Proliferation of Extended Spectrum β–Lactamase (ESBL) producing Gram negative bacteria, diagnostic inputs and impact on selection of Antimicrobial therapy


Abstract
Irrational use of Extended Spectrum Beta Lactamase (ESBL) inducers like ceftazidime in yesteryears contributed to emergence and then proliferation of multidrug resistant ESBL producing microorganisms especially Klebsiella and Escherichia coli (E. coli) in hospitals and community. These enzymes hydrolyse the beta lactam ring of 3rd generation cephalosporins like Ceftriaxone, ceftazidime, Cefoperazone, cefotaxime etc. Role of 3rd generation cephalosporins in other gram negative and overall gram positive bacterial infections has also thus become questionable. Alternative therapeutic agents like extended spectrum cephalosporins with beta lactamase inhibitor combinations i.e. tazobactam, sulbactam, clavulanic acid etc. along with carbapenems are also showing a rise in resistance trend. Incorporation of screening and confirmatory methods for ESBL detection vis-à-vis Klebsiella and E. coli species have become mandatory for rationalizing the use of third generation cephalosporins for more appropriate patient care.

Key words: ESBL, Cephalosporins, Klebsiella spp, E. coli, MDR

Introduction
Antibiotic drug resistance in general and multidrug resistance in particular loom large on our head as never before. The war waged between micro-organisms and antimicrobials continues to flare up unabated with each developing new weaponry and seeking novel ways of combat. Beta lactamas continue to be the leading cause of resistance to beta lactam antibiotics in Gram negative bacteria. In recent years there has been an increase in incidence and prevalence of ESBL producing microbial diseases. These enzymes hydrolyze beta lactam ring and cause resistance to oxyimino-Cephalosporins and aztreonam1,2. ESBLs are more prevalent in Klebsiella pneumoniae and E. coli than in any other gram negative bacterial species and outbreaks of infections caused by ESBL producing strains have been reported widely3. Klebsiella spp and E. coli are very frequently isolated in every hospital setting and a significant proportion is multidrug resistant posing a formidable challenge.

Extended spectrum beta-lactamases
ESBLs are most often associated with Klebsiella pneumoniae and E. coli. They are plasmid mediated and get transferred to genera of other enteric bacilli including Proteus mirabilis, Citrobacter and Serratia. Global prevalence of ESBL producing organisms presently varies from <1% to 74%4,5. Initially the beta-lactamases produced were active against only a few beta-lactams but over the years microorganisms have learnt to elaborate newer beta-lactamases with extended substrate profile and such important group of enzymes are active against virtually all beta-lactams except the carbapenems6. ESBL production varies from hospital to hospital because of variation in selection of type of antibiotics and the antibiotic selection pressure thereof. Patients having infections by ESBL producing organisms are at higher risk of treatment failures with 3rd generation cephalosporins, thus making it mandatory for all microbiology laboratories to screen such bacterial strains for ESBL production using reliable tests recommended by NCCLS (National Committee for Clinical Laboratory Standards or as per new CLSI (Clinical and Laboratory Standards Institute guidelines)7,8 and if an isolate is confirmed to be ESBL producer, 3rd generation cephalosporins should be prescribed with caution in such cases, and incorporation of screening and confirmatory methodologies for ESBL detection vis-à-vis Klebsiella and E. coli species in our systems is necessary for rationalizing third generation Cephalosporin usage.
ESBLs and metallo-beta-lactamase (MBLs) are enzymes which hydrolyse beta-lactam ring of beta-lactam group of antibiotics. Resistance to such commonly used antibiotics leads to treatment failures. This public health risk has become a global problem with some countries seriously affected\(^9\). Members of the family Enterobacteriaceae, including \textit{E. coli}, are among the most important human pathogens accounting for the majority of bacterial strains isolated from clinical patient samples. Natural or acquired mutations in bacteria after human involvement are caused by the uptake of foreign genetic material, either incorporated into the bacterial chromosome or existing in an independent, stable form\(^{10}\). Extended Spectrum beta-Lactamase (ESBL) production by bacteria is an important threat to clinical therapeutics\(^{11,12}\). These organisms elaborate plasmid encoded beta-lactamases, a variety of which have been described among members of the family Enterobacteriaceae\(^{13}\). First described in 1983, ESBL producers have contributed to the dramatic increase in recent years to resistance among gram-negative bacteria to beta-lactam agents. Plasmid borne genes code for enzymes that hydrolyze penicillins, cephalosporins, Aztreonam, and are inhibited by clavulanic acid\(^{10}\).

Reporting of ESBL production from microbiology laboratory has to be interpreted scientifically as in vitro sensitivity to 3\(^{rd}\) generation cephalosporins in ESBL positive organisms’ amounts to in vivo resistance. These ESBL producing resistant strains which emerge out of irrational use of antimicrobials are difficult to treat and thus pose a big challenge for the future especially in tertiary care setups.

