CHAPTER 2

LITERATURE REVIEW

2.1 Urinary Tract Infection (UTI)

The urinary tract is comprised of the kidneys, ureters, bladder, and urethra. A urinary tract infection is an infection caused by pathogenic organisms (bacteria, fungi, or parasites) in any of the structures that comprise the urinary tract. UTI have different names, depending on what part of the urinary tract is infected (Canton, 2006):

- Bladder -- an infection in the bladder is also called cystitis or a bladder infection
- Kidneys -- an infection of one or both kidneys is called pyelonephritis or a kidney infection
- Ureters -- the tubes that take urine from each kidney to the bladder are only rarely the site of infection.
- Urethra -- an infection of the tube that empties urine from the bladder to the outside is called urethritis.

2.1.1 Prevalence

The prevalence of UTIs in children is 1–5%. The prevalence increases greatly in young sexually active women, up to 20% of who have acute cystitis each year. This prevalence is increased by a previous UTI, and in some populations nearly one-half of women with cystitis had one or more recurrences within 1 year of follow-up. Fifty to sixty percent of women report having had a UTI

sometime during their life. Overall prevalence of UTI was 3.3%. Higher prevalence occurred in whites (10.7%), girls (4.3%), uncircumcised boys (8.0%), and those who did not have another potential source for their fever (5.9%), had a history of UTI (9.3%), malodorous urine or hematuria (8.6%), appeared “ill” (5.7%), had abdominal or suprapubic tenderness on examination (13.2%), or had fever ≥39°C (3.9%). White girls had a 16.1% prevalence of UTI (Cheesbrough, 2006).

2.1.2 Etiology

Although some cases of UTI are due to fungus or a virus, most are caused by one of several types of bacteria. Approximately 80% of acute uncomplicated UTIs are caused by *Escherichia coli (E.coli)*. *E. coli* clones cause the majority of uncomplicated infections and consist of only a few serogroups. Staphylococcus saprophyticus accounts for 5–15% of uncomplicated UTIs. Enterococcus and a variety of other primarily Gram-negative aerobes account for the other 5–10% of UTIs: Proteus mirabilis, Klebsiella species, and Pseudomonas species. More than 95% of UTI’s are caused by a single bacterial species. Complicated UTIs are defined as those occurring in a functionally, metabolically, or anatomically abnormal urinary tract or caused by bacteria resistant to antibiotics. Basically, complicated UTIs occur in anyone who is not young, healthy, and non-gravid. In complicated UTIs, E. coli is recovered in only approximately one-third of cases (34.5%); Streptococcal faecalis is recovered in 16%, Staphylococcus epidermidis in 13% and *Klebsiella Pneumoniae* in 13%. Other bacteria involved in complicated UTIs include Pseudomonas sp.(5%),Staphylococcus aureus (4%),Enterobacter sp.(2.5%), Serratia

sp.(1.5%),Streptococcus sp.(1.5%), Acinetobacter sp. (1.5%), Citrobacter sp.(1.0%), Providencia sp.(0.25%), Morganellamorganii (0.25%), and Candida sp.(0.25%) (Laraki, 1999).

2.1.3 Route of infection

The first consideration in discussing the pathogenesis of UTI is the route by which microorganisms, especially bacteria, reach the urinary tract in general and the kidney in particular. Four potential routes have been proposed: the ascending route, from the urethra to the bladder, then by the ureters to the kidneys; the hematogenous route, with seeding of the kidney during the course of bacteremia; intestine to kidney by way of lymphatic's; and direct infection.

There is considerable clinical evidence that most infections of the kidney result from ascension of fecal derived organisms from the urethra and periurethral tissues into the bladder and then by the ureter to the renal pelvis, with subsequent invasion of the renal medullae at this site. In discussing the pathogenesis of ascending infection, it is useful to examine the various steps necessary for the spread of organisms from the periurethral region to the kidney.
In humans, blood-borne infection of the kidneys and urinary tract accounts for less than 3% of the cases of UTI and pyelonephritis because the kidneys receive 20 to 25% of the cardiac output, any microorganism that reaches the bloodstream can be delivered to the kidneys (Fleiszig, 2002).

