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Review Article

FECAL CARRIAGE OF EXTENDED-SPECTRUM BETA-LACTAMASESPRODUCING *ESCHERICHIA COLI* IN HOSPITALS AND COMMUNITY SETTINGS: A REVIEW

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ABSTRACT

Antibiotic resistance is an emerging threat worldwide, endangering the treatment of serious diseases. Widespread resistance to beta-lactam antibiotics among Gram-negative bacteria (GNB) is a serious threat to the currently used antibacterial therapy. Extended-spectrum beta-lactamases (ESBLs) are a group of enzymes capable of hydrolyzing extended-spectrum cephalosporins and are inhibited by clavulanic acid. These enzymes are a major cause of hospital-acquired infection and community-acquired infections caused by *E. coli* (*Escherichia coli*). *E. coli* forms a part of normal intestinal flora and is an important reservoir of the antibiotic-resistant genes for ESBLs. These resistant antibiotic genes can be easily spread among GNB as these are carried on plasmids. Fecal carriers of ESBL producing *E. coli* in hospitalized patients and in community can be a reservoir for person-to-person transmission strengthening their dissemination. Over the last few decades, there had been a considerable increase in the emergence and spread of antimicrobial-resistant enzymes favoring the predominance of antibiotic-resistant bacteria which results in morbidity, mortality, and increased hospital expenditure. The gastrointestinal tract plays an important role in development of antibiotic-resistant microorganism and harboring the microorganisms as commensal. Antibiotic consumption may lead to alteration in the genome of the microorganisms leading to emergence of resistant microorganisms. The resistant microorganisms may then spread into the environment through faces aiding dissemination of the resistant genes.

Keywords: Morbidity, Mortality, Extended-spectrum beta-lactamases, *Escherichia coli.*

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INTRODUCTION

Resistance to antibiotics has posed a serious threat to both healthcare settings and community. The overuse and misuse of antibiotics has led to the bacteria to counter the effects of antibiotics by developing different resistant strategies [1]. A major consequence of antibiotic selection pressure is the emergence and spread of multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus*, Metallo-beta-lactamases (MBL) *Pseudomonas*, vancomycin-resistant *S. aureus*, extended-spectrum beta-lactamases (ESBLs)-producing bacteria, *Klebsiella pneumonia* carbapenemase-producing bacteria, vancomycin-resistant *Enterococcus*, and multidrug-resistant *Mycobacterium tuberculosis* [2]. Among all these, the ESBL-producing Gram-negative bacteria (GNB) have disseminated across the globe since its first description in 1983 [3].

Beta-lactam antibiotics such as penicillins and cephalosporins work by inhibiting enzymes responsible for the cell wall synthesis of bacteria. Beta-lactam antibiotics were the most common antibiotics used widely to treat infections caused by GNB. An excessive and continuous use of these antibiotics had promoted genetic mutations in the bacteria that benefitted them to expand their activity against several classes of beta-lactam antibiotics by producing ESBLs [4]. ESBLs are a group of enzymes conferring resistance to beta-lactam ring-containing antibiotics such as penicillins; first-, second-, and third-generation cephalosporins and aztreonam (but not the cephamycins or carbapenems) and are inhibited by beta-lactamase inhibitor such as clavulanic acid, sulbactam, and tazobactam [5]. The genes encoding for ESBLs are located on plasmids and thus can be transferred within and between different bacterial species by transformation, conjugation, or transduction process [6]. The plasmid-mediated transfer of ESBLs has resulted increase resistance to non-beta-lactam antibiotics such as trimethoprim-sulfamethoxazole, aminoglycosides, and fluoroquinolones, tetracyclines as plasmids may carry genes for conferring multidrug resistance presenting an additional challenge for controlling infections caused by ESBL-producing pathogens [7].

Escherichia coli is a GNB commonly found both in pathogenic as well as non-pathogenic feces. The normal commensal *E. coli* may carry genes for antimicrobial resistance that can be transferred to the pathogenic *E. coli*, so the commensal resistant bacteria of the gut are also a reason for concern as they can be a cause of dissemination of the antibiotic-resistant genes [8,9].

