CHAPTER 1

1. INTRODUCTION

The modern antimicrobial revolution can be assumed with the accomplishment of the first natural antibiotic Penicillin and the synthetic antibiotic Sulfonamides in the early 90’s (Cantas et al., 2013). In spite of knowledge about several antibiotics as significant milestones of today’s medicine for the past fifty years, to treat bacterial infections is once again getting increasingly tough because of antibiotic not responding to these antibiotics globally (Ashbolt et al., 2013). Among the global challenges, it became a challenge for the whole world that affects all pharmaceutically used antibiotics. It has become the prime health care concern with a rapid increase in the past ten years, to treat the severe cases of infections among patients. A research on determinants of resistance to antibiotic revealed that resistance has covered and affected all the classes of society; not like VIP syndromes where only high class of society is affected (Rossolini et al., 2010). Microbes with an increasing rate of non-susceptibility to empirically used antibiotics include Staphylococcus aureus which is resistant to Methicillin (MRSA), Shigella species, new strains of non-susceptible Enterococci that is non-susceptible to Vancomycin (VRE), and Salmonella species non-susceptible to many antibiotics, other GNB’s of colon vis a vis. Klebsiella and Enterobacter species non-susceptible to the beta lactem antibiotics that are for wide usage and Streptococcus Pneumonia that is non-susceptible to Penicillin (PRSP) (Conly, 2002).

Worldwide, antibiotic resistance is the current issue of serious concern. It is more important when not one or two but multi drug resistance is threatening the world. The most common reason of raising the antibiotic non-susceptibility is unbalanced use (non required or wrong prescription) of antimicrobials globally. Antibiotics are popped up and equally discarded in very unnatural and unethical ways by human race. Hence humans themselves provide the antibiotics or their remnants in the environment to microbes, so that they offer sensitization to bacteria to change their genes for resistance. Each microbe has a different resistance mechanism for particular antibiotic, which may involve an intrinsic method, where bacteria or any other microbe itself has resistance genes naturally in it, or it may be acquired which results in gaining resistance on arrival of
adverse conditions for survival (Girgis et al., 2009). Apart from this, in adaptive mode exists, when the microorganism comes in contact with the antibiotic specifically, it starts some down regulation of its physical properties so that the microbe may not receive the antibiotic (Girgis et al., 2009; Hollenbeck et al., 2012). If all medical practioners, nurses, midwives or any kind of health care professionals, and even common man behave sensibly and sensitively on consumption and discard of antibiotics the problem can be half solved. At the same point of time, it is highly recommended to speed up the studies at genetic and molecular level to understand the regulation of genes responsible for antibiotic resistance (Frye et al., 2013). Furthermore, biological solutions of antibiotic resistance need to be searched.

There is no doubt that special health care units like premature infant care, organ transplant, surgery cannot work efficiently without antibiotics. Only effective antibiotic therapy results in such successful procedures. If effective measures are not taken to combat antibiotic resistance, it will lead to raised mortality and morbidity (Sandoz et al., 2010).

There are many new potent synthetic antimicrobials that are active against Gram-positive cocci have been commercialized for treatment in the last few decades, but there is no advancement seen in the case of Gram-negative bacteria and almost no new antibiotic class has been seen potent against GNB that are non-susceptible to many drugs more than one or could have been expected long. Although tough to think, the truth is this more health care people will have to see a cutting edge in the therapy of many types of serious bacterial diseases. We may soon face the post antibiotic era with a stressing factor that may take us retrospectively to the the 1930s and early 1940s, the time when antibiotics were not in use (Carlet et al., 2012).

Enhancement in antimicrobial surveillance system has taken keen interest of government worldwide. There are various government institutes like Infectious Diseases Society of America’s (IDSA) and World Health Organization (WHO) and that have stepped forward to combat with antibiotic resistance. The activities of management of antibiotic resistance should be enhanced, strengthened and stringent. Big investments and research on antimicrobial resistance is the need of the hour. The investments in diagnostics Research and development and its integration to clinical practice must also be raised so that the discovery of new drugs can be increased to cope up resistance. Important measure that need to be taken to eliminate insane use of antibiotics in animals, marine environment and plants.
At present, biological solutions for antibiotic resistance are in trend and could offer justified solutions. Use of products of other bacteria/algae for inhibition of pathogenic microorganisms could be a safe and smart strategy. One major benefit of it will be that if a bacterium mutates somehow to change its genes to create resistance, on the other way the bacteria/algae that produces bacteriocins will also be able to produce the new allo-chemical by being in the same environment naturally to that particular resistant bacteria (Kohanski et al., 2010). There are so many other microbes that produce bacteriocins to help in control of microbial growth. Moreover, phages can also be used in killing the bacteria in spite of using antibiotics (Lawrence et al., 2013). Other new and clear policies on management of resistant microorganisms should be created which may focus on hygienic and sanitation standards for the society. The advisories to keep vigil eye on all infections and their susceptibility patterns must be released in all government and private sectors.

1.a. ANTIMICROBIAL SUBSTANCES

These are the agents that today are the main tools of treatment to cease or cure almost all bacterial infectious diseases. The word antibiotic is referred to state to some chemical or biological agents used to stop or to kill the growth of microorganisms basically, same as antimicrobial agent (William, 2009). Antibacterials are the secondary metabolites of bacteria with specific antimicrobial properties against bacteria (Aminov, 2010). These antimicrobial agents in form of natural or synthetic antibiotics are being used in management and cure of bacterial infections regularly all over the world (Aminov, 2010). The role of antibiotics is either to kill or to stop the growing population of bacteria. The action of antibiotics may be versatile in nature effecting broad range of microbes such as fungi, protozoa but not on viruses. Though taken for wellness of humans, some of antibiotics may be toxic to humans and animals, even under prescription and may cause harm when used unfairly. There are some studies that have distinguished between antibacterials and antibiotics, with antimicrobials that are used in detergents, soaps etc., but not in medicine (Aminov, 2010).

The effectiveness of antimicrobials with antibacterial compounds in treating various bacterial infections depends on a long list of factors. These factors may be host immunity, the predilection or of location pathogen/ infection, and the antibacterial properties of the pathogen (bactericidal
or bacteriostatic)(Pankey et al., 2004). Some laboratory and clinical studies have shown that a bactericidal property of antibiotics often requires the exponential phase of bacteria, with running metabolism and division of bacterial cells(Pelczar et al., 1999; Pankey et al., 2004; Mascio et al., 2007). Since the property and activity of antibiotics also depends on the concentration in which the antibiotic is being used.(Rhee et al., 2004) so to determine the type of antibacterial activity in vitro detection by MIC and MBC is done usually (Pankey et al., 2004; Wiegand et al., 2007).

1.b. EVOLUTION AND HISTORY

Antibiotics entered the health care services in the 20th century, and with development of vaccines simultaneously, that was nearly to eradicate the diseases or infections like tuberculosis in the developed countries. The progressive achievements in curing diseases by antibiotics, their quick effectiveness and easy availability led to overuse/misuse, of these drugs/antibiotics, which encouraged the bacteria to develop resistance. The widespread problem of antibiotic resistance is so large that the World Health Organization declared antibiotic non-susceptibility as a threatening hazard. This has no anymore been future forecast, but has happened right now globally with potency to hit anyone, with different age groups, any geography (Aminov, 2010).

Before 20th century, therapies for microbial infections were predominantly based on traditional beliefs. Combinations of some therapeutic agents with antimicrobial qualities which were in use of treating many infections have been discussed and reported as old as 2000 years ago (Lindblad, 2008). Certain age old societies, inclusive the cultures of Egypt and Greece, used few selected fungi and plant extracts and other materials of the plants to treat infections (Forrest, 1982; Wainwright et al., 1989). Later the laboratory researches revealed antagonist association among microbes and helped in the finding of natural antibiotics synthesized from the microbes. In 1877, Louis Pasteur and Robert Koch found an interesting fact that the growth of Bacillus anthracis can be inhibited by an airborne bacillus (Landsberg, 1949), which became the first found antibiosis study. Later on, Selman Waksman, who was an American microbiologist, in year 1942 renamed these drugs as antibiotics. In Germany, in the late 1880’s Paul Ehrlich started development of Synthetic antibiotic chemotherapy (Calderone et al., 2007). Ehrlich observed that some coloring agents would color all types of living or dead prokaryotic or eukaryotic cells, while some coloring agents may not. Thereafter an idea was proposed by him was to produce
some synthetic chemicals that might be selective to kill pathogenic bacteria without wrongdoing with the human host. After working on many and many of dyes against many microorganisms, in year 1907, a medicinally useful drug was developed or discovered by him. The synthetic antibacterial Salvarsan that is now called Arsphenamine (Limbird, 2004; Calderon et al., 2007; Bosch et al., 2008).

Looking into history of antibiotics, we found that the very first antibiotic, Arsphenamine revolutionized the period of antibacterial treatment. In year 1907, Alfred Bertheim with the cooperation of Paul Ehrlich, synthesized it for treatment of this STD (Goodman et al., 1941; William, 2009). In 1933, Gerhard Domagk discovered the first systemically effective antibiotic, Prontosil (Goodman et al., 1941; Aminov, 2010).

