, but specialty tubes constructed of silicone rubber, latex rubber, or stainless are also widely available. Most tubes have an inflatable cuff to seal the trachea and bronchial tree against air leakage and aspiration of gastric contents, blood, secretions, and other fluids. Uncuffed tubes are also available, though their use is limited mostly to pediatric patients (in small children, the cricoid cartilage, the narrowest portion of the pediatric airway, often provides an adequate seal for mechanical ventilation).

Types of endotracheal tube include oral or nasal, cuffed or uncuffed, preformed (e.g. RAE (Ring, Adair, and Elwyn) tube), reinforced tubes, and double-lumen endobronchial tubes. For human use, tubes range in size from 2 to 10.5 mm in internal diameter (ID).

Types of catheter and its composition

Catheters	Composition
Endotrachial	PVC
Intravenous	Latex
Urinary tract	Silicon

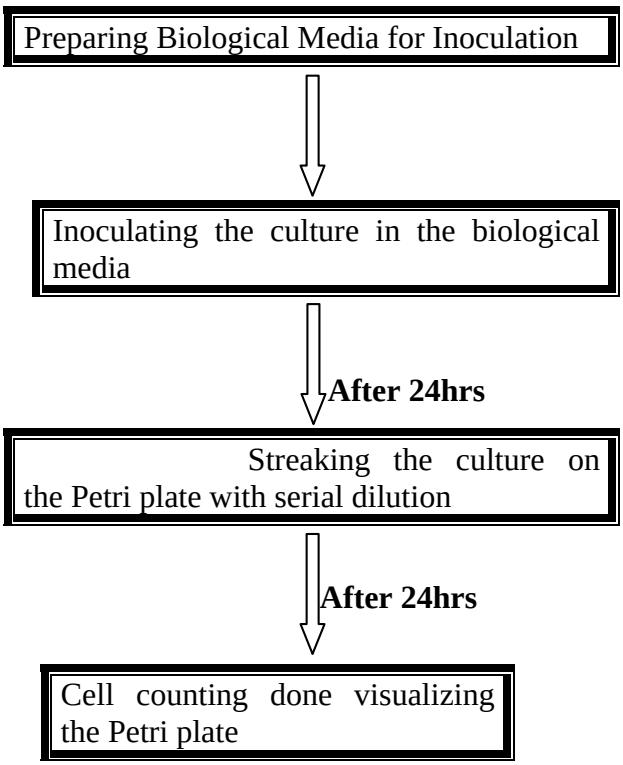
Materials and Method

Description

To measure the antimicrobial resistance of bacteria found on medical implants, bacteria is tested against an anti-microbial agent at a variety of concentration. The output from testing the effectiveness of sample is a minimum inhibitory concentration (MIC) value of anti-microbial agent is determined by broth dilution method which tests the sensitivity of bacteria in the plank tonic phase. MIC values are important in laboratories diagnostic and monitoring the activity of new anti-microbial agents. The minimum biofilm inhibitory concentration (MBIC) value determines the sensitivity of bacteria in their biofilm phase (sessile phase) of development.

Biofilm quantification was done using crystal violet assay for biofilms. The cells which are attached to the surfaces were stained purple with CV whereas abiotic surfaces are not stained. In the assay the CV gives a reliable method for biofilm quantification without any disruption during process of quantification. Antibiotics and clinical materials: Standard powders of antimicrobial agents (amoxicillin, oxytetracycline, and tetracycline) will be used (Fig. 7). These antibiotics are common for the treatment of bacterial infections. The antibiotic powder will be weighed and a stock solution of 5 mg/ml prepared in distilled water. Different externally implanted devices used were Urethral catheter & Rubber catheter. The drugs used against the biofilm growth will be amoxicillin, oxytetracycline and tetracycline. They are water soluble drugs be amoxicillin, oxytetracycline and tetracycline are antibiotics, used for the treatment of many types of bacterial infections, particularly those caused by Gram-negative organisms.

Cell Count



Drugs

Amoxicillin ($C_{16}H_{19}N_3O_5S$)

A broad-spectrum semisynthetic antibiotic similar to ampicillin except that its resistance to gastric acid permits higher serum levels with oral administration (Fig. 10).

Tetracycline ($C_{22}H_{24}N_2O_8$)

Tetracycline is a broad spectrum polyketide antibiotic produced by the Streptomyces genus of *Actinobacteria*. It exerts a bacteriostatic effect on bacteria by binding reversible to the bacterial 30S ribosomal subunit and blocking incoming aminoacyl tRNA from binding to the ribosome acceptor site. It also binds to some extent to the bacterial 50S ribosomal subunit and may alter the cytoplasmic membrane causing

intracellular components to leak from bacterial cells (Fig. 11).

Oxytetracycline ($C_{22}H_{24}N_2O_9$)

It is a tetracycline analog isolated from the actinomycete *Streptomyces rimosus* and used in a wide variety of clinical conditions (Fig. 12).

Drugs	Range	MIC	MBIC$_{50}$	MBIC$_{90}$
Amoxicillin	8-22 µg/ml	12 µg/ml	60 µg/ml	120 µg/ml
Tetracycline	1-16 µg/ml	8 µg/ml	40 µg/ml	80 µg/ml
Oxytetracycline	10-30 µg/ml	15 µg/ml	75 µg/ml	150 µg/ml

The above analysis determines the effectiveness and high efficiency of tetracycline drug. It is the most effective and target oriented drug with minimum range in it. The drug is specific in action and has the maximized eradication of biofilm through its action (Fig. 8 and 9).

Biofilm formation and quantification

Three EID is available for biofilm formation. MIC and MBIC analysis of *E. coli* against two antibiotics are used for quantification. Intravenous catheter is more prone for biofilm formation. Maximum biofilm formation occurs in intravenous catheter after 48 h while in intravenous catheter blood transfusion unit and endotrachial tube catheter after 24 h.

In crystal violet (CV) assay for biofilm the cells which are attached to the surfaces were stained purple with CV whereas antibiotic surfaces are not stained.

In the assay the CV gives a reliable method for biofilm quantification without any disruption during process of quantification. The data was further confirmed by sessile CFU assay and found to be similar as in case with CV assay.

Minimum biofilm inhibitory concentration (MBIC) value

MBIC$_{50}$ and MBIC$_{90}$ were calculated for 3 drugs (Amoxicillin, Tetracycline, and Oxytetracycline) used against biofilm. The value was calculated for the concentration of respective drug needed for 50% and 90% biofilm reduction on different EID that are represented in Table 1 and Fig. 8.

MBIC assay was performed, the MBIC was defined as the lower concentration of antibiotics/antimicrobials which shows 50% and 90% reduction in biofilm formation. Since, biofilms are structural communities which are unable to show any explanatory drug resistance by any single mechanism, complete reduction or inhibition is difficult to achieve.

MBIC 50% for amoxicillin in case of intravenous catheter, urinary tract catheter, endotrachial catheter was found to be 60 µg/ml, 55 µg/ml and 50 µg/ml respectively while MBIC 90% for amoxicillin in case of intravenous catheter, urinary tract catheter and endotrachial catheter was found to be 120 µg/ml, 110 µg/ml and 100 µg/ml respectively.

MBIC 50% for tetracycline in case of intravenous catheter, urinary tract catheter, endotrachial catheter was found to be 40 µg/ml, 35 µg/ml and 30 µg/ml respectively while MBIC 90% for tetracycline in case of intravenous catheter, urinary tract catheter and endotrachial catheter was found to be 80 µg/ml, 70 µg/ml and 60 µg/ml respectively.
MBIC 50% for oxytetracycline in case of intravenous catheter, urinary tract catheter, endotrachial catheter was found to be 75 µg/ml, 70 µg/ml and 600 µg/ml respectively

while MBIC 90% for oxytetracycline in case of intravenous catheter, urinary tract catheter and endotrachial catheter was found to be 150 µg/ml, 140 µg/ml and 120 µg/ml respectively.

Externally Implanted Devices (EID) are commonly used in care of patient in routine hospital practices. The infections related to these devices are matter of serious concern which leads to patient discomfort and serious consequences in terms of blood streams infections. The increasing use of EID has lead to a concomitant increase in incidences of biofilm associated infections, with the most common colonizer *E. coli*.

E. coli addition and colonization depend on many factors including both the biotic and abiotics. Material surface properties and chemistry are among the important abiotic factor affecting microbial colonization.

Biofilm development causes various chronic, intractable infections like biomaterial associated infection, infection of prosthetic device causes serious problem in patient. Implanted device like catheters, heart valves, replacement of joint provide surface of pathogenic microorganisms on which they can form biofilm.

Usually the treatment of this problem is to remove the infected material and replacement with new device. Bacteria in biofilm stage become several fold more resistant to antibiotics (Costerton *et al.*, 1999).

The MIC for the drugs Amoxicillin and Oxytetracycline was calculated by Broth dilution assay method. Oxytetracycline was found to be most effective while for amoxicillin, microbes show about twenty times resistance than Amoxicillin.