BETA-LACTAM ANTIBIOTICS: MECHANISM OF ACTION

Antibiotics have long been considered as the "magic bullet" that would end infectious diseases. They act against bacteria by one of the several ways such as inhibition of cell wall synthesis, inhibition of protein synthesis, impeding nucleic acid synthesis, cell membrane disorganization, or inhibition of a metabolic pathway [10]. Beta-lactam antibiotics include penicillins, cephalosporins, carbapenems, and monobactams. These antibiotics have in common a chemical structure that includes a three-carbon, one-nitrogen cyclic amine structure known as the beta-lactam ring. The side chain associated with the beta-lactam ring is a variable group attached to the core structure by a peptide bond; the side chain variability contributes to antibacterial activity [11]. They act by irreversible inhibition of carboxypeptidases and transpeptidases. These enzymes, also called as penicillin-binding proteins (PBPs) as they can bind irreversibly to the beta-lactam ring, help synthesis of cell wall of bacteria by catalyzing the cross-linking of D-alanine-D-alanine amino acid residues which present in the nascent peptidoglycan layers. Beta-lactam antibiotics share a structural similarity with the D-alanine amino acid residue present in the cell wall. Beta-lactam ring binds to the serine residue of the active site of PBP impeding the binding of D-alanine residue. This irreversible inhibition of PBPs inhibits cell wall synthesis, ultimately resulting in death of bacterial cell by activation of an autolytic system [12,13].

The resistance to beta-lactam antibiotics may be natural or may be acquired through genetic mutations or gene transfer. GNB have developed resistance to beta-lactam antibiotics by producing beta-

lactam hydrolyzing enzymes called as beta-lactamases and Gram-positive bacteria have conferred resistance by producing an altered PBP which is insensitive to beta-lactams [14].

AN INSIGHT INTO THE HISTORY OF ESBLS

The beginning of antibiotic era with the discovery of penicillin by Alexander Fleming in the year 1928 is marked by the consecutive development of antimicrobial resistance among different pathogens. Alexander Fleming in 1928 observed the growth of a mould of the genus *Penicillium* inhibiting the growth of bacteria in close vicinity of the mould. He identified that the mould produces an antibacterial substance called penicillin [15]. Even before the introduction of penicillin for the therapeutic use, a bacterial enzyme penicillinase was discovered by Abraham and Chain in 1940. Only after few years of introduction of penicillin for clinical use, penicillin-resistant beta-lactamases were reported in various Gram-positive and GNB. These beta-lactamases were thought to break down the beta-lactam ring of the antibiotic resulting in ineffective antibiotic [12]. The first plasmid-mediated β-lactamase in GNB was temoniera (TEM)-1 which was described in the early 1960s.

To counter the effect of beta-lactamases, broad-spectrum cephalosporin was introduced and they remained the first line of treatment for over 20 years. However, soon after the availability of these antibiotics, enzymes resistant to broad-spectrum cephalosporins emerged known as ESBLs. In 1983, the first report of bacteria producing ESBLs was published. TEM was named so as it was first found in *E. coli* isolated from the blood of a patient named Temoneira from Greece [5]. In these 20 years, many new antibiotics were developed to treat infections to resist the action of beta-lactamases. However, with the discovery of each new antibiotic, a new class of beta-lactamases has emerged such as the use of broad-spectrum cephalosporins has lead the development of broad-spectrum beta-lactamases (TEM-1, SHV-1) which have emerged as a result of single nucleotide mutation in the TEM or SHV beta-lactamases. Widespread use of third-generation cephalosporins and aztreonam is believed to be the predominant cause of mutations in the earlier discovered broad-spectrum beta-lactamases (TEM, SHV) and has led to the development of ESBLs. The term ESBL was first used by Phillippon, so that they can be differentiated from the broad-spectrum beta-lactamases [16]. By the end of 20th century, many studies have been published studying the recovery of ESBLs from various clinical isolates and in different parts of the world [17]. Some of the studies have also proposed the use of inhibitors of beta-lactamases such as tazobactam for treating infections caused by ESBL-producing bacteria [12].

ESBL-PRODUCING *E. COLI* IN THE GUT: A SERIOUS THREAT

Human gastrointestinal tract (GIT) harbors a large number of bacteria, and *E. coli* forms one of the largest groups, so there is an important role played by the gut in the acquisition and transmission of resistant pathogens [9]. *E. coli* is a GNB belonging to the family *Enterobacteriaceae* and colonizes the GIT of the human forming the most abundant facultative anaerobe of the human intestinal microflora. Although *E. coli* is found as a commensal microorganism in the intestine of human, there are variants of *E. coli* that are pathogenic as well. Pathogenic strains can be classified as intestinal pathogens causing diarrhea and extraintestinal pathogens causing a variety of other infections including urinary tract infections, meningitis, and septicemia [18]. *E. coli* presents as gut normal microflora may carry antibiotic-resistant genes on plasmids. Pathogenic diarrhea causing strains of *E. coli* can acquire resistant genes from the commensal *E. coli* in the intestine. Due to the augmented use of antibiotics, the sensitive strains of *E. coli* are killed allowing a more resistant ESBL-producing *E. coli* to survive and grow rapidly to increase in number. These resistant bacteria may then spread to the other person through environment contaminated with faces of these persons [19]. Poor hand hygiene and close contact with the cattle harboring the resistant *E. coli* can be an important contributing factors for the spread of ESBL-producing *E. coli* from a common source or from person to person [20].

