ß-lactam Antibiotics Resistance Pattern and its Development in Indigenous Clinical Isolates

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MICROBIOLOGY

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IN THE NAME OF ALLAH
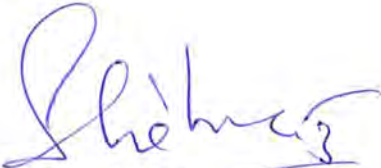
THE MOST MERCIFUL AND

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DEDICATED

TO

MY FAMILY

DECLARATION

The material contained within this thesis is my original work and has

not been previously submitted to this or any other university.

Dr Shamim Mumtaz

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LIST OF ABBREVIATIONS AND SYMBOLS

ATCC	American Type Culture Collection
API	Analytical profile index
ß	Beta
CAZ	Ceftazidime
CDC	Centers for Disease Control and prevention
CLED	Cystine-Lactose-Electrolyte Deficient
CoNS	Coagulase -Negative Staphylococci
CTX	Cefotaxime
°C	Degree Celsius
DNA	Deoxyribonucleic acid
E. aerogenes	*Enterobacter aerogenes*
E. coli	*Escherichia coli*
EGNR s	Enteric Gram negative rods
ESBL	Extended spectrum ß-lactamase
GNB	Gram–negative bacilli
EGNR	Enteric Gram negative rods
ICU	Intensive Care Unit
K.. pneumoniae	*Klebsiella pneumoniae*
MRSA	Methicillin Resistant *Staphylococcus aureus*
MRVP	Methyl Red Voges Proskauer
MIC	Minimum Inhibitory Concentration

MBC	Minimum Bactericidal Concentration
NCCLS	National Committee for Clinical Laboratory Standards
NIH	National Institute of Health
Omp	Outer membrane protein
OPD	Out Patient Department
OXA	oxacillin
P. aeruginosa	*Pseudomonas aeruginosa*
PBPs	Penicillin-binding proteins
p-CMB	p-chloromercuribenzoate
PCR	Polymerase Chain Reaction
%	Percentage
±	Plus-minus
P	Probability
P. mirabilis	*Proteus mirabilis*
:	Ratio
spp.	Species
S. aureus	*Staphylococcus aureus*
SHV	Sulph-hydral variant
TEM	Temoniera
URTI	Upper respiratory tract infections
UTI	Urinary Tract Infection
WHO	World Health Organization
x	Multiplication
Zn^{2+}	Zinc ion

ABSTRACT

The emergence of resistance to antimicrobial agents is a global public health problem. Although a number of factors can be identified which contribute to this problem, ß-lactamases of Gram-negative bacteria are the most important mechanism of resistance against ß-lactam drugs .

This study was carried out to evaluate the prevalence of the infection and the development of resistance in clinically significant bacteria against commonly used antibiotics with special reference to ß-lactam agents. In addition, the study was also aimed to determine the frequency of Extended-Spectrum ß-Lactamase (ESBL) production among Enteric Gram-negative rods (EGNRs) and their sensitivity pattern as well.

A total of 9712 samples, received in the Clinical Microbiology Laboratory of Fauji Foundation Hospital, Rawalpindi during April 2004 to March 2006, both from in-patients and out-patients, were processed and subjected to culture and sensitivity, followed by ESBL detection by Double Disk Diffusion Synergy Test .

The incidence of bacterial infection was 43.3%. The Gram- negative rods (GNRs) were most prevalent (57.5%) followed by Gram-positive cocci (40.1%). *Staphylococcus aureus* (32.6%) was most prevalent organism, followed by *Escherichia coli* (24.7%) and *Pseudomonas aeruginosa* (15.9%).

The frequency of ESBL in Gram-negative rods was 38.9%. The most common ESBL-producing EGNR was *E. coli* (47.5%) followed by *K. pneumoniae* (45.0%). Highest resistance of ESBL-producing *E. coli* was noted against 3rd generation cephalosporins {ceftazidime (97.1%), cefotaxime (97.2%)}, followed by aztreonam (96.1%), co-

trimoxazole (89.9%), piperacillin/tazobactam (21.1%) and imipenem (1.9%). Similar pattern of resistance was noted for ESBL-producing *K. pneumoniae* ceftazidime (96.8%), cefotaxime (98.1%), followed by aztreonam (92.2%), ciprofloxacin (89.6%), gentamicin (89.1%), piperacillin/tazobactam (9.1%) and imipenem (3.1%).

The sensitivity of ESBLs-producing *K. pneumoniae and E. coli* was reduced not only towards 3rd generation cephalosporins but cross-resistance was noted against other antibiotics as well like co-trimoxazole, doxycycline, co-amoxiclav, norfloxacin and gentamicin. Carbapenems, ß-lactam/ ß-lactamase inhibitors and fosfomycin were found most effective against both ESBL-producing and ESBL non-producing Gram-negative rods.

In conclusion, considerable resistance was demonstrated amongst the isolated organisms against all the commonly used antibiotics including ß-lactams. So it is important to avoid the misuse of antibiotics, as well as to screen for ESBLs routinely by all the laboratories. If an isolate is found to be an ESBL-producer, it should be considered resistant to all β-lactam drugs including third generation cephaolosporins and aztreonam. Administration of these antibiotics as an empirical therapy could be disastrous in these cases because these would not only be ineffective thus causing increased mortality but would also promote the ESBL-production. The best empirical therapy for these cases would be carbapenems, ß-lactam ß-lactamase inhibitor combinations and fosfomycin. On the other hand, regarding ESBL non-producer, most of the conventional cheap antibiotics would be effective to combat the infection.

CHAPTER 1

INTRODUCTION

INTRODUCTION

The human life has always been in danger from diseases caused by microorganisms. The history still mourns the death toll of epidemics of influenza, plague and malaria which occurred during the 20th century. Nosocomial or hospital acquired infections are major cause of morbidity and mortality among hospitalized patients. The mortality of bacteremia remains approximately 20-40% despite the availability of effective antimicrobials.

Staphylococcus aureus being the most versatile human pathogen in both hospital and community acquired infections is one of the major causative agent in bacterial infections, because of its impressive capacity to colonize and persistence in a range of diverse environments. (Baldwin *et al*, 1990; Aftab and Iqbal, 2006; Butt *et al*, 2004, Sader *et al*, 2002; Asrat and Amanuel, 2001, Mahmood, 2001; Sader *et al*, 1999). *S. aureus* especially Methicillin–resistant (MRSA) frequently causes disease outbreaks and has become endemic in many regions, adding to the morbidity, mortality and cost of care associated with hospital- acquired infections.

Gram–negative bacilli (GNB) are a common cause of sepsis, pneumonia, urinary tract infections and post surgical infections in patients in acute care hospitals (Liverelli *et al*, 1996; Prescott *et al*, 1999; Yan *et al*, 2001; Beck-sague *et al* , 1995).

There has been a major shift in the etiology of hospital-acquired infections during 1980s, leading to an increase in the laboratory isolation of *Coagulase-Negative Staphylococci* , *Candida, S. aureus, Enterococci, Pseudomonas aeruginosa (P. aeruginosa)* and *Enterobacter spp* (Banerjee *et al*, 1991; Schaberg *et al*, 1991). *S. aureus*, coagulase-negative *Staphylococci* and *E. coli* have been considered as the major isolates (Decousser *et al*, 2003). Etiologic shifts in nosocomial infections and an upsurge of antimicrobial resistance among these pathogens, are impressive and alarming. (Hsueh *et al*, 2002).

Taken as a whole, the shifts are away from more easily treated towards more resistant pathogens with fewer options left for therapy (Schaberg *et al*, 1991).

The search for antibiotics began in the late 19th century, with the growing acceptance of the germ theory of disease, which linked bacteria and other microorganisms to the causation of a variety of ailments. As a result, scientists began to devote time to searching for drugs that would kill these pathogens. The goal of such research was to find "magic bullets" that would destroy microbes without harming the person taking the drug (Alcamo, 1994).

Antimicrobial agents are among the most dramatic advances of modern medicine. Many infectious diseases once considered incurable and lethal are now amenable to treatment. During the last 50 years, mankind has observed a tremendous decrease in mortality and morbidity especially from bacterial diseases because of these antimicrobial agents.

 A single injection of penicillin could eradicate a life threatening infection. Unfortunately, due to malpractices or natural causes, most of them have lost their efficacy (Norby, 1990). As a result expensive and complicated antibiotics has been introduced and marketed to combat simple infections (Irvani, 1992; Mumtaz *et al*, 2002).

The discovery of the ß-lactam antibiotics was one of the major achievements of medical science in 20th century and they proved to be the most useful chemotherapeutic agents . But their efficiency is continuously being challenged by the emergence of resistant bacterial strains (Gold & Moellering, 1996).

The ß-lactam group of antibiotics includes an enormous diversity of natural and semi-synthetic compounds that inhibit several enzymes associated with the final step of synthesis in the bacterial cell wall. Clinically useful families of this group include penicillins, cephalosporins, monobactams and carbapenems (Chambers, 2004). Some of

these have limited use as therapeutic agents but may be used in combination with other ß-lactams to act as ß-lactamase inhibitors (Jones *et al*, 1985).

Antimicrobial resistance was first realized in 1940's with the discovery of penicillinase in *E. coli.* (Tenover & Hughes, 1996). Thus even before the wide spread use of antibiotics, the resistance mechanism had already been detected in the bacteria (Fred & James, 1996). Cephalosporins were considered as alternatives for those bacterial infections, non-responsive to standard treatments, but now most of the gram-negative rods have gained resistance against them (Aftab and Iqbal, 2006; Butt *et al*, 2004; Iqbal *et al*, 2002; Zafar 1999).

In the late 1950s, it was revealed that the resistant strains of *Shigella* species in the mixed cultures were capable of transferring their resistance pattern to previously sensitive strains of *E. coli* (Watanabe, 1963). This type of resistance towards ampicillin, trimethoprim-sulphamethoxazole & chloramphenical has been reported in an outbreak in Mexico in 1972 involving more than 10,000 cases (Olarte *et al*, 1973). This resistance is plasmid mediated and can be transferred more rapidly to other bacteria (c Akhtar *et al*, 1997).

Acquired bacterial resistance is common among clinical isolates from both hospital and community acquired infections in developing countries (Kunin, 1993). It is particularly increasing, among the diarrhoeal, respiratory and commensal enteric pathogens, towards first-line, inexpensive, broad-spectrum antibiotics (Rahal *et al*, 1997).

Many clinically important bacteria produce enzymes that are capable of chemically modifying or destroying antibiotics. These include ß-lactamases, aminoglycoside modifying enzymes, chloramphenicol acetyl transferase, erythromycin estrases (Quintiani & Courvalin, 1995).

ß-lactamases destroy ß-lactam ring of antibiotics, which become so changed in their chemical structure, that they are no longer recognized by the enzymes responsible for making the peptidoglycan layer of the bacterial cell wall. (Frere, 1995; Shah *et al*, 2004). Some of these have a preferential activity against penicillins , while others are active against cephalosporins. Whereas broad-spectrum ß-lactamases have activity against both penicillins and cephalosporins. β-lactams are the most widely used antibiotics & β-lactamases are the greatest source of resistance to them. β-Lactamase- producing bacteria are increasing in number and cause more severe infections because of their mutation. Extended mutation has led to the emergence of Extended-spectrum ß-lactamase enzymes, the incidence & types of which vary with geographical location & time (Shah *et al,* 2004). Elaboration of structurally & mechanically novel ß-lactamase enzymes by Gram-negative pathogens is the most important means by which resistance occurs (Shah *et al,* 2004).

Two types of ß-lactamases can confer resistance against 3rd generation cephalosporins. Chromosomally mediated ß-lactamases which are present in Gram-positive bacteria, Gram-negative bacteria, Mycobacteria & Nocardia (Neu, 1984; Shah *et al*, 2004). They are either inducible or constitutive and are not inhibited by clavulanic acid. Resistance due to these enzymes is non-transferable (Livermore, 1995 ; Shah *et al* 2004).They are almost ubiquitous in Enterobacteria, except for *Salmonellae* but vary greatly in amount, mode of production and consequently in their contribution to resistance (Sykes & Methew, 1976).

The second type of enzyme is plasmid-mediated ß-lactamases, which are more common in *Staphylococci, Enterobacter, Haemophilus influenzae,* and *Niesseria gonorrhoeae.* T hese confer resistance to broad-spectrum ß-lactam antibiotics. Aminoglycoside and trimethoprim sulphamethoxazole resistance are co-transferred on the same plasmid (Patterson, 2000; Shah *et al,* 2004). Over 75 different plasmid mediated ß- lactamases have been recorded in Gram-negative bacilli (Bush *et al,* 1995) . The most common among Enterobacteriaceae is TEM-1(Temoniera), others include TEM-2, SHV-1(sulph-hydral variant), and OXA -1 (Sanders

and Sanders, 1992).

Secondary ß-lactamases have been reported widely in *P. aeruginosa* but are much rare than in Enterobacteriacae. Incidence of 13% from France (Tirado *et al*, 1986), 7% from Spain 0.7% and 2.5% from England has been reported (Livermore 1995; William *et al*, 1984).

The extended-spectrum ß-lactam agents (extended-spectrum penicillins, cephalosporins and monobactams) were first introduced into the general clinical practice in late 1970s. Initially, they were fully active against Enterobacteriaceae but in the mid 1980s, due to their intensive use in hospital, resistance emerged against these antibiotics (Sanders and Sanders, 1983, Pfaller and Segreti, 2006). This resistance was transferable and clinically much more significant and appeared due to the mutant forms of ß- lactamases such as TEM-1, TEM-2, and SHV-1. These mutant forms were known as Extended-spectrum ß-lactamases (ESBLs).

These strains are resistant to a wide variety of commonly used antimicrobials such as ß-lactam antibiotics including extended-spectrum penicillins, cephalosporins and monobactams (Pfaller and Segreti, 2006). While carbapenems, cephamycins and temocillins are stable against these ESBLs-producers and have been successfully used against these ESBLs-producers (Jacoby & Carreras, 1990; Pangon *et al*, 1994; Ahmed & Salam, 2002 ; Iqbal *et al*, 2002).

ESBLs are encoded by genes located on very large plasmids, which often carry genes for resistance to other classes of antimicrobial agents as well (like aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenical (Thomson *et al*, 1996).

ESBL-producing organisms pose a major problem for the clinical therapeutics. ESBLs-producing Enterobacteriaceae have been responsible for numerous outbreaks of infection throughout the world and pose challenging infection control issues. Nursing home patients may be an important reservoir. Use of broad-spectrum oral antibiotics and probably poor infection control practices may facilitate spread of this plasmid-mediated resistance. In

addition to known populations at risk, ambulatory patients with chronic conditions represent another patient population that may harbor these pathogens .

ESBL-producing strains of Enterobacteriaceae have emerged as a major problem in hospitalized as well as community based patients (Ananthkrishnan *et al*, 2000;Chaudhury, 2004; Rodriguez-Bano *et al*, 2004; Bhattacharya, 2006). Major outbreaks have been reported from all over the world, thus making them emerging pathogens (Ananthkrishnan *et al*, 2000). These are responsible for a variety of infections like urinary tract infection (UTI), septicemia, hospital acquired pneumonia, intra-abdominal abscess, brain abscess and device related infections (Bhattacharya, 2006).

The incidence of ESBL-producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitation of therapeutic options (Ananthkrishnan *et al*, 2000). Prevalence of these strains in various species of *Enterobacteriaceae* differs in different countries & in different hospitals. Usually one of the three species (*K. pneumoniae, E. coli, Enterobacter*) predominates (Luzzaro *et al*, 2006; Shah *et al*, 2002;Sorlozano et *al*, 2006; Chow et *al*, 2005; Shah *et al*, 2004).

ESBL-producing strains can survive in the hospital environment and can be transmitted from patient to patient, through the hands of hospital staff and are usually found in those areas of hospitals, where antibiotic use is heavy and patient's condition is critical (Thomson *et al*, 1996;Coulter *et al*, 1995; Hobson *et al*. 1996).

ESBL-producing organisms may appear susceptible to some extended-spectrum cephalosporins, however, treatment with such antibiotics has been associated with high failure rates (Paterson and Bonomo, 2005; Grover *et al*, 2006). There are very limited drugs to choose from for treating patients with ESBL-infection, the antibiotics like cefotaxime, ceftazidime, ceftriaxone, aztreonam, tiarcillin, mezlocillin, piperacillin have poor or have lost their activity (Singh, 1999). Therefore, antibiotic options in the treatment of ESBL-producing organisms are becoming extremely limited (Paterson

and Bonomo, 2005; Nathisuwan *et al*, 2001).

With the spread of these ESBL-positive strains in the hospitals all over the world, it is necessary to know the prevalence of these strains , so as to formulate a policy of empirical therapy in high risk units where infections due to these resistant organisms is high. The routine susceptibility tests fail to detect ESBL-positive strains and can erroneously detect the isolates sensitive to the broad-spectrum cephalosporin (Mathur *et al*, 2002), leading to the misuse of extended-spectrum cephalosporins, which remain an important component of antimicrobial therapy in high-risk wards (Mathur *et al*, 2002).

The other factors involved in the development of resistance include (a) transfer of resistance genes among bacteria that transform susceptible strains to resistant ones (b) dosage and types of antibiotics that cause the selection pressure to certain species of bacteria and (c) level of organization and strict adherence to hygiene and anti-epidemic regimen starting with the entry of patients into hospital. Prevention and control measures are also important because of the multiresistant nature of these pathogens (Blahova *et al*, 2001).

In Pakistan, unhygienic conditions and injudicious use of antibiotics in hospitals and their easy availability without prescription at drug stores have lead to the enhanced rate of resistance (Haneef and Khan, 1990; Sturm *et al*, 1997). Lack of education and absence of regulatory laws compound the situation. Medical practitioners are actively encouraged by pharmaceutical industry to over prescribe and also to prescribe expensive medications. Government agencies are being persuaded not only to register drugs at the rate of 500 a year, but also put in to use obsolete drugs or those of questionable value (Akhtar, 1999).Self-medication initiated by over-the-counter availability of drugs is unlikely to be a major factor. Control of use and misuse of antimicrobial agents is a complicated issue, especially in developing countries (Kunin, 1993 ; Khan and Bangash, 2003). Education of the medical profession regarding the use of antimicrobial agents seems to be the single most important tool in avoiding further development of resistance through misuse (Samper and Sturm, 1988). This has not been successful to date and innovative approaches to achieve this goal are urgently needed.

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Mode of action of antimicrobial agents

Antimicrobial agents are among the most dramatic examples of the advances of modern medicine. Many infectious diseases once considered incurable and lethal are now amenable to treatment with few pills. The remarkably powerful and specific activity of antimicrobial drugs is due to their selectivity of targets that are either unique to microorganisms or much more important in them than in human beings. Among these targets are bacterial cell wall synthesizing enzymes, the bacterial ribosomes, the enzymes required for nucleotide synthesis and DNA replication (Chambers, 2004).

The antimicrobials exert their action via one of the several pathways:

1. Inhibition of bacterial cell wall synthesis

2. Inhibition of the nucleic acid synthesis

3. Inhibition of the protein synthesis

4. Alteration of cell membrane function

5. Other mechanisms of action

INHIBITORS OF CELL WALL SYNTHESIS

ß-lactam antibiotics

Integrity of the cell wall is a pre-requisite for the bacterial survival. The major component of the bacterial cell wall, the peptidoglycan chains, is cross-linked between short peptide side chains by an amide linkage. ß-lactam antibiotics inactivate enzymes located in bacterial cell wall, thereby preventing cross linkage & hampering the osmotic stability.

This group of antibiotics includes an enormous diversity of natural and semi-synthetic compounds , derived from a ß-lactam structure: a four-membered ring in which the ß-lactam bond resembles a peptide bond. The multitude of chemical modifications based on this four-membered ring permits the astonishing array of antibacterial and pharmacological properties within this valuable family of antibiotics. Clinically useful families of ß-lactam compounds include the penicillins, cephalosporins, monobactams and carbapenems (Chambers, 2004).

Penicillins

Undoubtedly, one of the greatest accomplishments of modern medicine has been the development of antimicrobials for the treatment of infectious diseases. The first antibiotic, penicillin was discovered by Alexander Fleming in 1928 (Sritharan and Sritharan, 2004). The penicillins share features of chemistry, mechanism of action, pharmacologic & clinical effects and immunologic characteristics with cephalosporins, monobactams, carbapenems and ß-lactamase inhibitors (Chambers, 2004).

Chemistry

All penicillins have the same basic structure, 6-aminopenicillanic acid nucleus, which is composed of a thiazolidine ring, attached to a ß-lactam ring that carries a secondary amino-

group. Substituents can be attached to this amino-group. Structural integrity of the 6-aminopenicillanic acid is essential for the biologic activity of these compounds. If the ß-lactam ring is enzymatically cleaved by bacterial ß-lactamases, the resulting product, penicilloic acid, lacks antibacterial activity (Chambers, 2004; Levinson, 2004).

Classification

The clinically important penicillins fall into following principal groups:

1. Penicillin-G

These have the greatest activity against Gram-positive organisms, Gram-negative cocci, and non-ß-lactamase-producing anaerobes. However, they have little activity against Gram-negative rods. They are susceptible to hydrolysis by ß-lactamases and are acid-labile (Chambers, 2004).

2. Anti-Staphylococcal penicillins

These include methicillin, naficillin and isoxazolyl penicillins, which are resistant to Staphylococcal ß-lactamases. They are active against *Staphylococci* and *Streptococci* but inactive against *Enterococci,* anaerobic bacteria, Gram-negative cocci and rods (Chambers, 2004).

3. Extended-spectrum penicillins

These include aminopenicillins and antipseudomonal penicillins. These drugs retain the antibacterial spectrum of penicillin G, differ in having improved activity against Gram-negative bacteria due to their enhanced ability to penetrate the Gram-negative outer membrane. Like penicillin G, they are inactivated by ß-lactamases (Khan and Bangash, 2003; Chambers, 2004). Extended-spectrum penicillins are active in vitro against most Gram-positive and Gram-negative aerobic cocci, some Gram-positive aerobic bacilli and many Gram-negative aerobic or anaerobic bacilli. Because of the propensity of *P.*

aeruginosa to develop resistance during single drug therapy, antipseudomonal penicillin generally is used in combination with an aminoglycoside for pseudomonal infections (Khan and Bangash, 2003).

Mechanism of Action

Penicillins, like all ß-lactam antibiotics, inhibit bacterial growth by interfering with a specific step in bacterial cell wall synthesis. The targets for ß-lactam drugs are the penicillin-binding proteins (PBP's) in the cytoplasmic membrane. These target proteins catalyze the synthesis of peptidoglycans in the cell wall, providing structural stability to the bacterial cell (Waxmann and Strominger, 1983; Levinson, 2004; Livermore, 1991).

After a ß-lactam antibiotic has attached to the PBP's, the transpeptidation reaction is inhibited, peptidoglycan synthesis is blocked leading to the cell death. Whereas at the sub lethal concentrations may lead to the alterations in cellular morphology. The exact mechanism responsible for cell death is not completely understood but autolysins are involved. Penicillins and cephalosporins are bactericidal only if cells are actively growing and synthesizing cell wall (Chambers, 2004; Levinson, 2004).

Clinical uses

Penicillin G is the drug of choice for infections caused by *Streptococci, Meningococci, Enterococci*, penicillin-susceptible *Pneumococci* and non-ß-lactamase-producing *Staphylococci* (Chambers, 2004). Anti-*Staphylococcal* penicillins are indicated for infections by ß-lactamase producing *Staphylococci*. An isoxazolyl penicillin such as oxacillin, cloxacillin or dicloxacillin is suitable for treatment of mild, localized Staphylococcal infections. For serious systemic Staphylococcal infections, oxacillin or nafcillin is indicated (Chambers, 2004).

Extended-spectrum penicillins in general retain the spectrum of activity of penicillin G, differ in having greater activity against Gram-negative bacteria. Aminopenicillins like ampicillin and amoxicillin have the same spectrum and activity as that of penicillin G. Many strains of Gram-negative species which produce ß-lactamases, are resistant to ampicillin, precluding its use for empirical therapy of urinary tract infections, meningitis and typhoid fever (Chambers, 2004). Carbenicillin, the first antipseudomonal carboxypenicillin, has become obsolete as a parenteral agent with the advent of more active and better-tolerated agents.

Cephalosporin derivatives and related compounds

Cephalosporins are ß-lactam antibiotics that are structurally and pharmacologically related to penicillins. The nucleus of the cephalosporins (7- aminocephalosporanic acid) bears a close resemblance to 6-aminopenicillanic acid. The difference in antimicrobial activity and stability to ß-lactamases, is due to the addition of various chemical entities to two positions on the cephalosporins nucleus (Karchmer, 1995).

