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ORIGINAL ARTICLE

Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment

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Abstract Antibiotic resistance is a problem of deep scientific concern both in hospital and community settings. Rapid detection in clinical laboratories is essential for the judicious recognition of antimicrobial resistant organisms. Production of extended-spectrum β-lactamases (ESBLs) is a significant resistance-mechanism that impedes the antimicrobial treatment of infections caused by *Enterobacteriaceae* and is a serious threat to the currently available antibiotic armory. ESBLs are classified into several groups according to their amino acid sequence homology. Proper infection control practices and barriers are essential to prevent spread and outbreaks of ESBL producing bacteria. As bacteria have developed different strategies to counter the effects of antibiotics, the identification of the resistance mechanism may help in the discovery and design of new antimicrobial agents. The carbapenems are widely regarded as the drugs of choice for the treatment of severe infections caused by ESBL-producing *Enterobacteriaceae*, although comparative clinical trials are scarce. Hence, more expeditious diagnostic testing of ESBL-producing bacteria and the feasible modification of guidelines for community-onset bacteremia associated with different infections are prescribed.

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1. Introduction

Resistance of pathogenic organisms to countenance antibiotics has become a worldwide problem with serious consequences on the treatment of infectious diseases. The heightened use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to the phenomenon. There

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is an alarming increase of antibiotic resistance in bacteria that cause either community infections or hospital acquired infections. Of particular interest are the multidrug resistant pathogens, *e.g. Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant *Enterococcus*, and extensively drug-resistant *Mycobacterium tuberculosis* (Alekshun and Levy, 2007).

Beta-lactam antimicrobial agents exhibit the most common treatment for bacterial infections and continue to be the prominent cause of resistance to β-lactam antibiotics among Gram-negative bacteria worldwide. The persistent exposure of bacterial strains to a multitude of β-lactams has induced dynamic and continuous production and mutation of β-lactamases in these bacteria, expanding their activity even against the newly developed β-lactam antibiotics. These enzymes are known as extended-spectrum β-lactamases (ESBLs) (Pitout and Laupland, 2008; Paterson and Bonomo, 2005). Treatment of these multiple drug resistant organisms is a deep scientific concern. At the level of a wider geographic scale, the incidence of ESBL-producing organisms is difficult to resolve due to various reasons, difficulty in detecting ESBL production and inconsistencies in reporting (Steward et al., 2000). Recently, a significant increase in the incidents of ESBL-related infections has been observed throughout the globe (Rupinder et al., 2013; Abhijit et al., 2013; Majda et al., 2013; Meeta et al., 2013; Kritu et al., 2013; Fatemeh et al., 2012; Gupta, 2007).

2. How do antibiotics work?

There are five major modes of antibiotic mechanisms of activity and here are some examples.

2.1. Interference with cell wall synthesis

Beta-lactam antibiotics like penicillin and cephalosporin impede enzymes that are responsible for the formation of peptidoglycan layer (Benton et al., 2007).

2.2. Inhibition of protein synthesis

Oxazolidinones, the newest class of antibiotics, interact with the A site of the bacterial ribosome where they should interfere with the placement of the aminoacyl-tRNA. Tetracyclines interfere with protein synthesis by binding to 30S subunit of ribosome, thereby weakening the ribosome-tRNA interaction. Macrolides bind to the 50S ribosomal subunit and inhibit the elongation of nascent polypeptide chains. Chloramphenicol binds to the 50S ribosomal subunit blocking peptidyl transferase reaction. Aminoglycosides inhibit initiation of protein synthesis and bind to the 30S ribosomal subunit (Leach et al., 2007).

2.3. Interference with nucleic acid synthesis

Rifampicin interferes with a DNA-directed RNA polymerase. Quinolones inhibit DNA synthesis with interference of type II topoisomerase, DNA gyrase and type IV topoisomerase during replication cycle causing double strand break (Strohl, 1997).

2.4. Inhibition of a metabolic pathway

Sulfonamides (e.g. sulfamethoxazole) and trimethoprim each block the key steps in the folate synthesis, which is a cofactor in the biosynthesis of nucleotides, the building blocks of DNA and RNA (Strohl, 1997).

2.5. Disorganizing of the cell membrane

The primary site of action is the cytoplasmic membrane of Gram-positive bacteria, or the inner membrane of Gram-negative bacteria. It is hypothesized that polymyxins exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial content. The cyclic lipopeptide daptomycin displays rapid bactericidal activity by binding to the cytoplasmic membrane in a calcium-dependent manner and oligomerizing in the membrane, leading to an efflux of potassium from the bacterial cell and cell death (Straus and Hancock, 2006). Antibiotic resistant versus antimicrobial activity mechanism is shown in Fig. 1.

3. Antibiotic resistance mechanism

Antibiotic resistance is the reduction in effectiveness of a drug such as an antimicrobial or an antineoplastic in curing a disease or condition. When the antibiotic is not intended to kill or inhibit a pathogen, then the term is equivalent to dosage failure or drug tolerance. More commonly, the term is used in the context of resistance that pathogens have "acquired", that is, resistance has evolved. When an organism is resistant to more than one drug, it is said to be multidrug-resistant (Fisher and Mobashery, 2010). Bacterial strains may possess different types of resistant mechanisms which are shown in Fig. 2 and are explained as follows.

