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REVIEW ARTICLE

Impact of Infection caused by Extended Spectrum β-Lactamse Producing Bacteria: A Review Update

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Abstract

Emergence of bacterial resistance causes limitation of the action of antimicrobial agents. Frequency of extended spectrum β-Lactamase (ESBL) production is high among *Escherichia coli* (*E.* coli) and *Klebsiella* species. This has created a worldwide problem resulting in treatment failure. ESBLs have become widespread throughout the world. Microbes undergo mutation of genes, which can spread from cell to cell by mobile genetic elements such as plasmids, transposons and bacteriophages. Resistant bacteria flourish in areas of heavy antibiotic use such as hospitals and ICU. With widespread use of antibiotic, the frequency of penicillinase producing *staphylococci* increased. The availability of the second-generation cephalosporin, such as cefamondole, cefoxitin, and cefuroxime, or 3rd generation cephalosporin, such as cefotaxime, ceftazidime, ceftriaxone has been the leading cause of potential resistances in nosocomial Gram-negative bacilli. Hospital outbreaks of multi-drug resistant Enterobacteriaceae are now being frequently caused by extended spectrum beta-lactamase (ESBL) producers. Incidence of ESBL producing strains among clinical isolates has been steadily increasing over past years resulting in limitation of therapeutic options. Bacterial antibiotic resistance has become a major clinical concern worldwide including Bangladesh. Recently, the use of second and third generation cephalosporin has led to the selection of Gram-negative organisms resistant to β-lactamase stable cephalosporin. This resistance is attributed to the production of extended spectrum β-lactamases.

Keywords: ESBL, *Escherichia coli*, *Klebsiella* species, extended spectrum β-Lactamase

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Introduction

Drug resistance is a burning problem in the field of medicine[1]. There are many ways which mediate resistance, among them beta-lactamases play a major role in developing resistance against Gram negative organisms[2]. During the last three decades many beta-lactam drugs have been used against the hydrolytic action of beta-lactamases for the treatment of Gram negative bacterial infections. Oxyimino Cephalosporins (3rd generation) are the most widely used beta-lactam drugs having broad spectrum antibacterial activity[1].

Microorganisms are gradually developing resistance to these betalactam antibiotics by producing beta-lactamases. Due to increased spectrum of activity of beta-lactamases especially against the oxyiminocephalosporins, they are called extended spectrum beta-lactamases (ESBLs)[2].

Definition of ESBL

ESBLs are defined as β-lactamases capable of hydrolyzing oximinocephalosporins that are inhibited by clavulanic acid. Recently, a classification scheme was devised by Bush, Jacoby, and Medeiros that uses the biochemical properties of the ESBL enzyme plus the molecular structure and nucleotide sequence of the genes to place β-lactamases into functional groups. Using this scheme ESBL are placed into functional group[3].

Epidemiology

ESBLs are now a problem in hospitalized patients worldwide. The ESBL phenomenon began in Western Europe, most likely because extended-spectrum β-lactam antibiotics were first used their clinically. However, it did not take long time before ESBLs had been detected in the United States and Asia. The prevalence of ESBLs among clinical isolates varies from country to country and from institution to institution[4]. In the United States, occurrence of ESBL production in Enterobacteriaceae ranges from 0 to 25%, depending on the institution, with the national average being around 3%[5]. Among isolates of *K. pneumoniae*, the percentage of ceftazidime resistance ranges from 5 to 10% for non-intensive care unit (non-ICU) and ICU isolates, respectively[6].

