CHAPTER 2

2. REVIEW OF LITERATURE

2.a. HISTORY OF RESISTANCE

Antibiotics originally are structural and molecular agents with their potency to ability to cease the growth of bacteria at high concentrations (Cantas et al., 2013). The history shows that along with the discovery of antibiotics, the resistance also emerged. Antibiotic-resistant pathogens have soon been reported after the introduction of new drugs in hospitals where antibiotics were intensively used. In year 1929, Alexander Flaming discovered antibiotic Penicillin (Franco et al., 2009). But in year 1940, before the known use of penicillin in therapy, bacterial penicillinases, genes encoding for these enzymes, responsible for resistance to penicillins, were discovered (Schechner et al., 2013). In 1944 antibiotic Streptomycin was discovered to cure of Tuberculosis, which was further seen changed by simultaneous discovery of resistant genes for Streptomycin, confirming that the resistance comes along with antibiotic. Another study in year 1942 showed the resistance for particular antibiotic, where a many people were cured by Penicillin. 4 out of them, whose treatment was being done, were taken for serial cultures, showed decreased sensitivity to the antibiotic. In year 1947, a British physician showed resistance in Staphylococcus pyogenes (Schechner et al., 2013). In year 1981 Canada’s first Methicillin Resistant Staphylococcus aureus was emerged. Then first Vancomycin Resistant Enterococcus was discovered in 1993 in Canada. It soon became evident that development of resistance would present a clear serious therapeutic dilemma to the clinical community (Barber, 1947; Schechner et al., 2013).

2.b. COMMENSALS: RELATION TO INFECTIONS

When two different species live together for the benefit of each other this is called symbiotic relationship. Because of outnumbering of the symbiotics, pathogens are unable to colonize in humans generally. These bacteria also help in breaking down the nutrients or by producing some enzymes in maintenance of health. Symbiotics are also helpful in formation of immunity by imparting similar antigenic characters to that of pathogenic bacteria (Allen et al., 2008). Although, all symbiotic normal flora adds value to human health, but there are a small number of bacteria in symbiotic microbiota that may be harmful e.g., Staphylococcus aureus, Escherichia
coli and members of Enterobacteriaceae like Klebsiella *pneumonia* (O’Hara and Shanahan, 2006; Cogen *et al.*, 2008). These micro-organisms are causative agents for many infections like, nosocomian and community acquired infections.

No child can be assumed sterile once it is out of uterus. At the time of delivery the child starts getting contact with microflora and it can never be reverted again (Berg, 1996; Fanaro, 2003). Approximately $10^{14}$ bacteria are carried by each human being in the intestines alone, which outnumbers ten folds of the human cells (Berg, 1996). All these bacteria present in our body together are known as commensal microflora or commensal microbiotas which are different according to the different sites of body. For an instance the commonest microflora of skin is *Staphylococcus epidermidis* (Cogen *et al.*, 2008), while in the colon it is *Bacteroides spp* (Berg, 1996). However, colon has the highest number of colonizing bacteria (O’hara and Shanahan, 2006).

There have been a lot of discussion on wiser use of antibiotics to prevent antibiotic resistance, but the suitability of an antibiotic for the treatment of an infection is sometimes skipped to discuss. The suitability of an antibiotic is determined chiefly by the location of infection, the responsible bacteria and the resistance pattern of this pathogen to particular antibiotics. Initially the treatment of many infections is started based on observations, like kidney and bladder infections or cellulitis/ fascitis cases. Where hit and track antibiotics that are started for treatment, the susceptibility of pathogen to them are not known. Hence, the probable causative agent and the susceptibility should be taken into account to make a suitable choice of antibiotics.

Urinary Tract Infections vary from mild self-limiting cystitis to prostatitis and pyelonephritis and end into urosepsis (Johnson *et al.*, 2005). Actually UTI’s being very common infection among patients of all populations and have become one of the main indicators for antibiotic treatment.

The most commonly found bacteria responsible for urinary tract infections include *E. coli*, Klebsiella *pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus*, *Proteus* and *Enterobacter cloacae* (Das *et al.*, 2009; Den *et al.*, 2010). However, the species distribution differs widely as per patient population and locality, in UTI’s *E. coli* is the number one causative organism in any patient population whereas, *S. saprophyticus* is more often associated with young population. *K. pneumoniae* is isolated often from elderly patients (Den *et al.*, 2010). Some bacteria such as
Proteus spp. and Enterobacter spp. also with Klebsiella spp. are observed causing UTI more among hospital admitted patients and the people who live in nursing homes (Das et al., 2009; Swab, 2011).

Whereas, in epideral infections or and some other tissue infections the most common bacterial pathogens are GPC’s like S. aureus and Streptococcus pyogenes. The infections of skin are often mild up to impetigo or cellulitis but these infections can be more severe lead to necrotizing fasciitis and sometime can be followed with signs of systemic involvement (Stevens et al., 2005). In mild skin infections like impetigo, often topical antimicrobial agents are suggested (Schofer et al., 2010). The most common commensal bacteria are discussed below:

I. Staphylococcus aureus

Staphylococcus aureus is the bacteria first described by Sir Alexander Ogston in year 1880 (Lowy, 1998). It is a facultative anaerobe, potentially pathogenic, Gram positive cocci and normal flora of skin and mucosa. It is normally found in the anterior nares. The pathogen is cultured from many body sites, as there are many locations for the bacteria to colonies. Staphylococcus aureus is responsible for causing many infections including community acquired, nosocomian infections and cellulitis or fasciitis mainly mild epidermal infections, like erysipelas, cellulitis, severe to fatal ones, such as necrotizing pneumonia, bacteremia and cardiac infections (Lowy, 1998). The persistent carriers of Staphylococcus aureus are at more risk of getting infections than intermittent or noncarriers. Generally among normal flora S. aureus infections are the commonest infections (Von et al., 2001; Wertheim et al., 2005; Bode et al., 2010).

II. Enterococcus

These are GPC, diploid, in smaller chains Enterococci are Gram-positive, diplococci that exist in pairs or short chains, and are tough to differentiate from streptococcus relying on morphology only (Fisher and Phillips, 2009). E. faecalis and E. faecium are the two clinically important species of these bacteria that are normal flora of human intestine (Fisher and Phillips, 2009). Underlying conditions like low immunity or age factors may contribute to the infections caused by Enterococci. This spp. mainly causes urinary tract infections, bacterial endocarditis, bacteremia, and may affect intestine or brain as well (Zhanel et al., 2001, Kurup et
Most appropriate antibiotics that are used enterococcal infections are Ampicillin, Penicillin and Vancomycin (Tüngeret al., 2004). The cases where resistance to Vancomycin is reported, Nitrofurantoin can be given as an alternative (Guardado et al., 2006).

Natural resistnace in this bactria is very high. Many of this bacteria resistnac to penicllins naturally and many aminoglycosides. Since last two decades, some strains of Enterococci have shown resistance to Vancomycin These cases are found mainly in USA and other developed countries responsible for nosocomial infections (Jin et al., 2004).

III. Escherichia coli

Escherichia coli are the organism first described by Theodor Escherich in 1885 (Shulman et al., 2007). It is a facultative anaerobe, potentially pathogenic, Gram negative bacilli and normal flora of intestine. E. coli has been found responsible for intra as well as extra intestinal infections. These infections are also caused by patients own microflora (Yamamoto et al., 1997; Beerepoot et al., 2012). E.coli infection severity depends upon virulence factors that are associated with the pathogen. Depending upon these virulence factors, the extra intestinal infections caused by E. coli vary from mild to severe infections i.e., self-limiting cystitis, urosepsis and meningitis (Levinson, 2010). The virulence factors are encoded by various genes that are located on pathogenicity islands (Escobar-Paramo et al., 2004). These Extra intestinal E. coli survive outside the intestine with the help of many adhesins and iron acquisition systems, whereas intra intestinal (diarrhoea causing) E. coli cause infection by injecting toxins into the host cells that are expressed with the help of specific secretion systems (Wiles et al., 2008).

IV. Klebsiella pneumoniae

Klebsiella pneumoniae was first described by Carl Friedlander, named after German bacteriologist Edwin Klebs in year 1883. This is a Gram negative rod, an opportunistic but a potential pathogen with facultative anaerobic properties (Wiles et al., 2008). Unlike E. coli, this bacterium has to put special systems for survival anywhere, also commonly found in the environment (Podschunet al., 1998). Like E.coli; it has many virulence factors that are responsible for causing infections. The range of infections is not limited to urinary and respiratory tract infections only. In lowered cared of patient with compromised immunity, the Infections can be severe as bacteremia also (Levinson, 2010).
V. Proteus

Proteus is a Gram-negative bacillus. Proteus bacilli are broadly distributed in nature as saprophytic bacteria, being found in decomposing animal matter, sewage and in human and animal faeces. Proteus is opportunistic pathogen, causing urinary and septic infections in hospital admitted patients (Matsuyama, 2000). P. vulgaris is normal flora of human intestine and manure. P. vulgaris often affects immune suppressed patients. Proteus has three species that are infective to humans: P. vulgaris, P. mirabilis, and P. penneri. The main infection caused by this species is urinary tract infections. Proteus species is responsible for skin and urine infections. Generally, the strains of P. mirabilis are Ampicillin, Cephalosporins susceptible (Rauprich et al., 1996). However, P. vulgaris is not sensitive to these antibiotics. Whereas, P. mirabilis, though found in soil and water normally, once attached to the urinary tract, it is more prone to infect kidneys than E. coli (Guentzel, 1996).

VI. Pseudomonas

Pseudomonas is aerobic Gram-negative bacilli (Euzeby, 1997). These bacteria have property of colonizing in a wide range of niches. The most common species of these bacteria is Pseudomonas aeruginosa. This is also known as an opportunistic human pathogen. The intrinsic resistance has been found to Penicillin and beta lactems likewise rest of Gram Negative Bacteria. These pathogens are found generally sensitive to Piperacillin, Imipenem, Ticarcillin, or Ciprofloxacin (Palleroni, 2010). Aminoglycosides such as Tobramycin, Gentamicin, and Amikacin can also be used for therapy (Van, 2003).

Pseudomonas aeruginosa possesses property of low antibiotic susceptibility. Pseudomonas species has also been seen showing resistance to many antibiotics being Multi drug resistant (MDR). This occurs due to many genetic alterations acquired or intrinsic mainly by horizontal transfer of antibiotic resistant genes (Van, 2003).

VII. Acinatobacter

Acinatobacter is a Gram-Negative Bacilli, seen in pairs under microscope (Viscaet et al., 2011). These bacteria are non-motile and oxidase-negative. A. baumannii has always been reported as one of the causative bacteria responsible for nosocomian infections like bacteremia,
urinary tract infections (UTIs), secondary meningitis and infective endocarditis. Wound and burn infections are also caused by these bacteria (Rokhbakhsh-Zamin et al., 2012). Studies have reported the bacteria responsible for ventilator-associated nosocomial pneumonia. According to the studies, this bacterium is resistant to a wide range of antibiotics. Resistance to Carbapenems has also been reported (Hu et al., 2006).

Acinatobacter baumannii infections in seriously ill patients during their stay in hospitals has mortality rates ranging from 20% to 60% (Falgas et al., 2006).

