CHAPTER 2

LITERATURE REVIEW

2.1 Respiratory Tract Infection (RTI)

RTI is any infection of the respiratory tract (nose, throat, pharynx, larynx, trachea, brounchus, lung). It is a pathological state resulting from the invasion of the body by pathogenic microorganisms. Common respiratory tract infections include the common cold, pneumonia and influenza (Todar, 2004).

2.1.1 Prevalence

RTI continues to be the leading cause of illnesses worldwide and remain the most important cause of infant and young children mortality, accounting for about two million deaths each year and ranking first among causes of disability-adjusted life-years (DALYs) lost in developing countries. The populations most at risk for developing a fatal respiratory disease are the very young, the elderly, and the immune compromised. *Pseudomonas aeruginosa* is the leading etiology for Gram-negative bacteria at most medical centers, carrying a 40-60% mortality rate. it attacks up two thirds of the critically-ill hospitalized patients, and this usually portends more invasive diseases. *Pseudomonas aeruginosa* cases occur mostly in United States (40 million), China (21 million), Indonesia and Nigeria (34 million each) in year 2009. Pneumonia is responsible for about 21% of all deaths in children aged less than 5 years, leading to estimate that of every 1000 children born alive. Ventilator-associated pneumonia (VAP) caused by *Pseudomonas aeruginosa* has been associated with higher case fatality rates than VAP caused by other bacterial etiologies (Garibaldi, 2003).

2.1.2 Division of Respiratory Tract Infection

Respiratory tract infections (RTIs) are classified into two division by location: the lower respiratory tract infection (LRTI) or the upper respiratory tract infection (URTI). Lower respiratory infections, such as pneumonia, tend to be far more serious conditions than upper respiratory infections. Pneumonia is further divided into Hospital-acquired pneumonia (HAP) and Community-acquired pneumonia (CAP). When pneumonia occurs in a hospitalized patient who is on a ventilator, it is known as ventilator-associated pneumonia (VAP) (Todar, 2004).

2.1.2.1 Upper respiratory tract infection (URTI)

A nonspecific term used to describe acute infections involving the nose, paranasal sinuses, pharynx, and larynx is known as Upper respiratory tract infection (URTI).The prototype is the illness known as the common cold, which will be discussed here, in addition to pharyngitis and sinusitis. Influenza is a systemic illness that involves the upper respiratory tract and should be differentiated from other URTIs (Mossad, 2005).

Common viruses causing most URTIs includes *rhinovirus, parainfluenza virus, coronavirus, adenovirus, respiratory syncytial virus, coxsackievirus,* and *influenza virus* accounting for most cases. Human metapneumovirus is a newly discovered agent causing URIs. *Group A beta-hemolytic streptococci* (GABHS) cause 5% to 10% of cases of pharyngitis in adults.Other less common causes of bacterial pharyngitis include group C *beta-hemolytic streptococci, Corynebacterium diphtheriae, Neisseria gonorrhoeae, Arcanobacterium haemolyticum, Chlamydia pneumoniae, Mycoplasma*

pneumoniae, and *herpes simplex virus. Streptococcus pneumoniae, Haemophilus influenza* and *Moraxella catarrhalis* are the most common organisms that cause the bacterial superinfection of viral acute sinusitis. Less than 10% of cases of acute trachea bronchitis are caused by *Bordetella pertussis, M. pneumoniae*, or *C. pneumonia* (Mossad, 2005).

2.1.2.2 Lower respiratory tract infection (LRTI)

Lower respiratory tract is the part of the respiratory tract below the vocal cords. LRTI usually cause disease in the alveolar sacs, and the resulting infections are called pneumonia. Symptoms include shortness of breath, weakness, high fever, coughing and fatigue. There are a number of acute and chronic infections that can affect the lower respiratory tract. The two most common infections are bronchitis and pneumonia. Influenza affects both the upper and lower respiratory tracts. Antibiotics are often thought to be the first line treatment in LRTI however these are not indicated in viral infections. It is important to use appropriate antibiotic selection based on the infecting organism and to ensure this therapy changes with the evolving nature of these infections and the emerging resistance to conventional therapies. It has also become apparent the importance of atypical pathogens such as *C. pneumoniae, K. pneumoniae, M. pneumonia,L. pneumophila, E. coli and P. aeruginosa* (Mossad, 2005).