Worldwide Prevalence

Most of large outbreaks of nosocomial infection due to ESBL-producing *Klebsiella* have been reported from Europe, especially in France. Largest *outbreak* of ceftazidime-resistant *Klebsiella* occurred in general hospital of North America. Outbreak coincided with increasing use of ceftazidime and declined after restriction of ceftazidime, implying a causal relationship[7]. In Europe, the prevalence of ESBL production among isolates of *Enterobacteriaceae* varies greatly from country to country. In the Netherlands, a survey of 11 hospital laboratories showed that <1 % of *E. coli* and *K. pneumoniae* strains possessed an ESBL[8]. However, around 40% of *Klebsiella pneumoniae* isolates were found to be ceftazidime resistant in France[9]. Across Europe, the incidence of ceftazidime resistance among *Klebsiella pneumoniae* strains was 20% for non-ICU isolates and 42% for isolates from patients in the ICU[4]. In Japan, the percentage of β-lactam resistance due to ESBL production in *E. coli* and *Klebsiella pneumoniae* remains very low, In a recent survey of 196 institutions across the country, <0.1% of *E. coli* and 0.3% of *Klebsiella pneumoniae* strains possessed an ESBL[10]. Elsewhere in Asia, the percentage of ESBL production in *E. coli* and *K. pneumoniae* varies, from 4.8% in Korea[11] to 8.5% in Taiwan[12] and up to 12% in Hong Kong[13]. Sporadic infections or nosocomial outbreaks caused by ESBL producing *Salmonella* have been reported in numerous countries of Latin America, Africa, Asia, and Europe[14].

Bangladesh Situation

A common theme among hospitals plagued by organisms that produce ESBLs is the high volume and indiscriminate administration of expanded-spectrum cephalosporins[15]. In Bangladesh extended spectrum β-lactamase mediated third generation cephalosporin resistance in *Shigella* isolates has been reported

by ICDDRB, Dhaka[10]. Two *S. sonnei* and one *S. boydii* were positive by double disc diffusion synergy test (DDST) and were resistant to β-Lactam, but susceptible to β- lactam/β-lactamase inhibitor combination, cefoxitin, and imipenem indicating the presence of a class A ESBL. Molecular characterization was not done, but the resistance phenotypes of *S. boydii and S. sonnei suggest* the ESBLs as CTX-M types. ESBL producing MDR *Shigella spp.* possesses an important threat in the treatment of dysentery especially in children[16]. In Bangladesh a study was done on prevalence of ESBL producing *E. coli* and *Klebsiella pneumoniae* in an urban hospital in Dhaka. Double disc method was performed. *E. coli* (43.2%) and *Klebsiella pneumoniae* (39.5%) were found as ESBL positive[16]. Another two studies were done in BSMMU, one by Alim[17] and found 40.9% *Klebsiella* species and 26.9% *E. coli* to be the ESBL producers. Other by Mostaquim[18] found 43.47% *Klebsiella spp.* and 35.4% *E. coli* to be the ESBL producers.

Emergence of ESBL

Emergence of bacterial resistance causes limitation of the action of antimicrobial agents. After the discovery of Sulfonamide and Penicillin in twentieth century, it was thought that all infectious diseases were conquered. Within a short period of time microbes underwent mutation of gene and started to produce penicillinase enzyme increasingly and finally Methicillin Resistance *Staphylococcus aureus* (MRSA) came into the picture. *Escherichia coli* and *Moraxella catarrhalis* produce beta-lactamases very frequently but previously they were occasional beta-lactamase producers. At the same time beta-lactamase negative *H. influenzae* and *Neisseria spp.* started to produce the enzyme increasingly[19].

ESBL Producing Bactria

ESBL was first reported in 1983 from Germany in isolates of *Klebsiella pneumoniae*[20]. Gradually more than 150 different ESBLs have been described[4] ESBL producing organisms are *E. coli, Klebsiella* species, *Salmonella* species, *Morganella morganii, Proteus mirabilis, Serratia marcescens, Pseudomonas aeruginosa* etc. Frequency of ESBL production is high among *E. coli and Klebsiella* species[21]. ESBLs have become widespread throughout the world and arc now found in significant percentage of *E. coli* and *Klebsiella* spp. They have been found in other Enterobacteriaceae and *Pseudomonas aeruginosa*[4]. This has created a worldwide problem resulting in treatment failure. In general ESBLs are most frequently identified in *Klebsiella pneumoniae* and *E. coli,* but in recent years incidence is increasing in other organisms of family Enterobacteriaceae, including *Salmonella* spp. As ESBLs are frequently encoded by genes located on different transferable genetic elements, a variety of epidemiological situations have been identified, ranging from sporadic cases to large outbreaks[22]. Whereas ESBLs were initially associated with nosocomial outbreaks caused by single enzyme producing strains, study has been revealed more complex situations, with a significant increase of in community isolates[23]. Majority of ESBL producing strains are *K. pneumoniae, K. oxytoca* and *E. coli.* Other organisms reported to harbor ESBLs include *Enterobacter spp., Salmonella spp., Morganella morganii, Proteus mirabilis, Serratia marcescens, Shigella dysentriae, Pseudomonas aeruginosa, Burkhoderia cepacia* and *Capnocytophaga ochracea*[24].