VIII. Enterobacter

Enterobacter is a facultatively anaerobic GNB. The genus Enterobacter is a part of the Coliforms. This is a non-sporeing bacterium (Tan et al., 2014). Two significant strains of Enterobacter responsible for causing illness in humans are E. aerogenes and E. cloacae. There have been many Enterobacter strains causing infections to humans like nosocomial infections in immuno-compromised and hospitalized patients. It may also cause infection to the patients, who are on mechanical ventilation. Enterobacter spp. mainly causes urinary and respiratory tracts infection (Cabral, 2010).

IX. Citrobacter

Enterobacteriaceae family also includes Citrobacter species that are Gram negative in their staining reaction. The clinically significant species of these bacteria are C. amalonaticus, C. koseri, and C. Freundii. This bacterium uses citrate for its energy consumption in form of carbon. These are the normal flora in human intestine and rarely causes urinary tract infections that too in immuno compromised patients. Some cases of infant meningitis and sepsis caused by these bacteria have also been reported. C. freundii has resistance genes to Ampicillin and first-generation Cephalosporins. The plasmid encoded resistance too may be responsible for resistance to many other antibiotics too. These bacteria have inducible ampC genes encoding resistance to antibiotics. (Badger et al., 1999).

These bacteria are found in soil, water and wastewater, etc. They can also be found in the human intestine.

As antibiotics are being used in drastic ways these days, there is a strong need of antibiotics with selective toxicity that harms pathogens only but non-pathogenic bacteria or human cells. But
antibiotics do not differentiate between pathogenic bacteria and nonpathogenic bacteria or human cells (Willing et al., 2011). These results in a disturbance of the normal microbiota of human body, however, the usage of antibiotic and type of antibiotic used effects on the degree of disturbance (Willing et al., 2011). The commonly used antibiotics to treat infections include Nitrofurantoin, Fosfomycin, the Fluoroquinolones and Amoxicillin-Clavulanic Acid for UTI and Flucloxacillin, the Macrolides, Clindamycin and Fusidic Acid for the treatment of epidermis infections or necrotizing tissue infections.

2.c. ANTIBIOTICS FOR EMPIRIC TREATMENT

I. Nitrofurantoin

Nitrofurantoin is commercially available antibiotic since 1953. The antibiotic is activated by the enzyme produced by bacteria that is known as nitroreductases immediately after influx into the bacterial cell and then only it can start inhibiting many bacterial enzymes thus interfering in the nucleic acid synthesis, carbohydrate metabolism and other metabolic processes (Guay, 2001). Nitrofurantoin ahs wide range of a target, that is why it is very useful in treating both GNB and GPC. GNBlike Pseudomonas spp, Serratia spp. and Proteus spp. are non-susceptible to resistant to Nitrofurantoin naturally (Guay, 2001; Gilbert et al., 2009). Excretion of the antibiotic Nitrofurantoin (unchanged), via the kidneys, is approximately 40%. The highest concentration of this antibiotic in urine is reached after 4-5 hours. Its properties of low tissue penetration and high concentration in the urine make it a suitable agent suitable for uncomplicated cystitis (Charalabopoulos et al., 2003). The specific severe side effects of this antibiotic include polyneuropathy in old age people and in those with renal impairment; another side effects include nephrotoxicity or hepatotoxicity among patients (Karageorgopoulos et al., 2012).

II. Fosfomycin

Fosfomycin is one of the oldest antibiotics that have been used for many years mainly to cure uncomplicated cystitis (Wiles et al., 2008). Fosfomycin also has a broad spectrum antibacterial activity on both GPC like Staphylococcus and Enterococcus and Gram Negative Rods e.g. enterobacteriaceae and Pseudomonas aeruginosa. Antibiotic Fosfomycin interferes with the synthesis of N-acetylmuramic acid that is a precursor of peptidoglycan, thus the cell wall synthesis is inhibited (Garau, 2008). It also helps in reducing infection by decreasing the
adhesion of the bacteria to the epithelia of bladder (Garau, 2008). It is excreted in urine at a high rate. The antibiotic Fosfomycin has a good tissue penetration and overall fewer side effects. Resistance to Fosfomycin has been low globally. (Naber et al., 2008) Fosfomycin has also shown results against Extended Spectrum Beta Lactamases producing strains. (Garau, 2008; Karageorgopoulos et al., 2012) Despite its favorable characteristics, Fosfomycin is only directed to treat simple urine infections and it is not indicated for other infections.

III. Fluoroquinolones/Quinolones:

Quinolones are the antibiotics that have been commercially available since 1962 and many comparable antibiotics have been developed since then, including fluoroquinolones. Quinolones are broad spectrum antibiotics, prescribed for many diseases in which the causative organisms could be GPC and GNB and is prescribed in long-term care centers (Nicolle et al., 2000; Daneman et al., 2011). For GPC, The treatment include Levofloxacin and Moxifloxacin and for GNB ciprofloxacin and Norfloxacin (Ruiz, 2003). Quinolones act on bacteria by inhibiting bacterial gyrase and topoisomerase IV. There are needed to coil or uncoil and linkage of bases of the DNA strand at the time of replication (Hooper, 1999; Oliphant and Green, 2002).

IV. Amoxicillin-Clavulanic Acid and Flucloxacillin:

These antibiotics belong to Beta-Lactam group of antibiotics. Amoxicillin-Clavulanic Acid is a combination antibiotic with a beta-lactam antibiotic with a beta-lactamase inhibitor. Flucloxacillin is also not inhibited by enzyme penicillinases. These antibiotics act by inhibiting transpeptidase or Penicillin Binding Protein (PBP), thus inhibiting formation of the peptidoglycan layer, which with the help of transpeptidase is responsible for the cross linkage the different peptidoglycan layers of the bacterial cell wall. Amoxicillin-Clavulining antibiotic that has ability to treat multiple bacterial infections, mild to severe and is also used in complicated cases of UTI (Hooper, 1999). Flucloxacillin is a narrow spectrum antibiotic, which is mainly used to treat Staphylococcus aureus infections. Otherwise, both antibiotics are prescribed in all types of hospital settings (Hooper, 1999).

V. Macrolides and Clindamycin:
Macrolides block the attachment of the next tRNA and translocate ribosomes at 50S RNA. Thus they inhibit the protein synthesis essential for bacteria to survive (Roberts, 2004). Both the Macrolides and Clindamycin are mainly effective against Gram positive infections (Leclercq, 2002). These agents are not effective against facultative anaerobic Gram negatives bacteria i.e., enterobacteriaceae (Gilbert et al., 2009).

VI. Fusidic Acid:

Fusidic Acid is the bacteriostatic antibiotic that basically inhibits bacterial growth, by preventing the protein synthesis. Protein synthesis is prevented by binding of elongation factor G on the ribosome (Howden et al., 2006). Fusidic acid is potent in killing Gram positive aerobes as well as anaerobes such as Staphylococci, Corynebacterium. The Enterobacteriaceae are intrinsically resistant to this antibiotic and the effect of this antibiotic towards Streptococci and Enterococci is also limited (Howden et al., 2006). Fusidic acid is used both in systemic and topical formulations. In Netherland, systemic Fusidic Acid is used in lesser frequencies (Swab, 2012), whereas the topical use of Fusidic Acid is much higher (Rijinders et al., 2012). The general use of Fusidic Acid is to cure epidermal illness topical formulations (Rijinders et al., 2012). Overall the uses of this antibiotic are high with fewer side effects (Schofer et al., 2010).

2.d. MODE OF ACTION OF ANTIBIOTICS-OVERVIEW

Antibiotic groups Beta-Lactams apply their antimicrobial action by inhibiting cell-wall synthesis thus disturbing transpeptidase and carboxypeptidase enzymes activities. These enzymes alter the penicillin binding proteins (PBP) of S.aureus to impart resistance to Methicillin. The altered PBP is known as PBP2aor PBP2. PBP is encoded by mecAgene in bacteria that has reduced binding potency for beta-Lactams; hence drugs like Methicillin such as Cloxacillin become unable to disrupt cell-wall synthesis. The reason of Penicillin resistance in pneumococci is also altered PBP. Generally Pneumococci have 6 PBPs, resistance results from altered pbp1a, pbp2b, and pbp2x low-affinity, encoded by complex genes that are supposed to have genetic material acquired from other species of bacteria like Streptococcus mitis (Fluit et al., 2001). The glycopeptides class of antibiotics e.g., Vancomycin and Teicoplanin, act on bacteria by attaching to the D-alanyl–D-alanine side chains of peptidoglycan of cell wall of bacteria, which prevents the cross-linkage of the peptidoglycan chain that ultimately results in disrupted cell wall synthesis. The production of a new ligase, VanA, that is encoded by the vanAgene that helps in
producing peptidoglycan side chains with less binding tendency for glycopeptide antibiotics imparts resistance to Vancomycin and Teicoplanin. The gene vanB1-3 imparts resistance to Vancomycin only. Though Vancomycin-Resistant Enterococci are very common pathogens till date, responsible for nosocomial infections in immunocompromised hosts, Vancomycin-resistance has also arrived into much more virulent strains of S. aureus (VRSA) (Bartley et al., 2002; Chang et al., 2003). For antibiotics like Aminoglycosides (Gentamicin, Tobramycin, Streptomycin), Tetracyclines and the MLS group of antimicrobial Macrolides, Lincosamins and Streptogramins, protein synthesis inhibition in the ribosomes is the main target, where some genes encode specific enzymes that include and alter ribosomal targets that confer resistance to these drugs. MLS antibiotics have a broad selection of resistance mechanisms. In case of Gram-Positive bacteria, erm genes alter in 23S rRNA that confers non-susceptibility to Lincosamides, Macrolides and Streptogramin B, except Streptogramin A (Fluit et al., 2001). Other enzymes like Aminoglycoside modifying enzymes impart resistance to aminoglycosides. These enzymes are effective only in a good quantity and they act by altering rRNA. In Gonococcus and S. aureus Tetracycline resistance is caused by altered ribosomal target that is encoded by Tet M gene. The action of antibiotic group Quinolones against bacteria is by stopping the bacterial gyrase that is the principle element to coil the DNA. In Gram-Negative Rods, specifically in the GyrA subunit encoded by the gyrA gene is altered by DNA gyrase. Whereas, in Gram-Positive bacteria, fluoroquinolones resistance results by the alteration in topoisomerase IV enzyme. The antibiotic classes of folate inhibitors i.e., Trimethoprim and Sulfonamides inhibit the function of bacterial cell by inhibiting folic acid synthesis. In bacterial cell, altered target enzymes DHFR and DHPS impart resistance to folate antagonists; for Trimethoprim for Sulphonamide respectively.

2.e. BACTERIAL ANTIBIOTIC RESISTANCE

Since late 60’s, the unbalanced use of antibiotics in medicinal world has proceeded into the prominence of many bacterial strains that show non-susceptibility to some of the antibiotics (McDermott et al., 2003). Antibacterial resistance progresses by different mechanisms: a) cell wall or membrane of bacteria, permeability changes, reducing the access of bacteria to active sites; b) working efflux action of the antibiotics out of bacteria; c) mutations of the target sites;
d) degradation or changes in the antibiotic enzymatically; e) adaptation of new, changed metabolic pathways, which are inhibited by the specific antibiotic.