3.1. Antibiotic inactivation

3.1.1. By hydrolysis

Many antibiotics have chemical bonds such as amides and esters which are hydrolytically susceptible. Several enzymes are known to ruin antibiotic activity by targeting and cleaving these bonds. These enzymes can often be excreted. Extended-spectrum β-lactamases (ESBLs) mediate resistance to all penicillins, third generation cephalosporins (e.g. ceftazidime, cefotaxime, and ceftriaxone) and aztreonam, but not to cephamycins (cefoxitin and cefotetan) and carbapenems (Bonnet, 2004).

3.1.2. By redox process

The pathogenic bacteria infrequently exploited oxidation or reduction of antibiotics. However, there are a few examples of this strategy (Yang et al., 2004). One is the oxidation of tetracycline antibiotics by the TetX enzyme. *Streptomyces virginiae*, a producer of the type A streptogramin antibiotic virginiamycin M1, protects itself from its own antibiotic by reducing a critical ketone group to an alcohol at position 16.

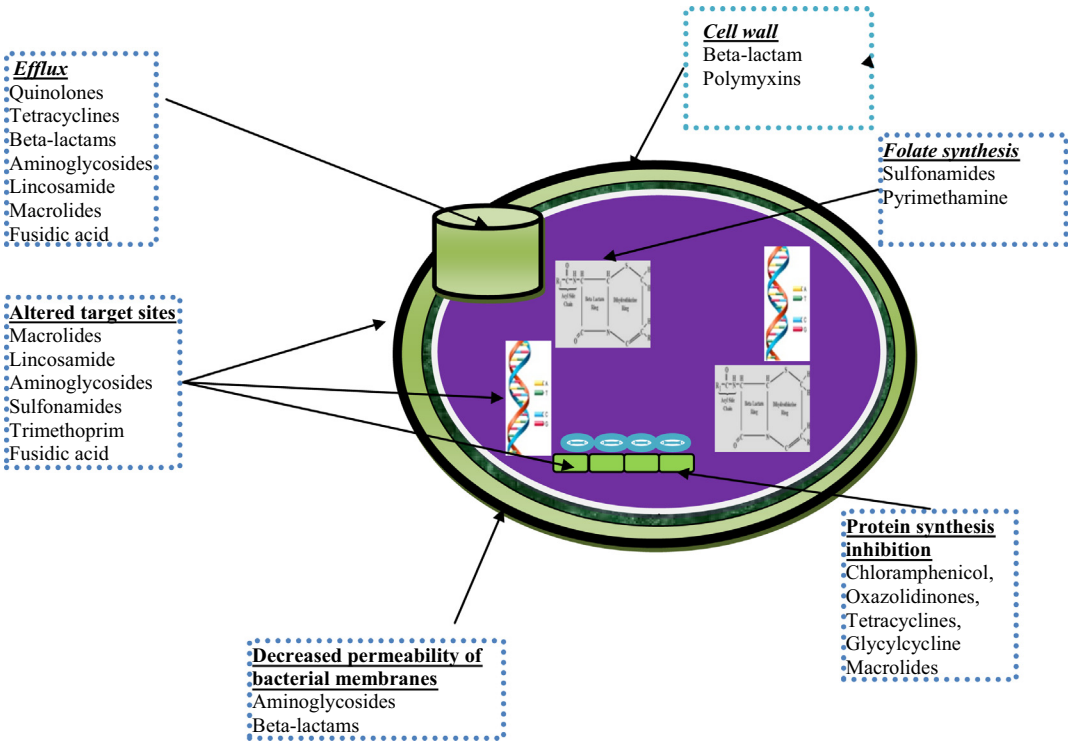

Figure 1 Antibiotic resistance vs. antimicrobial activity mechanism.

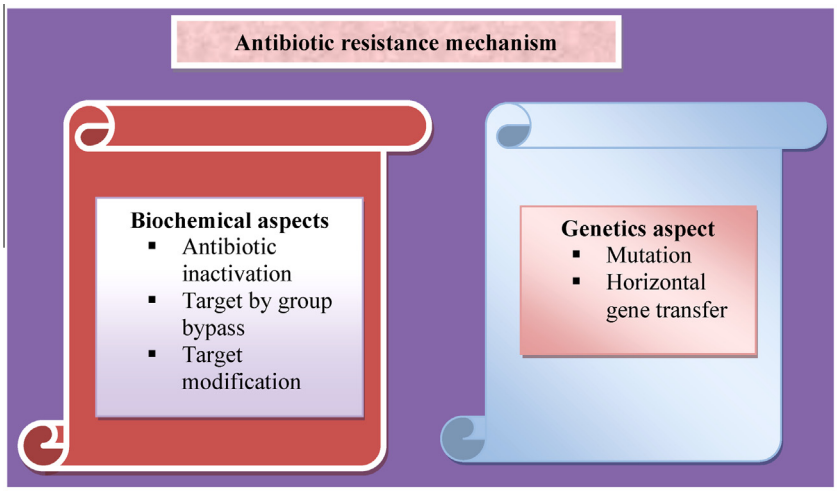

Figure 2 Biochemical and genetic aspects of antibiotic resistance mechanisms.

