CHAPTER 6

6. SUMMARY AND CONCLUSION:
The modern antimicrobial revolution can be assumed from the success story of the penicillin antibiotic produced naturally and the antimicrobial agent sulfonamide (synthetically produced) in the 20th century’s first half. (Cantas et al., 2013). In spite of knowledge about several antibiotics, the resistance shown by various microbes against the current varieties of antimicrobial agents it has become important corner to be considered of modern medicine industry globally for the past fifty years, it is once again getting tough to treat infections or diseases caused by microbes (Ashbolt et al., 2013). Resistance to Antibiotic is now a global challenge that impact whole medical industry. It has now the prime health care concern with a rapid increase in the last 10 years, creating a serious warning to the treatment of bacterial infections. A study on evaluation of antimicrobial resistance 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). Vancomycin Resistant Enterococci (VRE), Shigella and Salmonella species resistant to multiple antibiotics, Methicillin-Resistant Staphylococcus aureus(MRSA), penicillin-resistant Streptococcus pneumonia (PRSP), GNB from colon non-susceptible to prolonged and wide range of β-lactams are microorganisms for which rate of resistance to antimicrobials is increasing gradually (Conly, 2002).

Worldwide, antibiotic resistance is an 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 resistance is due to overuse and misuse of antibiotics 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 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 downregulation 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 other 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. In absence of effective antibiotics in future, no one can imagine the extents and end of bioterrorism. If effective measures are not taken to combat antibiotic resistance, it will lead to raised mortality and morbidity (Sandoz et al, 2010). Since last few years a lot new strong compounds have been made available that are active against Gram-positive cocci, but for Gram-negative bacteria the case is different and there is almost no new antibiotic can be anticipated in the near future, which will be active against multi-resistant Gram-negative bacilli. Although hard to imagine, the truth of the scene is that many physicians will face a dead end in the treatment of various types of microbial infections in the near future. We may soon face the post antibiotic era with a daunting situation which takes us back to the back 1930s and early 1940s in pre-antibiotic era of the years (Carlet et al., 2012).

Enhancement in antimicrobial surveillance system has taken keen interest of government worldwide. There are various government and private organizations that have stepped forward to combat with antibiotic resistance. The activities to prevent and resistance to antimicrobials should be enhanced. 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 may be taken to eliminate insane use of antibiotics in animals, marine environment and plants is awareness program at ground level.

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 was 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. Antibiotics originally are structural and molecular agents with their ability to hinder microbial growth at higher concentrations (Cantas et al., 2013). The history shows that the resistance has emerged along with the antibiotics discovery. Just after new drugs introduced in hospitals where antimicrobials were used frequently, number of antimicrobial-resistant pathogens have been observed. 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 responsible for resistance to Penicillins, were also discovered (Schechner et al., 2013). In 1944 antibiotic streptomycin was discovered for the treatment of Tuberculosis, resistance genes for streptomycin also were discovered simultaneously, showing resistance comes along with antibiotic. Another study in year 1942 showed the resistance for particular antibiotic, where a number of patients with staphylococcus aureus infection that was cured by penicillin. 4 patients from them, whom treatment was being done, were taken for serial cultures showed decreased sensitivity to the antibiotic (Schechner et al., 2013). 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 first discovered in 1993 in Canada. It soon became clear that development of resistance would present a clear serious therapeutic dilemma to the clinical community (Schechner et al., 2013; Barber, 1947). It has been observed in various studies that lifestyle, personal habits and hygiene are associated with antibiotic resistance (Padarukhet et al., 2014). Where these factors are found poor and unhealthy, resistance has been seen raised by flourishing the growth of multiple microorganisms there. 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 play some role in raising resistance. The prolonged the stay in hospital, more the chances of getting nosocomial infections, however, it is known that nosocomial bacteria are highly resistant towards particular antibiotics (Davies et al., 2010).
The most important determinant of resistance found to be is inappropriate use of antibiotics, which can be either misuse or overuse of antibiotics (Caron et al., 2010). A study conducted in Mexico showed 40% of antibiotics 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).

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 raised resistance to higher antibiotics like Linezolid, tigecycline (Long et al., 2012). Carbepenems like Ertapenem, Doripenem are now a day no more sensitive drugs. (Fernandez et al., 2013). Seeing 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 abovementioned general reasons of resistance, 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 to be resistant to particular antibiotics (Donkar et al., 2013; Hawkey, 2008).