Cephalosporins are among the most frequently prescribed antibiotics because of their broad spectrum of antimicrobial activity, favorable pharmacokinetics, low incidence of adverse reactions and proven efficacy against variety of infections (Klein and Cunha ,1995). Cephalosporins are traditionally classified into four classes or generations (Karchmer, 1995). Major differences in the three generations is increasing activity against a variety of Gram-negative species and decreasing susceptibility to ß-lactamases. (Karchmer, 1995; Khan and Bangash ,2003).

First-generation cephalosporins

This group includes cefadroxil, cefazolin, cephalexin, cephalothin, cephapirin, and cephradine. These drugs are very active against Gram-positive cocci, including

Pneumococci, Streptococci and Staphylococci. Gram-negative rods like *E. coli, K. pneumoniae,* and *P. mirabilis* are often sensitive (Chambers, 2004; Khan and Bangash 2003).

Second-Generation Cephalosporins

Members of this group include cefaclor, cefamandole, cefonicid, cefuroxime, cefprozil, loracarbef, ceforanide and the structurally related cephamycins e.g cefoxitin, cefmetazole, cefotetan, which have activity against anaerobes. In general, they are active against organisms affected by first-generation drugs but in addition, they have extended Gram-negative coverage (Khan and Bangash, 2003). Modifications of the basic cephem nucleus (true" cephalosporins), lead to the development of the newer derivatives, the cephamycins and oxacephems, which contain ß-lactam ring, a requirement for their activity. Cephamycins A, B and C are naturally produced cephalosporin-type antibiotics. Cephamycins A and B are found to be more active against Gram-positive organisms, while cephamycin C is more active against Gram-negative organisms (Miller *et al,* 1972).

Third-Generation Cephalosporins

Third-generation agents include cefoperazone, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, cefixime, cefpodoxime proxetil, cefditoren pivoxil, ceftibuten and moxalactam.The major features of these drugs are their expanded Gram-negative coverage and the ability of some to cross the blood-brain barrier (Khan and Bangash, 2003 ; Chambers, 2004). Third-generation cephalosporins are used to treat a wide variety of serious infections caused by organisms that are resistant to most other drugs. Because of their ability to penetrate central nervous system, third-generation cephalosporins can be used to treat meningitis, caused by *Pneumococci, Meningococci, H. influenzae,* and susceptible Enteric Gram-negative rods. (Chambers, 2004).

Fourth-Generation Cephalosporins

The fourth-generation agents, cefepime and cefpirome are classified as such because they have exceptional Gram-negative activity as well as having good activity against Gram-positive cocci. (Levinson, 2004). They are more resistant to hydrolysis by chromosomal ß-lactamases and some extended-spectrum ß-lactamases, that inactivate many of the third-generation cephalosporins. Its clinical role is similar to that of third-generation cephalosporins & their present use is in serious nosocomial infections (Chambers, 2004).

Other ß-lactam drugs

Monobactams

These drugs possess monocyclic ß-lactam ring which are relatively resistant to ß–lactamases. Aztreonam is the only monobactam available. They resemble aminoglycosides in their spectrum of activity. They are active against Gram-negative rods (including *Pseudomonas* and *Serratia*) but possess no activity against Gram-positive bacteria or anaerobes. (Chambers, 2004; Levinson, 2004).

ß-Lactamase inhibitors

These include clavulanic acid, sulbactam and tazobactam .These compounds have limited antimicrobial activity but their major value is an inherent ability to limit the destructive action of ß -lactamases against more active ß-lactam compounds such as penicillins and cephalosporins (Williams, 1997). For example co-amoxiclav, is a combination of amoxicillin and a ß-lactamase inhibitor clavulanic acid, whereas tazobactam, is combined with piperacillin and cefoperazone is combined with sulbactam (Williams, 1997; Livermore, 1987). These combinations are indicated as an empirical therapy for infections caused by a wide range of potential pathogens in both immunocompromised

and immunocompetent patients and for the treatment of mixed aerobic and anaerobic infections, such as intra-abdominal infections (Chambers, 2004).

Carbapenems

These are structurally related to ß-lactam antibiotics which include ertapenem, imipenem and meropenem . They penetrate body tissues and fluids well, including the cerebrospinal fluid (Chambers, 2004). Imipenem has a wide spectrum with good activity against many Gram-negative rods, including *P. aeruginosa*, Gram-positive organisms and anaerobes. These are indicated for infections caused by susceptible organisms that are resistant to other available drugs and for the treatment of mixed aerobic and anaerobic infections (Chambers, 2004). Imipenem is resistant to most ß-lactamases but not metallo-ß-lactamases. It is inactivated by dehydropeptidases in renal tubules, resulting in low urinary concentrations. Consequently, it is administered together with an inhibitor of renal dehydropeptidase (cilastatin) for clinical use. Meropenem is similar to imipenem but it is not significantly degraded by renal dehydropeptidase and does not require an inhibitor (Chambers, 2004; Levinson, 2004).

Other inhibitors of cell wall synthesis

Vancomycin

Vancomycin is a glycopeptide which inhibits cell wall synthesis by blocking transpeptidation but a mechanism different from that of the ß-lactam drugs (Levinson, 2004). It is particularly useful in the treatment of serious *Staphylococcal* infections. (Chambers, 2004).

Teicoplanin

It is a glycopeptide antibiotic that is very similar to vancomycin in its mechanism of action and antibacterial spectrum. Unlike vancomycin, it can be given intramuscularly as well as intravenously.

Fosfomycin

Fosfomycin trometamol inhibits a very early stage of bacterial cell wall synthesis. It is an analog of phospho-enolpyruvate. It is active against both Gram-positive and Gram-negative organisms at concentrations $\leq$ 125-úg/mL. In vitro synergism occurs when it is combined with ß-lactam antibiotics, aminoglycosides or fluoroquinolones. There is a rapid selection of resistance to fosfomycin, rendering it unsuitable for most clinical purposes (Chambers, 2004).

BACTERIAL RESISTANCE TO ANTIBIOTICS

Microorganisms can adapt to environmental pressures in a variety of effective ways and their response to antibiotic pressure is no exception. An inevitable consequence of antimicrobial usage is the selection of resistant microorganisms. Overuse and inappropriate use of antibiotics has fueled a major increase in prevalence of multidrug - resistant pathogens, leading some to speculate that we are nearing the end of the antibiotic era (Chambers, 2004).

The mechanisms by which bacteria resist the destructive effect of antibiotics are many and vary according to both the antibiotic and microorganism involved. They can be grouped under the two broad headings:

1. Non–enzyme mediated
2. Enzyme mediated

Non-enzyme mediated resistance

This type of resistance results from the intrinsic ability of the bacterial cell to interfere with the process by which the antibiotic has its effect (Brooks *et al*, 1995; Levinson, 2004). These include:

(1)   Modification of target penicillin-binding proteins (PBPs).

(2)   Impaired penetration of the drug to the target PBPs.

(3)   Presence of an efflux pump.

1. Modification of target Penicillin Binding Proteins

Alteration of PBPs can lead to ß-lactam antibiotic resistance (Maluoin and Bryan, 1986). It is responsible for methicillin resistance in *Staphylococci* and penicillin resistance in *Pneumococci* and *Enterococci*. These resistant organisms produce PBPs that have low affinity for ß-lactam antibiotics and as a result, they are not inhibited except at relatively high drug concentrations, which may exceed what is clinically achievable (Chambers, 2004).

2. Impaired penetration of drug to target PBPs

Resistance caused by impaired penetration of antibiotics to target PBPs, occurs only in Gram-negative species and is due to the impermeability of an outer limiting membrane that is present in Gram-negative but not in Gram-positive bacteria (Nikaido, 1988). ß-lactam antibiotics cross the outer membrane and enter the organisms via outer membrane protein channels (porins). Absence of the proper channel or down-regulation of its production can prevent or greatly reduce drug entry into the cell. Impaired penetration alone is usually not sufficient to confer resistance because enough antibiotic eventually enters the cell to inhibit growth. However, this barrier can become important in the presence of a ß-lactamase, which hydrolyzes antibiotic as it slowly enters the cell. Permeability changes and decreased affinity of PBPs are the mechanisms jointly found in clinical isolates of *P. aeruginosa* (Mirelman *et al*, 1981).

3. Presence of an efflux pump

Gram-negative organisms may produce an efflux pump, which consists of cytoplasmic and periplasmic protein components that efficiently transport some ß-lactam antibiotics from the periplasm, back across the outer membrane eg, extrusion of nafcillin by *Salmonella typhimurium* (Chambers, 2004).

Enzyme mediated resistance mechanisms

This type of resistance is due to the production of various enzymes by bacteria, which are capable of inactivating a particular antibiotic. These include ß-lactamases, aminoglycoside-modifying enzymes, chloromphenical acetyl transferase and erythromycin estrases (Quintiani & Courvalin, 1995).

Inactivation of ß-lactam antibiotics by ß- lactamases

In clinically significant bacteria, the most important mechanism of resistance is the production of one or more ß-lactamase (penicillinases & cephalosporinases) enzymes that hydrolyze the ß-lactam bond characteristic of ß-lactam antibiotics. (Heritage *et al*, 1999; Livermore, 1995; Levinson, 2004). Since their introduction into the clinical practice, the effectiveness of ß-lactam antibiotics has been reduced. The ability of a ß-lactamase to cause resistance varies with its activity, quantity , cellular location and the permeability of the producer strain (Livermore, 1995).

Action of β–lactamases

ß-lactamases destroy the ß-lactam ring of the antibiotics. They bind to and prevent the action of penicillin binding proteins (PBPs), which are responsible for building and maintenance of peptidoglycan layer (Livermore, 1991).The ß-lactam agent become so changed in its chemical structure that it is no longer recognized by the enzymes

responsible for making the peptidoglycan layer of the bacterial cell wall. (Chambers, 2004). In case of Gram–negative bacteria , ß-lactamases are retained within the bacterial cell in the periplasmic space. Therefore, the ß-lactam agents must penetrate into the bacterial cell wall before they are exposed to the action of ß-lactamases (Livermore ,1991). If the ß-lactam compound readily passes through the outer membrane into the periplasm and reaches the PBPs in concentrations that will inhibit peptidoglycan synthesis, the bacterial cell will die. Conversely ,the ß-lactam is inactivated in the periplasm by ß-lactamases and the cell will survive (Frere,1995).

Distribution of ß –lactamases

Bacterial ß –lactamases are mediated by either chromosomes or plasmids.

1. Chromosomal mediated ß –lactamases

These enzymes are produced by bacteria encoded by a gene on the bacterial chromosome. They are present in Gram-positive & Gram-negative bacteria, *Mycobacteria* and *Nocardia* (Neu, 1985). They are either inducible or constitutive (Livermore, 1995) and most of them are not susceptible to inhibitors (Sykes & Mathew, 1976).

2. Plasmid mediated ß-lactamases

The genes encoding the ESBLs are present on plasmids, facilitating their spread in nosocomial pathogens (Livermore, 1995; Paterson and Bonomo, 2005)· Like their parental TEM and SHV enzymes, all ESBLs are highly susceptible to ß-lactamase inhibitors such as clavulanic acid, sulbactam & tazobactam (Philippon *et al,* 1989; Bush *et al,* 1995; Sykes & Mathew, 1976). Over 75 different plasmid –mediated ß-lactamases have been recorded in Gram-Negative bacilli (Bush *et al,* 1995). Transposons that encode ESBLs have also been described (Heritage *et al,* 1999).

History of ß- lactamases

Abraham and Chain isolated an enzyme, penicillinase (an antibiotic destroying component from a strain of *Escherichia coli)* in 1940. Thus even before the widespread use of antibiotics, the resistant mechanism had already been detected both in Gram- positive and Gram-negative bacteria (Fred & James, 1996).

In the late 1950s, it was found that during mix cultures, the resistant strains of *Shigella* spp. were capable of transferring their resistance pattern to previously sensitive strains of *E.coli* (Watanabe, 1963). This resistance was shown to be due to the presence of a transmissible plasmid.

In 1965, the first plasmid mediated ß -lactamase was isolated from an appendicectomy wound of a Greek girl Temoniera and was called TEM enzyme, so named after the name of the girl (Datt & Kontomichalou, 1965). The most common plasmid mediated ß-lactamase is TEM-1, which has been reported in about 75-80% of plasmid- mediated ß-lactamase resistances (Matthew, 1979; Simpson *et al.* 1980; Roy *et al.* 1983).

The first extended-spectrum SHV enzyme was described in the Federal Republic of Germany in 1983 from clinical isolates of *K. pneumoniae, Klebsiell a ozaenae* and *Serratia marcescens* (Knothe *et al,* 1983; Heritage *et al,* 1999) and was related to SHV-1 (sulph-hydral variant), which was resistant to ceftazidime (Dubois *et al,* 1995). Because of its similarity to SHV-1, the new enzyme was named as SHV-2 (Kliebe *et al* 1985). A single amino acid substitution alters the spectrum of activity of the SHV-1 ß-lactamase to encompass extended-spectrum cephalosporins. (Barthélémy *et al,* 1988).Later these mutated ß-lactamase enzymes were named as extended-spectrum ß-lactamases (ESBLs).

What are Extended Spectrum ß-Lactamases (ESBLs)?

These are a rapidly evolving group of ß-lactamases, which share the ability to hydrolyze

third-generation cephalosporins and monobactams , yet are inhibited by clavulanic acid. A point mutation which alters the configuration around the active site of the TEM and SHV type enzymes has led to ß-lactamases that are now known as "Extended Spectrum ß-Lactamases (Paterson and Bonomo, 2005).

Among the first of the extended-spectrum ß-lactamases to cause significant clinical problems were mutants derived from the narrow-spectrum SHV-1 or TEM-1 ß-lactamases (Knothe *et al* 1983; Jarlier, *et al* 1988; Jacoby & Medeiros, 1991).

The first ESBL , cefotoximase TEM/CTX-1 was produced by *K. pneumoniae* at the teaching hospital of Clermont-Ferrand, France in July 1984 (Sirot *et al*, 1987). ESBLs are most commonly seen in *E.coli* and *K. pneumoniae* but also have been described in other members of Enterobacteriacae as well . Many of these enzymes are TEM or SHV derivatives and other newly emerging class A enzymes such as PER-1 and CTX-M (De Champs *et al*, 2000). When ESBLs appeared, they were predominantly TEM-type enzymes (Goldstein *et al*, 1993, De Champs *et al*, 2000), but in Europe since the mid-1990s, the SHV-type ESBLs are more frequent ((Babini and Livermore. 2000). The greater incidence of the SHV-type ESBLs seems related to the predominance of *K. pneumoniae* among the ESBL-producing strains (Buré *et al*, 1988; Chanal, *et al*, 1996).To date more than 20 variants of TEM-1 and TEM-2 and more than six variants of SHV-1 have been described (Philippon *et al.* 1989).

Classification of ß-lactamases

The classification and nomenclature of ß-lactamases has always proved problematic. Several schemes have been proposed for the classification of this large family of enzymes. The first proposal was to divide ß-lactamases into the penicillinases (that hydrolyze penicillins) and cephalosporinases (that attacked cephalosporins). The biochemical activity and substrate profiles of different enzymes formed the basis of early classification schemes. (Richmond, & Sykes, 1973; Jack *et al*, 1970). Later, the location

of the genetic determinants (whether plasmid mediated or chromosomal) became incorporated into classification schemes. Data from isoelectric focusing studies & enzyme kinetics were also considered important and these too formed the basis of subsequent classifications. (Matthew & Hedges, 1976; Sykes & Matthew ,1976).

These schemes all had major anomalies but following rapid developments in molecular biology, sequence homology studies were able to resolve difficulties with previous classification schemes. (Ambler, 1980; Jaurin & Grundström, 1981; Huletsky et al 1990; Bush, 1989; Bush et al, 1995).

Classification of ß-lactamases (on the basis of phenotypic characters)

It was first proposed by Jack et al in 1970 and was modified by Richmond and Sykes in 1973. It is based on phenotypic characters such as substrate profile and susceptibility to inhibitors such as isoxazolyl penicillins (like oxacillin & cloxacillin), clavulanic acid and p–chloromercuribenzoate (p-CMB)]. This scheme divides the ß-lactamases from Gram-negative bacilli into five major classes (Bryan ,1988) .

Class I.   Enzymes, which are primarily cephalosporinases.

Class 2.   Enzymes, which are primarily penicillinases

Class 3.   Enzymes ,which are active against broad spectrum penicillins and
            cephalosporins, while resistant to inhibition by p-CMB and
            sensitive to cloxacillin.

Class 4.   Enzymes, which have substrate profile similar to Class 3 but are resistant to
            inhibition by cloxacillin and sensitive to p-CMB.

Class 5.   Penicillinases which have broader spectrum than that of Class 2.

The phenotypic classification faces the problem that point mutation can greatly alter the substrate specificity and inhibitor susceptibility of the enzyme.

Classification of ß-lactamases (based on amino acid & nucleotide sequence)

ß-lactamases are now classified by amino acid and nucleotide sequence. Such classification is stable and cannot be distorted by mutations. This scheme separates ß-lactamases into four major classes. Classes A, C and D comprise evolutionarily distinct groups of serine enzymes and class B contains Zn^{2+} types (Livermore, 1995).

Class A

These enzymes are most prevalent plasmid–mediated ß-lacatmases of Gram–negative rods. These include TEM-1, TEM -2 and their subsequent mutants (Mayer *et al* ,1995). These enzymes destroy penicillins and are becoming increasingly important, as mutations in these have led to increase in their spectrum. For example, MEN-1 confers resistance to cefotaxime, while NMC-A and SME-1 has carbapenemase activity.

Class B

This class of ß- lactamases is unique because they contain a metal ion (Zn^{2+}) at the active site (i.e they are metaloproteases) rather than a serine residue, which is found in all the other ß -lactamases. These enzymes are broad-spectrum and usually have a greater activity against carbapenems, penicillins and have lesser activity against cephalosporins. In addition to carbapenems, the isolates were resistant to other ß-lactams and ß-lactamase inhibitors with the exception of aztreonam (Mayer *et al,* 1995).

Class C

Class C enzymes are primarily chromosomal–mediated but some of them have migrated to plasmid-mediated (Mayer *et al*, 1995).The most prevalent enzyme in this group, found among the *Enterobacteriaceae* and *P. aeruginosa,* is Amp C (Naumovski *et al,* 1992; Emery & Weymouth, 1997). They are more effective against cephalosporins and

are not susceptible to inhibition by ß-lactamase inhibitors (Mayer *et al*, 1995). However, mutations in the regulatory gene, which occurs at a high frequency among *Enterobacter cloacae*, can result in high-level constitutive production, resulting in resistance to all ß-lactams except carbapenems. (Naumovski *et al*, 1992; Emery & Weymouth, 1997).

Class D

Enzymes of this class are usually more active against penicillins than other ß-lactam drugs and have a wide spread substrate spectrum. Unlike other serine-based enzymes, these are also active against oxacillin. Class D enzymes are normally plasmid-mediated in Gram-negative bacteria (Mayer *e t al,* 1995).

ß-lactamases of Gram–positive bacteria are classified separately from those of Gram–negative bacteria. Type A, B and C are inducible enzymes , while type D is constitutively produced (Bryan, 1988).

Other Secondary ß-lactamases

Secondary ß-lactamases have been reported widely in *P. aeruginosa* but are much rare than in *Enterobacteriaceae* . Incidence of 13% from France, 7 % from Spain and 0.7% and 2.5% from England has been reported (William et *al,* 1984; Livermore, 1995). In contrast to the predominance of TEM & SHV types in *Enterobacteriaceae,* these are rarely seen in   *P. aeruginosa* (Livermore ,1995).

Nomenclature

There has been considerable confusion over the nomenclature of ß-lactamases. There is no rational basis for the naming of these enzymes. By nucleotide sequencing and hybridization, most of the ESBLs have been determined to be derived from TEM-I, TEM-2 or SHV-I enzymes. Consequently, most have been given TEM or SHV designation. The name `TEM' is a contraction of Temoniera, while `SHV' is a contraction of sulphydryl variable (a description of the biochemical properties of this ß-

lactamase). Furthermore, ß-lactamases may be given one name when first identified, only to change, after subsequent studies have allowed a more complete characterization of its properties. CTX-1 was so called because it conferred resistance to cefotaxime but nucleotide sequence analysis showed that, this enzyme had arisen by the accumulation of point mutations in the gene encoding a TEM ß-lactamase. Consequently, CTX-1 is now named TEM-3. Similarly SHV-1 had been called as PIT-2 as it was described by Pitton for the first time, in 1972. (Pitton, 1972) .Similarly TEM-5 is also called CAZ-1 (Philipon *et al,* 1989). In few cases, a second name describes the local, where the enzyme was first discussed. For example, TEM-9 is also called RHH-1, which stands for Royal Hampshire Hospital, England (Spencer *et al,* 1987).

Effect of ESBLs on resistance

The presence of ESBLs carries tremendous clinical significance. They can confer resistance to broad-spectrum ß-lactam antibiotics, including 3rd and 4th generation cephalosporins, monobactams and extended -spectrum penicillins. (Jacoby & Medeiros, 1991). A very broad-antibiotic resistance pattern extending to many classes of drugs is a frequent characteristic of ESBL producers. These include aminoglycosides, trimethoprim, sulfonamides, tetracyclines, chloramphenicol and fluoroquinolones (Thomson *et al,* 1996; Paterson and Bonomo, 2005). Hence a more appropriate name would be "multidrug resistant organisms." (Nathisuwan *et al*, 2001). Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited (Paterson and Bonomo, 2005).

To date none has been described that are able to hydrolyze cephamycins or carbapenems. The carbapenems, cephamycins (cefoxitin & cefotetan) and temocillin are stable against ESBLs (Jacoby & Carreras, 1990),therefore imipenem has been successfully used against ESBL-producers *in vivo* (Pangon *et al*, 1994; Philippon *et al*, 1989).

ESBL-producing strains have been isolated from abscesses, blood, catheter tips, lungs, peritoneal fluid, sputum, and throat culture ((Naumovski *et al*, 1992; Emery & Weymouth, 1997)· The lower digestive tract of colonized patients is the main reservoir of these organisms (Quinn, 1994).

Other problems due to ESBL-producing bacteria are difficulty in detecting the presence of ESBLs, limited treatment options and deleterious impact on clinical outcomes. Clinicians should be familiar with the clinical significance of these enzymes and potential strategies for dealing with this growing problem (Nathisuwan *et al*, 2001).

DETECTION OF ESBLS

The problem of resistance mediated by ESBLs has been compounded by the lack of detection methods of ESBLs. Therefore, detection of ESBL-producing Gram-negative rods (GNRs) remains a challenge for the microbiology laboratory. Many ESBL-producing strains of Enterobacteriaceae do not show resistance to newer cephalosporins or aztreonam in routine susceptibility tests. Therefore, a clinical microbiology laboratory must not rely solely on routine susceptibility tests but should also use a more accurate method of detecting ESBLs (Thompson *et al*, 1996). The most reliable approach to detect ESBLs-producer is the use of special tests for ESBLs detection.

Special Tests of ESBLs Detection

Various tests have been developed to detect ESBLs, the main aim is to detect ESBLs in *Klebsiella*, the main host genus, but are equally applicable to other Enterobacteriaceae e.g *E. coli* and *P. mirabilis.* (Livermore and Brown, 2001). The National Committee for Clinical Laboratory Standards (NCCLS) has recently published performance standards for screening and confirmatory tests for ESBLs in publication M7-A5, January 2000 (Singh, 1999). In common to all ESBLs-detection methods, is the general principle that the activity of extended-spectrum cephalosporins against ESBL-producing organisms

will be enhanced by the presence of clavulanic acid (Paterson and Bonomo, 2005). These tests include double disc diffusion test, ESBL E test, MIC Determination, genetic method and isoelectric focusing (IEF) (Thomson *et al*, 1996).

1. Double Disk Diffusion Technique

This is the most widely used test for ESBL detection. In this test the discs of 3rd generation cephalosporins, aztreonam and clavulanic acid are placed on a lawn of test organism, 30mm apart. The disc of clavulanic acid alone is not available hence, a disc of augmentin (20µg of amoxicillin plus 10 µgof clavulanic acid) can be used (Miles and Amyes, 1996). Zones of inhibition around these discs are observed. Enhancement of the zone of inhibition or a so-called ghost zone between the cephalosporins discs and clavulanate-containing disc indicates the presence of an ESBL. (Jarlier *et al*, 1988)..This technique is cost effective and simple to perform. Neither any specialized equipment nor any professional expertise is required .It can be handled by any person trained to perform disc diffusion testing. Only discs of 3rd generation cephalosporins, aztreonam and a disc of co-amoxiclav are required, which are usually available in a microbiology laboratory.

