4.1. PRELUDE

An antibiotic is a substance produced by various species of microorganisms (bacteria, fungi, actinomycetes), that suppresses the growth of other microorganisms and may eventually destroy them. However, common usage often extends the term antibiotic to include synthetic antibacterial agents, such as sulfonamides and quinolones, which are not products of microbes. Therapeutic science has come a long way from the insight and experiments of Ehrlich and Flemming. In the last two decades the list of antimicrobials has expanded exponentially, with the introduction of many new groups like quinolones, polypeptides etc. or improved derivatives of the major groups.

The introduction of antibiotics in 1940’s revolutionized the practice of medicine. The discovery, development and clinical exploitation of antibiotics can arguably be counted as one of the most significant medical advances of the twentieth century. Their potential for misuse was also recognized soon. It is therefore sobering that at the beginning of the twenty first century, articles abound concerning resistance. These super bugs with no new drugs to treat them, question the prospects of a post antibiotic era! Thereafter this has steadily increased with each passing decade. Widespread emergence of resistant organisms is the biggest fallout of irrational antibiotic use. Penicillin resistant pneumococcal infections, methicillin resistant Staphylococcus aureus, multi-drug resistant Klebsiella pneumoniae, vancomycin
resistant *Enterococci*, extended spectrum beta-lactamase producing *Enterobacteriaceae* complicate therapy immensely in hospitalized patients (New, 1992). Antibiotic resistance is believed to have evolved millions of years ago in soil bacteria as a means of protecting themselves against other, antibiotic-producing bacteria or their own antimicrobial products (Davis et al., 1971).

At present, medical practice at social and private level cannot be understood without taking into account the quality control and cost-benefit of medical activities. These parameters are essential for program assessment, implementation of therapies and for individual or collective decisions. Antibiotic resistance is believed to have evolved millions of years ago in soil bacteria as a means of protecting themselves against other, antibiotic-producing bacteria or their own antimicrobial products (Davies et al., 1971). However, most researchers believe that the current crisis of antibiotic resistance is bred from the intense selection pressure posed by wide spread use and misuse of antibiotics; indeed, organism archived prior to the current age of antibiotic excess do not display nearly the number of diversity of hydrolyzing enzymes present in recent clinical isolates (Hughes et al., 1983).

Among the many factors that have contributed to the emergence and spread of multiple-resistant organisms, three are of key importance: mutations in common resistance genes that have extended their spectrum of resistance; exchange of genetic information among microorganisms,
transferring well-known genes into new hosts and the increase in selective pressure in hospitals, other institutional settings and communities that allow resistant organisms to proliferate. The first two reflect the ability of bacteria to adapt to changing environments, while the last emphasizes that environmental conditions often enhance the emergence of novel phenotypes. All three are clearly interrelated (New, 1992; Murray, 1991).

Mutations in common genes:

One example of the role of mutations in emerging resistance concerns changes in β-lactamases that extend their spectrum of activity. β-Lactamases are enzymes that inactivate, β-lactam drugs, such as penicillin, ampicillin, and cephalothin (Bush et al., 1995). Until recently, these enzymes were not able to hydrolyze newer extended-spectrum cephalosporins such as cefotaxime and ceftazidime. However, in 1982, mutant forms of the β-lactamases were reported that were capable of inactivating the extended-spectrum cephalosporins and monobactams, such as aztreonam (Knothe et al., 1983). The enzymes called extended-spectrum β-lactamases (ESBLs), have now been isolated from organisms throughout Europe, Japan and the United States. At least 30 such ESBLs have now been reported (Phillipon et al., 1989, Jacoby et al., 1997). When the amino acid sequences of the first ESBLs were examined, only three differences were found between the ESBL and the wild-type β-lactamase that is found in 70% of ampicillin-resistant, but extended-spectrum cephalosporin susceptible, E. coli (Agarwal et al., 1997).
These amino acid changes were the reflection of point mutations in the coding sequence of the β-lactamase gene. Recent experience suggests that under the selective pressure of high cephalosporin usage in a hospital ward, organisms with mutations that extend the spectrum of activity of β-lactamases can emerge and disseminate (Meyer et al., 1993). The development and spread of ceftazidime-resistant *Klebsiella pneumoniae* is perhaps the best example of this phenomenon (Rice et al., 1990). These organisms, which contain variations of common *bla*TEM or *bla*SHV β-lactamase genes (bla for beta-lactamase), have caused a number of outbreaks in the United States (Burwen et al., 1994). Mutations in β-lactamase genes, however, are but one-way mutations can lead to resistance. Reduced membrane permeability limiting a drug’s access into the cell and changes in a drug’s target site can also be a result of mutation.

**Genetic exchange:**

Bacteria can exchange genetic information by transformation (the uptake of naked DNA), transduction (transfer of DNA by bacteriophage), and conjugation (cell-to-cell contact) (Johnson et al., 1998). Although conjugation was previously though to be limited to Gram-negative bacilli, extensive data now confirm the existence of a similar transfer process among Gram-positive organisms whereby plasmids or independent transposable elements, often carrying multiple-resistance genes, move from one organism to another. The transfer process extends even to highly unrelated groups of
organisms, such as Campylobacter coli and Enterococci (Trieu et al., 1985), which have been shown to exchange aminoglycoside resistance genes.

Enterococci demonstrate how organisms can gradually accumulate resistance genes by genetic exchange and develop into multi-resistant nosocomial pathogens causing untreatable infections. Enterococci accounted for 16571 nosocomial infections in hospitals reporting to the national nosocomial infection surveillance system during the period from January 1, 1989, to March 31, 1993 (CDC, 1993). Over the last decade, Enterococci have become increasingly resistant to multiple antimicrobial agents. For example, the genes encoding resistance to gentamicin and the production of β-lactamase were acquired from staphylococci and when coupled with resistance to streptomycin and vancomycin, resulted in organisms that were very difficult, if not impossible (Handwerger et al., 1993, Montecalvo et al., 1994; Vashishtha, 2000), to treat. Streptomycin resistance is mediated primarily by an aminoglycoside-modifying enzyme ANT, although resistance due to ribosomal mutation has also been noted. Vancomycin resistance can be mediated by several genes, including van A, van B, van C1, or van C2 (Handwerger et al., 1993, Montecalvo et al., 1994), that function in concert with other genetic loci frequently located on the same transposable element. The alarming proportion of nosocomial enterococcal isolates resistant to vancomycin, particularly from patients intensive care units,
which rose from 0.4% to 13.6% in the 4-year period from 1989 to 1993, continued to rise in 1994 and 1995.