The effects of some kind of molds as antibacterials for some infections had been observed many a times. In year 1928, Mr. Fleming found similar response in a test plate, here some pathogenic bacteria were eradicated or killed by a fungus Penicillium. Fleming theorized this act of killing the pathogens is encouraged by an antibacterial amalgam that can be used for chemotherapy and he named it Penicillin. At first Fleming identified few of biological qualities of Penicillium, and also tried to make a basic composition to treat some infections, but Fleming could not follow its next level development without the support of expert chemists (Fleming, 1980; Sykes, 2001).

The first antimicrobial compound used in modern medicine was produced biologically, for an instance the Penicillins from fungi Penicillium and Streptomycin produced by bacteria Streptomyces. The development of organic chemistry escorted many antimicrobial agents to be synthesized chemically, e.g., sulpha drugs and Quinolones. Bactericidal or bacteriostatic are the two terms that classify the antibacterial agents into two. Bactericides kill bacteria directly whereas bacteriostatic antibacterials stop the bacteria from growth and division. However, both of these lead to cessation of bacterial infections in any way (Hancock, 2005).

The very first commercially available antibacterial, Prontosil, and first sulfonamide were developed by a researcher Gerhard Domagk and his teammates in Germany. This sulphonamide had a wide range of effects against Gram-Positive Cocci. These effects were not seen against Gram-Negative bacteria (Bosch et al., 2008). This finding after its successful venture, was
provoked quickly in the pharmaceuticals. evolvement of the Sulphonamide drug i.e., prontosil created opportunities to newer antibiotics.

Tyrothricin, which was the first antibiotic that was synthesized naturally from Bacillus brevis was discovered by Rene Dubos in year 1939. It was one of the first antibiotic produced at large scales for commercial use. This antibiotic was globally. This antibiotic responded very well in treatment of lesions, abscesses (Van, 2006).

In 1942, the first Penicillin, Penicillin G was successfully purified with qualities of very low toxicity and wide spectrum effect by Florey and Chain. Though discovered with additional non-degradability and enhanced sustainability in biological constituents like pus, unlike the synthetic sulfonamides, this antibiotic did not make place commercially outside some branches of defense system earlier in 1945. However, this discovery was considred as milestone. This discovery created and renewed interest of researchers through the paths for finding appropriate antibacterial agents (Flory, 1945).

1.c. The term “antibiotics”

In 1942, Selman Waksman used the term antibiotic in research papers to refer the chemical compound produced by microbes, which suppressed the population of another microbes. According to this term the products that used to kill the microbes were not synthesized form microbes itself e.g., gastric juices and hydrogen peroxide were not termed as antibiotics (Waksman, 1947).

1.d. SYNTHESIS

As the medical sciences developed, almost all the antibiotics have been produced semi-synthetically with various changes in natural antibacterial compounds (Von et al., 2006). For an instance, the Beta-Lactem antibiotics, including the Penicillins (produced by fungi Penicillium), the Cephalosporins, and the Carbapenems. There are certain antibacterial agents, which are isolated from living organisms only e.g., Aminoglycosides, however, Sulfonamides, Quinolones and Oxazolidinones are some examples of antibiotics that are purely synthetic. In relevance to
this, antibacterial compounds are categorized into natural, semisynthetic, and synthetic antibiotics, depending upon their chemical/biosynthetic origin.

1.e. CLASSIFICATION OF ANTIBIOTICS

Classification of antibiotics is done depending upon their mode of activity against bacteria (Tenover, 2006). These methods involve disturbance in normal synthesis of cell wall (i.e., β-Lactams), protein synthesis inhibition (Macrolides), disruption in nucleic acid synthesis (Quinolones), disruption in metabolic pathways of bacterial cell (Trimethoprim-Sulfamethoxazole), and other antibiotics like polymixins interfere with cell membrane structure of bacteria.

Antibacterials may also be classified according to their chemical compositions. Most of antibiotics aim inhibition of growth and bacterial metabolic activities (Calderon et al., 2007). The antibiotics that attack on the cell wall of bacteria (e.g., Penicillin and Cephalosporin), the bacterial membrane by antibiotics like Polymyxins, or disrupt cellular enzymatic activities of bacteria (Rifamycin, Lipoarmycin, Quinolone, and Sulfonamides) are known for their bactericidal properties. The antibiotics that disturb the proteins synthesis in bacterial cells e.g., Macrolides, Lincosamides and Tetracyclines, are known for their bacteriostatic property (except bactericidal Aminoglycosides)(Finberg et al., 2004).

Targets ob bacterial sites, that are specified to be attacked by the becomes the part of other type of classification like GNB or GPC. When these antibiotics attack on specific are known as "Narrow-spectrum" antibiotics, whereas, when an antibiotic affects a wide range of bacteria, irrespective to its cell wall structure, is called “Broad-spectrum” antibiotic.

One more classification of antibiotics is set upon the activity, where, two broad categories have been used to segregate antibacterial agents observing upon their inhibition effect on microbes: Bactericidal and bacteriostatic agents.

1.f. ANTIMICROBIC RESISTANCE
Resistance is a property of a microbe, to resist against the antimicrobial agent that was once able to treat or kill that microbe (Kirkil, 2007).

Very clearly, the antibiotic resistance by almost all clinically important bacteria has become a general occurrence and it is assumed that an evolutionary process occurs during antibiotic treatment that could raise initiation of resistance. Enhanced capacity by bacteria to survive high doses of antibiotics may depend on the antibiotic treatment given and may be because of physiological or genetic alterations in the strains. Moreover, in some situations, some of other susceptible bacteria are inhibited by the antibiotic that was supposed to kill the responsible pathogen, which may lead to the favored growth of resistant bacteria (Levy, 1994). Antibiotics like Penicillin and Erythromycin that were once used as highly effective drugs against many bacterial infections are now considered to be very less effective, because of resistance developed in many bacteria to these antibiotics (Pearson et al., 2007).

Antibacterials may also be degraded under the process of resistance e.g., Sulfamethazine-degrading bacteria from soil were experimentally transferred to Sulfamethazine in swine faeces (Topp et al., 2013). Resistance in bacteria in most of the cases is genetic in origin (Witte, 2004); however, resistance is also acquired through transfer if genes horizontally. Horizontal gene transfer happens commonly in geographical sites where usage of antibacterial agents is frequent (Dyer et al., 2003).

Antibiotic resistance may cost the lives of patients by enhancing survival of resistant strains, which can be controlled by ceasing the spread of antibiotic resistant bacteria. Still some mutations, however, may enhance the survival of these bacteria (Andertsson, 2006).

Historical data showed the mechanisms of antibiotic resistance are as old as the antibiotic itself. (D’Costa et al., 2011). The antibiotics that target specific sites of bacteria (with no impact of mutations on bacterial viability or reproducibility) are considered to be as useful antibiotics (Gladkiet et al., 2013).

Apart from physiological mechanisms many genetically driven modes of resistance for antibiotics are also known. This Intrinsic resistance to antibiotics helps in forming resistance genes during genetic makeup of bacteria (Alekshuni et al., 2007). These genes either deplete an
antibiotic target from the bacterial genome resulting in non effectiveness of that antibiotic to that particular strain or acquired resistance comes as a result of a genetic change in the cellular genome. Otherwise the change in the genome could be by the accession of extra-chromosomal genes in the genome, that is why, more particularly known as acquired resistance (Alekshuni et al., 2007). Antibiotic-producing bacteria have also shown resistance to the antibiotic they produce themselves. In some cases it has also been seen that they acquire resistance genes from other antibacterial-resistant strains to the antibiotic they produce (Marshall et al., 1998; Nikaido, 2009). Apart from HGT or recombinations of DNA, Antibiotic resistance also can be spread through vertical transmission of mutations at the time of log phase (Witte, 2004). The studies showed that genes of antibiotic resistance may transfer among various other species or strains of bacteria with the help of plasmids that carry and transport these resistance genes (Witte, 2004; Baker et al., 2006). Some Plasmids carry many different resistance genes at a time and may grant resistance to multiple antibiotics (Baker et al., 2006). When a single gene transfers resistance to multiple antimicrobial compounds, the phenomenon is known as Cross-resistance to many antibacterial (Baker et al., 2006).

Any bacteria that were once sensitive to the particular antibiotic get resistance due to genetic reasons also (Williams, 2009). This resistance due to genetic mutation can be instantly acquired by change in the transfer of genes. By adopting one of these mechanisms, the bacteria get resistance to antimicrobial agents. There are many bacteria, which show resistance towards two or more than two antibiotics are termed as Multi Drug Resistant (MDR) and commonly called as superbugs (Wilson et al., 2007).

The bacterial strains like Mycobacterium that were initially sensitive but now are getting resistant to same antibiotics are referred to as "superbugs". A study showed that almost 5 lac of new cases of multidrug-resistant tuberculosis bacteria that is resistant to many antibiotics has been reported increasingly. Another instance, NDM-1 is a newly found enzyme that is responsible for conveying resistance in many bacteria to many of penicillin antibiotics (Boseley, 2010).