CLASSIFICATION OF BETA-LACTAMS AND TYPES OF ESBL

More than 200 types of ESBL are known till now. There are different schemes for the classification of beta-lactams. The two major classification schemes followed for classifying beta-lactams into different categories are the Ambler molecular classification and the Bush-Jacoby-Medeiros functional classification [21]. Ambler classification scheme divides β-lactamases into Class A, Class B, Class C, and Class D enzymes on the basis of protein sequence (amino acid similarity). In the Ambler classification scheme, β-lactamases of Classes A, C. and D are serine β-lactamases. In contrast, the Class B enzymes are MBL. With the exception of OXA-type enzymes (which are Class D enzymes), the ESBLs are of molecular Class A [3].The majority of ESBLs identified in clinical isolates to date have been SHV or TEM types, which have evolved from narrow-spectrum β-lactamases such as TEM-1,2 and SHV-1. The Bush-Jacoby-Medeiros classification scheme also called as functional classification scheme classifies beta-lactamase based on substrate and inhibitor profile. According to this scheme, ESBL belongs to the "2be" Group or 2d Group (OXA type ESBL). The 2be designation shows that these enzymes are derived from Group 2b beta-lactamases (for example, TEM-1, TEM-2, and SHV-1); the "e" of 2be denotes that the beta-lactamases have an extended spectrum. The ESBLs derived from TEM-1, TEM-2, or SHV-1 differ from their progenitors by as few as one amino acid. This has resulted in a profound change in the enzymatic activity of the ESBLs so that they can now hydrolyze the third-generation cephalosporins or aztreonam, hence the extension of spectrum compared to the parent enzymes [21]. Table 1 shows the Bush Jacoby functional classification and Amber molecular classification of ESBL along with their characteristics.

EPIDEMIOLOGY OF ESBL-PRODUCING *E. COLI*

Antimicrobial resistance is rapidly spreading across the globe and entails a significant threat to public health. Antibiotic resistance increases the morbidity, mortality, and costs of treating infectious diseases [22,23]. The gut plays a vital role in the development of antibiotic resistance, and the emergence of resistant microorganisms in the gut may be related to ingestion- or antibiotic-induced alterations in microorganisms. The resistant organisms then contaminate the environment. Asymptomatic fecal carriage of ESBL-producing bacteria had been reported from several countries and continents with wide differences in carriage rates between geographic areas. Over the years, prevalence of ESBL-producing *E. coli* has increased enormously. Various studies have documented the prevalence and susceptibility of ESBL-producing *E. coli*, and each one had reported quite different ESBL rates. As mentioned earlier also, ESBLs were first described in 1983 from Germany and England. Various surveys conducted across the region had demonstrated the geographical variation. In a survey of laboratories in the Netherlands, <1% of *E. coli* and *K. pneumoniae* strains possessed ESBL [24]. While in another study conducted in France and Italy, ceftazidime resistance was observed in as many as 40% of strains of *K. pneumonia* [25]. The first ESBL-producing organisms were first reported in the US in 1988 [26].

A study conducted in Vietnam observed a prevalence of 87.4% of GNB from various clinical specimens out of a total 350 isolates. Of these GNB isolates, 88.9% were *Enterobacteriaceae*, of which 14.7% were ESBL-positive [27]. A study conducted by Ko *et al*., at South Korea, documented 22.4% of *K. pneumoniae* isolates and 10.2% of *E. coli* isolates as ESBL producers [28]. In a study from India, the percentage of ESBL-positive isolates was found to be elevated, with 23.1% of isolates being ESBL-positive [29]. Of the isolates from India, 48.4% of isolates were *E. coli* and 51.6% were *K. pneumoniae* [30]. ESBL prevalence of 30-60% of from intensive care units in Brazil, Colombia, and Venezuela had been reported [31-34]. Moreover, several studies had been conducted to test the prevalence fecal carriage of ESBL-producing GNB as these carriers can form important reservoirs for the transmission of hospital-acquired infection (HAI) and community-acquired infections caused by these bacteria. In a study conducted at South Africa by Mahomed and Mahomed out of

Table 1: Bush-Jacoby functional classification and Amber molecular classification

