The resistance to β-lactam antibiotics is an increasing problem worldwide and β-lactamases production is the most common mechanism of drug resistance. Both global and Indian figures showed a marked increase in the number of β-lactamases producing organisms. These enzymes extended spectrum β-lactamases (ESBLs) are numerous and continuous mutation has led to the development of enzymes having expanded substrate profile. To date, there are more than 130 TEM type and more than 50 sulphydryl variable (SHV) type β-lactamases found in Gram negative bacilli. ESBL producing Enterobacteriaceae are, as a rule, resistant to all cephalosporins and extended spectrum penicillins including the monobactam, aztreonam, while resistance to trimethoprim - sulphamethaxazole and aminoglycosides is frequently co-transferred on the same plasmid. Many ESBL producing organisms also express Amp C β-lactamases. Amp C β-lactamases are clinically significant, as these confer resistance to cephalosporins in the oxyimino group, 7α-methoxy cephalosporins, and are poorly inhibited by clavulanic acid. Carbapenems are the drugs of choice for the treatment of infections caused by ESBL producing organisms but carbapenemases (MBLs) have emerged and have spread from Pseudomonas aeruginosa to Enterobacteriaceae. The routine clinical microbiology laboratories should employ simple methods to recognize these enzymes using various substrates and inhibitors. These organisms may lead to therapeutic dead ends. Presently, the therapy relies on β-lactam/β-lactamases inhibitor combinations, carbapenems and piperacillin – tazobactam plus aminoglycoside combination. Proper infection control practices and barrier precautions are essential to contain the organisms producing β-lactamases.

Key words β-lactamases - ESBL - MBL - resistance - SHV - TEM

The first β-lactamase was identified in Escherichia coli prior to the release of penicillin for use in medical practice. In Gram negative pathogens, β-lactamase production remains the most important contributing factor to β-lactam resistance.

The four major groups of β-lactams penicillin, cephalosporins, monobactams and carbapenems have a β-lactam ring which can be hydrolysed by β-lactamases resulting in microbiologically ineffective compounds. The persistent exposure of bacterial strains to a multitude of β-lactams has led to overproduction and mutation of β-lactamases. These β-lactamases are now capable of hydrolyzing penicillins, broad-spectrum cephalosporins and monobactams. Thus these are new β-lactamases and are called as extended spectrum beta lactamases (ESBLs). In Gram negative bacteria these enzymes remain in the periplasmic space, where they attack the antibiotic before it can reach its receptor site. The first plasmid mediated β-lactamase was described in early 1960. ESBLs have been isolated from a wide variety of Enterobacteriaceae, Pseudomonas aeruginosa and Capnocytophaga ochracea.
On the basis of mechanism of action, most common β-lactamases are divided into three major classes (A, C & D) depending on amino acid sequences. These enzymes act on many penicillins, cephalosporins and monobactams. Class B β-lactamases called as metallo beta lactamases (MBLs), act on penicillins, cephalosporins and carbapenems but not on monobactams. MBLs differ from other β-lactamases in using metal ion zinc, linked to a histidine or cysteine residue to react with the carbonyl group of the amide bond of most penicillins, cephalosporins and carbapenems. Another class, Amp C-β-lactamases are also clinically significant, since they confer resistance to cephalosporins in the oxyimino group, 7α-methoxy cephalosporins and are not affected by available β-lactamase inhibitors. Amp C β-lactamases have been reported in *E. coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *Citrobacter freundii*, *Enterobacter aerogenes* and *Proteus mirabilis*.13-15.

