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Extended spectrum Beta lactamase (ESBL) Mechanism of antibiotic resistance and Epidemiology

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Abstract: The antibiotics are widely utilized to control pathogenic microbes causing infectious diseases, including Urinary tract infection (UTI), Bacteremia, pneumonia, Septicemia, diarrhea, caused by the gram negative bacteria, such as *Enterobacteriaceae, Klebsiella species and E.coli.* The extended spectrum β-lactmase (ESBL) producing strains of *Enterobacteriace*ae with diverse types of ESBL genes are resistant to antibiotics, which create serious global health problem especially in the hospital, set up. The mechanisms of ESBL production among *Escherichia coli, Klebsiella species and Enterobacteriaceae* are discussed.
Keywords: *ESBL, E-Coli, Klebsiella sp, Enterobacteriaceae.*

Introduction:

The discovery of antibiotics is playing a critical role in the treatment of bacterial infections. However the continuous usage of antibiotics resulted in the emergence of antibiotic resistance or multidrug resistance in various species of bacteria. The drug-resistant pathogens are of a major concern, since it is very difficult to identify by routine diagnostic techniques and lack of new antibiotics[1], especially for multidrug-resistant gram-negative bacteria which produce extended spectrum beta-lactamases (ESBLs).

The Enterobacteriaceae is the largest family of Gram-negative, rod shaped, non fermenting facultative, anaerobic bacteria. The majority of the Enterobacteriaceae strains are residing in the intestine of human and animals and few species are found in water and soil. The human pathogens, including *Escherichia coli* and *Klebsiella pneumonia* are playing critical roles since they cause various types of infections, such as bacteremia, infection in central nervous system, Urinary tract infection (UTI), Diarrhea and severe hospital-acquired infection[2] (Table.1).

Table1. Clinically important members of the family Enterobacteriaceae commonly causing infections

Clinically important strains	Common type of infections
Citrobacter *freundii*	UTIs (Urinary tract infections), pneumonia, meningitis, septicemia wound infections
Enterobacter. *aerogenes, E. cloacae*	UTIs, pneumonia, septicemia, wound infections
Escherechia *coli*	UTIs, diarrhea, septicaemia, meningitis
Klebsiella *pneumoniae, K. oxytoca*	UTIs, pneumonia, septicemia
Morganella *morganii*	UTIs, septicemia
Plesiomonas *shigelloides*	Diarrhea, septicemia
Proteus. *mirabilis, P. vulgaris*	UTIs, pneumonia, septicemia, meningitis, wound infections
Providencia *rettgeri, P. stuartii*	UTIs
Salmonella *enteritica*	Diarrhea, typhoid fever, septicemia, UTIs, osteomylitis
Serratia *marcescens, S. liquefaciens*	UTIs, pneumonia, wound infections, s septicemia
Shigella *sonnei, S. flexneri*	Diarrhea, dysentery
Yersinia *pestis, Y. enterocolitica*	Plague, enteritis, diarrhea, septicemia

Infectious diseases caused by members of *Enterobacteriaceae* is frequently treated with beta-lactam antibiotics. The beta-lactam is a broad spectrum antimicrobial agent consists of four atom ring called as beta-lactam ring in their molecular structure and inhibits cell wall synthesis in bacteria. This group includes penicillin**,** cephalosporins, e.g. cefotaxime, ceftriaxone, ceftazidime, monobactams, imipenem, oxyimino monobactam and carbapenems. The Resistant strains of *Escherichia coli* and *Klebsiella pneumonia* inactivate these antibiotics by hydrolysis of the beta-lactam ring (Fig.1). The ESBLs are able to break down penicillin's, cephalosporins, monobactams, rarely carbapenemase and New Delhi metallo beta-lactamase (NDM).[3]

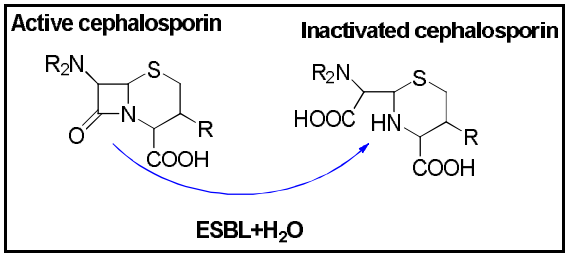

Figure 1: Inactivation of cephalosporin through hydrolysis of beta-lactam Ring by an ESBL