3.2. Antibiotic inactivation by group transfer

The most diverse family of resistant enzymes is the group of transferases. These enzymes inactivate antibiotics (aminoglycosides, chloramphenicol, streptogramin, macrolides or rifampicin) by chemical substitution (adenylyl, phosphoryl or acetyl groups are added to the periphery of the antibiotic molecule). The modified antibiotics are impaired in their binding to a target. Chemical strategies include O-acetylation and N-acetylation (Blanchard, 2004; Schwarz et al., 2004), O-phosphorylation (Matsuoka and Sasaki, 2004), O-nucleotidylation (Brisson-Noel et al., 1988), O-ribosylation, O-glycosylation, and thiol transfer. These covalent modification strategies all require a co-substrate such as ATP, acetyl-CoA, NAD^+, UDP-glucose, or glutathione for their activity and consequently these processes are restricted to the cytoplasm.

3.3. Antibiotic resistance via target modification

The second major resistance mechanism is the modification of the antibiotic target site so that the antibiotic is impotent to bind properly. However, it is possible for mutational changes to occur in the target that reduce susceptibility to inhibition while retaining cellular function (Spratt, 1994).

4. Genetics of antibiotic resistance

4.1. Antibiotic resistance via mutations

There is a substantial number of biochemical mechanisms of antibiotic resistance that are based on mutational events, like the mutations of the sequences of genes encoding the target of certain antibiotics (e.g. resistance to rifampicin and fluoroquinolones is caused by mutations in the genes encoding the targets of these molecules, RpoB and DNA-topoisomerases, respectively) (Ruiz, 2003). The variation in the expression of antibiotic uptake or of the efflux systems may also be modified by mutation (e.g. the reduced expression or absence of the OprD porin of *Pseudomonas aeruginosa* reduces the permeability of the cell wall to carbapenems) (Wolter et al., 2004).

4.2. Antibiotic resistance via horizontal gene transfer

A principal mechanism for the spread of antibiotic resistance is by horizontal transfer of genetic material. Antibiotic resistance genes may be transferred by different mechanisms of conjugation, transformation or transduction. Over the last 15 years, β-lactamase enzymes that have an extended spectrum of activity (ESBL) against the majority of β-lactams, including cephalosporins but not carbapenemases, have evolved. One of these, CTX-M-15, initially found in *E. coli* but now found in other members of *Enterobacteriaceae* and frequently associated with a specific lineage, uropathogenic clone ST131 (Bush and Fisher, 2011; Woodford et al., 2011), has spread worldwide. It is often located on highly mobile IncFII plasmids and associated with mobile genetic element IS26. The risk of infection is particularly high in individuals in association with prolonged hospitalization, catheterization, nursing home residency, previous antibiotic treatment, underlying renal or liver pathology, and travel to high-risk areas (Nordmann et al., 2011).

5. ESBL definition and classification

There is no consensus of the precise definition of ESBLs. ESBLs are a group of enzymes that break down antibiotics belonging to the penicillin and cephalosporin groups and render them ineffective. ESBL has generally been defined as transmissible β-lactamases that can be inhibited by clavulanic acid, tazobactam or sulbactam, and which are encoded by genes that can be exchanged between bacteria. The currently most common genetic variant of ESBL is CTX-M (Paterson and Bonomo 2005; Walsh, 2003).

Beta-lactamases are commonly classified according to two general schemes: the Ambler molecular classification and the Bush–Jacoby–Medeiros functional classification (Bush et al., 1995; Ambler, 1980). The Ambler scheme classifies β-lactamases into four classes according to the protein homology of enzymes. Beta-lactamases of class A, C, and D are serine β-lactamase and class B enzymes are metallo-β-lactamases. The Bush–Jacoby–Medeiros functional scheme is based on functional properties of enzymes, i.e. the substrate and inhibitor profiles.

5.1. SHV type

The SHV family of β-lactamases appears to be derived from *Klebsiella* spp. The progenitor of the SHV class of enzymes, SHV-1, is universally found in *K. pneumoniae*. In many strains of *K. pneumoniae*, the gene encoding SHV-1, or its apparent precursor, LEN-1, resides within the bacterial chromosome too; it may be that the gene for SHV-1 β-lactamase evolved as a chromosomal gene in *Klebsiella* and was later incorporated into a plasmid which has spread to other *enterobacteria* species. SHV-1 confers resistance to broad-spectrum penicillins such as ampicillin, tigecycline and piperacillin but not to the oxyimino substituted cephalosporins (Livermore, 1995). The SHV-1 β-lactamase is responsible for up to 20% of the plasmid-mediated ampicillin resistance in *K. pneumoniae* species (Tzouvelekis and Bonomo, 1999).