If we talk about Gram Negative Bacilli, all GNBs have also become resistant to 3rd generation cephalosporins now (Khan et al., 2012). Almost all bacteria have got resistance genes now and it for atleast one antibiotic it becomes resistant. For instance, E. coli, the most common commensal flora of gut, is having predominant genes for résistance (Jakobsson et al., 2010). Once acquired these 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. have emerged as nosocomians 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 BL et al., 2012). A data collected from sickle cell anemia patients (children) on prophylactic antibiotics that are given to the Sickle cell anemia patients by collecting their nasal swabs and nasopharyngeal swabs, showed isolated Streptococcus pneumonia resistance (0-11%) and Staphylococcus aureus resistance (40%) in Ghana (Sandoz et al., 2010). Lactobacillus also has shown resistance to newer drugs, like Vancomycin Resistant Enterococcus, Methicillin Resistant Staphylococcus aureus (Colomer et al., 2011; Devirgiliis et al., 2013).

The effects of antibiotics can be strain specific as a study on antibiotic combination on Multi drug resistant Acinetobacter baumannii showed the response towards particular antibiotic to be strain specific (Dent et al., 2010; Kmeid et al., 2013; Lawrence et al., 2013). A study in Vietnam showed that Helicobacter spp. (Binh et al., 2013). Resistant to clarithromycin and metronidazole suggested that standard triple therapies may not be useful as first line treatment in Vietnam (Sandoz et al., 2010). Basically, we can say 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; Bakhshi et al., 2014). The common mobile genetic elements (MGE) are plasmids, transposones or resistomes (Pehrsson et al., 2013). The basic genes for resistance are resistome 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 (Donkar et al., 2013). The evolution of resistance to a single antibiotic is frequently accompanied by raised resistance to multi drugs (Erez et al., 2010).

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).

In another study that was conducted to standardize the antibiogram and biochemical tests of S. aureus, duplicates of Staphylococcus aureus from same patient were taken to observe the
impact of repeat isolates on MRSA and on antibiogram changes. It also helped in the cost
efficacy of laboratory and better results of antibiotic susceptibility pattern (Rebecca et al., 2003).
According to a study by Melaku et al people in developing countries are prone to Urinary tract
infections most with one or more bacteria (Bjorkman et al., 2013). *E.coli* is the most common
pathogen found in Urinary tract infections, according to a study in Ethiopia. The study included
all adult patients with clear 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 infection
got from community, whereas the most prominent in iatrogenic infections was found to be as
Klebsiella (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 than one antibiotic.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).

Miscellaneous bacteria commonly are also found in buccal cavity apart from Gram positive and
gram negative bacteria. A study in year 2013 stated that at root canal of teeth acute apical
abscess is the most common infection. 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 (Jose et al., 2013).

All gram positive bacteria and gram negative bacteria are isolated by their morphological and
colony characteristics. *E.coli* and other Gram –ve pathogen were recognized by using
biochemical tests like hydrogen Sulphide, urease, and IMViC (Indole, Methyl Red, Vogus Proskeur and Citrate) tests (Ashley et al., 2011). Gram positive cocci from various clinical samples that are isolated on blood agar are characterized by their property to hemolyse RBC’s on blood agar, catalase test and coagulase test mainly (Ruoff, 2002).

The present work was undertaken to study the bacteria present in clinical samples and their antibiogram. Data from patients for the appreciable period was retrieved from laboratory records for retrospective analysis. Thus the present study describes the isolation, identification and surveillance in clinical samples. The study was conducted in a private laboratory of Ludhiana, Punjab. Blood, urine, stool and pus samples were included in the study. The vein from where the blood is to be drawn was identified before the skin was disinfected. In patients with venous catheter, blood was withdrawn from fresh site (McFadden et al., 1980). Brain Heart infusion broth was dispensed in a special flat or round blood culture bottle of about 100-120ml capacity for blood culture and fitted with a screw cap with a central hole (Collee et al., 1974). After the vein was selected, the skin site was defatted with 70% isopropyl alcohol and an antiseptic i.e. tincture iodine was then applied to kill surface and subsurface bacteria (MacFaddin et al., 1980). Only 1 to 5 ml of blood was drawn for bacterial culture depending upon the age of patient. The ratio of blood volume:broth was 1:5 (MacFaddin et al., 1980). Freshly passed stool are collected in a leak proof sterile container. Stools were transferred to alkaline peptone water and Salmonella Shigella medium as soon as possible for enrichment of bacteria. In a clean, sterile, leak proof container, midstream urine was collected. The samples were placed on suitable media immediately after collection. After collecting blood sample was incubated at 37˚C for 24 hour. For other samples, there was no need of prior incubation.