**Origin & spread**

Many Gram negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase. These enzymes may have evolved from penicillin binding proteins with which they show some sequence homology. This development was likely due to selective pressure exerted by β-lactam producing soil organisms found in the environment. The TEM 1 enzyme was originally found in *E. coli* isolated from a patient named Temoniera hence named as TEM. Being plasmid and transposon mediated has facilitated the spread of TEM 1 to other species of bacteria. The second common plasmid mediated β-lactamase found in *K. pneumoniae* and *E. coli* is SHV 1 (sulphydryl variable). The SHV 1 β-lactamase is chromosomally encoded in the majority of isolates of *K. pneumoniae* but is usually plasmid mediated in *E. coli*. The first real ESBL to be described in 1983 was TEM 3, and now over 100 additional TEMs have been isolated.7 Unlike the TEM type β-lactamases, there are fewer derivatives of SHV 1. However, a multi-resistant transferable plasmid encoding the SHV 5 β-lactamase causing unusually high resistance to ceftazidime and aztreonam and combination of acetylating enzymes producing resistance to all clinically available aminoglycosides was identified in *K. pneumoniae*. *K. pneumoniae* has been considered as the main agent producing ESBL. This may be attributed to presence of large mult-iresistant plasmids and presence of ESBL genes on these plasmids. Also *Klebsiella* is more adapted to hospital environment and longer survival of this organism on hands, environmental surfaces facilitates cross infection within hospitals.

An individual acquires an ESBL strain either due to contact with the colonized health care worker or contaminated fomites. After this, the isolate emerges as a result of selective effect of antibiotic use. Removal of selective pressure by drug class restriction leads to the disappearance of ESBL producing strains. Some important factors related to acquisition and infection with ESBL-producing organisms are seriously ill patients with prolonged hospital stays, in whom invasive medical devices are present. Heavy antibiotic use is also a risk factor for acquisition of an ESBL producing organism. Use of many third generation cephalosporins, ceftazidime, quinolones, and aminoglycosides has been shown to be associated with emergence of ESBLs. Patient to patient transmission has also been described. However, same ESBLs in a particular unit of a hospital may be mediated by different plasmids and many different ESBLs may be found in the same unit at a same time. Intensive care units are usually the epicenters of ESBLs production. Transfer of genotypically related ESBLs may occur from hospital to hospital, from city to city, country to country and even intercontinental transfer has been described. Community acquired infection with ESBLs have also been found.

**Classification**

β-lactamases are increasing in number and diversification of the group of enzymes is occurring that inactivates β-lactam type of antibacterials. These can be classified based on two major approaches. One is based on the biochemical and functional characteristics of the enzymes and the second is based on the molecular structure of the enzyme.

Functional classification of the β-lactamases is based on spectrum of antimicrobial substrate profile, enzyme inhibition profile, enzyme net charge, hydrolysis rate and other parameters. Bush et al37 presented the classification based on 4 major groups (1-4) and subgroups (a-f). According to this classification, most ESBLs belong to group 2 B e, which is β-lactamases inhibited by clavulanic acid, which can hydrolyze penicillins, narrow and extended spectrum cephalosporins and monobactams.

**Characterization and types of ESBLs**

Classical ESBLs have evolved from the widespread plasmid-encoded enzymes families TEM, SHV, cefotaxime (CTX-M) and oxacillin (OXA).
TEM (Class A): TEM 1 is capable of hydrolyzing penicillins and first generation cephalosporins but is unable to attack the oxyimino cephalosporin. The first TEM variant with increased activity against extended spectrum cephalosporins was TEM 3. To date, there are > 130 TEM type and > 50 SHV type β-lactamases, and it appears that new ones are being found every week. They are mainly found in E.coli, K.pneumoniae and P. mirabilis but can occur in other members of the Enterobacteriaceae family and in some non-enteric organisms such as Acinetobacter species.

SHV (Class A): The progenitor of the SHV class of enzymes, SHV-1, is universally found in K. pneumoniae. SHV 1 confers resistance to broad spectrum penicillins such as ampicillin, ticarcillin and piperacillin but not to oxyimino substituted cephalosporins. In 1983, a new SHV derived from a mutation in SHV 1 which is called plasmidic SHV 2, was isolated from three isolates of K. pneumoniae which demonstrated, transferable resistance to cefotaxime as well as to other newer cephalosporins. To date, more than 50 SHV type β-lactamases have been described.