Although resistance genes from gram-positive organisms may be transferred into Gram-negative organisms or vice versa, differences in the genetic control mechanisms present in various species may limit the ability of the new resistance genes to be expressed and to mediate (Davies et al., 1971) resistance. Thus, there appear to be some limits to the constellation of resistance genes that can be assembled and expressed in bacteria. Nonetheless, the ability of bacteria to acquire resistance genes from other members of the normal bacterial flora under the selective pressure of antimicrobial use should not be underestimated. It has been shown, for example, that the van A vancomycin resistance gene cluster in Enterococcus faecalis can transfer to aureus via conjugation and can express high-level resistance (Noble et al., 1992). Although an S. aureus isolate with high-level vancomycin resistance has yet to be encountered in nature, the possibility clearly exists that the genetic unit that includes van A, or perhaps one of the other vancomycin resistance genes, will be transferred under natural conditions to S. aureus. Such an occurrence would have serious public health implications, since nosocomial strains of S. aureus, which tend to be resistant to multiple drugs, could acquire the vancomycin resistance gene, rendering current treatment ineffective.
Selective pressures in institutional and community settings

The concept of selective pressure refers to the environmental conditions that encourage or enhance the proliferation of strains of bacteria that develop resistance to antimicrobial agents through spontaneous mutation or by acquisition of new DNA. It is hypothesized that organisms with new mutations or genes would likely not survive were it not for the selective pressures that encourage their emergence. In addition to communications or hospital settings expanded use of antimicrobial agents in sites outside the hospital, including nursing homes, day care centers and animal feedlots, increases the selective pressure for resistant organisms to emerge (American Society for Microbiology, 1995). The use of broad-spectrum antimicrobial agents is increasing in outpatient settings. Antimicrobial drugs are often used for common conditions for which their effectiveness is unclear (Knothe et al., 1983; Gonzales et al., 1995).

*Streptococcus pneumoniae* serves as an example of a community-acquired organism that has become increasingly resistant to a wide variety of antimicrobial agents (Knothe et al., 1983; Reinert et al., 1995). The metropolitan Atlanta, Ga, area noted that 25% of invasive pneumococcal isolates from that region were no longer susceptible to penicillin and 9% were no longer susceptible to extended spectrum cephalosporins (Hofmann et al., 1995) and pneumococcal isolates in Franklin Country, Ohio, were penicillin-resistant. The development and spread of multiply-resistant
*Pneumococci* can be a major problem among children in day care centers when antimicrobial agent use is often high (Knothe *et al.*, 1983), in part because children have clinically confirmed, or suspected, otitis media receiving antimicrobial agents the likelihood increases that multi-resistant organisms will be found in their throats that may disseminate to other children. Among three children with pneumococcal meningitis caused by strains resistant to cefotaxime and ceftriaxone, all had received prior cephalosporin therapy for otitis media (Sloas *et al.*, 1992). Thus, control of antimicrobials, particularly those used prophylactically in children attending day care centers, is in issue that must be reassessed.

While resistance is increasing in many pathogens, the number of new antimicrobial agents approved for use in the United States has slowed. In 1993 only a single antibacterial agent was approved for use in the United States by the Food and Drug Administration and in 1994, no new antibacterial agents were approved (National Committee for Clinical Laboratory Standards, 1993). Thus, our ability to control outbreaks of infectious diseases through antimicrobial use alone is diminishing. A recent report from the Hospital Infection Control Practices Advisory Committee on preventing the spread of vancomycin resistance (FDA, 1994) stresses the need for professional and public educational programs, enhanced microbiological surveillance, enhanced surveillance among patients, effective implementation of infection control procedures and perhaps most important,
prudent use of antimicrobial agents for treatment and prophylaxis. Whether these recommendations can be generalized to aid in the control of other types of resistant organisms found in the hospital environment remains to be seen. Studies to validate the effectiveness of the guidelines in controlling the spread of vancomycin-resistant *Enterococci* are in progress. The report also notes that our major surveillance system in the United States for the detection of novel resistant organisms is the network of hospital microbiology laboratories, since it is these data that feed into the National Nosocomial Infections Surveillance System and other such programs. However, with the exception of *M. tuberculosis* and *N. gonorrhoeae*, there are no national surveillance systems that systematically monitor resistance in community-acquired infections. While some states are initiating surveillance of penicillin resistance in *Pneumococci, H. influenzae*, it is up to infectious disease specialists, clinical microbiologists, pharmacists and public health personnel to be vigilant for organisms with novel resistance patterns so that control measures can be effectively implemented. Emergence of drug-resistant organisms also has clear implications for vaccine and antimicrobial drug development priorities.

Certain non-fastidious, Gram-negative bacilli possess the ability to rapidly develop resistance to many of the newer “enzyme stable” β-lactum antibiotics. This finding poses many clinical problems including emergence of resistance during therapy with the drugs. Therapeutic alternatives for
patients are severely limited which this problem occurs because multiple drug resistance may arise simultaneously. To date, two mechanisms have been found to be responsible for this resistance. The first, which produces multiple β-lactum resistance, is the induction of chromosomal β-lactamases that mediate resistance to non-substrate drugs by the creation of a non-hydrolytic barrier that blocks access to target patients within the cell.

The second mechanism, which produces β-lactam/aminoglycoside resistance, involves a change in outer membrane permeability.

Many problems have been encountered with the newer generation of β-lactam antibiotics. Most of these problems have involved the ability of certain non-fastidious, Gram-negative bacilli to rapidly develop resistance to the drugs. Such resistance was unanticipated when these antibiotics were developed since they had been designed to solve the problems posed by the β-lactamase that had limited efficacy of older penicillin’s and cephalosporins. However, stability of β-lactamases does not guarantee producing bacteria rather; this characteristic appears to be responsible for many of the problems that have occurred with these new drugs.

Many major improvements in antimicrobial agents have been based upon knowledge of the mechanisms responsible for microbial drug resistances, microbial resistance due to production of drug inactivating enzymes has been circumvented by the design of new enzyme stable compounds. Such “antibiotic engineering” led to the development of
penicillinase resistant penicillin and several new aminoglycosides that are resistant to the diver’s aminoglycosides inactivating enzymes of bacteria. These drugs represented major improvements in our antimicrobial armamentarium. When the new β-lactamase-stable cephalosporins were developed, it was only logical to expect a similar improvement with these agents. However, is much respect they have not lived up to expectations?

**Extended Spectrum Beta Lactamase Mediated Resistance**

ESβL was first detected in Europe in 1978, in USA 1981 and in Germany during 1983 (Medeiros, 1997). These resistant strains are established in many hospitals, producing epidemic diseases. These ESβL producing strains have become more and more important because of their ability to adopt in the hospital environment and cause more outbreaks in hospitals (Guillume *et al.*, 1993, Chaibi *et al.*, 1999).