The ESBLs are broadly classified into three groups: TEM (Temorina *Escherichia coli* mutant), SHV (Sulfhydryl variant), and CTX-M (Cefotaximase-munich) types**.** *Klebsiella pneumoniae* and *Escherichia coli* are the major ESBL-producing organisms around the globe. The infections caused by ESBL-producing Enterobacteriaceae are more severe than non ESBL producing strains. ESBL genes are transferable between bacteria by horizontal transfer through plasmid which is double-stranded, extra chromosomal genetic material that replicate independently**.** Plasmid genes are not essential for bacterial survival; however they encode genes for virulence, adaptation, and resistance to heavy metals and antibiotics. Some plasmids are able to transfer them to other bacteria through bacterial conjugation [4].

ESBLs are primarily produced by the Enterobacteriaceae family of Gram-negative microbes, in particular *Klebsiella pneumonia* and *Escherichia coli.*[5-6] They are also produced by non fermentative Gram-negative organisms, such as *Acinetobacter baumannii* and *Pseudomonas aeroginosa and E.coli* .[7]

3.0 Classification of extended spectrum beta-lactmases(ESBLs).

The two popular classification schemes of beta-lactamase enzymes are Bush-Jacoby scheme which is based on functional characteristics of the enzymes and Ambler's scheme which is based there sequence similarity. In pursuance to Ambler's Molecular taxonomy scheme, beta-lactamases are divided in four classes (Table: 2)[9].

Table2. Taxonomy of beta-lactamases in pursuance to the Amber molecular scheme

	Class	β - lactamases	Examples
Serine β - lactamases	A	Broad spectrum β - lactamases	TEM-1, TEM-2, SHV-1
		ESBL TEM – type	TEM-3
		ESBL SHV – type	SHV-5
		ESBL CTX-M – type	CTX-M1, CTX-M9
		Carbapenemases	KPC
	C	AmpC cephamycinases (chromosomal encode)	AmpC
		AmpC cephamycinases (plasmid encode)	CMY, DHA
	D	Broad spectrum β - lactamases	OXA-1, OXA-9
		ESBL OXA – type	OXA-2, OXA-10
		Carbapenemases	OXA-48, OXA-23
Metallo β - lactamases	B	Metallo β - lactamases	VIM, IMP

TEM: Temorina *E-coli* variant, SHV: Sulfhydryl variant, ESBLs: Extended spectrum β-lactamases. OXA: Oxacillanase

Classes A, C, and D are serine-beta-lactamases which employ an active-site serine to catalyses hydrolysis, while class B, beta-lactamases are metalloenzymes requiring one or two zinc ions for their activity. The Bush-Jacoby Medeiros schemes group, these enzymes according to functional similarity (substrate and inhibitor profile). This classification scheme is more relevant to physicians or microbiologists in diagnostic laboratory because it considers beta-lactamase inhibitor and beta-lactam substrates that are clinically relevant **(Table 3)**

Table 3: Modified Bush –Jacoby Medeiros Classification of beta-Lactamases[9]

Functional group	Substrate profile	Molecular Class	Inhibitor	Representative enzyme(s)
1	Cephalosporinase	C	OXA	AmpC,MIR-1
2a	Penicillinase	A	Clav	*S.aureus*
2b	Broad spectrum	A	Clav	Tem-1/2,SHV-1
2be	Extended	A	Clav	Tem-3-29,Tem-46,Tem 104, SHV 2-28, CTX-M types
2br	Inhibitor resistant	A	-	Tem-30-41(IR 1-12)
2c	Carbenicillinase	A	-	AER-1 (C), CARB-3
2d	Oxacillinase	D	Clav	PSE-1
2e	Cephalosporinase	A	Clav	OXA-1, OXA-2,10
2f	Carbepenemase		Clav	IPM-1,NmcA, Smc1-3
3	Metalloenzymes	A	-	*S. maltophilia*
4	Penicillinase	B	-	B. cepacia (c)