CTX-M (Class A): A new family of β-lactamases that preferentially hydrolyzes cefotaxime has arisen. It has been found in isolates of Salmonella enterica serovar. Typhimurium and E.coli mainly and some other species of Enterobacteriaceae. These are not very closely related to TEM or SHV β-lactamases. In addition to the rapid hydrolysis of cefotaxime, another unique feature of these enzymes is that they are better inhibited by the β-lactamase inhibitor tazobactam than by sulbactam and clavulanate.

Recently CTX-M enzymes have been recognized in a number of focal outbreaks from many parts of the world, e.g. in Japan (Toho-2), India (CTX-M-15) and UK suggesting their wide dispersal.

OXA (Class D): The OXA type oxacillin hydrolyzing enzymes are another family of β-lactamases and have been found mainly in P. aeruginosa. OXA β-lactamases belong to Ambler class D. These enzymes confer resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin and are poorly inhibited by clavulanic acid. Many of the newer members of the OXA β-lactamase family have been found in bacterial isolates originating in Turkey and in France.

Other unusual enzymes having ESBL have also been described (e.g., BES-1, CME-1, VE-B-1, PER, SFO-1, GES-1). These novel enzymes are found infrequently.

Genetics

The spread of β-lactamases may be chromosomal or plasmid mediated. The genes encoding some β-lactamases are carried by transposons. Many genes may be found in integrons. Sometimes, β-lactamase resistance markers are a part of the integron that has gene cassettes encoding resistance genes for other classes of antibiotics as well. Decreased porin production and overproduction of β-lactamases can also result in enhanced resistance, e.g. in K. pneumoniae K 6 strain there is novel ESBL - SHV 18 and it also exhibits the loss of two outer membrane porins. In this case although the enzyme hydrolyzed cephapaxime more than the ceftazidime but K6 strain was more resistant to cefotaxime because of possible increased penetration of cefotaxime or increased efflux of ceftazidime. This combination of mechanisms for resistance have been described in group 1 cephalosporinases as well.

The gene for TEM 1 and TEM 2 are carried by transposons. The gene encoding SHV 1 is found on the chromosome of most isolates of K. pneumoniae. SHV genes may also occur on transmissible plasmids.

Genes for the remaining new types of β-lactamases are usually found incorporated into integrons. Integrons are involved in the acquisition of AmpC type β-lactamases by plasmids. Carbapenemases of the Imimenem (IMP) and Verona integrons encoded (VIM) families are also found within integrons. Conjugal transfer of wide host range R-plasmids bearing blaIMP gene is the mechanism of dissemination of blaIMP gene cassettes onto various Gram negative bacterial species. Another mode of resistance is that impenem and meropenem resistant mutants of Enterobacter cloacae and Proteus rettgeri lack porins.

Epidemiology

Due to difficulties in detecting ESBL producers and inconsistencies in reporting it is difficult to determine the true prevalence of ESBL producing organisms. However, the ESBL producing organisms are distributed worldwide and their prevalence is increasing. The incidence of ESBL varies depending on the area of the world the isolates belong to. The first ESBL producing organism was detected in Europe. Although, the initial reports were from Germany and England, later on many reports came from France. The proliferation of
of ESBLs in France was quite dramatic. Up to 35 per cent of hospital acquired *K. pneumoniae* were found to be ESBL producers by early 1990s. Strict infection control interventions led to the decline in the incidence of ESBL producers. However, ESBL producing *K. pneumoniae* are rising in Eastern Europe, in addition 30.2 per cent of *Enterobacter* are showing ESBL production. In USA, the first report occurred in 1988. National Nosocomial Infections Surveillance (NNIS) figures revealed 6.1 per cent of *K. pneumoniae* isolates from ICU while only 1.8 per cent of outpatient isolates of *K. pneumoniae* to be ESBL producers. CTX-M type ESBL has recently been described in USA and Canada. Various ESBL types reported from South America are SHV 2, SHV 5, CTX-M 2, GES-1 and BES 17th-22. As high as 32 to 60 per cent of *Klebsiella* isolates from ICU in Brazil, Columbia and Venezuela have been found to be ESBL producers. From Africa and Middle East, the ESBL production has been reported in 6.1 per cent of *K. pneumoniae* isolates in a single South African Hospital. CTX-M-12 has been found in Kenya. TEM and SHV types of ESBLs have been characterized from South Africa. GES-2 has also been reported from *P. aeruginosa*. In Australian hospitals the proportion of ESBL GES-2 producing *K. pneumoniae* isolates was about 5 per cent. From China, the figures of ESBL producers varies between 25-40 per cent. SHV-2 has also been found in China. About 12-24 per cent of isolates from Thailand, Taiwan, Philippines and Indonesia have been described by national surveys to be ESBL producers. In Japan, 5 per cent of *K. pneumoniae* are ESBL producers. The various lineage of SHV viz., SHV 2, SHV 5, SHV 12 and others have been described from Japan. Recently, CTX-M types have been reported from China, Japan, Korea and Taiwan. The SENTRY Surveillance Program covered the period 1997-1999 and MYSTIC covered 1997-2003. The latter included a greater number of Intensive Care Units, the figure of MYSTIC program showed an overall increase over SENTRY Surveillance Program. Apart from *K. pneumoniae*, *E. coli*, *K. oxytoca*, ESBLs have become common in *P. mirabilis*. ESBLs in *Salmonella* spp. are also growing.