Gram-negative bacteria are an important hospital acquired pathogen that causes severe morbidity and mortality in neonatal and pediatric patients. Several out breaks of infection caused by *K. pneumoniae* isolates that are simultaneously resistant to broad-spectrum cephalosporins and aminoglycosides have been reported (Arlet *et al.*, 1990; Bush *et al.*, 1995, 2001). Over the last 20 years, many new β-lactam antibiotics have been developed that were specifically designed to be resistant to the hydrolytic action of β-lactamases. However, with each new class that has been used to treat patients, new β-lactamases emerged that caused resistance to this class
of drug. Not surprisingly, resistance to these expanded spectrums $\beta$-lactam antibiotics due to $\beta$-lactamases emerged quickly. The first of these enzymes capable of hydrolyzing the newer $\beta$-lactam, SHV-2, was found in a single strain of *Klebsiella ozaenae* isolated in Germany (Kim et al., 2000). Because of their increased spectrum of activity, especially against the oxyiminocephalosporins these enzymes were called extended spectrum $\beta$-lactamases (ES$\beta$Ls). The organisms that are resistant to third generation cephalosporins also shows high degree of resistance to other class of antibiotic such as cephlothin, piperacillin and ampicillin etc, therefore they were named as extended broad-spectrum $\beta$-lactamases. Although the original definition of what constituted an ES$\beta$L was primarily based on the substrates hydrolyzed by the enzymes, more recently the term ES$\beta$L has been limited to those $\beta$-lactamases that are inhibited by clavulanic acid in addition to showing the enhanced spectrum of activity. During early 1980, strains of *Klebsiella* with reduced susceptibility to third generation cephalosporins were noted in Europe. Today over 150 different ES$\beta$Ls have been described. These $\beta$-lactamases have been found worldwide in many different genera of *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Resistance was mediated by new $\beta$-lactamases present on plasmids. These resistances were soon passed on to *E. coli*, which later showed resistance to the same antibiotic for which *Klebsiella* was resistant. These enzymes were derived from plasmid mediated
class A, B lactamase in Gram-negative bacilli such as TEM-1 and SHV-1 (Chaibi et al., 1999; Heritage et al., 1999).

In the absence of specific surveillance ESβL producing strains in our country are probably more prevalent than currently recognized, because they are often undetected by routine susceptibility testing methods (Agarwal et al., 1997). Recent reports have highlighted the emergency of ESβL producing strains endowed with an extremely wide spectrum of antibiotic resistance including resistance to trimethoprim, amikacin, gentamicin and streptomycin. The increased prevalence of Enterobacteriaceae producing ESβL creates a great need for laboratory testing methods that will accurately identify the presence of these enzymes in clinical isolates.

Functional group 1 β-lactamases are described as cephalosporinases which are not inhibited by clavulanate (Bush et al., 1995). Originally, these enzymes were naturally occurring, chromosomally encoded AmpC β-lactamases found in genera such as Enterobacter, Citrobacter, Serratia and Pseudomonas. The genes encoding these enzymes were found on the chromosome and are inducible by certain beta lactam antibiotics (Sanders et al., 1988). However, in recent years some of these genes have found their way onto plasmids and are being expressed constitutively at high levels in Klebsiella pneumoniae and Escherichia coli. Whereas the extended-spectrum β-lactamases in these organisms confer resistance to the expanded-spectrum β-lactam antibiotics such as ceftazidime, cefotaxime and aztreonam, the
plasmid-mediated AmpC-type enzymes also confer resistance to the cephemycins. The first of these to be described was the MIR-1 enzyme which, on the basis of a partial DNA sequence, appears to have originated from the AmpC β-lactamase of Enterobacter cloacae (Agarwal et al., 1997). Since that time a number of other enzymes such as BIL-1, CMY-1, CMY-2, LAT-1, LAT-2 (Gazouli et al., 1997), FOX-1, and MOX-1 (Horii et al., 1992) have been described. These enzymes are now being found on plasmids with increasing frequency.

**Metallo beta lactamases**

The increase in antibiotic resistance among Gram-negative bacteria is a notable example of how bacteria can procure, maintain and express new genetic information that can confer resistance to one or several antibiotics. This genetic plasticity can occur both inter- and intragenerically. Gram-negative bacterial resistance possibly now equals or usurps that of gram positive bacterial resistance and has prompted calls for similar infection control measures to curb their dissemination (Poole, 2003). Reports of resistance vary, but a general consensus appears to prevail that quinolone and broad-spectrum β-lactam resistance increasing in members of the family *Enterobacteriaceae* and *Acinetobacter* spp. and that treatment regimes for the eradication of *Pseudomonas aeruginosa* infections are becoming increasingly limited (Maniatis et al., 2003, Neuhauser et al., 2003). For example, a 5-year longitudinal study involving many centres from Latin
America indicated that year after year, *P. aeruginosa* resistance has continually risen to the point where approximately 40% are resistant to “antipseudomonal” drugs, including carbapenems (Andrade *et al.*, 2003). While the advent of carbapenems in the 1980s heralded a new treatment option for serious bacterial infections, carbapenem resistance can now be observed in *Enterobacteriaceae* and *Acinetobacter* spp. and is becoming commonplace in *P. aeruginosa*. Gram-negative bacteria have at their disposal a plethora of resistance mechanisms that they can sequester and/or evince, eluding the actions of carbapenems and other β-lactams. The common form of resistance is either through lack of drug penetration (i.e., outer membrane protein [OMP] mutations and efflux pumps), hyper-production of an AmpC-type-lactamase and/or carbapenem-hydrolyzing-lactamases. Based on molecular studies, two types of carbapenem-hydrolyzing enzymes have been described: serine enzymes possessing a serine moiety at the active site, and metallo-β-lactamases (MBLs), requiring divalent cations, usually zinc, as metal cofactors for enzyme activity (Bush *et al.*, 1995; Bush, 2001, Buynak *et al.*, 2004).

This is the first extensive study to prospectively look for ESβL, AmpC and metallo beta lactamase producing bacteria in pyogenic meningitis cases.
Detection of bacterial drug resistance in the clinical laboratory:

Several of the new resistance mechanisms recognized in Gram-positive and Gram-negative organisms are difficult to detect with current laboratory methods. For example, vancomycin resistance in Enterococci can be difficult to detect by automated methods, such as Vitek (BioMerieux USA, Hazelwood, Mo) and Microscan walk-away (Dade International, West Sacramento, Calif), but is readily detected by most other broth and agar-based methods (Tenover 1991). A vancomycin resistance agar screen test has been developed as a backup to traditional susceptibility testing methods for the detection of vancomycin-resistant Enterococci (Knothe et al., 1983).

With regard to resistance in Gram-negative bacilli, a major problem noted recently is the detection of cefotaxime ceftriaxone and ceftazidime resistance in K. pneumoniae and E. coli, particularly when resistance is mediated by ESBLs. In part, the inability of the laboratory to recognize resistant strains is due to the minimal inhibitory concentration (MIC) breakpoints chosen by the National committee for Clinical Laboratory standards to define “resistance” (1993). Then breakpoints, which for extended-spectrum cephalosporins are usually 32 or 64 μg/mL, were chosen based on population distributions of bacteria before ESBLs became widely disseminated. Depending on the antimicrobial agent tested in the laboratory, MICs for ESBL-containing strains may vary anywhere from 4 μg/mL (susceptible) to 256 μg/mL (highly resistant). Variation depends on the type
of ESBL (of which there are over 30), the antimicrobial agent tested, and the method of testing. Given that the usual MIC of *K. pneumoniae* to ceftazidime is 0.06 µg/mL, it is possible to achieve a 50-fold increase in the MIC and still be in the “susceptible” MIC range. However, if cefotaxime were tested in lieu of ceftazidime, resistance may well be detected. New laboratory tests to confirm the presence of ESBLs in *K. pneumoniae* and *E. coli*, such as disk diffusion assays performed in parallel with and without the addition of the enzyme inhibitor clavulanic acid may aid in the detection of such organisms, other commercially prepared tests are under evaluation. Studies sponsored by the National Committee for Clinical Laboratory Standards are currently under way to develop alternate testing procedures to detect ESBL-mediated resistance. Because laboratories need to screen a variety of bacterial pathogens that were once considered uniformly susceptible, a guide to appropriate susceptibility testing methods is presented in the Table. While new testing methods may aid in identifying organisms with novel resistance genes, growing restraints on the personnel and supply budgets in hospital based microbiology laboratories may hinder the widespread implementation of the tests. In fact, new laboratory information systems often mask important quantitative information, such as the actual MICs of antibiotics, in lieu of reporting only the interpretive categories of susceptible, intermediate and resistant. Thus, physicians who suspect the presence of new resistant organisms in their hospitals should consult with the hospital’s
microbiologists regarding the optimal approach to identifying and testing of such organisms.