Bush-Jacoby group	Ambler class	Characteristics	Examples of enzymes
1	C	Cephalosporinases, chromosomal enzymes, resistant to clavulanic acid	*E. coli* AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
2a	A	Penicillinase, greater hydrolysis of benzylpenicillin than cephalosporins, inhibited by clavulanic acid	PC1
2b	A	Broad-spectrum beta-lactamases inhibited by clavulanic acid and but not by EDTA	TEM-1, TEM-2, SHV-1
2be	A	ESBLs, inhibited by clavulanic acid	TEM-3, SHV-2, CTX-M-15
2br	A	Penicillinases not inhibited by clavulanic acid and EDTA	TEM-30, SHV-10
2c	A	Enzymes hydrolyzing carbenicillin, inhibited by clavulanic acid but not by EDTA	PSE-1, CARB-3
2d	A	Enzymes hydrolyzing cloxacillin	OXA-1, OXA-10
2e	A	Extended-spectrum Cephalosporins, inhibited by clavulanic acid but not by aztreonam	CepA
2f	A	Enzymes hydrolyzing carbapenems, inhibited by clavulanic acid	KPC-2, IMI-1, SME-1
3	B	Metalloenzymes that hydrolyze carbapenems and other beta-lactams except monobactams. Not inhibited by clavulanic acid	CphA, Sfh-1
4	D	Miscellaneous enzymes that do not fit into other groups	

EDTA: Ethylenediaminetetraacetic acid, ESBLs: Extended-spectrum beta-lactamases

Table 2: Screening by disc diffusion method

Antibiotic (mcg)	Diameters of zone of inhibition (mm)
Cefpodoxime, 10	≤17
Ceftazidime, 30	≤22
Cefotaxime, 30	≤27
Ceftriaxone, 30	≤25

300 samples analyzed, *E. coli* isolates were obtained from 97 stool samples and *K. pneumoniae* from 12 patients only. ESBL production was positive only in 3 *E. coli* isolates (3.1%) and 11 isolates out of 12 *K. pneumoniae* were ESBL-positive. The combined fecal carriage of ESBL-producing *E. coli* and *K. pneumoniae* was 4.7% [35]. Reuland *et al*. in a study determined the rate of fecal carriage of ESBL-producing *Enterobacteriaceae* in the Dutch community in the Netherlands. Out of the total 720 samples, 73 of them were tested positive for ESBL-producing *Enterobacteriaceae,* among which the predominant was *E. coli* [36]. In contrast, rates reported in Europe never exceeded 10%, with the exception of a recent report of 11.6% observed in 2011 among patients on admission to a geriatric unit in Belgium. Community carriage in Africa has been studied very poorly. Reported rates appear to be quite high, from 10.0% in Senegal [37] to 30.9% in Niger [38]. In a study conducted in a Tertiary Care Hospital in Chennai, India, a high prevalence of ESBL producers was observed. Higher resistance rate was noted for amoxyclav, ciprofloxacin, cefotaxime, ceftazidime, and ertapenem [39]. A study carried out in Coimbatore, India, showed that among 49 isolates, *E. coli* registered its prevalence in about 44 samples followed by *Klebsiella* spp. and *Pseudomones* spp. [40]. Thus, the reported prevalence of carriage of ESBL-producing bacteria may be influenced by characteristics of the population under study such as the geographic area, previous use of antibiotics, and healthcare environment.

PHENOTYPIC METHODS FOR THE DETECTION OF ESBL PRODUCTION IN *E. COLI* ISOLATES

Phenotypic detection of ESBL-producing *E. coli* isolates in clinical laboratory can be done by performing various screening and confirmatory tests according to the guidelines lead by clinical laboratory standard institute [41].

Screening methods for ESBL producers

Susceptibility of positive *E. coli* to third-generation cephalosporins is detected by performing antibiotic susceptibility test on Mueller-Hinton agar using a 0.5 McFarland standard inoculum. Screening method for detection of ESBL production is based on measuring the zone of inhibition. *E. coli* isolates may be regarded as positive for screening test for ESBL production under the following conditions as shown in Table 2.

BROTH MICRODILUTION METHOD

Broth microdilution test can be performed with Mueller-Hinton broth to determine the minimum inhibitory concentration. A positive test for ESBL-producing *E. coli* isolates is indicated by a MIC ≥8 µg/ml for cefpodoxime, MIC ≥2 µg/ml for ceftazidime, aztreonam, cefotaxime, or ceftriaxone.