A latest study from US reported 4.9 per cent of all Enterobacteriaceae to be ESBL producers. These isolates occurred at 74 per cent of the ICU and 43 per cent of the non ICU sites. Transferable Amp C beta lactamases were detected in 3.3 per cent of *K. pneumoniae* isolates.

Carbapenem resistant *Serratia marcescens* and *P. aeruginosa* emerged in Japan nearly 10 years ago. These strains produced IMP 1 which is plasmid mediated. Strains carrying bla (IMP-1) with a class 1 integron are the most prevalent types in Japan. However, now these have also been identified in Europe and Singapore.

In the last 4-5 yr, new transferable MBLs have spread rapidly. In some countries, *P. aeruginosa* possessing MBLs constitute nearly 20 per cent of all nosocomial isolates, whereas in other countries the number is comparatively low. The spread of MBL genes is likely to rise further highlights the importance of reporting and studying the epidemic spread of these enzymes by various surveillance projects like SENTRY, MYSTIC, Alexander and EARSS.

Many bacterial species like *Enterobacter*, *S. marcescens*, *E. coli*, *P. aeruginosa* and *C. freundii* possess beta-lactamases of the Amp C type. The product of Amp C gene is an enzyme that is broadly active against cephalosporins but is not inhibited by clavulanate. This differentiated Amp C enzymes from ESBLs. Further, migration of chromosomal Amp C genes into plasmids poses a serious threat. Usually, the plasmid encoded Amp C beta-lactamases are present in species that do not possess a chromosome encoded version of the enzymes. CTX-M types of ESBLs are regarded as emerging pathogens. A recent study from Spain showed 70 per cent of the ESBL producing *E. coli* from bacteraemia cases to be of CTX-M types.

Indian scenario

In India, ESBL producing strains of *Enterobacteriaceae* have emerged as a challenge in hospitalized as well as community based patients. In 1997, from Nagpur 17 out of 66 *Klebsiella* isolates showed ESBL production. In 2002, 68 per cent of Gram negative bacteria were found to be ESBL producers in a study from New Delhi in which 80 per cent of *Klebsiella* were ESBL. In 2004 two other studies from Delhi showed 70.6 and 12.6 per cent *Klebsiella* isolates to be ESBL producers. In 2005 from New Delhi, showed 68.78 per cent of the strains of *P. aeruginosa* from bacteraemia cases to be of CTX-M types.