A kind of LRTI is pneumonia. Pneumonia is an infection of the lungs that is caused by bacteria, viruses, fungi, or parasites. It is characterized primarily by inflammation of the alveoli in the lungs or by alveoli that are filled with fluid (alveoli are microscopic sacs in the lungs that absorb oxygen). At times a very

serious condition, pneumonia can make a person very sick or even cause death. Although the disease can occur in young and healthy people, it is most dangerous for older adults, babies, and people with other diseases or impaired immune systems. In the United States, more than 3 million people develop pneumonia each year, and about 17% of these receive treatment in a hospital. Most people with pneumonia recover, but about 5% will succumb to the condition. Pneumonia is divided into Hospital-acquired pneumonia (HAP) and Community-acquired pneumonia (CAP) (Victor, 2008).

2.1.2.2.1 Community acquired pneumonia

Community-acquired pneumonia refers to pneumonia acquired outside of hospitals or extended-care facilities. The signs and symptoms of acute pneumonia develop over hours to days, whereas the clinical presentation of chronic pneumonia often evolves over weeks to months (Schmitt, 2001).

2.1.2.2.2 Hospital acquired pneumonia

Hospital-acquired pneumonia develops at least 48 h after hospital admission. The most common pathogens are gram-negative bacilli. Symptoms and signs are the same as those for community-acquired pneumonia, but in ventilated patients, pneumonia may also manifest as worsening oxygenation and increased tracheal secretions. Diagnosis is suspected on the basis of clinical presentation and chest x-ray and is confirmed by blood culture or bronchoscopic sampling of the lower respiratory tract. Treatment is with antibiotics. Overall prognosis is poor, due in part to co-morbidities. Important pathogens include enteric gram-negative bacteria (mainly *Enterobacter sp, Klebsiella pneumonia,*

Escherichia coli, Pseudomonas aeruginosa,Proteus sp, Acinetobacter sp). Ventilator associated pneumonia refers to pneumonia that occurs more than 48 hours after endotracheal intubation (Bartlett, 2004).

2.1.5.3.2.1 Ventilator-associated pneumonia (VAP)

Ventilator-associated pneumonia (VAP) is a sub-type of hospital-acquired pneumonia (HAP) which occurs in people who are receiving mechanical ventilation. VAP is not characterized by the causative agents. Instead definition of VAP is restricted to patients undergoing mechanical ventilation while in a hospital. Ventilator associated pneumonia refers to pneumonia that occurs more than 48 hours after endotracheal intubation. A positive culture after intubation is indicative of ventilator-associated pneumonia and is diagnosed as such. In order to appropriately categorize the causative agent or mechanism it is usually recommended to obtain a culture prior to initiating mechanical ventilation as a reference (Kollef, 2008).

2.1.3 Etiology

Respiratory tract infection usually caused by viruses. However, a bacteria or fungus can be its cause as well. Most common infecting agents includes:

- *Streptococcus pneumoniae* (35-50%),
- *Chlamydia pneumoniae* (14-20%),
- *Haemophilus influenzae* (5-6%),
- *Mycoplasma pneumonia*(14%),
- *Pseudomonas aeruginosa* (6%).