5.2. TEM type

TEM-1, first reported from an *E. coli* isolate in 1965, has substrate and inhibition profiles similar to those of SHV-1 (Datta and Kontomichalou, 1965). 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 (Soughakoff et al., 1988; Sirot et al., 1987). TEM-2 the first derivative of TEM-1, had a single amino acid substitution from the original β-lactamase (Barthelemy et al., 1985). This caused a shift in the isoelectric point from a pI of 5.4–5.6, but it did not change the substrate profile. TEM-3, originally reported in 1989, was the first TEM-type β-lactamase that displayed the ESBL phenotype (Soughakoff et al., 1988). In retrospect, TEM-3 may not have been the first TEM-type ESBL. *Klebsiella oxytoca*, harboring a plasmid carrying a gene encoding ceftazidime resistance, was first isolated in Liverpool, England, in 1982 (Du Bois et al., 1995). The responsible β-lactamase was what is now called TEM-12. Interestingly, the strain came from a neonatal unit which had been stricken by an outbreak of *K. oxytoca* producing TEM-1 (Du Bois et al., 1995). This is a good example of the emergence of ESBLs as a response to the selective pressure induced by extended-spectrum cephalosporins.

5.3. CTX type

A new family of β-lactamases that preferentially hydrolyzes cefotaxime has arisen. It has been found in isolates of *Salmonella enterica* serovar, *Typhimurium*, *E. coli* mainly and some other species of *Enterobacteriaceae* (Gazouli et al., 1998; Knothe et al., 1983). These are not very closely related to TEM or SHV β-lactamases (Tzouvelekis et al., 2000). 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 (Bradford et al., 1998; Ma et al., 1998).

CTX-M β-lactamases are found exclusively in the functional group 2 (Bush and Jacoby, 2010) and thought to originate from chromosomal ESBL genes found in *Kluyvera* spp. (Bush and Jacoby, 2012), an opportunistic pathogen of the *Enterobacteriaceae* found in the environment. The first

CTX-M proteins were discovered in the late 1980s and today more than 100 variants have been sequenced (Bonnet, 2004). Based on their amino acid sequences, they can be divided into five groups (CTX-M group 1, 2, 8, 9, and 25) (Bonnet, 2004).

The origin of the CTX-M enzymes is different from that of TEM and SHV ESBLs. While SHV-ESBLs and TEM-ESBLs were generated by amino acid substitutions of their parent enzymes, CTX-M ESBLs were acquired by the horizontal gene transfer from other bacteria using genetic apparatuses such as conjugative plasmid or transposon. The gene sequences encoding CTX-M enzymes show a high similarity to those of β-lactamases of *Kluyvera* species. In addition, the gene sequences adjacent to the CTX-M genes of *Enterobacteriaceae* are also similar to those surrounding the β-lactamase genes on the chromosomes of *Kluyvera* species (Olson et al., 2005; Humeniuk et al., 2002; Poirel et al., 2002).

Kinetic studies have shown that the CTX-M-type β-lactamases hydrolyze cephalothin or cephaloridine better than benzyl penicillin and they preferentially hydrolyze cefotaxime over ceftazidime (Tzouvelekis et al., 2000; Bradford et al., 1998). Although there is some hydrolysis of ceftazidime by these enzymes, it is usually not enough to provide clinical resistance to organisms in which they reside. It has been suggested that the serine residue at position 237, which is present in all of the CTX-M enzymes, plays an important role in the extended-spectrum activity of the CTX-M-type β-lactamases (Tzouvelekis et al., 2000). Although it has been shown not to be essential, the Arg-276 residue lies in a position equivalent to Arg-244 in TEM- or SHV-type ESBLs, as suggested by molecular modeling, and may also play a role in the hydrolysis of oxyimino cephalosporins (Gazouli et al., 1998). Recent crystallographic data for the Toho-1 enzyme suggested that there was increased flexibility of the interacting β 3 strands and ω loop of this enzyme in comparison to other class A β-lactamases. Furthermore, the lack of hydrogen bonds in the vicinity of the ω loop could account for the extended-spectrum phenotype (Ibuka et al., 1999).

5.4. OXA type

The OXA-type β-lactamases are so named because of their oxacillin-hydrolyzing abilities. These β-lactamases are characterized by hydrolysis rates for cloxacillin and oxacillin greater than 50% as that for benzyl penicillin (Bush et al., 1995). They predominantly occur in *P. aeruginosa* (Weldhagen et al., 2003) but have been detected in many other Gram-negative bacteria. In fact, the most common OXA-type β-lactamase, OXA-1 has been found in 1–10% of *E. coli* isolates (Livermore, 1995). The OXA-type ESBLs were originally discovered in *P. aeruginosa* isolates from a single hospital in Ankara, Turkey. In France, a novel derivative of OXA-10 (numbered OXA-28) was found in a *P. aeruginosa* isolate (Poirel et al., 2001). A novel ESBL (OXA-18) and an extended-spectrum derivative of the narrow spectrum OXA-13 β-lactamase (numbered OXA-19) have also been discovered in France in *P. aeruginosa* isolates (Philippon et al., 1997). The evolution of ESBL OXA-type β-lactamases from parent enzymes with narrower spectra has many parallels with the evolution of SHV- and TEM-type ESBLs. Unfortunately there are very few epidemiologic data on the geographical spread of OXA-type ESBLs (Philippon et al., 1997).