The results shows the variation in microbes resistance in response to different drugs which depend on microbial physiology, strain type, exopolysaccharides production (Jee-Hoon Ryu), different environmental factor (Akinobu Ito) and gene expression (S.V.Lynch, L).

This work demonstrates that polymer surfaces are not only differentially susceptible to microbial colonization but also they show differential response against the antibiotics. The study also clearly demonstrates that the MBIC values are independent of pattern of biofilm development polymeric surfaces.

During the MBIC study it was found that in the entire cases Intravenous catheter was showing highest MBIC 50 & 90.

The differential drug response to the biofilm varies with material and generally does not follow any order or have relationship with biofilm development.

This work demonstrates that polymer surfaces are not only differentially susceptible to microbial colonization but also they show differential response against the antibiotics. The study also clearly demonstrates that the MBIC values are independent of pattern of biofilm development on polymeric surfaces.

The differential drug response to the biofilm varies with material and generally does not follow any order or have relationship with biofilm development.

Material properties are directly related to microbial adhesion and colonization is followed by biofilm formation process. The Scanning electron microscope images proved that irregularities of polymeric surface promote biofilm formation because

of increase surface area associated with rough surfaces and depression in the rough surfaces which provide shelter and favorite site of microbial colonization. Our data support the finding of Boyd *et al.*, 2002, who showed increase microbial adherent in case with increase in surface roughness. Earlier researchers have showed that when the microorganism reached the proximity of a surface, attachment is determined by physical and chemical interaction which may be attractive and repulsive, depending upon the complex interplay of the chemistries of the microorganism and substratum surfaces and the aqueous phase. It has also been reported that surface materials with different porosity, groove and braid are with higher infection rate than flat ones, probably due to increase surface area. Microbes preferentially adhere to irregularities that confirm to their size since this maximizes microbial surface area. Grooves or scratches that are an order of microbial size increase the contact area and hence the binding potential and thus the extent of biofilm formation in vitro studies perform by laboratories have determine that microbial adherence to biomaterial occur in the following order: latex > silicon > PVC > Teflon > polyurethane > stainless steel for gram negative *E. coli.*

Nanoparticle encapsulated drugs were found to be more effective against biofilm i.e. 5 to 10 times better than normal drug thus it can be open a new horizon for pharmaceutical industries as well as human health. As for as future possibilities are concerned, there is a wide scope of combining various combination of polymeric nanoparticles to get the better result.

Table.1 Minimum biofilm inhibitory concentration (MBIC) value

DRUGS	MATERIAL	$MBIC_{50}$	$MBIC_{90}$
Amoxicilin	Intravenous Catheter,	60µg/ml	120 µg/ml
	Urinary tract Catheter,	55 µg/ml	110 µg/ml
	Endotract Catheter	50 µg/ml	100 µg/ml
Tetracycline	Intravenous Catheter,	40 µg/ml	80 µg/ml
	Urinary tract Catheter,	35 µg/ml	70 µg/ml
	Endotract Catheter	30 µg/ml	60 µg/ml
Oxytetracycline	Intravenous Catheter,	75 µg/ml	150 µg/ml
	Urinary tract Catheter,	70 µg/ml	140 µg/ml
	Endotract Catheter	60 µg/ml	120 µg/ml