2. The ESBL E test

The newest approach has been to use commercially available products of ESBL detection. The ESBL screening E test (AB Biodisk Solna Sweden) strips are based on recognition of a reduction in ceftazidime MICs in the presence of a fixed concentration (2 µg /ml) of clavulanic acid. This test is highly reliable for the detection of ESBLs in *E. coli*, *K.pneumoniae* and *Klebsiella oxytoca*. However, it is not a reliable method for other species of Enterobacteriaceae (Ferraro & Jorgenson, 1995)."E"test strips are plastic strips with a fixed gradient of drug. These strips are applied on an inoculated plate in a similar way as discs are applied in disc diffusion testing. MICs are read directly where ellipse of inhibition intersects the strips. (Fig.1). (Cormicon *et al*, 1996).

ESBL DETECTION BY 'E' TEST
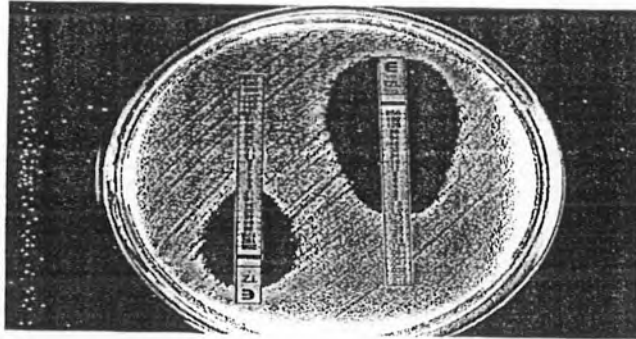
ESBL production is indicated by an obvious fall in MIC when clavulanic acid is added to ceftazidime.
Key: TZ – ceftazidime, TZL – ceftazidime plus clavulanic acid

Fig 1.ESBL Detection by E test

3. MIC Determination

MICs are determined for cefotaxime (CTX) or ceftazidime (CAZ) with or without clavulanic acid. In the presence of clavulanic acid, MICs for 3rd generation cephalosporins are reduced considerably for the ESBL-producers (Swenson *et al*, 1995).

Other Methods for ESBLs Detection

1. Genetic Methods

a. DNA Probe and Hybridization Studies

Several DNA probes and PCR primer sets have been developed to detect the genes encoding for TEM, SHV, OXA and other ß-lactamases present in Gram-negative bacteria. This method is very helpful for epidemiological studies of ESBLs. The main disadvantage is that, it is very tedious to perform since more than 20 different hybridization reactions must be completed for each strain. However, the system is more sensitive than isoelectric focusing for identifying ß-lactamase genes (Tenover *et al*, 1995).

b. Plasmid DNA Analysis

It is another genetic method used for epidemiological studies. Plasmid DNA is extracted and clear lysates are used directly for electrophoresis on agarose gel. Size of the plasmid is determined by using standard plasmids of known size (Sirot *et al*, 1987).

2. Isoelectric Focusing (IEF)

ß-lactamases for isoelectric focusing are prepared by ultrasonic disintegration of the test strains .The enzyme activities are located in the gels and are detected by an iodometric method or by nitrocefin, a chromogenic cephalosporins for ß-lactamase detection. (Chanal *et al*, 1996).

Risk factors and transmission of organisms harboring ESBLs

Known risk factors for colonization and/or infection with organisms harboring ESBLs include admission to an intensive care unit, instrumentation, prolonged hospital stay , antibiotic exposure, especially to extended-spectrum ß-lactam antibiotics (Quinn, 1994) and recent surgery, some investigators have identified abdominal surgery as the major risk factor (Jonson & Woodford, 1993). It is known that ESBL-producing strains can survive in the hospital environment (Hobson *et al*, 1996). These strains are usually found in those areas of hospitals where antibiotic use is heavy and patient's condition is critical (Thomson *et al*, 1996). The length of stay in ICU is also important. In one study, more than half of the patients were colonized after 30 days stay in hospital (Spencer *et al*, 1987). Apart from ICUs, ESBL-producing strains have also been isolated from patients in general wards and nursing homes. Patient to patient transmission of these strains occur via the hands of hospital staff (Coulter *et al*, 1995). Whereas the use of 3rd generation cephalosporins is the most important factor for acquiring ESBLs.

Nosocomial transmission of ESBLs-producing organisms
Nosocomial infections in patients occur through the administration of extended-spectrum ß-lactam antibiotics or via transmission from other patients through health care workers.

Prevention

Spread of ESBL-producing GNRs can be controlled by good infection control practices (Gaillot *et al,* 1998; Burwen *et al,* 1994), especially by good hand washing technique, although Lucet *et al* (1996) showed that stressing good hand washing practice was not sufficient to control transmission of ESBL-producing strains. They combined education of staff with careful review of nursing care practices to minimize the risk of transmission (Gaillot *et al*, 1998). Other experts are advocating the role of antibiotic manipulation and restriction to control ESBL outbreaks.

Improved laboratory detection and reporting of ESBL-producing strains is needed. Laboratories should test for susceptibility of all *K. pneumoniae* and *E. coli* isolates to extended-spectrum ß-lactam antibiotics and ESBLs. NCCLS guidelines recommend both screening and confirmatory tests be used ((National Committee for Clinical Laboratory Standards.1999). Monitoring and control of usage of extended-spectrum cephalosporins and regular surveillance of antibiotic resistance patterns as well as efforts to decrease use as empirical therapy is indicated (Emery and Weymouth, 1997; Naumovski, 1992)

Treatment

There are very limited drugs, to choose from, for treating patients with ESBL infection. Cefotaxime, ceftazidime, ceftriaxone, aztreonam, tiarcillin, mezlocillin, piperacillin have poor or no activity. Although penicillins, cephalosporins, or aztreonam will appear to be susceptible *in vitro*, ESBL-producing *E. coli* or *Klebsiella* spp. may be clinically resistant to therapy with these antibiotics. Infectious disease specialists are good resources when consultation for therapy of ESBL-producing organisms is needed (Singh, 1999).

Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, yet carbapenem-resistant isolates have recently been reported. ESBL-producing organisms may appear susceptible to some extended-spectrum cephalosporins. However, treatment with such antibiotics has been associated with high failure rates (Paterson and Bonomo, 2005).

CHAPTER   3

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

Resistance to antimicrobial agents has become a serious problem in the treatment of bacterial infections In contrast to developed countries, the antibiotic resistance in Pakistan is expected to be much higher due to indiscriminate use of antibiotics by the physicians and public .Further more there is not much data available on antibiotic resistance patterns of commonly isolated bacteria due to lack of facilities for bacterial culture and antibiotic sensitivity testing. The microbial pathogens as well as their sensitivity pattern keep on changing from time to time and place to place, therefore knowledge of the current drug resistance pattern of the common pathogenic bacteria in a particular region is useful in clinical practice and is important for implementation of effective hospital infection control policies as well.

Considering these facts, the present study was designed to determine the magnitude and current trends of antibiotic resistance and their development among clinically important isolates against commonly used antibiotics with special reference to ß. lactam antibiotics in Pakistan. Accordingly, some recommendations may be formulated for the control, prevention and empirical antibiotic treatment.

The following objectives were set to achieve the goal.

1.   Isolation, characterization and preservation of clinically important bacterial isolates.

2.   Evaluation of the epidemiological data regarding the prevalence of clinically significant isolates in different patients presenting at various departments of the Fauji Foundation Hospital, Rawalpindi, Pakistan.

3.   Study of the resistance pattern of clinically significant isolates against commonly used antibiotics with special reference to ß- lactam drugs.

4.   Study and determination of prevalence of extended-spectrum ß -lactamase - producing strains among Gram-negative bacilli as multiple antibiotic resistance is often a characteristic of ESBL- producing Gram –negative bacteria .

CHAPTER 4

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was conducted at Clinical Microbiology Laboratory, Fauji Foundation Hospital Rawalpindi, Pakistan, during the period from April 2004 to March 2006. A total of 9712 samples from in-patients and out-patients were received in the microbiology laboratory. Out of 9712 samples, 2877 were pus samples, 2757 were urine samples, 1923 were high vaginal swabs, 1550 were blood samples and 605 were sputum samples.

Collection & Transportation of the Samples

Each sample was labeled properly & a request form accompanied the sample with the following points.

1. Name, age & sex of the patient

2. Number of samples

3. Registration No, Ward and Bed number of the patient

4. Type of sample

5. Time & date of collection of sample

6. Investigations required

7. Any antibiotic taken

1. Pus Samples

The pus samples were either aspirated by disposable syringe or collected on sterile cotton wool swabs, after proper cleaning of the wound or the infected area. About 2-5 ml of pus was aspirated. Swab was sufficiently wet with the sample and was transported immediately to the laboratory to prevent the dryness of the sample.

2. Blood Samples

Blood culture bottle was prewarmed to 37 °C or to room temperature. Venepuncture site was cleansed with 2% iodine, followed by 70% alcohol & allowed to dry. About 3-5 ml of blood was drawn at the height of pyrexia and before the start of antibiotic therapy. Two to three samples were collected within 24 hours, as bacteremia is intermittent in the majority of infections.

3. Sputum Samples

Sputum was collected in wide mouthed, sterile, leak proof container. The samples were collected early in the morning and before any mouthwash was used. The patient was asked to cough deeply to produce sputum. It was taken care that specimen was sputum and not saliva.

4. Urine Samples

A mid stream urine was collected in a sterile container. It was immediately delivered to the laboratory & if not possible, than it was refrigerated.

5. Vaginal Samples

Samples were collected on sterile swabs & immediately transported to the laboratory to prevent dryness of the swabs.

Processing of Samples

1. Pus Samples

Pus samples were directly inoculated on Blood agar (CM55 and SR50-OXOID) and MacConkey's agar (CM7-OXOID) plates and incubated for 24 to 48 hours at 37 °C aerobically. Next day the plates were examined for bacterial growth.

2. Blood Samples

Blood collected under aseptic condition was immediately transferred to 50 ml of Brain Heart Infusion (BHI) broth (CM225-OXOID) and incubated at 37 °C for 24 hours. In case of no growth, incubation period was extended for another 24 hours. Growth was sub-cultured on blood agar (CM55 and SR50-OXOID) and MacConkey's agar (CM7-OXOID) plates and incubated for 24 hours at 37 °C. For broth with no growth even after 48 hours, incubation was extended to ten days. The samples were considered negative only, if there was no turbidity or growth on tenth day.

3. Sputum Samples

The samples were inoculated on blood agar and Mac Conkey's agar plates and incubated for 24 to 48 hours at 37 °C under aerobic conditions. Chocolate agar plates were also inoculated and incubated at 37 °C in a 7% CO_2 atmosphere. The organisms were identified after 24-48 hours of incubation.

4. Urine Samples

Urine samples were inoculated on Cystine Lactose-Electrolyte Deficient (CLED) medium (CM301-OXOID), with the help of calibrated filter paper method. The plates were incubated aerobically for 24 to 48 hours at 37 °C and examined next day for bacterial count & growth.

Colony Count

The colonies were counted over the entire inoculated area. The number of colonies was multiplied by 1000 to obtain an estimate of the number of organisms per ml of urine, if there were 10 colonies, bacterial count will be 10x1000 =10,000 colonies per ml. Ten colonies were considered significant (Sleigh and Duguid, 1989).

5. Vaginal Samples

The samples were inoculated on Blood agar & MacConkey's agar and incubated for 24 to 48 hours at 37 °C, under aerobic conditions. Chocolate agar plates were also inoculated and incubated at 37 °C in a 7% CO_2 atmosphere. The organisms were identified after 24 hours of incubation.

Identification of Bacterial Isolates

Bacterial isolates from all type of samples processed were identified and characterized up to genus and species level with the help of following tests:

1. Morphology of Isolated Colonies

2. Gram Staining

3. Biochemical Analysis

The Gram-positive organisms were identified using catalase, coagulase & DNase tests, optochin, and bacitracin and novobiocin sensitivity. While Gram- negative rods were identified on the basis of oxidase test, citrate utilization test, methyl-red test, voges-proskauer test (MR-VP), triple sugar iron (TSI) test, indole-production test, urease and motility test (Collee and Miles, 1989).

4. Biochemical profile using API20E for Enteric Gram-negative rods were also used depending on their availability (Fig 2).
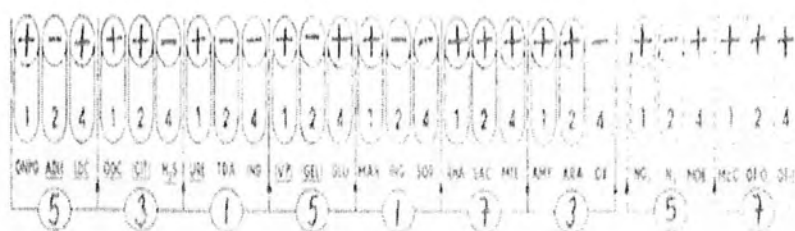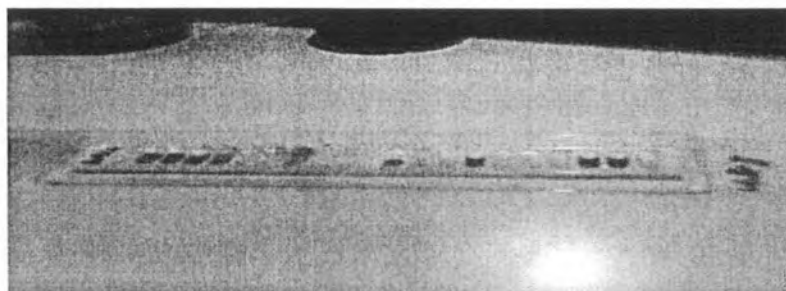
Fig 2. Analytical profile index 20 E

(API. 20 E Strip)

Susceptibility Testing of the Isolates

Isolated organisms after identification were subjected to sensitivity testing by Kirby-Bauer disc diffusion method (Bauer *et al*, 1966), using National Committee for Clinical Laboratory Standards criteria (NCCLS, 1993) to interpret diameter of inhibition zone.

Preparation of Mc Farland Turbidity Standards

Barium chloride standard was prepared, against which the turbidity of the inoculum was compared. The reagents were sulphuric acid (1%) and barium chloride dihydrate 1.175% (2.35 gm, barium chloride plus 200 ml water).

Method

About 0.5 ml of barium chloride dihydrate was added to 99.5 ml of 1% sulphuric acid. Solution was dispensed into tubes, comparable to those used for inoculum preparation, which were sealed tightly and stored in dark at room temperature. The McFarland 0.5 standard provides turbidity comparable to a bacterial suspension containing 1.5×10^8 cfu/mL (NCCLS, 1993).

Control Strains

The following control strains were used for the study;

1. *Escherichia coli*   (ATCC 25922)

2. *Pseudomonas aeruginosa* (ATCC 27853)

3. *Klebsiella pneumoniae* (ATCC 700603)

4. *Staphylococcus aureus* (ATCC 25923)

5. *Staphylococcus aureus* (ATCC 25823)

Kirby-Bauer Disc Diffusion Susceptibility Testing

Mueller-Hinton agar (CM337-OXOID) was used as the growth medium, which was prepared according to the manufacturer's instructions. Sterilized medium was cooled to 45-50 °C in a water bath. About 25 ml of medium was poured into 90 mm diameter sterile petri dishes to a depth of 4 mm on a level surface to make the depth of the medium uniform and left at room temperature overnight to check sterility. The plates were stored at 2-8 °C in sealed plastic bags to be used within two weeks.

Antibiotics Discs

Antibiotics tested with specified potencies are shown (Table 1). The discs were stored in a refrigerator at 4 °C under anhydrous conditions to prevent loss of potency. Before use, the working stock of the discs was allowed to warm at room temperature to minimize condensation of moisture, which leads to hydrolysis of the antibiotics.

Table 1 Antimicrobial agents discs along with code and potencies

S.No.	Antimicrobial Agents	Code	Disc Potency	Manufacturer
	β-Lactams			
1	Penicillin G	P	10 IU	Oxoid
2	Ampicillin	AMP	10 ug	Oxoid
3	Amoxicillin	AML	10 ug	Mast Diagnostic
4	Methicillin	MET	1 ug	Oxoid
5.	Co-amoxiclav	AUG	30 ug	Oxoid
6.	Piperacillin/tazobactam	TZP	75.1 + 10:1	Oxoid
7.	Sulbactam/cefoperazone	SCF	10^5 ug	Oxoid
8.	Cephradine	V	30 ug	Oxoid
9.	Cefotaxime	CTX	30 ug	Oxoid
10.	Cefuroxime	CXM	30 ug	Oxoid
11.	Ceftriaxone	CRO	30 ug	Becton Dickinson
12.	Ceftazidime	CAZ	30 ug	Oxoid
13.	Cefoperazone	CFP	75 ug	Oxoid
14.	Meropenem	MEM	10 ug	Oxoid
15.	Imipenem	IMP	10 ug	Becton Dickinson
16.	Vancomycin	Va	30 ug	Oxoid
17.	Teicoplanin	TEC	30 ug	Oxoid
18.	Aztreonam	ATM	30 ug	Mast Diagnostic
19.	Fosfomycin	FOS	50 ug	Oxoid
	Quinolones			
20.	Ofloxacin	OFX	5 ug	Oxoid
21.	Enoxacin	ENX	10 ug	Becton Dickinson
22.	Norfloxacin	NOR	10 ug	**BBL**
23.	Ciprofloxacin	CIP	5 ug	Oxoid
24.	Pipemidic acid	UR	50 ug	Mast Diagnostic
	Aminoglycosides			
25.	Amikacin	AK	30 ug	Oxoid
26.	Gentamicin	CN	10 ug	Oxoid
	Other drugs			
27.	Trimethoprim/ sulphamethoxazole	SXT	1.25 ug + 23.75 ug	Oxoid
28.	Erythromycin	E	15 ug	Oxoid
29.	Doxycycline	DOX	30 ug	Oxoid
30.	Lincomycin	L	2 ug	Oxoid

Preparation of Inoculum

For inoculum, Tryptone Soya broth (CM129-OXOID) was prepared according to the manufacturer's instructions and 5 ml of sterile broth was dispensed in screw cap test tubes. The test tubes were kept in an incubator for 24 hours at 35 °C to check sterility. About 5-10 colonies of already identified clinical isolates were inoculated in the sterilized test tubes containing the medium and placed in an incubator overnight at 35 °C. The turbidity of broth cultures were adjusted according to 0.5 McFarland standards by adding sterile saline against a white background with contrasting black line.

Secondary sensitivity was set up on the day of isolation of the organisms. Pure culture of these organisms was used as the inoculum. A sterile cotton swab was saturated by dipping into standardized bacterial suspension and excess material was removed by turning the swab against the side of the tube. Inoculum was spread evenly over the entire surface of the Mueller-Hinton agar plates by swabbing back and forth across the agar in three directions to give a uniform inoculum to the entire surface. The plates were allowed to dry. Within 15 minutes, discs of given potency were applied on the inoculated plates with the help of forceps. The discs were 15 mm from the rim of petri dish and 20 mm of space was kept between discs to avoid overlapping of the zone of inhibition or extension of the zone to the edge of the plate. The plates were incubated aerobically at 37 °C overnight. The results were read by measuring the diameter of the circular area of the growth inhibition around each of the disc including the diameter of the disc. Based on the diameter of the zone of inhibition, the organisms were categorized as sensitive, intermediately sensitive or resistant.

Detection of Extended-Spectrum ß-lactamases

Double Disc Diffusion Technique

In the present study, this technique was used because it is cost effective and simple to perform. Neither any specialized equipment nor any professional expertise is required

It can be handled by any person trained to perform disc diffusion testing. Only discs of 3rd generation cephalosporins, aztreonam and a disc of co-amoxiclav are required.

After identification procedures, the clinical isolates were tested for the production of ESBLs. Along with the setting up of the secondary sensitivity of the test organism, a disc of co-amoxiclav (20µg amoxicillin and 10µg clavulanic acid) was placed in the center of lawn of test organism on agar surface. The discs of cefotaxime, ceftriaxone, ceftazidime and aztreonam (30µg) each were placed around the disc of co-amoxiclav. These discs were arranged in such a way that the distance between the central and surrounding discs was approximately 30mm. After overnight incubation, the zones around 3rd generation cephalosporin discs and aztreonam were observed. If the inhibition zone around one or more cephalosporin discs and aztreonam was extended on the side nearest to the co-amoxiclav disc, the organism showing this synergism was considered as ESBLs-producer. If there was no extension of zones, the test was repeated by reducing the distance between the co-amoxiclav, cephalosporin and aztreonam discs to 20mm or even less. Zones of inhibition were again observed on the next day. If there was no extension of zones of 3rd generation cephalosporins and aztreonam towards co-amoxiclav disc, they were considered as ESBLs non-producer (Fig 3).

Maintenance of Bacterial Strains

ESBLs-positive isolates were subcultured and preserved for further studies. For short-term storage of bacterial culture, bacterial isolates were subcultured on nutrient agar slants and maintained at 4 °C and subcultured monthly for routine use. Whereas, for long-term storage mid-exponential phase isolates were stored in Tryptone Soya broth (CM129-OXOID) containing 20% glycerol in screw capped tube and were kept at minus 70 °C.

Statistical Analysis

Chi-square test was used to compare the categorical data and p- value of <0.05 was taken as significant.
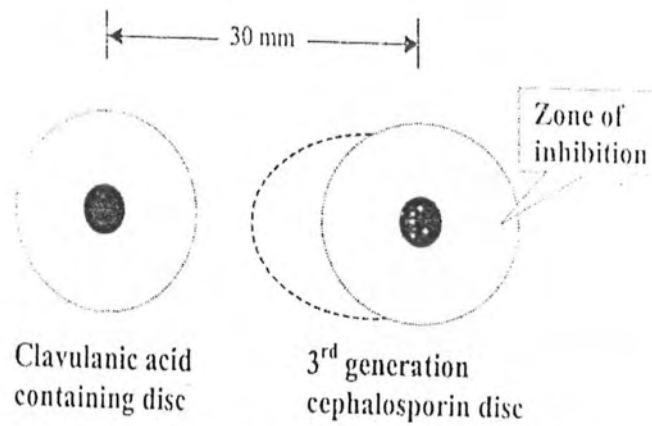
Demonstration of ESBL by double disc diffusion technique. If the isolate is an ESBL producer, the zone of inhibition around the third generation cephalosporin disc extends towards the disc containing clavulanic acid.

Fig 3. ESBL detection: Double Disc Diffusion Technique

CHAPTER 5

RESULTS

RESULTS

This study was carried out in Microbiology Laboratory of the Pathology Department, Fauji Foundation Hospital Rawalpindi. It is a teaching hospital attached with Foundation University Medical College, Rawalpindi, Pakistan.

A total of 9712 samples, both from in-patients and out- patients, were received during a period of 2 years from April 2004 to March 2006. These samples comprised of 2877 pus samples, 2757 urine samples, 1923 high vaginal swabs (HVS), 1550 blood samples and 605 sputum samples.

Frequency of Positivity

Out of 9712 samples processed, 4204 (43.3%) showed significant growth of pathogenic organisms. Highest frequency of infection was found in pus samples, 1562 out of 2877 (54.3%) followed by high vaginal swabs (912 out of 1923) and urine samples 1303 out of 2757 (47% each), sputum samples 262 out of 605 (43.3%) and blood stream samples 165 out of 1550 (10.7%) (Fig 4 & 5).

Out of 4204 positive isolates, 2660 isolates from patients presenting to the hospital were further analyzed in terms of location. Unfortunately, the remaining isolates from outside referrals could not be analyzed due to incomplete clinical information . Two-third of samples, 1758 (66.1%) were from in-patients, while one-third, 902 (33.9%) samples were from out-patients .