5.5. PER type

The PER-type ESBLs share only around 25–27% homology with known TEM- and SHV-type ESBLs (Bauernfeind et al., 1996). PER-1 β-lactamase efficiently hydrolyzes penicillins and cephalosporins and is susceptible to clavulanic acid inhibition. PER-1 was first detected in *P. aeruginosa* (Neuhauser et al., 2003), and later in *S. enterica* serovar *Typhimurium* and *Acinetobacter* isolates as well (Vahaboglu et al., 2001). In Turkey, as many as 46% of nosocomial isolates of *Acinetobacter* spp. and 11% of *P. aeruginosa* were found to produce PER-1 (Vahaboglu et al., 1997). PER-2, which shares 86% homology to PER-1, has been detected in *S. enterica* serovar *Typhimurium*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, and *Vibrio cholerae* O1 El Tor (Petroni et al., 2002).

5.6. GES type

GES-1 was initially described in a *K. pneumoniae* isolate from a neonatal patient just transferred to France from French Guiana (Poirel et al., 2000). GES-1 has hydrolytic activity against penicillins and extended-spectrum cephalosporins, but not against cephamycins or carbapenems, and is inhibited by β-lactamase inhibitors. These enzymatic properties resemble those of other class A ESBLs; thus, GES-1 was recognized as a member of ESBLs.

5.7. VEB-1, BES-1, and other ESBL type

Other unusual enzymes having ESBL have also been described (e.g. BES-1, CME-1, VE-B-1, PER, SFO-1, and GES-1) (Bradford, 2001). These novel enzymes are found infrequently, details of these enzymes are reviewed elsewhere (Naas et al., 2008).

6. Detection

Observation of organisms harboring ESBLs provides clinicians with helpful information. Treatment of infections caused by ESBL-producing organisms with extended-spectrum cephalosporins or aztreonam may result in treatment failure even when the causative organisms appear to be susceptible to these antimicrobial agents by routine susceptibility testing (Paterson and Bonomo, 2005; Paterson et al., 2001). Additionally, patients colonized or infected with ESBL-producing organism should be placed under contact precautions to avoid hospital transmission (Siegel et al., 2006). These benefits summon the detection of ESBL-producing organisms in clinical laboratories. Further, revision of cephalosporin breakpoints has been achieved by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and is under way by the Clinical and Laboratory Standards Institute (CLSI) for better prediction of clinical outcome by MIC values (Kahlmeter, 2008). It is still controversial whether this revision might allow clinical laboratories to dispense with ESBL detection (Paterson and Bonomo, 2005; Kahlmeter, 2008).

6.1. Phenotypic detection

The US Clinical and Laboratory Standards Institute (CLSI) and the UK Health Protection Agency (HPA) have published guidelines for ESBL detection in *Enterobacteriaceae* specifically for *E. coli*, *Klebsiella* spp., and *Proteus* spp. (CLSI, 2002; HPA, 2008). The HPA guidelines also include other species, such as *Salmonella* spp. These guidelines are based on the principle that most ESBLs hydrolyze third-generation cephalosporins although they are inhibited by clavulanate, and recommend initial screening with either 8 mg/L (CLSI) or 1 mg/L (HPA) of cefpodoxime, 1 mg/L each of cefotaxime, ceftazidime, ceftriaxone, or aztreonam, followed by confirmatory tests (including the E-test ESBL strips) with both cefotaxime and ceftazidime in combination with clavulanate at a concentration of 4 μg/mL. Automated systems that use similar detection principles have proved to be popular in clinical laboratories, especially those in North America and certain European countries (Spanu et al., 2006). If clinical laboratories adhere to the published guidelines for detecting ESBLs, the CLSI and HPA published methods show high sensitivity of up to 94% and specificity of 98% for detecting ESBLs in *E. coli*, *Klebsiella* spp. and *Proteus* spp. (Wiegand et al., 2007).

6.2. Genotypic detection

The determination of whether a specific ESBL present in a clinical isolate is related to TEM and SHV enzymes is a complicated process because point mutations around the active sites of the TEM and SHV sequences have led to amino acid changes that increase the spectrum of activity of the parent enzymes, such as in TEM1, TEM2, and SHV1 (Bradford, 2001). The molecular method commonly used is the PCR amplification of the bla_{TEM} and bla_{SHV} genes with oligonucleotide primers, followed by sequencing. Sequencing is essential to discriminate between the non-ESBL parent enzymes (e.g. TEM1, TEM2, or SHV1) and different variants of TEM or SHV ESBLs (e.g. TEM3, SHV2, etc.) (Bradford, 2001).

The PCR amplification of CTX-M-specific products without sequencing, in an isolate that produces an ESBL, usually provides sufficient evidence that a $bla_{CTX\text{-}M}$ gene is responsible for this phenotype. This is unlike TEM and SHV types of ESBLs. Several recent studies have described various molecular approaches for the rapid screening of ESBL-positive organisms for the presence of different $bla_{CTX\text{-}M}$ genes. This involved a PCR assay that used four sets of primers to amplify group specific CTX-M β-lactamase genes (Pitout et al., 2004), amplification of a universal DNA fragment specific for most of the different groups of CTX-M β lactamases (Batchelor et al., 2005), duplex PCR (Pitout et al., 2007), multiplex PCR (Woodford et al., 2006), real-time PCR (Birkett et al., 2007), pyrosequencing (Naas et al., 2007), and reverse-line hybridization (Ensor et al., 2007). Molecular techniques undoubtedly have the potential to play an essential part in the laboratory setting for the screening, tracking, and monitoring of the spread of large number of organisms producing CTX-M enzymes from the community and hospital settings in real time.