Fig.1 The technique of usage of intravenous catheters

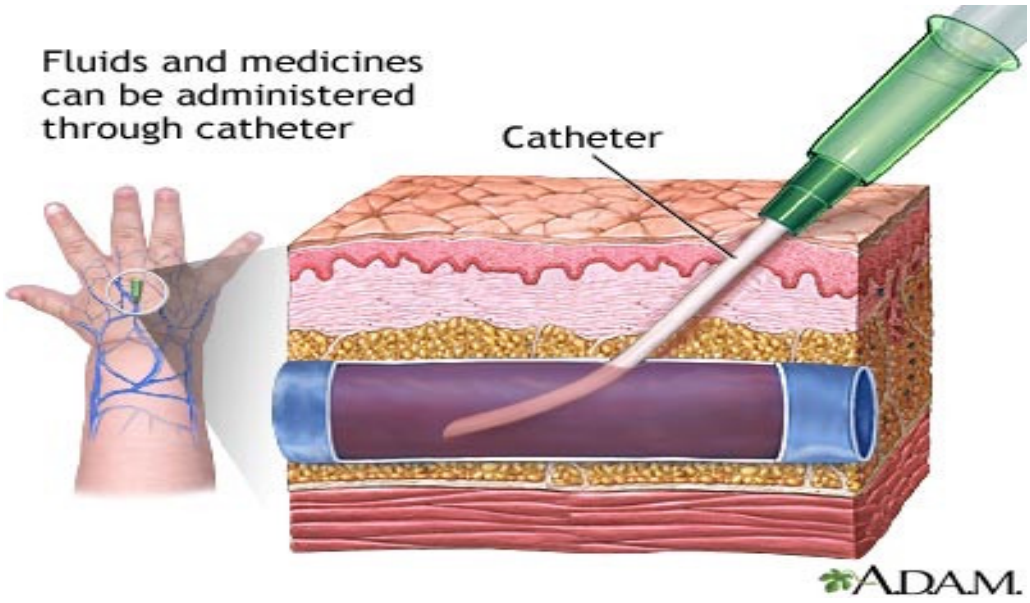


Fig.2 Intravenous catheter

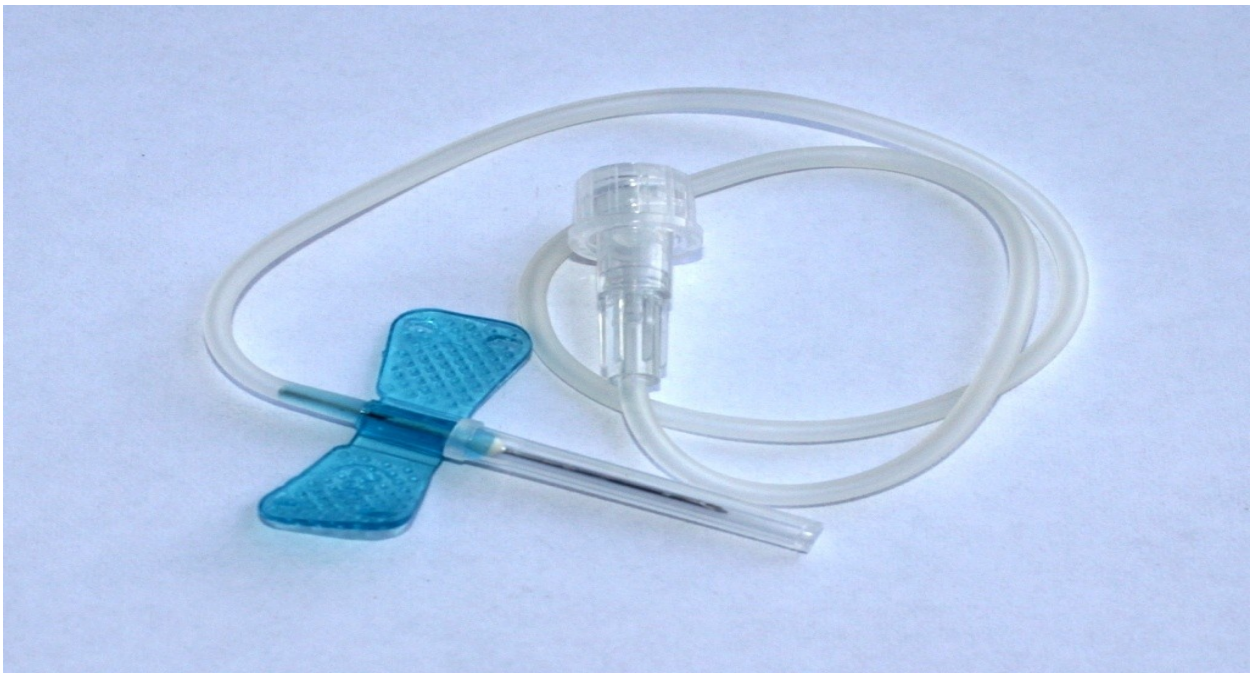


Fig.3 Foley catheter

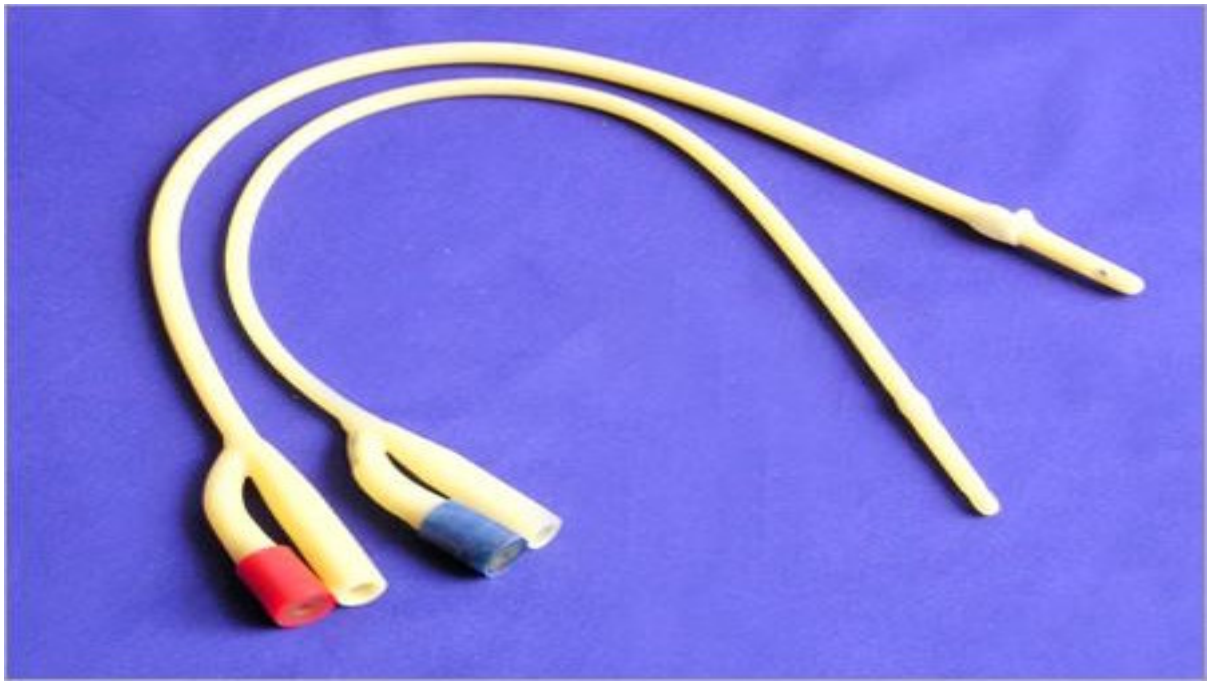


Fig.4 Endotracheal Catheter

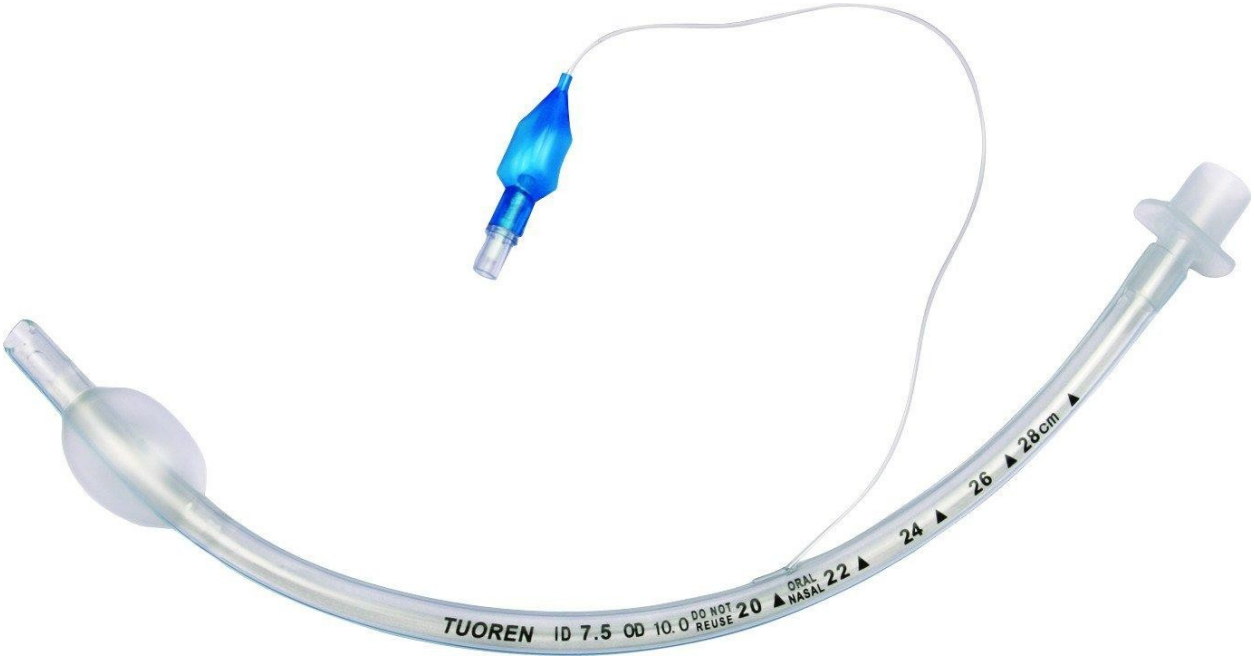


Fig.5 Colony counter

Fig.6 Bacterial colonies

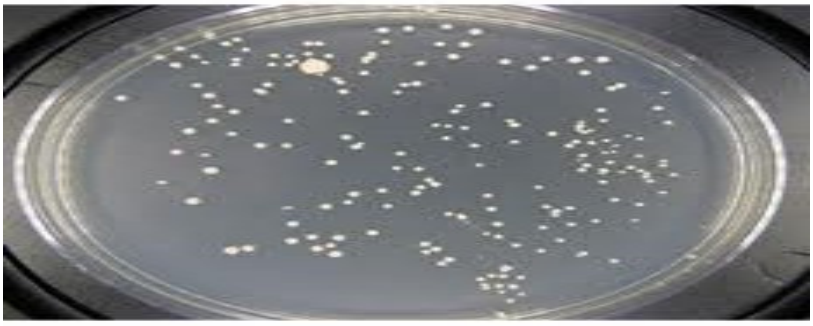


Fig.7 MIC for different antibiotics

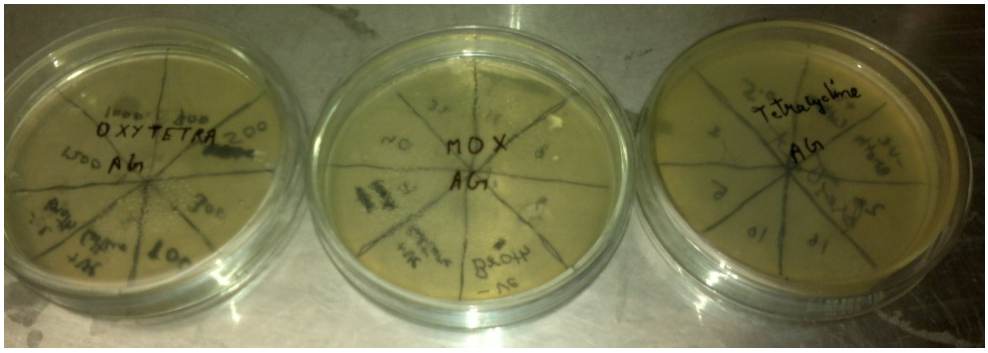