**Objectives**

1. To evaluate the susceptibility pattern of the organisms isolated from pyogenic meningitis.
2. To evaluate extended spectrum of beta lactamase production.
3. To evaluate AmpC production in gram negative bacilli.
4. To evaluate metal beta lactamase production.
5. To evaluate methicillin resistant among *Staphylococcus aureus*.

### 4.2. MATERIALS AND METHODS

The primary purpose of antimicrobial susceptibility testing is to provide clinicians with information that will assist in the choice of an appropriate antibiotic for the patients. Laboratory report indicating that an organism is sensitive to an antibiotic implies that the organism is relevant to the patient’s clinical condition and that the infection is treatable under a given set of pharmacological conditions. Reporting of resistance implies that infection is not treatable with the antibiotic.

A total of 61 consecutive clinical isolates of which *Streptococci pneumoniae* (89), *H. influenzae* (n=51), Group B *Streptococci* (19), *N. meningitides* (13), *Staphylococcus aureus* (n=11), *Klebsiella pneumoniae* (n=08), *E. coli* (n=05) and *Pseudomonas aeruginosa* (n=03), obtained from pyogenic meningitis cases...
were identified by standard methods were included in this study. Susceptibility to antibiotics was tested by disk diffusion method (Kirby Bauer’s disc diffusion method). Extended spectrum beta lactamases was detected by double disk diffusion synergy test (Bauer et al., 1966, NCCLS, 1993, 1997, 1998, 2000, 2004).

4.2.1. Disk diffusion by Kirby Bauer’s disc diffusion method:

The basic principle of disc diffusion method is the antibiotic-impregnated disc absorbs moisture from the agar and antibiotic diffuses into the agar medium. The rate of extraction of antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases, there is a logarithmic reduction in the antibiotic concentration. The depth of the agar affects the extent of antimicrobial diffusion. Visible growth of bacteria occurs on the surface of the agar where the concentration of antibiotic has fallen below its inhibitory level of the test strain. The results were interpreted as per Kirby-Bauer’s chart provided by the procured company (Hi-media Ltd. Mumbai).

All the organisms isolated from pyogenic meningitis were subjected for antimicrobial susceptibility testing. The medium (Muller-Hinton) was prepared and sterilized and was dispensed into flat-bottomed 9 cm glass petri dishes. The pH of the medium was checked when medium was prepared. Inoculum was prepared by picking 3–4 morphologically similar
colonies from the culture plates and inoculating into a test tube containing peptone water. The tubes were incubated for 2 hrs at 37°C to produce a bacterial suspension of moderate turbidity. The inoculum size was adjusted by comparison with a barium sulfate standard, 0.5 Mac Farlands standard units (Bauer et al., 1966).

A sterile swab was dipped into the inoculum and excess of inoculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid. Swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60° after each application. The inoculum was left to dry for few minutes at room temperature with the lid closed. The antibiotics used were penicillin, ampicillin, erythromycin, gentamycin, amikacin, ciprofloxacin, cephalexin, cefotaxime, ceftazidime, cefotaxime, ceftriaxone, cefuroxime and ofloxacin (Hi-media laboratories, Mumbai). Discs were placed on the inoculated plates using a pair of sterile forceps. The plates were incubated at 35°C for 18-24 hrs. After overnight incubation, the diameter of each zone (including the diameter of the disc) was measured and recorded in mm. The size of the zone of inhibition is an indication of the susceptibility of the pathogen. The zone sizes were compared with the standard chart and reported accordingly.
4.2.2. Antimicrobial susceptibility testing of *H. influenzae* by the disk diffusion method

Prepare the inoculum for seeding the antimicrobial susceptibility media with *H. influenzae* from fresh, pure cultures of *H. influenzae* (i.e., from isolates grown overnight on supplemented chocolate agar). Prepare cell suspensions of the bacteria to be tested in broth or sterile physiological saline; use a suspension equal to a density of a 0.5 McFarland turbidity standard for the inoculum.

a) Suspend viable colonies from an overnight chocolate agar plate in a tube of broth to achieve a bacterial suspension equivalent to a 0.5 McFarland turbidity standard; be careful not to form froth or bubbles in the suspension when mixing the cells with the broth. This suspension should be used within 15 minutes.

b) Compare the suspension to the 0.5 McFarland turbidity standard by holding the suspension and the McFarland turbidity standard in front of a light against a white background with contrasting black lines and compare the density. If the density of the suspension is too heavy, the suspension should be diluted with additional broth. If the density of the suspension is too light, additional bacteria should be added to the suspension.
c) When the proper density is achieved, dip a cotton swab into the bacterial suspension. Press the swab on the side of the tube to drain excess fluid.

d) Use the swab to inoculate the entire surface of the HTM plate three times, rotating the plate 60 degrees between each inoculation. Use the same swab with each rotated streak, but do not re-dip the swab in the inoculum (i.e., the bacterial cell suspension).

e) Allow the inoculum to dry before the disks are placed on the HTM plates. Drying usually takes only a few minutes and should take no longer than 15 minutes. (If drying takes longer than 15 minutes, use a smaller volume of inoculum in the future).

f) After the plate is dry, antimicrobial disks should be placed on the HTM plate. The disks should be placed on the agar with sterile forceps and tapped gently to insure adherence to the agar. Diffusion of the drug in the disk begins immediately; therefore, once a disk contacts the agar surface, the disk should not be moved.

g) Invert the plate and incubate it in a CO₂-enriched atmosphere (5% CO₂-incubator or candle-extinction jar) for 16–18 hours at 35°C.

h) After overnight incubation, measure the diameter of each zone of inhibition. The zones of inhibition on the media containing blood are measured from the top surface of the plate with the top removed.
Use either calipers or a ruler with a handle attached for these measurements, holding the ruler over the center of the surface of the disk when measuring the inhibition zone. Care should be taken not to touch the disk or surface of the agar. Sterilize the ruler occasionally to prevent transmission of the bacteria. In all measurements, the zones of inhibition are measured as the diameter from the edges of the last visible colony. Record the results in millimeters (mm).

i) Interpretation of the antimicrobial susceptibility is obtained by comparing the results obtained and recorded (in the manner described in this protocol) to the NCCLS standard inhibition zone diameter sizes.