2.1.4 Categorization of Urinary Tract Infection

Urinary tract infections (UTIs) are classified by location: the lower urinary tract (which includes the bladder and structures below the bladder) or the upper

urinary tract (which includes the kidneys and ureters). They can also be classified as uncomplicated or complicated UTI's (Walter, 2008).

2.1.4.1 Lower Urinary Tract Infections

Several mechanisms maintain the sterility of the bladder; such as the barrier of the urethra, urine flow, uretero-vesical junction competence, various antibacterial enzymes and antibodies and anti-adherent effects mediated by the mucosal cells of the bladder. Abnormalities or dysfunctions of these mechanisms are contributing risk factors for lower urinary tract infections.

Sign and symptoms of an uncomplicated lower urinary tract infection (cystitis) include burning on urination, frequent voiding, urgency, nocturia (awakening at night to urinate), incontinence and suprapubic or pelvic pain. Other presenting complaints were hematuria and back pain.

In patients with complicated urinary tract infections, manifestations can range from asymptomatic bacteriuria to gram-negative sepsis with shock. Complicated urinary tract infections often are caused by a broader spectrum of organisms, have a lower response rate to treatment and tend to recur (Scholar, 2000).

2.1.4.2 Upper Urinary Tract Infections

Pyelonephritis is a bacterial infection of the renal pelvis, tubules and interstitial tissue of one or both kidneys. The causes are either the upward spread of bacteria from the bladder or spread from systemic sources reaching the kidney via the bloodstream.

The patient with acute pyelonephritis is acutely ill with chills, fever, leukocytosis, bacteriuria and pyuria. Other common findings are such as low back pain, flank pain, nausea and vomiting, headache, malaise and painful urination. Physical examination reveals pain and tenderness in the area of the costovertebral angle. In addition, symptoms of lower urinary tract involvement, such as urgency and frequency, are common.

Patients with chronic pyelonephritis usually exhibit no symptoms of infection unless an acute exacerbation occurs. Noticeable signs and symptoms may include fatigue, headache, poor appetite, polyuria, excessive thirst and weight loss (Kalsi, 2003).

2.1.4.3 Community Acquired Urinary Tract Infection

The clinical and epidemiologic spectrum of 175 cases of community-acquired urinary tract infection (UTI) was evaluated. Patients were grouped in five different categories of which complicated UTI was the most common (39%). Bacteremia was detected in eight patients (18%) of this group and in five (12%) with acute uncomplicated pyelonephritis.

A single organism was isolated in 166 cases (95%). The rate of Escherichia coli bacteriuria ranged from 60% (asymptomatic bacteriuria) to 94% (uncomplicated cystitis). Of the 184 isolates, 92% were susceptible to ciprofloxacin and significantly high rates of resistance were found for ampicillin, cefazolin, cefuroxime, and co-trimoxazole. Isolates causing uncomplicated UTI had significantly high rates of resistance to ampicillin, amoxycillin-clavulanate and co-trimoxazole and those causing complicated UTI, had significantly high rates of resistance to most oral antibiotics tested, except quinolones and nitrofurantoine.

Community-acquired UTI requiring hospital evaluation occurs in a complex group of patients, and current patterns of antibiotic resistance make it difficult to suggest empiric oral treatments in this setting (Finkelstein, 2009).

2.1.4.4 Hospital Acquired Urinary Tract Infection

Nosocomial urinary tract infections (UTIs) account for up to 40% of all hospital-acquired infections. The associated morbidity and mortality are a major drain on hospital resources. Patients with indwelling urinary catheters, patients undergoing urological manipulations, long-stay elderly male patients and patients with debilitating diseases are at high risk of developing nosocomial UTIs.