Among the in-patients positive samples, pus samples were most frequent, 848 (48.2%), followed by urine samples, 563 (32.0%). While in out-patients positive samples, high vaginal swabs, 550 (61.0%) and urine samples, 168 (18.6%) were the most frequent positive samples (Fig 6).
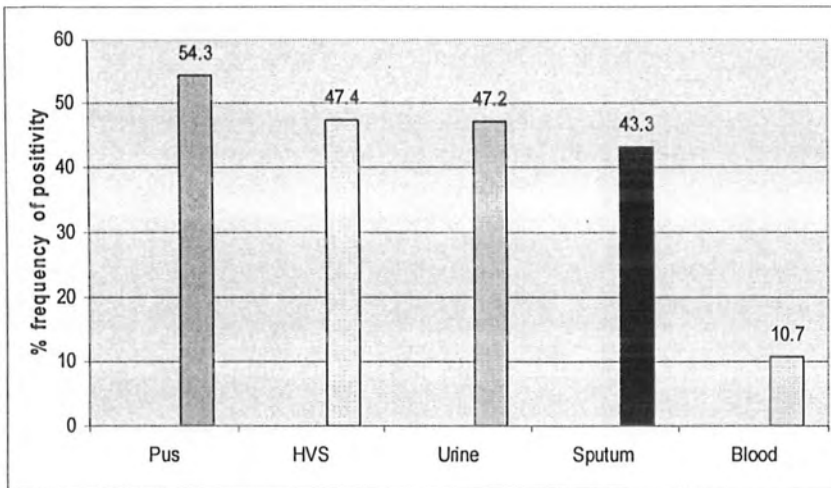
Figure 4 . Percentage Frequency of positivity in different
types of samples
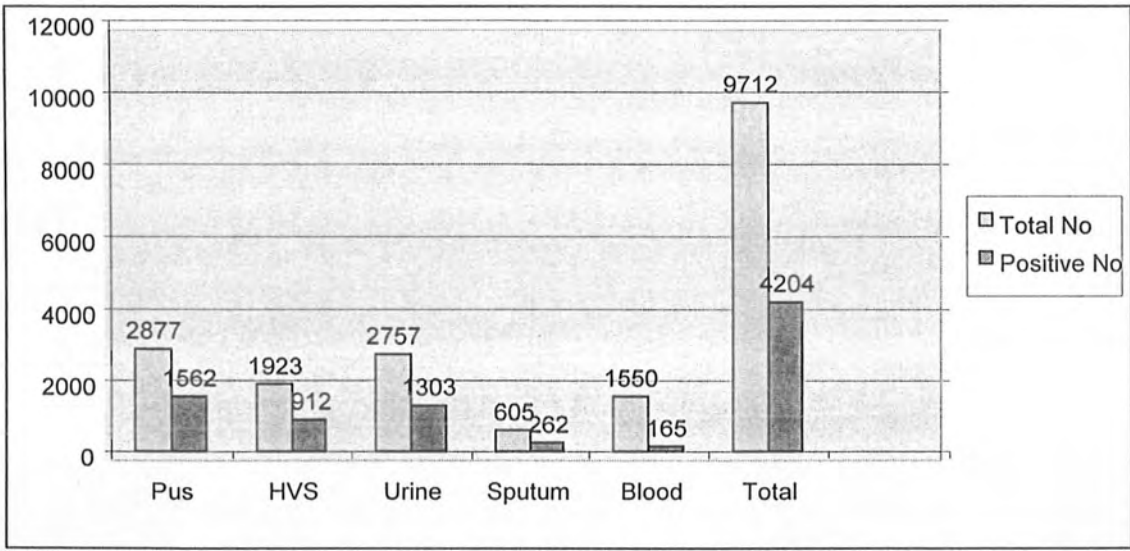
Figure 5. Frequency of positivity in different types of samples

	Pus	Urine	High vaginal swabs	Sputum	Blood
□ Indoor No	848	563	171	94	82
▣ Indoor %	48.2	32	9.7	5.4	4.7
□ Outdoor No	150	168	550	28	6
□ Outdoor %	16.6	18.6	61	3.1	0.7
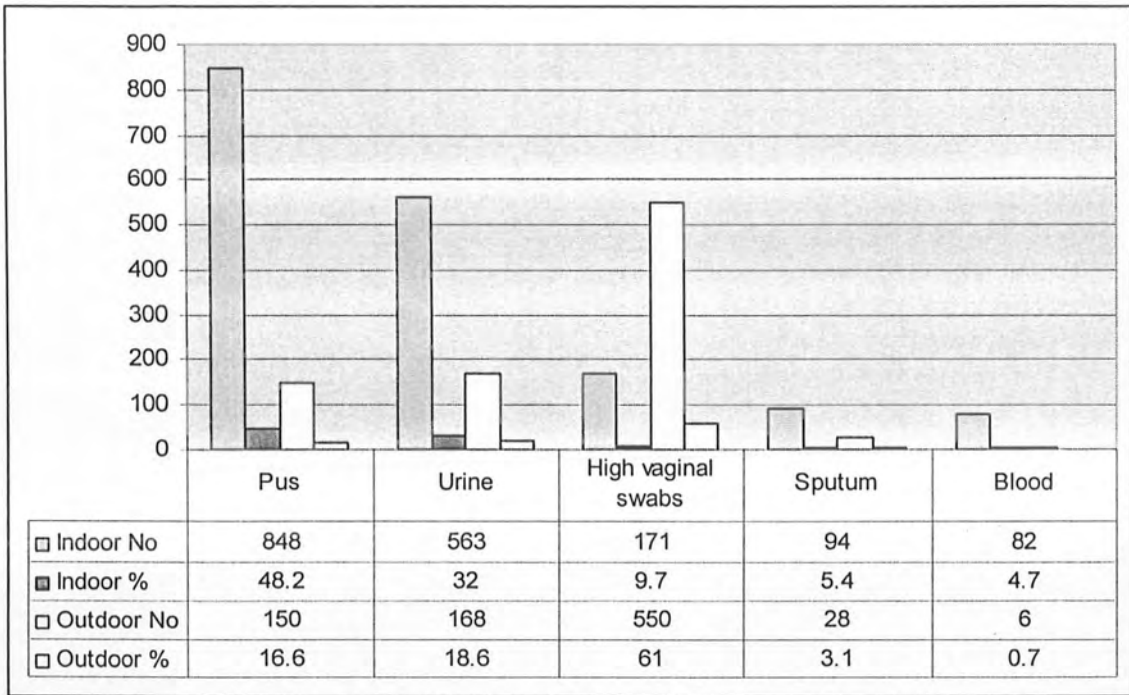
Fig 6 ; In-patients and Out-patients distribution in different types of positive samples

Gender Distribution of Samples

In 2979 positive samples, patients gender was known, 2299 were from female patients (77.2%) and 680 (22.8%) were from male patients. Female to male ratio was 3.4:1. Out of 2299 positive samples from females, 912 (39.7%) were   high vaginal swabs, 824 (35.8%) were urine samples, 362 (15.8%)   pus samples, 120 (5.2%)   sputum samples and 81 (3.5%) were   blood samples .Out of 680 positive samples from males, 322 (47.4%) were urine samples, followed by pus 255 (37.5%), sputum 61 (9.0%) and blood samples 42 (6.1%) (Table 2).

Age wise Prevalence of Infections

In 1992 samples, the age of the patients was known which varied between 3 months to 70 years. The samples at different age groups in both females and males were analyzed for the frequency of infections. The highest frequency of infection (21.9% and 22%) was found at two age groups (31-40 and 41-50 years) followed by a decline in the frequency. The least frequency was found at earlier age group (0-10years) , may be due the fact that least number of samples were of this age group. (Figure 7).

It was found out that in females highest frequency of infection, 95.9% and 94.1% was observed at two age groups, 31-40 and 41-50 years respectively. In males, the highest frequency of infection 64.7% and 52.9% was at the age of 0-10 years and 11-20 years followed by 61-70 years of age group (35.3%) (Fig 8). This trend of infections in relation to age groups is quite obvious in Fig 9

The frequency of different type of infections was analyzed in terms of different age groups in both females and males. In females, urinary tract infections were the predominant type of infections between the age group 41-50, pyogenic infections between 11-20 and 51-60 years and vaginal infections between 31-50years (Table 3). In males pyogenic infections were the predominant type of infections at all groups followed by urinary tract infections (Table 4).

Table 2. Types & frequency of infections among positive
samples in female & male patients

Samples	Females		Males		Female: Male ratio
	No	%	No	%	
HVS	912	39.7	-	-	-
Urine	824	35.8	322	47.4	2.5:1
Pus	362	15.8	255	37.5	1.4:1
Sputum	120	5.2	61	9.0	2:1
Blood	81	3.5	42	6.1	1.9:1
Total	2299	100.0	680	100.0	-
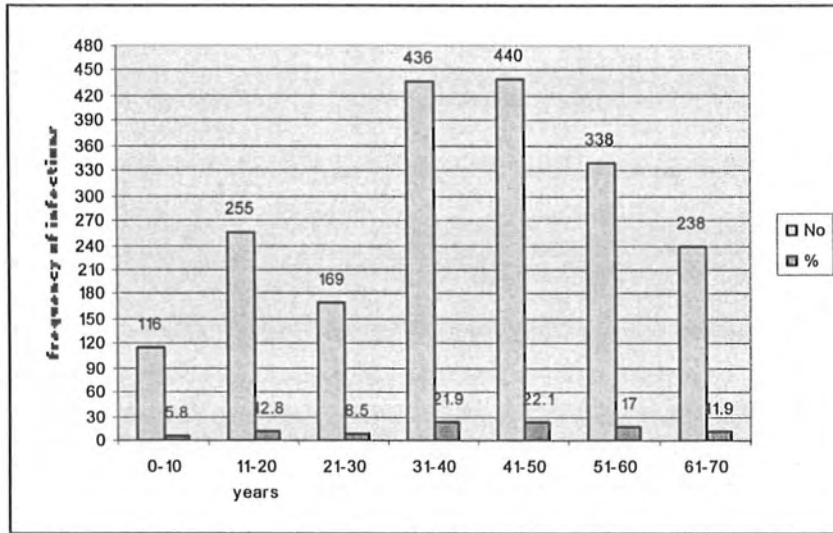
Fig 7 ; Frequency of infections at different age groups
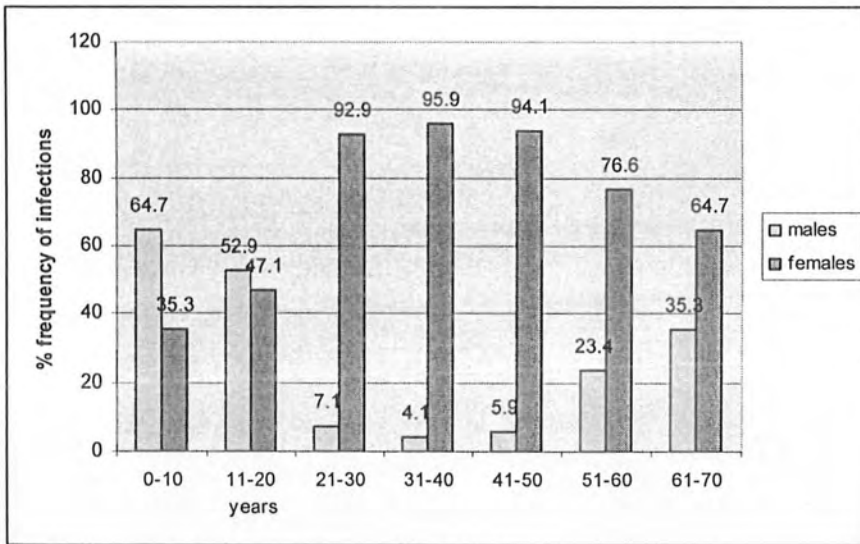
Fig 8 ; Frequency of infections at different age groups in female & male Patients
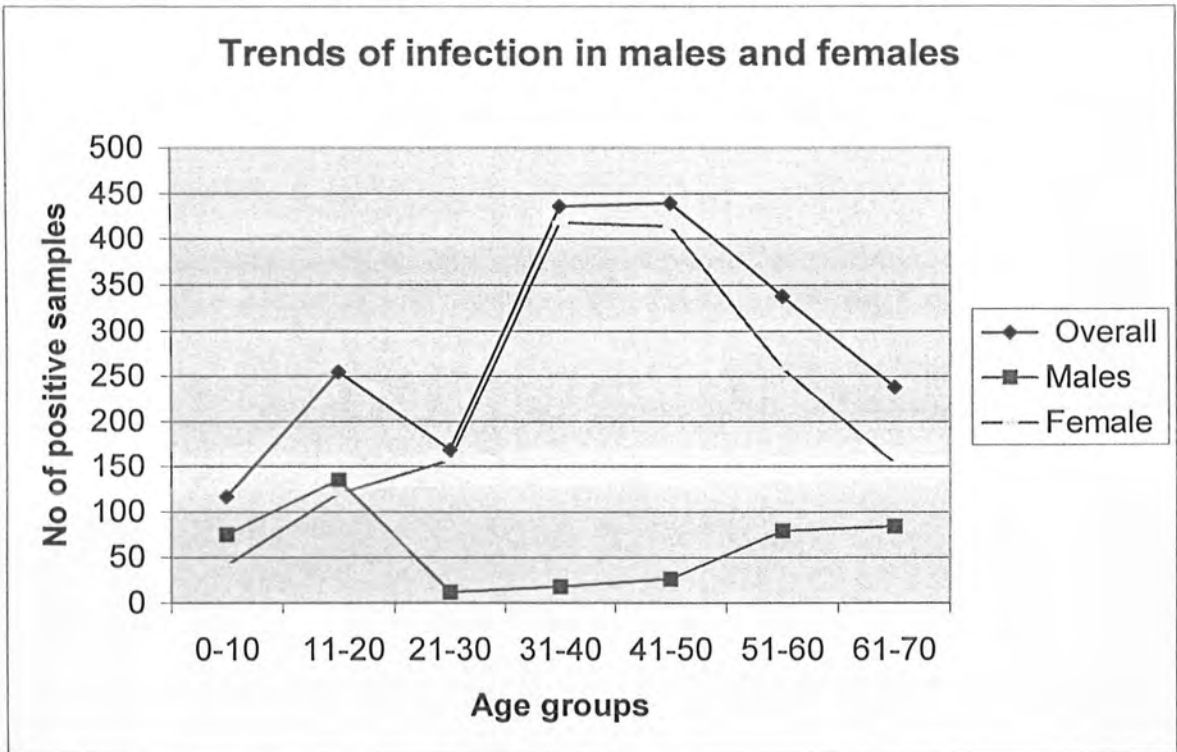
Figure 9. Frequency of infections in females
and males in relation to age groups

Table 3. Frequency of different types of infections at different age groups in female patients

Age groups (Yrs)	Samples					Total	
	Urine (No)	Pus (No)	Sputum (No)	HVS (No)	Blood (No)	No	%
0-10	20	12	4	2	3	41	2.6
11-20	26	72	4	15	3	120	7.7
21-30	33	47	2	71	4	157	10.0
31-40	52	67	3	289	7	418	26.7
41-50	62	70	14	262	6	414	26.5
51-60	51	104	5	96	3	259	16.6
61-70	44	63	8	33	6	154	9.9
Total	288	435	40	768	32	1563	100.0

Table 4. Frequency of different types of infections at different age groups in male patients

Age group (Years)	Samples				Total	
	Urine (No)	Pus (No)	Sputum (No)	Blood (No)	No	%
0-10	12	56	2	5	75	17.5
11-20	14	107	3	11	135	31.4
21-30	1	11	0	-	12	2.8
31-40	6	9	3	-	18	4.2
41-50	10	12	3	1	26	6.1
51-60	15	55	8	1	79	18.4
61-70	22	52	10	-	84	19.6
Total	80	302	29	18	429	100.0

Prevalence of Mixed Infections

Overall prevalence of mixed infections was 9.0% (363 out of 4039). Respiratory tract infections showed the highest rate of mixed infections, 19.5% (51 out of 262) followed by pyogenic infections, 13.9% (217 out of 1562) (Table 5).

Seasonal Variations in the Occurrence of Infections

For the last 12 months of the study period, 2239 samples were analyzed month-wise and it was found out that the infections were most common in the month of September, (13.1%), followed by April (12.0%), May (11.8%) and so on (Table 6, Figure 10).

Season-wise data (spring, summer, autumn and winter) was also evaluated and it was found out that infections were more common ($p < 0.05$) in the changing weathers like spring (43%) and autumn (43.1%) as compared to summer (32.9%) and winter season (36.4%). Infections were also higher ($p < 0.05$) in winter season as compared to summer (Table 7).

Prevalent Organisms in Different Samples

Out of 4204 isolates, Gram-negative rods were most prevalent organisms (57.5%), followed by Gram-positive cocci (40.1%), *Candida* spp (1.3 %), Gram-negative cocci (1.0%) and Gram-positive rods (0.1%) (Table 8).

Gram-negative rods were most prevalent in urinary isolates (87.6%), followed by sputum (51.9%), blood stream and pyogenic isolates (49.7% each), and high vaginal swabs isolates (30.8%). Gram-positive cocci were most prevalent in vaginal isolates (66.0%), followed by in blood (49.7%), pus (49.5%), sputum (31.0%) and urinary isolates (11.2%). Candidiasis was most prevalent in vaginal isolates (3.1%), followed by sputum isolates (2.3%), urinary (1.2%) and pyogenic isolates (0.4%). Gram-negative cocci were most prevalent organisms in sputum isolates (14.9%) followed by

Table 5. Frequency of mixed growth in different samples

Samples	Total (No)	Pure growth		Mixed growth	
		No	%	No	%
Sputum	262	211	80.5	51	19.5
Pus	1562	1345	86.1	217	13.9
HVS	912	830	91.0	82	9.0
Urine	1303	1290	99.0	13	1.0
Total	4039	3676	91.0	363	9.0

Table 6. Month wise distribution of positive samples

Months	Positive samples	
	No	%
February	176	7.9
March	168	7.5
April	268	12.0
May	264	11.8
June	217	9.7
July	75	3.3
August	205	9.2
September	294	13.1
October	105	4.7
November	114	5.1
December	180	8.0
January	173	7.7
Total	2239	100.0
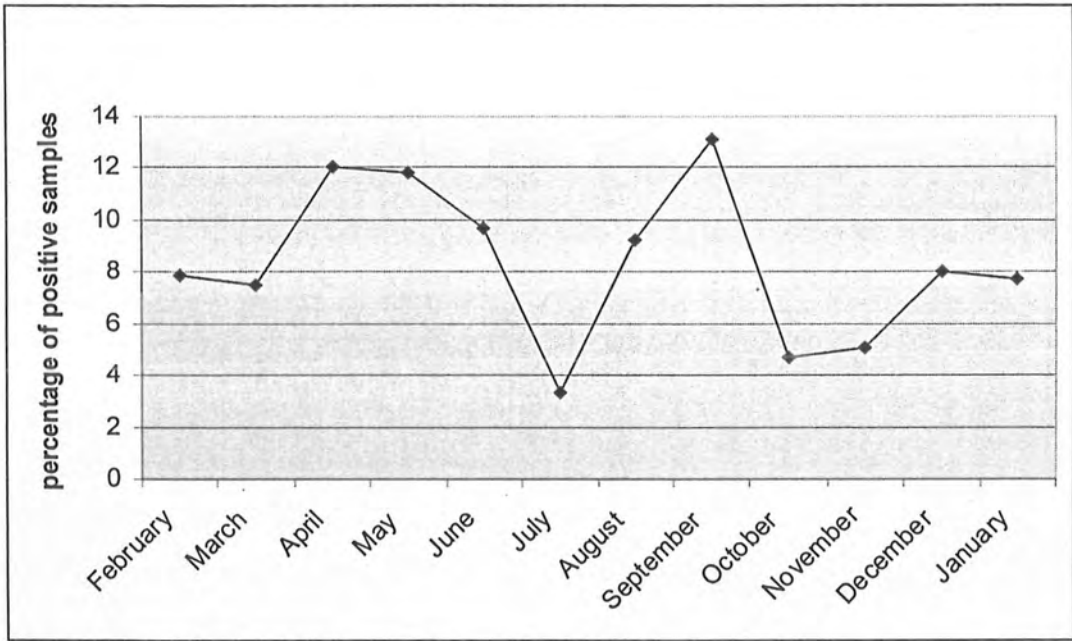
Figure 10. Annual trend of infection

Table 7. Season wise frequency of infections

Seasons	Samples				
	Total	Positive		Negative	
	No	No	(%)	No	(%)
Spring March-April	1012	436	43.0	576	57.0
Summer May- August	2311	761	32.9	1550	67.1
Autumn Sept-Oct	925	399	43.1	526	56.9
Winter Nov-Feb	1915	697	36.4	1218	63.6
Total	6163	2293	37.2	3870	62.8

Table 8. Prevalent type of organisms in 4204 isolates

Type of organisms	No	%
Gram-negative rods	2417	57.5
Gram-positive cocci	1684	40.1
Candida species	56	1.3
Gram-negative cocci	41	1.0
Gram-positive rods	6	0.1
Total	4204	100.0

blood (0.6%) and high vaginal isolates (0.1%) (Table 9).

Prevalent Organisms in Different Samples

Among 4204 isolates, *Staphylococcus aureus* was found to be the most prevalent organism (32.6%), followed by *Escherichia coli* (24.7%), *Pseudomonas aeruginosa* (15.9%) and *Klebsiella pneumoniae* (11.6%). These four organisms in combination constitute about 85% of the total isolates. *Streptococcus pyogenes, Enterococci, Proteus* species, *Candida* species and *Acinetobacter* species formed another 10.5% of the total isolates (Table 10). Different types of rare organisms constituted another 4.5 % of the total isolates (Table 11).

Staphylococcus aureus was the commonest organism isolated from high vaginal swabs (48.7%), pus (44.6%) and blood samples (40.6%). *Escherichia coli* was the most prevalent Gram-negative rod isolated from urine samples (47.7%). *Klebsiella pneumoniae* was the most common Gram-negative rod isolated from the sputum samples (21.4 %) (Table 12).

The frequency of different organisms in different samples varied. *Staphylococcus aureus* was the commonest organism isolated from pus (50.8%), high vaginal swabs (32.4%), urine (8.9%), blood (4.9%) and sputum samples (3.0%). *Escherichia coli* was the most prevalent Gram-negative rod isolated from urine samples (59.3%), followed by pus (21.6%) and high vaginal swabs (12.4%). *Klebsiella pneumoniae* was the most common Gram-negative rod isolated from the urine samples (38.6%). *Staphylococcus aureus* (50.8%), *Pseudomonas aeruginosa* (42.8%) and *Proteus species* (53.2%) were the organisms most commonly found in the pus samples. *Escherichia coli* (59.3%), *Klebsiella pneumoniae* (38.6%), *Acinetobacter* (40%), *Enterobacter* (38.2%) and *Providencia spp* (51.4%) were most commonly found in urine samples. *Streptococcus*

Table 9. Distribution of type of organisms in various samples

Samples	Total (No)	Gram-negative rods		Gram-positive cocci		Candida species		Gram-negative cocci		Gram-positive rods	
		No	%	No	%	No	%	No	%	No	%
Urine	1303	1141	87.6	146	11.2	16	1.2	-	-	-	-
Pus	1562	777	49.7	773	49.5	6	0.4	-	-	6	0.4
Blood	165	82	49.7	82	49.7	-	-	1	0.6	-	-
Sputum	262	136	51.9	81	31.0	6	2.3	39	14.9	-	-
HVS	912	281	30.8	602	66.0	28	3.1	1	0.1	-	-
Total	4204	2417	-	1684	-	56	-	41	-	6	-

Table 10. Prevalent organisms in 4204 isolates

Organisms	No	%
Staphylococcus aureus	1370	32.6
Escherichia coli	1039	24.7
Pseudomonas aeruginosa	667	15.9
Klebsiella pneumoniae	490	11.7
Streptococcus pyogenes	147	3.5
Enterococci	133	3.2
Proteus species	62	1.5
Candida species	56	1.3
Acinetobacter species	40	1.1
Others rare organisms	200	4.5
Total	**4204**	**100.0**

Table 11. Rare occurring organisms

Organisms	No	%
Morexalla catarrhalis	39	0.9
Providencia spp	35	0.8
Enterobacter spp	34	0.8
Coagulase negative Staphylococci	20	0.5
Streptococcus species	14	0.3
Citrobacter spp	9	0.2
Salmonella species	8	0.2
Morganella spp	8	0.2
Salmonella typhi	6	0.1
Corynebacterium species	6	0.1
Haemophilus influenzae	4	0.1
Hafnia spp	4	0.1
Serratia spp	4	0.1
Aeromonas spp	4	0.1
Niesseria meningitidis	1	0.0
Yersinia atypical	1	0.0
Niesseria species	1	0.0
Fusobacteria	1	0.0
Xanthomonas	1	0.0
Total	200	4.5

Table 12. Most prevalent organisms in various samples

Samples	Total (No)	Prevalent organisms		
		Type	No	%
HVS	912	*Staphylococcus aureus*	444	48.7
Pus	1562	*Staphylococcus aureus*	696	44.6
Blood	165	*Staphylococcus aureus*	67	40.6
Urine	1303	*Escherichia coli*	622	47.7
Sputum	262	*Klebsiella pneumoniae*	56	21.4

pyogenes (44.9%) and *Enterococci* (52.6%) were most commonly found in high vaginal swabs (Table13). The overall frequency of different organisms with their distribution in different samples is shown in Table 14.