Fig.8 Minimum Inhibitory Concentration

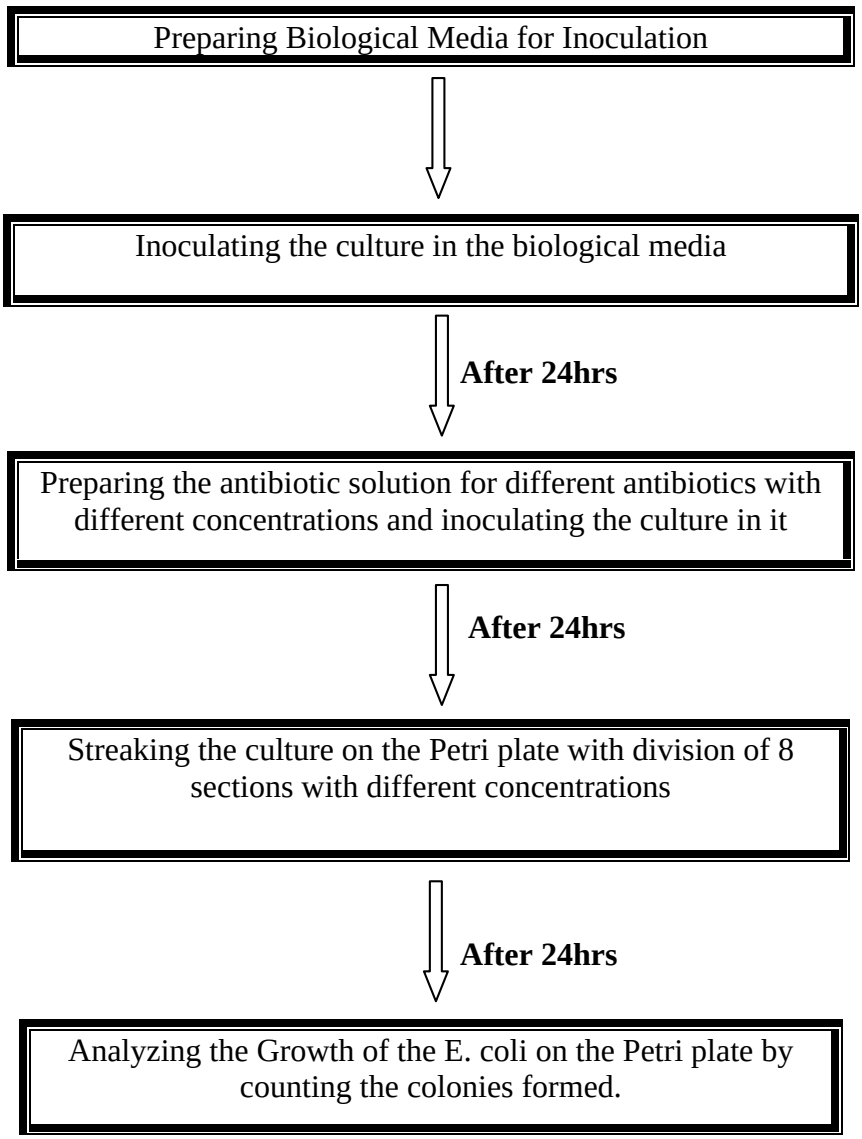


Fig.9 Eppendorfs containing different catheter

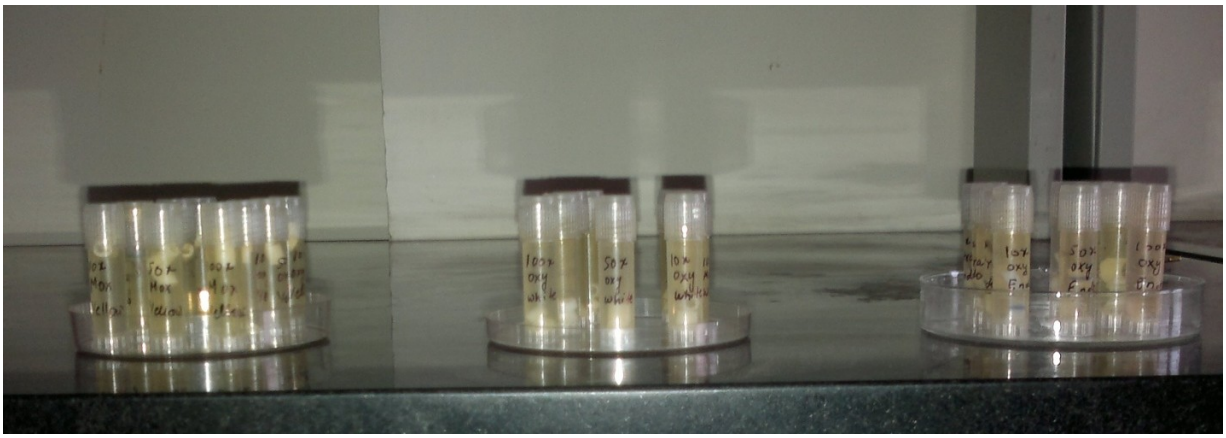


Fig.9 Minimum biofilm inhibitory concentration

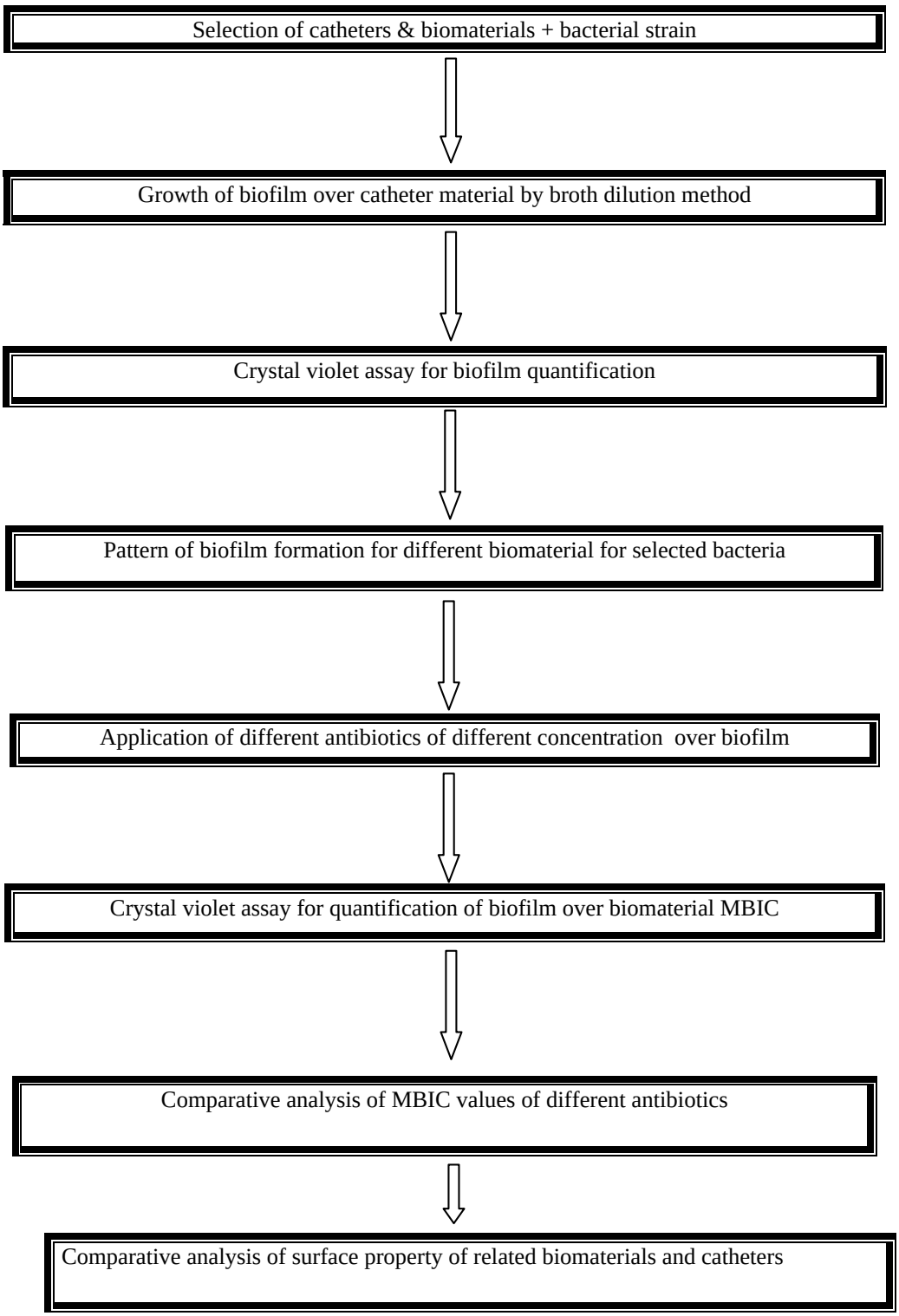


Fig.10 Chemical structure of amoxicillin

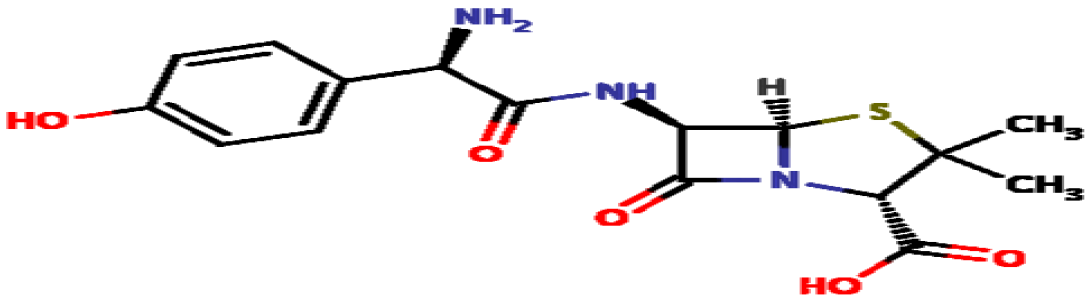


Fig.11 Chemical structures of tetracycline

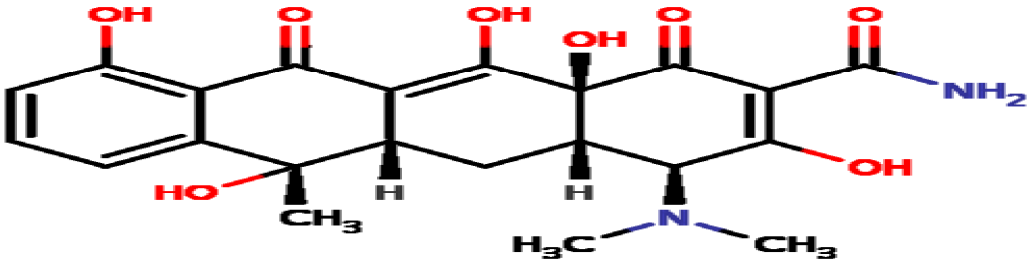
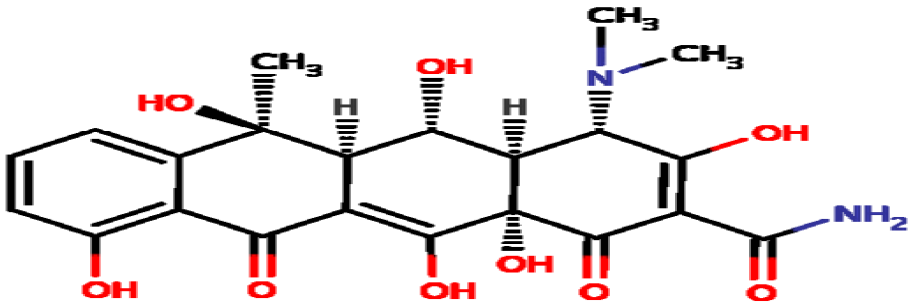