Bacteria could be resistant to many of the antibiotics. As we know that the mechanism of resistance to could be adapted or intrinsic in which the resistance is. Where as in acquired the resistance is imparted to bacteria by HGT from the resistant bacteria to sensitive organisms rendering the sensitive one to be resistant (Tenover, 2006). Intrinsic resistance may be explained as a natural event which is a feature of physiology or genetic constitution and is represented by all members of same species (Harbottle et al., 2006). Vis a vis, Enterococcus species that are naturally non-susceptible to cephalosporins because of reduced binding tendency to the binding proteins that bind to penicillins. Whereas, acquired resistance may comes as the adaptation of an external resistance gene or some alterations in the structural DNA of the bacteria (Harbottle et al., 2006). Acquired resistance may not only be available within whole populations of bacteria but also within a specific sequence of species from sensitive mother strain.

2.f. GENERAL REASONS OF ANTIBIOTIC RESISTANCE

Discovery of antibiotics in medical sciences had been proven to be a boon. As the time is passing it has drastically entered into a threat of resistance. It has been observed in various studies that lifestyle, personal habits and hygiene are associated with antibiotic resistance (Padarukh et al., 2014). Where these factors are found poor and unhealthy, resistance has been seen risen by flourishing the growth of multiple microorganisms. A study in Canada revealed that 30% -40% antibiotic resistance is due to unwashed hands of clinical or hospital personnel (Johnson et al., 2013).The length of stay of patients in hospital also plays a role in raising resistance. The prolonged the stay in hospital, more the chances of getting nosocomial infections and it is known that nosocomial bacteria are highly resistant towards particular antibiotics (Davies et al., 2010).

Many studies have been initiated to find out the reasons of non-susceptbility. The commonest Case of non-susceptibility found was inappropriate use of antibiotics, which can be either misuse or overuse of antibiotics (Caron et al., 2010). For antibiotic overuse, at least three minimum interdependent actions are the main causes. First action is when patient gets antibiotic prescription for any mild infection even simple fever where according to the physicians; there is no need of antibiotics. Physicians also fear about uncontrolled spread of infection, so directly broad spectrum or third generation antibiotic are given to the patients without paying attention
what harms it may give to the patient. Third and important mode of resistance is that many of the countries allow antibiotic sale without any prescription slip. A study in Mexico showed 40% of antibiotic are consumed randomly by people without any prescription or advice by physician. Generic drugs due to cheaper rates are often chosen by middle class population worldwide without thinking of the quality or the adversity related to resistance of the drug (Donkar, 2013). Bacterial resistance attributes to: 1. ability of antibiotic prescriber: a physician, a nurse or a quack. 2. Socioeconomic facilities in a region 3. Poor pharmacodynamic principles and 4. Dose variation by patient (Fernández et al., 2013). Mobility of population, increased industrialization has also imparted a significant role in microbial resistance (Donkar et al., 2013). The negligence or irrational use of antibiotics has also a role in raising antibiotic resistance (Pathak et al., 2013). Lack of urine testing, and non selective use of prophylaxis has raised the broad spectrum antibiotic usage, where microbes are directly made resistant to lower generation drugs (Pathak et al., 2013). In practice of ocular diseases generally antibiotic is given directly to control the infection without knowing identity or susceptibility of bacteria (Bertino et al., 2009), which is a direct invitation to antibiotic resistance. These factors have somehow a role in risen resistance to higher antibiotics like Linezolid, Tigecycline (Long et al., 2012). Carbapenems like Ertapenem, Doripenem are now day’s no more sensitive drugs (Fernandez et al., 2013). Considering so many factors for resistance it is observed that resistance is directly or indirectly proportional to consumption of antibiotics (Hollenbeck et al., 2012, Bell et al., 2014). Moreover, antibiotics like oxytetracycline, tetracycline has also been found in waste water, soil water, animal farms, and rivers, proving their presence in environment (Chancey et al., 2012; Suzuki et al., 2012).

Apart from the general reasons of resisrance mentioned above, the main genetic cause of resistance is that many bacteria that produce antibiotics are resistant to that particular one but they can spread these resistant genes to other susceptible organisms and impart resistance to them. The genes that are present in microenvironment are up taken by susceptible bacteria and become resistant to particular antibiotics (Hawkey, 2008; Donkar et al., 2013).

If considering Gram Negative Bacilli, all GNBs have become resistant to 3rd generation cephalosporins now (Khan et al., 2012). Almost all bacteria have got one or other resistance genes and are non-susceptible to minimum one antibiotic. For an instance, Escherichia coli, the most common commensal flora of gut, has got predominant genes for resistance (Jakobsson et
Once acquired, these genes can very easily be transferred to pathogenic ones (Hoffmann et al., 2011). From 1983-2001, E. coli resistance for Trimethoprim has risen from 0% to 12% (Jungermann et al., 2012). Enterococcus spp. has emerged as nosocomial is also alarming a high risk of spreading resistance genes in hospital admitted patients (Baquero et al., 2011). Enterococcus spp. has drawn a potential role in virtually all clinical antibiotics resistance (Hollenbeck et al., 2012). A data collected in Ghana from sickle cell anemia patients (children) on prophylactic antibiotics that were given to the Sickle cell anemia patients by collecting their nasal swabs and nasopharyngeal swabs, showed isolated strains of Streptococcus pneumoniae with up to (0-11%) resistance and Staphylococcus aureus resistance (40%) (Sandoz et al., 2010). Like Vancomycin Resistant Enterococcus, Methicillin Resistant Staphylococcus aureus, (Colomer et al., 2011; Devirgiliis et al., 2013).

A study on antibiotic combination therapy on Multi drug resistant Acinatobacter baumannii showed that the response towards particular antibiotic can be strain specific (Dent et al., 2010; Kmeid et al., 2013; Lawrence et al., 2013). A research conducted on Helicobacter spp. concluded that standard triple therapies may not be useful as first line treatment in Vietnam (Binh et al., 2013). It is estimated that transfer of genes to clinical pathogens might have occurred by horizontal gene transfer, which is considered as the most appropriate way of gene transfer (Palmer et al., 2010; Bakhshiet al., 2014). The common mobile genetic elements (MGE) are plasmids, transposons or resitomes (Pehrsson et al., 2013). The basic genes for resistance are resitome genes, but there are a few genes which are even not sequenced by sequencers (Davies et al., 2010). Few reports have shown phages for gene transfer options also (Donkar et al., 2013). The evolution of resistance from a single antibiotic frequently leadsto risen resistance to multi drugs (Erez et al., 2010).

2.g. GENETIC METHODS OF RESISTANCE
The main methods of antibiotic non-susceptibility are mainly divided into three broad categories 1) enzymatic inactivation: antibiotic drugs, 2) drug target site alterations, replacements or amplifications to reduce the bonding of the drug to the target 3) with the help of efflux pumps reduction in approach of the antimicrobial agents to the target (Fluit et al., 2001; Sundsfjord et al., 2004).
I. **Enzymatic inactivation of the antimicrobial agent**

There are some of enzymes that inactivate the antimicrobial drugs, the classic example of is the betalactamases. Theses enzymes distort beta-lactams group of antibiotics. Theses enzymes can be chromosomal or plasmid-mediated. Betalactamases are involved in imparting resistance to *S. aureus*, Gram-negative bacilli, Gonococcus and Haemophilus *influenzae*. These enzymes have different antibacterial spectrums arising from penicillinases to complex Betalactamases like TEM-1. Simple penicillinases can hydrolyze Benzylpenicillin, whereas, broad-spectrum Beta-Lactamases, like TEM-1, act by hydrolyzing Oximino-Cephalosporins and hydrolyze Ampicillin, Beta-Lactamases that act onb wide range of bacteria. One more type of BetaLactamases; AmpC Beta-Lactamases and Carbapenemases can neutralize carbapenems also. There are some antibiotics that can be enzymatically modified by certain cytoplasmic modifying enzymes. Aminoglycosides in Gram-Negative Bacilli and Enterococcus are enzymatically degraded by aminoglycoside modifying enzymes (Aminoglycoside Phosphotransferases APH, Acetyltransferases AAC and nucleotidyltransferases ANT). The enzyme Chloramphenicol acetyl transferase in enterobacteriaceaeand Staphylococcus enzymatically modify or inactivate Chloramphenicol and can pose resistance to Chloramphenicol. *EreA, EreB* encode some enzymes that impart resistance for Macrolides. Acetyl transferase can cause resistance to Streptogramin A. Some hydrolyzing enzymes coded by genes like *vgb, vgbB* impart resistance to Streptogramin B. Tetracycline-inactivating enzyme is encoded by the *tetX*gene, but its impact on tetracycline and clinical significance is not clearly known (Fluit *et al.*, 2001).

II. **Impaired access of the antimicrobial agent**

The accessibility of drug to bacterial cell can be reduced by bacteria in two principle ways, 1) by reduction in the permeability for the drug by bacterial cell or 2) with the help of efflux systems that pump out the drugs from the cell. The reduction in the permeability helps Gram-Negative bacteria to be resistant to Macrolides, Lincosamines and Streptogramines, inherently. In *Pseudomonas aeruginosa* and *E. cloacae* this helps in imparting resistance to Beta-Lactam antibiotics and Aminoglycosides (Fluit *et al.*, 2001). Specific efflux pumps to Macrolide and Tetracycline cause resistance to both antibiotics in Staphylococci. Tetracycline efflux pumps help imparting non-susceptibility to Tetracycline in both GNB and GPC with help by TetA-E and Tet G-H genes that encode them. The Efflux pumps that are encoded by NorA confer non-susceptibility to fluoroquinolones in Gram-Negative bacilli and *S. Aureus*. 
2.h. GENETIC TOOLS OF RESISTANCE

I. Horizontal transfer of antimicrobial resistance
Almost genes in cells, which were acquired from other sources have been sequenced (Normark & Normark, 2002). In the study by Nomark and Nomark, it was reported that the DNA that is horizontally acquired normally operates the tasks, which are selectively advantageous to the bacteria e.g., antibiotic resistance, virulent tendency, biodegradation etc. The three basic methods of horizontal gene transfer are: (1) Transformation, the method in this procedure the organism accepts the free DNA directly from the surroundings, (2) Transduction, in this procedure, with the help of bacteriophages, the bacterial DNA is shifted from one cell to other cell of bacteria (3) Conjugation, an activity in which genetic material is transferred from one bacterium to another by cell-to-cell contact. There are various DNA elements that have been reported playing important roles in the building resistance in bacteria (Normark & Normark, 2002). These are plasmids, transposons, genomic islands, phage, integrons and gene cassettes.

II. Plasmid
they are the material that lie extra-chromosomally in the cell and replicates in the bacterial DNA without the aid of chromosomal material (Baron et al., 1996). Plasmids are characteristicly circular, super helical, double-stranded DNA elements. Some non helical, linear plasmids are also studied in some bacteria like Streptomyces and Borrelia (Baron et al., 1996). Plasmids may lie in bacterial cell as many as one to hundred copies, depending upon the size of plasmid. A bacterial cell can also have more than one type of plasmid in it. Size of plasmids is variable from around 5->100 kilo base pairs. Plasmids generally do not participate in growth of bacteria (Baron et al., 1996). The bacteria that carry them are conferred with advantages like antibiotic resistance. Plasmids confer other functions also. The toxins of bacteria and proteins of cell are also made with the help of plasmids. Plasmid also impart role in consumption of hard carbon source for energy production, the synthesis of few antibiotics is also aided by plasmids. Plasmids help in producing many enzymes that help the bacteria to impart resistance to heavy metals.