Resistant Pattern of Most Prevalent Organisms from Various Samples

Results of present study indicate that *Staphylococcus aureus* strains were highly resistant to commonly used antibiotics like ampicillin, amoxicillin, co-trimoxazole, doxycycline and co-amoxiclav .While vancomycin and teicoplanin are still the most effective agents against them.(Table 15)

Gram-negative bacteria showed a high rate of resistance to many of the commonly prescribed antimicrobials like ampicillin, amoxicillin, amoxicillin/clavulanic acid . While carbapenems, β-lactam β-lactamase inhibitor combinations proved to be most effective agents against these organisms. (Tables 16-25)

Prevalence of Extended-Spectrum ß-Lactamases (ESBLs) in Gram-Negative Rods

About 38.9% of the Enteric Gram-negative rods (EGNRs) were found to be ESBL-producers. About 51% of the Enteric Gram-negative rods (EGNRs) from urinary source and 41.6 % from pus samples were ESBL- positive (Table 26).

Table 13. Prevalence of organisms in different types of samples

Samples	Staphylococcus aureus		E.coli		Pseudomonas		Klebsiella pneumoniae		Streptococcus pyogenes		Enterococci		Proteus species		Acinetobacter spp		Enterobacter spp		Providencia spp	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Pus	696	50.8	227	21.6	290	42.8	171	34.9	39	26.5	32	24.0	33	53.2	12	30.0	12	35.3	11	31.4
HVS	444	32.4	130	12.4	51	7.5	68	13.8	66	44.9	70	52.6	11	17.7	11	27.5	4	11.8	3	8.5
Urine	122	8.9	622	59.3	260	38.4	189	38.6	-	-	23	17.4	13	20.9	16	40.0	13	38.2	18	51.4
Blood	67	4.9	40	3.8	36	5.3	6	1.2	7	4.8	3	2.3	4	6.5	1	2.5	4	11.8	-	-
Sputum	41	3.0	30	2.9	40	5.9	56	11.5	35	23.8	35	3.7	1	1.7	-	-	1	2.9	3	8.7
Total	1370	100	1049	100	677	100	490	100	147	100	133	100	62	100	40	100	34	100	35	100

Table 14. Distribution of organisms in different samples

Organisms	HVS (No)	Sputum (No)	Pus (No)	Urine (No)	Blood (No)	Total (No)
Staphylococcus aureus	444	41	696	122	67	1370
Escherichia coli	130	30	227	622	30	1039
Pseudomonas aeruginosa	51	40	290	-	26	667
Klebsiella pneumoniae	68	56	171	189	6	490
Streptococcus pyogenes	66	35	39	-	7	147
Enterococci	70	5	32	23	3	133
Proteus species	-	1	33	-	4	62
Candida species	-	6	6	-	-	56
Acinetobacter species	-	-	12	-	1	40
Morexalla`species	-	39	-	-	-	39
Providencia species	-	-	11	18	-	35
Enterobacter species	-	-	12	13	4	34
Coagulase negative Staph	-	-	6	1	5	20
Streptococcus species	-	-	-	-	-	14
Citrobacter species	1	-	2	6	-	9
Salmonella species	-	-	6		2	8
Morganella species	1	-	7		-	8
Salmonella typhi	-	-	-	-	6	6
Haemophilus influenzae	-	-	-	-	-	4
Niesseria species	1	-	-	-	-	1
Corynebacterium species	-	-	6		-	6
Hafnia species	1	-		3	-	4
Serratia species	-	1	2	1	-	4
Aeromonas species	-	-	1	-	3	4
Neisseria meningitidis	-	-		-	1	1
Yersinia atypical	-	-	1	-	-	1
Fusobacteria species	-	-	1	-	-	1
Xanthomonas species	-	-	1	-	-	1
Total	912	262	1562	1303	165	4204

Table 15. Resistance pattern of *Staphylococcus aureus* isolates to various antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Cefoperazone/ sulbactam	16	-	-	16	0
Teicoplanin	34	-	1	35	2.8
Vancomycin	125	5	3	133	2.2
Piperacillin/ tazobactam	255	6	7	268	4.9
Imipenem	248	11	24	283	13.4
Fosfomycin	457	65	82	604	24.3
Erythromycin	5	-	2	7	29.6
Methicillin	504	12	190	706	29.6
Cefuroxime	18	-	7	25	28.0
Cefotaxime	349	35	113	497	29.8
Ciprofloxacin	413	35	205	653	36.8
Gentamicin	349	59	152	560	37.7
Norfloxacin	92	15	45	152	39.5
Ceforanide	10	-	7	17	41.2
Penicillin	8	-	7	15	46.8
Amikacin	45	18	21	86	47.7
Lincomycin	149	30	122	301	50.5
Cephradine	13	1	13	27	51.9
Enoxacin	11	-	14	25	56.0
Co-amoxiclav	214	169	128	511	58.1
Doxycycline	189	72	194	455	58.5
Ceftazidime	8	1	11	20	60.0
Amoxicillin	53	28	62	143	62.9
Co-trimoxazole	213	85	326	624	65.9
Aztreonam	63	1	243	307	79.5
Ampicillin	52	119	218	389	86.6

Table 16. Resistance pattern of *Escherichia coli* isolates to various antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Meropenem	16	-	-	16	0
Imipenem	265	6	9	280	5.4
Piperacillin/tazobactam	225	7	19	251	10.4
Cefoperazone/sulbactam	8	1	-	0	11.1
Fosfomycin	473	17	64	554	14.6
Amikacin	59	5	20	84	29.8
Ceftazidime	61	11	56	128	52.3
Cefotaxime	212	28	219	459	53.8
Aztreonam	224	30	242	496	54.8
Ciprofloxacin	235	22	285	542	56.7
Lincomycin	6	-	8	14	57.1
Gentamicin	177	29	231	437	59.5
Ofloxacin	4	-	6	10	60.0
Norfloxacin	122	19	183	324	62.3
Pipemidic acid	9	-	15	24	62.5
Cefuroxime	30	1	56	87	65.5
Co-amoxiclav	119	127	213	459	74.1
Co-trimoxazole	98	23	405	526	81.4
Cephradine	10	1	51	62	83.9
Amoxicillin	13	3	83	99	86.9
Doxycycline	39	13	268	320	87.8
Ceftriaxone	1	3	6	10	90.0
Ampicillin	21	11	220	252	91.7

Table 17. Resistance pattern of *Pseudomonas aeruginosa* isolates to various antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Piperacillin/tazobactam	135	13	12	160	15.6
Imipenem	227	13	29	269	15.6
Meropenem	156	18	15	189	17.5
Amikacin	265	60	41	366	27.6
Ciprofloxacin	271	24	108	403	32.8
Piperacillin	44	-	23	67	34.3
Aztreonam	222	33	99	354	34.3
Norfloxacin	68	3	65	136	50.0
Ceftazidime	146	61	162	369	60.4
Gentamicin	128	67	147	342	62.6
Fosfomycin	135	58	180	373	63.8
Pipemidic acid	7	-	14	21	66.7
Cefotaxime	33	35	67	135	75.6
Co-trimoxazole	7	-	22	29	75.9
Co-amoxiclav	11	1	66	78	85.9

Table 18. Resistance pattern of *Klebsiella pneumoniae* isolates to various antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Meropenem	13	-	-	13	0
Imipenem	197	1	3	201	2.0
Piperacillin/tazobactam	137	6	1	144	4.9
Fosfomycin	205	16	30	251	18.3
Amikacin	24	-	7	31	22.6
Ciprofloxacin	199	14	136	349	43
Cefotaxime	166	17	123	306	45.8
Aztreonam	190	21	142	353	46.2
Ceftazidime	78	3	74	155	49.7
Norfloxacin	81	13	95	189	57.1
Gentamicin	126	28	143	297	57.6
Co-trimoxazole	131	9	203	343	61.8
Co-amoxiclav	131	68	149	348	62.4
Doxycycline	40	18	76	134	70.1
Cephradine	2	1	33	36	94.4
Ampicillin	9	16	137	162	94.4

Table 19. Resistance pattern of *Streptococcus pyogenes* isolates to
various antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Piperacillin/tazobactam	28	-	-	28	0
Imipenem	80	-	-	80	0
Ampicillin	117	2	3	122	4.1
Ceftazidime	9	-	1	10	10.0
Cefotaxime	108	4	11	123	12.2
Penicillin	90	7	10	107	15.9
Amoxicillin	13	-	3	16	18.7
Ciprofloxacin	93	17	8	118	21.2
Co-amoxiclav	7	-	2	9	22.2
Norfloxacin	16	5	3	24	33.3
Fosfomycin	78	20	31	129	39.5
Doxycycline	48	17	20	85	43.5
Erythromycin	7	4	2	13	46.2
Lincomycin	35	11	23	69	49.3
Gentamicin	48	31	38	117	59.0
Cephradine	2	-	5	7	71.4
Co-trimoxazole	27	11	104	142	81.0

Table 20. Resistance pattern of *Enterococci* isolates to various antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Vancomycin	14	1	1	16	12.5
Piperacillin/tazobactam	48	6	4	58	17.2
Co-amoxiclav	37	4	5	46	19.6
Ampicillin	63	3	13	79	20.3
Ciprofloxacin	72	13	25	110	34.5
Imipenem	25	-	14	39	35.9
Fosfomycin	51	19	24	94	45.7
Cefotaxime	56	8	42	106	47.2
Doxycycline	20	2	20	42	52.4
Aztreonam	9	-	10	19	52.6
Gentamicin	32	18	27	77	58.4
Penicillin	10	11	8	29	65.5
Co-trimoxazole	34	8	57	99	65.7
Lincomycin	9	-	18	27	66.7
Norfloxacin	15	12	29	56	73.2
Amikacin	2	2	4	8	75.0

Table 21. Resistance pattern of *Proteus spp.* to different antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Amikacin	10	-	-	10	0
Piperacillin/tazobactam	39	2	1	42	7.1
Ciprofloxacin	49	4	5	58	15.5
Imipenem	39	-	7	46	15.2
Ceftazidime	24	1	5	30	20.0
Aztreonam	38	2	8	48	20.8
Fosfomycin	42	4	11	57	26.3
Cefotaxime	28	3	7	38	26.3
Co-amoxiclav	31	1	16	48	35.4
Norfloxacin	8	1	6	15	46.7
Gentamicin	25	3	18	46	45.7
Ampicillin	12	1	18	31	61.3
Cotrimoxazole	16	4	33	53	69.0
Doxycycline	1	4	19	24	95.8

Table 22. Resistance pattern of *Acinetobacter spp.* isolates to different antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Imipenem	12	-	3	15	20.0
Piperacillin/tazobactam	7	1	2	10	30.0
Amikacin	7	2	2	11	36.4
Ciprofloxacin	13	-	11	24	45.8
Gentamicin	15	-	18	33	54.6
Cefotaxime	9	2	9	20	55.0
Co-amoxiclav	6	-	9	15	60.0
Co-trimoxazole	5	-	11	16	68.8
Aztreonam	5	2	12	19	73.7
Ceftazidime	4	2	11	17	76.5

Table 23. Resistance pattern of *Morexalla spp.* isolates to different antibiotics

Antibiotics	Sensitive	Intermediately sensitive	Resistant	Total	Resistance %
	(No)	(No)	(No)	(No)	
Amikacin	5	-	-	5	0
Piperacillin/tazobactam	24	-	-	24	0
Co-amoxiclav	27	2	-	29	6.9
Imipenem	19	2	-	21	9.6
Cefotaxime	34	2	2	38	10.6
Ciprofloxacin	28	-	5	33	15.2
Gentamicin	25	3	4	32	21.9
Ampicillin	30	7	2	39	23.1
Ceftazidime	6	-	6	12	50.0
Fosfomycin	12	6	12	30	60.0
Doxycycline	6	6	9	21	71.5
Aztreonam	5	-	17	22	77.3
Co-trimoxazole	2	4	32	38	94.7

Table 24. Resistance pattern of *Providencia spp.* isolates to different antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Imipenem	29	-	-	29	0
Gentamicin	7	-	2	9	22.2
Piperacillin/tazobactam	21	7	-	28	25.0
Ciprofloxacin	16	5	4	25	36.0
Cefotaxime	18	3	6	27	33.3
Norfloxacin	17	7	3	27	37.0
Amikacin	10	3	3	16	37.5
Co-trimoxazole	20	4	9	33	39.4
Co-amoxiclav	10	6	4	20	50.0
Fosfomycin	14	14	5	33	57.6
Ceftazidime	6	4	5	15	60.0
Ampicillin	7	10	3	20	65.0
Aztreonam	10	13	7	30	66.7

Table 25. Resistance pattern of *Enterobacter spp.* isolates to different antibiotics

Antibiotics	Sensitive (No)	Intermediately sensitive (No)	Resistant (No)	Total (No)	Resistance %
Imepenem	8	-	1	9	11.1
Piperacillin/tazobactam	6	1	-	7	14.3
Norfloxacin	4	-	1	5	20.0
Amikacin	3	1	-	4	25.0
Ciprofloxacin	9	-	7	16	43.8
Gentamicin	6	1	4	11	45.5
Fosfomycin	8	2	5	15	46.7
Cefotaxime	8	2	7	17	52.9
Aztreonam	5	3	3	11	54.5
Ceftazidime	3	-	4	7	57.1
Co-amoxiclav	2	1	3	6	66.6
Doxycycline	1	-	4	5	80.0
Co-trimoxazole	2	-	10	12	83.3

Table 26. Prevalence of ESBLs in Gram-negative rods from various samples

Samples	Total (No)	Positive		Negative	
		No	%	No	%
Urine	163	83	51.0	80	49.0
Pus	183	76	41.6	107	58.4
Sputum	93	37	39.8	56	60.2
Blood	46	15	32.6	31	67.4
High vaginal swabs	124	26	21.0	98	79.0
Total	609	237	38.9	372	61.1

Prevalent ESBLs-Producing EGNRs

The most prevalent ESBLs-producing EGNR was *Escherichia coli*, 125 out of 263 (47.5%), followed by *Klebsiella pneumoniae*, 87 out of 193 (45%) and *Enterobacter*, 2 out 9 (22.2%) (Table 27).

Prevalence of ESBL-Producing EGNRs
in Different Samples

Escherichia coli

Most prevalent ESBL- producing organism was *Escherichia coli* with the highest frequency from pus isolates, 37 out of 63 (58.7%), followed by sputum, 21 out of 37 (56.7%), blood, 8 out of 15 (53.3%) and urinary isolates 43 out of 87 (49.4%) (Table 28).

Klebsiella pneumoniae

The second most prevalent ESBL-producing organism was *Klebsiella pneumoniae* with the highest frequency from the urinary isolates, 40 out of 58 (69.0 %) followed by pus, 27 out of 56 (48.2%) and sputum isolates, 13 out of 43 (30.2%) (Table 29).

Pseudomonas aeruginosa

The highest frequency of ESBL-producing *Pseudomonas aeruginosa* was from the sputum isolates, 3 out of 14 (21.4%) followed by vaginal, 3 out of 14 (21.4%) and pus isolates, 6 out of 43 (13.9%) (Table 30).

Table 27 . Prevalent ESBL-producing Gram-negative rods

Organisms	Total (No)	Positive	
		No	%
Escherichia coli	263	125	47.5
Klebsiella pneumoniae	193	87	45.0
Enterobacter spp	9	2	22.2
Pseudomonas aeruginosa	98	14	14.3
Proteus spp	15	2	13.3
Acinetobacter spp	14	1	7.1
Citrobacter spp	3	0	-
Salmonella species	1	0	-
Aeromonas spp	7	4	57.1
Morganella spp	2	1	50.0
Providencia spp	4	1	25.0
Total	609	237	38.9

Table 28. Prevalence of ESBL-producing *Escherichia coli*

in different samples

Samples	Total No	Positive	
		No	%
Pus	63	37	58.7
Sputum	37	21	56.7
Blood	15	8	53.3
Urine	87	43	49.4
High vaginal swabs	62	16	25.8
Total	263	125	47.5

Table 29 . Prevalence of ESBL-producing *Klebsiella pneumoniae* in different samples

Samples	Total (No)	Positive	
		No	%
Urine	58	40	69.0
Pus	56	27	48.2
Sputum	43	13	30.2
Blood	8	2	25.0
High vaginal swabs	28	5	17.8
Total	193	87	45.0

Table 30 . Prevalence of ESBL-producing *Pseudomonas aeruginosa* in different samples

Samples	Total No	Positive	
		No	%
Sputum	14	3	21.4
High vaginal swabs	14	3	21.4
Pus	43	6	13.9
Blood	15	2	13.3
Urine	12	0	0
Total	98	14	14.2

Prevalent ESBLs-Producing Organisms

The most prevalent ESBLs-producing organism was *Escherichia coli* (52.8%), followed by *Klebsiella pneumoniae* (36.8%) and *Pseudomonas aeruginosa* (5.9%).These three organisms constitute more than 95% of the total ESBLs-producing isolates (Table 31).

Prevalent ESBLs-Producing Organisms in Various Samples

Pus

Out of 76 ESBLs-producing EGNRs, 37 were *Escherichia coli* (48.7%), 27 were *Klebsiella pneumoniae* (35.5%) and 6 were *Pseudomonas aeruginosa* (7.9 %)

Urine

Out of 83 ESBLs-producing EGNR, 43 were *Escherichia coli* (51.8%) and 40 were *Klebsiella pneumoniae* (48.2%).

Sputum

Out of 37 ESBLs-positive isolates, 21 were *Escherichia coli* (56.8%), 13 were *Klebsiella pneumoniae* (35.1%) and 3 were *Pseudomonas aeruginosa* (8.1%)

Table 31. Distribution of ESBL-producing
Gram-negative rods

Organisms	No	%
Escherichia coli	125	52.8
Klebsiella pneumoniae	87	36.8
Pseudomonas aeruginosa	14	5.9
Aeromonas spp	4	1.7
Enterobacter spp	2	0.8
Proteus spp	2	0.8
Acinetobacter spp	1	0.4
Morganella spp	1	0.4
Providencia spp	1	0.4
Total	**237**	**100.0**

High vaginal swabs

Out of 26 ESBLs-positive isolates, 16 were *Escherichia coli* (61.6%), 5 were *Klebsiella pneumoniae* (19.2%) and 3 were *Pseudomonas aeruginosa* (11.5%)

Blood

Out of 15 ESBLs-positive isolates, 8 were *Escherichia coli* (53.3%), while *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were equally prevalent, 2 out of 15 (13.3%) .

The number and percentage of most prevalent ESBLs-producing organisms, *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in different samples is summarized in Table 32   .

In-patients and Out-patients Distribution in ESBL-producing Isolates

In 176 ESBL-producing isolates, source of samples in term of in-patients/out-patients was analyzed. Out of these 176 isolates, 155 (88.1%) were from in-patients while 21 (11.9%) were from out-patients .

Out of 155 in-patients ESBL-producing isolates, 72 were from pus samples (46.4%), 63 were from urine (40.6%), 15 were from sputum samples (9.8%) and 5 were from high vaginal swabs (3.2%). Out of 21 out-patients ESBL-producing isolates, 8 were from urine samples (38.0%), 6 were from pus and high vaginal swabs (28.5% each) and one was from sputum samples (4.8%) (Table   33).

Table 32. Comparison of most common ESBL-producing organisms in various samples

Organisms	HVS		Sputum		Urine		Pus		Blood	
	No	%	No	%	No	%	No	%	No	%
Escherichia coli	16	61.6	21	56.8	43	51.8	37	48.7	8	53.3
Klebsiella pneumoniae	5	19.2	13	35.1	40	48.2	27	35.5	2	13.3
Pseudomonas aeruginosa	3	11.5	3	8.1	-	-	6	7.9	2	13.3

Table 33. In-patients and Out-patients distribution of ESBL-producing isolates in different samples

Type of samples	Indoor		Outdoor	
	No	%	No	%
Pus	72	46.5	6	28.5
Urine	63	40.6	8	38.1
Sputum	15	9.7	1	4.8
HVS	5	3.2	6	28.6
Total	155	100.0	21	100.0

Regarding in-patients, out of 155 ESBL-positive isolates, *E.coli* was found to be most prevalent organism, 79 (51%) followed by *K. pneumoniae,* 62 (40%) and *P. aeruginosa* 9 (5.8%). In case of out-patients, the most prevalent ESBL-producing EGNR was *K. pneumoniae* 10 out of 21 (47.1%) followed by *E.coli,* 8 out of 21 (38.1%), *P. aeruginosa,* 2 out of 21 (9.52%) and *Salmonella spp* 1 out of 21 (4.76%) (Table 34).

Gender wise Distribution of ESBL-producing Isolates

In 165 ESBL-producing isolates, patients gender was known, 106 were females (64.3%) and 59 were males (35.7 %) . Out of 106 ESBLs-producing isolates in females, 44 were from urinary EGNRs (41.5%), 42 from pyogenic isolates (39.6%), 10 each from vaginal and sputum isolates (9.4% each). Out of 59 ESBL-producing isolates in males, 32 were from pus isolates (54.2%), 23 from urinary isolates (39%) and 4 were from sputum samples (6.8%) (Table 35).

Age wise Prevalence of ESBL-producing EGNRs

In 165 ESBL-producing isolates, the age of the patients was known which varied from 3 months to 70 years. ESBLs-producing GNRs were most frequent in 61-70 years of age group, 46 out of 165 (27.9%), followed by 41-50 years of age group, 33 out of 165 (20.0%) and 11-20 years of age group, 22 out of 165 (13.3%) (Table 36). Trends of prevalence of ESBLs-producing GNRs at different age groups is shown in Fig 11.

In case of female patients, ESBL-producing isolates were most frequent at 41-50 years of age group, 31 out of 106 (29.2%) followed by 61-70 years, 27 out of 106 (25.5%), and 51-60 years, 16 out of 106 (15.1%) (Table 37). In case of female patients, in 41-50 years of age group, ESBL-producing isolates were most frequent in urinary isolates, 14 out of 31 (45.1%) followed by ESBL-producing isolates from pus samples, 10 out of 31 (32.2%). At the age group of 61-70 years, ESBL- producing isolates were more

Table 34. Distribution of ESBL-producing organisms

Organism	In-patients	%	Out-patients	%
Escherichia coli	79	51.0	8	38.1
Klebsiella pneumoniae	62	40.0	10	47.6
Pseudomonas aeruginosa	9	5.8	2	9.5
Acinetobacter	1	0.6	-	-
Salmonella spp	-	-	1	4.8
Proteus spp	1	0.6	-	-
Providencia spp	1	0.6	-	-
Aeromonas spp	2	1.4	-	-
Total	155	100.0	21	100.0

Table 35. Gender distribution of ESBL-producing isolates

Samples	Females		Males	
	No	%	No	%
Urine	44	41.5	23	39.0
Pus	42	39.6	32	54.2
HVS	10	9.4	-	-
Sputum	10	9.4	4	6.8
Total	106	100	59	100.0

Table 36 . Overall Prevalence of ESBL-producing organisms at different age groups

Age groups (years)	No	%
0-10	9	5.5
11-20	22	13.3
21-30	18	10.8
31-40	9	5.5
41-50	33	20.0
51-60	28	17.0
61-70	46	27.9
Total	165	100.0
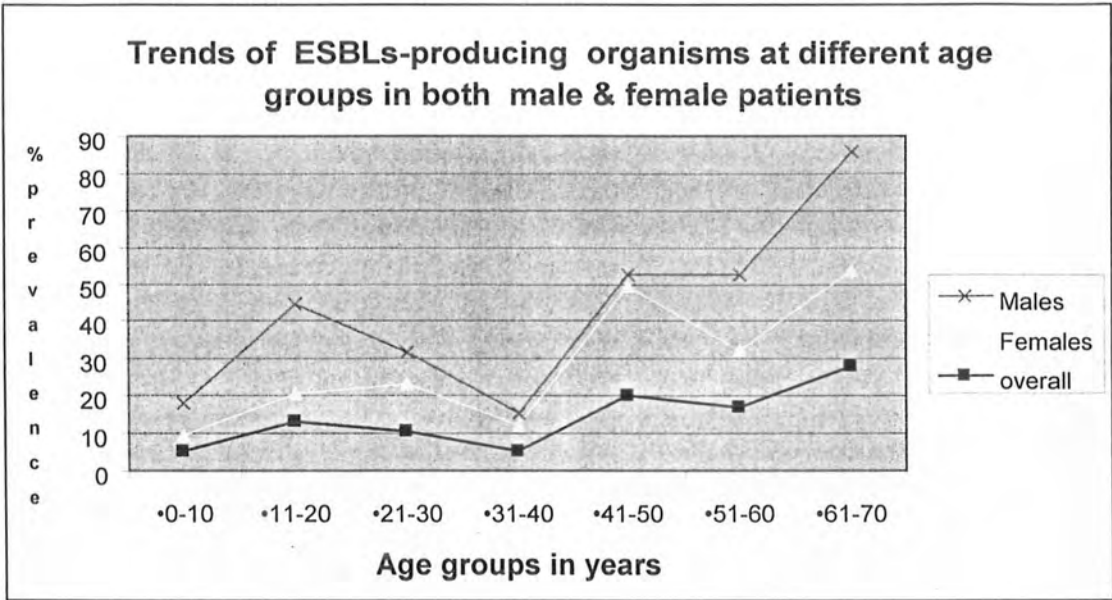
Fig 11. Trends of ESBL-producing organisms at different

age groups

Table 37 . Prevalence of ESBL-producing organisms at

different age groups in female patients

Age Groups (years)	No	%
0-10	4	3.8
11-20	8	7.5
21-30	13	12.3
31-40	7	6.6
41-50	31	29.2
51-60	16	15.1
61-70	27	25.5
Total	106	100.0

frequent in pus isolates, 13 out of 27 (48.1%) followed by urinary isolates, 9 out of 27 (33.3%) (Table 38).