From liquid media, subcultures were done on mac-conkey and blood agar to observe colony characteristics for final diagnosis. Blood culture was examined once during the first day and therefore at intervals. Subculture was done after 48, 72 hours and 7 days. It was usual to continue incubation and inspection for up to 5-7 days with a final subculture then. Prolonged incubation was recommended, before the bottles are discarded, in the investigation of some infections such as bacterial endocarditis, or if patient is on antibiotics (Baron et al., 2005). With aseptic technique described above the samples was plated on Mac-conkey agar and Blood agar and incubated at 37˚C overnight isolated pure colonies were further identified by morphological
colonies, characteristics and biochemical and gram staining tests (Collee et al., 1971; Ibrahim et al., 2014).

Colony Morphology of all pathogenic bacteria was observed and then these bacteria were tested for biochemical tests for identification. For identification and confirmation of the isolated organisms gram staining, vogus proskauer test, methyl red test, indole test, oxidase test, urease test, citrate test, motility, catalase, coagulase test and TSI tests were conducted. Kirby beaur method. Was used for antimicrobial susceptibility test. The organisms were defined as sensitive, intermediate sensitive and resistant by breakpoints defined by CLSI. (Ananthnarayan and Paniker., 2005)All data was analyzed in MS-EXCEL.

Results obtained from various clinical samples indicated that microbes varied in different samples. There was no uniformity in microbes in different samples. Maximum no. of samples obtained were urine sample that were 1172 out of 1673 number of total clinical samples (70%). whereas, samples of body fluids received (7.4%) were found to be least i.e., 124 among 1673. However, the positive results from both types of samples were 29.94% (351 positive out of total 1172 urine samples) and 54.5% (12 positive out of total 124 patients). These results show that urine infections are more prevalent in the particular region, but the microbial isolation techniques may not be sufficient in diagnosing UTI’s. Other types of samples like pus and blood for culture and sensitivity tests received were 12.49% (209 out of 1673) and 7.47% (125 out of 1673) respectively. The positivity rate of both types of samples was 61.72% (129 out of 209) and 17.6% (22 out of 125) respectively.

Since urine samples received were highest in number among other samples, we must know if there are any particular causative organisms responsible for UTI in specific region. These isolates of urine samples must be kept in vigil to control UTI’s as per their mode of transmission, their quantization on culture plate i.e., scanty or confluent growth and antibiotic susceptibility pattern, etc. The results of urine culture showed that the majority of isolate was E.coli(5.11%) i.e., 60 number of Enterococcus from total 1172 urine samples in contrast with Proteus spp.,which was only 0.42% (5 among 1172 urine samples) in all urine culture positive isolates. Other Gram negative bacteria isolated were Klebsiella spp., i.e., 2.9% (34 in 1172 urine samples) and 19 Enterobacter. Non fermenter bacteria, Pseudomonas spp. and Acinatobacter spp. remained were found as 25 and 19 in UTI’s respectively i.e., 1.6 % and 2.1% each.
Out of Gram positive bacteria isolated in urine cultures, Enterococcus *spp.* was predominant i.e., 138 in 1172 urine samples (11.77%). Whereas, other Gram positive cocci like *Staphylococcus aureus* contributed just 3.83% to UTI’s (45 in 1172 urine samples).

For patients with UTI’s, age and gender wise analysis of patients discovered the incidence to be highest in female adult patients (89.0%) being 1044 female adults among 1172 total urine samples.. Out of total samples collected for the study, only 10.92% (128 out of 1172) males adult patients were infected with UTI. In female patients, *E. coli* and Enterococcus were the most predominant pathogen responsible for UTI. Whereas, in male patients, Enterococcus *spp.* and *Pseudomonas spp.* were equally responsible for causing UTI.

In pus cultures, the most affected category of patients was females (55.98%) being 117 out of total 209 pus samples. male adult patients that showed 92 out of 209 pus samples (44.01%) also showed not a less number of infections. In both sexes, *Staphylococcus aureus* Enterococcus *spp.* were the most common isolate found in pus cultures almost same like of urine culture, where after *E. coli* has found to be second important pathogen causing pus infections.

