

A STUDY OF β -LACTAMASES IN CEFTAZIDIME AND ITS EFFECT IN *E. COLI* & *KLEBSIELLA PNEUMONIAE* ISOLATES

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ABSTRACT

Long-spectrum β -lactamases (ESBLs) are a group of plasmid-mediated, diverse and rapidly developing enzymes that present a major therapeutic challenge for hospitalized and community patients today. ESBL infections range from uncomplicated infections in the urinary tract to life-threatening sepsis. Derived from the older TEM, the patient from whom the strain was isolated in India. The β -lactamases of these enzymes have the ability, and are still inhibited by clavulanic acid, to hydrolyze cephalosporins and aztreonam of the third generation. Moreover, ESBL organisms are co-resistant to many other classes of antibiotics, thereby restricting therapeutic choices. Their detection is also a major challenge, because of the inoculums and their substrate specificity. However, currently, the detection guidelines for ESBLs in the *Klebsiella pneumoniae*, *K. oxytoca*, *Escherichia coli* and *Proteus mirabilis* are provided by organization such as the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards). In conjunction with all methods of ESBL-detection, the general principle is that the presence of clavulanic acid will increase the activity of the extended-spectrum cephalosporins against organisms producing ESBL. Carbapenems are the preferred treatment for severe inflammation due to organisms that make ESBL, but newly reported carbapenem isolates. The ability of gram-negative bacteria to develop new mechanisms of antibiotic resistance against the introduction of new antimicrobial agents is an impressive example for ESBLs. Efficient infection control practices are therefore needed to contain outbridges, and intervention policies, such as antibiotic rotation, are needed to reduce the further selection and spread of these pathogens that are increasingly resistant.

KEYWORDS: Extended-spectrum β -lactamases, Gram negative bacteria (GNB) and Antimicrobial resistance

INTRODUCTION

Extension β -lactamases (ESBLs) are a fast-development group of β -lactamases that are capable of hydrolyzing cephalosporins and aztreonam in third generations, but are inhibited by clavulanic acid. They are a first

example of β -lactamase resistance to antibiotics by β -lactam as a result of fundamental changes in the enzyme substrate spectrum.

Now, the total number of ESBLs is over 200. These can be found in detail on George Jacoby and Karen Bush's authoritative website on ESBL nomenclature. Research on ESBL's has now been published in more than 30 countries and reflects the genuinely global distribution of ESBL organisms.

Since the 1980s, the most significant cause of hospital-acquired infections has been found for enterobacteriaceae, in particular *Klebsiellaspp.* producing ESBLs such as HVD and TEM. In the late 1990s, however, ESBL manufacturers were also found to be several acquired pathogens that usually cause urinary tract disease and diarrhea. These comprise *Escherichia coli*, *Salmonella*, *Shigella* and *Cholera Vibrio*.

The ESBL is frequently encoded with genes on major plasmids that also contain genes that are resistant to other antimicrobials, such as aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenicol. Recent studies have shown that the ESBL producing plasmids are resistant to fluoroquinolone through the co-transfer of the Qnr determinant. So a very broad antibiotic resistance to various antibiotic classes is now a common trait of enterobacterial isolates produced by ESBL. This creates a major problem for clinical therapeutics for ESBL-producing organisms. Based on the literature available on this diverse, complex and fast-developing group of enzymes, this review aims to present a comprehensive picture.

RESISTANCE TO β -LACTAMS

β -lactams are a bacterial cell wall group of antibiotics. Penicillins, cephalosporins, carbapenems and monobactams are included. They bind to carboxypeptidases and transpeptidases and inhibit them. These are the cell-wall enzymes that synthesize the D-ala cross-linkages of the peptidoglycan wall that surrounds bacteria, also called penicillin-binding proteins or PBP. As a result, the cell wall structure has decreased and cell lysis has occurred.

Resistance of β -lactams has probably developed during the bacterial history, but is now a useful and consequently selected feature since antibiotics β -lactam was used clinically. These drugs have been used to select Darwinians, kill sensitive bacteria, and enable them to survive.

A certain specimen, as seen in enterococci, with inherently insensitive PBPs may have resistance to β -lactams. In addition, spontaneous mutation or transmission of DNA can be acquired. β -lactam resistance can functionally result from β -lactam production, impermeability, efflux and change in targets. These can occur individually or in various combinations.

Changes in normal PBPs and additional PBPs insensitive to β -lactam are the most common cause of resistance in gram-positive cocci such as pneumococcal and MRSA. However, resistance in gram negative bacteria is

due in particular, together with natural up-regulated impermeability and efflux, to the combination of acquired β -lactamases.