In case of males, the ESBL-producing organisms were most prevalent at 61-70 years of age group, 19 out of 59 (32.2%), followed by 11-20 years, 14 out of 59 (23.7%) and 51-60 years ,12 out of 59 (20.3%) (Table 39).

In case of males, at the age group 61-70 years, ESBL-producing organisms were most common in urinary isolates, 13 out of 19 (68.4%) followed by pus isolates, 5 out of 19 (26.3%). At the age group of 11-20 years and 51-60 years, ESBL-producing organisms were most common in pus isolates, 10 out of 14 (71.4%) and 6 out of 12 (50%) (Table 40).

Sensitivity pattern of ESBL-producing and non-ESBL-producing organisms

The sensitivity pattern of ESBL-producing *E.coli* and *K.pneumoniae* to different antibiotics is summarized in Table 41 & 42. The sensitivity pattern of non-ESBL-producing *E.coli* and *K. pneumoniae* to different antibiotics is summarized in Table 43 &44. The comparison of sensitivity pattern of ESBL-producing and non-ESBL-producing *E. coli* and *K.. pneumoniae* to different antibiotics is summarized in Fig 12&13 respectively. The number of other ESBL-producing organisms was too small, so their sensitivity pattern is not mentioned.

Table 38. Distribution of ESBL-producing Organisms at different age groups in Females

Samples	Age Groups (years)						
	0-10	11-20	21-30	31-40	41-50	51-60	61-70
Urine	1	4	5	2	14	9	9
Pus	2	3	6	3	10	5	13
HVS	-	-	1	2	3	1	3
Sputum	1	1	1	-	4	1	2
Total	4	8	13	7	31	16	27

Table 39. Prevalence of ESBL -producing

organisms at different age groups in males

Age groups (years)	No	%
0-10	5	8.5
11-20	14	23.7
21-30	5	8.5
31-40	2	3.4
41-50	2	3.4
51-60	12	20.3
61-70	19	32.2
Total	59	100.0

Table 40 . Distribution of ESBLs-producing Organisms at different

age groups in different samples in males

Samples	Age Groups (Years)						
	0-10	11-20	21-30	31-40	41-50	51-60	61-70
Pus	2	10	5	2	2	6	5
Urine	3	3	-	-	-	4	13
Sputum	-	1	-	-	-	2	1
Total	5	14	5	2	2	12	19

Table 41. Sensitivity pattern of ESBL-producing *Escherichia coli*

Antibiotics	Sensitive (No)	Intermediate (No)	Resistant (No)	Total (No)	Sensitivity %
Meropenem	18	-	-	18	100.0
Imipenem	51	1	-	52	98.1
Fosfomycin	54	5	6	65	83.1
Piperacillin/tazobactam	15	1	3	19	78.9
Cefoperazone/sulbactam	12	1	3	16	75.0
Amikacin	9	7	2	18	50.0
Ciprofloxacin	13	2	38	53	24.5
Gentamicin	9	2	58	69	13.0
Doxycycline	3	2	19	24	12.5
Co-trimoxazole	4	-	32	36	11.1
Norfloxacin	1	-	15	16	6.2
Aztreonam	3	2	71	76	3.9
Co-amoxiclav	2	8	48	58	3.4
Ceftazidime	1	2	31	34	2.9
Cefotaxime	2	1	68	71	2.8
Amoxicillin	-	-	10	10	0

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Table 42. Sensitivity pattern of ESBL-producing
Klebsiella pneumoniae

Antibiotics	Sensitive (No)	Intermediate (No)	Resistant (No)	Total (No)	Sensitivity %
Cefoperazone/sulbactam	8	-	-	8	100.0
Imipenem	31	1	-	32	96.9
Fosfomycin	44	1	3	48	91.7
Piperacillin/tazobactam	10	1	-	11	90.9
Meropenem	5	-	2	7	71.4
Amikacin	6	2	2	10	60.0
Ciproflaxacin	7	3	26	36	19.4
Co-trimoxazole	4	-	22	26	15.4
Gentamicin	5	3	38	46	10.9
Norfloxacin	1	1	9	11	9.1
Aztreonam	4	2	45	51	7.8
Co-amoxiclav	2	6	31	39	5.1
Ceftazidime	1	1	29	31	3.2
Cefotaxime	1	2	49	52	1.9
Doxycycline	0	0	5	5	0
Amoxicillin	-	-	6	6	0

Table 43. Sensitivity pattern of non-ESBL-producing
Escherichia coli

Antibiotics	Sensitive (No)	Intermediate (No)	Resistant (No)	Total (No)	Sensitivity %
Meropenem	11	-	-	11	100.0
Imipenem	54	-	1	55	98.2
Piperacillin/tazobactam	22	1	-	23	95.7
Cefoperazone/sulbactam	12	1	-	13	92.3
Fosfomycin	56	-	5	61	91.8
Aztreonam	49	-	6	55	89.1
Cefotaxime	62	1	7	70	88.6
Ceftazidime	32	-	8	40	80.0
Gentamicin	57	7	12	76	75.0
Norfloxacin	12	-	7	19	63.1
Ciprofloxacin	40	2	13	55	72.7
Amikacin	6	2	1	9	66.7
Co-amoxiclav	33	12	18	63	52.4
Doxycycline	7	-	7	14	50.0
Co-trimoxazole	8	2	18	28	28.6

Table 44. Sensitivity pattern of non-ESBL-producing *Klebsiella pneumoniae*

| Antibiotics	Sensitive (No)	Intermediate (No)	Resistant (No)	Total (No)	Sensitivity %
Meropenem	4	-	-	4	100.0
Cefoperazone/ sulbactam	6	-	-	6	100.0
Imipenem	37	-	-	37	100.0
Norfloxacin	10	-	1	11	90.9
Fosfomycin	48	1	5	54	88.9
Piperacillin/tazobactam	16	3	-	19	84.2
Aztreonam	42	3	8	53	79.2
Cefotaxime	28	1	7	36	77.8
Ceftizidime	31	-	9	40	77.5
Ciprofloxacin	35	-	12	47	74.5
Gentamicin	28	7	17	52	53.8
Co-amoxiclav	30	4	17	51	58.8
Co-trimoxazole	15	-	16	31	48.4
Doxycycline	13	2	13	28	46.4
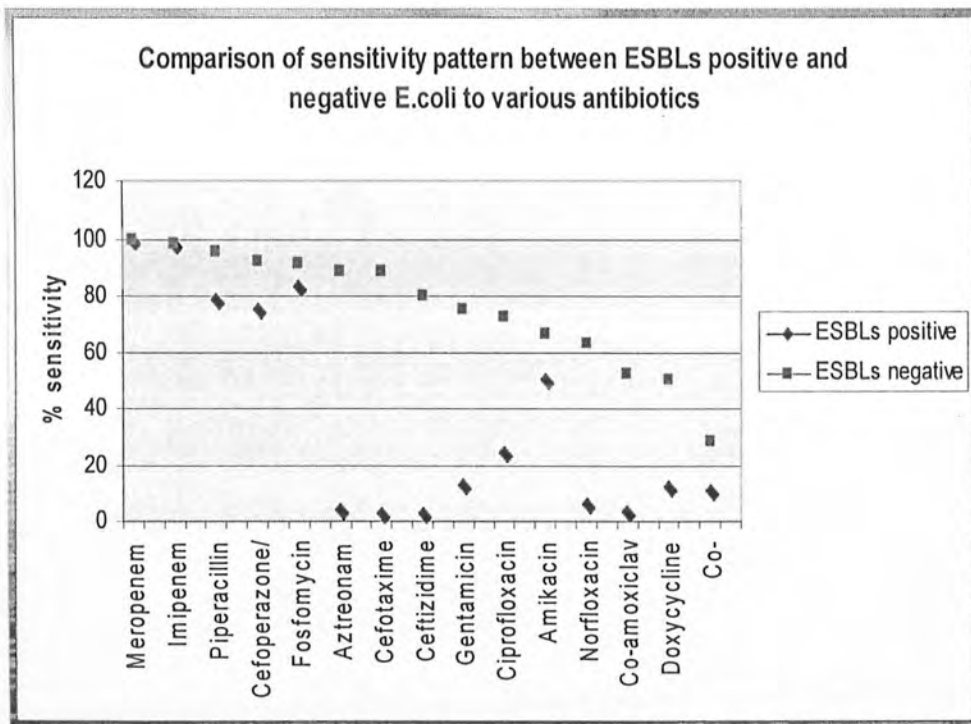
Fig 12. Comparison of sensitivity pattern between ESBL-producing
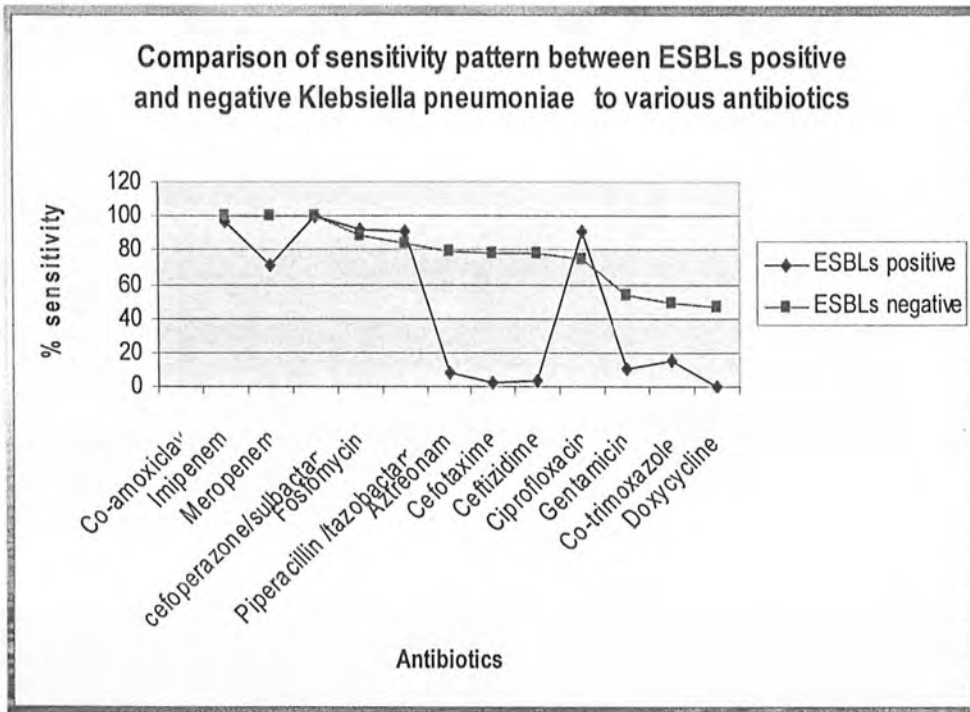and non-ESBL-producing *E.coli* to various antibiotics

Fig 13. Comparison of sensitivity pattern between ESBL-producing
and non-ESBL-producing *Klebsiella pneumoniae* to various antibiotics

CHAPTER 6

DISCUSSION

DISCUSSION

Bacterial resistance is common among clinical isolates from healthy as well as persons having community-acquired infections in developing countries. Over the past several decades, the frequency of antimicrobial resistance and its association with serious infectious diseases have increased at alarming rates. The resistance is increasing, particularly to first-line, inexpensive, broad-spectrum antibiotics.

The local trends in the development of antimicrobial resisance among the bacterial pathogens recovered from different patients with infections in the Fauji Foundation Hospital Rawalpindi were assessed during the period of 2004-2006. Surprisingly, the antimicrobial resistance rates remained relatively constant for the majority of the organisms–antimicrobial combinations examined in this study. In general carbapenems and β-Lactam β- Lactamase inhibitor combinations were found to be active agents against Gram–negative bacilli as well as Gram–positive cocci in Pakistan .While fosfomycin a drug not used routinely in clinical practice but frequently used in Fauji Foundation Hospital has a good sensitivity against both groups of organisms but *P. aeruginosa* has started gaining resistance against this rare antibiotic, pointing towards the fact that frequent use of antibiotics leads to the development of antimicrobial resistance giving a clue towards the restriction of antibiotics in routine use.

Prevalence of Infections

The overall prevalence of bacterial infection was 43.3% in Fauji Foundation Hospital Rawalpindi during the study period , comparable to the findings by Vincent *et al* (1995). Most prevalent infections were pyogenic infections (54.3%), comparable to previous findings (50.6% & 52.4%) of Asif (2003).The prevalence of pyogenic infections was 47% (Anwar *et al*, 1998) and 81.6-87.0% in various studies (Arshad *et al*, 2004; Mashita

et al, 1999; Akhter *et al,* 1997). Urinary tract infections and vaginal infections were the next most common infections (47% each). The bacteriurea in different studies ranged from 17.6-43% (Samsygina *et al,* 2000; Vincent *et al,* 1995; Farooqi *et al.* 1989; Hafiz and Lyall, 1989; Khan *et al,* 1984) and a prevalence of vaginal infections was 29% in a study by Rao *et al* , (2004). The incidence of respiratory tract infections was 43.3% in this study ,while in other studies ,the incidence was in a range of 7.4-80% (Khan *et al,* 2003; Najam *et al,* 2000; Samsygina *et al,* 2000; Saqib *et al,* 1999; Strauss *et al,* 1998; Qureshi *et al,* 1997; Vincent *et al,* 1995; Mastro *et al,* 1993; Naseer, 1992; Ghafoor *et al,* 1984). Blood stream infections were least common (10.6%) comparable to previous studies , 10.2-54 % (Aftab and Iqbal 2006; Asif. 2003; Kiani *et al,* 2002; Vincent *et al,* 1995).

Seasonal Variations and Month Wise Frequency of Infections

Many infectious diseases exhibit seasonal dynamics. Seasonal cycles of infectious diseases have been variably attributed to changes in atmospheric conditions, prevalence, virulence & transmission rates of the pathogen and the behavior of the host (Koelle *et al,* 2005).

Seasonal evaluation of the data revealed that infections were most common ($p<0.05$) in the changing weathers like spring (43%) and autumn (43.1%) as compared to summer (32.9%) and winter (35.8%). This is in good agreement with other reported studies where the peak incidence of upper respiratory tract infections, were from early fall until spring. Impetigo, a common skin infection, had shown seasonal variation in African, Australian and Indian studies. The number of impetigo cases was always higher in late summer than in winter (Loffeld *et al,* 2005; Macfarlane *et al,* 2001; Peltola, 1982). Comparatively high frequencies of urinary tract infections were found during the fall and winter (Vorland *et al.* 1985; Latham *et al,* 1983; Peltola, 1982). UTI was presented to the general practitioners more frequently in the summer and in the third calendar quarter of each year

(Anderson, 1983). Hospitalization for community-acquired pneumonia showed a significant seasonal variation with peak admission rates in March and April (Al-Muhairi *et al*, 2006). Other studies revealed no definite trend of seasonal variations and the occurrence of clinical isolates found to be scattered throughout the year (Asif 2003; Loeb *et al*, 2000; Kohn *et al*, 1995). According to Abussaud (1996), monthly infection rates in case of pyogenic infections varied, the highest rates occurring in July, incomparable to the present study, where infections were most common in September.

Prevalent Organisms

Bacterial infections are rapidly growing in the developing countries and are one of the major contributors to the burden of diseases (Raza *et al*, 2001). The distribution of pathogens, and their resistantce pattern, changes with time & among hospitals (Schaberg 1991).

More than 50% of the pathogens isolated in this study, belong to Gram-negative rods, comparable to the previous studies (Chow *et al* 2005; Schaberg *et al*, 1991). In 1970s and early 1980s, resistant Gram-negative bacteria were the major scourges and were common causes of sepsis, pneumonia, urinary tract infections and postsurgical infections.

The Gram-negative bacilli (87.5%) are still the most common pathogens in the urinary tract infections in agreement with the previous studies, (70.7-92.5%) (Khan & Shah 2000; Lazarevic *et al*, 1998; Kumamoto *et al*, 1999; Mumtaz, 1995). Major sources of uropathogenic organisms appear to be the patient's own rectal flora and the hospital environment.

Gram-negative rods and Gram-positive cocci in pyogenic infections collectively accounted for 99% of the total isolates in this study, comparable to the previous studies (Ahmad *et al*, 2005, Asif, 2003; Mumtaz *et al*, 2002 ,Mashita *et al*, 1999). Postoperative infections are a nightmare that no surgeon wants to see. They cause significant morbidity

and mortality, also increase the economic burden significantly on the patients as well as increasing the workload on the staff.

The female genital tract is a microbiologist's nightmare and usually the etiology of gynecological infections is complex. The lower genital tract contains contaminating organisms; therefore, it is very difficult to isolate the exact causative agent from vagina. Many studies carried out in the world have reported the incidence of specific and non-specific organisms in their population. The same is true from this study as well where predominant vaginal isolates were Gram-positive cocci (66.0%), Gram- negative rods (30.8%) and *Candida* spp (3.1%). This study coincided well with that of Khan & Khan (2004) where Gram-positive organisms were more common (71%) as compared to Gram-negative organisms (29%).

Blood stream infections remain one of the major challenging problems in the ICUs and are associated with significant mortality and morbidity. (Mahmood, Butt and Anwar, 2002). Blood stream infections were caused by Gram-positive cocci and enteric Gram-negative rods (49.7% each) in this study in comparable to other studies, where Gram-positive organisms were 18-72 % and Gram-negative organisms were 28-81%. (Aftab and Iqbal, 2006; Mathur *et al*, 2005; Mehta *et al*, 2005; Mamishi *et al*, 2005; Butt *et al*, 2004; Asrat and Amanuel, 2001).

The Most Prevalent Pathogens

The most prevalent pathogen isolated in this study was *S. aureus* (32.6%), followed by *E. coli* (24.7%), *P. aeruginosa* (15.9%) and *K. pneumoniae* (11.7%). *S. aureus* has an impressive capacity to colonize and persist in a range of diverse environments. It can be isolated from fomites in the hospital environment, as well as from niches in the human host, where it can exist harmlessly as a commensal, inhabiting skin or mucous membranes. It can cause a wide variety of infections in various body sites, ranging from

superficial skin infections to deep-seated infections. It was first described by Ogston in1880, since that time it has remained one of the most common cause of infection, incidence of which has been steadily increasing.

The spectrum of bacteria isolated in this study with predominance of *S. aureus* is in good agreement with other reported studies, where *S.aureus* (18.4-60%) was most predominant, followed by *E. coli* (16.2%) and *Klebsiella* spp (13.7%) (Sattar *et al*, 2005; Fluit *et al*, 2001; Mahmood, 2001; Cheong *et al*, 1995). Whereas other reported studies showed that most frequently reported bacteria were *Enterobacteriaceae* (34.4%), followed by *S. aureus* (30.1%), *P. aeruginosa* (28.7%), coagulase-negative *Staphylococci* (19.1%) and fungi (17.1%) (Vincent *et al*, 1996). In another study, *P.aeruginosa* (30.3%) was the most frequent , followed by *E. coli* (18.6%), *K. pneumoniae* (16.9%), *Acinetobacter baumannii* (8.8%) and *Enterobacter cloacae* (7.1%) (Kiffer *et al*, 2005). Other studies reported *E. coli* (25-45%) to be the most frequent isolates , followed by *K. pneumoniae* (18-25%), *P. aeruginosa* (22-28.7%), *Acinetobacter spp* (7%) and *Enterobacter spp* (7-11%) (Chow *et al*, 2005; Izhar *et al*, 2001; Karamat *et al*, 1999 ; Zafar, 1999; Omari *et al*, 1997). *E. coli* was the leading uropathogen accounting for 47.7% of the total isolates, followed by *P.aeruginosa* (20.0%), *K. pneumoniae* (14.5%), *S. aureus* (9.4%) and *Enterococci* (1.8%). This matched well with other studies (Khan & Shah 2000; Kumamoto *et al*, 1999; Barnett and Stephens 1997; Cheong *et al*, 1995; Nicolle *et al*, 1988; Gerdezi *et al* 1983). In most cases of UTI, the reservoir for uropathogenic *E.coli* is faecal flora, from which it spread to the urogenital mucosa, ascend to involve the ureters and kidneys. (Langermann and Ballou, 2001).

The most frequently isolated pathogen from wounds and abscesses in this study was *S. aureus* (44.6%) followed by *P. aeruginosa* (18.6%), *E. coli* (14.5%) and *K. pneumoniae* (10.9%), comparable to the previous reported studies, where *S. aureus* were 35- 49%, *E. coli* were 25-31%, *K. pneumoniae* were 9.5-10% & *P.aeruginosa* were 8.6-38% (Arshad *et al*, 2004; Mumtaz *et al*, 2002; Abussaud, 1996; Cheong *et al*, 1995). *S. aureus* and *P. auroginosa* together made up 83.0% of the total organisms (Ahmad *et al* 2005; Arshad *et*

al 2004). The most common pathogen isolated from brain abscess was *S. aureus* followed by *Proteus* and *E. coli* (Anwar *et al*, 1998). Asif, (2003) reported *E. coli* (34.5%), to be the most prevalent pathogen followed by *P.auroginosa* (19.6%), *K. pneumoniae* (12.7%) & *Enterobacter* (5.9%).Yura *et al,*(1986) reported *E. coli* (25.6%) to be the most prevalent pathogen followed by *anaerobes* (21.1%), *Streptococcus spp.* (14.3%), and *Staphylococcus spp.* (11.3%) . Arya *et al*, (2005) reported *S. aureus* to be the most prevalent pathogen followed by *E. coli, P. aeruginosa, Acinetobacter, K. pneumoniae* .

The most prevalent pathogen causing respiratory tract infections was *K. pneumoniae* (21.4%) followed by *S. aureus* (15.6%) & *P. aeruginosa* (15.3%). The prevalent pathogens in other studies were *H. influenzae* (73%), *Moraxella catarrhalis* (12%), *S. pneumoniae* (10%) and *H. parainfluenzae (5%)* (Butt *et al*, 2005). Qureshi *et al*, (1997) *S. pyogenes* to be the most prevalent pathogen (75.9%) followed by *S. aureus (10.3%), Klebsiella* spp *(7.7%), P. aeruginosa* (2.9%) and *H. influenzae* (1.85%). Khan *et al*, (2003) reported *S. pneumoniae* as the prevalent pathogen, followed by *Moraxella catarrhalis, H. influenzae.* As the majority of the patients in this study were in-patients, so the nosocomial pathogens were isolated more ferquently as compared to the primary pathogens (like *H.influenzae* and *S. pneumoniae)* .

The microbial flora of vagina present as extensive and diversified spectrum of pathogenic and nonpathogenic organisms as any other human tissue.The most predominant pathogen isolated from high vaginal swabs was *S. aureus* (48.7%) followed by *E coli* (14.3 %), *Enterococci* (7.7%) and *Candida species* (3.1%). In other reported studies, the most prevalent pathogen was *Enterococci* (31%), followed by *S. pyogenes* (22%), *E coli* (21%) and *candida* (11%) (Khan & Khan 2004) incomparable to the present study. Other studies showed *Candida* spp *(*21.3%-72.5%) to be the most prevalent pathogens. (Tariq *et al*, 2006; Rizvi *et al*, 2003; Balaka *et al*, 2003; Bhatti *et al*, 1995). Majority of the above organisms are known to colonize the female genital tract and may not be explained implicated with disease production and moreso in our area, where good hygiene practices are not followed. Furthermore these organisms may themselves produce disease under

certain circumstances if introduced into the foreign locations in large numbers and if predisposing factors present.