4.2.3. **Antimicrobial susceptibility testing of *N. meningitidis* by E test**

All the isolates were subjected to antibiotic susceptibility testing based on the almost 100% susceptibility of *N. meningitidis* to commonly used antibiotics. The antibiotic susceptibility patterns of 100 *Neisseria meningitidis* strains to cotromixazole, chloramphenicol, cefotaxime and penicillin were determined by a disc diffusion method using Mueller Hinton II agar with 5% sheep blood. The table used for interpretation of disc diffusion results was that recommended by the Clinical and Laboratory Standards Institute (CLSI) 2005.
Either 150-mm or 100-mm plates can be used for the E-test®, depending on the number of antimicrobial agents to be tested per isolate. Two different E-test® antimicrobial strips can be placed in opposite gradient directions on a 100-mm plate and although the manufacturer states that up to six E-test® strips can be used on a 150-mm plate, this laboratory manual suggests that in order to avoid overlapping zones of inhibition of growth, not more than five E-test® strips be used on a 150-mm plate. Mueller Hinton chocolate agar was used.

4.2.4. Tests for ESBL production

Double disk approximation test for screening:

The test organisms were applied on to a Mueller Hinton agar plate by adjusting turbidity to McFarland no 0.5 tube. Antibiotic discs of amoxicillin/clavulanic acid (20/10 µg) and cefotaxime (30 µg) were placed at a distance of 15 mm apart and incubated. Organisms that showed a clear extension of cefotaxime inhibition zone towards the disc containing Clavulanate were considered as ESBL producer. The organisms which were screened and found positive for ESBL production were subjected to confirmatory test.

NCCLS phenotypic confirmatory test:

Ceftazidime (30 µg) and ceftazidime plus Clavulanic acid (30/10 µg) were placed on Mueller Hinton agar and incubated. Organism was considered as ESBL producer if there was a ≥ 5mm increase in diameter of ceftazidime plus clavulanic disc and that of ceftazidime disc alone.
4.2.5. Amp C Disk Test:

Isolates were tested for AmpC activity by a three-dimensional extract method, which was an adaptation of procedures described previously for the detection of extended-spectrum beta-lactamases (ESBLs) (Agarwal, 1997). Briefly, 50 µl of a 0.5 McFarland bacterial suspension prepared from an overnight blood agar plate was inoculated into 12 ml of tryptic soy broth and the culture was grown for 4 h at 35°C. The cells were concentrated by centrifugation and freezing-thawing the cell pellets five times made crude enzyme preparations. The surface of a Mueller-Hinton agar plate was inoculated with one each of two E. coli strains (ATCC 25922 and ATCC 11775) as described for the standard disk diffusion method (NCCLS, 1999); a 30-µg-cefoxitin disk was placed on the inoculated agar. With a sterile scalpel blade, a slit beginning 5 mm from the edge of the disk was cut in the agar in an outward radial direction. By using a pipette, 25 to 30 µl of enzyme preparation was dispensed into the slit, beginning near the disk and moving outward. Slit overfill was avoided. The inoculated media were incubated overnight at 35°C. Enhanced growth of the surface organism at the point where the slit intersected the zone of inhibition was considered as positive for three-dimensional test result and was interpreted as evidence for the presence of AmpC beta-lactamase.
4.2.6. **Metallo β-lactamase (MBL) production**

Gram negative organisms that showed resistance to Imipenem were selected for MBL production.

**Imipenem-EDTA combined disc test**

This test was performed according to Yong *et al.* test organisms were inoculated onto Mueller Hinton agar plates as per the CLSI recommendations. Two 10µg imipenem disks were placed on the plate and 10 µl of sterile 0.5 M EDTA solution was added to one of the imipenem disk. The inhibition zones of the imipenem and imipenem plus EDTA disks were compared after inoculation. If the increase in inhibition zone with the imipenem plus EDTA disc was ≥ 7mm than the imipenem disc alone, it was considered as MBL positive.

4.2.7. **Detection of methicillin resistant *Staphylococcus aureus***

Methicillin resistance was detected by disc diffusion technique using 30µg cefoxitin disc (Hi-Media, Mumbai). The suspension for inoculation was prepared from the colonies from an overnight growth on nutrient agar plate. The growth was suspended in 0.5 ml of sterile saline and the turbidity was adjusted to 0.5 Mcfarland’s units. A sterile swab dipped into this suspension and the excess of inoculum was removed by pressing it against the sides of the tube. This swab was used to inoculate on Mueller-Hinton agar plate. Cefoxitin disc was applied within 15 min after the inoculation. The plates were incubated for 24 hrs at 37°C. The diameter of the zone around the disc
was measured and the results were interpreted. *Staphylococcus aureus* NCTC 12493 was used as the control strain.

4.3. RESULT

Of 236 samples cultured, 199-yielded growth of microorganisms. Of these, 68 were Gram-negative bacilli and 119 were Gram-positive cocci and 12 were Gram-negative cocci. Gram-negative bacteria isolated were *H. influenzae* (n=51), *Klebsiella* sps (n=08), *E. coli* (n=06), *Pseudomonas aeruginosa* (n=03). Gram negative cocci isolated is *N. meningitides* (02). Among Gram-positive bacteria *Streptococci pneumoniae* (89), Gp B *Streptococci* sps (19) were the predominant organism followed by *Staphylococcus aureus* (11).

6.3.1. Disc diffusion method (Kirby Bauer’s disc diffusion method)

Antibiotic resistant pattern of Gram-negative organisms isolated from pyogenic meningitis cases (Table 4.1) (Plate 4.1, 4.2 and 4.3).

Out of 51 *H. influenzae*, 66.7% were resistance to more then three groups of antibiotics. Among cephalosporins, all the isolates of *H. influenzae* were sensitive to cefotaxime, ceftriaxone and ceftazidime. Among aminoglycosides, maximum sensitivity was observed to amikacin (84.6 %), 34 (66.7%) of *H. influenza* was resistant to gentamicin, 34 (66.7%) to tetracycline, 42 (82.3%) to ampicillin and 43 (84.6%) to amoxicillin.

Out of 08 isolates of *Klebsiella* sps, 07 (87.5%) to tetracycline, 04 (50.0%) to amikacin, 05 (62.5%) to chloramphenicol, 07 (87.51%) to ceftazidime. Maximum sensitivity was seen to cefotaxime 06 (75.0%).
Table - 4.1: Antimicrobial susceptibility pattern of the Gram negative bacilli isolated from pyogenic meningitis