DEFINITION OF EXTENDED-SPECTRUM β -LACTAMASES

The exact definition of ESBL is not agreed. A common definition of work is that, as a result of hydrolysis of such antibiotics, ESBLs are β -lactamases able to confer bacterial resistance to penicillins; cephalosporins of first and second and third generations; and aztreonam (but not cephamycins or carbapenems), which are inhibited by β -lactamases such as clavulanic acid.

The Molecular classifications Ambler and the functional classification Bush-Jacoby-Medeiros are the two most commonly used β -Lactamase's classifications. Ambler divides β -lactamases in 4 main classes (A to D). Protein homology, (similarity with amino acid) and not phenotypic properties are the basis of this classification scheme. β -lactamases of classes A, C and D are serine β -lactamases in the Ambler classification scheme. The class B enzymes, by contrast, are metallo- β -lactamases. The ESBLs are of molecular class A with the exception of enzymes of type OXA (which are class D enzymes).

According to functional similarities the Bush Jacoby Medeiros classification scheme groups β -lactamases (substrate and inhibitor profile). This system contains four main groups and several subgroups. In a diagnostic lab it takes into account the relevance of β -lactamase-and β -lactam substrates that are clinically relevant to the physician or microbiologist. ESBLs are classified under group 2be or group 2d (OXA type), which, although resistant, have the most essential properties of group 2be enzymes.

The designation 2be shows that these enzymes come from the group 2b β -lactamases (for instance, TEM-1, TEM-2 and SHV-1); the 'e' 2be denotes the widespread spectrum of β -lactamases. TEM-1, TEM-2, SHV-1 derived ESBLs vary as little as one amino acid compared to their progenitors. The enzyme activity of the ESBLs will therefore change profoundly so that they can hydrolyze cephalosporins or aztreonam of third generation (hence the extension of spectrum compared to the parent enzymes).

An inhibition of inhibitors of β -lactamase, such as clavulanic acid and inability to hydrolyze cephamycins, distinguishes ESBL from AmpC β -lactamases (group 1), which are non-clavulanic acid inhibited by cephalosporins of the third-generation. The choice of stably de-pressured mutants producing β -lactamase-type ampCs was linked to clinical failure when cephalosporin from the third generation is used to treat serious organismal infections that produce these enzymes. Generally, cefalosporin, cefepime in 4th generation (AmpC), is clinically useful in the treatment of ESBL-producing organisms against organisms that produce AmpC-type β -lactamases. In addition, third generation of cephalosporins (and Carbapenemic Acid, ETTA) a heavy metal chelator is inhibited, but not clavulanic acid, by the metalloenzymes (Group 3) produced by organisms such as *Stenotrophomonas maltophilia*.

EVOLUTION AND DISSEMINATION OF ESBLs

Cellular encoded and universally present in a species or mediated plasmid can be β -lactamases. It is thought that the chromosomal enzymes have developed out of PBPs that show homology in the same sequence. This was probably due to the β -lactam-producing soil organisms that exercise selective pressure in the environment.

In the beginning of the 1960s, the first plasmid mediated β -lactamase was described in gram-negative bacteria TEM 1.

It was so named as isolated from a blood culture in Greece called Temoniera. TEM-1 enzymes are spread throughout the world and are present in numerous species within the Enterobacteriaceae family, *Pseudomonas aeruginosa*, *Hemophilus influenzae* and *Neisseria gonorrhoea*. *Pseudomonas aeruginosa* are mediated. Another β -lactamase found commonly in *Klebsiella* and *Escherichia coli* is SHV-1 (for type 1 sulfhydryl variable). Over the years, new variants of β -lactam have been chosen using new antibiotics.

In the early 1980s, in response to the increasing prevalence and propagation of β -lactamases, cephalosporins of the third generation or of oxy-minos were introduced into clinical practice. Resistance to these widespread cephalosporins quickly arose and the first report from Germany in 1983 about the enzyme SHV-2 capable of hydrolyzing these antibiotics.

Due to their increasing spectrum of activity, particularly oxyiminocephalosporins, these enzymes have been called expanded spectrum β -Lactamases. There are a number of ESBL groups with similar conduct but different history. TEM and SHV β -lactamases, with 150 members, are mutants in the biggest groups. Mutations affecting a small group of critical amino acids enlarge the active site of the enzyme so that the substitutes are dislocated and the β -lactam ring is normally protected. In this way, whereas the classical enzymes TEM and SHV can't hydrolyze oxyiminocephalosporins significantly, the mutants can resist their host strains.