The most prevalent pathogen in bloodstream infections (BSI) in this study was *S. aureus* (40.6%) , followed by *E. coli* (18.2%) and *P. aeruginosa* (15.8 %). The results were comparable to other reported studies where *S. aureus* (20.5-32%), *E. coli* (17.2- 37%), *Klebsiella spp* (6.3-9.6%), coagulase-negative *Staphylococci* (15.6%), and *P. aeruginosa* (6.5%) were predominant pathogens (Aftab and Iqbal, 2006; Sader *et al*, 2002; Mahmood, 2001; Sader *et al*, 1999). Other studies reported Coagulase-negative *Staphylococci* to be the most prevalent pathogen (26-48.4%), followed by *S. aureus* (8-16.7%), *Klebsiella spp* (8.5-31%), *E. coli* (8.1-21%), *Pseudomonas* spp (6.7-17%), *Acinetobacter* spp (5-10%), *Salmonella* spp (3.8%) (Asrat and Amanuel, 2001; Butt *et al*, 2004). In a study by Mamishi *et al*, (2005) *Klebsiella* spp (31%) were most predominant, followed by *E. coli* (21%) and *P. aeruginosa* (17%). Kiani *et al*, (2002) recovered *S. epidermidis* as the major isolate (43%), followed by *Klebsiella spp* (28%). Decousser *et al*, (2003), established *E.coli*, *S. aureus* and coagulase-negative *Staphylococci* as the three major bacterial isolates in BSI. In a study by Mehta *et al*, (2005), the most predominant species were *P. aeruginosa* (19.7%), *E. coli* (15.2%), *K. pneumoniae* (14.9%) and *Salmonella typhi* (12.8%), followed by *S. aureus* (13.8%) and *Enterococcus feacalis* (2.3%). This variation in the types of the organisms isolated from BSI is probably of geographical distribution.

Resistance Pattern of Most Prevalent Organisms

In 20th century, resistant Gram-positive bacteria are become increasingly important pathogens. Methicillin-resistant *Staphylococci* , penicillin–resistant *Pneumococci* and vancomycin-resistant *Enterococci* are providing major challenges to the present day clinicians. *S. aureus* being the most versatile human pathogen in both hospital and community acquired infections is a major causative agent in surgical wound infections and epidemic skin diseases in new born infants (Baldwin *et al*, 1990). The infections may also be

superimposed on superficial dermatologic diseases as well (Kloos and Bannerman., 1995). Several studies have been conducted to find out the antimicrobial resistance pattern of *S. aureus* and it has been found resistant towards ß-lactam antibiotics, aminoglycosides and macrolides (Maple *et al,* 1989). More than 90% of MRSA in our clinical practice have acquired resistance to penicillinase-susceptible penicillins. ß-lactamase-resistant penicillins turned out to be the solution to this therapeutic problem in 1960s. However the situation has changed with the passage of time and a significant population of MRSA has developed resistance to this group of drugs over a period of time. About 46.8% of *S. aureus* were penicillin-resistant in this study. The prevalence of resistance is less as compared to other reported studies ,where.resistance ranged from 63-100% (Bataineh, 2006; Sattar *et al,* 2005; Shoaib *et al,* 2005; Anupurba *et al,* 2003; Akbar *et al,* 2002; Qureshi *et al,* 1997). About 86.6% of S. *aureus* was resistant towards ampicillin in the present study, comparable to previous studies (Aftab and Iqbal 2006; Orrett and Land , 2006 ; Sattar *et al,* 2005; Akbar *et al,* 2002; Garau *et al,* 2001). Mahmood *et al,* (2002) reported that none of the S *aureus* were ampicillin sensitive. Amoxicillin resistance for S *aureus* was 62.9% in this study. Moderate activity of ampicillin and amoxicillin against *S. aureus* was reported by Shoaib *et al,*(2005) and Ahmad *et al,* (2002) .The higher resistance to ampicillin is probably due to more frequent use of ampicillin then amoxicillin and with increasing use concomitant resistance also increases.

Among the aminoglycosides tested against *S. aureus,* gentamicin showed the least resistance (37.7%) followed by amikacin (47.7%) and lincomycin (50.5%), incomparable to a study by Anupurba *et al* (2003), where 39.5% of S. *aureus* were resistant to amikacin. Fifty percent of *S. aureus* were resistant towards lincomycin in this study, comparable to the previous studies (33.3%-53%) (Sattar *et al,* 2005; Anwar 2003). The other reported studies showed a variable range of gentamicin resistance (39.1%-90.5%) (Hafeez *et al,* 2004; Anwar and Bokhari, 2003; Anupurba *et al,* 2003 ; Qureshi *et al,* 2003; Mahmood *et al,* 2002; del Valle *et al* ,1999).Although the usual pattern of bacterial resistance towards gentamicin is higher then other aminoglycosides; this higher resistance to amikacin may be due to increasing use of this antibiotic in our setup.

About 65.9% of *S. aureus* were resistant to co-trimoxazole; comparable to other reported studies, where the resistance ranged from 4.7-97% (Anupurba *et al*, 2003; Qureshi *et al*, 2003; Mahmood *et al*, 2002; Garau *et al*, 2001; del Valle *et al*, 1999 ; Qureshi *et al*. 1997). The resistance of *S. aureus* towards doxycycline was 58.5% comparable to other reported studies where it ranged from 21.3-100% (Orrett and Land 2006; Anupurba *et al*. 2003 ; Qureshi *et al*. 2003 ; del Valle *et al*, 1999; Qureshi *et al*. 1997). The resistance of erythromycin towards *S aureus* was 29.6%. In other studies the resistance varies from 50-88% incomparable to the present study (Orrett and Land 2006; Hafeez *et al*, 2004; Qureshi *et al*. 2003; Anwar, 2003; Anupurba *et al*, 2003; Mahmood *et al*, 2002; Akbar *et al*. 2002; Asrat and Amanuel, 2001;del Valle *et al*, 1999).

Cephalosporins are among the most frequently prescribed antibiotics because of their broad-spectrum of antimicrobial activity and proven efficacy against variety of infections (Klein and Cunha 1995). Gram-positive bacteria are usually sensitive to first-generation cephalosporin (Fong *et al*, 1976; Chambers, 2004; Khan and Bangash 2003). But in this study 51.9% of S. *aureus* isolates has gained resistance to cephradine, due to the increasing use of this antibiotics in resistant strains of S. *aureus* . About 28.0% of them were found resistant to cefuroxime. This resistance is much more as compared to a study by Garau *et al* (2001), where resistance was 13.5%. Third generation cephalosporins showed less activity against S *aureus* (cefotaxime 29.8% and ceftazidime 60.0%) in the present study, indicating that cefotaxime was found to be more effective as compared to ceftazidime. This finding was consistence with other findings (Jones and Thornberry, 1982). Experimental models show that the selective pressure exerted by broad-spectrum cephalosporins brings about a rapid overgrowth of *Staphylococci* that are resistant to the antibiotics used (Edlund and Nord 1991).

Methicillin- sensitive *Staphylococci are* generally susceptible to the flouroquinolones, but methicillin-resistant *Staphylococci* are often resistant. Among flouroquinolones, the resistance of *S. aureus* was highest towards enoxacin (56.0%) followed by norfloxacin (39.5%) and ciprofloxacin (36.8%). Variable results were observed in other reported studies, regarding the resistance of *Staphylococci* towards ciprofloxacin (13.4-89.7%)

(Aftab and Iqbal, 2006; Mehta *et al,* 2005;.Hafeez *et al,* 2004; Qureshi *et al* 2003; Anupurba *et al,* 2003; Mahmood *et al,* 2002; Garau *et al,* 2001; del Valle *et al,* 1999).

Carbapenems has a wide spectrum with good activity against many Gram-negative rods, Gram-positive organisms and anaerobes. These are indicated for infections caused by susceptible organisms, which are resistant towards other available drugs and for the treatment of mixed aerobic and anaerobic infections (Chambers, 2004). Even the carbapenems have not been immune to bacterial resistance. The organisms with β-lactamases (especially with zinc metalloenzymes) are now capable of hydrolyzing even the most potent carbapenems such as imipenem and meropenem. This resistance is evident from this study as well , where resistance has been gained by 13.4% of *S. aureus,* comparable to previous studies, where the resistance ranges between 10.9-18% (Bataineh, 2006; Aftab and Iqbal, 2006). Fosfomycin, a rarely used drug in clinical practice has gained resistance towards *S. aureus* (24.3%), which is comparable to a study by Ahmed *et al* (2003), from Karachi where resistance was 38.5%. The resistance was very high when compared to del Valle *et al* (1999), where only 1.2% of *S. aureus* were resistant to fosfomycin. The increasing resistance to imipenem and fosfomycin is due to increasing use of these antibiotics in resistant strains of S. *aureus* during the last few years.

ß-lactam ß-lactamase inhibitor combinations are indicated as an empirical therapy for infections caused by a wide range of potential pathogens in both immunocompromised and immunocompetent patients (Chambers, 2004). These compounds have limited activity but have an importance because of their ability to limit the destructive action of ß-lactamases against more active ß-lactam compounds. (Williams, 1997). In this study 58.1% of S. *aureus* were found resistant to co-amoxiclav, comparable to the previous studies, where it ranged from 61-90% (Shoaib *et al,* 2005; Mumtaz *et al,* 2002; Ahmad *et al,* 2002; Garau *et al,* 2001). While piperacillin/tazobactam showed very low resistance against S. *aureus* (4.9%) and no resistance was noted against cefoperazone/sulbactam

comparable to other studies (Akbar *et al*, 2002), may be due to infrequent use of these drugs against *S. aureus*.

The worldwide incidence of antibiotic-resistant bacteria e.g methicillin-resistant *S. aureus* (MRSA) and vancomycin–resistant *Enterococci* (VRE) is increasing. MRSA is the most problematic nosocomial pathogen by virtue of its multiple drug resistance. The effectiveness of penicillin & cephalosporins along with the other drugs like quinolones, aminoglycosides, macrolides, tetracycline and sulphonamides is reduced in such settings. MRSA is commonly isolated from post-operative infections. Such patients become a real problem for the treating clinicians as the bacterium is very difficult to be eradicated from the hospital wards (Livermore, 1991). The incidence of MRSA in this study was 29.6% comparable to previously reported studies (4.3-54.8%) (Orrett and Land, 2006; Bukhari *et al* , 2004; Hafeez *et al*, 2004; Anupurba *et al*, 2003; Hsueh *et al*, 2002; Akbar *et al*, 2002; Mahmood *et al*, 2002; Khatoon *et al*, 2002; Fluit *et al*, 2001; Asrat and Amanuel, 2001). About 2.2% of *S. aureus* were vancomycin- resistant and 2.8% of them were teicoplanin resistant in this study. No resistance to vancomycin was reported in other studies (Majeed and Izhar, 2005; Mehta *et al*, 2005; Bhateja *et al*, 2005; Kacmaz and Aksoy, 2005; Hafeez *et al*, 2004; Butt *et al*, 2004; Ahmad *et al*, 2003; Anupurba *et al* 2003; Qureshi *et al*, 2003; del Valle *et al*, 1999; Hsieh, 2000). However only 0.12-0.3% resistance was reported by Anwar and Bokhari (2003) and Jones *et al*, (2002). A still higher (4%) resistance to vancomycin was reported by Bukhari *et al*, (2004). Our results showed that not all *Staphylococci* were sensitive to the glycopeptide antibiotics. This indicates that moderate-level resistance to glycopeptide antibiotics in these organisms does exist in our hospital. Because glycopeptides are the main drugs with reliable activity, against methicillin- resistant strains of this organism, the emergence of *S. aureus* strains with intermediate resistance to glycopeptides has aroused concern about the development of strains resistant to all available antibiotics. The only choice left for these strains will be linezolid and quinopristin-dalfopristin.

Results of present study indicate that *S. aureus* strains were highly resistant to commonly used antibiotics like ampicillin, amoxicillin, co-trimoxazole, doxycycline and co-amoxiclav. According to other studies multidrug resistant strains have been reported with increasing frequency world wide, including those resistant to methicillin, macrolides, amino-glycosides, fluoroquinolones or combinations of these antibiotics (Mumtaz *et al* 2002; Akhtar et *al*, 1997). The emerging resistance in *Staphylococci* against these drugs worldwide necessitates strict surveillance of these organisms, institution of effective infection control policies and judicious use of antibiotics.

The emergence of resistance to antimicrobial agents is a global public health problem and it results in increased illness, deaths and health-care costs (Fridkin *et al*, 1999; Emery and Gaynes, 1993). The increasing number of immunocompromised patients and increasing use of indwelling devices as well as widespread use of antimicrobial agents in hospital settings, particularly in intensive care units contributes to antimicrobial resistance among bacterial pathogens (Fridkin *et al*, 1999). Inducible ß-lactamases have been responsible for multiple ß-lactam resistance among the isolates of *Enterobacteriaceae* and *P. aeruginosa*. Penicillinase was first described in a strain of *E. coli* in 1940. Since that time Gram-negative bacilli are gaining resistance day by day, acquiring even new means of resistance .Mutated ß-lactamases (i.e Extended-spectrum beta-lactamase enzymes) are continuously transffered by transmissible plasmids to other sensitive enteric Gram–negative bacilli so transferring the resistance among them. These enzymes are of tremendous clinical significance as they can confer resistance to broad-spectrum ß-lactam antibiotics, including 3rd & 4th generation cephalosporins, monobactams and extended-spectrum penicillins. Outbreaks were caused by multidrug resistant *Klebsiella* carrying a TEM-3 gene (Brun-Buisson *et al*, 1987). Patients with septicemia due to ESBL-producing organisms had a significantly higher fatality rate than those with non-ESBL isolates (71% *vs* 39%) (Blomberg *et al*, 2005).

Of major concern for physicians and the public, is the emergence of drug-resistant strains of *E. coli, K. pneumoniae* and *P. aeruginosa*. The prevalence of such bacteria has dramatically increased worldwide. Although resistance is highest to penicillin, it is

increasing rapidly towards other antibiotics as well. *E. coli* resistance in Pakistan is much higher than reported from western literature. Multidrug-resistant isolates are very common. The orgainisms have gained resistance against most of the commonly prescribed broad-spectrum ß-lactam antibiotics, including 3rd & 4thgeneration cephalosporins, monobactams and extended-spectrum penicillins.Ampicillin was found to be the most resistant antibiotic against *E coli* (91.7%) in the present study. These findings are in agreement with other reported studies where 78.5-100% of *E. coli* were found resistant towards ampicillin and amoxicillin (Butt *et al,* 2004; Mehmood *et al*, 2002 ; Iqbal *et al* 2002) . Ampicillin was widely used in our country resulting in the development of resistance against EGNR. Of all antibiotic drugs, ampicillin had a very significant role in the therapy of urinary tract infections. However, its long-term usage led to increased resistance (Lazarevic *et al,* 1998).

A high rate of resistance (70%) was observerd for co-amoxiclav by *E coli* in the present study, comparable to a previous study (Asrat and Amanuel, 2001) . Co-amoxiclav being the most commonly prescribed antimicrobial drug and because of its wide usage, Gram-negative bacteria have started gaining high rate of resistance. Other β-lactam β-lactamase inhibitor combinations, like cefoperazone/sulbactam and piperacillin/ tazobactam as they are relatively new antibiotics and are infrequently used , the organisms has developed least resistance (10.4%- 11.1%) towards them comparable to the previous studies (17%) (Mehta *et al,* 2005; Fluit *et al,* 2001).

Cephalosporins, either second or third generation were considered as alternatives for infections, non-responsive to standard treatments but now most of *E. coli* and *K.pneumoniae* isolates have gained multiresistance including third generation cephalosporins. In this study among 3rd generation cephalosporins, *Escherichia coli* showed highest resistance towards ceftriaxone (90.0%), followed by cefotaxime (53.8%) and ceftazidime (52.3%), incomparable to a previous study where at least 99% of *E. coli* isolates were susceptible towards ceftriaxone (Fluit *et al,* 2001). In other reported studies resistance of *E coli* was 25-34% (ceftriaxone), 36% (cefotaxime) and 28% (ceftazidime)

respectively (Aftab and Iqbal, 2006; Butt *et al*, 2004; Iqbal *et al*, 2002). The resistivity of all the cephalosporins against the pathogens has dramatically increased in the previous years e.g. resistance of cefotaxime, has increased from 0% against all the Gram-negative urinary pathogens to 61.8 % against *E. coli*, 65.5% against *K pneumoniae*, 51.3% against *P.aeruginosa* and 55.6% against *Acinetobactor* spp (Khan & Ahmed, 2001; Goldstein, 2000; Khan & Shah, 2000; Lazarevic et *al*, 1998; Mumtaz, 1995; Farooqi *et al*, 1989). Ceftazidime had a resistivity of 29.4% and ceftriaxone had a resistivity of 14% against Gram-negative species (Mamishi *et al*, 2005; Sader *et al*, 1999).

Monobactams possess ß-lactam ring which are relatively resistant to ß–lactamases. They are active against Gram-negative rods but possess no activity against Gram-positive bacteria or anaerobes. Fifty five percent of *E. coli* were resistant to monobactams incomparable to previous studies (17 %- 25%) Garrabe *et al* (2000; Iqbal *et al* 2002).

A very broad-antibiotic resistance pattern extending to many classes of drugs (aminoglycosides, trimethoprim, sulfonamides, tetracyclines and fluoroquinolones) has been found in majority of Gram-negative rods in this study pointing towards the fact that majority of them are ESBLs-producers, leading to the development of "multidrug resistant organisms," having extremely limited antibiotic options for their treatment.

In case of co-trimoxazole, 81.4% of the *E. coli* were resistant, comparable to previous studies, where most of *E. coli* were resistant towards trimethoprim/sulfamethoxazole (Mamishi *et al*, 2005; Rafiq *et al*, 2002; Mehmood *et al*, 2002; Iqbal *et al* 2002; Asrat and Amanuel, 2001). Doxycycline resistant *E. coli* were 87.8 %, higher than the previous studies, where 61% of EGNR were resistant (Asrat and Amanuel, 2001).

Among fluoroquinolones, *E. coli* showed the highest resistance against norfloxacin (62.3%) , ofloxacin (60.0%) and ciprofloxacin (56.7%) , comparable to the previous studies (Aftab

and Iqbal, 2006; Iqbal *et al* 2002; Quereshi *et al*, 1997). These findings are not in line with results of Rafique *et al* (2002) who reported 47.0% resistance in *E. coli* isolated from UTI.

Aminoglycosides are most widely used against gram-negative enteric bacteria, especially *Pseudomonaus, Enterobacter, Serratia, Acinetobacter* and *Klebsiella*. At present gentamicin is employed mainly in severe infections e.g sepsis and pneumonia by Gram-negative bacilli that are likely to be resistant to other drugs. In this study, 59.5% of *E. coli* were resistant to gentamicin followed by amikacin (29.8%). The results in terms of other studies were 35-86% (gentamicin) and 53-72% (amikacin) (Butt *et al*, 2004; Mehmood *et al*, 2002). In different studies the range of activity of amikacin against *Enterobacteriaceae* was 76.6-99% (Mehta *et al*, 2005; Mamishi *et al*, 2005; Chow et *al* 2005; Fluit *et al* 2001; Asrat and Amanuel, 2001; Zafar, 1999).

About 14.6% of *E. coli* has gained resistance to fosfomycin. A study by Garau *et al* (2001) showed that fosfomycin is the antibiotic with the highest activity against *E. coli* (95.5%). Fosfomycin is a good alternative that should be considered for the treatment of non-complicated lower UTI (Garau *et al*, 2001). Because of infrequent usage of fosfomycin, the organism have gained least resistance and can be a good alternative for the treatment of serious, life -threatening infections in addition to carbapenems and ß-lactam ß –lactamase inhibitor combinations. But there is a rapid selection of resistance to fosfomycin, rendering it unsuitable for most clinical purposes (Chamber 2004). So this antibiotic needs to be used cautiously and in selected patients, otherwise the result will be like that of rifampicin in tuberculous patients.

Carbapenems have a wide-spectrum with good activity against many Gram-negative rods, including *P. aeruginosa*. These are indicated for infections caused by susceptible organisms that are resistant to other available drugs. Imipenem is resistant to most extended-spectrum ß-lactamases; therefore it has been successfully used against ESBL-producers *in vivo*. In this study, none of the *E.coli* showed resistance towards meropenem, while 5.4% of the *E.coli* were resistant towards imipenem.. Aftab and Iqbal

(2006) reported that 12-20% of *E.coli* were resistant towards imipenem and meropenem . Similar pattern of results were obtained by other researchers (Iqbal *et al*, 2002; Izhar *et al*, 2001; Fluit *et al*, 2001; Karamat *et al*, 1999). Imipenem was the most effective antibiotic against Enterobacteriaceae (100% susceptibility), reported by Garrabe *et al*, (2000).

Among most commonly used antibiotics, *K. pneumoniae* showed highest resistance against ampicillin and cephradine (94.4% each), which is a well known fact. The next being doxycycline (70.1%), followed by co-amoxiclav (62.4%), co-trimoxazole (61.8%), gentamicin (57.6%), norfloxacin (57.1%), ceftazidime (49.7%), aztreonam (46.2%), cefotaxime (45.8%), ciprofloxacin (43%) and amikacin (22.6%). Fosfomycin (18.3%), piperacillin/tazobactam (4.9%), imipenem (2.0%), meropenem (0%) were found to be most effective antibiotics against *K. pneumoniae* in this study.

K. pneumoniae is found in human and animal gastrointestinal tract and is associated with UTI, wound infection, bacteremia and nosocomial infections. Predisposing factors frequently include pregnancy, urinary tract instrumentation, long-term bladder catheterization, manipulation or obstruction and underlying conditions such as diabetes mellitus (Connier and Manuselis, 1995 ; Liverelli *et al*, 1996). Hundered percent resistance against ampicillin has been observed among *Klebsiella* spp from blood isolates in a tertiary care hospital (Jain *et al*, 2003). These findings are in line with the results of Masood *et al* (2002), who isolated *K pneumoniae* from patients with urinary tract infections associated with long-term catheterization and spinal cord injuries, which were 100% resistant to ampicillin. Whereas 99 -100% resistance was seen in other studies (Butt *et al*, 2004). According to Asrat and Amanuel, (2001) Gram-negative bacteria showed a high rate of resistance towards many of the commonly prescribed antimicrobial drugs: amoxicillin/clavulanic acid (65%), ampicillin (87.5%), and amoxicillin (91.7%) . *Klebsiella spp* showed a resistance rate of 61.8% against trimethoprim / sulfamethoxazole in the present study ,comparable to previous studies (39-64%) (Mamishi *et al*, 2005; Jain *et al*, 2003; Iqbal *et al*, 2002; Asrat and Amanuel, 2001).

In case of fluoroquinolones, ciprofloxacin was found to be more effective with the resistance rate of 43% as compared to norfloxacin (57.1%). These findings are not in agreement with Al-Lawati *et al* (2000), who reported 20% resistance in *K. pneumoniae* against ciprofloxacin. Jain *et al* (2003), reported 18.5% resistance in *Klebsiella* spp, isolated from septicaemic neonates.

Among aminoglycosides, amikacin was found to be an effective antibiotic,(resistant rate 22.6%) as compared to gentamicin, (resistance rate 57.6%). While Al-Lawati *et al* (2000), reported 10% resistance rate. Masood *et al* (2002) claimed 100% susceptibility rate among *K. pneumoniae* isolated from urine samples. Elhag *et al* (1999), reported 36.0%. of *K. pneumoniae* resistant towards gentamicin .

Among cephalosporins, cephradine exhibited marked resistance (94.4%) in the present study. The resistance rate of 45.8% and 49.7% was observed for cefotaxime and ceftazidime respectively. Other studies reported about 60% resistance in *Klebsiella spp*, against both cefotaxime and ceftazidime (Jain *et al* 2003; Elhag *et al,* 1999). The susceptibility of *Klebsiella spp* to ceftriaxone was 47%, (Mamishi *et al,* 2005).   According to Zafar (1999), most of the isolates of *E. coli* and *K. pneumoniae* were multiresistant including third generation cephalosporins.

In this study, imipenem an.d meropenem proved to be the most effective antibiotics against *K. pneumoniae*, with resistance rate of 0-2% respectively. Similar findings were also reported by other researchers (Al-Lawati *et al,* 2000; Elhag *et al,* 1999). *E. coli* and *K. pneumoniae* were highly susceptible to carbapenem and their resistance among the *Enterobacteriaceae* is still rare in this region. Since they play an important role in nosocomial infections in this environment, the use of empirical combination therapy to treat these pathogens may be justified (Kiffer *et al,* 2005, Karamat *et al,* 1999).