There are few plasmids that can be transferred to other bacteria while others cannot be. These plasmids contain genes 

itra genes are the genes responsible for making the plasmid transferrable among bacterial cells (Snyder &Champness, 2007). These plasmids use the process of
Transconjugation most commonly. On the other hand, some other plasmids are mobile only; they impart a few. Rest of the functions that are needed to make the plasmid transferrable to cells are not found in other plasmids (Snyder & Champness, 2007). R plasmids is a type of plasmid that is carry resistance genes (Harbottle et al., 2000). They were actually R factors that were initially isolated from Shigella flexneri in the 1950’s (Watanabe & Fukasawa, 1961). From that time, R plasmids have been found in both GPC and GNB. Scientists have found Plasmid-associated resistance genes against almost antibiotics and with their very common property to mediate resistance from one bacterium to multiple antimicrobials simultaneously among different bacterial genera have evolved the resistance very much (Harbottle et al., 2000). Plasmids also help in movement of other genetic elements of antibacterial non-susceptibility, e.g., transposons and integrons.

III. Transposable elements

They are the metrial that is moveable or mobile with features of encoding an enzyme that is quite specific for its site. This enzyme is called as transposase with main feature of required additions and cuttings (Normark & Normark, 2002). There are main 3 kinds of transposones found: 1. Insertion sequence element transposones, 2. composite transposons, and 3. non-composite transposones. Length of IS elements is normally seven hundred fifty bp to two thousand basepairs and they have a gene that encodes transposase enzyme which helps in the transposition and two inverted repitions of DNA at the ends (Snyder & Champness, 2007). That’s why, scientist have identified thousands of different IS elements in bacteria. Generally Plasmids are also constituted of Insertion sequence elements. Two different types of IS are required to form a Composite transposon to bracket the rest of the genes (Snyder & Champness, 2007). In various bacterial species, different determinant of resistance are found in composite transposones. However, non composite transposones are studied to have inverted repeated shorts at ends. Moreover, they are insertion sequences elements completely (Snyder & Champness, 2007). They contain transposase gene which is encoded by tnpA, the resolvase gene encoded by tnpR, and res gene where the resolvase gene acts binds. Noncomposite transposons may also have integrated resistance genes. At the time of transfer of random plasmids or via phage Transposones may move into various genera of bacteria. Few transposones have been reported to be conjugative power. These sometimes can help in forming a bacteriophage also (Harbottle et al., 2006).
They act by changing the whole DNA of the bacterial cell in which the action needs to occur. These transposones are able to enhance the conjugation of the neighboring bacteria and thus the required genetic material is transferred from the neighboring bacteria to recipient cell.

**IV. Gene cassettes**

They are circular, non-replicating and discrete genetic materials that exist freely, when DNA materials move (Bennett, 1999). This, when is integrated in integron, only is considered to be part of integron. They consist of one gene only with a small genetic sequence, known as fifty nine base elements. This base element performs as a particular recombination site, known as $attC$ functioning for binding of Cassette. These gene cassettes are composed of short usually of 500–1000 bp. These genes do not have any kind of promoters and thus they are expressed with the help of a promoter on the integron. Integrones are genetic materials that are having ability of catching and moving these cassettes. Although the inegrone are unable to move themselves (Bennett, 1999; Fluit & Schmitz, 2003).

**V. Integrons:**

It is the genetic element helps in encoding an enzyme known as integerase. This also proceeds the particular binding sites tasks. This enzyme works by excising or cutting the gene cassettes from integrons. They also help in attaching the integrones to new additional DNA with the help of gene $attI$ that helps in attachment. The gene that code for promoters is $intI$.

These integrones are of two types: one type of integrones provide the gene cassettes that impart resistance genes to bacteria, these are called resistance integrones. Another type of integrones is capable of imparting various functions in bacterial cells. These are only located on the bacterial gene. They are called as super integrones (Fluit & Schmitz, 2003). Resistance integrons have been classified into three distinct classes based on the integrase gene sequence. Till date in pathogenic bacteria, Type 1 of the integrones is the most common type. 3'-CS regions are found, with gene cassettes with variable regions and structurally class1 integrones possess 5'-CS region (Bennett. 1999). The gene called as integrase, $intI$ is present in 5'-CS region, gene cassettes, $attI$ will get inserted into promoter region that is located within integrase gene $intI$. Sulphonamides impart resistance in the 3'-CS region, with the help of $sulI$ gene. The other important thing to consider is that, because of the internal stop codon the $intI2$ integrase
may not function. IntI3 integrase functions similar to the IntI1 integrase.

First super integrons in First bacterium was detected is *V. cholera* and initially it was seemed to be as a class 4 integron (Fluit & Schmitz, 2003). With hundreds of ORFs structure of super-integrons can be very large, integron of *Shewanella oneidensis* is an exception of the super integrones which includes 3 gene cassettes. For resistance integrones these are considered to be as the reservoir of gene cassettes.

2.i. EMERGENCE AND SPREAD OF RESISTANCE

1. Selection pressure and risk factors

The fungus that grew and inhibited bacteria on agar plates (Fleming *et al.*, 1929) produced the bactericidal agent, Penicillin, for its own survival by competing with many other organisms to survive in a natural environment. To this, in the same way, bacteria also started producing some protective, toxic agents as defense against anyone that nature throws on them. So phenomenon of antimicrobial resistance is a quite natural that helps microbes in their survival in an environment with toxic substances. The bacteria in environment that are free of any antimicrobial agent will cost for it by being attacked by other antimicrobial agents. Whereas, the environment where antimicrobial agents are present, like hospital settings, bacteria start harboring resistance mechanisms for survival thus getting an advantage in surviving by Darwinian law (Darwin, 1859). As a result, usage of antimicrobial drugs, whether suitable or not, has the strength to lead to the non-susceptibility (*Austin et al.*, 1999; Livermore, 2005). In European countries, it is alarming that outpatient antibiotic consumption and antibiotic resistance rates are higher in Southern European countries in contrast to Northern Europe (Goossens *et al.*, 2005). Even if the suitable antibiotic usage is considered good but the problem is also associated with non-judicial use of drugs to great extent. Usage of narrow spectrum antibiotics in an appropriate dose, within correct duration not only kills off the desired bacteria, but also leaves the least possible effect on the normal flora of the host. On the other hand, unnecessary usage of broad-spectrum antimicrobials damages body cells to higher extent in terms of adverse ecological effects, develops resistant bacteria and leads to colonization of these bacteria in patients along with secondary infections with resistant bacteria (Paterson, 2004). Clinicians have started using Cephalosporin and fluoroquinolones in combinations for their broad spectrum actions of
bactericidal properties towards a wide range of relevant clinical pathogens by ignoring the infrequent side effects. There are studies that have reported the use of cephalosporin in infections of Vancomycin-Resistant Enterococci, ESBL–Producing Klebsiella pneumoniae, and Clostridium difficile. Likewise, use of fluoroquinolones is reported in the methicillin resistance Staphylococcus aureus infections. Increasing levels of non-susceptibility to fluoroquinolones in Gram Negative Rods, including Pseudomonas aeruginosa has also been reported (Van et al., 2001).

Since it is known that the sub-therapeutic drug treatment only suppresses bacteria, but does not eradicate them completely, thus the number of bacteria that are exposed to the drug and the time of exposure are increased that allows them to survive longer. Inadequate consumption is common, which is prompted by imprecise prescription and easy availability of antibiotics in market without prescription (Uplekar et al., 1991; Van et al., 2001). In economically poor countries, the use of low quality and forged drugs is an extremely serious problem which is disturbing the world widely (Taylor et al., 1995; Kapp, 2002; Newton et al., 2006). Actually, if everyone starts using poor quality drugs available at cheaper prices, even the best ventures to cope with the antibiotic resistance would not be able to give good results. There are many factors responsible for antibiotic resistance other than antibiotic use for acquiring infections with resistant bacteria (mainly with ESBL-producing bacteria). These factors include intensive care units admission, receipt of parenteral nutrition, usage of indwelling catheters, kidney failure and burns (Paterson et al., 2004). Outside hospital, the factors that are responsible for acquiring infections with ESBL-producers are antibiotic treatment during the last three months, especially with cephalosporins, old age, and history of underlying disease like diabetes and a history of recent hospitalization (Colodner et al., 2004). To an extent, increasing cases of HIV may also add to the current global scenerio of antibiotic non-susceptibility (Jones et al., 1998; Madhi et al., 2000; Wininger et al., 2002).

2. Acquisition, spread of resistance characters

There are some inherent traits coding resistance in particular bacteria e.g., Klebsiella pneumoniae for Ampicillin-resistance, Enterococcus for Cephalosporin-resistance and in some Gram-negative bacteria for Erythromycin resistance. Apart from inherent traits, there are some traits of resistance that are acquired by bacteria in three principal ways: 1) addition of mutations in the
bacterial cell chromosome, e.g., mutations in gyrA, gyrB, parC, parE genes leading to Fluoroquinolones resistance and the rpoB gene in \textit{M. Tuberculosis} leading to Rifampicin resistance 2) insertion of a new gene, which occurs by different methods like plasmid transfer and conjugation in both GPC and GNB with transformation and transduction in Gram-positive bacteria 3) recombination of genes intra-genetically to form mosaic genes that are responsible for encoding resistance traits e.g., emergence of penicillin-resistance in pneumococci (Maiden \textit{et al}., 1998). Resistance traits can be spread by vertical transfer i.e., proliferation of the resistant bacteria by bacterial multiplication and transfer of similar resistance gene to offspring. It is observed that poor hygiene is a good source for bacteria to spread resistant. In health care institutions, where the antibiotics are used at high, resistant bacteria get a favorable condition to survive over susceptible bacteria. Moreover, resistance genes can be spread horizontally among bacteria, e.g. via plasmids. According to a study, coliforms can exchange plasmids with resistance genes even in the gut. (Platet \textit{et al}., 1986; Marchandin\textit{et al}., 1999).