The CTX-M enzymes are the second largest ESBL group. These groups are divided into five subgroups with approximately 40 members based on sequence homology. The chromosomal β -lactamase genes that have escaped *Kluvera* spp., an enterobacterial genus of small clinical importance, have developed most of these subgroups. The CTX-M β -lactamases can evolve further after migration to mobile DNA. CTX-M enzymes have been identified, primarily from within the community as the cause of urinary tract infections, by Enterobacteriaceae (mainly *Escherichia coli*). Several reports show that CTX-M ESBLs are the most common ESBL type in the world.

The oxacillin-hydrolyzing capability of the OXA-type β -lactamases (group 2d) is named like this. It is mostly found in *Pseudomonas aeruginosa*, however, in many other gram-negative bacteria. In *Pseudomonas aeruginosa* isolates from Turkey, the ESBLs of OXA were originally discovered. Evolution by parent enzymes

with narrower spectra of ESBL OXA-type β -lactamases has many parallels to developments in ESBLs of the SHV type and TEM type. OXA-10 hydrolyses (weakly) cefotaxime, ceftriaxone, and aztreonam, which give many organisms less vulnerability to antibiotics, confer frank resistance to cefotaxim and, sometimes, ceftazidim and aztreonam, but OxA-11, -14, -16, -17, -19, -15, -18, -28, -32, -35 and -45. The simulcast of an aztreonam hydrolyzing OXA enzyme and a carbapenem-hydrolyzing metalloenzyme can easily lead to resistance against all β -lactam antibiotics. A range of other plasmid-mediated or Integrase-related class A enzymes (PER, VEB, GES, BES, TLA, SFO, IBC groups) have been found. They are not simple point mutant products of any known β lactamases found in a wide variety of geographical locations. There were also described new chromosomally encoded ESBLs.

PROBLEMS IN DETECTION

For the clinical microbiology laboratory, identification of ESBL-producing organisms is a major challenge. This involves the production of various types of β -lactamases by one bacterial isolate and the creation of ESBLs by organizations which constitute AmpC β -lactamases, varying substrate affinity, and inoculum action. This involves multiple factors.

In comparison to genotypical tests, phenotypic confirmatory tests are highly sensitive and specific. There are, however, several instances of falsely positive or negative phenotypic confirmatory tests.

Isolates that do not contain the ESBLs, but which produce SHV-1, may produce false positive confirmational evidence for *Klebsiella pneumoniae* or *Escherichia coli*. The ceftazidim MICs of such isolates can reach a height of 32 $\mu\text{g}/\text{mL}$.

Numerous reports now contain plasmids-mediated AmpC-type β -lactamases in *Klebsiella pneumoniae* isolates. Some of these organisms contain β -lactamases and ESBLs in type AmpC. In addition to high cephalosporin MICs, the coexistence of the two types of enzymes in the same strain can give false negative evidence for the detection of ESBLs. It is likely that the β -lactamases AmpC-type are resistant to clavulanate inhibition and therefore obscure the synergistic effect of clavulanate and cephalosporins on ESBL.

The MIC in extended spectrum cephalosporins for ESBL producing bacteria has dramatically increased as the inoculum has been increased beyond that used for routine susceptibility tests. The same isolates test in the standard inoculum and in a higher inoculum resistant. Thus, with both screening and confirmatory testing, false-negative results can occur when lower inoculum is used.

Some ESBL isolates may seem susceptible to cephalosporin in vitro in third generation, especially in cases of relatively high breakpoints. The treatment of ESBL-produced infections with third generation cephalosporin may, however, lead to clinical failure even if the MIC is below its breakpoint and when MIC testing is

performed with heavy inoculum, the ability of these enzymes to confer resistance to low-substrate cephalosporin is clear. This can be due to their varying affinity with various substrates and inoculum effects.

Despite being sensitive in vitro, several ESBL producers are resistant to combinations. This can be caused by hyperproduction, so that the host's relative impermeability or co-production of inhibitor Resistant penicillanases is overwhelmed (e.g., OXA-1).

Since ESBL production is normally mediated by plasmid, both ESBL producing and non-ESBL-producing cells of the same species can be found in one specimen. This suggests that several colonies from a primary culture plate have to be tested for optimal detection.

Some antibiotics, amino acids or body fluids may induce ESBL enzymes. Organisms with β -lactamases inducible genes have an untrusted sensitivity when tested.