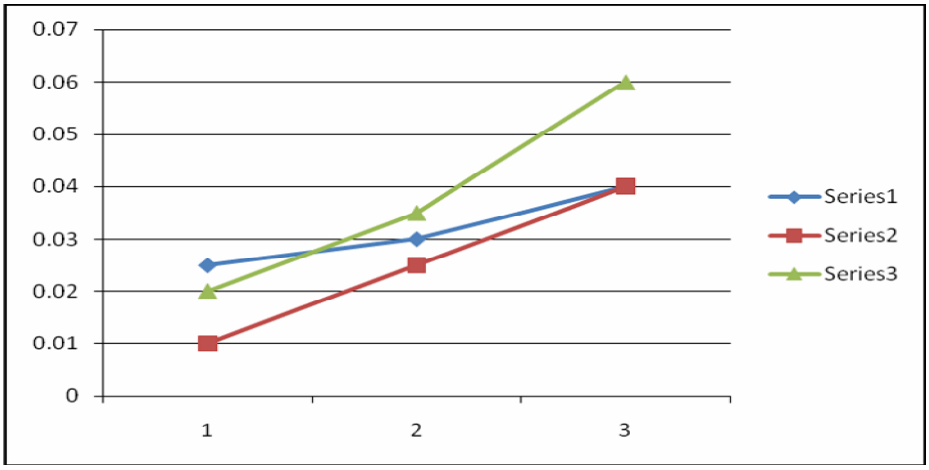
Fig.12 Chemical structures of oxytetracycline



Endotrachial catheter

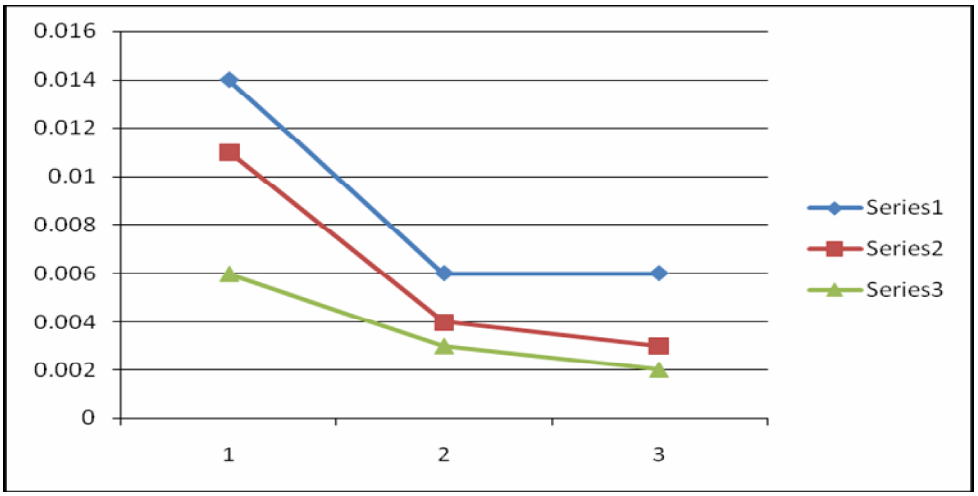
S.NO.	HOURS	O.D at 570 nm
1.	24	0.050
	48	0.040
	72	0.031
2.	24	0.060
	48	0.040
	72	0.025
3.	24	0.090
	48	0.035
	72	0.020

Fig.13 The graph showing OD at different hours for endotrachial catheter



S.NO.	DRUGS (CONCENTRATION)	O.D at 570nm
1.	AMOXICILLIN 10X	0.008
	AMOXICILLIN 50X	0.006
	AMOXICILLIN 100X	0.004
2.	TETRACYCLINE 10X	0.005
	TETRACYCLINE 50X	0.004
	TETRACYCLINE 100X	0.003
3.	OXYTERTACYCLINE 10X	0.003
	OXYTERTACYCLINE 50X	0.002
	OXYTERTACYCLINE 100X	0.001

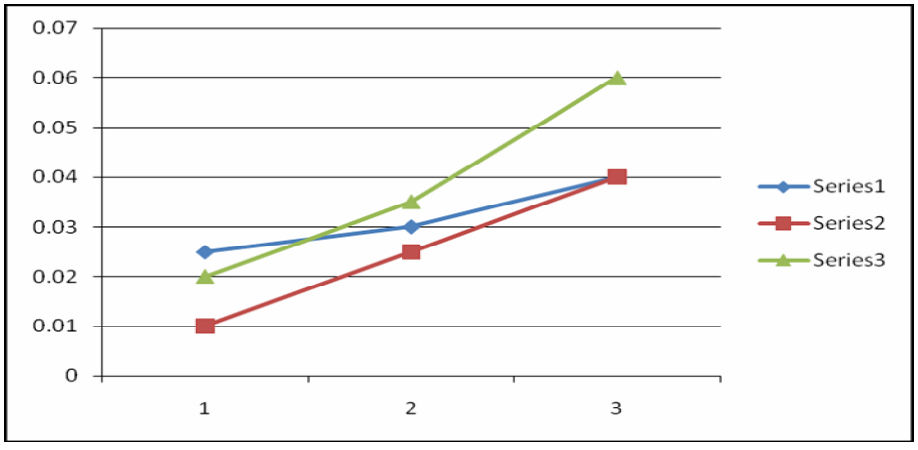
Fig.14 The graph showing OD of different antibiotics for endotrachial catheter



Urinary Catheter

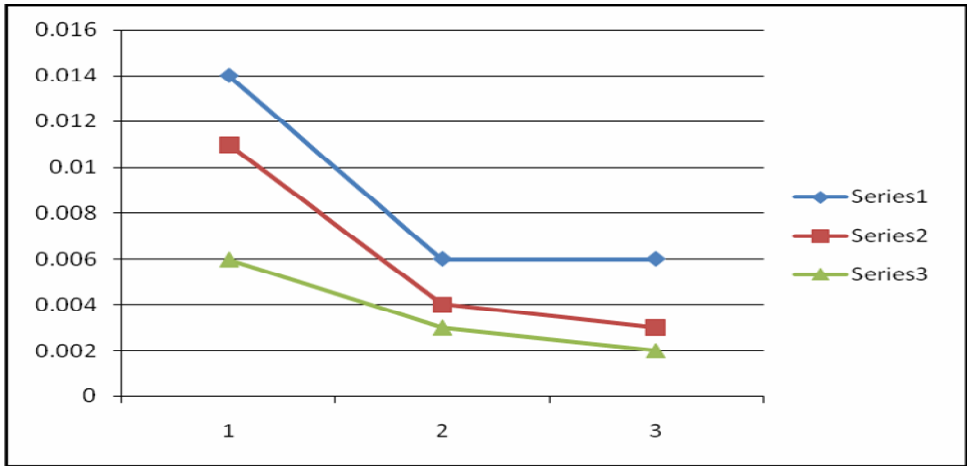
S.NO.	HOURS	O.D at 570nm
1.	24	0.025
	48	0.030
	72	0.040
2.	24	0.010
	48	0.025
	72	0.040
3.	24	0.020
	48	0.035
	72	0.006

Fig.15 The Graph showing OD for different hours for urinary tract catheter



S.NO.	DRUGS (CONCENTRATION)	O.D at 570nm
1.	AMOXICILLIN 10X	0.010
	AMOXICILLIN 50X	0.008
	AMOXICILLIN 100X	0.005
2.	TETRACYCLINE 10X	0.006
	TETRACYCLINE 50X	0.005
	TETRACYCLINE 100X	0.004
3.	OXYTERTACYCLINE 10X	0.004
	OXYTERTACYCLINE 50X	0.003
	OXYTERTACYCLINE 100X	0.002