The organisms responsible usually originate from patients' endogenous intestinal flora, but occasionally from a moist site in the hospital environment. Nosocomial pathogens causing UTIs tend to have a higher antibiotic resistance than simple UTIs. Infection control policies are important in limiting the number of hospital-acquired UTIs.

Other important points include catheterization using an aseptic technique and sterile equipment and the use of closed drainage systems. UTIs should be treated only after a urine sample has been sent and the advice of a microbiologist sought. In the future catheters impregnated with antibiotics, and the use of newer materials, may lead to further reductions in the incidence of nosocomial UTIs (Kalsi, 2003).

2.2 *Klebsiella pneumoniae*

2.2.1 Biology Properties

Klebsiella pneumoniae a member of the family Enterobacteriaceae is an organism named after Edwin Klebs, a 19th century German microbiologist. *K. pneumoniae* is non-motile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule. *K. pneumoniae* is among the most common gram-negative bacteria encountered by physicians worldwide for respiratory tract infection. It is a common hospital-acquired pathogen, nosocomial pneumonia. *K. pneumoniae* is also a potential community-acquired pathogen (Wen, 2002).

2.2.2 Morphology

The genus *Klebsiella* belongs to the tribe *Klebsiellae*, a member of the family Enterobacteriaceae. The organisms are named after Edwin Klebs, a 19th century German microbiologist. *Klebsiellae* are non-motile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms. The genus was originally divided into 3 main species based on biochemical reactions. Today, 7 species with demonstrated similarities in DNA homology are known. These are (1) *Klebsiella pneumoniae,* (2) *Klebsiella ozaenae,* (3) *Klebsiella rhinoscleromatis,* (4) *Klebsiella oxytoca,* (5) *Klebsiella planticola,* (6) *Klebsiella terrigena* and (7) *Klebsiella ornithinolytica*. *K. pneumoniae* is the most medically important species of the group. *K. oxytoca* and *K. rhinoscleromatis* have also been demonstrated in human clinical

specimens. In recent years, Klebsiellae have become important pathogens in nosocomial infections (Umeh, 2009).

2.2.3 Pathogenesis and Virulence Factors

One or more virulence factors may contribute to pathogenicity in humans. Few factors that may mediate virulence: cell wall receptors, capsular polysaccharide, lipopolysaccharide, aerobactin, mucoidphenoytype plasmid, endotoxin and multiple adhesion.First, the presence of cell wall receptors enables K. pneumoniae to attach to the host cell, thereby altering the bacterial surface so that phagocytosis by polymorphonuclear leukocytes and macrophages is impaired and invasion of the non-phagocytic host cell is facilitated (Umeh, 2009).

Second, invasion of the host cell is also facilitated by the large polysaccharide capsule (K antigen) surrounding the bacterial cell. In addition this capsule acts as a barrier and protects the bacteria from phagocytosis. Polysaccharide capsule also triggers extensive lung tissue damage and data indicate that there might be a correlation between the production of this extracellular complex and *Klebsiella* virulence. *This* polysaccharide capsule is responsible for resistance to complement mediated killing and impedes adhesion to and invasion of epithelial cells by sterically preventing receptor-target recognition of bacterial adhesin. Recently we have demonstrated that polysaccharide capsule mediates resistance to antimicrobial peptides (APs), trapping APs and thus acting as a bacterial decoy. Lipopolysaccharides (O antigen) are another bacterial pathogenicity factor. They are able to activate complement, which causes selective deposition of C3b onto LPS molecules at sites distant from the bacterial cell membrane. This inhibits the formation of the

membrane attack complex (C5b-C9), which prevents membrane damage and bacterial cell death (Highsmith, 2007).