*Enterobacter*, being 4, out of 209 pus samples are least responsible organisms (1.91%) for causing pus infections. Data of pus culture showed variety of organisms (*Acinobacter spp.*, *Citrobacter spp.* and *Proteus spp.* *Klebsiella*, *Pseudomonas* ;2.8%, 1.91%, 3.34%, 3.34%, 9.56% respectively) causing infection of skin and wound.

*Proteus spp.* was found significant 7 in number among total positivity of isolates; 129 (5.42%) in causing all types pus infections in both genders, same as *Citrobacter spp.*, *Acinobacter* *spp.* and *Klebsiella spp.* Here in this case, although both organisms have been reported very few in numbers (3.1%, 4.6% and 5.42%) among all bacteria isolated from pus samples i.e. 129 in number.

As samples for blood culture and sensitivity tests received were very less (125 in number among all 1673 clinical samples), their positivity rate was 17.6% (22 out of 125). 9 male patients has reported any infection in blood out of 125 samples(7.2%). 6 isolates of *Staphylococcus aureus*(4.8%) were isolated. 3 (2.4%) isolates of *Pseudomonas* were reported from blood culture. Both of the organisms may signify hospital acquired infections, as both organisms are considered to be nosocomians. This data itself has queried the concerned hospital personnel or physicians
that why and how the nosocomial infections arise in blood cultures. The influence and impact of pathogens on particular society has been documented in various studies but in Punjab, not too many studies on microbial surveillance have been done. The research mainly yields the point that in common infections, there is geographical and climatic difference that creates difference in species of pathogens in similar sample (Kumar, 2013). There is prevalence of some strains in Our study showed that in this region of Punjab though urinary tract infections are most common the positivity rate is very less, leading to the suggestion that the diagnosis and treatment should be strengthened to rule out true urine infections. In case of positivity rate of body fluids, it is very much exposed to sight that the positivity rate is higher due to severe or chronic illness. lesser percentage of fluid culture tests may not only signify that people in particular geography are less afflicted with fluid infections, but it may also be considered that due to inaccessible sample collection sites for body fluids in patients, the samples of body fluids cannot be collected as frequently as other common samples like urine, pus or blood. Hence a single number of pathogen may be considered as critical for selection of antibiotics severe illness.

The difference in microbial distribution in each sample in this study was striking. As noted, the frequency of the bacteria is likely to be influenced by many predisposing / underlying factors. A study documented by Ramsamy et al., in trauma patients, Klebsiella spp. were isolated maximum (25%) in contrast with E.coli (19%), where as in our study we report more E.coli(14.1%) than Klebsiella (8.16%). According to Ramsamy’s study Acinetobacter spp. were considered to be colonizers unless they showed a role in severe sepsis. In contrast with our study, Acinetobacter in was observed as sole organism in pus and urine infections. Given this observation, differences in society, living style, and geography, age and sex are responsible for difference in species in particular samples all over world. The study shows that the main victim of UTI is adult females in contrast with female children and male patients. This may be due to human anatomy that why females catch UTI more often. Moreover, in South Africa traumatic patients showed that there were more than 15 species of gram negative bacilli isolated; indicating that sudden distressed immunity may encounter rare and vast number of bacteria. Other supporting factors of encountering with rare pathogens may be patient’s hospital admission, nosocomial infections or iatrogenic infections. No fungal infection was detected in this study in contrast with Ramsamy’s study.
In comparing these data it is first and foremost to understand clearly that the results of most surveillance studies have thinkable biases. In this the contemplations should be made about the population surveyed, geography surveyed or methods used for surveillance. Significant differences may exist in microbial patterns and these differences may likely to affect data to compare different studies. Thus similar methods, population and sites may enhance the affectivity of surveillance and help in real eradication multi drug resistance and common bacterial infections due to unawareness of common man. Ultimately, this study has provided the basic trend of pathogens in particular region in specific infections. That will help clinicians to rule out some super bugs from easily handled bacteria causing infections with precluding any broad spectrum antibiotics.

Ideally antimicrobial surveillance should include collection of clinical and epidemiological data. There is facts that appropriate use of antimicrobials may decrease the rate of emergence of resistance. The surveillance studies have been conducted at so many places at large scales, but no significant studies have been made in Punjab. This study is oriented on various different antibiotic resistance patterns in Nawanshahr region of Punjab. The results of isolates show that there is not a big difference between the infection rates of both GPC and GNB. The little difference that has come out may be due to type of clinical samples. The present study reveals that Enterococcus spp. is the most commonly infecting pathogen in all types of clinical samples. The second most commonly occurring pathogen in infection was found to be E. coli that coincides with study by Rao et al. There have been studies that show second dominance of GNB’s in many kinds of clinical samples. Such GNB dominance in the aerobic growth in pus culture has been highly seconded by this study. Though antibiotic class Penicillins showed variable results between Penicillin and Ampicillin, their combinations are not up to satisfactory mark.