7. Epidemiology

The epidemiology of ESBLs is quite complicated. Initially, there are certain levels to consider: the wider geographical area, the country, the hospital, the community, and the host (in most cases a single patient or a healthy carrier). Moreover, these are bacteria (*E. coli* is more endemic, and *K. pneumoniae* is more epidemic) and their mobile genetic elements, usually plasmids. Additionally, there are various reservoirs, including the environment (e.g. soil and water), wild animals, farm animals, and pets. The final component entails transmission from food and water, and via direct or indirect contact (person to person) (Carattoli, 2008). The first ESBL to be identified was found in Germany in 1983, but it was in France in 1985 and in the United States at the end of the 1980s and the beginning of the 1990s that the initial nosocomial outbreaks occurred (Rice et al., 1990). Soon thereafter, it was discovered that many of the *K. pneumoniae* strains that caused nosocomial infections in France in the early 1990s were ESBL producers (Sirot et al., 1987).

From an international aspect, the use of antibiotics, especially broad-spectrum agents, is narrow in Sweden (Cars et al., 2001). Since February 2007, clinical laboratories are required to report all cases involving ESBL-producing *Enterobacteriaceae* strains to the Swedish Institute for Infectious Disease Control, and the number of such cases increased by 100% from 2008 to 2011 (SIIDC, 2012). In recent years, there have also been larger nosocomial outbreaks of clonally ESBL strains: one at a neonatal care unit with ESBL-related mortalities, a large outbreak in Uppsala involving *K. pneumoniae* with CTX-M-15, and in Kristiansand caused by a multi resistant CTX-M-15-producing *E. coli* strain (Alsterlund et al., 2009). According to data from the European Antimicrobial Resistance Surveillance System (EARSS), 2.6% of *E. coli* and 1.7% of *K. pneumoniae* strains in Sweden were resistant to third-generation cephalosporins in 2010 (EARSS, 2011).

New TEM and the SHV enzymes are still emerging in Europe, and distinct epidemic clones have been found, for example *Salmonella* isolates with TEM-52 in Spain (Fernandez et al., 2006) and *E. coli* and *K. pneumoniae* isolates with SHV-12 in Italy (Perilli et al., 2011). Isolates with the CTX-M-9 group are common in Spain and strains with the CTX-M-3 enzymes have been described chiefly in Eastern Europe, although clones producing the CTX-M group 1 (including the CTX-M-15 type) are the most widespread throughout Europe (Coque et al., 2008a,b; Canton et al., 2008). Today, *E. coli* and the CTX-M enzymes are not uncommon in outpatients. Moreover, the resistance exhibited by *K. pneumoniae* has reached a higher level with emergence of carbapenemases such as OXA-48, which was first found in Turkey (Aktas et al., 2008).

Another investigation was conducted at a tertiary hospital in Nigeria, among the overall ESBL producing isolates, 35% being community origin and 65% from hospitals. The ESBL isolates showed high resistance to tetracycline, gentamicin, pefloxacin, ceftriaxone, cefuroxime, ciprofloxacin and Augmentin (Amoxicillin and clavulanic acid combination). Conjugation studies for Resistance plasmid transfer showed non-transference of resistance determinants between the ESBL transconjugants and recipient strains. Correspondingly, the plasmid curing studies revealed that the acridine orange could

not affect a cure on the isolates as they still retained high resistance to the antibiotics after the treatment (Ruth et al., 2011).

A study conducted at the National Public health laboratory (NPHL), Kathmandu, Nepal reported that 31.57% of *E. coli* were confirmed as Extended Spectrum β-lactamase producers, these isolates further exhibited co-resistance to several antibiotics (Thakur et al., 2013).

In another research conducted at a tertiary hospital in Mwanza, Tanzania, the overall prevalence of ESBLs in all Gram-negative bacteria (377 clinical isolates) was 29%. The ESBL prevalence was 64% in *K. pneumoniae* but 24% in *E. coli* (Mshana et al., 2009). Dramatic figures were also obtained in a small study at an orphanage in Mali, where 63% of the adults and 100% of the children were found to carry ESBL-producing *Enterobacteriaceae* that showed extensive co-resistance to other antibiotics (Tande et al., 2009). Moreover, in Madagascar, Herindrainy et al. (2011) observed that 10% of non-hospitalized patients carried ESBLs, in the majority of the cases CTX-M-15, and these investigators also found that poverty was a significant risk factor for carriage. Fatemeh et al. found that 26.5% of *E. coli* and 43% of *K. pneumonia* were ESBL positive in their study conducted at the Imam Reza hospital of Mashhad, IR Iran. They indicated the high prevalence of ESBL producing *Enterobacteriaceae* family especially in inpatients (Fatemeh et al., 2012).