\textbf{2.j. EPIDEMIOLOGY}

As scientists began to unravel the events, which we believed responsible for acquisition of resistance by organisms, a rare opportunity was presented to explore the epidemiology of resistance. Till date no studies in Punjab have been conducted on survey of antibiotic susceptibility. Worldwide reports have been published on resistance data. In 1990, where Penicillin could not be used among some patients due to its allergic properties, Erythromycin was selected to treat patients. The study showed that the organisms non-susceptible to Erythromycin also responded similar to Clarithromycin and Azithromycin in 1989-1994 from 0.31% to 2.5 \% for bacitracin and macrolides in US (Shakya \textit{et al}., 2013). In America, MRSA death rate is more than HIV, Parkinson’s, Emphysema and homicides (Jacobsson \textit{et al}., 2010). A study conducted in Sweden and Ujjain on antenatal cases, showed at least one antibacterial resistance in 94\% patients (Jungermann \textit{et al}., 2011). In USA 72\% resistance to multi drugs and 58\% resistant to Imipenem, Amikacin, and Ampicillin-Sulbactem salt in Acinatobacterbaumannii has been observed (Carlet \textit{et al}., 2012). In Canada 8\% of Staphylococcus \textit{aureus} and 11\% of \textit{Streptococcus pneumoniae} were found resistant to beta Lactems drugs (Cantas \textit{et al}., 2013). \textit{Bacteroidsfragilis} are also seen with resistance profile for Imipenem is 1.5\% Doripenem 1.9\% and Ertapenem 2.4\% for the first time in Argentina.
Bacteroidesfragilis showed most resistance to Moxifloxacin (15.1%) (Conly et al., 2012). A study conducted in Australia, showed that 19% of E.coli do not get affect by the use of fluoroquinolones and 27% are non-susceptible to Trimethoprim Sulfamethoxazole (Cantas et al., 2013). Chlamydiaespp. that mainly causes ocular, genital, zoonotic respiratory, respiratory, and veterinary infections, has been found resistant to Tetracyclines, Rifampicins, Fluoroquinilones, Aminoglycosides, Sulphonamides, Macrolides, Lincosamides (Caron et al., 2010). Fluoroquinolones resistant in GNB were seen resistant 6.3% - 62% and 20-100% in MSSA, 59% in Enterococci in healthcare associated UTI in Germany (Bjorkman et al., 2013). A study conducted on children in Vietnam, 79% to Tetracycline, 68% to Cotrimoxazole, 65% to ampicillin, 40% to chloramphenicol, 27% to nalidixic acid came out to be resistant (Hoffman et al., 2011). Another study conducted on in 3-14 years of children with isolates from stool samples showed Nalidixic Acid Tetracycline, Ampicillin 37%, Amoxicillin- Clavulinic Acid 29% resistance in Ujjain, India (Edlin et al., 2014). Some papers revealed that the antibiotic Ciprofloxacin was used in large amounts by clinicians in Ujjain and its remnants were found in waste water, lead could have led to risen resistance in susceptible organisms under tolerance and pressure (Sahoo et al., 2010). Bacteria may also release many volatile compounds in environment while food transportation and fermentation. e.g. E.coli and other GNB release Trimethylamine, which leads to resistance gene stimulation in bacteria (Davies et al., 2010).

2.k. EPIDEMIOLOGY OF ANTIBIOTIC RESISTANCE:
AT LOCAL LEVEL, NATIONAL LEVEL, AND INTERNATIONAL LEVELS

At one stage, the epidemiological data of resistance is highly limited. Generally breakouts of similar resistance with clusters of resistance affect a small number of infected people in a small number, and the predominant non-susceptibility are generally highest in the groups or component where the highest sensitive patients are forgathered and the antimicrobial therapy thereupon is heaviest. A study on epidemiology of resistance by Archibald et al documented that in comparison to general patient wards or outpatient departments at the hospitals, indoor unit patients showed twice the rates of resistance for antibiotic Vancomycin among Enterococcus, Methicillin among Staphylococcus, Ceftazidime seen in E. cloacae and P. aeruginosa, and Imipenem seen in P. aeruginosa. In nearly all European countries, ICU’s reported high generality in cases of MRSA (Methicillin Resistant S.aureus) than the general patients wards (Voss et al., 1994; Archibald et al., 1997).
At another stage, the epidemiology of resistance is purely nationwide. It has been seen in Europe that the prevalence of common resistance patterns increases to the southward direction. Whereas in North America, it is found that United States have high resistance rates than in Canada. There have been documented that some newly developed countries of East Asia and South America have one of the worst resistance rates. The reports state about 30%–45% of MRSA of all bacteremia cases in Spain, Portugal, Italy, France, that were higher than the rates (10%–15%) prevailing in United Kingdom, Germany and Austria. The lowest MRSA cases were found in Netherlands and Scandinavia (1%).

In case of Penicillin resistance in *S. pneumoniae*, 70%–80% of *S. pneumoniae* cases were non-susceptible in Korea, Japan, Taiwan, and Vietnam, whereas, the rates were lesser up to 30%–40% in France and Spain. Penicillin resistance in *S. pneumoniae* was documented just 5%–10% in UK, only 1%–2% within Scandinavia (Song et al., 1999). Among Southern European countries and United States where Gentamicin resistance in *E. coli* was studied, the prevalence remains to be very high considerably, United Kingdom reported surprisingly very less resistance rate for i.e., 3% only (Reacher et al., 2000).

The epidemiology of resistance is noticeably halfway international, as some principal causations are transferable worldwide that rise with the prevalence. The prevalence of resistance has become international to an extent because the resistance genes are spread worldwide among countries and continents. A study documented those children returning from holidays, through nasopharyngeal carriage imported serotype 6B of Multi drug-resistant Pneumococci from Spain possibility to Iceland, possibly (Kristinsson, 1995). Then after some time, Pneumococci were detected in iceland among some child care units, with a rise on the rate of non-susceptibility of penicillin from year 1988 to 1993, ranging from 1% to 17% respectively. Available data reports that serotype 23F of Penicillin-Resistant Pneumococci spread from Spain to the Eastern Spain, some part of America, and South Africa (Munoz et al., 1991). There are evidences of importing of some AmpC B-lactamases having *E. coli* and Klebsiella *spp.* from subcontinent of India, Punjab to United Kingdom. There, after sometime, the same were found in local population of UK (M’Zali et al., 1997; Child, 2001). From France first recorded PER-1 ESBL in *P. aeruginosa* and was soon isolated in many cities in Turkey in many strains of *P. aeruginosa*, Salmonella, and Acinetobacter *spp* (Nordmann et al., 1993; Danel et al., 1995). Furthermore, the study confirmed
its significance by enquiring that the first patient with PER-1 ESBL in P. aeruginosa in France was a Turk originally, who used to visit France for some treatment (Vahaboglu et al., 1997).

2.1. EPIDEMIC RESISTANT STRAINS

The strains that spread successfully in the form of epidemic become seriously responsible for the accumulation of resistance. The vectors for the carriage of resistance that are common in hospitals are basically contacts with non sterile devices/ procedures or dealing with staff members. The spread of resistance is accelerated in the community by the factors; crowding and travelling have also helped the epidemics to spread. Generally the strains, resistant or sensitive are able to spread locally, but some strains achieve a large scale. The Penicillin-Resistant Pneumococcal lineages spread internationally. A study reported the increasing trend of MRSA in England and Wales, from year 1989 to 1993, from 1%–3% respectively that shotup till year 2000 up to 42% (Reacher et al., 2000). According to another report, the emergence of two new strains responsible for causing epidemics (E), named as EMRSA 15 and EMRSA 16, has reached up to 93% of total Staphylococcus aureus bacteremia cases in Britain (Johnson et al., 2001). For the first time the resistant strain of K. pneumoniae, serotype K25 with resistance genes SHV-4 B-Lactamases for resistance to Amikacin and Ciprofloxacin was found in Paris, which subsequently was found in hospitals of the Mediterranean Atlantic coast and also from Belgium Ghent and then it was imported to France in year 1988 (Bure et al., 1988; Arlet et al., 1994). In year 1994, a survey on ESBL’s among 966 Klebsiella spp. was conducted that included 35 centers, there out of only 5 centers from France, the single strain was found responsible for fifty two of two hundred twenty Extended spectrum beta lactamases producers were chosen (Yuan et al., 1998). In some parts of Europe, strain with the enzyme TEM-24 B-lactamases was isolated from the bacteria Enterobacter aerogenes that featured multi resistance genes to Aminoglycosides, Quinolones, was established widely that also showed Carbapenems resistance through porins also (Bosiet et al., 1999). The strains of Burkholderia cepacia in cystic fibrosis and Salmonella typhimurium strains were found to have a major clonal element, which have been responsible for mushrooming of multidrug-resistant strains of specific type DT104 inter-continentally (Casin et al., 1999; Threlfall et al., 2002).

The epidemic strains of Vancomycin-Resistant Enterococcus faecium were found to be closely related to each other when tested by process of amplification of DNA with RFLP (restriction fragment-length polymorphism). This research suggested the idea of those bacteria though
collected from various parts of European countries, American countries, and Australia were genetically similar rather being sporadic and agricultural isolates (Willems et al., 2001). There were some other bacterial strains that had similar resistance characters to those of epidemic prone bacteria are documented generally in the hospitals from similar characters and patient groups, but these strains were unsuccessful to expand abundantly. The reason for epidemic success of some organisms have been unclear, but possible factors may include: (1) more bonding to cells of the host or artificial grafting in the host body things, (2) higher resistance to desiccation methods, (3) increased non-susceptibility to disinfectants, (4) high development levels, (5) acquisition of the charges for the health of resistance.

There are few evidences of the role of above mentioned factors for many strains. From France and Belgium, one example of the well to do epidemic of serotype K25 K. pneumoniae strain isolation, one study documented that the fimbrial antigen that is mediated by plasmid, helps in holding of the bacteria to the mucosal wall of gut was the one factor responsible for the spread (Di et al., 1996). There must be a difference kept in mind about Local and national variations in epidemics, and the part of epidemic bacteria ought to be considered when executing a prevalence survey. A study showed the regional variation on resistance that was greater in Boston and San Francisco than Stockholm and Madrid. Since ICU’s borne bacterial strains have a high resistance rates, hence decisions of the normal dispensation of antibiotic susceptibility must not be understood from the data collected from ICUs (Archibald et al., 1997). There is a big concern to discuss about the bacteria isolated from community is that microbiological tests to investigate the infections are generally to be performed for unmanageable infections, which may be uncontrollable since these infections are caused by pathogens that are resistant. Thus study in Bristol by MacGowan and his coworkers found that infected people representing with signs of disease of respiratory illness to health care personals were tested for sputum culture were only 3% and out of which that the possible presence of Ampicillin resistant H. influenzae strains dropped down from 22% to 11% (MacGowan et al., 1998).

2.m. EFFECT OF ANTIBIOTIC RESISTANCE

The after-effects of resistance are tough to be summarized by any medical personnel. There are some patients that recover themselves even under inadequate antibacterial therapy, just like as some people recovered before antimicrobial era. On the other hand, there are some people that
don’t recover from infection despite appropriate therapy. It has always been a topic of debate about immune compromised patients; whether they die of new infections or underlying disease. It might be cleared in the example of response of gonorrhea to Penicillin. Where, Classical strains of gonorrhea respond to Penicillin with MIC of 0.06 mg/at 1000 ml. lower potencies and resistance that is mediated through the chromosomes (Minimum inhibitory concentrations from 0.25mg/1000 ml–2 mg/1000ml) strains reacted to high levels of Penicillin; and the gonorrheal strains with presence of B-Lactamase enzymes were not be treated with Penicillins of different types. Somewhere, relationships are not clear for in vivo and in vitro efficacy e.g., in case of cystic fibrosis with pseudomonal infection where antibiotics that respond in vitro become unsuccessful to treat infection, and some other drugs like Erythromycin that are completely resisted by organism often improve the symptoms of infection (Doring et al., 2000). Many of the connections come between these ends. A research by Mosdell et al documented that chances of severities like abscess production, infections of wound or lesions, reoperation etc. may raise two times if generalized antimicrobial treatment of sepsis spreading from abdomen failed to be broad spectrum (Mosdell et al., 1991). The chances of complications may rise further if the previously given generalized antimicrobial therapy is not changed even when resistant pathogens are isolated. Another study by Kollef and Ward (Kollef and Ward, 1998) and Ibrahim et al (Ibrahim et al., 2000) documented that there was rise in 2-times death rate in Intensive care units where patients suffer with ventilator-associated pneumonia where the isolated organisms found to be non-susceptible to the Antimicrobials used generally. Most of the cases in the situations like this were caused by P. aeruginosa or Staphylococcus aureus that were once not under the cover of the general treatment generally used. There were some Risk issues for growth and identification of these bacteria and the same for futile results that involved last use of antibiotics and last hospitalization. These points, bacterium and its general resistance patterns of limited local region, should always be kept in consideration when framing empirical drug therapy for any hospitals. A note must be taken into that the medicinal science is now more precisely framing cure of the particular pathogen in a particular infection, keeping in account that for many infections, generally in vitro susceptibility is indicative of in vivo activity of drugs. Apart from Increased morbidity and mortality there are some other dangerous and dramatic outcomes of resistance. Sometimes, health care professional have to treat the infection by previously reserved agents as first-line therapy forcefully. The antibacterials that are administered to patients may be
toxic or inherently less potent e.g., as antistaphylococcal drug, vancomycin is used often as first-line treatment, which is not as much bactericidal as the semisynthetic antistaphylococcal penicillins, which also can be replaced by Benzyl Penicillin that are 100-fold more potent against fully susceptible Staphylococci. The old antibiotics that have been used since date may have been wiped away by resistance. There are reasons for using more potent antibiotics when the lesser potent antibacterial are available. One of the examples is the increased Cephalosporin-resistance in bacteria that drives clinicians to use carbapenems early and that is the reason of the production of carbapenems that could be given orally with long shelf-life. No doubts, the pressure exerted by selection that is raised by the mass use of antibiotics will be worrying, when coming carbapenems are being developed so fast (Drusano, 2002). We know that resistance costs to regimen failures that in turn prolong the hospital stay of patients, by this hospital beds are occupied by the older patients and are blocked to new ones thus high yielding time is lost for newer one in form of late treatment. If new or before reserved antibiotics are required for treatment, they are usually quite expensive than old and regularly used ones. The cost of these antibiotics seems nor reduced in next years, especially with development of genomics-based drugs.