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Tetracycline</th>
<th>Ampicillin</th>
<th>Gentamicin</th>
<th>Amoxicillin</th>
<th>Amikacin</th>
<th>Chloramphenicol</th>
<th>Cefotaxime</th>
<th>Cefazidime</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. Influenzae (51)</td>
<td>R</td>
<td>34 (66.7)</td>
<td>42 (82.3)</td>
<td>34 (66.7)</td>
<td>43 (84.6)</td>
<td>08 (15.4)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>17 (33.3)</td>
<td>09 (17.7)</td>
<td>17 (33.3)</td>
<td>08 (15.4)</td>
<td>43 (84.6)</td>
<td>34 (66.7)</td>
<td>51 (100)</td>
<td>51 (100)</td>
</tr>
<tr>
<td>Klebsiella sps (08)</td>
<td>R</td>
<td>07 (87.5)</td>
<td>06 (75.0)</td>
<td>05 (62.5)</td>
<td>05 (62.5)</td>
<td>04 (50.0)</td>
<td>05 (62.5)</td>
<td>02 (25.0)</td>
<td>07 (87.5)</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>01 (12.5)</td>
<td>02 (25.0)</td>
<td>03 (37.5)</td>
<td>03 (37.5)</td>
<td>04 (50.0)</td>
<td>03 (37.5)</td>
<td>06 (75.0)</td>
<td>01 (12.5)</td>
</tr>
<tr>
<td>E. coli (06)</td>
<td>R</td>
<td>03 (60.0)</td>
<td>04 (80.0)</td>
<td>03 (60.0)</td>
<td>03 (60.0)</td>
<td>02 (40.0)</td>
<td>05 (100)</td>
<td>04 (80.0)</td>
<td>03 (60.0)</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>02 (40.0)</td>
<td>01 (20.0)</td>
<td>02 (40.0)</td>
<td>02 (40.0)</td>
<td>03 (60.0)</td>
<td>--</td>
<td>01 (20.0)</td>
<td>02 (40.0)</td>
</tr>
<tr>
<td>Pseudomonas sps (03)</td>
<td>R</td>
<td>02 (66.7)</td>
<td>03 (100)</td>
<td>03 (100)</td>
<td>03 (100)</td>
<td>02 (66.7)</td>
<td>03 (100)</td>
<td>02 (66.7)</td>
<td>03 (100)</td>
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<tr>
<td></td>
<td>S</td>
<td>01 (33.3)</td>
<td>-</td>
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<td>--</td>
<td>01 (33.3)</td>
<td>--</td>
<td>01 (33.3)</td>
<td>--</td>
</tr>
</tbody>
</table>
Plate - 4.1: AmpC production by three dimensional diffusion method for *Klebsiella pneumoniae*
Plate - 4.2: Antibiotic susceptibility testing by Kirby-Bauer’s disc diffusion method for *Pseudomonas aeruginosa*

Plate - 4.3: Antimicrobial susceptibility pattern of *N. meningitidis* by E test
The response of other organisms varied differently with other antibiotics tested. Out of 06 E. coli isolates, 02 (40.0%) were resistant to amikacin, 06 (100%) to chloramphenicol 04 (80%) to cefotaxime and 03 (60%) each to ceftazidime and ceftriaxone and all the isolates were sensitive to chloramphenicol (Table-4.1).

Out of 03 Pseudomonas isolates, 100% of organisms were resistant to gentamicin, ampicillin, chloramphenicol, ceftazidime and ceftriaxone. 02 (66.7%) were resistant to cefotaxime and 02 (66.7%) to amikacin.

Antibiotic resistant pattern of Gram-positive bacteria is depicted in Table-4.2. Out of 11 Staphylococcus aureus, 100% were resistant to penicillin and 06 (>50%) to ampicillin. In aminoglycosides group, 02 (33.3%) were resistant to gentamicin and 04 (66.7%) to amikacin. In quinolones, maximum resistance was observed to each of cephoxitine, chloramphenicol and ceftriaxone with 06 (54.5%) resistance and ceftriaxone.

Among Streptococcus pneumoniae 23.6% were sensitive to penicillin, and 100% of the isolates were sensitive to ampicillin.

All the Gp B Streptococci were sensitive to penicillin.

Among N. meningitides, all the isolates were resistant to tetracycline, amoxicillin and sensitive to ampicillin, gentamicin, amikacin, chloramphenicol, cefotaxime, ceftazidime, ceftriaxone (Table-4.3).
Table - 4.2: Antimicrobial susceptibility pattern of the Gram positive cocci isolated from pyogenic meningitis

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Penicillin</th>
<th>Tetracycline</th>
<th>Ampicillin</th>
<th>Gentamicin</th>
<th>Cephalotin</th>
<th>Amikacin</th>
<th>Chloramphenicol</th>
<th>Cefotaxime</th>
<th>Ceftazidime</th>
<th>Ceftriaxone</th>
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</thead>
<tbody>
<tr>
<td><strong>Streptococcus Pneumoniae (89)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>21 (23.6)</td>
<td>NT</td>
<td>--</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>S</td>
<td>68 (76.4)</td>
<td>--</td>
<td>89 (100)</td>
<td>--</td>
<td>89 (100)</td>
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<tr>
<td><strong>Gp B Streptococci (19)</strong></td>
<td></td>
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<tr>
<td>R</td>
<td>NT</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>00</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>19 (100)</td>
<td>--</td>
<td>--</td>
<td>19 (100)</td>
<td>--</td>
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</tr>
<tr>
<td><strong>Staphylococcus aureus (11)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>R</td>
<td>11 (100)</td>
<td>07 (&gt;50.0)</td>
<td>06 (33.3)</td>
<td>02 (33.3)</td>
<td>04 (66.7)</td>
<td>06 (54.5)</td>
<td>03 (27.3)</td>
<td>05 (45.5)</td>
<td>06 (54.5)</td>
<td>06 (54.5)</td>
</tr>
<tr>
<td>S</td>
<td>04 (36.4)</td>
<td>05 (45.5)</td>
<td>08 (72.7)</td>
<td>05 (45.5)</td>
<td>03 (27.3)</td>
<td>05 (45.5)</td>
<td>08 (72.7)</td>
<td>06 (54.5)</td>
<td>05 (45.5)</td>
<td>05 (45.5)</td>
</tr>
</tbody>
</table>
Table 4.3: Antimicrobial susceptibility pattern of *N. meningitidis* isolated from pyogenic meningitis

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Penicillin</th>
<th>Tetracycline</th>
<th>Ampicillin</th>
<th>Gentamicin</th>
<th>Amoxicillin</th>
<th>Amikacin</th>
<th>Chloramphenicol</th>
<th>Cefotaxime</th>
<th>Ceftazidime</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. meningitidis (13)</td>
<td>R</td>
<td>13 (100)</td>
<td>-</td>
<td>--</td>
<td>13 (100)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
<td></td>
<td>S</td>
<td>--</td>
<td>13 (100)</td>
<td>13 (100)</td>
<td>--</td>
<td>13 (100)</td>
<td>13 (100)</td>
<td>13 (100)</td>
<td>13 (100)</td>
<td>13 (100)</td>
</tr>
</tbody>
</table>
Extended spectrum beta lactamase production in pyogenic meningitis

Out of 51 *H. influenzae* isolated 24 (41.2%) were found to produce ESBL, similarly out of 08 *Klebsiella pneumoniae*, 06 (80.0%) were found to produce ESβL and out of 05 *E. coli* 03(60.0%) were found to produce ESβL and out of 03 *Pseudomonas aeruginosa* 02(66.7%) were found to produce ESβL (Table-4.4).

**Amp C production in pyogenic meningitis**

Out of 51 *H. influenzae* isolated 14 (27.5%) were found to produce AmpC, similarly out of 08 *Klebsiella pneumoniae*, 03 (37.5%) were found to produce ESβL and out of 05 *E. coli* 2 (20.0%) were found to produce ESβL and out of 03 *Pseudomonas aeruginosa* 01 (33.3%) were found to produce ESβL (Fig. 4.1).