Among the penicillin group, co-amoxiclav, piperacillin, piperacillin /tazobactam were tested against *P. aeruginosa* isolates. Most of the isolates (85.9%) in this study were co-amoxiclav

resistant comparable to Akhtar (1999), where co-amoxiclav resistance was 82.4%. The present study shows that piperacillin is no longer an effective antipseudomonal agent among penicillin group with resistance of 34.3%, incomparable to previous studies, where piperacillin was found to be one of the most effective drugs against *P. aeruginosa* isolates (Al-Lawati *et al*, 2000; Elhag *et al*, 1999). In the present study the sensitivity of piperacillin towards *Pseudomonas* was 65.7% while when combined with tazobactam, the sensitivity was increased to 84.4%. The results in terms of other studies were 63.8-85% (Kiffer *et al*, 2005; Fluit *et al*, 2001) comparable to the present study. However, incomparable to the results of Karamat *et al* (1999), where piperacillin/tazobactam showed an activity of 36% towards nosocomial *P. aeruginosa.*

Gram-negative bacteria showed a high rate of resistance to many of the commonly prescribed antimicrobial agents. Aminoglycosides once used for infections by *P. aeruginosa* isolates are no more effective,evident from the present study, where 27.6-62.6% of *P. aeruginosa* isolates were resistant towards amikacin and gentamicin. A resistance rate of 8-40% has been reported in other studies (Kiffer *et al*, 2005; Fluit *et al*, 2001; Garrabe *et al*, 2000; Akhtar, 1999).

Among third generation cephalosporins, cefotaxime (resistance-75.6%) was found to be less active against *P. aeruginosa* isolates, as compared to ceftazidime (resistance-60.4%). Other workers have obtained variable results with this antibiotic(Murray *et al*, 1993). One of the previous study showed, percentage susceptibility of 31.8-79.4% (cefotaxime *versus* ceftazidime) for *P. aeruginosa* isolates (Akhtar, 1999).According to Manchanda et *al*, (2005) multi-drug resistance was noted in 94 % of isolates .During the last two decades, the sensitivity of third generation cephalosporins, cefoperazone and cefotaxime have dramatically decreased against *P aeruginosa* in developed countries. (Pfaller and Jones, 2000; Goossens, 2000).This development of resistant by *P aeruginosa* might be due to wide spread use of these compounds (Fung-Tome *et al*, 1989).

Thirty four percent of *P. aeruginosa* isolates were resistant towards aztreonam (34.3%), incomparable to previous reported studies where 81.2-90% of isolates were sensitive to aztreonam (Akhtar, 1999; Qureshi *et al*, 1997). Aztreonam which had 100% sensitivity against urinary pathogens particularly *P.ae*ruginosa in the previously reported studies has decreased to 10-64.3% (Mumtaz, 1995; Farooqi *et al*, 1989).

About 63.8% of the *P.aeruginosa* isolates were resistant to fosfomycin, while other organisms like *E.coli* (14.6%), *K.pneumoniae* (18.3%) and *S. aureus* (24.3%) showed less resistance towards fosfomycin. *P.aeruginosa* is among those organisms which develop resistance more commonly towards antibiotics. In this study the higher resistance may be due to the fact that this antibiotic is more commonly used in our set up.

About 15.6-17.5% of *P.aeruginosa* isolates were resistant towards imipenem & meropenem. Whereas other studies have reported 100% sensitivity towards to imipenem (Japoni *et al* 2006; Chow *et al* 2005; Butt *et al*, 2004; Fluit *et al* 2001; Zafar, 1999; Karamat *et al*, 1999). According to Sader *et al*, (1999) the most active antibiotics against *P. aeruginosa* isolates were meropenem (94.1%) and piperacillin/tazobactam (84.3%). According to Sader *et al*, (2002) *P.aeruginosa* resistance rates to meropenem and piperacillin/tazobactam showed a significant increase and resistance varied according to the countries.

Most of the isolates of dominant species among Enteric Gram-negative bacilli (like *E. coli* , *K. pneumoniae, P.aeruginosa*) were found multiresistant in this study. But they are still sensitive towards two groups of antibiotics like carbapenems and β-lactam- β-lactamase inhibitor combinations which can be used in the initial empiric therapy in any life threatening bacterial infections caused by these bacilli comparable to the conclusions of Zafar, 1999 ; Garrabe *et al*, 2000). However, overuse of these drugs again is not without risk so should be cautiously used to prevent the development of resistance against them (Elhag *et al* , 1999).

Extended-Spectrum ß-lactamases (ESBLs)
in Gram-Negative Rods

The incidence of ESBL-producing strains among clinical isolates has been steadily increasing over the past few years. Major outbreaks involving ESBLs strains have been reported from all over the world, thus making them emerging pathogens. The increased incidence of infections due to these organisms is the result of frequent use of broad-spectrum ß-lactams..With the spread of ESBL-producing strains in the hospitals all over the world, it is necessary to know the prevalence of these strains in a hospital so as to formulate a policy of empirical therapy in high risk units where infections due to resistant organisms is much higher .

Prevalence of ESBLs in Gram-Negative Rods

Out of 609 Enteric Gram- negative rods from various sources, the prevalence of ESBL was 38.9%. Variable results have been reported in other studies, 4.3- 89% (Japoni *et al*, 2006 ; Sorlozano *et al*, 2006; Grover *et al*, 2006; Yan *et al*, 2006 ; Luzzaro *et al* ,2006; Manchanda *et al*, 2005; Mathur *et al*, 2005; Shah *et al*, 2004 ; Ali *et al*, 2004; Jabeen *et al*, 2003; Zaman *et al* 1999;Coudron *et al*, 1997). According to Touati *et al* (2006), the out of 365 isolates of Enterobacteriaceae, only five cases were confirmed as ESBL - producers. During a five-year surveillance study in northern France, the overall proportion of ESBL-producers was 11.4% *Klebsiella species* and 47.7% in the 2353 strains of *E. aerogenes* (Albertini, 2002).

About 51% of the isolates from urine cultures, 41.6% from pus isolates, 40% from sputum, 32.5% from blood and 21.0% from vaginal isolates were ESBL-producers in the present study. According to El-Khizzi & Bakheshwain, (2006) from Riyadh, Saudi Arabia., 15.8% of the isolates from blood cultures were ESBL-producers. In another

study most ESBL-producing pathogens were obtained from urinary tract infections, comparable to the present study (Luzzaro *et al*, 2006). According to Pena *et al* (2006), from Barcelona, Spain, infection developed in 68% of the hospitalized patients, with surgical site (44%) and urinary tract (17%) infections being the most frequent.

The highest incidence of infection by ESBLs-producing EGNRs was seen at 61-70 years of age group, followed by 41-50 . The persons are immunocompromised at later life and any infection occurring will be more serious and life threatening at this age.

Prevalence of ESBL-producing Strains in Various Species of Enterobacteriacae

Prevalence of ESBL- producing strains in various species of Enterobacteriacae differs in different countries & in different hospitals. Usually one of the three species (*Klebsiella spp, E. coli, Enterobacter spp*) predominates. The most prevalent EGNR producing-ESBLs in this study was *E. coli* (47.5%), followed by K.*pneumoniae* (45%), *Enterobacter spp* (22.2%), *P. aeruginosa* (14.2%), *Proteus spp* (13.3%) & *Acinetobacter* spp (7.14%). The frequency of ESBLs-producing Gram-negative bacilli in the present study is similar to that reported by Shah *et al* (2002), from Islamabad, Pakistan with *E. coli* (48%) being the most prevalent organism. In other repoted studies, the prevalence of ESBL-producing *E. coli* ranges from 7-28.5 % (Sorlozano et *al*, 2006; Chow et *al*, 2005; Shah *et al*, 2004).

There is a marked increase in the incidence of infections due to ESBL-producing *E coli*. Calbo *et al*, (2006) reported that ESBLs were present in 25% of *E. coli* and 17% of *K. pneumoniae* isolates. *Klebsiella* spp (80%) were the most common ESBL-producing organisms reported from Armed Forces Institute of Pathology, Rawalpindi and all India institute of Medical Sciences, New Delhi (Zaman *et al*, 1999; Mathur *et al*, 2002) . In a study by Coudron *et al*, (1997), ESBL-producing EGNR include *K pneumoniae, E coli,*

P. mirabilis, Enterobacter species, Citrobacter freundii, P. aeruginosa, Acinetobacter and *Stenotrophomonas maltophilia* . Lucet *et al* (1996) reported *K. pneumoniae* as the most prevalent ESBLs-producing organism.Shah *et al* (2004) from Quaid-i-Azam University Islamabad, reported 70% of *K. pneumoniae* isolates to be ESBL-producers. El-Khizzi & Bakheshwain (2006) from Riyadh Saudi Arabia reported 48.4% of *K. pneumoniae* isolates from blood cultures were ESBLs-producers, followed by *E. coli* (15.8%) and *E. cloacae* . *E. cloacae* (79%) was the most frequent ESBL-producer in other reported studies (Ali *et al*, 2004; Chanal *et al*, 1996).

Secondary β-lactamases has been reported in *P. aeruginosa* but rarer than in other EGNR. According to Yan *et al* (2006) 3.5% of *P aeruginosa* were found to be ESBLs-producer. Incidence rate of 13% from France, 7% from Spain and 2.5% from England (William *et al*, 1984) have been reported. In the present study, the incidence of ESBLs in *Pseudomonas* spp was 14.2% which is comparable to the above mentioned studies.

Distribution of ESBL-producing Isolates in In-patients & Out-patients

ESBL-producing strains of Enterobacteriaceae have emerged as a major problem in hospitalised as well as community based patients. (Chaudhury, 2004; Rodriguez-Bano *et al*, 2004; Bhattacharya, 2006). Over the past decade, these have emerged as serious nosocomial pathogens throughout Europe (Livermore, 1996). Outbreaks have occurred among the critically ill patients in intensive care units (Jacoby, 1997). The ESBLs–producing strains are usually found in those areas of hospitals, where antibiotic use is heavy and patient's condition is critical (Thomson *et al*, 1996).Their survival in the hospital environment lead to nosocomial infections (Hobson *et al*, 1996). These infections occur in the patients through the administration of extended-spectrum ß-lactam antibiotics or via transmission from other patients, as well as through health care workers. (Coulter *et al*, 1995) . In the present study the ESBL-producing isolates were more

commonly isolated from in-patients (88.1%) as compared to out-patients (11.9%). According to Spencer *et al,* (1987), more than half of the patients were colonized after 30 days stay in the hospital. Apart from ICUs; ESBL-producing strains have also been isolated from patients in general wards and nursing homes. According to Luzzaro *et al* (2006), the prevalence of ESBLs was 7.4% among in-patients and 3.5% among out-patients.

ESBL-producing *E. coli* was the most prevalent organism (51%) in in-patients, followed by *K. pneumoniae* (40%) and *P. aeruginosa* (5.8%).While in out-patients *K. pneumoniae* (47.1%) was the most prevalent ESBLs-producers, followed by *E coli* (38.1%) and *P. aeruginosa* (9.52%).According to Calbo *et al* (2006), the prevalence of community-onset ESBL-producing *E coli* in UTI increased from 0.4% in 2000 to 1.7% in 2003,showing that it has shifted from 50% in the first period to 79.5% in 2003 . According to Pena *et al* (2006), from Barcelona, 68% of the hospitalized patients develop infection, yielding one or more clinical isolates of ESBLs-producing *E coli*. A significant increase in the incidence of ESBL-producing *E coli* colonization or infection was observed by him during his study period. Luzzaro *et al,* (2006) reported that among hospitalized patients, the most prevalent ESBL-producer was *E coli*, while in his previous study in 1999, *K. pneumoniae* was most prevalent ESBL-producer . Sorlozano *et al,* (2006) reported 16.3% of ESBL-producing *E coli* from out-patients. Whereas Lin *et al*, (2006) reported that ESBL was rare in community-acquired *K pneumoniae* infection .

Cumulative Sensitivity Pattern of ESBL-producing
E. coli & K. pneumoniae

ESBL-producing strains are resistant to a wide variety of commonly used antimicrobials (Pfaller and Segreti, 2006). With the ability to produce highly effective ß-lactamase enzymes, these organisms were considered resistant to all ß-lactam antibiotics including

extended-spectrum penicillins, cephalosporins and monobactams but ß-lactamase-stable ß-lactams (carbapenem) and cephamycins were active in *vitro* and also appeared to be clinically effective (Ahmed & Salam,2002; Iqbal *et al*, 2002).

Among ESBL-producing *E coli*, none of the organism was sensitive to amoxicillin. Least sensitivity was observed for 3rd generation cephalosporins, cefotaxime (2.8%) and ceftazidime (2.9%) followed by co-amoxiclav (3.4%), aztreonam (3.9%), norfloxacin (6.2%), co-trimoxazole (11.1%), doxycycline (12.5%), gentamicin (13.0%), ciprofloxacin (24.5%) and amikacin (50.0%). Maximum sensitivity was observed for cefoperazone/sulbactam (75.0%), followed by piperacillin/tazobactam (78.9%), fosfomycin (83.1%), imipenem (98.1%) and meropenem (100.0%).

Regarding the sensitivity of ESBL-producing *K. pneumoniae*, none of the organism was sensitive to amoxicillin and doxycycline.Least sensitivity was observed for 3rd generation cephalosporins, cefotaxime (1.9%) and ceftazidime (3.2%) followed by co-amoxiclav (5.1%), aztreonam (7.8%), norfloxacin (9.1%), gentamicin (10.9%), co-trimoxazole (15.4%), ciprofloxacin (19.4%) and amikacin (60.0%). Maximum sensitivity was observed for meropenem (71.4%) followed by piperacillin/ tazobactam (90.9%), fosfomycin (91.7%), imipenem (96.9%) and cefoperazone/sulbactam (100%).The sensitivity of other ESBL-producing EGNR could not be discussed because of their rare occurrence.

It is evident from the present study, that ESBL-producing *E. coli* & *K. pneumoniae* showed least sensitivity towards third generation cephalosporins (cefotaxime, 1.9-2.8%) & (ceftazidime, 2.9-3.2%). According to Grover *et al* (2006), from India, overall higher frequency of resistance to cephalosporins was seen for cefotaxime (19.7-85.9%) and ceftazidime (51.7-100%). Twenty-one strains were resistant to cefotaxime and/or ceftazidime (Touati *et al*, 2006)). In a national surveillance program conducted in 1996 in Argentina, conducted by Bantar *et al*, (2000). 48% of *K. pneumoniae*, 26% of

P.mirabilis and 8% of *E. coli* isolates respectively were resistant towards extended-spectrum cephalosporins .

Second and third generation cephalosporins were commonly used for the treatment of *K. pneumoniae* infections but resistance among these strains is on the rise in this country, which has been attributed to emergence of strains expressing ESBL (Grover *et al*, 2006). ESBL-producing organisms may appear susceptible to some extended-spectrum cephalosporins, however, treatment with such antibiotics has been associated with high failure rates (Paterson and Bonomo, 2005). There are very limited drugs to choose from for treating patients with ESBL-infection, the antibiotics like cefotaxime, ceftazidime, ceftriaxone, aztreonam, tiarcillin, mezlocillin, piperacillin have poor or have lost their activity (Singh, 1999).

Regarding the sensitivity of ESBL-producing EGNR towards other antibiotics, it was noted that they have gained resistance towards other antibiotics as well like gentamicin (10.9-13%) and amikacin (50-60%). Comparison to the previous studies showed that sensitivity of these ESBL-producers towards aminoglycosides has decreased further, 72.3% for ESBL-producing *K pneumoniae* and 81.3% for ESBL-producing *E coli* (Liao *et al*, 2006). According to Luzzaro *et al* (2006), susceptibility to gentamicin and amikacin was 48.0-84.7%.

Sensitivity towards co-trimoxazole ranged from 11.1-15.4% & for doxycycline, it ranged from 0- 12.5% . According to Hoffmann *et al* (2006), all ESBL-producing isolates showed co-resistance to sulphonamides .

Regarding quinolones activity against ESBL-producing EGNRs, 19.4% of *K. pneumoniae* and 24.5% of *E. coli* were sensitive towards ciprofloxacin while 6.2% of *E. coli* and 9.1% of *K. pneumoniae* were sensitive towards norfloxacin in this study. According to Liao *et al* (2006), from Taiwan, only 30.0% of ESBL-producing *E. coli* and 36.6% of ESBL-producing *K. pneumoniae* were susceptible to ciprofloxacin.

According to Luzzaro *et al* (2006), susceptibility to ciprofloxacin was 32.8%. Elhag *et al*, (1999) reported that imipenem and ciprofloxacin are the most suitable antimicrobial agents for empirical treatment of serious Gram-negative infections in their settings, being the most effective agents against the ICU isolates. However, overuse of these drugs is not without risk.

From the above sensitivity pattern, it is clear that ESBL-producing organisms are not only resistant to ß-lactam drugs but show cross-resistance towards other antibiotics as well, like co-trimoxazole, doxycycline, quinolones & aminoglycosides, comparable to the previous studies. Three antibiotics showing best activity against ESBL-producing *E. coli* and *K. pneumoniae* in the present study are β-lactam β-lactamase inhibitors, carbapenems & fosfomycin (83.1-91.7%).

About 78.9- 90.9% of ESBL-producers were sensitive towards piperacillin /tazobactam & 75-100% were sensitive towards cefoperazone/ sulbactam in this study , comparable to other reported studies, where susceptibility ranged from 60-80% (Luzzaro *et al*, 2006; Yu *et al* 2006).

The sensitivity of ESBL-producing *K. pneumoniae* & *E coli* towards imipenem ranged from 96.9-98.1% & for meropenem from 71.4-100%. According to Liao *et al* (2006), from Taiwan, the MICs of all carbapenems were relatively low, with almost all isolates being susceptible. According to Luzzaro *et al*, (2006) with the exception of *P. mirabilis* and *Providencia stuartii* isolate, carbapenems were active against all ESBL-producing enterobacteria. According to Yu *et al* (2006), the isolates were 100% susceptibile to imipenem. ESBL-producers had a more antibiotic-resistant profile than non-producers but were usually susceptible to carbapenems. According to Chow et *al*, (2005) the carbapenems were consistently active *in vitro* against *Enterobacteriaceae* worldwide, including ESBL-producers.

Carbapenems are the treatment of choice for serious, life-threatening infections due to ESBL-producing organisms (Paterson and Bonomo, 2005; Poirel *et al*, 2003; Singh,

(1999), yet carbapenem-resistant isolates have recently been reported (Paterson and Bonomo, 2005). Carbapenems have the highest induction potential of class 1 chromosomal ß-Lactamase, leading to high resistance to cephalosporins and penicillins. (Elhag *et al*, 1999). Carbapenems appear to be the drug of choice and ß-lactam/ß-lactamase inhibitor combinations represent an alternative in non-life-threatening infections (Luzzaro *et al*, 2006).

Since the hydrolytic profile of ESBLs includes extended-spectrum cephalosporins, the use of 3rd generation cephalosporins for treating serious infections caused by an isolate with a confirmed ESBL phenotype should be avoided. However, treatment with such antibiotics has been associated with high failure rates and the mortality rate in patients who have such infections and who are treated with extended-spectrum cephalosporins is high (Tumbarello *et al*, 2006; Poirel *et al*, 2003 ;Paterson and Bonomo, 2005).

Although penicillins, cephalosporins, or aztreonam will appear to be susceptible *in vitro*, ESBL-producing *E. coli* or *Klebsiella* spp may be clinically resistant to therapy with these antibiotics (Singh, 1999). Monitoring and control of usage of extended-spectrum cephalosporins and regular surveillance of antibiotic resistance patterns as well as efforts to decrease use as empirical therapy is indicated (Naumovski, 1992; Emery and Weymouth, 1997). Although cephalosporins are essential drugs in the treatment of a variety of infections, their overuse can result in widespread resistance. Indeed, when ceftazidime was used in excess in the hospital environment, a resistant sub-population of ß-lactamase overproducing mutants was selected (Elhag *et al*, 1999). Complete removal or diminished use of this compound can result in a decline in resistance rates (Burwen *et al*, 1994).

CHAPTER 7

CONCLUSIONS

CONCLUSIONS

Considerable resistance was demonstrated amongst the organisms against all the commonly used antibiotics including ß-lactams. Comparative sensitivity pattern of ESBL-producing and non-ESBLs -producing EGNRs showed that their sensitivity was reduced not only towards 3rd generation cephalosporins but revealed cross- resistance towards most of the other antibiotics like co-trimoxazole, doxycycline, co-amoxiclav, norfloxacin and gentamicin as well. Only some of the antibiotics exhibited high sensitivity against both ESBLs-producing and non-ESBLs-producing *K. pneumoniae* and *E. coli* like carbapenems, ß-lactam/ ß-lactamase inhibitors and fosfomycin.

Overall, almost 38.9% (51% from urinary isolates) of the EGNRs were ESBL-producers, *E.coli and K. pneumoniae* being the most common one. Administration of 3rd generation cephalosporins and aztreonam as empirical therapy would be disastrous, because this would not only be ineffective thus causing increased mortality but would also promote the ESBLs-production. At the same time long-term use of carbapenems as blind empirical therapy would be very expensive, thus leading to increased carbapenems resistance. Therefore, it is very important to screen for ESBLs-production routinely by all the laboratories. The most reliable approach to detect ESBLs- production is the use of special tests like double disk diffusion tests.

For the treatment of serious life threatening infections by ESBLs-producers, best antibiotics are carbapenems, β-lactam–β–lactamase inhibitor combinations and fosfomycin. On the other hand, regarding non- ESBLs-producer, most of the conventional cheap antibiotics would be effective to combat the infection.

CHAPTER 8

RECOMMENDATIONS

RECOMMENDATIONS

Based on our results we recommend the empirical therapy with carbapenems, ß-lactam ß-lactamase inhibitor combinations and fosfomycin in serious life- threatening infections.

The infections by multi-resistant organisms are a serious threat to our surgical patients. There is an urgent need to adopt basic principles of asepsis and sterilization to prevent the development of infections by these organisms. Judicious use of antibiotics for prophylactic and therapeutic purposes is therefore recommended.

Infections due to ESBL- producing *E.coli* and *K. pneumoniae* have become an important clinical problem. Therefore local knowledge of antimicrobial susceptibilities of these organisms is important for implementation of effective hospital anti-infective policies.

Because ESBL-producing strains are resistant to wide variety of commonly used antimicrobials, their proliferation poses a serious global health concern that has complicated treatment strategies for a growing number of hospitalized patients. There is a need for development of new agents due to the increasing resistance to antibiotics.

Many ESBL-producing strains of Enterobacteriaceae do not show resistance to newer cephalosporins or aztreonam in routine susceptibility tests so may be missed on routine disc diffusion susceptibility testing. Therefore, a clinical microbiology laboratory must not rely solely on routine susceptibility testing but should use a more accurate method of detecting ESBLs .

Recent technological improvements in testing & in the development of uniform standards for both ESBL-detection and confirmatory testing, promise to make accurate identification

of ESBL-producing organisms more accessible to clinical laboratories. Microbiology laboratories should start reporting ESBL-producing Enterobacteriaceae due to their importance in respect to antibiotic therapy and infection control aspects.

Screening method for ESBLs -production should be easy & time effective so that it can be used by every laboratory conviently & should be able to provide a guideline for starting the therapy. Laboratories can detect ESBL- production by simple technique of double disc diffusion technique. Any strain showing reduced zones against 3rd generation cephalosporins or aztreonam should be suspected of harboring ESBLs. Aztreonam & ceftazidime are better agents for screening of ESBL producers, at least one of these agents should be routinely used for ESBLs detection against Enterobacteriaceae. If an isolate gives positive resulting synergy test, it should be considered resistant to all β-lactam drugs (except cephamycins, carbapenems).

Although cephalosporins are essential drugs in the treatment of a variety of infections, their overuse can result in widespread resistance. Complete removal or diminished use of these compounds can result in a decline in resistance rates.

There is an urgent need of setting up a national quality control laboratory to provide the performance standards, reference quality control strains and quality antibiotic discs to ensure reproducible and reliable results. Further, there is a need of greater awareness amongst microbiologists regarding the performance of antimicrobial sensitivity testing as per the NCCLS method and hence to conduct Continuing Medical Education Programme to train them in this regard. These initiatives will contribute to generate a reliable data for emerging bacterial antibiotic resistance needed for framing the drug policies and preventing indiscriminate use of antibiotics.

CHAPTER 9

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