Government and other private agencies started worrying about antibiotics resistance since the late 1990s. Since then, many government organizations in concern to this have published reports regarding guideline of usage of antibiotics (Shlaes et al., 1997; Nash, 2001). Generally all of these reports say about (1) precise use of antibiotics, (2) suitable selection of antibiotics, (3) caseation of spread of infection, and (4) production of new antibiotics against resistance. To check the effects of these steps requires good quality surveillance of prevalence of resistance and on the prescription of antibiotics.

There have been evidences that by decrease in antibiotic usage, prevalence in non-susceptibility can also be reduced. According to one study conducted in Iceland between 1993 to 2000, when penicillin was lesser advised and usage was reduced up to 12.9%, the presence of Penicillin-Resistant Pneumococci was reduced from approx. 19% to 14% (Austin and Anderson, 1999). In Finland, when national advisory (from year 1988 to year 1994) suggested the reduced usage of macrolides, by prescribing every month from three doses among one thousand number of population to 1.1 doses in one thousand number of sample, the successful results were also observed by getting decreased presence of Streptococcus pyogenes that were resistant to
erythromycin from 19% to 8% approximately from year 1993 to 1996. When by the year 1998, Macrolide use was again raised per month to 2.1 doses among one thousand samples of population; the rates of non-susceptibility among Streptococcus pyogenes again were raised to 18% (Kataja et al., 1998). Displacement of any resistance gets tougher in case of multiple antibiotic non-susceptibility disseminated among different strains. In spite of practical mismanagement of Streptomycin and Chloramphenicol in humans with compromised actions of bacteria to these drugs, resistance for these antibiotics remains unchangeable in Gram Negative Bacteria (Chiew et al., 1998). In United Kingdom from years 1991 to 1999, to hamper Sulphonamide resistance in E.coli, use of Cotrimoxazole was reduced, but the results were not as expected likewise other studies. The resistance to this antibiotic still remained 39%–45%, making the strategy unsuccessful (Enne et al., 2001). After investigating the reasons, two causes that failed the plan were explored: first cause was the Potential use of antibiotic in agricultural activities in London and rest of UK (Kollef et al., 1998). Another reason that was considered responsible for this was the multi drug resistance plasmids present as resistance determinants within large population of E.coli.

It is an evolutionary process that favors those determinants and cost the minimum fitness on their host bacterium and the strain variants. Therefore, if antibiotics are used heavily for longer times, resistant strains have to be honed by evolution. There have been some laboratory researches that describe how this process can happen. Initially when the strain of an E. coli constituted with a gene or plasmid resistant to Tetracycline was grown in laboratory; its growth was quite slower than its parent that was without plasmid, later on by its 500 successions, the strain started growing more rapidly by 6% rise in speed (Bouma and Lensky, 1988). Another experiment to describe the changes by evolution was conducted to detect the rates of growth of an E.coli that was non-susceptible to Streptomycin. At the time of starting the test, the mutant of E.coli in culture had a hindered growth rate up to 14%, slowly in next levels of repeated subcultures, the suppression rate reduced to 6% only, which was quite slowly than the parent strain (Lenski, 1997). The strains that are well-adapted cannot be easily displaced. The plasmids play very important role in survival of resistant strains. There are some plasmids that specifically bind sites to the chromosome, which helps the plasmid to segregate with each daughter cell. Another type of plasmids encodes a “plasmid addition” system that determines toxin with a long shelf-life and another less stable, antitoxin that was homologous. Thus as the maximum time the plasmid
persists in the cell, antitoxin helps the bacteria to survive, but the stronger remnant toxin kills any next generation bacteria which is unable to derive a copy of resistant plasmid (Rawlings, 1999). This plasmid carriage within populations is maintained, that increases the pressure to reduce the fitness cost.

2.n. EFFECTS OF RESISTANCE: FATALITY
Most often, the duration of stay in hospital by patient is used to represent morbidity. It is still a conception that inadequate drugs treatment may increase the suffering of the patient. There are many studies that have documented the association between prolonged stay in hospital and resistant pathogen infections (Cosgrove, 2006). A study signifies this by documenting that *S. aureus* infections caused by Methicillin resistant strains are increased p<0.001 in cases of prolonged stay in hospital (Engemannet al., 2003). Another study states the resistant pathogen infections prolong the patient suffering and duration of stay in hospital e.g., Penicillin Resistant pneumococcal infected patients have to stay in hospital longer than that of patients with Penicillin-Susceptible pneumococcal infections (Rowland et al., 2000). One more study adds to this statement by documenting the cases of ESBL-producing *E. coli* and Klebsiellaspp. Patients with ESBL-producing *E. coli* and Klebsiellaspp. infection also have to stay prolonged in the hospital for treatment (Lautenbach et al., 2001).

2.o. IMPACT OF ANTIMICROBIAL RESISTANCE ON MORTALITY
In some cases, the bacteria have a little virulence power but are foremost to cause disease in patients that are admitted with serious predisposing factors or many underlying diseases e.g., highly resistant bacteria like Enterococcus. In such cases, it becomes difficult to conclude whether the serious consequences are due to the antibiotic resistance or due to the underlying conditions. There are many prospective (Linden et al., 1996; Bhavnani et al., 2000) and retrospective studies (Edmond et al., 1996; Stosor et al., 1998) that have documented the increased risk of death from Enterococcus infections if the pathogen is resistant one especially when the causative organism of infection was VRE (Vancomycin Resistant Enterococcus) (Pelz et al., 2002; Carmeli et al., 2002).

The available studies on *S. aureus* bacteremia documented that MRSA infections in patients had more chances to be fatal than patients with Methicillin-Sensitive *S. aureus* (Whitby et al., 2001;
Cosgrove et al., 2003). A research on haemodialysis patients with bacteremia due to S. aureus also stated the increased risk of fatality in MRSA infections as compared to the patients with MSSA infections. Likewise, in some prospective studies of MRSA infections at surgical sites, have documented the increased risk of fatal outcomes as compared to MSSA (Engemann et al., 2003). There are some pathogens, whose association with resistance and increased case-fatality rates have not been established yet. For example, pneumococcal infections with Penicillin resistance have not shown any associations with increased fatality (Pallares et al., 1995; Einarsson et al., 1998; Yu et al., 2003). The reason for this is not that Penicillin-Resistant Pneumococci may be less virulent, but there may be the reasons that 1. The patients generally acquire pneumococcal infections from the community hence they may have less underlying diseases to worsen the condition or 2. In case of Penicillin Resistance pneumococcal infections, appropriate antimicrobial agents, like Vancomycin, are generally used that are empirical treatment of Penicillin resistance.

Many studies on Gram-negative rods have reported the effect of resistance on consequences of infections caused by Pseudomonas aeruginosa (Carmeli et al., 1999). For Enterobacter spp., increased patient fatality rates have been found to have contributed to emergence of non-susceptibility in the causative pathogen during treatment. (Lautenbach et al., 2001). However, in South Korea, a univariate analytical study in pediatric cases reported increased cases of fatality in infections by ESBL-producing E. coli and Klebsiella spp. E.g., bacteremia as compared to the bacteraemia caused by non ESBL-producing pathogens (Kim et al., 2002). A study conducted in Chicago stated the infections/ bacteremia caused by E. coli or Klebsiella spp. resistant to ceftazidime and were more likely to survive if appropriate treatment within 3 days of onset of the bacteremia was given (Schiappa et al., 1996).

2.p. COST INFERENCE OF ANTIBACTERIAL RESISTANCE

Increased stay in hospital for curing the disease caused by resistance pathogens increases the cost in two ways that are hospitality and expenses on purchasing the expensive second-line drugs. Noteworthy, relationship between resistant microbial infection and increased cost has been seen in Penicillin-Resistant Pneumococci (Rowland et al., 2000), Methicillin-Resistant S. aureus infections (Reed et al., 2005) and E. coli and Klebsiella able to produce ESBL. (Lautenbach et al., 2001).
2.q. IMPACT OF FORMULARY CHANGES ON RESISTANCE
As there are a small number of examples of reducing resistance at large scale by changing the prescription, there are a large number of examples of changed (than reduced) prescription that is observed by alterations in the epidemiology of resistance at local level. To reduce Gentamicin resistance among opportunistic Gram-Negative Bacteria, Amikacin was formulary changed, for several years (King et al., 1992; Betts et al., 1984). According to study by Bradley et al, in fevers with neutropenia, a formulary change in actual Ceftazidime to Pipercillin-Tazobactam not only worked effective in infection control, but also helped in reducing the illness caused by Enterococcus that are resistant to Vancomycin (Bradley et al., 1999). Then when researchers returned to older Ceftazidime, Vancomycin Resistant Enterococcus was re-emerged in spite of continuing weight on the containment of infections. Another instances of better management were seen when ceftazidime was replaced with cefepime, the reduction in the presence of AmpC-depressed Enterobacter was seen (Levison et al., 2000) and of Cephalosporin formulary switch to Pipercillin-Tazobactam or Carbapenems that reduced the prevalence of Cephalosporin-Resistant Enterobacteriaceae (Rahal et al., 1998; Rice, 1999). Though these examples spot light positive consequences of displacement, but also alarm about many things. First of all, the changes due to which the positive consequences are achieved must be recorded than the changes that get unable to do so. Another, the other scientists when followed these strategies could not found same results. Negative impact of Formulary changed was noticed when Gentamicin was Formulary switched to Amikacin that increased Amikacin resistance (Friedland et al., 1992). The new respiratory Quinolones put a new quandary. As outcome, of more antipneumococcal activity, these respiratory Quinolones generally do not opt for the mutant of S. pneumonia that was resistant to first-step Quinolone-Resistant other than Ciprofloxacin. Quinolones, are not as good as others but also can provide resistnace to bacterial microbiota of gut (Kern et al., 2000).