Antibiotic used in both worlds of medicine between human and animal are facing high rates of resistance. The growth of bacteria that are resistant is flourished by the usage of antibiotics that
increase selective pressure for bacteria in both humans and animals resulting in or by causing the death of susceptible bacteria.

Although the researchers have been advancing towards the eradication of resistance but by time resistance to antibiotics are being hardened. Though there is a hightime for newer antibiotic therapies, new antibiotics are continuously being rejected by bacteria. Therefore, with an alarming increase in antibiotic resistance there is a strong need for alternative treatments. Staphylococcus aureus has been found resistant to Methicillin and Vancomycin MRSA, gram negatives are found resistant to other antibiotics like ESBL producers are the latest examples of non-susceptibility. Moreover, Enterococcus has been seen resistant to Vancomycin and Acinatobacter to many antibiotics are the common examples of drug-resistant bacteria. The trend is also observed in other microbes like non cellular Viruses or eukaryotic cells like fungi and protozoa of gaining resistance to the antibiotics that once upon a time were effective against these microbes.

1. g. CONTRIBUTING FACTORS TO ANTIBIOTIC RESISTANCE

Some studies revealed that the antibiotic resistance existed since the time of discovery of antibiotics. despite of the knowledge of already present antibiotic resistance, more bacteria are becoming resistant due to widespread antibiotic use during past few decades. This process is commonly known as evolutionary pressure(Spanu et al., 2008).

There have been many reasons that indicate the widespread usage of antibiotics:

1. The easy and increased availability of antibiotics globally since the 1950s,

2. Many developing countries with poor income sources allowed pharmacies to sell the antibiotics without prescription, which resulted in tremendous use of antibiotics even when they are not required and it could have resulted in emergence of resistance in rest of the bacteria (Sharma et al., 2002).

3. Treatment of any bacterial infections with broad-spectrum antibiotics, this could have induced the resistance of narrow-spectrum antibiotics.
4. The countries, where livestock feed is conducted at industrialized scale, accept the use of low doses of antibiotics for growth promotion of livestock feed, which directly leads to resistance (Srivastava et al., 2011).

5. Pharmaceutical companies during manufacturing of antibiotics, release antibiotics at large scales into the environment because of improper treatment of wastewater (Larson, 2007).

6. The certainty of antimicrobial agents in detergents and other disinfectant or antiseptics has lead to antimicrobial resistance, the usage of these products is still not appreciated by scientists (Larson, 2007).

1.h. ANTIBIOTIC RESISTANCE IN HUMAN MEDICINE

According to the studies on antibiotic resistance in human, it has been found that the intensity of resistance in few of the bacteria is found more than that in other bacteria. Some bacteria may show resistant to one antibiotic only, while others have potency to impart resistance to more than one antibiotic. In this context, multi-drug resistant Mycobacteria are the example that carries many resistance genes and is resistant to more than one antibiotic (Thacker, 2012). In the patients that use Glycopeptide, Cephalosporins, and Quinolones have shown increased rates of MRSA infections (Al-Habib and Al-Saleh., 2010). Clostridium difficile has been found more likely to be colonized with the use of some antibiotics like Cephalosporins, Clindamycin and specifically Quinolones (Smullen et al., 2007). One study showed that antibiotic resistance corresponds to the amount of antibiotics consumption or noncompliancein consumption of antibiotics (D’Costa et al., 2011). Inappropriate prescription of antibiotics is represented in many reasons, which includes a) patients themselves pop up drugs, b) medical health care personnel suggest the patients when they really run short of time to describe to patients that why antibiotics are not necessary, c) when physicians do not have knowledge of appropriate antibiotic prescription (Alekshun and Levy, 2007). For an instance, approximately 35% of population think that the use of antibiotics in common cold will be effective to treat the symptoms (Van, 2006) however, this viral infection is the most common illness that is wrongly prescribed for antibiotics even after knowing that antibiotics are of no use against viral infections (Limbird, 2004). In the patients, a single
prescribed course of antibiotics ranging from a month to a year possibly may increase the chances of bacteria to become resistant to antibiotics given (Lindblad, 2008).

As mentioned above, duration of treatment has an important role in acquiring antibiotic resistance; therefore, if effective minimal course of antibiotics is maintained, it can decrease the rates of resistance. This will also reduce the cost of the treatment, and will show better outcomes in short durations. There are some examples of infections like Community-Acquired Pneumonia, middle ear infection and throat infections, where short courses of antibiotics are adopted (Boucher et al., 2013). Though a study suggested a wise usage of antibiotics by stopping the use of antibiotics after 3 days of course within which the symptoms disappear (Metlay et al., 2007), but in some cases short course is not considered to be fruitful e.g., Tuberculosis (Ong et al., 2007).

Frequency at which antibiotic resistance is adopted by pathogens depends on transmitting the resistant bacteria to healthy individuals through surroundings or interactions with patients. A factor that is included in mushrooming of antibiotic resistance is the intensive care unit settings where mechanical ventilation and underlying diseases in the patients have come into sight in contribution to the spread of bacterial resistance (Marquez, 2005). The resistant bacteria are speeded to patients by hospital staff because of Poor hand hygiene has also been found related, this was proved by a test given to hospital staff where the increase in hand washing resulted in lowering the rates of the resistant organisms (Sabuncu et al., 2009).

The factors like racialism and poverty are some of the socioeconomic factors that affect availability and compliance to drug therapy, which leads to drug resistance. Against this, some structural sadism improvement programs may contribute to the effective treatments for drug-resistant strains (Ambroi et al., 2010).

1.i. ANTIBIOTIC RESISTANCE IN VETERINARY MEDICINE

For the, use of antibiotics in animals of live stocking or for the purposes of animal husbandry could also be one of the reason of emergence of antibiotic-resistant bacteria in human medicine (Everette et al., 1996). It has been found that tremendous usage of antibiotics in the field of animal livestock or husbandry has evolved since late 20th century. In animals, the antibiotics are
used as 1. Therapeutic agents, 2. prophylactic agents, 3. metaphylactic agents, and 4. growth promoters (Everette et al., 1996). These all four uses contribute to bacterial resistance, by being released in the environment through one or more ways e.g., any release in water, soil and air. The surroundings of bacteria get in contact with the remnants of antibiotics released, which slowly induce resistance genes in the bacteria present in environment. This is how a new sensitive bacterium becomes a resistance strain to the antibiotic by vertical gene transfer. As we know that antimicrobial non-susceptibility is a slowly emerging phenomenon that occurs naturally process, the uses of antibiotics among animals that increases their exposure to animals and thus to bacteria, for more and long promoted durations at smaller levels. In this way, the use of antibiotics in animals, nontherapeutically, greatly increases the non-susceptibility (Everette et al., 1996). A study in U.S. reported that in year 2013 out of total antibiotics usage of antibiotics rose to 80% from 50-% since year 1997. This has endangered the environment by antibiotic remnants being released into surroundings (Maeshall et al., 1997). There are some antibiotics that are not considered to be important and are not used in humans, because of the deficiency of efficiency or due to non-fulfillment of human purposes like, ionophores in ruminants (Livermore and Yuan., 1997). Some other antibiotics like Penicillin and some of Tetracyclines are used in both humans and animals (Landsberg, 1949).

Among the effective measures to combat with resistance, in spite of dealing directly with resistant bacteria, food animals for their antibiotic consumption has been regularized to control the drug remnants in poultry or dairy products.

A team of scientists, in 2001, estimated that U.S. uses more than 50% of the antibiotics animals that are to nourished to be food for humans (pork, canines like beef and chickens) without any signs or symptoms of disease in them (Ian et al., 2001). The amounts, in which the antibiotics were given to those animals, were termed as sub-therapeutic i.e., inadequate to tackle or cure disease. In spite of no evidence of disease, these drugs generally help in reducing morbidity and mortality with flourished growth of the animals, when given. It is postulated that the sub-therapeutic dosages kill some sensitive bacteria in the animal and leaves the bacteria that are non-susceptible to antibacterial agents intrinsically. Due to this the number of bacteria in surroundings gets always constant; the jumbled bacteria are affected only. The basic mode of action of antibiotics to which sub-therapeutic dosages of antibiotics enhance the growth of
animals is still not clear. A research study have theorized that some animals or birds could be the victims of non symptomatic infections that would be cured by giving them sub therapeutic dosage of antibiotics in feed, thereby allowing them to survive longer. In United States, most of these antibacterial agents are used in animals to promote the production strength of animals also (Ian et al, 2001).

In some studies, drug-resistant organisms transmitting from animal to human has been revealed. The bacteria that are non-susceptible to many antibacterial agents get crosstransferred from canine to humans in basic modes three in number: by utilization or consumption of animal products like poultry and dairy products, cattle or fowl handling or indirect contact with animals through surroundings (Rowland and Turnidge, 2000). These three mechanisms of spreading bacteria from animal to human are the main cause of zoonotic diseases. The spreading through animal products can be reduced or eliminated by food preservation methods that prohibit the growth of these bacteria in foods. But the proofs for the transmission of antibiotic-resistant bacteria from zoologically is found few, with many evidences that prove that these microbes limit within humans (Lautenbach et al., 2001).