The overall data on ESBL-producing *Enterobacteriaceae* in the countries of the Middle East are extremely worrisome, and this region might indeed be one of the major epicenters of the global ESBL pandemic. Investigation conducted in that country showed that 61% of *E. coli* produced ESBLs of the CTX-M-14, CTX-M 15, and CTX-M 27 types, and all of strains harbored the TEM enzyme (Al-Agamy et al., 2006). In a study of inpatients in Saudi Arabia in 2008, Tawfik and colleagues found that 26% of *K. pneumoniae* isolates produced ESBLs, the majority of which were SHV-12 and TEM-1 enzymes, and 36% were CTX-M-15 (Tawfik et al., 2011). Another investigation conducted in the same country in 2004–2005 showed that 10% of clinical urinary *E. coli* isolates from inpatients and 4% of such isolates from outpatients were ESBL producers (Khanfar et al., 2009). Moubareck and colleagues analyzed fecal samples in Lebanon in 2003 and noted that ESBL carriage differed somewhat between patients (16%), healthcare workers (3%), and healthy subjects (2%), and also that there was a predominance of the CTX-M-15 enzyme (83%) (Moubareck et al., 2005). Other researchers in Lebanon (Khanfar et al., 2009) observed that the proportion of ESBL-producing isolates was significantly larger among inpatients (15.4%) than in outpatients (4.5%). Moreover, data collected over three years in Kuwait showed that the levels of ESBLs were lower in community isolates of *K. pneumoniae* (17%) and *E. coli* (12%) than in the corresponding hospital isolates (28% and 26%, respectively) (Al Benwan et al., 2010).

Only lately have we begun to understand the extent of the ecological disaster related to ESBL-producing *Enterobacteriaceae* in parts of Asia and the Indian subcontinent, and the number of reports of very high frequency of such bacteria in those regions continues to rise. It is likely that some of the successful ESBL-producing clones originate from Asia. Deficient sewage routines (the "Delhi belly") and poor quality of drinking water, in combination with a lack of control over prescription and sales of antibiotics, are probably major factors that have promoted the development of resistance. The United Nations has estimated the population of Asia to be 4.2 billion in 2012, and hence it is a very challenging task to try to stop the growing resistance to antibiotic stemming from this part of the world as exemplified by the rapid spread of the carbapenemase NDM-1 (Kumarasamy et al., 2010). A few articles published as early as the end of the 1980s and the beginning of the 1990s have reported occurrence of the SHV-2 and Toho-1 (CTX-M-44) enzymes in China and Japan (Hawkey, 2008). According to the SENTRY surveillance program there have been rapid increase in ESBL-producing *K. pneumoniae* (up to 60%) and *E. coli* (13–35%) in different parts of China, with a predominance of the CTX-M-14 and CTX-M-3 enzymes (Hawkey, 2008; Hirakata et al., 2005). It has been 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 found (Ensor et al., 2006), and an investigation of 10 other centers in that country showed that rates of ESBL-producing *Enterobacteriaceae* reached 70% (Mathai et al., 2002). Recently ESBL production was observed in 48% of *E. coli*, 44% of *K. pneumoniae* and 50% of *P. aeruginosa* isolates in a tertiary hospital in Patiala, Punjab (Rupinder et al., 2013). In other recent studies, authors observed ESBL rates of 46% and 50% in out- and inpatients, respectively (Sankar et al., 2012), and Nasa and co-workers detected ESBL production in almost 80% of clinical isolates (Nasa et al., 2012). Investigations from India and Pakistan show an alarming and rapid increase in the prevalence of *Enterobacteriaceae* with NDM-1 with prevalence rate from 6.9% in a hospital in Varanasi, India, to 18.5% in Rawalpindi, Pakistan (Perry et al., 2011) and perhaps the spread of these enzyme could be even more rapid than the spread of the CTX-M enzymes.

Majda et al. reported that 72% of *E. coli* and 65.8% of *K. pneumoniae* were ESBL producers at the Microbiology laboratory of Shalamar Medical College, Lahore. Sensitivity testing showed a multidrug resistance in ESBL producing *E. coli* and *K. pneumoniae*. Maximum resistance was recorded in *E. coli* (ESBL) as cefotaxime (98.9%), Ceftazidime (96.7%) and Cefuroxime (93.4%) while minimum resistance was seen with Imipenem (0.8%), fosfomycin (1.2%) and Nitrofurantoin as well piperacillin/tazobactam (2.2%) each. The ESBL producing *Klebsiella* showed maximum resistance to ceftazidime (100%), cefotaxime (89%), and Cefuroxime (84%) while minimum resistance was seen with imipenem (4%), Nitrofurantoin and Piperacillin/Tazobactam (8%) (Majda et al., 2013).

In a most recent study Shakti et al. reported 12.11% ESBL positive among ICU and NICU isolates and 22.47% ESBL positive from nosocomial isolates. The author further statically confirmed that ESBL strains were equally distributed in community or hospital units. Antibiogram of 23 antibiotics revealed progressive increase in drug resistance against each antibiotic with the maximum resistant values recorded against gentamycin: 92% and 79%, oxacillin: 94% and 69%, ceftriaxone: 85% and 58%, and Norfloxacin 97% and 69% resistance, in nosocomial and community isolates respectively (Shakti et al., 2014).

Although there are some differences between countries, the highest prevalence of ESBL-producing *K. pneumoniae* in the world is seen primarily in Latin America, data from 33 centers in Latin America collected over the period 2004–2007 within the Tigecycline Evaluation and Surveillance Trial (TEST)

Table 1 Epidemiology of ESBL producing organisms.