2.r. SOCIAL CONTEXT
The responses to the queries on resistance are quite apprehensive on scientific grounds, and it is also considered that the whole problem of resistance and antibiotic use in complicated matters is twined together with political and commercial issues more than moral or social concerns. Matters
around resistance are used as proofs for other topics, which but obviously includes selling skills by the commercial production and cost control among governed health system (Livermore, 2000). Many a times, single patient gets a third generation antibiotics at early stages, but the risks of resistance are raised for society. But the method of antibiotic selection have become complex. In many cases, old and simple antibiotics may fail to the treatment of infection, which in turn leads to worsened condition of the patient or the infection is moven around, thus this condition may require higher therapy. Along with this the possible, spread of foreseen selection pressure forces the health care people to directly put the patient on stronger and new antibiotics helplessly. Even after launching and following a confined list of antibiotics for community prescription in Hungary, before 1989, the country then had the highest prevalence rates for Penicillin Resistant Pneumococci of world (Martonet al., 1999).

2.s. CONSEQUENCES OF ANTIMICROBIAL RESISTANCE

Instinctively, due to antimicrobial resistance the health of patient falls by inefficacious and inadequate chemotherapy that is subsequently led to unsuccessful treatment with high morbidity rates and increased cost (Cohen, 1992; Tenover et al., 1996; Acar, 1997). A study by WHO states that the infections that are caused by resistant pathogens generally lead to lengthy illness and mortality (Livermore et al., 1996). Because of the prolonged sickness, there are much chances of spreading the infection from the patients to other healthy people. Expenditure of treatment is increased, due to the more expensive antibacterial drugs usage and because of prolonged duration of hospitalization for patient care. Serious consequences of illness and death can be prevented by appropriate antibiotics that are essential specifically in complications like bloodstream infections. While the reason of resistance and complications seems to be obvious, there are not much scientific proofs of this association and the research of evaluating this relationship are being challenged for many reasons (Cosgrove, 2006). There are many surprising factors that interfere with patient’s health and may influence the outcomes. For the first reason particularly, many underlying conditions are needed to be considered. Sometimes it becomes difficult to judge whether the harmful results are the outcome of an underlying disease or it is due to antimicrobial resistance in the pathogen causing the infection. The studies on resistance that are published are retrospective mainly, but reliability of information can be obtained from prospective studies. Furthermore, the size of study decides the connectivity between resistance
and its results where, size is considered to limit the potential of detecting associations, if the sample number in the study is too small, the biological difference may not be observed. In developed countries, the cost of treatment and wellness, morbidity are more concerned tools of resistance than the impact of resistance on mortality (Cosgrove, 2006). Due to low incomes, the gush in antibiotic resistance is considered as potentially disastrous due to the expenses on purchasing expensive second-line drugs among poor regions of the world (Hart et al., 1998; Okeke et al., 2005).

2.1. ISOLATION, IDENTIFICATION AND CHARACTERISATION OF PATHOGENS

Isolation and characterization of clinical pathogens are the two basic units of surveillance. Although, microbiological identification and characterization depends mainly on the phenotypic and biochemical characters of particular organism, but a study conducted on Gram positive organisms shows that a single phenotypic test may not be sufficient to characterize the organisms. For an instance, the tube coagulase test may better be used by testing the isolated organism with Mannitol salt agar and DNAase test along with Tube coagulase, for characterization of Staphylococcus aureus (Kateete et al., 2010).

Gram positive cocci from various clinical samples are isolated on blood agar, depending upon their requirement to lyse RBCs on blood, GPC grow on blood agar (Ruoff, 2002).

Another study was conducted to standardize the antibiogram and biochemical tests. According to the study, duplicates of Staphylococcus aureus from same patient were taken to observe the impact of repeat isolates MRSA and antibiogram changes. It also helped in the cost efficacy of laboratory and better results of antibiotic susceptibility pattern (Lawrance et al., 2003).

According to some studies by Melaku et al people in developing countries are prone to Urinary tract infections generally with one or more bacteria (Björkman et al., 2013). E.coli is the commonest bacteria responsible for Urinary tract infections, according to a study in Ethiopia. The study included all adult patients with prominent signs of Urinary tract infection. Midstream urine samples were collected aseptically and were directly inoculated on suitable media plates. The common isolates that were found to cause UTI were E.coli, Klebsiella spp., Staphylococcus spp. (Melaku et al, 2012).

E.coli was also found to be the most common isolate in blood. In Zambia, Democratic Republic of Congo, Mozambique and Tanzania, E.coli was the most common organism causing
community acquired infections, whereas Klebsiella *spp.* was found to be most prominent in iatrogenic infections (Mshana *et al*., 2012).

Even in diarrhea *E. coli* plays an important role (Isenbarger *et al*., 2002; Shakya *et al*., 2013). It was again the most common isolate in stool samples of diarrheal kids under age of 6 years in Tehran (Mshana *et al*., 2012).

In another study conducted in 710 self employed women in India, 710 *E. coli* were isolated. Out of which, approximately 667 were resistant to one or more antibiotics. 15.3% were extended Beta Lactamases producers, whereas, 1.26% were found to be multidrug resistant *E. coli* (Pathak *et al*., 2013, Cagan *et al*., 2014).

Apart from Gram positive and gram negative, miscellaneous bacteria are commonly found in buccal/oral cavity. A study in year 2013 stated that acute apical abscess is the most common infection at root canal of teeth. The microorganisms commonly isolated were *Parvimonas, Fusobacterium, Treponema, Prevotella, Porphyromonas, Dialister,* and *Streptococcus.* Culture techniques are the most suitable techniques for isolating pathogens of oral samples. The organisms isolated are identified by similar or dissimilar phenotypic characters, by controlling the changed reproducibility of results, taking the whole procedure of isolation and characterization of particular organisms in mind as very fine or minute change in procedure can affect the test result, leading to name or report wrong causative bacteria (Ashley *et al*., 2011; Jose *et al*., 2013).

**2.u. ANTIBIOTIC RESISTANCE IN URINARY INFECTIONS**

In year 2009, a study on UTI’s conducted in Chennai and Haryana, reported 4% of Gram Negative Bacilli in Chennai, in which, 2.1% *E. coli, 0.8% Klebsiella spp,* and 0.17% other enterobacteriaceae were found resistant to carbapenems from total 3521 (4%) Enterobacteriaceae isolated in urine samples. Out of these carbapenems resistant Enterobacteriaceae, 31.2% of GNB’s were New Delhi Metallo beta lactamases (NDM)-1 positive, which constituted of *E. coli* (19%), *K. pneumoniae*(14%), Enterobacter *cloacae*(7%), *Proteusspp.* (2%), *Citrobacterfreundii*(1%), and *Klebsiellaoxytoca*(1%). Simultaneously, the same study was conducted in Haryana, where 24% Carbapenem-Resistant GNB’s were isolated of all Enterobacteriaceae and NDM-1 was found to be positive in 13% cases. The only organism that was found positive for NDM-1 was *K. pneumoniae.* According to the study these isolated from
Chennai and Haryana basically were community acquired. These isolates were found in Urinary Tract Infections, pneumonia, and blood infections.

According to a study conducted in UK in year 2008–09, where first NDM-1 positive GNB was isolated in year 2008, the rate of NDM-1 positive cases increased in year 2009 by 44% among all carbapenemase producers. Across England, Scotland and Northern Ireland total 37 Enterobacteriaceae isolates with the NDM-1 enzyme were referred for confirmation. These were confirmed as K. pneumoniae (56.7%), E.coli (18.9%), Enterobacter spp.(13.5%), Citrobacter freundii (5.4%), Morganella morganii (2.7%), and Providencia spp. (2.7%).

NDM-1 Carbapenemases that contribute to carbapenems resistance in Enterobacteriaceae, also impart resistance for many other antibiotic classes with potential indication of high resistant to many antibiotics or may be the end of treatment e.g., Aminoglycosides, Fluoroquinolones and with β-Lactems antibiotic groups, that are the main antibiotic classes for the treatment of Gram-Negative Bacilli infections. According to the study only a few GNB’s remained sensitive to a single Aminoglycosides antibiotics and Aztreonam, which could be due to the absence of genetic material responsible for resistance. Colistin and Tigecycline produced good results by killing GNB’s in most isolates.

To identify the similarities between E. coli and K. pneumonia between India and UK or between Northern and Southern India, typing of E.coli and Klebsiella was tried out. The experiment concluded the strains from two different parts similar. However, the isolates of K. pneumoniae with NDM-1 positive among isolates from Haryana were sequenced, indicating that some of these strains are strongly responsible for clustered cases of illness. The reason for this was that most of the blaNDM-1positive plasmids were easy to transfer, and were prone to rearrangements like, deficiency of genes; that is rare DNA during moving of genes. The quality of plasmid to transmission with plasticity indicates the alert on definite potential to transmit with variability among many other populations among different bacteria (Renuart et al., 2013).

As China is one of the prone countries for antibiotic resistance, because of the rapid emergence of blaNDM-1 gene, resistance has also been highlighted as a concern there. Many studies highlight the use of antibiotics without prescription in India, which directly leads to selection pressure by which it can be foreseen that the NDM-1 problem will be valuably worsened in the near future. The problem is very much disturbing because a very few antibiotics against Gram-negative rods
are in clinical trials. Moreover, out of them not a single antibiotic is active against NDM-1 producers. Moreover, most of the Indian isolates that were studied were community acquired, indicating that $bla_{NDM-1}$ gene pervasive in the surroundings (Kumarasamy et al., 2010).

UK, keeping in minds the historical connections between UK and India, has taken the introduction of NDM-1 in the country very seriously. The credit of registering the first NDM-1-positive bacteria in the country with generalised presence of it, goes to UK among all western countries. Nevertheless, there are other countries that registered these cases soon. These included the first a NDM-1-positive K. pneumoniae from Sweden, here the pathogen was isolated from patient of with originality of India and residency of Australia. The patient visited Punjab in year 2009. The isolate had $bla_{NDM-1}$ gene and it was highly resistant to almost all antibiotics. The gene cloning revealed the same isolated types in India and UK. The spread of this gene is result of many UK source patients going to India for aesthetic surgeries. India also provides electric surgeries including aesthetic procedures for rest part of the world, his become the possible reason of spread of blaNDM-1 worldwide (Kumarasamy et al., 2010).

As the antibiotic non susceptibility is a global concern, both Gram positive and Gram negative bacteria have been reported non susceptibility from various surveillance studies. This mainly includes Escherichia coli that are the commonest pathogen of urine infections (UTIs). Moreover, Extended Spectrum Beta lactamase (ESBL) and carbapenemase producing drug resistant E. coli clones have become prevalent not only in the hospitals but in the general population also. Being one of the prevalent infections, for UTI treatment, the antibacterial agents that are suggested are based on the observations by physicians. Thus an incorrect prescription with combination of the highly prevalent UTIs is accounted as a precipitating factor for uropathogenic E. coli to enhance the antibiotic resistance among. This also favours the transmission of resistant clones of pathogens e.g., E. coli ST131. The antibiotic resistance is varied among the hospitals among various countries in terms of ESBL positive bacteria. Prevalence between health care institutions and between countries. The problem to handle the infection, manage the treatment of the patient among hospital or countries arises from multiple dissimilarities. Cross border spread of these pathogens have also been reported. The information available currently on the prevalence of antimicrobial resistance with special attention on the prevalence of E. coli strains with ESBL production can help the physicians in selecting the appropriate empiric treatment (Renuart et al., 2013).
In Euregio Meuse-Rhine in year 2013 conducted a study on E. coli in nine urology centers and provided data on antimicrobial resistance. In the study they found notable contrasts in resistance among the Dutch, German, Belgian isolated pathogens. However, highest prevalence rate of antimicrobial non-susceptibility was found in the bacteria isolated from Belgium. The lowest prevalence of the resistance was seen in Dutch isolates, the maximum number of ESBL positive bacteria was from the bacteria isolated from Germany. Very significantly, the conclusions of the study were that the commonly used antibiotics as the first choice antibiotics for the empiric treatment of urine infections vis a vis. Amoxicillin-Clavulanic Acid, Fluoroquinolones and Trimethoprim-Sulfamethoxazole were of no use. The study concluded these findings when the non-susceptibility to antibiotics was seen resistance exceeded from 10% to 18% (Donk, 2013).