According to World Health Organization, insufficient usage of antimicrobial agents in livestock is an elementary involvement that has a key role in exposure and spread of bacteria that are resistant to antibiotics. The WHO concluded by stating that the antibiotics should not used as animal growth enhancing agents (Rowland and Turnidge, 2000).

1.j. NATURAL OCCURRENCE OF ANTIBIOTIC RESISTANCE

Environmental resistome is the resistance gene that imparts the resistance naturally to the bacterial cell towards particular antibiotics. The antibiotic resistance that occurs naturally is very common (Reed et al., 2005). The environmental resistome genes that can be spread from environmental non clinical bacteria to clinically important pathogenic ones, which lead to serious concern about resistance to clinically important antibiotic (Linden et al., 1996). In year 1952, the existence of Penicillin-Resistant bacteria before the effective treatment with Penicillin was started was discovered (Bhavnani et al., 2000); moreover, resistant genes to Streptomycin also existed before (Edmond et al., 1996). In 1962, the enzyme Penicillinase existence in spores of
Bacillus *licheniformis* were also detected from soil of plant that were revived (Stosor *et al.*, 1998; Pelzet *et al.*, 2002; Carmeli *et al.*, 2002). From the bowels of two persons, six strains of *Clostridium* were found, which were found resistance to *Clindamycin* and *Cefoxitin* (Whitby *et al.*, 2001). It is theorized that emergence of Penicillinase could have developed as a protective lifestyle of microbes in their niches, for an instance, *Staphylococcus aureus* that is rich in penicillinases, lives with *Trichophyton* that produces Penicillin. But this is not necessary to happen in all cases (Whitby *et al.*, 2001). Search for a class of proteins that primarily are capable to combine with Penicillin to increase its efficacy has given a new direction in the study of Penicillinases ancestors also (Cosgrove *et al.*, 2003). There is a documentation that some pollutants and heavy metals may be chosen as reservoirs for antibiotic-resistant small amounts (Einarsson *et al.*, 1998).

1.k. ROLE AND IMPACT OF ENVIRONMENT

It is evident that the environment has been polluted by antibiotics since the introduction of antibiotics to surrounding through animals, pharmaceutical industry, farming and last but not least human waste post medication (Pallares *et al.*, 1995). Simply, resistant bacteria also are released along with that antibiotic waste. Thus bacteria that are non-susceptible to antibacterial agents are introduced to surroundings directly. As short as the time taken by bacteria to replicate, the resistance genes are distributed in the environment rapidly. It has already been mentioned that during replication, the bacteria dividing their genes, can transfer their resistance genetic elements to rest of the bacteria via HGT and through vertical gene transfer also. From this we can infer that, the particular antibiotic-resistance genes will persist in the environment, when the antibacterial agent is no more present in surroundings (Pallares *et al.*, 1995).

1.l. GENERAL MECHANISMS OF ANTIBIOTIC RESISTANCE

There are basically four methods by adopting which the bacteria represent resistance to antibiotics:

a. antibiotic modification or inactivation
Penicillin G is deactivated by enzymes ß-lactamases in Penicillin-resistant bacteria. Generally, the antibiotic is deactivated by addition of an specific group to the particular antibacterial agent by the protective biological catalysts that are formed in bacteria that reduce the capability of antibiotic for ribosomal attachment of bacteria to inhibiton of production of proteins (Carmeli et al., 1999).

b. Changes in target site

In the bacteria resistant to Penicillin and MRSA, the binding site targeted for Penicillin is PBP; the Penicillin binding protein is altered by protective enzymes to reduce the binding ability of drug. Another example of the protective mechanism is found among bacteria whose ribosomes are attacked by drug to interrupt protein synthesis. Here the bacterial cells start producing ribosomal protection proteins. These ribosomal protections bind to the ribosomes of the bacteria that in turn change in the conformation of ribosomes. By this mechanism the ribosomes keep on synthesizing essential proteins thus by preventing drugs to attach to the bacterial ribosome to stop the production of essential proteins in bacterial cell.

c. Metabolic pathway Alteration

Some of the bacteria alter their metabolic pathways to avoid their antibiotic dependent requirements. E.g., the important precursor for the folic acid synthesis and nucleic acid synthesis is para-aminobenzoic acid (PABA). In altered metabolic activity, if PABA not provided, the bacteria can survive without it e.g., sulfonamide-resistant bacteria.

d. Reduced drug accumulation

Some bacteria decrease drug permeability or increase active efflux pumps by decreasing the cell surface (Kim et al., 2002). In the cellular menembrane of some bacteria, there are specialized structures know as efflux pumps are set. The main role of these pumps to push the antimicrobial agent out of the bacterial cell immediately so that the bacteria may not face any damage. The efflux pumps are generally activated after introduction of an antibiotic along with a specific substrate(Schiappaet al., 1996).
In many cases, horizontal gene transfer leads to Antibiotic resistance (Sundsfjord et al., 2004). There are some point mutations in the bacterial genome that also carries antibiotic resistance but at as low rate as 1/108th of DNA production. Though the genetic changes are rarely found according to the figure but the rate of reporsuvctio of bacteria that is high enhances the distribution of genes also A mutation may either hinder the bacterial sites to attach to the antibiotic or can make the binding site different for antibiotic. This will allow the bacterial cell site to carry on the activity very well under the antibiotic pressure.

There are some examples of genes that are responsible for imparting resistance to bacteria:

Lex A gene has been studied and observed to be the main key for acquiring genetic changes so that Quinolones and Rifampicin may not be effective against bacterial cells. When the bacterial genes in the cell are damaged, the gene LexA that is an SOS gene repressor undergoes autoproteolytic activity. Following this, three nonessential DNA polymerases genes, that are required for mutation after DNA damage, are transcripted (Fluit et al., 2001). The bacteria that are mutated have the benefit of prolonged survival in environment. The bacteria reproduce and then pass their traits with mutations to their offsprings, hence leading to the evolution of completely resistant bacteria. Though the chromosomal mutations appear to help and ease pathogens. This is done by providing the non-susceptibility gens to the bacteria, but a bacterial health is also granted. For an instance, a mutation in ribosome protects bacteria by altering the targets the antibiotics by this the process of synthesis of protein is slowed down also (Carmeli et al., 1999). A distinct study particularly compared the few typhoid causing bacteria and Escherichia coli that were resistant towards antibiotics for overall fitness to the strains that were once sensitive to antibiotics. After the study it was observed that the fitness rate in all parameters like growth was decreased in antibiotic resistant ones (Livermore, 2005).

Fluoroquinolones resistance occurs by three mechanisms. There are some efflux pumps that help in reduction of concentration of quinolone intracellularly. They act by directly pumping the drug out (Livermore, 2005). Plasmid-mediated resistance genes have been found to produce some proteins that attach to DNA gyrase e.g., in Gram-negative bacteria thus protecting the GNB’s from the action of Quinolones. In other words, changes in the primary binding targets in DNA
gyrase or topoisomerase IV reduce the attaching capability for Quinolones; thus antibiotic effectivity is decreased (Austin et al., 1999).

For many laboratory protocols, Antibiotic resistance is artificially introduced as a selected marker into a microorganism to observe the methods of transfer of resistance gene or to identify some of bacteria that absorb a segment of DNA that includes the resistance gene or other useful genes. A research documented that Staphylococcus acquires the genetic genes much more than older ones by horizontal gene transfer and it also provides genetic elements apart from virulence and antibiotic non susceptibility. Apart from this, genes apart from mobile genetic elements are also introduced to species (Goossens et al., 2005).

Since long time, it had been assumed that, a microbe can get resistance only when large population of microbe is available, but the recent studies has proven that even a small population can get resistance genes and then transfer it to next generations at the rate of its reproduction(Goossens et al., 2005).

1.m. PATHOGENIC BACTERIA AND RESISTANCE PATTERNS

Pathogenic bacteria show enormous variety in their resistance pattern, frequency and mechanisms. Some of the known pathogens which are of prime interest in terms of antibiotic resistance are discussed here as specific

a) *Staphylococcus aureus*

*Staphylococcus aureus* (informally called "Staph aureus" and its infection as “Staph infection") has now days become one of the predominant resistant pathogens. This is found as one third of the total microbiota of exteriors of human like epidermis and mucus membranes thus antibiotic oppressions in terms of imparting resistance or some cellular changes in bacteria have a significant impact on this bacterium. *Staphylococcus aureus* was the bacterium that was found to be resistant to Penicillin at very beginning stages; in year 1947, the production was strated at high levels in large quantities. After this being noticed by scientists, *Methicillin* was then the antibiotic of choice for *Staphylococcus aureus* treatment. But due to notable kidney toxicity caused by Methicillin, it was replaced by the antibiotic *Oxacillin*. Thereafter, in year 1961, in
Britain, the first *Staphylococcus aureus* that was non-susceptible to Methicillin was detected. Afterwards this bacterial isolation, The bacteria was detected in hospitals very often. In year 1999, one report from UK documented by year 1999, the fatal cases of sepsis due to MRS infections reached up to 37% that were around 4% in year 1991. At present, US reports nearly 50% of all *S. aureus* infections as MRSA infections with resistance to antibiotics like Erythromycin, Penicillin, and Tetracycline. Thus Vancomycin was the only effective agent found at the time. But this was not the end, some strains started showing intermediate levels of resistance, or complete resistance towards Vancomycin (VISA or GISA). *Staphylococcus aureus* that was non-susceptible to Vancomycin was found in 1996 in Japan for the first time, and then the resistant strains started appearing in hospitals across Britain, French and the American populations. Resistance to Vancomycin was termed as Vancomycin-Resistant *Staphylococcus aureus* (VRSA) and it was reported first in the United States in 2002 (Takahashi *et al.*, 2002). To combat with this, in 2011, a variant of Vancomycin with quality of binding to the different lactates of bacteria or to the basic sites meant to be of mutated *Staphylococcus aureus* was discovered. This helped in restoring efficacy of antimicrobial activity of Vancomycin (Kloos *et al.*, 1998).