- *Rhinoviruses* (52%),

- *Coronavirus OC43 or 229E* infection (8%),

- *Influenza A or B virus* (6%),

- *Parainfluenza virus*(7%) (Flaherty, 2003)

2.2 *Pseudomonas aeruginosa*

2.2.1 Definition

Pseudomonas aeruginosa is member of the Gamma Proteobacteria class of Bacteria. It is a Gram-negative, aerobic rod belonging to the bacterial family *Pseudomonadaceae.* It is about 1-5 µm in length and about 0.5-1.0 µm in breadth and is an obligate aerobe, which means it requires oxygen and uses aerobic respiration as its choice of metabolism. *Pseudomonas aeruginosa* can also proliferate in anaerobic conditions. *Pseudomonas aeruginosa* is an opportunistic human pathogen because it seldom infects healthy individuals. Instead, it often colonizes immunocompromised patients. *Pseudomonas aeruginosa* is the second most common Gram-negative bacteria encountered by physicians worldwide for respiratory tract infection. It is a common hospital-acquired pathogen, nosocomial pneumonia (Joshua, 2000).

2.2.2 Morphology and Cell metabolisme

Pseudomonas aeruginosa is a Gram-negative rod. It is an obligate aerobe, which means it requires oxygen and uses aerobic respiration as its choice of metabolism. Due to its capability to synthesize arginine, *Pseudomonas aeruginosa* can also proliferate in anaerobic conditions. This, then, makes *Pseudomonas aeruginosa* a very ubiquitous microorganism, for it has been found

in environments such as soil, water, humans, animals, plants, sewage, and hospitals (Irvin, 2003).

Since *Pseudomonas aeruginosa* is a Gram-negative microbe, it has an outer membrane which contains Protein F (OprF). OprF functions as a porin, allowing certain molecules and ions to come into the cells, and as a structural protein, maintaining the bacterial cell shape. Because OprF provides *Pseudomonas aeruginosa* outer membrane with an exclusion limit of 500 Da, it lowers the permeability of the outer membrane, a property that is desired because it would decrease the intake of harmful substances into the cell and give *Pseudomonas aeruginosa* a high resistance to antibiotics (Irvin, 2003).

Pseudomonas aeruginosa uses its single and polar flagellum to move around and to display chemotaxis to useful molecules, like sugars. Its strains either have a-type or b-type of flagella, a classification that is based primarily on the size and antigenicity of the flagellin subunit. The flagellum is very important during the early stages of infection, for it can attach to and invade tissues of the hosts. Similarly to its flagellum, *Pseudomonas aeruginosa* pili contribute greatly to its ability to adhere to mucosal surfaces and epithelial cells. Specifically, it is the pili's tip that is responsible for the adherence to the host cell surface. Overall, *Pseudomonas aeruginosa* flagellum and pili have similar functionality (for attachment) and structure (both are filamentous structures on the surface of the cell) (Irvin, 2003).

2.2.3 Pathogenesis and Virulence Factors

Pseudomonas aeruginosa rarely causes disease in healthy humans. It is usually linked with patients whose immune system is compromised by diseases

or trauma. First, *Pseudomonas aeruginosa* adheres to tissue surfaces using its flagellum, pili, and exo-S. Then, it replicates to create infectious critical mass and lastly, it makes tissue damage using its virulence factors. Since the powerful exotoxins and endotoxins released by *Pseudomonas aeruginosa* during bacteremias continue to infect the host even after *Pseudomonas aeruginosa* has been killed off by antibiotics, acute diseases caused by *Pseudomonas aeruginosa* tend to be chronic and life-threatening (Irvin, 2003).

Pseudomonas aeruginosa secrets many virulent factors to colonize the cells of its host. For example, exotoxin A, the most toxic protein produced by *Pseudomonas aeruginosa*, catalyzes the ADP-ribosylation to form ADP-ribosyl-EF-2, which inhibits the protein synthesis of the host's cells. Moreover, elastase, an extracellular zinc protease, attacks eukaryotic proteins such as collagen and elastin and destroys the structural proteins of the cell. It also breaks down human immunoglobin and serum alpha proteins (Ramphal, 2008).