**Metallo beta lactamase production in pyogenic meningitis**

Out of 51 *H. influenzae* isolated 09 (17.54%) were found to produce MBL, similarly out of 08 *Klebsiella pneumoniae*, 02 (25%) were found to produce MBL and out of 03 *Pseudomonas aeruginosa* 01 (33.3%) and out of 05 *E. coli* 02 (20%) were found to produce MBL (Fig. 4.2).

**Methicillin resistant *Staphylococcus aureus***

Out of 11 *Staphylococcus aureus* isolated, 4 (36.4%) were resistant to methicillin (Fig. 4.3).
Table - 4.4: Distribution of ESβL producing strains among different organisms isolated

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. of organisms isolated</th>
<th>ESβL producing isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. influenzae</em></td>
<td>51</td>
<td>24 (41.2%)</td>
</tr>
<tr>
<td><em>Klebsiella</em> sps</td>
<td>08</td>
<td>06 (80.0%)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>05</td>
<td>03 (60.0%)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sps</td>
<td>03</td>
<td>02 (66.7%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>67</strong></td>
<td><strong>35 (52.2%)</strong></td>
</tr>
</tbody>
</table>
Fig. 4.1: Incidence of Amp C production among Gram negative bacilli

Fig. 4.2: Percentage of metallo beta lactamase among Gram negative bacilli
Fig 4.3. incidence of Methicillin resistant Staphylococcus aureus

Number of isolates

- Number of Staphylococcus aureus
- Methicillin resistant Staphylococcus aureus

Organisms
4.4. DISCUSSION

Bacterial meningitis is a fatal infectious disease in majority of the cases, caused by a variety of microorganisms. Even among survivors, morbidity rates in terms of neurological sequel are fairly high. Chemotherapy for bacterial meningitis has steadily progressed through successive eras beginning with sulphonamide followed by penicillin’s, Beta-lactams, amino glycosides and quinolones. The new potent agents offered a wide choice for therapy with their broad spectrum and effectiveness against multi-resistant organisms. However, clinicians by and large, would agree that despite the developments in the management, the improvement in the outcome from bacterial meningitis is marginally significant. Some of the attributable reasons could be approaches in diagnosis, delay in the management, newer emergence of resistant bacterial strains, choice of empirical antimicrobial regimens and path physiological events that occur due to host inflammatory reactions.

These results signify the varying levels of drug resistance amongst the gram positive and the gram negative microbes and the need to control the spread of these resistant strains before they reach the alarming levels in this region. It is particularly useful for the clinicians to possess the susceptibility data on Gram positive and Gram negative bacteria rather than for particular organisms only.

This problem of antibiotic misuse may lend credence to the increasing resistance of the etiology to the common anti-meningitic drugs i.e.,
ampicillin, penicillin and chloramphenicol, in Nigeria (Akpede et al., 1999). The high resistance of the pathogens to penicillin and ampicillin and the sensitivity to Chloramphenicol was not strange in Nigeria (Akpede et al., 1999; Green et al., 1993). Chloramphenicol appears to be the only one of the three traditional drugs which can safely be used for empirical treatment of meningitis without significant risk of treatment failure. In our experience, there had been no major untoward effect of chloramphenicol use in children. Ciprofloxacin achieves good CSF penetration in meningitis (Green et al., 1993) and this had been shown to translate to less mortality among meningitic children (Kiwanuka et al., 2001). On the other hand, the major problem with ceftriaxone and ciprofloxacin is the high cost which technically reduces their usefulness in the developing world where most patients are poor.

Ampicillin resistance was found in 82.3% of H. influenzae isolates. Resistance to chloramphenicol was noted in 33.3% of isolates. Resistance to chloramphenicol was associated with resistance to ampicillin in 14.3% of strains. These findings are similar to prevalences among isolates from different types of specimen in nine European countries (1988-89) and South Africa (1991-92) (Hussey et al., 1994). In the USA ampicillin resistance in H. influenzae occurred twice as often, yet chloramphenicol resistance was rare (Kayser et al., 1990). A significantly higher prevalence of ampicillin resistance was found among our type b as compared with non-type b strains. This was
also seen in the USA, but not in previous studies from Europe and South Africa (Kayser et al., 1990; Hussey et al., 1994). One study mentioned highest rates of ampicillin resistance among children aged less than 4 years, comparable to our findings. As the outcome of patients with *H. influenzae* meningitis caused by strains which were resistant to initial therapy consisting of ampicillin and chloramphenicol appeared to be worse in one study, this regimen cannot be recommended now for *H. influenzae* meningitis (Green et al., 1993).

Analysis of our *N. meningitidis* isolates revealed an overall prevalence of intermediate penicillin resistance of 30.7%, comparable to prevalences found in the UK (1980s), USA (1991) and Canada (1991-1992), but far lower than the 20-40% prevalence in Spain (1989-1990) (Jones and Sutcliffe, 1990; Saez-Nieto et al., 1992; Ringuette et al., 1995). Among our intermediately resistant strains, serogroup B:4:P1.15 was relatively often present, as found in Spain (Saez-Nieto et al., 1992). Penicillin is still regarded as first choice for the treatment of meningococcal meningitis with intermediate resistant strains (Jones and Sutcliffe, 1990). *N. meningitidis* was sensitive to chloramphenicol, amikacin, but strains resistant to tetracycline and amoxicillin have been reported.

Resistance to penicillin or chloramphenicol in *S. pneumoniae* CSF isolates was very rare. With the recently suggested change in susceptibility categories for pneumococci, 6 (6.7%) strains would be classified as
intermediately resistant and 21 (23.6%) as resistant (Scheel et al., 1995). The rare occurrence of resistance cannot be explained by a different sero-
distribution compared with other regions, as the four serogroups known for high rates of resistance (6, 14, 19, 23) represent 40% of CSF isolates. A more likely explanation is that in India antibiotics are available without prescription, and guidelines recommending restricted use of antibiotics in various bacterial infections are not widely used.

In fact, the prevalence of resistance found in our pneumococcal CSF isolates is among the lowest in the world. In other countries, the prevalence of penicillin resistance in CSF and blood isolates were 4.3% (Belgium, 1986-1993) and 1.8% (Germany, 1992-94) as compared with 0.5% in our isolates. Chloramphenicol resistance was found in 2.7% and 1.9% of isolates from Belgium and Germany, respectively, as compared with 0.3% of isolates in this study (Verhaegen et al., 1995). The prevalence of penicillin resistance in CSF isolates was 40% and 25% in Spain (1979-89) and France (1993), respectively; 25% of isolates were chloramphenicol resistant (Linares et al., 1992; Olivier et al., 1994). In a recent population-based surveillance of invasive pneumococcal infections in the USA 25% of isolates were resistant to penicillin and 3% to chloramphenicol (Hofmann et al., 1995). The findings in the present study do not justify a change in initial therapy of presumptive pneumococcal meningitis.
Present work showed relatively high incidence of MRSA infection in meningitis. The prevalence rate of MRSA was found to be 36.4%, which was slightly higher, compared to other reports where it ranged between 20 to 32.8% (Udaya et al., 1997). Methicillin resistance in *Staphylococci* is due to the production of an additional non-native penicillin-binding protein PBP2a). PBP2a is coded by the MecA gene and has low affinity for beta-lactam antibiotics. The epidemiology of MRSA is gradually changing since its emergence. Initially, there were occasional reports, but now it has become one of the established pathogens. Moreover, association of multidrug resistance with MRSA has added to the prevailing problem. β-lactam antibiotics like penicillin and cephalexin were not found to be effective against MRSA. Penicillin was 100% resistance. Over 72.7% of MRSA strains were resistant to amikacin however, 72.7% of the strains were susceptible to cefotaxime. To date, there have been no isolates of MRSA with documented *in-vitro* resistance to Vancomycin in India. Even in our study, none of the MRSA isolates was resistant to vancomycin. Hence, it may be used as the drug of choice for treating multidrug resistant MRSA strains isolated from pyogenic meningitis.