Third, the presence of a 180-kilobase plasmid encoding production of aerobactin was correlated with the virulence of *Klebsiella pneumoniae. K. pneumonia* strain becomes a virulent when it has lost this plasmid. In addition to aerobactin production, another phenotype could be correlated with the presence of this virulence plasmid: the mucoid phenotype of the bacterial colonies. Participation of this phenotype in the virulence of *K. pneumoniae* was demonstrated by constructing a mutant altered in the plasmid gene encoding this phenotype. The resulting strain demonstrated a 1,000-fold decrease in virulence. On the other hand, neither the overproduction of capsular polysaccharide nor the presence of colanic acid was detected in mucoid strains of *K. pneumoniae*. We conclude that this mucoid phenotype is definitely an important virulence factor of *K. pneumoniae*. It is due to the plasmid-encoded production of a substance which is different from colanic acid and the capsular polysaccharide of *K. pneumonia* (Chen, 2004).

Finally, *K. pneumoniae* produces an endotoxin that appears to be independent of factors that determine receptors and capsular characteristics. Marked interspecies differences in endotoxin production may correlate with virulence.The bacteria also produce multiple adhesins. These may be fimbrial or nonfimbrial, each with distinct receptor specificity. These help the microorganism to adhere to host cells, which is critical to the infectious process (Cano, 2009).

2.2.4 Clinical Manifestation

The most typical symptoms of UTI are:

- frequency for micturition by day and night
- painful voiding (dysuria)
- suprapubic pain and tenderness
- haematuria
- smelly urine

These symptoms relate to bladder and urethral inflammation, commonly called ‘cystitis’, and suggest lower urinary tract infection. Loin pain and tenderness, with fever and systemic upset, suggest extension of the infection to the pelvis and kidney, known as pyelitis or pyelonephritis.

However, localization of the site of infection on the basis of symptoms alone is unreliable.UTI may also present with minimal or no symptoms or may be associated with atypical symptoms such as abdominal pain, fever, haematuria in the absence of frequency or dysuria. In children, who cannot complain of dysuria, symptoms are often ‘atypical’. The possibility of UTI must always be considered in the fretful, febrile sick child who fails to thrive (Fihn, 2003).

2.2.5 Clinical Diagnosis

Based on quantitative culture of a clean-catch midstream specimen of urine and the presence or absence of pyuria. Diagnosis of ‘low count bacteriuria’ ($\geq 10^2$ organisms) demands additionally the presence of pyuria. The criteria for the diagnosis of UTI, particularly in symptomatic women, are shown below:

- Symptomatic young women:

≥10^2 coliform organisms/mL urine plus pyuria (>10 WBC/mm^3) or ≥10^5 any pathogenic organisms/mL urine or any growth of pathogenic organisms in urine by suprapubic aspiration.

- Symptomatic men: ≥10^3 pathogenic organisms/mL urine
- Asymptomatic patients: ≥10^5 pathogenic organisms/mL urine on two occasions.

Dipsticks tests can be used to detect nitrites in urine. Most Gram-negative organisms reduce nitrates to nitrites and produce a red colour in the reagent square. False-negative results are common. Dipsticks that detect significant pyuria depend on the release of esterase's from leucocytes. Dipstick tests positive for both nitrite and leucocyte esterase are highly predictive of acute infection (sensitivity of 75% and specificity of 82%) (Cameron, 2004).

2.3 Antimicrobial

2.3.1 Definition

Antimicrobial is an agent that kills microorganisms or suppresses their multiplication or growth. Antimicrobial drugs exert an effect in the patient that is either bactericidal or bacteriostatic. Those antimicrobial drugs that are generally bacteriostatic at concentrations that are achieved clinically (e.g. chloramphenicol, erythromycin, and tetracycline's) inhibit bacterial cell replication but do not kill the organism. Other antimicrobial drugs (e.g. penicillins, cephalosporins, and aminoglycosides) are usually bactericidal: they cause microbial cell death and lysis. A few compounds (e.g. sulfonamides) are either cidal or static according to the composition of the environment (blood, pus, urine, etc.) in which the infecting organisms are growing (Scholar, 2000).