Table 2.1 Main Putative Virulence Factors of *Pseudomonas aeruginosa*

Substance /Organelle	Function	Virulence in Animal Disease
Pili	Adhesion to cells	Unknown
Flagella	Adhesion motility,inflammation	Yes
Lipopolysaccharide	Antiphagocytic activity,inflammation	Yes
Type 3 secretion system	Cytotoxic activity (ExoU)	Yes
Proteases	Proteolytic activity,cytotoxity	Unknown
Phospholipases	Cytotoxicity	Unknown
Exotoxin A	Cytotoxicity	Unknown

(Ramphal, 2008)

2.2.4   Clinical Manifestation

Pseudomonas aeruginosa pneumonia described patients with acute clinical syndrome of fever,chills,cough,and necrotizing pneumonia.The traditional accounts described a fulminant infection,with cyanosis,tachypnea,copious sputum,and systemic toxicity. A sputum Gram's stain showing mainly polymorphonuclear leukocytes (PMNs) in conjuction with a culture positive for *Pseudomonas aeruginosa* pneumonia (Ramphal, 2008).

2.2.5 Clinical Diagnosis

Sputum is material coughed up from the lungs and spit out through the mouth. A sputum culture is done to find and identify the germ causing an infection such as pneumonia. If a specific germ is found, more testing is done to determine which antibiotic will best treat the infection. Sputum must be carefully collected into a sterile container so that germs normally in the mouth don't contaminate the sample. Once in the laboratory, each culture type is handled differently (Rank, 2005).

To confirm the diagnosis of a Pseudomonas infection in the laboratory is done by Gram staining of the sputum. Gram staining (or Gram's method), an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. Gram-postive have thick layer of peptidoglycan over inner cytoplasmic membrane. In Gram-negative bacteria the peptidoglycan layer is thinner and is located between space of the outer and inner cytoplasmic membrane. There are four basic steps of the Gram stain, which include applying a primary stain crystal violet(purple) to a heat-fixed (death by heat) smear of a

bacterial culture, followed by the addition of a trapping agent (Gram's iodine), rapid decolorization with alcohol or acetone, and counterstaining with safranin (red). Bacteria that stain purple are called gram-positive, those that stain red are called gram-negative. (Rank, 2005).

Besides, *Pseudomonas aeruginosa* grows on most common laboratory media, including blood and Mac Conkey agars.Two of identifying biochemical characteristics of *Pseudomonas aeruginosa* are an inability to ferment lactose on Mac Conkey agar and positive reaction in the oxidative test (Rank, 2005).

2.3 Antimicrobial Therapy

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, tetracyclines) inhibit bacterial cell replication but do not kill the organism. Other antimicrobial drugs (e.g. penicillins, cephalosporins, aminoglycosides) are usually bactericidal: they cause microbial cell death and lysis. A few compounds (e.g. sulfonamides) are either bactericidal 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 bacterial infection:

Table 2.2 Classification of Antibiotics

Class	Groups	Examples
β-lactam	Penicillin	Amoxycillin
	Cephalosporin Generation I	Cephalothin, Cefazolin
	Cephalosporin Generation II	Cefuroxime, Cefoxitin
	Cephalosporin Generation III	Cefotaxime, Ceftriaxone
	Cephalosporin Generation VI	Cefepime, Cefpirome
	Carbapenem	Meropenem
Aminoglyco-side		Kanamycin, Gentamycin, Netilmycin, Amikacin.
Quinolone		Ciprofloxacin, Ofloxacin, Norfloxacin
Tetracyclines		Tetracycline, Doxycycline
Others		Chloraphenicol, cotrimoxazole, Sulfonamide

(Port, 2006)

2.3.2 Recommended antibiotics for *Pseudomonas aeruginosa* infection

Most experts recommend starting with 2 antipseudomonal antibiotics and then de-escalating to monotherapy. Deciding when to switch from combination therapy to monotherapy according to the American Thoracic Society-Infectious Diseases Society of America guidelines for ventilator-assisted pneumonia, start with combination therapy that includes a beta-lactam and

aminoglycoside for 5 days and de-escalate to monotherapy based on organism culture sensitivity (Qarah, 2009).