The high rate of ESBLs among hospitalized patients is a global problem. It is generally thought that patients infected by an ESBL-producing organism are at an increased risk of treatment failure with an expanded-spectrum beta-lactam. ESβL producing *K. pneumoniae* have emerged as one of
the major multidrug resistant organisms. ESβL mediated resistance to 3GC was found in 52.2% in the pyogenic meningitis. This prevalence rate is higher than other reports from India and abroad. Since the isolates were obtained from infection in different age group they might be wide disparity in the prevalence rate of ESβL producing Gram-negative bacteria when compared to other reports. During past decade, ESβL producing *K. pneumoniae* have emerged as one of the major multi drug organism (Agarwal *et al.*, 1997; Jerestin *et al.*, 1997). The incidence of ESβL producing *Klebsiella* isolated in the United States has been reported to be 5% in France and England 14-16% ESβL producers among clinical, *Klebsiella* isolates has been reported. In a previous study in central India, 76.5% of *Klebsiella* isolates resistant to 36°C antibiotics was found to produce ESβL by DDST. In our study, 80.0% of *Klebsiella pneumoniae* were ESBL producers.

The incidence of ESβL producing strains among clinical *Klebsiella* isolates has been steadily increasing over the past years and accounts for 6-17% of all nosocomial urinary tract infection. In the present study *Klebsiella* accounted for 80.0% ESβL producer. According to Vinodkumar *et al.*, 2004 the total 96 strains isolated from blood specimen were found to be resistant to a minimum of 3 antibiotics, hence these were considered multidrug resistant. 93.8% of isolates showed resistance or decreased susceptibility to at least one of the 3GC and 68.8% to all the 36°C.
We found that the ESβL producing isolates were conferred with resistance or decreased susceptibility to various third generation cephalosporins. The DDST detected ESβL in 52.2% of the isolates. The specificity of DDST is well documented. In view of its simplicity, it may be undertaken in a routine diagnostic laboratory for detecting ESβL producing strains with due consideration to factors like precise placement of the discs, correct storage of the clavulanate containing discs and performance of appropriate control tests, which are critical to the sensitivity of the DDST. The other Gram negative bacteria producing ESBL are *E. coli*, *Pseudomonas aeruginosa* and *H. influenzae*. Study conducted by Enting at Netherlands, showed 30% of *H. influenzae* produced beta lactamases, but in our study the incidence of beta lactamases was 41.2%.

Study carried out by Vishwanath *et al.*, the influenza isolates were sensitive to ciprofloxacin, 66% of the isolates were sensitive to ampicillin and cefotaxime and 50% of the isolates were sensitive to cephalexin, gentamicin and co-trimoxazole. 83% of *Streptococci pneumoniae* were resistant to gentamicin and 67% to ciprofloxacin. All the isolates were resistance to cephalexin, ampicillin and co-trimoxazole and all the isolates were sensitive to amikacin and vancomycin.

AmpC β-lactamases are clinically important cephalosporinases encoded on chromosomes of many of the *Enterobacteriaceae* and a few other organisms, where they mediate resistance to cephalothin, cefazolin, cefoxitin,
most of the penicillins and β-lactamase inhibitor. In many bacteria Amp C enzymes are inducible and can be expressed at high levels by mutation. Over expression confers resistance to broad spectrum cephalosporins. In the present study 17.9% were AmpC producers and *H. influenzae* was the predominant Amp C producer followed by *Klebsiella pneumonia*. There are no reports to compare the incidence of Amp C mediated resistance among pyogenic meningitis infection in India and abroad.

Information on the prevalence of Amp C β-lactamase producing strains in India is very limited, and no data’s are available on the prevalence of Amp C production in pediatric group. In a recent study showed that 20.7% of the clinical isolates were found to harbor Amp C enzymes in contrast to reported values of 1.2 and 4.2% (Philippon et al., 1989) by other workers in different clinical conditions. In the present study 12 (17.9%) isolates were resistant to cefoxitin were positive by 3-dimensional test, negative for inducible β-lactamases by disc diffusion test and sensitive to imipenem. Due to above phenotypic characters these isolates were considered to be harboring plasmid encoded Amp C β-lactamases. It has been stated that detection of AmpC enzymes is quiet challenging since; hyper-production of chromosomal Amp C with OMPF porin loss in *E. coli* or porin deficiency in *K. pneumoniae* can produce similar resistant phenotype. Further not all strains with AmpC enzymes meet NCCLS criteria for resistance to cephemycins and oxyimino-cephalosporins (Philippon et al.,
Hence a reference laboratory for β-lactamase iso-electric focusing or gene localization is needed. This will help us to know the actual prevalence of these enzymes and characterize them.

Metallo β-lactamase (MBL) is a group of carbapenem hydrolysing β-lactamase. They have been reported from many countries, as well as from different parts of Indian subcontinent, particularly in multidrug resistance pathogens like *Pseudomonas aeruginosa* and *Acinetobacter* species. The MBLs are inhibited *in-vitro* by CuCl$_3$, FeCl$_3$, EDTA and thiol compounds like 2 mercaptopropionic acid, sodium mercaptoacetoic acid and 2 mercaptoethanal, but not by β-lactamase inhibitors like clavulanic acid, sulbactum or tazobactam. Detection of MBL production in MDR organisms from burn infection has tremendous therapeutic consequences, as the treatment option for such isolates are aztreonam or potentially toxic polymyxin B and colistin. In the present study not all gram negative bacteria were tested for MBL production. Only those gram negative bacilli resistant to imipenem were screened for MBL production and 29.5% of them were metallo β-lactamase producers. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, were the predominant MBL producers.

Thus, the study clearly highlights the rising level of drug resistance amongst the meningitis pathogens and hence the need to update and formulate newer drug policies. Good infection control practices, rational antibiotic policies, judicious use of interventions and implementation of
standard of isolation precautions are of vital importance today. Unless there are strategies to optimize effective use of antibiotics, very few options will be left in future in the antibiotic armamentarium and it might herald an era of medical disaster with strains virtually untreatable with current spectrum of antimicrobials.

Stress should be given on the restrained and rationale use of antimicrobials both in and outside the hospital. This study also indicates the urgent need for more of such studies in the patients of meningitis vis-a-vis aetiology and drug resistance along with the need for the in-house review of drug policy within hospitals at least once in every five years. There is also an urgent need to develop institutional programs to enhance antimicrobial stewardship thus minimizing the emergence and spread of antimicrobial resistance.