In the 1990s, Oxazolidinones, a new range of antibiotics, were commercially synthesized for health systems and Linezolid, analogous to Vancomycin in effectiveness against MRSA was launched, which was the first Oxazolidinone antibiotic. But resistance to this antibiotic too was seen after short period in year 2001 (Svec *et al.*, 2004).

One more complication in the way to deal with *Staphylococcus aureus* is Community-acquired MRSA (CA-MRSA). This is becoming out as outbreak at smaller levels, that lead to life threatening illness that are rapidly escalating in severe blood infections along with soft tissue infections also, the lung infection of this pathogen also becomes fatal. Very often isolated bacteria that are non-susceptible to antibiotics in American regions among many hospitals are MRSA. An incidence and distribution of MRSA infections are changed highly. The infections that were once confined to the patients for last 10 years, has jumped into community as CA-MRSA. The clustering of CA-MRSA cases are being commoner these days in various communities like rehabilitation centers, in newborn nurseries, sports, defense, and among gay
communities. Community acquired MRSA illness has turned out as endemic in various non-rural regions of world (Lowdy, 2003).

b) Streptococcus and Enterococcus

Streptococcus pyogenes cured by using a large number of different antibiotics normally. Especially in invasive GAS infections, early treatment can reduce the risk of death. Helping care in an ICU may be needed in cases of severe illness (Tiemersma et al., 2004). Though very easily killed bacteria, but the emergence of macrolide antibiotics resistant Strains of *S. pyogenes* have been reported. However, all strains of Streptococcus continue to exist consistently sensitive to Penicillin (Hiramatsu et al., 1997).

Some of the studies reported that Streptococcus pneumoniae’s non-susceptibility to Penicillin and rest of the Beta-Lactams worldwide has become a serious concern. Majorly, genes that encode Penicillin-Binding Proteins are presented to some mutations. Selective pressure is considered to be the major mechanism of resistance and furthermore, use of more beta-lactam antibiotics has been considered for imparting resistance to Streptococcus pneumoniae (Rice, 1999).

Associated with nosocomial infections, Enterococcus faecium and Enterococcus faecalisi that was non-susceptible to many antibiotics are known to be the main pathogens. Among these strains, Penicillin-Resistant Enterococcus, Vancomycin-Resistant Enterococcus and Linezolid-Resistant Enterococcus have also been reported in years 1983, 1987, in the late 1990s respectively (Okuma et al., 2002).

c) Pseudomonas aeruginosa

Exploiting immediate opportunities, Pseudomonas aeruginosa has become highly frequent pathogen. *P. aeruginosa* has presented its one of the most daunting character i.e., it’s very low antibiotic susceptibility. The main explanation of cause goes with a joint action by efflux pumps meant for resistance to many drugs and intrinsic genes encoded for non-susceptibility to antibiotics. However, the bacterial cells envelopes by the virtue of the low permeability also attributes to the resistance (Lomovskaya et al., 2001). 4-hydroxy-2-alkylquinolines (HAQs) that
have pro-oxidant effects (inhibiting oxidant systems) along with tendency to overexpress the increased susceptibility to antibiotics are also been found in Pseudomonas aeruginosa. Basically, in Pseudomonas aeruginosa relA and spoT genes are disrupted that help stringent response (SR) in cells under nutrition deprived conditions, leading to antibiotic resistance (Kriengkauykiat et al., 2005).

d) **Clostridium difficile**

Clostridium difficile is a hospital acquired clinically important bacteria that is responsible for diarrhea worldwide (Morena et al., 2013). The use of antibiotics like Fluoroquinolones, Cephalosporins, Carbapenems, and Clindamycin attribute to enhanced growth of *C. difficile* leading to the infection *C. difficile* colitis (Binion and David., 2010). There are some studies that declare that the overuse of antibiotics in the animal husbandry confers to outbreaks of *C. difficile* infections (Johnston et al., 2012).

The reason of overuse of antibiotics leading to *C. difficile* infections is that the normal flora of intestine is de-arranged by these broad spectrum antibiotics like Clindamycin. This condition, flourishes the growth of *C. difficile*. Pseudomembranous colitis caused by this pathogen can lead to the inflammation of the colon with progression of "pseudomembrane", which is a thick, sticky dump of dead, inflamed pus cells and fibrin (Just et al., 1995). In years from 1989 to 1992, diarrhea associated with *C. difficile* that was resistant to Clindamycin was detected in forms of large outbreaks in various regions of America. In year 2005, Fluoroquinolones-Resistant *C. difficile* (resistant to floxacin drugs) have also been reported in northern regions of USA (Bomers and Marije., 2015).

e) **Salmonella and E. coli**

It is very well reported that infected, non-potable water and food ingestion is the main cause of *Escherichia coli* and *Salmonella* Infections. Since the strains have been found in waters frequently, these strains have acquired the antibiotic resistant genes due to wide spread use of antibiotic and presence of their residuals in environment. These are the most commonly known bacteria, responsible for causing hospital-acquired infections. The reasons that have made them very much responsible in causing hospital linked infections could be their adaptations to
antibiotics used in hospitals (Lautenbach et al., 2001). Both of these bacteria are concerned with serious health conditions. There are reports of many outbreaks worldwide with fatality due to both of these pathogens. Fluoroquinolones antibiotics have been found not effective by these bacteria through some reports in the year 1993.

Though it was considered that mutations alone have an important role in imparting antibiotic resistance to these pathogens, a study in year 2008 revealed that survival of these bacteria after exposure to antibiotics is attributed not alone by mutations (Kim et al., 2002). The study emphasized on the non-susceptibility of bacteria to Ampicillin and many drugs like NA and tetracyclines. This study resulted that in E. coli little part of non-susceptibility resulted from acquired mutations in spite of common intrinsic mutations in DNA. These results again were confirmed with reports representing the antimicrobial susceptibility could be reverted too (Schuappa et al., 1996). When a gene expression is altered in spite of changing the gene code, the phenomenon is called epigenetics. There are many mechanisms of by which epigenetics may happen. This involves methylation of bacterial DNA and alteration in proteins like histone; whereas, the point of significance is that succession of mutations that occur randomly and the epigenetic markers, both end in the change of expression of gene, thus expressing antibiotic resistance genes (Schiappa et al., 1996).

f) **Klebsiella pneumoniae**

Another nosocomian infections agent is Klebsiella pneumoniae. Klebsiella pneumoniae has been studied to produce some carbapenemase, known as Klebsiella Pneumonia Carbapenemases (KPC). These KPC’s impart resistance to all or one Carabapenem antibiotics. Klebsiella pneumoniae includes many mechanisms for antibiotic resistance other than KPC, where most of them locate on highly mobile genetic elements (Schiappa et al., 1996; Paterson et al., 2004).

g) **Mycobacterium tuberculosis**

Over the past few years, Tuberculosis is the infection that is the matter of concern globally, especially in developing countries. TB has shown resistance for many drugs at a time, which is globally termed as Multidrug Resistant TB or MDR TB. According to a report, 150,000 deaths
annually occur due to MDR TB Globally. HIV/AIDS epidemic has also contributed to this infection because of low immunity in HIV patients (Archibald et al., 1998).

Among much common disease in earlier times, this was among one of the non-curable diseases until the antibiotic Streptomycin was discovered (Saiman, 2004). Like other pathogens, these bacteria soon developed resistance to Streptomycin. Since then, to treat TB Infection, antibiotics like Isoniazid and Rifampin have been used. The reason of developing antibiotic resistance in tuberculosis is sudden genetic changes in the genome. Resistance of TB to one drug is often seen, thus multiple antibiotics are chosen for the treatment. There are reports of tuberculosis bacteria that is non-susceptible to drugs extensively, where the bacteria causing tuberculosis gets resistant to the second line of drugs too (Mann et al., 2009).

The antibiotics like Isoniazid, Rifampin has stopped working on Mycobacterium tuberculosis. This has challenged the clinicians (Flynn and Chan., 2003). The evidences of horizontal gene transfer for plasmid exchange or plasmid associated resistance are still not available for MTB (Gagneux, 2009).

h) Neisseria gonorrhoeae

This bacteria, is a GNC, diplococcus, causes genitourinary diseases with symptoms of pelvic pain, painful urination, genital discharge. Some systemic symptoms also appear in Neisseria infection (Biais et al., 2008).