TEM: Temorina *E-coli* variant, SHV: Sulfhydryl variant, ESBLs: Extended spectrum beta-lactamases. OXA: Oxacillanase

Group 1 are cephalosporinases not inhibited by clavulanic acid, related to the molecular class C. Group 2 are penicillinases, cephalosporinases, prevented by clavulanic acid, analogical to the molecular classes A and D reflects the original TEM and SHV genes. Additionally, many classes A TEM beta-lactamases are inhibited by beta-Lactamase inhibitor protein (BLIP). Because of the increasing number of TEM- and SHV-derived beta-lactamases, these were divided into two subclasses, 2a and 2b. The 2a subgroup restrains only penicillinases.

In resistance to 2a and 2b are broad-spectrum beta-lactamases, significance that they are capable of inactivating penicillin's and cephalosporins at the same way. Even, maiden subgroups were divided from subgroup 2b. Subgroup 2be, with the letter "e" for an extended spectrum of activity, represent the ESBLs, those are adequate of inactivating third-generation ceftazidime, cefotaxime, and Cefpodoxime also aztreonam. The 2br Enzymes, with the letter "r" enlighten reduced binding to Clavulanic acid and sulbactam, these are also called inhibitor-resistant TEM-derivative enzymes; whatever, they are commonly still susceptible to Tazobactam, where an amino acid substitution exists at position met69. Subgroup 2c was segregated from group 2 because these enzymes inactivate carbenicillin more than benzyl penicillin, with some effect on Cloxacillin. Subgroup 2d enzymes inactivate Cloxacillin greater than benzyl penicillin, as well as some activity against Carbenicillin and these enzymes are poorly inhibited by clavulanic acid, whatever, few of them are ESBLs the right term is "Oxacillinase". These enzymes are capacitated to inactivate the oxazolylpenicillins as oxacillin, cloxacillin. The enzymes belong to the molecular class D not molecular class A. Subgroup 2e enzymes are Cephalosporinases that can also hydrolyze monobactams, and they are inhibited by clavulanic acid. Subgroup 2f was added because these are serine-based carbapenemases, in resisting to the zinc-bound carbapenemases included in group 3. Group 3 is the zinc-based or metallo beta-lactamases, corresponding to the molecular class B, which are the only enzymes acting by the metal ion zinc. Metallo beta -lactamases are able to hydrolyze penicillin's, cephalosporins, also carbapenems hence carbapenems are inhibited by both groups 2f (serine-based mechanism) and group 3 (zinc-based mechanism). Group 4 is penicillinases that are unable to inhibit by clavulanic acid, and they do not have a corresponding molecular class[9].

4. Epidemiology

When ESBLs were first recognized in the year of 1980s, they were found out to be point mutations of the TEM and SHV enzymes, which resulted in resistance to the β-lactam class of antibiotics[10]**.** The mutations in the genes resulted in high catalytic activity for beta-lactams due to low Km values (i.e., high affinity) for the compounds TEM and SHV-types have been recognized worldwide with over 100 mutations being reported are responsible for resistance to the extended spectrum cephalosporins[11]**.** All around the globe 150 million of urinary tract infection per annum were reported and about 35% of those are suffering from nosocomial infection[22]**.** The prevalence of bacteria producing ESBL varies globally across the world including united state of America (North and South), Europe, Africa (South Africa, North, and East Africa) and Asian countries**.**

4.1 Africa

In Algerian hospitals, ESBLs are detected around 16.4-31.4% of the clinical samples. Class A ESBLs were most common, but plasmid-encoded AmpC (pAmpC) was also detected in the samples**.** In Egypt, ESBLs were found in 11-42.9% of samples in both hospitals and among the community[12]. In the city Guinea-Bissau and Libya, class A and D ESBLs and a carbapenemase were reported in 32.6 and 16%, respectively in fecal samples**.** In the city of Morocco, class A and D ESBLs, pAmpC, and carbapenemases were found in hospital settings[16-17]**.** In the community setting, class A and D ESBLs were found in acquired urinary tract infected urine samples. In Tunisia, class A and D ESBLs, pAmpC, and carbapenemases were present and the spreading ranges from 11.7 to 77.8% in city hospitals and was 0.7 and 7.3% in two communities[17]. In Cameroon, class A and D ESBLs were found in 55.3 and 82.8% of hospital fecal samples and in 17.2% of community fecal samples[18]**.**