A study from Coimbatore, Tamil Nadu, showed the presence of ESBL to be 40 per cent while from Nagpur this figure was 50 per cent in urinary isolates. In a study from...
Chennai, ESBL production was detected in 6 out of 90 isolates of *K. pneumoniae* in children under five with intestinal infections\(^\text{14}\). Further a study from Karnataka showed the frequency of ESBLs in neonatal septicemia cases to be 22.7 per cent\(^\text{15}\). A similar study from Lucknow showed high levels of ESBL production (63.6-86.6 %)\(^\text{16}\). Amp C enzyme has also been described in 3.3 per cent of isolates from Karnataka\(^\text{17}\). Metallo-beta-lactamase production is a significant problem especially in hospital isolates of *P. aeruginosa* and *Acinetobacter* species. Reports have described the prevalence of MBLs in *P. aeruginosa*\(^\text{18,19}\) and *Acinetobacters* species as well from India\(^\text{20}\).

The results of the initial studies for the MYSTIC programme in India confirmed the high levels of resistance and ESBL production in Gram-negative bacilli including *Salmonella*\(^\text{21}\). The latest report from National Institute of Communicable Diseases (NICD), New Delhi, India, shows PCR as a reliable method for detection of ESBLs as well as the rise of resistance to cefepime - a fourth generation cephalosporin\(^\text{22}\). CTX-M-15 has also been found in Indian *E. coli* and *K. pneumoniae* strains\(^\text{23}\).

**Methods of detection**

Different ESBL enzymes depict variable levels of resistance to third generation cephalosporins. According to National Committee for Clinical Laboratory Standards (NCCLS) now Clinical and Laboratory Standards (CLSI) interpretive definitions, ESBLs do not always increase MICs to levels characterized as resistant\(^\text{124,125}\). Now, it is mandatory that the routine clinical microbiology laboratory employs ESBL detection methods which are sensitive enough to recognize the level of resistance that would be achieved by the situation given *in vivo*.

**Screening tests:** Initial screening for reduced susceptibility to cefpodoxime, cefotaxime, ceftriaxone, ceftazidime or aztreonam and then performing phenotypic confirmatory test is recommended. As proposed by the CLSI, M100-S-16 document, the use of more than one of the five indicator cephalosporins suggested will improve the sensitivity of detection\(^\text{26}\). But, if it is necessary to rely on a single screening substance, ceftazidime or cefpodoxime would be the best choice\(^\text{127}\).

**Confirmatory tests**\(^\text{127}\): (i) The double disk approximation test: A susceptibility disk containing amoxicillin-clavulanate and a disk containing one of the oximino-beta-lactam antibiotics is used. Enhancement of the zone of ceftazidime disk on the side facing the amoxicillin-clavulanate disk is interpreted as a positive test\(^\text{129}\). (ii) Combined disk method - Commercialized disks containing clavulanate plus ceftazidime or cefotaxime (10 µg plus 30 µg respectively) are used in this method\(^\text{190}\). (iii) E test ESBL strip - In this method the zone of inhibition is read from two halves of the strip. A decrease in the MIC of ceftazidime of more than three dilutions in the presence of clavulanate is interpreted as a positive test. Sometime due to weak enzyme production, indeterminate results may be obtained. (iv) Three dimensional test- This method is very sensitive, but is technically difficult and labour-intensive requiring experienced clinical microbiologist to interpret the result\(^\text{131}\). (v) The automated ESBL microbial susceptibility test system- This method utilizes either ceftazidime or cefotaxime alone and in combination with clavulanic acid (4 µg/ml). A predetermined reduction in growth in wells containing clavulanate compared with those containing each single drug indicates the presence of an ESBL. Vitek ESBL test system has shown variable results\(^\text{132}\). (vi) Recently CLSI has recommended initial screening by testing for growth in a broth medium containing 1 µg/ml of one of five extended spectrum beta-lactam antibiotics\(^\text{128}\). For identification of specific ESBL expressed in a clinical isolate, the following molecular detection methods can be applied: Specific DNA probes, PCR with oligonucleotide primers oligotyping, PCR followed by restriction fragment length polymorphism analysis, ligase chain reaction and nucleotide sequencing. These techniques are available only in research centres and are beyond the scope of routine clinical microbiology laboratories.

Regarding the reporting of ESBL producing isolates, the microbiology laboratory report should state that the strain produced ESBL and should be considered resistant to all penicillins, cephalosporins and aztreonam.