According to this data, Penicillins should be kept aside in treating GPC infections in particular region. Though combination may work, but resistance genes may develop.

The resistance range of macrolides like erythromycin shows raising tolerance of GPC everywhere and has been observed in this study too. Judicial and timely usage of erythromycin may be helpful in diminishing the resistance.
A leading cause of extended spectrum beta lactamases (ESBL) production is that usage and resistance 3rd generation Cephalosporins has been observed. The resistance has been noted worldwide but shockingly, Ceftazidime showed resistance at very large scale i.e., 92%. The fluoroquinolones result was likely to be as expected as in trend globally.

Surveillance is an aid that can smoothen the way to prevention of infection and can make better use of its instant and long-term upshots by furnishing the required particular, facts and other information for measures. Hence by creating surveillance systems that integrate clinical (that is produced by clinicians, observing the symptoms of the disease) and laboratory data (confirming the isolate and its susceptibility patterns in laboratory with the help of various tests), not only can the vital data be collected but the strengths of both data sets can be pooled to combat with unnecessary existence antimicrobial resistance. This can help public by reducing morbidity and mortality furthermore with good and healthy life. Even though the specific antibiogram are selected but there is a need of revisions in the antibiogram looking upon the trends of antibiotic susceptibility. This can be achieved with the easy tools like surveillance of antibiotic resistance. There must be a data that focuses on infections that are leading to outbreaks and/or epidemics, including both currently established diseases and newly emerging diseases. There has been a high case-fatality rate of some infections or the potential for rapid spread of these infections by imposing antibiotic resistance pose a serious threat. Exact and in-time response and surveillance have great importance for the control and prevention of infections and antibiotic resistance. There must be system which gives you early response and warning for reporting and responding to infections and antibiotic resistance. These systems should include systems for immediate reporting of infections and antibiotic resistance to the higher level must have some procedures to deal with empiric treatment of infection according to guidelines.

Unfortunately there are very few mechanisms that take men and women differences satisfactorily into account before initiating the empiric treatment of infections. Moreover, the data about pregnant females can also be helpful in further control of infections, which is generally ignored or infrequently collected or reported. These factors bound or limit the potential for accepting the gender dynamics of infection including the process and course of infection by identifying vulnerable groups, and developing appropriate responses.
As discussed earlier, the basic differences between men and women lie at every biological stage, from whole body to single cell. It has been seen that anatomical and physiological differences including hormones between men and women influence the infectious disease process in many ways. Though not fully understood, major changes during gestation and lactation also affect the disease process in the women body.

Of course, there are few infections that are especially severe during gestation, and there are some infectious diseases that affect the unborn child. Vaccines and other medicinal agents that are synthesized chemically might have different results on non pregnant women than on pregnant women, or unfavorable effects on the fetus or breastfed baby.

If we discuss about gender related disease influence, The cure of the disease and pattern of exposure to infections differ in both gender. For an instance, gender roles influence when both male and female spend their time and the pathogenic agents they are exposed to, the nature of exposure, intensity and frequency differs in both genders. In the condition of treatment too, the differences among males and females are influenced that include the outcome and duration of infection. There are many reasons behind the infection interaction among both genders. The first and foremost reason is the time spent away from home and at home. There are some societies where males usually spend more time away from home. Thus male has to face a higher risk of exposure to outside infections, on the other hand inside the home females are in more exposure to infections. For an instance, males may go for a hunt for food or to log trees for fuel have to go into the forests. Here there are more chances for men to come into contact with wild animals. The consequences of this exposure pattern for both genders are complex, and are different for different pathogenic agents.

In another case, in most of the societies women are considered to be as caregivers for the sick more likely than men in both at home and in health-care premises. In this case, women are more exposed to infections than men.

There is a usual under-estimated male and female difference linked to the care and managing of (farm animals) livestock. Managing livestock is usually discriminated, with either men or women liable for different animals. In many societies, women generally take care of the animals such as
pigs, ruminants, poultry, etc (smaller ones). It has been observed that gender differences in taking care or managing of animals leads to differences in the threat of zoonotic infections.