4.2 USA

First reports of ESBLs in the USA in the late 1980s were reported with TEM-type and the major enzymes appeared to be the TEM and SHV types, with a minimum countenance of CTX-M types.

4.3 Europe.

In Europe, ESBL-producing Enterobacteriaceae has been spreading at a dangerous rate. Although there is comprehensive difference between European countries, nearly every European country has experienced outbreaks with ESBL-producing organisms. The first isolates were originally found in Germany and the UK; however, the first large outbreak was seen in France, where more than 50 patients in an intensive care unit (ICU) were infected with spread from other wards in the hospital.

4.4 Middle East (Arabian countries)

The studies in the Middle East revealed a higher prevalence of ESBL than in other parts of the world. A survey on *E. coli* ESBL producers in Egypt, conducted, during the period 1999 to 2000, indicated that, 38% of the *E. coli* tested positive for ESBLs. In another study in Iran, undertaken between 2007 and 2008, 45% of the *K.pneumoniae* isolated from urinary tract infections was found to be ESBL producers [19]. In the same study, it was detected that, 59.2% of *K. pneumoniae* of the clinical isolates from respiratory tract infections tested positive for ESBL production. In Iran, a one year study on *E. coli* collected from urinary tract infections showed that 25% of the isolates were ESBL producers. In 2007 in a study in K. pneumonia demonstrated a different range of ESBL production in different cities. In Saudi Arabia, about 26% of *K. pneumniae* isolated in 2008 produced ESBLs. In most of the isolates SHV-12, CTXM- 15 and TEM-1 were responsible for resistance to third generation cephalosporin[20].

4.5 Australia

The first report of an ESBL positive strain of *Klebsiella spp* was found in Australia in (gentamicin resistance) a study done between 1986 and 1988[21]. They later found that SHV was amenable for ESBL production in *Klebsiella spp***.** In the last decade, ESBL positive strains were also identified in all the regions in Australia. It is estimated that 5% of isolates in Australia are positive for ESBL production[22].

4.6 Asia

The first isolates of *K. pneumoniae* shelter from SHV-2 were reported from China in 1988 [23]. Lately the study of ESBLs in Asia showed a high prevalence among clinical strains. In 2001, the first CTX-M positive strains were reported in New Delhi. Some studies on limited isolates collected between 1998 and 1999, showed

that 30.7% of *K. pneumoniae* and 24.5% of *E.coli* isolates were ESBL producers. In China, between 1997-1999, 27% of *E. coli* and *K.pneumonia* were identified to be ESBL producers[24]. It is estimated that 5 to 8% of *E. coli* isolates from the countries, Korea, Japan, Malaysia also Singapore were positive for ESBL while it was 12 to 24% in Thailand, Taiwan, the Philippines, and Indonesia. However, the *K. pneumoniae* ESBL producers were less than 5% while in other Asian countries it was between 20 to 50%. There are variations between different hospitals. It has been reported that 1/4 of all the *K. pneumoniae* collected from various hospitals in Japan amid 1998-1999 tested positive for ESBL production[22].

The predilection of ESBLs for *K. pneumoniae* has never been clearly explained. It should be noted that the parent enzyme of TEM-type ESBLs, that is TEM-1, is widespread than many other species. Almost all the non-ESBL-producing *K. pneumoniae* isolates have chromosomally mediated SHV-1 beta-lactamases[24].