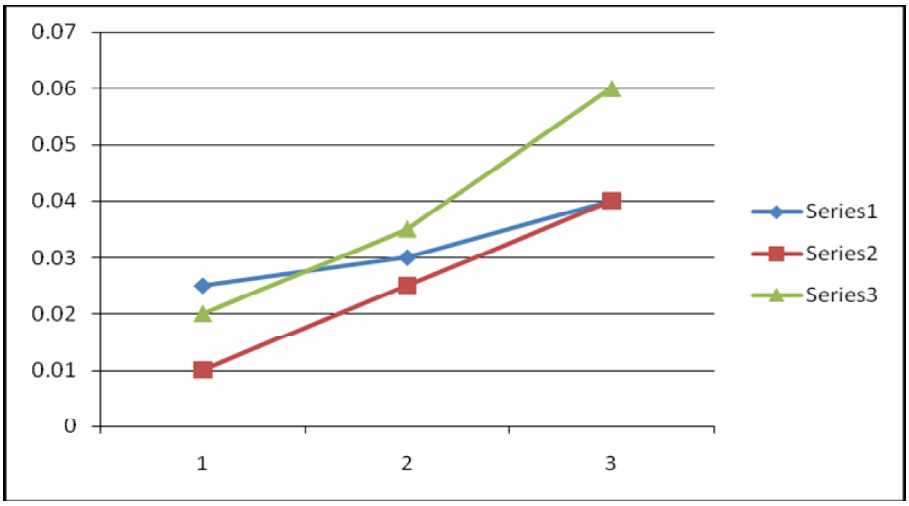
Fig.16 The graph showing OD at different antibiotics for urinary tract catheter



Intravenous Catheter

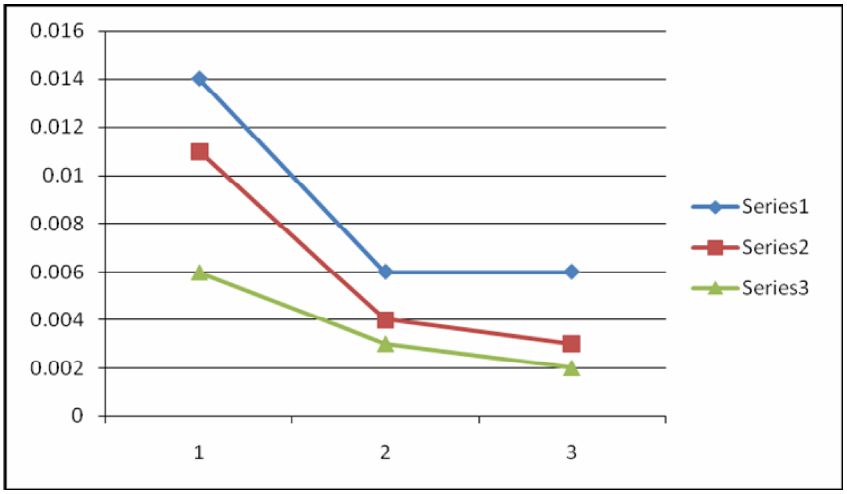
S.NO.	HOURS	O.D at 570nm
1.	24	0.036
	48	0.031
	72	0.025
2.	24	0.070
	48	0.060
	72	0.025
3.	24	0.094
	48	0.085
	72	0.054

Fig.17 The graph showing OD for different hours for intravenous catheter



S.NO.	DRUGS (CONCENTRATION)	O.D at 570nm
1.	AMOXICILLIN 10X	0.014
	AMOXICILLIN 50X	0.011
	AMOXICILLIN 100X	0.006
2.	TETRACYCLINE 10X	0.006
	TETRACYCLINE 50X	0.004
	TETRACYCLINE 100X	0.003
3.	OXYTERTACYCLINE 10X	0.006
	OXYTERTACYCLINE 50X	0.003
	OXYTERTACYCLINE 100X	0.002