Types of ESBLs

Most ESBLs are derivatives of' TEM or SHV enzymes[6]. There are now > 90 TEM-type and >25 SHV-type β-lactamases enzymes[7]. TEM and SHV ESBLs are most often found in *E. coli* and *K. pneumoniae;* however, they also been found in *Proteus spp*., *Providellcia spp*., and other genera of Enterobacteriaceae.

TEM

TEM-1 enzyme was first isolated from blood culture of a patient named Temoniera hence designation TEM. TEM-l is the most commonly encountered β-lactamase in Gram-negative bacteria. Up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1[25]. This enzyme is responsible for the

ampicillin and penicillin resistance that is seen in *H. influenzae and N. gonorrhoeae*[15]. TEM-l is able to hydrolyze penicillin and cephalosporin such as cephalothin and cephaloridine. TEM-2, the first derivative of TEM-l had a single amino acid substitution from the original β-lactamase. TEM-3, originally reported in 1989, was the first TEM-type β-lactamase that displayed the ESBL phenotype. A number of amino acid residues are especially important for producing the ESBL phenotype when substitutions occur at that position. They include glutamate to lysine at position 104, arginine to either serine or histidine at position 164, glycine to serine at position 238, and glutamate to lysine at position 240[4]. TEM-1, 2, SHV-I are non-ESBL and TEM-3, 4, 5, 6,7,8,9 etc. and SHV 2,3,4,5 etc. are ESBL[25].

SHV

SHV-1 β-lactamase is most commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance in this species. In many strains of *Klebsiella pneumoniae*, blaSHV-1 or a related gene is integrated into the bacterial chromosome[6]. It has been hypothesized that the encoding SHV -1 may exist as part of a transposable element. Unlike the TEM type β-lactamases, there are relatively few derivatives of SHV-1. Furthermore, the changes that have been observed in blaSHV to give rise to the SHV variants occur in fewer positions within the structural gene. The majority SHV variants possessing an ESBL phenotype are characterized by the substitution of a serine for glycine at position 238. A number of variants related to SHV -5 also have a substitution of lysine for glutamate at position 240. Both the Gly238Ser and Glu240Lys amino acid substitutions are similar to those seen in TEM-type ESBLs. The serine residue at position 238 is critical for the efficient hydrolysis of ceftazidime, and the lysine residue is critical for the efficient hydrolysis of cefotaxime. The majority of SHV-type ESBLs are found in strains of *K. pneumoniae.* However, these enzymes have also been found in *Citrobacter diversus, E. coli* and *P. aeruginosa*[4].

CTX-M

In recent years a new family of plasmid-mediated ESBLs, called CTX-M, that preferentially hydrolyze cefotaxime has arisen[26]. They have mainly been found in strains of *Salmonella enterica* serovar *typhimurium* and *E. coli,* but have also been described in other species of *Enterobacteriaceae.* They include the CTX- M type enzymes CTX- M-1 (formerly called MEN-1), CTX-M-2 through CTX- M-10[27].

OXA

The OXA-type enzymes are another growing family of ESBLs. These β-lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d[20]. The OXA type β-lactamases confers resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid. Most ESBLs have been found in *E. coli, Klebsiella pneumoniae,* and other *Enterobacteriaceae.* The OXA type ESBLs have been found mainly in *Pseudomonas aeruginosa*[4].