For clinical laboratories to adapt a method for screening for metallo beta-lactamases, the suggested methodology is as follows; firstly, the isolates are targeted based on ceftazidime and carbapenem MIC, *e.g.* *P. aeruginosa* with an imipenem MIC> 16 µg/ml and *Acinetobacter* spp. isolates with an MIC >2 µg/ml is appropriate. For ease of application, the E test MBL strip is recommended\(^\text{132}\). To increase the sensitivity of MBL detection, several substrates (imipenem, ceftazidime and meropenem) should be used preferably.
with more than one inhibitor (EDTA and mercaptopropionic acid)\textsuperscript{134}. It should be of help if clinical laboratories are able to detect organisms producing plasmid mediated Amp-C β-lactamases with a method, which is simple as well as inexpensive. CLSI has no recommendations available for detection of these organisms. A number of reports have been published based on different methodologies used\textsuperscript{135-137}.

**Treatment**

ESBL producing members of *Enterobacteriaceae* are, as a rule, resistant to all cephalosporins and extended spectrum penicillins, including the monobactam, aztreonam, while resistance to trimethoprim-sulfamethoxazole and aminoglycosides is frequently co-transferred on the same plasmid\textsuperscript{138}. Third and fourth generation cephalosporins should not be used even in the presence of apparent susceptibility. Cephamycins such as cefoxitin, cefotetan and moxalactam may be used for ESBL producing *E. coli* and *Klebsiella* spp. However, cephamycin therapy leads to emergence of plasmid mediated Amp C resistance. These enzymes are phylogenetically very distinct from the ESBL families and confer resistance to third generation cephalosporins as well as cephamycins\textsuperscript{139,140}. ESBLs are usually susceptible to β-lactam/β-lactamase inhibitor combinations, but these drugs can usually be overwhelmed by particularly large amounts of enzyme and thus show in vivo resistance. Treatment with β-lactam/β-lactamase inhibitor combination was shown to be inferior to treatment with imipenem or piperacillin/tazobactam plus aminoglycoside combination in an animal model\textsuperscript{140}. Currently the carbapenems, are regarded as the drugs of choice against ESBL producing organisms\textsuperscript{7}. Carbapenem treatment however, is not without its own complication because MBL producers which are carbapenem resistant, have already spread in various parts of the world\textsuperscript{98,99}. The only therapeutic option available for MBL producers is polymyxin\textsuperscript{141}. These molecules should not be used as monotherapy but may be combined with some aminoglycoside molecule. Also rifampin may be an interesting agent for treating multi-drug resistant *P. aeruginosa* infections. Thus, there is a need for developing novel agents in the near future otherwise these organisms may lead to therapeutic dead ends.

**Control measures**

Proper infection control practices and barriers are essential to prevent spread and outbreaks of ESBL producing bacteria. The reservoir for these bacteria seems to be gastrointestinal tract\textsuperscript{142}, oropharynx, colonized wounds and urine.

The colonized hands, equipment could help in spreading infection between patients. So, mandatory infection control practices would be hand washing, barrier precautions, isolation of colonized/infected patients. Surveillance of patients of ICUs will help in early detection and control practices related to ESBL production. Antibiotic restriction and antibiotic cycling especially the empirical use of higher generation cephalosporins and carbapenems are other measures which if monitored properly, could help in control of the emergence and spread of ESBL producing bacteria.

**Conclusion**

The incidence of infections due to organisms resistant to β-lactam agents due to production of various enzymes has increased in recent years. Detection of ESBL production is of paramount importance both in hospital and community isolates. Firstly, these strains are probably more prevalent than currently recognized. Secondly, ESBLs constitute a serious threat to currently available antibiotics. Thirdly, institutional outbreaks are increasing because of selective pressure due to heavy use of expanded spectrum cephalosporins and lapses in effective control measures. So vigilance and timely recognition of infection with resistant bacteria and appropriate antibiotic therapy, is the only answer to the current multi drug resistant bacterial population. Careful attention to barrier precautions and hand hygiene can help in preventing the spread of these, multi drug resistant Gram-negative microorganisms.

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