Pseudomonas aeruginosa is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed. The *P.aeruginosa* is already limited to only several antimicrobial classes, in addition emergence of multidrug resistance *P.aeruginosa* (MDRPA) compromises most of the antipseudomonals (except colistin and polymyxin B therapies) and combinations of antibiotics to which MDRPA strains are resistant (Marilee, 2005).

As the prevalence of MDRPA increases and treatment options become limited, various antimicrobial combinations have been proposed as an alternative in clinical practice despite resistance to one or both agents in the combination. Interactions between antibiotics are difficult to explore in patients, especially when bacteria are resistant to both agents within the combination (Marilee, 2005).

Because of differences in antimicrobial combinations, concentrations of these agents, and strains tested, reported results vary significantly. The evaluations indicate that rates of efficacy range from 0-25%, 0-30%, 0-17%, and 0-33% for two β-lactams, β-lactam plus fluoroquinolone, β-lactam plus aminoglycoside, and fluoroquinolone plus aminoglycoside combinations, respectively. Although only two studies examined triple combination therapies, they report higher rates of synergy if the combinations included two β-lactams. For example, rates of efficacy for β-lactam plus aminoglycoside combinations ranged from 0-71% in these studies. However, when two β-lactams were combined with an amino-glycoside, the rate of efficacy improved to 43-100% (Marilee, 2005).

2.3.2.1 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, 2002).

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, 2002).

This mechanism of resistance is believed to be acquired in 2 ways; hyper production of chromosomal β-lactamase and the production of inhibitor resistant TEM (Transposable Element) enzymes and deficiency in OMP F and/or OMP C porins (Marre, 2003).

2.3.2.2 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, 2008).

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 (Katzung, 2008).

2.3.2.3 Doxycycline

Doxycyclineis a member of the tetracycline antibiotics group and is commonly used to treat a variety of infections. Doxycycline is a semi-synthetic tetracycline invented and clinically developed in the early 1960s. Doxycycline inhibits cell growth by inhibiting translation process of protein synthesis. It binds to the 16S part of the 30S ribosomal subunit and prevents the amino-acyl tRNA from binding to the A site of the ribosome. The binding is reversible in nature (Fred, 2006).

Cells become resistant to Doxycycline by at least three mechanisms: enzymatic inactivation of doxycycline, antimicrobial efflux, and ribosomal protection. Inactivation is the rarest type of resistance, where an acetyl group is added to the molecule, causing inactivation of the drug. In efflux, a resistance gene encodes a membrane protein that actively pumps doxycycline out of the cell. This is the mechanism of action of the doxycycline resistance gene on the artificial plasmid pBR322. In ribosomal protection, a resistance gene encodes a protein that can have several effects, depending on what gene is transferred (Fred, 2006).

2.3.2.4 Amikacin

Amikacin is a semisynthetic derivate of kanamycin to which it resembles in pharmacokinetics, dose and toxicity. The outstanding feature of amikacin is its resistance to bacterial aminoglycoside inactivating enzymes. Thus it has the widest spectrum of activity, including many organisms resistant to other aminoglycosides. The range of conditions in which amikacin can be used is the same as for gentamicin. It is recommended as a reserve drug for hospital acquired gram negative bacillary infections where gentamicin/tobramycin resistance is high. Three mechanisms of resistance have been recognized, namely ribosome alteration, decreased permeability, and inactivation of the drugs by aminoglycoside modifying enzymes (Port, 2006).

2.4 Antimicrobial Susceptibility Testing

Antibiotic sensitivity testing is carried out to determine the appropriate medicine antibiotic agent to be used in a particular bacterial strain isolated from clinical specimens. Antibiotic sensitivity testing can be carried out by two broad methods which are disc diffusion tests and dilution tests (Parija, 2009).