The health-seeking behavior in many societies is also of importance for gender differences related to the access to health care facilities. In few societies there are differences in the health care facilities and in the type of care providing or utilization to males and females. An study in India (Kolkata), observed that boys suffering from diarrhea were more likely to be given better care and treatment than girls, and were sooner taken to doctors clinic for check up and treatment than girls. (Pandey et al., 2002). A similar evaluation was done in Bangladesh where the time gap in symptoms and treatment of diarrhea was considerably higher for female than male (Mitra, Rahman & Fuchs, 2000). As per various other evidences it is found in many societies that adult females are facing more social and economic barriers to be cared than adult males (WHO, 1998).

There is also lesser information about the different treatment both the genders. Only males subjects were included in many or female subjects only, or when the studies on both genders were conducted, during analysis no difference was seen between them. Even though the variations were seen, they were not noted or considered. Understanding of how various treatments interact with steps or stages of the reproductive cycle is scant. On the basis of ethical and financial grounds, pregnant women are generally not included in the clinical trials, which restrict the development of proper treatment policies and the different infectious agents that they would have been interacted.

Thus we can conclude that both men and women can get infection but the gender differences between can be obtained by calculating separate epidemiology rates, and also can be monitored during the course of infection by separately plotting curves for both genders.

However, the outcome and progression of an infection can be characterized by various factors like severity and nature of symptoms, the mortality rate and disease consequences. This also depends on both chemotherapy and biological responses to exposure. Differences between genders can be measured by separately calculating disability and case fatality rates among both genders, and by making symptom profiles separately.
Immune response difference between genders also influence the course and outcome of infection in general and the anatomical differences, especially important for sexually transmitted infections also are helpful in knowing different course of infections. The gender prejudice in access to immunization also draws the gender influences.

However because of gender roles, nutritional status also draws attention in both males and females. The access to preventive health care measures, including differences in the pace with which both genders get treatment outside their home also plays significant role in course of infection. Furthermore, the economic and social factors of diseases are often different among different genders. Infections in both males and females change over the life-cycle of human. The intensity of infectious diseases in kids is high in some cases whereas it’s low in so many other infections.

In childhood, despite maternal antibodies, children may get infections and that too in severe forms. In Adulthood, though there are many antibodies formed in the body, but infections are diverse and vary from place to place. In pregnancy, many changes are induced in the body in terms of immunity and for some other infections. Thus pregnancy becomes a risk factor for more serious course of infection. According to our life cycle, old age is also grouping of people those who are very prone to infections. According to research, in this group more female get infections than males.

Like many other geographical areas, in Botswana, the epidemiology of UTI pathogens was not known. The study conducted there by using laboratory the major organisms causing UTI and antibiotic non-susceptibility was tested. A total of 744 samples of urine were tested for UTI. According to that study, more than 10% resistance was noted for antibiotics like ciprofloxacin, amoxicillin-clavulanate, amoxicillin and co-trimoxazole. Resistance to isolates of E. coli to co-trimoxazole and ampicillin observed was more than 60%. According to current study, that antimicrobial resistance has already effected on many of the antibiotics, thus making effective treatment of UTIs hard. In this context, according to current study co-trimoxazole, Ampicillin and Nitrofurantoin should not be used as empiric treatment for Urinary track infections.
Though the infectious-disease epidemiology and control is understandable, but its mathematical modeling is to be implemented at so many places. In recent years the understanding of these mathematical models has been increased. The policy-making to control the infectious diseases should be with these models. Since these models are exciting and are now informing at the highest levels, these are getting increasingly-important and are playing an important growing role in research. Because the infectious diseases transmit from each other in different way than non-infectious diseases, the epidemiological techniques are changed to get the data for control of these infectious diseases. Mathematical modeling has important roles in so many programs like health-economic aspects, control-programme evaluation, emergency planning and risk assessment; monitoring of surveillance data and policy making.

To get interpretations from the findings of mathematical modeling studies and from critically-evaluate epidemiological data, Public health personals and pharmaceutical industry professionals with policy makers, and infectious disease researchers, with infectious diseases frequently need to understand the transmission patterns of infectious diseases. However, the spread of infectious diseases is dominating headlines in routine has become another significant factor to study the current topic. There are many techniques to assess the measurement and to analyze the outbreaks and emerging epidemics has been developed and are being developed rapidly. These techniques may be complex and need technical persons, but simple techniques to analyze the epidemiology of resistance and the pathogens still needs to be improvised.