Other ESBLs

While the majority of ESBLs are derived from TEM or SHV β– lactamases and others can be categorized with one of the newer families of ESBLs, a few ESBLs have been reported that are not closely related to any of the established families of β-lactamases[4].

Mechanism of Development of ESBL

Bacterial resistance to β-lactam drugs and their mechanism leading to resistance is gaining importance in the field of medical researchers throughout the world[28]. ESBLs are enzymes that mediate resistance to extended spectrum (3^{rd} generation) cephalosporins like ceftazidime, ceftriaxone, cefotaxime and monobactams like aztreonam but do not affect 2^{nd} generation cephalosporins such as cephamycin[24]. There has been an increased

incidence and prevalence of extended-spectrum beta-lactamases (ESBLs) enzymes that hydrolyze and cause resistance to penicillin, cephalosporins and aztreonam[29]. When the modern era of chemotherapy of infection has began in the 1930s with the clinical use of sulfonamides, infectious diseases appeared to be all conquered. The powerful arsenal of antibiotics appeared to be good, but they were spread around too widely, too cheaply and used indiscriminately. As a result of this widespread use antibiotic resistant organisms were generated[30]. Microbes undergo mutation of genes, which can spread from cell to cell by mobile genetic elements such as plasmids, transposons and bacteriophages. Resistant bacteria flourish in areas of heavy antibiotic use such as hospitals and ICU.

Genotypic Characteristics

Specific ESBLs appear to be unique to a certain country or region. For example, TEM-10 has been responsible for several unrelated outbreaks of ESBL producing organisms in the United States for a number of years[31]. However, TEM-l0 has only recently been reported in Europe with the same frequency[32]. Similarly, TEM-3 is common in France, but has not been detected in the United States[33]. In recent years, there have been reports of outbreaks of TEM-47-producing organisms in Poland[34] and the prevalence of TEM-52 in Korea is unique to that country[11]. Another recent survey of Korea revealed that the SHV-12 and SHV-2a β-lactamases are the most common ESBLs found in Korea[35]. In contrast, the SHV-5 β -lactamase is commonly encountered worldwide and has been reported in Croatia, France, Greece, Hungary, Poland, South Africa, the United Kingdom, and the United States[4]. ESBLs are most often encoded on plasmids, which can easily be transferred between isolates. In an outbreak of ESBL-producing *Klebsiella pneumoniae* and *E. coli* in Chicago, it was shown that a common plasmid expressing TEM-10 was found in isolates from numerous patients in several hospitals and nursing homes[36]. Because this plasmid was found in multiple different strain types, as demonstrated by pulse field gel electrophoresis (PFGE), it was presumed that this promiscuous plasmid expressing TEM-l0 was transferred to the normal flora of some of the patients. In another report from France, a 180-kb self-transmissible plasmid expressing TEM-24 was found in four different species of Enterobacteriaceae like *E. coli. K. pneumoniae, P. aerogenes*, and *P. rettgeri* isolated from a single patient[37].

Clinical Infection

Common organisms involved in nosocomial infections are Gram-positive cocci and Gram-negative bacilli. Most frequent among these Gram-negative bacilli are *Klebsiella, Enterobacter, Pseudomonas, Serratia, Proteus,* and *E. coli*[38]. With widespread use of antibiotic, the frequency of penicillinase producing *staphylococci* increased. A change in the penicillin binding protein led to methicillin resistant *staphylococci aureus* (MRSA) against which vancomycin is the only line of defense. Quinolones demonstrated an acceptable activity against (MRSA) when they became first available in the 1980s. Organisms previously known to produce β-lactamase occasionally such as *E. coli, M. catarrhalis* now are frequent. Additionally, those originally beta-lactamase negative, such as *H. influellzae* and *N. gonorrhoea* are now β-lactamase producers[19]. A high rate of resistance to erythromycin and clarithromycin has been reported among *Pneumococci*[39]. *Klebsiella pneumoniae,* which have extended spectrum beta-lactamases and aminoglycoside inactivating enzymes have been found. More recently, the availability of the second-generation cephalosporins, such as cefamondole, cefoxitin, and cefuroxime, or 3rd generation cephalosporins, such as cefotaxime, ceftazidime, ceftriaxone has been the leading cause of potential resistances in nosocomial Gram-negative bacilli[38]. Hospital outbreaks of multi-drug resistant *Enterobacteriaceae* are now being frequently caused by extended spectrum beta-lactamase (ESBL) producers. Incidence of ESBL producing strains among clinical isolates has been steadily increasing over past years resulting in limitation of therapeutic options. Bacterial antibiotic