The following table shows the antimicrobials that are commonly prescribed for patients with urinary tract infection:

Table 2.1 Classification of Antimicrobials

Class	Groups	Examples
β-lactam	Penicillin	Penicillin G, Ampicillin, Amoxycillin
	Penicillinase-resistant Penicillin	Methicillin, Nafcillin, Oxacillin, Cloxacillin, Dicloxacillin
	Broad Spectrum Penicillin	Carbenicillin, Piperacillin, Mezlocillin
	Cephalosporin Generation I	Cephalothin, Cephazolin
	Cephalosporin Generation II	Cefoxitin, Cefotetan, Cefuroxime
	Cephalosporin Generation III	Cefotaxime, Ceftazidime, Ceftriaxone
	Monobactam	Aztreonam
	Carbapenem	Imipenem, Meropenem
Aminoglyco-side		Gentamycin, tobramycin, netilmicin, amikacin, streptomycin, kanamicin
Glycopeptide		Vancomycin, teikoplanin
Quinolone		Norfloxacin, ciprofloxacin, ofloxacin
Others		Nitrofurantoin, nalidixic acid, cotrimoxazole

(Port,2006)

2.3.2 Amoxicillin

Amoxicillin is a penicillin-type antibiotic. Amoxicillin has a broad spectrum of activity. Amoxicillin's mechanism of action involves the inhibition of stage III of the bacterial cell wall biosynthesis, preventing cross-linking of peptidoglycan. It is an alternative substrate for transpeptidases because of its structurally similarity to

the transition state of the Ala-Ala terminal during cross-linking. The transpeptidases catalyzes the cross-linking of the peptidoglycan. The inability to synthesis the cell wall leads to cell lysis and thus amoxicillin is bactericidal. The C-6 substitute contains a secondary amine that can be protonated as well as a phenol group that can be deprotonated. The carboxylic acid at position 3 is also capable of deprotonation. When these groups are ionized, the molecule contains a net negative charge and therefore is less Zwitterionic and more capable of being orally absorbed. Amoxicillin, however, is still susceptible to inactivation by beta-lactamase enzymes, if produced by the bacteria (David, 2002).

Resistance to amoxicillin, as well as other beta-lactam type antibiotics, is due to the production of beta-lactamase enzymes by the bacterium through mutations in its genome. Beta-lactamases attack the beta-lactam ring at the carbonyl position opening the beta-lactam and thus inactivating the agent. Bulky substituents have been added to the C-6 position of the beta-lactam in hopes of protecting the carbonyl from beta-lactamase inactivation. However, in the case of amoxicillin, different measures have been taken. Administered along with amoxicillin is a type I mechanism-based beta-lactamase inhibitor called clavulanic acid. This inhibition is irreversible so the enzyme is tied up with the inhibitor while amoxicillin is capable of eliciting its antibacterial effects (David, 2002).

2.3.3 Amoxicillin-Clavulanic Acid

Amoxicillin is a semisynthetic antibiotic with a broad spectrum of bactericidal activity. Amoxicillin is, however, susceptible to degradation by β-lactamases, and therefore, the spectrum of activity does not include organisms which produce these enzymes. Clavulanic acid is a β-lactamase inhibitor,

structurally related to the penicillins, which possesses the ability to inactivate a wide range of β-lactamase enzymes commonly found in microorganisms resistant to penicillins and cephalosporins (French, 1998).

The formulation of amoxicillin and clavulanic acid in amoxicillin/clavulanate potassium protects amoxicillin from degradation by β-lactamase enzymes and effectively extends the antibiotic spectrum of amoxicillin to include many bacteria normally resistant to amoxicillin and other β-lactam antibiotics. Thus, amoxicillin/clavulanate potassium possesses the properties of a broad-spectrum antibiotic and a β-lactamase inhibitor (French, 1998).

These mechanisms of resistance is believed to be acquired in 2 ways; hyper production of chromosomal class C β-lactamase and the production of inhibitor resistant TEM (Transposable Element) enzymes and deficiency in OMP F and/or OMP C porins. Hyper production of chromosomal class C β-lactamase causes an increase of TEM-1 β-lactamase causing *Klebsiella Pneumoniae* to be less sensitive to clavulanate and thus it can hydrolyze amoxycilin .Deficiency in OMP F and/or OMP C porins causes decrease in intracellular concentration of antimicrobial, thus conferring resistance (Marre, 1997).