**Precipitating factors**
Mushrooming of fake antibiotic drug companies, faulty sterilization, disinfection, hospital waste management and hand hygiene practices, visitors rush to patients and lack of newer diagnostic facilities contributes to proliferation of drug resistant microorganisms. Self medication and use of high resistance potential antibiotics and prolonged hospital stay of critically ill patients, and prescription of antibiotics even for contaminants and normal flora also add to antibiotic resistance.

To make efforts for safeguarding the efficacy of antibiotic accessible to common man by rationalizing their usage and discouraging their overuse and underuse, steps have to be taken at an earliest. In such scenario, not only efficient detections, early reporting and rationale in treatment are important but aggressive infection control practices have to be employed. Incorporation of cost effective ESBL screening and confirmatory tests as discussed below at tertiary care setups will help in rationalizing the use of 3\(^{rd}\) generation cephalosporins to a great extent.

**Screening Tests**
Ceftazidime and cefotaxime are included in the primary panel for screening potential ESBL producers. Isolates with inhibition zone diameter of ≤22mm for ceftazidime and ≤27mm for cefotaxime is considered as potential ESBL producers as per the NCCLS guidelines and put to confirmatory testing by a double disk synergy test (DDST) and two phenotypic confirmatory disk diffusion tests (PCDDT A and PCDDT B)\(^{14,15}\).

**Double Disk Synergy Test:**
In the DDST ceftazidime, cefotaxime and Ceftriaxone 30 µg each a placed are at a distance of 15mm edge to edge from a centrally placed Co-AmoxylClav disk containing 20 µg of amoxicillin + 10 µg of clavulanic acid. ESBL production is inferred if the inhibition zone around the test antibiotic disk increases towards the Co-AmoxylClav disk\(^{4,16,17}\).
**Phenotypic Confirmatory Disk Diffusion Tests:**

Ceftazidime and cefotaxime 30 µg each will be used alone and in combination with 10 µg of clavulanic acid in the phenotypic confirmatory disk diffusion tests (PCDDT A) and B (PCDDT B) respectively. Individual discs are placed at least 3 cm centre to centre apart. An increase in zone diameter of either ceftazidime or cefotaxime by ≥ 5 mm with clavulanic acid versus its diameter when tested alone is considered as ESBL positive \(^{16,18}\)

**ETest**

ESBL strips of ceftazidime TZ (0.5-32 µg/ml) and TZL ceftazidime (0.064-4 µg/ml) plus 4 µg/ml clavulanic acid are used to confirm representative isolates positive for ESBL production by disk diffusion tests. The strips have a cephalosporin gradient at one end and a cephalosporin plus Clavulanate gradient at the other. ESBL production is inferred if the MIC ratio for cephalosporin alone: cephalosporin + Clavulanate ≥ 8 \(^{19}\). Positive control and negative control *E. coli* ATCC is also incorporated in the study.
Current Global Scenario