There is a need of moving fast about these mathematical models so that policy makers, public-health professionals and infectious disease scientists can be up-to-date.

It has been known about the mode or mechanism of resistance to antimicrobials by bacteria even before the time antibiotics were introduced as a source of treatment into routine clinical usage (Abraham and Chain 1940). Non-judicial rash and regular intake of antimicrobials without prescription has, however, increase the issue of resistant bacterial strains at the cost of antimicrobials which were sensitive (Lerner 1998; Hellinger 2000; Livermore 2000). With this increasing frequency human pathogens proteus and pseudomonas are getting resistance to many antibiotics, and the challenge of dealing with infections with gradually decreasing the number of effective antibiotics. It is clear those pathogens get resistant to antimicrobial agents by mutations.
in their genome or by acquiring some resistance mechanisms. Administration of antibiotics for prolonged period unnecessarily or by need helps in increasing the resistance mechanisms of the bacteria. Multi drug resistance Pseudomonas aeruginosa can increase its resistance capacity and potency to great extent. Thus there must be an accurate check on dosage and duration of P.aeruginosa infections treatment. Similarly, Proteus species isolated were found to have high antimicrobial resistance against many antibiotics. Proteus has also been studied to acquire resistance to multiple drugs. But Regular antibiotic administration to these strains would acquire multi-drug resistance property. Pseudomonas aeruginosa has been an emerging opportunistic pathogen of clinical relevance. Various epidemiological research showed its occurrence as a nosocomial pathogen in hospitals. These studies also showed that resistance to clinical isolates Pseudomonas is increasing.

There is technique that assesses the transfer of gene from one bacteria to another to detect mutations. In this technique, a gene which act as a selectable marker basically is introduced into cells in a culture or a bacterium cell. This refers a trait suitable for artificial selection. The gene that is introduced is a type of reporter gene which is used in molecular biology, genetic engineering and in microbiology laboratory, to indicate the success of a transaction or other method meant to introduce a cell with a foreign DNA. Selectable markers are often antibiotic resistance genes; bacteria that are subjected to introduce foreign DNA with any type of procedure are grown on a medium which is added with an antibiotic. The bacterial colonies that can grow in media with antibiotics indicate that they have successfully taken and expressed the foreign DNA/genetic material introduced in them. Normally the genes showing resistance to antibiotics such as kanamycin, chloroamphenicol, ampicillin, tetracycline or, etc., are considered as useful selectable markers for E.coli. Modus operandi.

In this technique, the non-recombinant genes are first separated from recombinants. i.e. introduction of A r-DNA in bacteria, there are some bacteria that remain non-transformed but some are transformed successfully. When these bacteria that have not taken up and expressed the new gene are grown on medium containing ampicillin, die because they are not resistant to ampicillin. Nitrocellulose paper is used to check the position and separated out to move them to medium containing nutrients for mass production of the product we need. Screenable marker is
an alternative to a selectable marker, which allows the researcher to distinguish wanted cells from unwanted ones, e.g. between blue and white colonies.

Based on the selection sought, different variety of markers are used for molecular biology research on antibiotic resistance. These include Positive selection markers also called selectable markers that confer selective advantage to the host organism an example of this is resistant antibiotic, which allows the hosting organism to survive antibiotic selection. another type of marker is counterselectable markers or Negative markers that inhibit growth or eliminate the growth of host organism upon selection. Thymidine kinase is an example of this type of negative marker, which makes the host sensitive to ganciclovir selection. Third type of markers is which can serve as both a positive and a negative marker, that shows an advantage to the host under one condition, and in other condition they inhibit the growth are known as Positive and Negative selectable markers. An example would be an enzyme that is able to convert a chemical to a toxic compound (negative selection) and can complement an auxotrophy (positive selection) (Jang et al., 2013).

There are many common selectable markers that help in infection control and antibiotic resistance: Beta-lactamases that confer resistance of ampicillin to bacterial hosts. Another example is Neo gene from Tn5, which confers resistance to kanamycin and geneticin in bacteria and in rest of the eukaryotic cells respectively. Another marker is which shows triclosan resistance to the host is named as Mutant FabI gene (mFabI) taken from E. coli genome, (Jang et al., 2013).

Patients undergoing cutting Open the body surgeries are more prone to massive risk of infections. Open-heart surgery is one common example. Surgeons can do operations that would have been deadly before, with antibiotics given before and after surgery.