2.4.1 Disc Diffusion Tests

Disc diffusion tests are most commonly used methods in a laboratory to determine susceptibility of bacteria isolates to antibiotics. In this method, the discs impregnated with known concentartions of antibiotics are placed on agar plate that has been inoculated with a culture of bacterium to be tested. The plate is incubated at 37˚C for 18-24 hours. After diffusion, the concentration of antibiotic usually remains higher near the site of antibiotics disc but decreases

with distance. Susceptibility to the particular antibiotic is determined by measuring the zone if inhibition of bacterial growth around the disc (Parija, 2009)

To evaluate whether the isolates microbes are sensitive of resistant to wards antimicrobial drug, the diameter of clear zone is compared using a standard table chart that is recommended by National Committee for Clinical Laboratory Standard (NCCLS).By using the NCCLS table microbes which are sensitive,intermediate-sensitive and resistant can be known.Results of disc diffusion tests are interpreted as follows:

a. Sensitive (S)   : Infection treatable by the normal dosage of the antibiotic.

b. Intermediate (I): Infection may respond to higher dosage

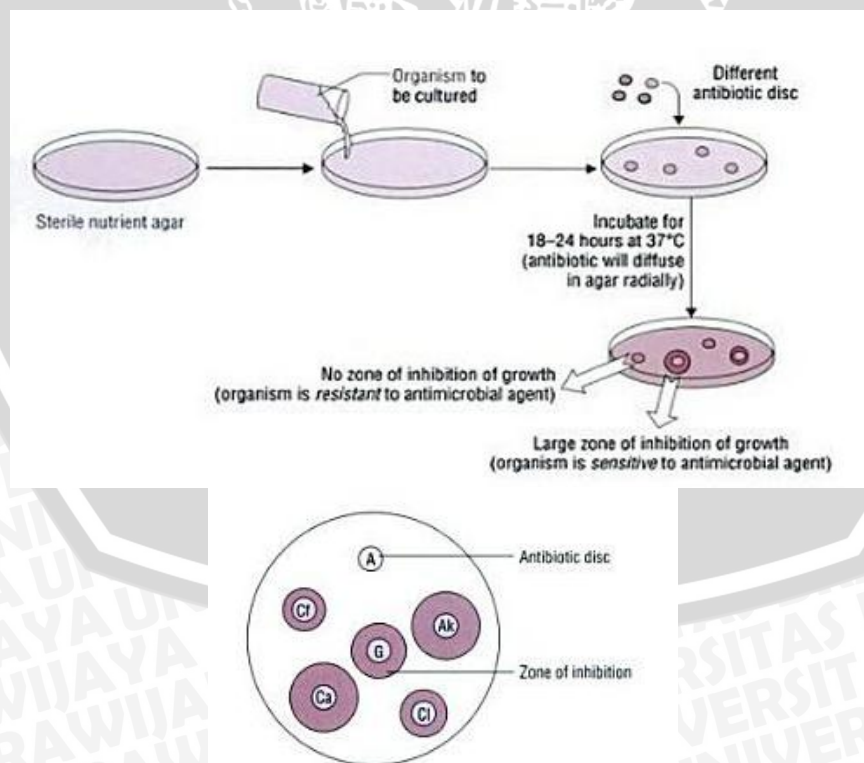c. Resistant (R)   : No respond to usual dosage of the antibiotic (Parija, 2009).

Figure 2.1 Schematic diagram showing the performance of antibiotic sensitivity testing by disc diffusion method (Parija, 2009).

2.4.2 Dilution Method

Dilution method can be done to identify the value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). MIC is defined as the lowest antimicrobial concentration that inhibits the bacterial growth while MBC is the lowest antimicrobial concentration that kills the bacteria. Estimation of these values is useful to regulate the therapeutic dose of the antibiotic accurately in the treatment of life threatening situation. Dilution method can be done by using either broth or agar method. In dilution method we use antimicrobial solution in decreasing concentration by serial dilution (Parija, 2009)