Antimicrobial surveillance program in 1997-99 from all over the world showed that ESBL producing *Klebsiella pneumoniae* account for about 45% in Latin America, 25% in western pacific, 23% in Europe and 8% in USA. Injudicious and rampant use of 3rd generation cephalosporins could be a leading contributory factor for high ESBL prevalence. Once an *ESBL* producing strain is detected, experts should report them resistant to all penicillins, cephalosporins and Aztreonam even if they test as susceptible. Treatment failures and death have occurred when cephalosporins were used against *ESBL* producers that appeared susceptible in vitro. Although beta lactamase inhibitors have tremendous activity against *ESBLs* in vitro, their clinical effectiveness against serious infections due to ESBL producing organisms is somewhat controversial. Hyper-producing strains may produce enough of beta lactamase to overcome the effect of the beta lactamase inhibitors. For these reasons beta lactamase inhibitors may not be optimal therapy for serious infections due to ESBL producing organisms. However they are useful for less serious infections such as urinary tract infections. Non-beta-lactam antimicrobial agents such as fluoroquinolones, aminoglycosides and Cotrimoxazole remain viable alternatives for the treatment of *ESBL* producing *Klebsiella* and *E. coli* strains. Carbapenems like meropenem, imipenem and ertapenem by far remain the drugs of choice for serious infections by *ESBL* producing *Klebsiella pneumoniae* and *E. Coli*. Faced with the global emergence of antimicrobial resistance, several studies have been undertaken to assess the susceptibility of bacterial pathogens to different antibiotics world over. These findings revealed that there is a widely spreading resistance to most of the available antibiotics. A susceptibility study conducted in California University on prospectively collected 255 *E. coli* isolates showed 22% resistance to trimetoprim-sulfamethoxazole. In a separate study in the US, three antimicrobial susceptibility-testing methods (Vitek, Microscan and Disk diffusion) were employed on 123,691 *E. coli* isolates, with similar results. Resistant patterns were observed to nitrofurantoin (1%), ciprofloxacin (3.7%), sulfamethoxazole (18.6%) and ampicillin (39.1%). To assess multi-drug resistance (MDR) patterns, 38,835 isolates were tested against five antimicrobials - 55.9% isolates were susceptible to all the drugs tested, 20% were predominantly resistant to ampicillin and 1.1% found to be MDR. In comparison to most of the prospective surveillance studies, a retrospective *in vitro* surveillance study was conducted in Saudi Arabia to assess antibiotic susceptibility patterns among *E. coli* isolates. Inpatients isolates were more likely to be antibiotic resistant than outpatient isolates, as observed in the
resistance patterns to Ampicillin (63% in inpatients, 50% in outpatients; sulfamethoxazol (44% in inpatients, 30% in outpatients); and ciprofloxacin (33% in inpatients, 14% in outpatients) 29. In a separate study, a double disc diffusion (Synergy test) was employed to study the prevalence of ESBL in nosocomial and outpatients in Islamabad University, Pakistan, delineating 70% of Klebsiella pneumoniae, 33.33% of Enterobacter cloacae, and 28.57% of E. coli with this property29. In neighboring Northern India, in a tertiary care hospital, a significant, overall resistance to carbapenems was reported - 22.16% to meropenem and 17.32% to imipenem. E. coli isolates showed lower resistant patterns as compared to the more resistant pseudomonas species (3.3% versus 37.6% to meropenem; 2.1% versus 30% to imipenem) 11,28

Kashmir: 5 years ESBL appraisal
Keeping in view the impact of ESBL burden at SKIMS and the impact on antimicrobial therapy, these need-based technologies were inducted into the system in Department of Microbiology at Sher-i-Kashmir Institute of Medical Sciences in a phased manner starting from year 2004. First study on ESBL producing Klebsiella pneumoniae at SKIMS-Kashmir,31 it was found that 72% of hospital acquired Klebsiella pneumoniae strains were ESBL positive (years 2005 and 2006). Continuing with Extended Spectrum-β-Lactamase (ESBL) mediated resistance to 3rd G cephalosporins in various isolates of E. coli in Kashmir32, during the period between 2005-2007, showed 60% of E. coli strains isolated from various sources as ESBL positive and both the studies should be taken as a quiet before a storm and need our immediate attention and also these need based latest technologies should be incorporated as quickly as possible in all of our Microbiology diagnostic systems including private sector as detection of ESBLs has a direct bearing on the use of 3rd generation cephalosporins namely Ceftriaxone, ceftazidime, cefotaxime and cefaperazone etc which are used extensively in Kashmir. Another study during the period of 2007-2009 on an equally important aspect of metallo-beta-lactamase (MBL) producing Pseudomonas aeruginosa33 at SKIMS showed 14% of the organism as MBL positive which has a direct bearing on the use of carbapenems such as imipenem, meropenem and doripenem.

Conclusions
To optimize the choice, dose and duration of antibiotics for therapeutic, prophylactic and empirical treatment of ESBL producing organisms, we need to understand the genesis of emergence and then proliferation of ESBL producing bacteria which have a direct bearing on the use of 3rd generation cephalosporins in patients. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitations of therapeutic options. Initially restricted to hospital acquired infections, they have also been isolated from infections in outpatients. Thus a systematic study with a focus on research acumen is an urgent need of the hour, with special attention to Extended Spectrum beta lactamase (ESBL) producing Gram negative bacteria. Threat posed by ESBLs should follow a multipronged approach. Not only efficient detection, early reporting and rationale in treatment important but efficient infection control policies cum practices, restricted and judicious use of cephalosporins are equally important. In developing countries this problem is compound by unawareness of most clinicians, poor infection control practices, and ‘over the counter’ availability of antibiotics. Combined efforts by trained clinicians and microbiologists to enforce strict infection control measures should be followed to decrease horizontally transferable resistance. Intensive screening of outpatients at the time of admission or during prolonged hospital stay will also help to curb these infections. Also judicious prescribing of antibiotics, antibiotic resistance surveillance program and antibiotic cycling should be tried.
References


27. Levy SB. The challenge of antibiotic resistance. Sci American 1989

**Note:** References number 31 to 34 can be got from the corresponding author.

**Conflict of Interest:** None

**Author Information:** Manzoor Ahmed Thokar is Professor Medical Microbiology, Faculty of Medicine, Al-Arab Medical University, Benghazi, Libya. **Email:** manzoor_thakur@rediffmail.com