Like for other pathogens, initially in 1940’s, Penicillin was used to treat this infection effectively. But soon by 1970’s the emergence of resistant strains was reported. These strains became resistant to penicillins through two modes: Penicillinase-Mediated Resistance and Chromosomally Mediated Resistance. PenA gene that encodes penicillin-binding protein PBP-2 is mutated stepwise in Penicillinases Mediated Resistance. Penicillinases-Mediated Resistance imparts the resistance by the acquisition of a Plasmid-Borne Beta-Lactamases Whereas, in Chromosomally Mediated Resistance the gene mtr is used to encode an efflux pump and another gene penB encodes the cell wall porins to pump the antibiotic out (Cahoon et al., 2011).
Till the time of achievable resistance by efflux pumps and mutations to genes that encode enzyme gyrase, Fluoroquinolones were used as successful next-line treatment (Cahoon et al., 2011). Since 2007, cephalosporins antibiotics of third generation were too been prescribed to cure gonorrhea, but resistance has also been emerged to this class of antibiotic. Some Neisseria gonorrhea Strains have been found to be resistant to aminoglycosides and tetracyclines also. According to studies on Neisseria gonorrhea, it has been seen that this pathogen has a high chances to be involved in HGT, therefore, if there is any strain, that is resistant to new antibiotic can spread the resistance genes very easily to susceptible strains (Detels et al., 2011).

1.n. MISMANAGEMENT OF ANTIBIOTICS IN INDIAN SCENARIO

Non-judicial antibiotic therapies and unnecessary usage of antibiotics is contributing maximum to the non-susceptibility of antimicrobial agents for its emergence. Consumption of antibiotics without consultant’s prescription in developing countries has a role in antibiotic resistant pathogens spread (Larson, 2007). In so many parts of world antibiotic agents are often suggested to cure any signs or diseases that are not required to be treated by antibiotics or likely to resolve themselves. (Slama et al., 2005; Larson, 2007). Some studies have reported the unnecessarily prolonged use of the drugs, like Penicillins is also linked with non-susceptibility to antibiotics. Likewise, Erythromycin have also been observed to emerge as non-effective drug to many bacteria since the 1950’s (Pearson et al., 2007; Hawkey, 2008). Increased prescriptions of antimicrobial agents in patient care centers is also connected to the rise in many bacteria that are further not sensitive to commonly used antibiotics nowadays (Hawkey, 2008).

The common reason of non-judicial use of these antimicrobial agents also seen is the unnecessary use of antibiotics as prophylaxis. This happens in the cases of travelers. Expertise of some health care workers to suggest required quantity of drugs by observing weight and height of the sick with proper history of illness also help in chosing the right antibiotic thus lowering the chances of antibiotic resistnace. Other forms of misuse include withdrawal of entire prescribed course of the antibiotic in between or when the symptoms disappear. Incorrect administration of antibiotics is also a form of misusage or mismanagement of antibiotic treatment. Failure to take rest for sufficient recovery has also been a reason for antibiotic misuse. Inappropriate antibiotic treatment in viral cases, such as the common cold has also found to be
the reason of antibiotic misuse. Awareness and judicial approach of both doctors and layman could help in reducing the unrequired usage of drugs (Metlay et al., 2007).

1.o) INTERNATIONAL SCENARIO

Since the year 1970, United Kingdom has restricted the use of antibiotics without a prescription (Swann report 1969). Likewise, the European countries also responded to antibiotic use in animal husbandry to stop the antibiotics for the enhancement in growth of animals and fowl since 2003. However, there are many governmental and nongovernmental organisations that have put stringent boundaries in using the antibiotics on the animals and birds that reused for food. This helped in stopping the antibiotics being used for nontherapeutic use. Still, there is a strong need to be fast at the regulatory and legislative ends for planning strict actions and mandatory rules so that the antibiotics use could be limited. Therefore, the pharmacies that have turned into commercialism for antibiotic production and marketing should be regulated, the time required to find the connection between the use and the resistance of antibiotics is the need of the hour to design next steps.

Animal husbandry has been using antibiotics at large scales. In America, Food and Drug Administration (FDA) questioned first about the usage of antibiotics in animal husbandry that linked to antibiotic resistance in year 1977. In an instance, in year 2012, the Newyork Court of USA had to order the Food and Drug Administration to cancellation of all approvals of using the antibiotics in animals (Gever, 2009).

1.p. ALTERNATE CANDIDATES FOR ANTIBIOTIC THERAPY

The antibiotic resistant strains have evoked the scientists to work on the development of alternatives to the conventional antibiotics to treat bacterial infections. The alternatives of antibiotics may follow the different methods to treat the bacterial infections:

1.q. RESISTANCE-MODIFYING AGENTS

The compounds that modify the genes of resistance to common antibiotics can be generated. For example, the genes encoding drug efflux system of the bacteria may be modified to reduce or
limit the drug efflux from the cell, which will inhibit the multidrug resistance mechanisms thus increasing the susceptibility of bacteria towards particular antibiotic. Sugar can also help removal of some types of antibiotic-tolerant bacteria being Metabolic stimuli and by keeping the bacterial metabolism active (Allison et al., 2011).

1. r. PHAGE THERAPY

Another alternative that may be chosen as alternative treatment for bacterial infections is Phage therapy. These days Phage therapy is gaining attention of scientists for treating resistant strains of bacteria. In this method, pathogenic bacteria are infected with the viruses that can infect bacteria themselves, basically called as bacteriophages. Bacteriophages are also known as phages, described as the viruses eating bacteria or their specific cells and disrupt their important lytic cycles of bacteria that are pathogenic to humans. By doing so, phages destroy the metabolism of bacteria, which at the end results in death of the cell (Sulakvelidze et al., 2001). Bacteriophages transfer their genetic material to bacteria, to construct their own DNA. When the phage genetic material is once transcribed into the bacterial cell, it starts producing new phages and eventually the cell will lyse to release the newly made phages. But the drawback due to which these phages have to be on back seat is that they don’t have differentiating power among "good" bacteria or bad or normal flora also. However, there are few studies that have reported the specificity of bacteriophages, that they can select the bacterium to be attacked. This report has shown the scientist the ray of hope for using the phages as alternate treatment to disease and to kill the antibiotic resistance bacteria, the task which is tough to do by any other technique (Sulakvelidze et al., 2001).

1.5) Global trends in antibiotic resistance

On April 2013, a research showed a matter of concern due to the lesser availability of present antibiotics to deal with antibiotic resistant Gram-Negative Bacilli (GNB). Studies reported that many new antibiotics that were annually approved for marketing is declining and only two new developed antibacterial agents were passed by America Since 2009, and the compiled data on this matter could only find few antibacterial agents at that time in in different phases of clinical
trials for GNB’s. Still the effectivity of these antibiotics was not against multi drug resistant bacteria (Steenhuysen et al., 2013; Boucher et al., 2013).

1.t. SURVEILLANCE ON ANTIBIOTIC RESISTANCE

Since we know that one of the global concerns about healthcare are the non effectivity of first line antibiotics to various pathogenic bacteria. The emergence of these pathogens leads to high fatality rates, which effect patients and health care services too. These results impact on the additional laboratory diagnosis, patient’s prolonged stay in hospital. The strength and duration of treatment is also increased (Pankey et al., 2004). Though mode of transfer of genetic material that imparts resistance characteristics to bacteria or intrinsic methods of resistance are the bacteria well elucidated, but the judicial use of antibiotics in terms of drug resistance has not yet been approached or understood completely. Some studies suggest that wise usage of antibiotics in treating human infections as well as animal and agriculture related microbial infections may help significantly. If the antibiotic resistance is truly to be controlled, the prevention, control and management of bacterial/communicable diseases, the programs must include containment and management illness infections caused by antibiotic resistant microorganisms overall (Cahoon et al., 2011). Hence by knowing the spread of illness due to particular non-susceptible pathogens among specific group of people, the pattern and signs of disease changed by time, we may get the vague idea to act co control the infections occurred due to antibiotic resistant microorganisms. This will become a tool in containing the emergence of resistance also. With the help of containment and management protocols for antibiotic resistant bacteria, the reports on the utilization or consumption of antibiotics may guide in developing new strategies to protect the public health immediately and in long terms (Andertsson, 2006).

1.u. IMPORTANCE OF LABORATORY DATA

The clinical diagnosis or prognosis is not confirmed without the data obtained from the laboratory. The power of laboratory data is not only when it confirms the prognosis by testing biological samples but also provides detailed characterization of the causative organism with more opportunities for the vast studies to understand the disease and/or its determinants. This type of characterization includes speciation, grouping or typing of pathogen, which facilitates a good analysis on the disease clusters (Garrec et al., 2011). As we know that in prevention and
control of any disease, the treatment is very important, the data on antibiotic sensitivity testing is quite significant for judging the most suitable and suitable antibiotic therapy to the patients with particular clinical syndromes (Garrec et al., 2011). In most cases, laboratory information is provided only when the patients consult doctors and the doctors require tests to diagnose infection with the help of suitable tests so that the treatment of patient may be provided with efficiency. So, we can say that the laboratory information will be available only where specific arrangements are made, to assist to the diagnosis of infection and to treat the sick people. Type of data provided is considered to be good and complete only when consistent technical standards are implemented with on time, accurate recording and reporting of results.