2.3.4 Ceftriaxone

Ceftriaxone is a third-generation cephalosporin based on its spectrum of activity. Ceftriaxone works by inhibiting the mucopeptide synthesis in the bacterial cell wall. The β-lactam moiety of ceftriaxone binds to carboxypeptidases, endopeptidases, and transpeptidases in the bacterial cytoplasmic membrane. These enzymes are involved in cell- wall synthesis and cell division. By binding to

these enzymes, ceftriaxone results in the formation of defective cell walls and cell death (Katzung, 2009).

It has been suggested that resistance in ceftriaxone can be due to acquisition of Extended Spectrum β-lactamases (ESBL), arising through mutation SHV-1 enzyme. In a research conducted by Centers for Disease Control and Prevention (CDC), it has been suggested that SHV-1 have mutated into SHV-8, thus increasing the level of resistance. In addition to acquisition of ESBL, *Klebsiella Pneumoniae* increase its resistance by down-regulating its cell wall porin(OmpF) thus decreasing or eliminating the flow of the antimicrobial drug (Fred, 2006).

2.3.5 Gentamicin

Gentamycin, a group of aminoglycoside acts by 2 ways; disrupting the protein synthesis and damaging the cell wall. Aminoglycosides bind to the bacterial 30S ribosomal subunit, thus inhibiting the translocation of tRNA during translation and leaving the bacterium unable to synthesize proteins necessary for growth. Aminoglycosides also damage the cell wall by displacing the cations in the bacterial cell biofilm that are responsible for linking the lipopolysaccharide (LPS) molecule characteristic of gram-negative bacterial cell walls. This creates holes in cell wall that may kill the bacteria (Port, 2006)

There are three mechanisms of aminoglycoside resistance: reduced uptake or decreased cell permeability, alterations at the ribosomal binding sites, or production of aminoglycoside modifying enzymes. Some strains of gram-negative bacilli exhibit aminoglycoside resistance due to a transport defect or membrane impermeabilization. This mechanism is likely chromosomally

mediated and results in cross-reactivity to all aminoglycosides.The level of resistance that is seen is moderate (i.e. intermediate susceptibility). (Mingeot-Leclercq, 1999)Mutations at the site of aminoglycoside attachment may interfere with ribosomal binding. Resistance to streptomycin can occur by this mechanism since this agent binds to a single site on the 30S subunit of the ribosome. Resistance to the other aminoglycosides by this mechanism is uncommon since they bind to multiple sites on both ribosomal subunits and high-level resistance cannot be selected by a single step (Kucers, 1997).

Enzymatic modification is the most common type of aminoglycoside resistance. Enzymatic modification results in high-level resistance.The genes encoding for aminoglycoside modifying enzymes are usually found on plasmids and transposons. Most enzyme-mediated resistance in gram-negative bacilli is due to multiple genes. It is hypothesized that the enzymes are derived from organisms that make the aminoglycoside or from the mutation of genes that encode the enzymes involved in cellular respiration (Davies, 1997).

2.3.6 Ciprofloxacin

Ciprofloxacin, a group of fluroquinolone acts directly on inhibiting the DNA synthesis. Inhibition appears to occur by interaction of the drug with complexes composed of DNA and either of the two target enzymes, DNA gyrase and topoisomerase IV. (Hooper DC, 1999) Resistance to fluoroquinolone group occurs through 3 mechanisms; mutation of topoisomerase, decrease in membrane permeability and active drug efflux. There are 2 types of topoisomerase in bacteria; DNA gyrase which is a primary target for gram negative bacteria and topoisomerase IV which is a primary target in gram positive