S. No.	ESBL producing organisms	Country/City	References
1	*E. coli, K. pneumoniae*	Sweden	Alsterlund et al. (2009)
2	*Salmonella* spp.	Spain	Fernandez et al. (2006)
3	*E. coli, K. pneumoniae*	Italy	Perilli et al. (2011)
4	*K. pneumoniae*	Turkey	Aktas et al. (2008)
5	*Enterobacteriaceae, P. aeruginosa*	Nigeria	Ruth et al. (2011)
6	*E. coli*	Nepal	Thakur et al. (2013)
7	*E. coli, K. pneumoniae*	Tanzania	Mshana et al. (2009)
8	*E. coli, K. pneumoniae*	Iran	Fatemeh et al. (2012)
9	*E. coli*	Middle East	Al-Agamy et al. (2006)
10	*E. coli*	Saudi Arabia	Tawfik et al. (2011)
11	*K. pneumoniae*	Saudi Arabia	Khanfar et al. (2009)
12	*E. coli, K. pneumoniae*	Lebanon	Moubareck et al. (2005)
13	*E. coli, K. pneumoniae*	Kuwait	Moubareck et al. (2005)
14	*E. coli, K. pneumoniae*	China	Hawkey (2008) and Hirakata et al. (2005)
15	*E. coli, K. pneumoniae*	India	Ensor et al. (2006) and Nasa et al. (2012)
16	*E. coli, K. pneumoniae*	Punjab	Rupinder et al. (2013)
17	*E. coli*	Odisha	Shakti et al., 2014
18	*Enterobacteriaceae*	Pakistan	Perry et al. (2011)
19	*E. coli, K. pneumoniae*	Lahore	Majda et al. (2013)
20	*E. coli, K. pneumoniae*	Latin America	Rossi et al. (2008)
21	*Enterobacteriaceae*	Canada	Pitout et al. (2005)
22	*E. coli*	United States	Winokur et al. (2001) and Sanchez et al. (2012)

showed ESBLs in 36.7% of *K. pneumoniae* isolates and in 20.8% of 932 *E. coli* isolates (Rossi et al., 2008).

A large variety of different types of SHV have also been described. One extensive outbreak of *Enterobacteriaceae* producing the CTX-M-14 enzyme occurred in Calgary, Canada (Pitout et al., 2005). In a large study performed in 2001, it was demonstrated that about 5.3% of *E. coli* in the United States harbored ESBLs (Winokur et al., 2001), and an investigation conducted in 2009 showed that 9% of *E. coli* isolates at a cancer center in Texas were ESBL producers (Bhusal et al., 2011). Sanchez et al., investigated data obtained from The Surveillance Network (TSN) concerning *in vitro* antimicrobial resistance in US outpatients between 2000 and 2010, and their results showed that resistance to ceftriaxone rose from 0.2% to 2.3% and resistance to cefuroxime increased from 1.5% to 5%, but the bacterial isolates in focus were not tested for ESBLs (Sanchez et al., 2012). The epidemiology of ESBLs producing organism is shown in Table 1.

8. Treatment

The carbapenems (imipenem, meropenem, ertapenem, doripenem) are still the first choice of treatment for serious infections with ESBL-producing *E. coli* and *K. pneumoniae*. It has been reported that >98% of the ESBL-producing *E. coli*, *K. pneumoniae* and *P. mirabilis* are still susceptible to these drugs (Perez et al., 2007). But with the emergence of the carbapenem-resistant Enterobacteriaceae, the "magic bullet" is actually difficult to find. There are some older drugs which can be used to treat the ESBL-producing *E. coli* or *K. pneumoniae* infections. Fosfomycin was reported of having admirable *in vitro* activity against the ESBL-producing *E. coli* or *K. pneumoniae*. In Hong Kong, most of the ESBL-producing *E. coli* isolates were reported to be sensitive to fosfomycin (Ho et al., 2010). Colistin is another choice which we can consider for the treatment of these organisms. Although once considered as quite a toxic antibiotic, it is a last resort that we can consider at the present moment as there is no new anti gram negative antibiotics available for the treatment of these multidrug resistant organisms. Other than ESBL-producing organisms, actually colistin is used in the treatment of multidrug resistant *P. aeruginosa*, carbapenem resistant *A. baumannii*. Close monitoring for the development of side effects can improve the safety margin when prescribing the drug. Tigecycline is also one of the drugs in the pipeline which can be considered for treatment (Perez et al., 2007).

9. Conclusion

The incidence of infections caused by beta-lactam-resistant organisms due to the production of various enzymes has increased in recent years. Detection of ESBL production is of paramount importance both in hospital and community isolates. Infection-control practitioners and clinicians need the clinical laboratory to rapidly identify and characterize different types of resistant bacteria. This in turn is required to minimize the spread of these bacteria and help select appropriate antibiotics. This is particularly true for ESBL-producing bacteria. The epidemiology of ESBL-producing bacteria is becoming more complex with increasingly blurred boundaries between hospitals and the community. The acquisition of efficient mobile elements has accelerated the transfer of various antibiotic resistance genes. Probably, a "super bug", resistant to relatively all licensed antibiotics, may rise in the future. Constant and careful worldwide surveillance for multidrug-resistant bacteria is urgently warranted.

Conflicts of interest

None declared.

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