1.v. IMPORTANCE OF SURVEILLANCE
The health care personnel responsible for control and prevention of infections must know about the distribution and the determinants of disease (surveillance) so that the services that are imparted to public become most suitable to public. To prevent the infection and its present/future effects, surveillance is the only tool that helps in the prevention of by furnishing the necessary data for action. To execute surveillance on antibacterial resistance, laboratory data are required and for communicable disease controls, clinical data is required.

1.w. SURVEILLANCE OF ANTIBIOTIC RESISTANCE
Preferably, for initiating the survey on antibiotic resistance microbiological laboratory data and its integration with clinical presumptive information data are required. By developing surveillance systems that collate both clinical and laboratory information, the necessary information from both can strengthen the actions. There are studies that describe how the wise consumption of antibiotics can help in reducing the resistance rate. Thus information on the antimicrobials usage with the antimicrobial resistance patterns can be an effective aid for the containment of resistance.

The reason to do surveillance of antimicrobial resistance provides necessary information that leads to an effective approach towards containment of community acquired infections so that death rates of public due to unawareness of both health care people and patients can be minimized. Furthermore, by knowing the data on antimicrobials, the emergence of antimicrobial
resistant pathogens can also be contained. The foremost purposes of the data gathered from the surveys conducted on antimicrobial resistance are have the motives to use the antibiotics in most suitable ways, to prevent, contain, manage and control the non-susceptibility of bacteria to antibiotic smaller geographies to larger geographical areas. This can be achieved by developing or updating the guidelines for factual, first hand, syndromic treatment or by identifying the necessity of imposing infection treatment measure at the local or regional levels. The last but not least by observing the effect of steps taken to make better antimicrobial use, the containment of antibiotic resistance can be done (Ian et al., 2001).

1.x. TYPES OF SURVEILLANCE
Surveillance is conducted in two ways discussed following:

• **Comprehensive surveillance**
The survey on a particular, specific illness or bacteria in the total population which is on the verge of getting infection that includes the gathering of information relevant on all reported incidences of same pathogen or disease. So this kind of surveillance requires the involvement of a big number of clinicians and laboratories. In this type of study only small pieces of information like age and sex of patient, address of the patient, sample type to be tested and resistance patterns are appropriate to collect (Mazaud et al., 2008).

• **Sentinel surveillance**
When a small unit or locality is selected study the matter, this is to present itself as indicator data that represents the whole of the population with same criteria. This is called as sentinel surveillance. This kind of study is usually done where a long term, detailed and lengthy information with extended time needs to be collected. The population chosen for the sentinel survey has to represent the whole of the population of the geography with similar traits, however in some incidences, when only the emergence of resistance is to be followed, the target approach is considered to be useful. Sample collection form all of the population may vary depending upon the settings and situations, moreover, the approach to data collection for comprehensive study sometimes be not feasible. Hence in the cases where the comprehensive study is not practicable, it is better to go for the survey by sentinel method approach, in which the most suitable information in brief is easy to gather. But there must be proper sample definition and
consistency in sample collection. The surveillance when done at specific intervals of time is called Episodic surveillance. Where there are limited sources of data are available, it is preferable to choose episodic surveillance. The continuous surveillance is done regularly with very good amount of recourses (Kwesigabo et al., 1996).

1.y) Establishment of surveillance

In today’s surveillance methods, the systems that help in explaining the trends of illness with specific symptoms of disease with the responsible organism’s resistance patterns are increasingly important parts. To be effective the main concern of surveillance should focus on the diseases of which the treatment options are very few due to antimicrobial resistance and because of that the mortality and morbidity rates are getting high. For a successful start of surveillance, primarily, local levels with very simple to handle procedures should be adopted. Therefore there is need of staring the systems that collect the smallest size of information required to execute a survey. It should move towards public health interventions with evidence. The systems should make it confirm that the collection and integration of the data at the smaller scales are very easy to perform as per standard operating procedure within prescribed timetable. The information that is provided for surveillance should be of good quality with very high reliability. The data or information that is provided should be clear and should lead to decision making. The data collected must show the limitations of the data for patient care also. As it is understood that surveillance of antimicrobial resistance is now an important step for improvement in judicial usage of antimicrobials, which is also required for containment of the threat of antimicrobial resistance. Depending upon surveillance, antibiotic resistance can be described as following:

1. **Prevalent**: In clinical use, when resistance to a specific antibiotic occurs to any height in any part of world at larger scales that impacts public or hospitals.

2. **Potential threat**: In clinical use, when resistance to a specific antibiotic occurs to any extent in whole world with its impact on patients or on health care, but with potential of spreading or arising within the country.

3. **Theoretical threat**: it is classified under non potential or non-prevalent but the bacteria has the notes expected threat of presenting non-susceptibility that may impact significantly overall
treatment of patients and alter the general health completely.

*Unknown*: There are countries where the predominance of antibiotic non-susceptibility is not known, even in clinically important bacteria that are responsible for causing illness or disease in the particular population.

**1.z. GENERAL DATA REQUIREMENT FOR SURVEILLANCE**

1. **Gap analysis:** Current surveillance systems should develop the techniques to get the surveillance done on bacteria that are resistant to antibiotics. Existing or potential problem or resistance in some diseases should be subjected to existing surveillance systems. To execute with surveillance, the first most step is to find and select available resources in form of the technicalities and data. Identification of most appropriate resources depends on particular disease and its prevalence (Fletcher et al., 2012).

2. **Population**

   Basically, Infections are categorized epidemiologically by the context in which the infection is transmitted i.e. from community or hospital acquired (nosocomian). There are evidences that the microbial species that cause the infections acquired from community and from hospitals, the trends in the antibiotic resistance is differed. Anyhow, the settings for executing the surveillance are not too different. For an instance, the Patients that face community-acquired infections can be treated in hospital but the patients that are contacted with nosocomian infections may not cure themselves until they return homes. The surveillance on antibiotic resistance should narrate the infection with reference to the settings from where the resistance was acquired. It should also include the health concerns of surveillance on population (Roussel and Touboul et al., 2011).

3. **Representation**

   When the surveillance is started, the patients and their clinical presentations vary with a rate of inconsistency. So this becomes significant to make out the terms of the representative population chosen for surveillance to the total samples. For reliability of data, even in many positive cultures, only the first culture that is positive came out form the sample to be tested for every episode of illness must be in the surveillance (Roussel and Touboul et al., 2011).

4. **Documentation and Maintenance:**
In order to ensure that the surveillance standards are being maintained, and the methods used, helps in important, in time, precise information about the disease, regular audit may be subjected to the system.

1.a.a. MICROBIOLOGICAL METHODS: ROLE IN SURVEILLANCE
Basically, for starting microbiological surveillance of infections due to resistant organisms, following steps should be followed throughout:
1. Appropriate specimen collection from appropriate patient
2. Isolation of causative organism
3. Antibiotic susceptibility testing
4. Gathering information, integration and analysis
5. Spread of suitable and useful data.

1.a.b. LABORATORY TESTING: SAMPLES FOR TESTING
The manner of gathering and processing and testing of clinical samples for microbiological surveys must include the appropriate quality standards and with consistency. If possible, the methods of sample collection should be clearly made understood and acceptable to the patient. It should involve easy and fast methods. Wherever possible, the method of collection of sample should be non-invasive. While collecting sample, contamination of other bacteria that could be commensals must be ruled out to minimize the false negative and false positive results. Generally sterile sites for sample collection needs the involvement of invasive procedures (for an instance, CSF or other body fluids), this could help in revealing the higher positive predictive value of disease that could be lesser if sample collected from other sites like epidermis, throat swab and sputum.

To make the testing produces in microbiology accurate and precise, data about the sample is required prior to the collection of sample is required:
• The most appropriate sample suitable to the symptoms of the disease should be collected. The preference should be given to the sample before the antimicrobial therapy gets started.
• The antibiotic susceptibility testing being the most important apart of the surveillance should be done with due care. The selection of bacterial colony from the culture must be the similar to the
characteristics to the rest of the colonies present on culture plate. The testing of antibiotic susceptibility *in vitro* should also represent the antimicrobial effect *in vivo*.

There is a need of a spectrum of access to check all the types of bacteria in surveillance, as it is not feasible to check all the microbes in one survey:

- **Comprehensive surveillance system** should strictly be adopted to check the antibiotic susceptibility at local levels.
- **Nonrandom surveillance systems** should be adopted where the patients fails to respond to any antibiotic treatment Due to any reason. Here in this case, in the surveillance the bacteria itself and the relevant data of the illness and laboratory investigations should be submitted. When there is surveillance conducted on the potential threat to be identified for resistance, the data should be submitted.