resistance has become a major clinical concern worldwide including Bangladesh. Recently, the use of second and third generation cephalosporins has led to the selection of Gram-negative organisms resistant to β-lactamase stable cephalosporins. This resistance is attributed to the production of extended spectrum β-Lactamases[20].

Antibiotics Used Against ESBL

Over the last 20 years, many new β-lactam antibiotics have been developed that were specially designed to be resistant to the hydrolytic action of β-lactamases. However, with each new class that has been used to treat patients, new lactamases emerged that caused resistance to that class of drug. Presumably, the selective pressure of the use and overuse of new antibiotics in the treatment of patients has selected for new variants of β-Lactamase. One of these new classes was the oxyimino cephalosporins, which became widely used for the treatment of serious infections due to Gram-negative bacteria in the 1980s[4]. Not surprisingly, resistance to these extended-spectrum β-lactam antibiotics due to β-lactamases emerged quickly. The first of these enzymes capable of hydrolyzing the newer β-lactams SHV-2 was found in a single strain of *Klebsiella ozaenae* isolated in Germany[29]. Because of their increased spectrum of activity, especially against the cephalosporins, these enzymes were called extended spectrum β-lactamases. Today, over 150 different ESBLs have been described. These β-lactamases have been found worldwide in many different genera of *Enterobacteriaceae* and *Pseudomonas aeruginosa*[4].

Biochemical characteristics

ESBLs contain a number of mutations that allow them to hydrolyze expanded- spectrum β-lactam antibiotics. While TEM and SHV type ESBLs retain their ability to hydrolyze penicillin, they are not catalytically as efficient as the parent enzymes[31]. In addition, the expansion of the active site that allows the increased activity against expanded-spectrum cephalosporins may also result in the increased susceptibility of ESBLs to β-Lactamase inhibitors[36]. ESBLs are not active against cephamycins, and most strains expressing ESBLs are susceptible to cefoxitin and cefotetan. However, it has been reported that ESBL-producing strains can become resistant to cephamycins due to the loss of outer membrane porin protein[4]. Because of their increased spectrum of activity, especially against the cephalosporin, these enzymes were called extended spectrum. These lactamases have been found worldwide in many different genera of *Enterobacteriaceae* and *Pseudomonas aeruginosa*[4].

Specific risk factors

The risk factors for acquisition of ESBL producing bacteria are ICU, severe illness, recent surgery, instrumentation like urinary or arterial catheterization, intubation and mechanical ventilation, prolonged hospital stay, antibiotic exposure, especially to extended spectrum antibiotics exerts a selective pressure for emergence of ESBL producing Gram-negative rods (Rice et al., 1990). Many of the patients infected with ESBLs are found in ICUs, but they can occur in surgical wards as well as most other areas of the hospital[38]. ESBLs are also being isolated with increasing frequency from patients in extended care facilities[21].

Conclusion

ESBLs are now a problem in hospitalized patients worldwide. However, it did not take long time before ESBLs had been detected in the United States and Asia. The prevalence of ESBLs among clinical isolates varies from country to country and from institution to institution. Among isolates of *K. pneumoniae*, the percentage of ceftazidime resistance ranges from 5 to 10% for non-intensive care unit (non-ICU) and ICU isolates, respectively.

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