SELECTIVE MEDIUM FOR DETECTION OF ESBL-PRODUCING *E. COLI*

Screening can also be made by the use of selective medium such as chrom ID ESBL agar, Brilliance ESBL agar, and HiCrome ESBL agar. ESBL-producing *E. coli* isolates produce blue-violet colonies on chrom ID ESBL agar and pink to burgundy colonies on Brilliance ESBL agar and either pink or purple colonies on HiCrome ESBL agar.

The isolates showing positive screening test are then tested further by confirmatory methods for ESBL detection as positive screening does not necessarily rule out the production of ESBL.

PHENOTYPIC CONFIRMATORY METHODS FOR THE DETECTION OF ESBL PRODUCTION

Double disc synergy test

Standardized inoculums of the test isolate are swabbed on the surface of a Mueller-Hinton agar plate. A combination disc, such as ceftazidime and clavulanic acid (30/10 mcg), piperacillin and tazobactam disc (100/10 mcg), was placed at the center surface of the plate. Disc containing 30 mcg of ceftazidime, cefotaxime, ceftriaxone, and 10 mcg of cefpodoxime was placed at a distance of 1.8 cm away from the central disc.

An extension in the zone of inhibition around the peripheral disc toward the centrally placed ceftazidime/clavulanic acid disc indicated ESBL production. An advantage of this method is that the method is relatively simple.

Combined disc test (inhibitor potentiated disc test)

Cephalosporins disc (cefotaxime 30 mcg, ceftazidime 30 mcg, and cefpodoxime 30 mcg) with or without clavulanic acid, 10 mcg, was placed on the Mueller-Hinton agar incorporated with the test organism. An increase in the inhibition zone diameter of ≥5 mm in cephalosporins

Table 3: Worldwide distribution of ESBL-producing *Enterobacteriacea*

Study group	Year of study	Study area	Clinical isolates	ESBL prevalence (%)	Risk factors
Blom *et al*. [41]	2016	Sweden	*E. coli*	10	Foreign travel
Asir *et al*. [42]	2015	India	*E. coli*	21	Invasive devices such as urinary catheters
Mahomed and Mahomed [34]	2014	South Africa	*E. coli* and *K. pneumoniae*	4.7	Contaminated food and water
Shakya *et al*. [43]	2013	India	*E. coli*	9	Higher socioeconomic status
Reuland *et al*. [35]	2012	Netherland	*Enterobacteriaceae*	10.1	Foreign travel
Wickramasinghe *et al*. [44]	2012	UK	*E. coli*	11.3	Foreign travel
Woerther *et al*. [37]	2011	Niger	*Enterobacteriaceae*	31	Transmission from hospitals
Peirano *et al*. [45]	2011	Canada	*E. coli*	14	Foreign travel
Herindrainy *et al*. [46]	2011	Madagascar	*Enterobacteriaceae*	10.1	Hospital acquisition

disc combined with clavulanic acid, compared to cephalosporins alone, indicated ESBL production.

Risk factors for acquisition of ESBL

The prevalence of ESBLs among clinical isolates varies between countries and from institution to institution. Several studies have already revealed various risk factors associated with colonization and infection with ESBL-producing organisms. In prolonged hospital stay, patients on medical devices such as urinary catheters, and central venous lines have been associated with infections by these organisms. Further, surgical procedures and indiscriminate use of antibiotics are added risk factors for the acquisition of ESBL-producing organisms. Presence of highly resistant pathogens in hospital sewage may result in transmission of resistant bacteria from environment to human.

Table 3 also describes various studies conducted across the world in different years to study the prevalence and the associated risk factors of ESBL-producing *E. coli* and other GNB.

CONCLUSIONS

Colonization with multidrug-resistant isolates, including ESBL producing isolates, is one of the significant risk factors for infection. Therefore, importance of detection of carriers of antimicrobial-resistant bacteria in hospitalized patients as well as in community is of utmost value. Antibiotic selection pressure in hospital may be the contributing factor for the presence of large number of carriers harboring resistant bacteria. By minimizing selective pressure through more judicious use of antibiotics, we may well be able to maintain antimicrobial susceptibility patterns at a level and we can tackle with. It was observed that healthy individuals also carried the commensal ESBL-producing *E. coli* in their gut in a high percentage. The occurrence of these ESBL-positive *E. coli* strains as colonizers in the community indicates a reservoir outside the hospitals that should be taken seriously regarding implementation of screening and hygiene precautions for prevention of infections with these drug-resistant bacteria.

Strict adherence to patient hygiene and infection control practices may be enforced to curtail hospital acquired infections. Rational use of antibiotics would substantially decrease pressure on the gut microflora and thereby limit acquisition of resistant genes among these microorganisms. There is a need to have stringent local and national research and surveillance efforts to monitor resistance pattern of commensal *E. coli.*

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