Fig.18 The graph showing OD at different antibiotics for urinary tract catheter



MINIMUM INHIBITORY CONCENTRATION

Fig.19 MIC for Amoxicillin

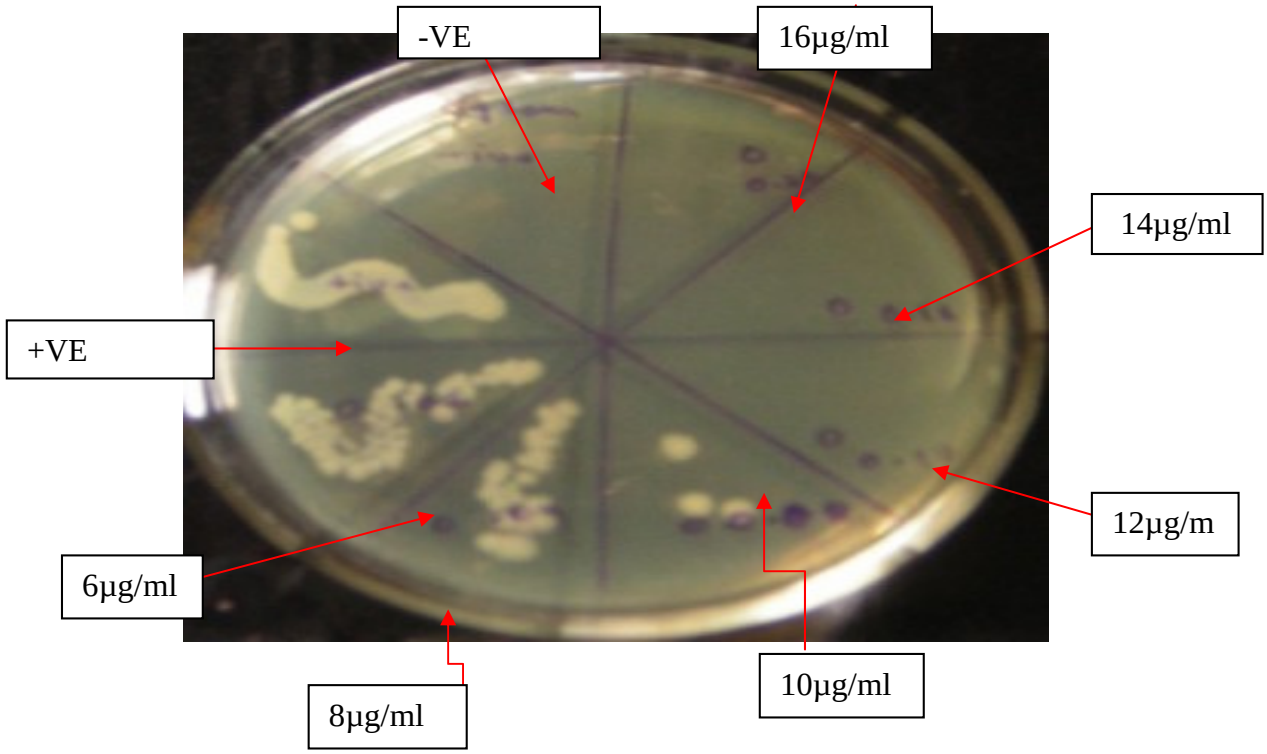


Fig.20 MIC for Tetracycline

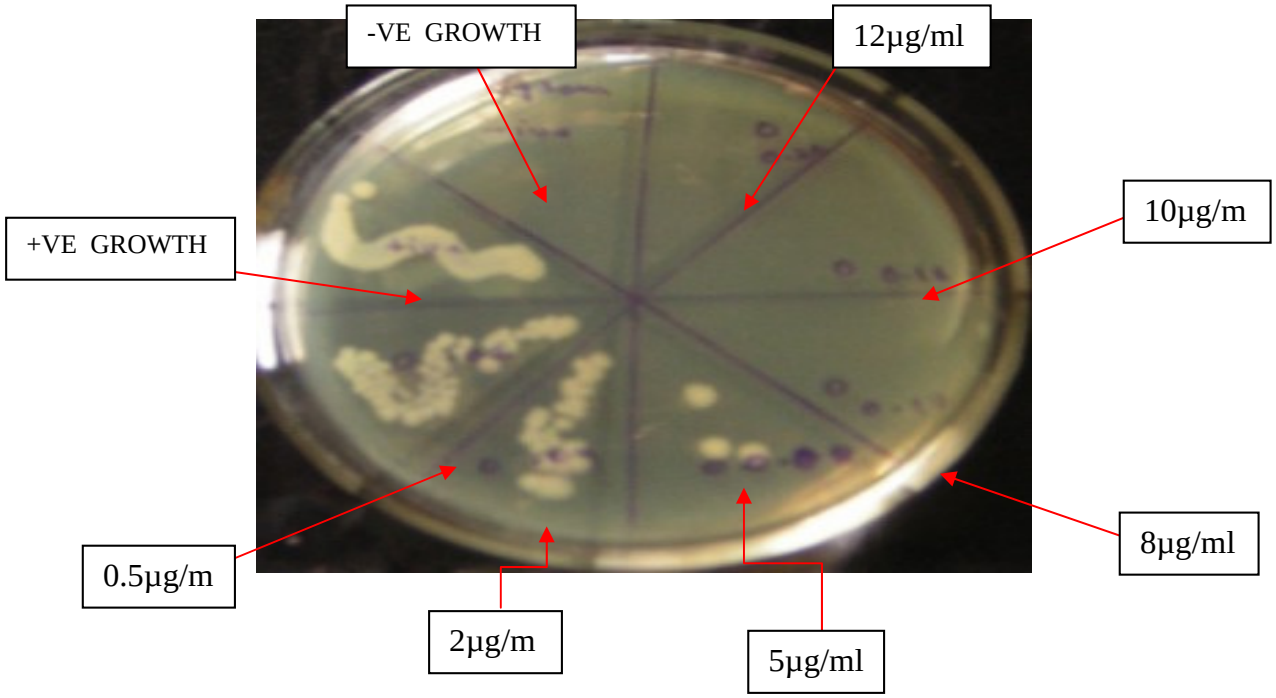


Fig.21 MIC for Oxytetracycline

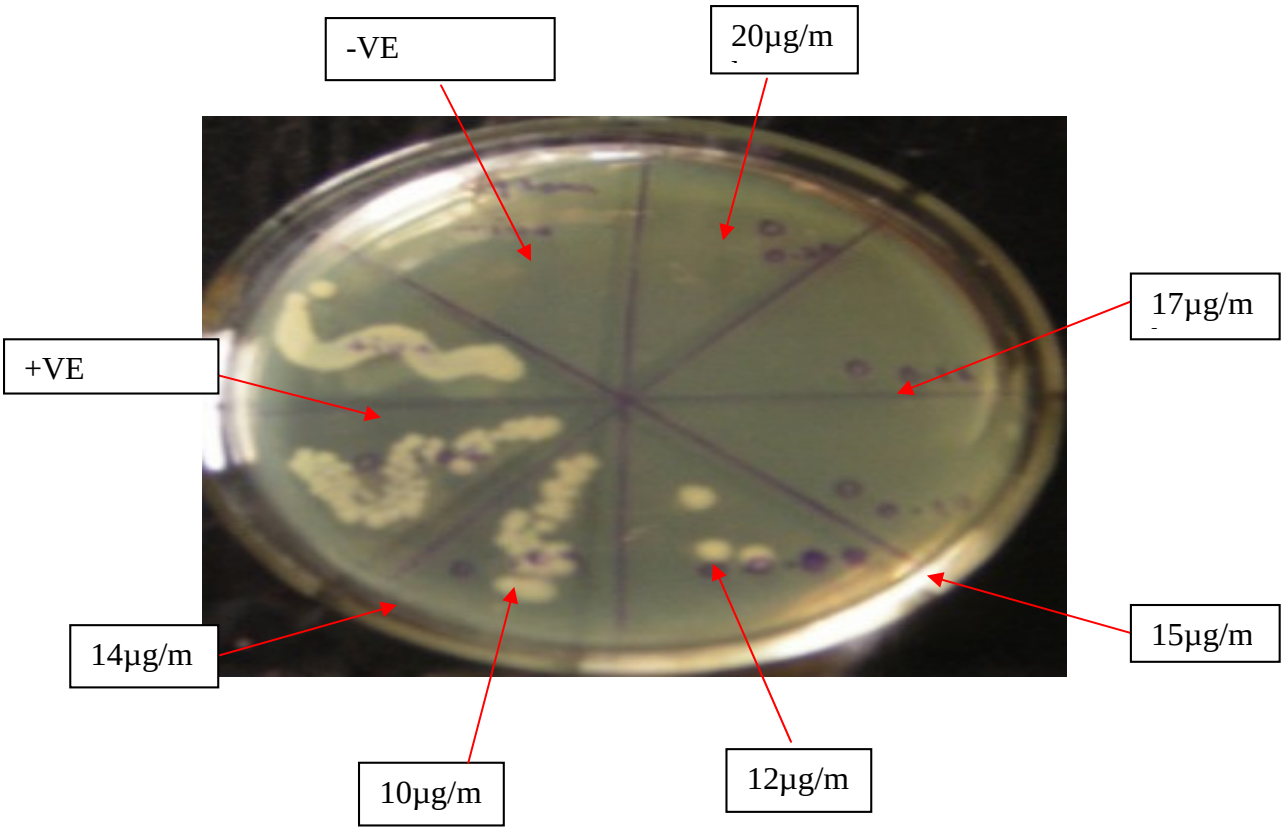
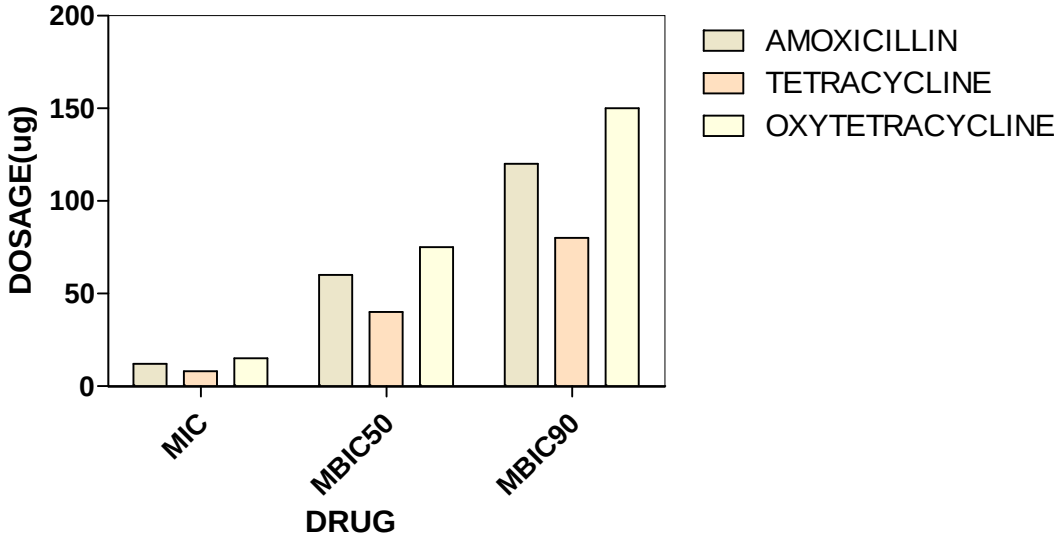
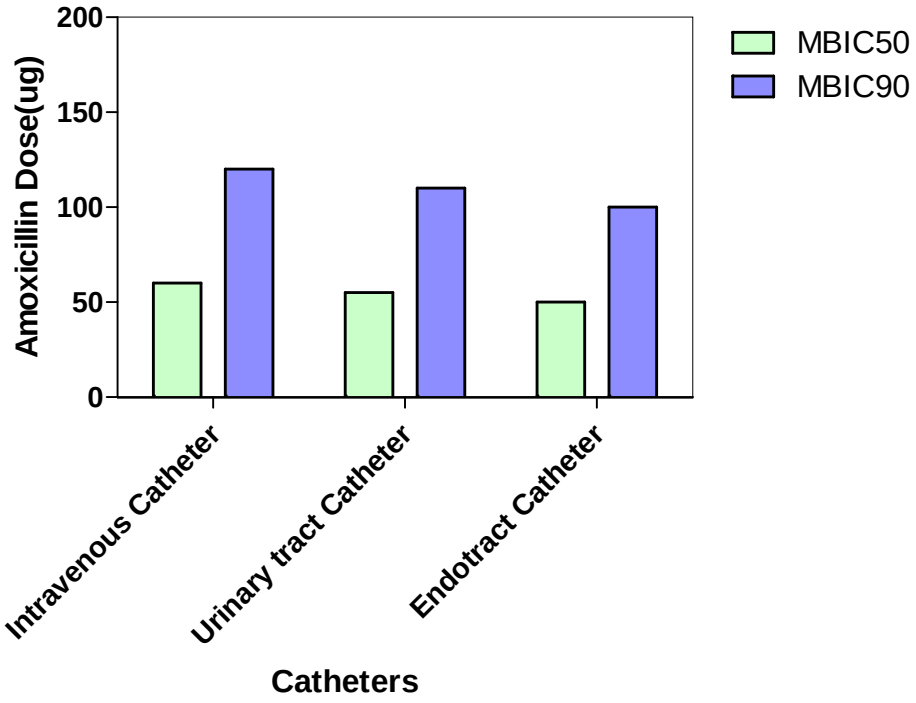
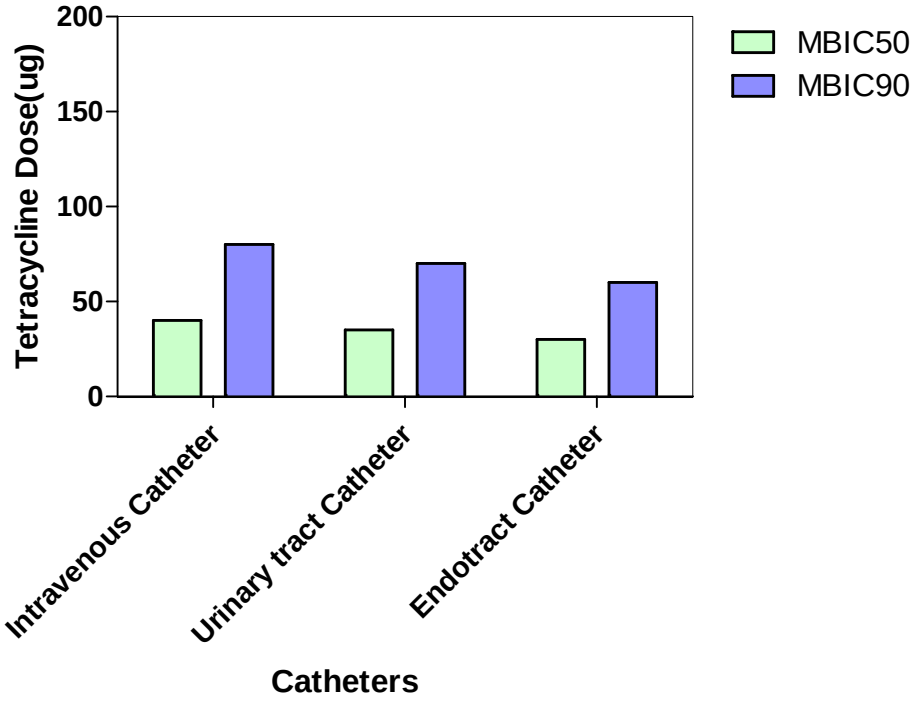
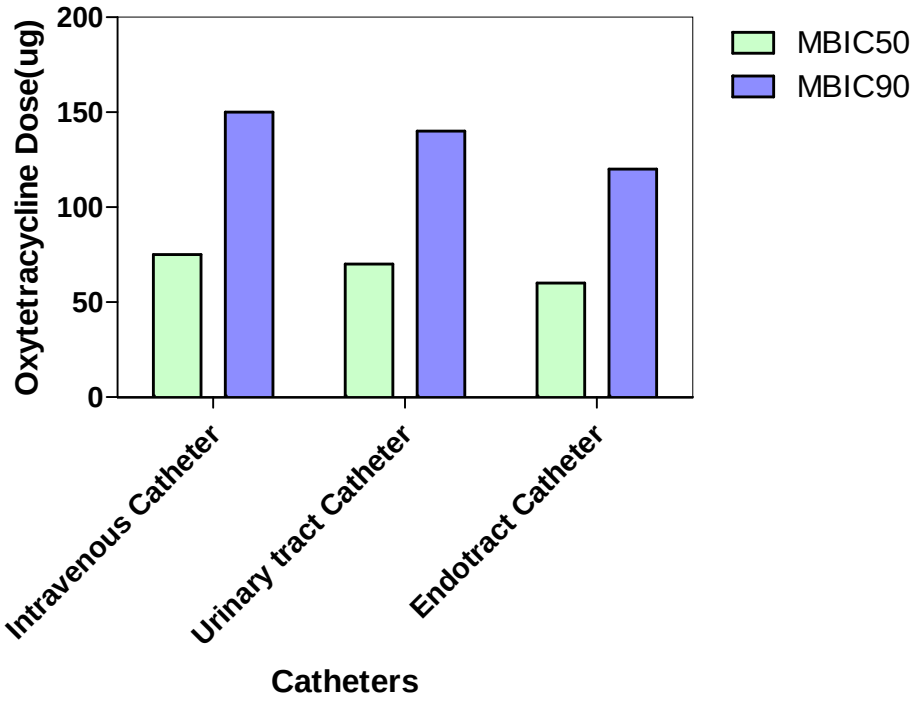