It is of importance that there is a candid need of going for the joint venture of the nation and rest of the globe to study on the complete profile of antimicrobial non-susceptibility, which should be done regularly and repeatedly to keep a check. There has been a significant rise in prevalence in the MRSA in some parts of Europe with increase in resistance to Ciprofloxacin and Gentamycin. In a study on the isolates of blood culture conducted in Europe by Dornbusch et al. 1990, the prevalence of GNB’s in blood was found highest with 52% of all isolates. The prevalence of Enterococcus faecium was lowest in blood infections being 2% only. Similar was in case of urine samples taken for study where GNB’s contributed 82% of all isolates in urine and Staphylococcus aureus (4%) contributed least. When the identified bacteria were tested to know their antimicrobial resistance or sensitive profiles by the microdilution method in Mueller-Hinton broth the resistance rate varied much depending on the location of laboratories. in northern Europe it was less than rest of the Europe. But the resistance rate of Carbapenems, Cefpirome and Cefepime was lesser and uniform throughout country. Resistance to Cefotaxime and Ceftazidime, Aminoglycosides also was raised in Europe (Dornbusch Ket al., 1990).

Cancers and before the transplantations of various totipotent cells like hematopoietic stem cell (HSCT), where myeloablative therapies are given, have many risks of bacterial blood infections results in Mucositis. One retrospective study among patients of febrile neutropenia (FN) was conducted by Mahallahawy in year 2013, indicated the pattern of bacteria and the resistance trends of pathogens responsible for antibiotic resistance in HSCT patients. This contributed to the knowledge of iatrogenic infections (Hospital acquired infections) with reference to the matter of
antimicrobial non-susceptibility in stem cell recipients. This study reported almost 78% positive blood cultures, in 50 patients of FN. Here also Gram negative bacilli were the primary pathogens, representing 67% of all isolates. Central venous line (CVL) is the main source of bacteria from where they can infect whole blood leading to bacteremia. Another cause of Bacteremia was bacteria that were predominantly found in gastric infections (diarrhea and vomiting). The isolates of bacteremia showed bacteria resistant to many antibiotics the isolates being 69% (Mahalllawy et al., 2013). Mortality and morbidity in health-care associated infections is mainly due to Blood stream infections. Since Bacteraemia varies from self-limiting infections to life-threatening sepsis, all blood stream infections are required to be treated rapidly and aggressively with antimicrobials. A retrospective study in university hospital was conducted in blood stream infections to know the pattern and trend of pathogens and their resistance respectively. In this study the rate of positive blood cultures was approximately 20.5%. Of all again the GNB’s accounted maximum of 67.5% and Pseudomonas being predominated in GNB’s (16%). Enterococcus faecalis had the lowest rate (3.7%) the sensitivity towards Cefoperazone-Sulbactam and Vancomycin was maximum for all GNB’s (81%) and GPC’s (100%) Vancomycin resistance was found in Enterococcus faecalis (12.7%) (Garg et al., 2007).

2.v. RESISTANCE IN STOOL INFECTIONS
Faecal bacteria like E. coli and Enterococci in healthy children could also contribute to spread and transmission of antimicrobial resistance genes. From a study on healthy children stool samples conducted in Portugal in year 2012, E. coli and Enterococcus were mainly isolated. Resistance to ampicillin by E. coli isolates was seen in nearly 40% of the E. coli strains. Whereas, resistance rates of other antibiotics like Tetracycline and Streptomycin were 25.0% and 26.1% respectively. Tetracycline resistance was of highest percentage in all Enterococcus isolates (28.7%). Antibiotics like Erythromycin and Kanamycin were also found to be of no effect on isolates. The resistance rate of isolates to both of the antibiotics was 21.8% and 8.9% respectively (Glatz, 2012).

In another example where stool samples from neutropenic cancer patients were taken, the resistance here were also high. Among a number of stool samples, 35 were found to be positive for Gram-Negative rods. The most common isolates were Escherichia coli (29%) was found to
be the first commonest rod found in stool samples, whereas, *Pseudomonas aeruginosa* (19%) was the second common bacteria isolated. There were few (13) isolates that persistently infected patients. There was most of *P. aeruginosa* isolated were non-susceptible to many antibiotics (Wingard *et al.*, 1986).

A study conducted on stool samples in year 2010 reported 50 Shigella isolates from 412 stool samples. The resistance of isolates to the antibiotics was very high. Ampicillin resistance was seen all the isolates, whereas, 96% of isolates were resistant to Nalidixic Acid, 94% to Tetracycline and 82% to Ciprofloxacin (Bhattacharya *et al.*, 2010).

### 2.w. RESISTANCE IN PUS AND WOUND INFECTIONS

Staphylococcus aureus has been considered as most common causative organism in wound and pus infections. There may be a reason that this pathogen is an endogenous source of infection. In infection of surgical site, nose contains *S. aureus* as normal flora, which precipitates the risk of infections. Another risk factor is contributed by its presence in environment, which can contaminate surgical instruments. Moreover, the disruption of natural skin barrier also helps *S. aureus* to easily find the way into surgical sites. In Raipur, India in year 2012, when pus infections were observed the main causative organism associated with these infections was found to be *Staphylococcus aureus* (45.1%), this pathogen in pus was found to be sensitive to almost all antibiotics like Amikacin, Azithromycin, Ciprofloxacin, Clindamycin, Cloxacillin, Chloramphenicol, Moxifloxacin, Linezolid, Gatifloxacin. These findings coincide with the findings of Taylor (1992) on surgical site infections where *Staphylococcus aureus* (50.32%) was mostly found. These findings also coincided with Buwembo where identified *Staphylococcus aureus* was the commonest causative agent of wound infections in Mulago hospital (Verma, 2012).

Antibiogram of local area can help in well judged and rational use of antimicrobial agents. A study conducted in Bhopal in year 2013 to set antibiogram of *S. aureus* in pus samples, a total of 216 pus samples were taken, from which 168 organisms were isolated including maximum of *S. aureus* (60), and minimum of *Streptococci spp.* (16) rest include GNB’s like Klebsiella spp., *Pseudomonas* and *Escherichia coli* with 32, 36 and 24 in number respectively. Sensitivity to Linezolid and Gentamicin for *S. aureus* remained 86.7%. According to the sensitivity patterns,
the most suitable antibiogram was Cloxacillin, Ampicillin-Sulbactam, Clindamycin, Chloramphenicol and Gentamycin (Kumar, 2013).

In this context, a study was conducted to set an antibiogram of Klebsiella pneumonia from pus isolates, out of total pus samples, S. aureus was isolated being the most common pathogen (36.7%) responsible for the pus and wound infections. K. pneumoniae (21.1%) accounted for 2nd most common organism of all pathogens. Here in the study the antibiogram that was set by disk diffusion method described by Kirby-Bauer (in year 1961) included to Amikacin, Gatifloxacin, Gentamicin and Chloramphenicol. Other organisms were Pseudomonas spp. (18.3%), Escherichia coli (12.7%), Streptococci aureus (9.8%) and Proteus spp. (1.4%) (Kumar, 2013).

When in Enugu and Abakaliki of Nigeria Pseudomonas aeruginosa was studied from pus samples for antimicrobial resistance, the results of the study included mainly P. aeruginosa strains (64%). The resistance rate was obtained highest for Amoxycillin (88.2%), followed by Cotrimoxazole (76.5%), which was considered to be 2nd most common antibiotic resisted by isolates. This was followed by resistance to Streptomycin, Gentamycin, Chloramphenicol and Ciprofloxacin to 67.6%, 58.8%, 58.8%, 23.5% by the isolates respectively. According to this study, Ciprofloxacin was the most appropriate drug opted in many countries like Abakaliki so that P. aeruginosa infections could be effectively managed (Amadi et al., 2008).

Another study carried out in Kingdom of Saudi Arabia revealed that Proteus spp. comprised of total 88% of all wound and pus infections. The study showed that Imipenem was the most sensitive drug (91%) for Proteus spp. this was followed by Amikacin (61%). infections rates by Proteus spp. during summer season were noted highest (Bahashwan and Shafey, 2013).

Another study conducted on pus isolates for antimicrobial resistance showed that the rate of positive pus cultures was 89.47%. Here also, like other studies, Staphylococcus aureus was the most common causative agent that was followed by other GNB’s like Pseudomonas aeruginosa, E. coli, K. pneumoniae, Proteus and GPC’s like Streptococcus pyogenes, S. epidermidis. For Gram positive pathogens, Vancomycin, Levofloxacan and Clindamycin came out to be the most
appropriate antibiotics. Among the Gram negative isolates, the most suitable drugs found were Imipenem, Amikacin, Piperacillin-Tazobactem and Levofloxacin (Rao et al., 2014).

2.x. IMPORTANCE OF THE STUDY

It has been seen that Bacterial infections are responsible of high rates of morbidity and mortality across globe, especially in developing countries (Bryce et al., 2005). antimicrobial non-susceptibility, when is emerged, erodes away the management of infectious diseases globally (Cohen, 1992; Tenover and Hughes, 1996). Already mentioned, easy availability of poor-quality antimicrobials at cheaper prices even without prescription, and many other factors support initiation of non-susceptibility, this may happen in low income countries more frequently (Taylor et al., 1995; Pillai et al., 1999; McIlleron et al., 2002; Paterson et al., 2004; Newton et al., 2006). Concurrently, results of antibiotic resistance may worsen either the settings with limited economical resources, because the substitutes for antimicrobial drugs are unavailable or unaffordable and sometimes both (Hart et al., 1998; Zaidiet et al., 2005). Spread of HIV also influences to some extent of bacteria causing infections (Archibald et al., 1998) and their antibiotic resistance patterns (Frieden et al., 1993; Wolday et al., 1998). Though it is important, but there is a little information published on antimicrobial resistance in the developing world. Available data from developing countries (Urassa et al., 1997; Belihu and Lindtjørn., 1999) suggested that the resistance rates in GNB’s were alarming over there. Since antimicrobial resistance varies greatly among geographical locations, it has become essential requirement for clinicians to shape factual therapy of serious infections like bloodstream infections on the basis of good knowledge of the ubiquity and patterns of antibiotic resistance of local bacterial isolates (O’Brien, 1997). The purpose of the of the study was to try to get a more clear vision in the epidemiology of specific bacterial infections and their resistance patterns in selected area of Punjab so that the evidences available can be increased to make final, judicial judgments on antibiotic therapy.