organisms. *Klebsiella Pneumoniae* DNA gyrase composed of two pair subunits, GyrA and GyrB which are encoded by *gyrA*and *gyrb*genes respectively. The intact enzyme is responsible for introducing and removing DNA supercoils and for unlinking interlocked DNA circles, thus restoring proper confirmation structure of DNA. Quinolones inhibits action of DNA gyrase and kill bacteria by binding to these enzyme-DNA complexes, thereby disrupting DNA replication. Mutations in the genes that encode for DNA gyrase can change the structure of one or more subunits of these enzymes. Norfloxacin resistance is obtained by alteration on the A subunit of DNA gyrase(*nfx A or nor A,* alleles of *gyrA*) (Bearden, 2001*).*

The mutation of topoisomerase, multiple antibiotic resistance (*mar*) genes acting on a variety of compounds decrease the efficacy of quinolones. The *mar* genes regulate the accumulation of quinolones by altering the expressions of porins and efflux pumps. This results in altering of quinolone concentration within the microorganism. An efflux pump, AcrAB can transport quinolones out of the bacteria. The pump is partly controlled by the *mar* genes and appears to be major mechanism of resistance for *mar* mutants.Additional nontopoisomerase resistance can change quinolone resistance patterns. The *nfxB*gene codes for an altered outer membrane protein, termed OMP F, thereby decreasing quinolone entry into the cell (Bearden, 2001)

2.4 Antimicrobial Susceptibility Testing

Drug sensitivity tests are also important in studies of the epidemiology of resistance and in studies of a new antimicrobial agents Mueller-Hinton agar media (4mm thickness plate)is considered best because (Satish Gupte, 2006):

i. Acceptable batch to batch reproducibility for susceptibility testing.

ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.

iii. It gives satisfactory growth of most non-fastidious pathogens.

iv. A large body of data and experience has been collected concerning susceptibility tests performed with the medium.

This medium should contain as low as possible thymidine or thymine (reverse the inhibitory effect of sulfonamide and trimethoprim).Zones of inhibition are measured to the nearest whole millimeter using sliding calipers, ruler and template prepared for this purpose which is held on the back of inverted petri plate (Satish, 2006).

These are applied to determine the susceptibility of pathogenic bacteria to antibiotics to be used in treatment. Antibiotic sensitivity tests are very useful for clinician and hence constitute important routine procedure in diagnostic bacteriology. Mainly they are two types (Satish, 2006):

(1) Diffusion test: The principle of it is to allow the drug to diffuse through a solid medium, concentration of drug being highest near the site of application of drug and decreasing with distance. There are many methods for implementation of this diffusion test and the most common, simple and easy method is to use filter paper discs impregnated with antibiotics (disc diffusion method).Here filter paper discs mm in diameter are charged with required concentration of drugs and are stored dry in the cold. Inoculation of pure bacterial growth in liquid medium may be done by spreading with swabs on solid medium. After drying the plate at 37°C for ½ hour antibiotic disc are applied with sterilized forceps. After overnight incubation at 37°C, zone of inhibition of growth around each antibiotic disc is noted. Inhibition zone shows degree of sensitivity of antibiotic for those particular

bacteria. The results are reported as sensitive or resistant. Disc diffusion test is done only after the pathogenic bacteria are isolated from clinical specimen in pure form. Sensitivity tests should be done only with pathogenic bacteria not with commensals. Further, nitrofurantin need to be tested only against urinary pathogens. Sensitivity tests on methanaminemandelate are not relevant as the drug is active only in vivo.

In case we require the drug sensitivity test sooner, clinical material is directly inoculated uniformly on the surface of solid media plate and discs are applied. This is done only in emergency and results are subsequently verified by testing the pure isolates.

(2) Dilution tests: These are quite laborious for routine use. However, these are useful where therapeutic dose is to be regulated accurately,e.g. in treatment of bacterial endocarditis and to find out small degree of resistance in slow growing bacteria like tubercle bacilli. In Dilution test, serial dilutions of drug are prepared and are inoculated with test bacterium. It may be done by the tube dilution or agar dilution methods.