Radiotherapy and chemotherapy which is being used for treatment of cancer damage patients immune system. During such type of treatments antibiotics are also prescribed to boost immune system of the patients. Immune system of the patients under going organ transplants needs to suppress with the help of drugs, otherwise immune system cab attack and reject the received organ. In these cases effective antibiotics are used to protect the body.
Among very strong antibiotics carbapenems is also showing resistance in various culture and sensitivity tests which is showing that medical uses of antibiotics is threatened. Number of tests showing resistance to carbapenems, has risen to more than 300 cases in 2010 from a handful of cases in 2003. Few drugs became resistant to many infections. A few cases of strains are even untreatable by any antibiotic.

**CONCLUSION**

Infectious diseses which were almost eradicated once are re-occurring. Gonorrhea the disease which is transmitted sexually has become increasingly difficult to cure. Extremely drug resistant and MDR (multi drug resistant) tuberculosis is an example of it, around the world. Only few number of antibiotics left which are working.

Older people which are being cured in the hospitals because of Infections, are one of the main concerns now days. E. coli and Klebsiella is opportunistic bacteria which live in the gut is the greatest threat in UK as during treatment of the patients in hospitals they are now becoming most common cause of infections. Many antibiotics are becoming resistant to infections.

According to the UK Cabinet Office, they have released an official warning for antibiotic resistance. Through the National Risk Register they have issued a warning for Civil Exigencies, which says over the next 20 years the counting of complicated infections due to anti microbial resistance could markedly increase. Even routine operations and small surgeries may be emerging as high risk procedures because of lack of effective antibiotics (National risk register, 2015).
6.2 RECOMMENDATIONS

The present work recommends the judicial use of antibiotics to control the antibiotic resistance. As seen in the present study the resistance rates of almost all bacteria are getting higher. So strong and stringent steps must be taken forward for containment of the current problem. Through the prudent use of current and future antimicrobials, the hope of controlling infectious diseases is and will continue to be in maintaining sensitivity against disease-causing bacteria.
6.3 FUTURE SCOPE

In lieu of present situation in context with resistance to antibiotic in clinical strains and the property of microbes for developing resistance to antibiotics that are commonly being used in community, it is clear that there is a need to call for cautious use of currently available and as yet effective antibiotics. Otherwise, resistance has become a common occurrence in infection and is not likely to disappear soon. This is the reason the resistance mechanisms are gaining ability, with multidrug efflux and generalized resistance mechanisms like membrane impermeability are the most common targets to manage resistance (Wright 2000).

The first revealed inhibitors of the largely specific multidrug efflux systems of P. aeruginosa have in recent times been reported (Renau et al. 1999). These inhibitors e.g. fluoroquinolones are effective by overcoming existing resistance. These agents have also shown effectiveness to prevent the exigency of resistance to fluoroquinolone in the first place (Renau et al. 1999). These inhibitors shown efficacy at compromising acquired biocide (i.e. triclosan) and intrinsic resistance in this microbe as well as antibiotic and biocide resistance in the bacteria that express homologous multidrug efflux systems (Blais et al. 1999). NorA multidrug transporter of Staphylococcus aureus inhibition have also been reported (Guz et al.,. 2001) where they are effective by enhancing susceptibility of fluoroquinolones (Markham et al. 1999) and preventing exigency of resistance to fluoroquinolone in vitro (Markham et al., 1999). Similarly, for microbial infections where outer membrane of bacteria is creating challenge for specific antibiotics, enhancement of use of antimicrobial agents that are able to permeabilize this type of bacteria is helpful (Ayres et al. 1998; Savage 2001; Poole 2001). Finally, strategies aimed at
interfering with biofilm formations seems to be useful in restricting clinical episodes of antibiotic resistance. Natural sensitivity in populations of pathogenic bacteria can be maintained by the wise use of present and future antibiotics. For biofilm formation of bacteria, bacterial cell signaling process is necessary and the molecules are identified that interfere that cell signaling processes (Rice et al., 1999) and these compounds are helpful in blocking biofilm formation process. Electromagnetic (McLeod et al. 1999) and Chemical (Armstrong et al., 2000) approaches to disrupt biofilm have also been reported.

6.4 LIMITATIONS OF RESEARCH WORK
1. Polymerase chain reactions to confirm the isolated bacterial characterisation
2. A wide range of area could be selected for the study.
3. More number of specimens could be taken.
4. Advanced diagnostic techniques could be used for identification of pathogenic bacteria.