As early as the mid 1990s, it was noted that 25% of the *Enterobacteriaceae* in Thailand were producing ESBLs, mainly different SHV enzymes[51-53]. Analyzed stool samples from healthy volunteers in Thailand in 2009, and the results showed that 30–50% of these subjects in three different regions were ESBL carriers (CTX-M types). The first report of CTX-M-producing *Enterobacteriaceae* in New Delhi was published in 2001[25]. Found that 66% of third-generation cephalosporin-resistant E. coli and K. pneumoniae from three medical Centers in India harbored the CTX-M-15 type of ESBL, which was also the only CTX-M enzyme showed, and an investigation of 10 another Center in that country showed that rates of ESBL producing Enterobacteriaceae reached 70%. Studies observed ESBL rates of 46% and 50% in out- and inpatients, respectively, and NASA co-workers[27]detected ESBL production in almost 80% of clinical isolates. Investigations from India and Pakistan shows an alarming and rapid increase in the prevalence of Enterobacteriaceae with NDM-1 with a prevalence rate from 6.9% in a hospital in Varanasi, India, to 18.5% in Rawalpindi, Pakistan [28] and perhaps the spread of these enzymes could be even more rapid than the spread of the CTX-M enzymes**.**

Recently *P. aeruginosa* isolate was obtained from endotracheal suction tip of 84 years old male patient diagnosed with CVA also hypertension. ESBL producing OXA β-lactamases was determined by PCR w *P. aeruginosa* producing OXA-4 ESBL [29] for the first time in the Indian subcontinent. A study from Tamilnadu, India reported a predominance of bacteria as follows :*E-coli* 31.5%, S. aurious 20.5%, Pseudomonas aeroginosa 7.5% , Proteus sp. 7.4%, These strains were resistant to antibiotics at decreasing levels of trimithoprin-sulphamethaxazole 83.3%,nalidaxic acid 67.37%,amoxicillin 67.3%,Co-trimoxazole, 61%,gentamycin 48.8%,ciprofloxacin 46%,and cefotaxime 43% in vitro[30].

5. Alternative anti microbial therapy

In recent days, topical therapy with antibiotics has become unpopular because of the development of resistance. One of the alternative approaches is to use photochemical to destroy bacteria infecting a wound in an animal model without damaging the surrounding host tissue. After topical application of a chlorine photosensitizer conjugated with poly-l-lysine, *E. coli* was rapidly killed upon exposure to selected visible light wavelengths. An alternative scenario was tested against acute bacterial skin and skin structure infection, where more patients switched to oral linezolid at discharge (60%) compared to vancomycin (36%) and daptomycin (4%). Mean time on antibiotic increased by 0.3 days due to additional time in the outpatient setting, given the longer duration of treatment with oral linezolid**.** A-thanatin is highly effective against extended spectrum beta-lactamases *E.coli* in vitro, with Minimum inhibitory concentration (MIC) rate is less than $4_{\mu g}$/mL. It has been confirmed that A-thanatin has little hemolysis and relatively high stagnation in plasma. Excellent in vivo therapeutic influence was also observed in a Septicemic animal model, with survival rates of 50.0%, 66.7%, and 91.7% in the low -dose, middle-dose, and high-dose groups, respectively. Membrane permeabilization may be a major biological action of A-thanatin. The bacteriophage-derived lysins can able to degrade bacterial peptidoglycans. Lysin CF-301 has been developed to treat Staphylococcus aureus because of its potent bacteriolytic effects on drug-resistant strains and eradicates biofilms, and utilized in combinational therapy with antibiotics [30].

6. Conclusion

ESBLs are known to cause problems in patients who are especially hospitalized. There have been reports of an increasing prevalence of ESBLs in different regions of the world. The high risk patients are known to be those who are contaminated with ESBL producing strains as this renders treatment to be ineffective for them. Thus there is an urgent need for immediate identification and appropriate policy directions to reduce the

prevalence of ESBLs. In dealing with the infected patient, priority must be given and along with antimicrobial therapy, proper hygiene must be maintained in the hospital during treatment. The molecular detection of the genes encoding ESBLs would be a reliable approach to investigate the mode of transmission in the hospitals. The alternative therapies including combinational therapy with herbs, natural compounds and small molecules will reduce the incidence of antibiotic resistance among the ESBL producers.

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