Fig.22 Comparative analysis of different drugs

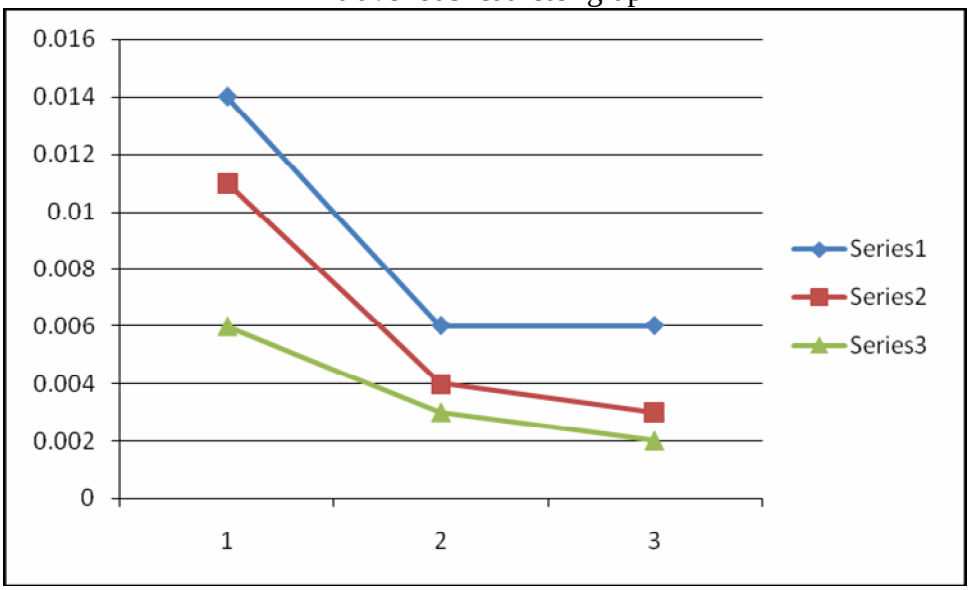



200
150
100
50
0
Amoxicillin Dose(ug)
MBIC50
MBIC90
Intravenous Catheter
Urinary tract Catheter
Endotract Catheter
Catheters

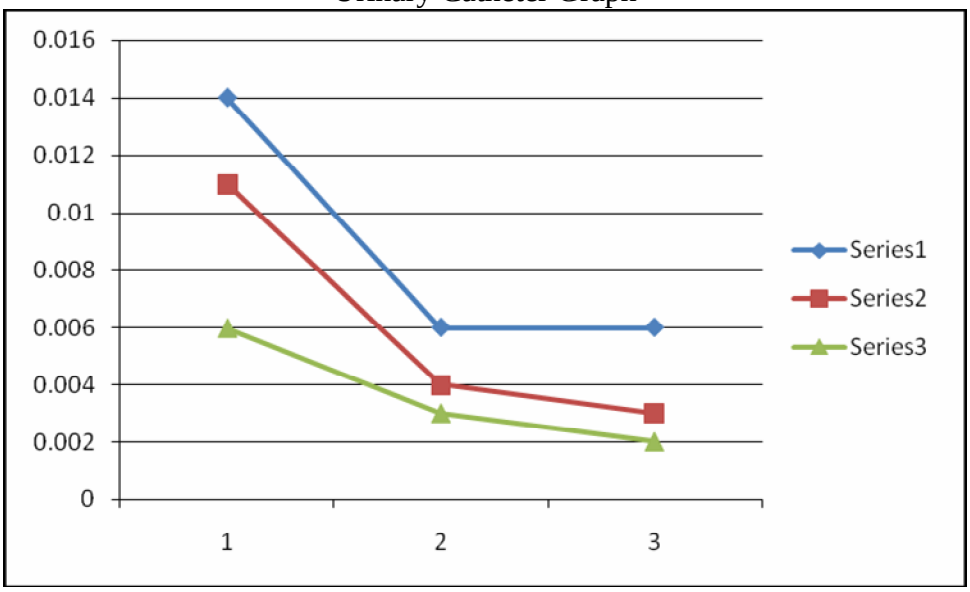

200
150
100
50
0
Tetracycline Dose(ug)
MBIC50
MBIC90
Intravenous Catheter
Urinary tract Catheter
Endotract Catheter
Catheters



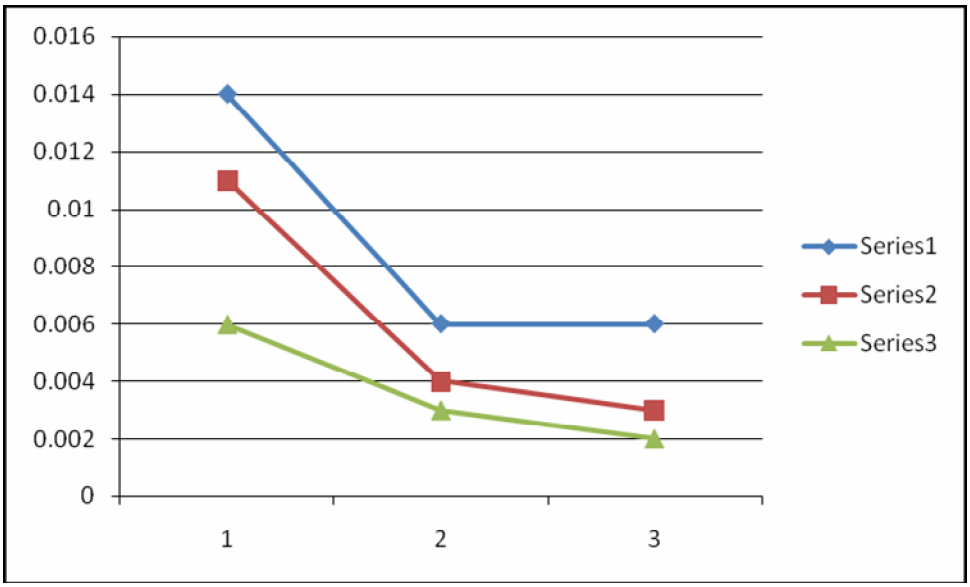
Intravenous catheter graph



Urinary Catheter Graph



Endotrachial Catheter



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