According to some studies, the antibiotic susceptibility of one strain specific to organ can indicate some information for the different strain that also affects the same organ and present same clinical symptoms. The sensitivity patterns of both bacteria can be same to an extent (e.g. *Streptococcus pneumoniae* sensitivity pattern can be similar to *Haemophilus influenzae*). But it should not always be considered that the patterns of sensitivity will be same or remain same in different bacteria (Ingham *et al.*, 2012; Ingham *et al.*, 2012; Varadi *et al.*, 2012).

1.a.c. **NO ISOLATES DETECTION**

General therapy, that is given without the microbiological testing of responsible pathogen by its isolation, identification and characterization and the methods that are other than culture techniques to identify infections persist in the patient, confront to the surveillance of antibiotic resistance. Here the above said practices are flourishing, surveillance studies, from collection to identification of pathogen from clinical sample needs to be re-considered. Where there is non-cultivable pathogen, methods apart from culture techniques like polymerase chain detection, antigen-antibody detection and so are to be followed (Ingham *et al.*, 2012; Ingham *et al.*, 2012; Varadi *et al.*, 2012).

1.a.d. **ANTIMICROBIAL SUSCEPTIBILITY TESTS**

The test to know the susceptibility of bacteria to particular antibiotic are the basic tests to know about the resistance patterns of any bacteria. These tests are the only procedures that aid the health care professional to choose the most appropriate antibiotic for treating of a specific
infection. For executing antibiotic susceptibility the suitable samples to detect the infection and the causative organisms has to be collected for isolation and identification purposes. Bacteria that are grown/cultured from the chosen specimens are to be tested for susceptibility towards antibiotics. There are many methods specified to detect the ABST but the common and reliable methods include minimum inhibitory concentration (MIC) of the antibiotics and Kirby beaur method that uses discs antibiotics loaded on it (Garrec et al., 2011).

1.a.e. ANTIBIOTICS FOR SURVEILLANCE

As we know that the fundamental cause of doing the surveillance on antibiotic resistance is to assist the health services to provide best solutions to treat the sick with minimum efforts and utilization of time. For this surveillance to be successful the antibiotics to be tested must be taken into account. Furthermore, the testing of antibiotics from any type of information should not affect the laboratories. Thus according to the policies the number of antibiotics to be tested is fixed depending upon the type of bacteria isolated. There are different antibiotics classified for different bacteria depending upon their Gram staining property. Last but not least, it one must take a note on the antibiotics chosen for testing in vitro should also be readily effective when given in vivo.

Different antibiotics should be used for various bacteria (e.g. GPC and GNB). For choice of these antibiotics, due concern is required on those that should show similar results in in vivo and in vitro (Garrec et al., 2011).

1.a.f. SIGNIFICANCE OF ANTIMICROBIAL SURVEILLANCE

The motive to do antibiotics resistant surveillance is to provide data and information for actions in prevention of resistance. Thus, this should be committed to the quality and timeliness of work for the information and datathat is required. The method of surveillance is required to be reviewed regularly to ensure that the procedures are providing quality public health services and the required information important for clinicians and policy-makers (Garrec et al., 2011).

1.a.g. TIME AND PLAN OF SURVEILLANCE

For antibiotic resistant bacteria, there are certain points to keep in mind that 1)what kind of surveillance is to be executed i.e., active or passive 2) what frequency of resistance in specific organisms is to be considered, 3). if there is a need of action there should be the percentage cases
of infection by resistant pathogen, 4) the number of isolates effects on frequency of occurrence of resistance.

1.a.h. IN DEVELOPING COUNTRIES A PLAN FOR SURVEILLANCE

There are two key points to consider:
1). the main and common infections,
2). the antibiotics that are used.

There must be an achievable and practicable plan where appropriate clinical specimens can easily be obtained from patients having concerned infections.

When there has not been any surveillance system in the region before, the first step is to establish the appropriate working conditions according to the plan.

Several new antibiotics have been raised from the fungi, which are in pipeline to act against MRSA. Streptomyces has proven to be a good source of antibacterial agents to treat MRSA and many more diseases caused by bacteria resistant to commonly-used antibiotics.

1.a.i. ROLE OF MICROBIOLOGICAL METHOD IN SURVEILLANCE

Clinical microbiology has with great enormity in past few decades. So as a part of results of this, the techniques of running the tests accurately and precisely should be at priority. Moreover, skilled technical hand is considered to be the responsible part of results in microbiology especially in surveillance. The methods of culture and identification techniques must meet the diagnostic expectations. The current progresses in diagnostic microbiology bring great automation in microbiology, a good level information generation systems for surveillance and last but not least reducing the time consumption in microbiology procedures. These advancements in microbiology improvise the surveillance units in hospitals. Other progresses like connectivity for data exchange between systems of laboratory to surveillance unit, between the microbiologist and the physician and related cost effectiveness also has a key role in surveillance of non-susceptibility to antibiotics. In surveillance of antibiotic resistance higher diagnostic techniques like imaging, mass spectrometry and sequencing that help in easy accessibility, accuracy, and a good correlation of AST to be made are too expensive to perform at a smaller unit.
Though advanced, novel diagnostic techniques have been introduced in microbiology; culturing has been the gold standard method in clinical microbiology since last century and will likely to be mainstay of microbiology for many decades. The reason behind this is that in many of the microbiology techniques, the need of living organisms in significant quantities remains constantly important. In surveillance study of antibiotic resistance the need remains same to get antibacterial susceptibility testing- not as much of culture methods are required when only detection of particular organisms is required, which can be done by strong alternative technologies like MALDI-TOF MS, but when AST is needed, one has to go for the gold standard method only. Bactericidal property of antibiotics is generally observed in liquid or solid growth media by monitoring the visible changes in density of media by bacteria in the presence or absence of gradient and strategic concentrations of particular antibiotics of clinical need. This read-out technology may be substituted in coming years but straightforward observation of living or dead organisms is still to be confirmed by suitable culture media with reproducibility and reliability of a single cell. In today’s, techniques, in many microbial assays, generally, up to 105 bacterial cells can be required and that requirement only can be satisfied by culture methods (Garrec et al., 2011).

However, the conventional culture techniques have been optimized and challenged by many advanced films, fibers and nano-porous carriers since long. These methods help in restricted growth development of bacteria on alumina oxide chips e.g., the agar media is loaded with some chromogenic dyes, which are used up by some bacterial species only (Ingham et al., 2012; Ingham et al., 2012; Varadi et al., 2012). This helps by a direct on plate visual observation in species identification that could have slowed down the process of culture results. The Visual reading by colour of the colonies, when used with antibiotics in the chromomeric and standard agar medium plates, presumptive identification of species identification with limited resistance screening can also be done (Van et al., 2011; Nordmann et al., 2012). Antibiotic susceptibility testing on solid agar media plate by observing growth inhibition in form of zone formation is still mainstay in microbiology. Disk diffusion test or Etesting are still considered as primary of AST in microbiology laboratories (Garrec et al., 2011). When large number of samples is received for surveillance studies, automation of sample inoculation on solid media can also be done. There have been many automated systems that keep a check on growing bacteria during incubation. These systems contain cameras for archiving culture growth, isolation and morphological
characters of microorganisms’ colonies and this helps in speeding up time of results (London et al., 2010). But it has been in the notice that it is accepted that such requirements are only available in big laboratories or laboratories in developed countries.

In microbiology techniques, Clinical specimens and management of clinical specimens play the role in quality determinants for surveillance. Since it is known that all microbiology depends on microbial viability, hence from the time of collection of sample the criticality of tests arrives. i.e., the longer the time of keeping clinical samples in growth-limiting conditions, the smaller the chance of getting accurate and quality assured results. Although the growth based methods are considered as gold standard, but this has a significant poor accuracy and precision of test, ending in poor results. Role of transportation of clinical samples is also important; otherwise unwanted organisms (contaminants) may overgrow hindering the growth of pathogens (Kerreman et al., 2009). Awareness policies that improve transportation, collection and rejection criteria are very important for surveillance studies. Appropriate media are required for transportation of clinical samples, if surveillance laboratory is established far. For some uncultivable bacteria, other diagnostic methods like DNA amplification are in trend but are very expensive, the essential growth factors containing media re in pipeline for this kind of bacteria (Griffiths et al., 2006).

The biological variety of different clinical samples is generally encountered in same clinical material and this makes the diagnosis procedures complex and confusing. Due to this, the equipments that in parallel facilitate the purification of host cells, bacteria form clinical samples have become need of clinical laboratories (Dauphin et al., 2011). The development of the methods that help in identification of potentially pathogenic microorganisms with the aid of stabilized nucleic acids in samples is required which are less susceptible to transportation.

1.2 PROBLEM IN HAND:
To know the susceptibility patterns of pathogenic bacteria those are isolated in Nawanshahr region.
1.3 OBJECTIVES

I. Isolation and biochemical characterization of pathogenic bacteria from various clinical samples.

II. Screening of antibiotic resistance profile.

III. Selection of broad antibiotic resistance strains and surveillance studies.
1.4 SCOPE OF RESEARCH WORK

The scope of research work is:

Understanding the resistance patterns of bacteria.

Awareness in Usage of appropriate antibiotics.