All of these factors make ESBL detection a complex task and improve the capability for ESBL detection in clinical laboratories.

The bad results of treatment of patients with an infection caused by an ESBL producing organism with cephalosporin that appears susceptible to this in vitro have resulted in two opposing perspectives. Some researchers believe that changing the cephalosporin breakpoints for Enterobacteriaceae by organizations like the Institute for Clinical and Laboratory Standards is more appropriate than increasing the efforts made to detect ESBLs. A benefit of this change would be that organisms like *Enterobacter spp.*, currently not covered in the ESBL detection guidelines for CLSI, would be covered.

Another view is that the effect of inoculum is important to organisms that produce ESBL. The inoculum of ESBL-generating organisms increases as the MICs of cephalosporins increase in vitro. Therefore, physicians should avoid cephalosporins when the ESBL producing agencies occur in the case of High-Inoculum infections (for example, intra-abdominal abscess, in some cases pneumonia) or in sites where medications penetrating poorly (e.g. meningitis, endocarditis, or osteomyelitis). In patients infected with higher MICs, the severity of disease could have been higher.

The importance of the infection control detection of multi-drug plasmid-mediated resistance is a favourable effort to detect ESBL. Epidemiologically, the significance of this resistance can only be as evident when organisms are simply reported as intermediate or resistant to individual cephalosporins, for the detection of ESBL-creating organisms. Outbreaks of ESBL organisms by appropriate infection control interventions may be abruptly halted. Infection control and antibiotic management interventions may also be used to curb the endemic transmission of ESBL producers. Detection of ESBL production from samples such as urine in organisms may be important as this is an epidemiologic colonizing marker (and therefore the potential for transfer of such organisms to other patients).

TREATMENT OPTIONS

The factors which determine the choice of antibiotics and other management options include a) site of infection; b) the site of the infection; c) severity of infection; d) presence of an implant or prosthetic device; e) metabolic parameters - liver or renal function; and f) patient-related elements, such as age, pregnancy or lactation are the factors determining the choice of antibiotics and any other management options. ESBL-producing organisms have very limited therapeutic possibilities. ESBLs enable them, with the exception of cephamycins and carbapenems, to be resistant to most β -lactam antibiotics. In addition, ESBL-encoding plasmids often have genes that encode resistance to other antimicrobials, such as aminoglycosides, trimethoprim, sulfonamides, tetracyclines and chloramphenicol.

Plasmid encoded decreases in quinolone susceptibility have also been reported to increase, often associated with plasmid mediated cephalosporin resistance.

The connection between the quinolone resistance and the production of ESBL, although the reason for this association is unclear, appears to be strong even in the absence of plasmid-encoded decreases in the susceptibility of quinolones. Increasing in-vitro Resistance of ESBL producers to quinolones will in the future limit the role of these Antibiotics. Fluorocinolones may be used in the treatment of uncomplicated urinary tract infections (UTIs) when found susceptible. Research has demonstrated that carbapenems are superior to quinolones in the treatment of severe ESBL-produced infections. [At the treatment of alternative cephalosporins, some organismal infections which were resistant to ceftazidim but susceptible to cefotaxime or ceftriaxone responded. However, the inoculum increases dramatically with the MICs of these agents.

Therefore, ESBLs are inferred from isolates giving a positive synergy test, and no susceptibility findings of any cephalosporins must be avoided.

Cefamycins such as cefoxitin and cefotetan are not recommended to treat those infections even when they are active in vitro, as they are relatively easy to decrease their external protein expression, making them resistant.

Even if ESBL activity is inhibited in clavulanic acid, the combinations of β -lactam β -lactamase inhibitors are not regarded as the optimal treatment for severe ESBL-causing infections, as the clinical effect of ESBL-producing organisms against severe infections is controversial. Most ESBL-produced organism, often in different amounts, produce more than one β -lactamase. It is also well known that organisms producing ESBL may continue to contain parent enzymes (for example, SHV-1 or TEM-1). The combination of β -lactamase production and porin loss may also result in reduced active inhibitors of β -lactamase, resulting in a hyperproduction of β -lactamas.

Increasing MICs as inoculum increases also involve β -lactam/ β -lactamase inhibitor combinations. As a result, the effects of the β -lactama-inhibitor may be associated with infected organisms with a high burden (intra-

abdominal sepsis) with sufficient β -lactamase production. They may, however, be helpful in less severe infections, such as a non-bacteremic infection in the urinary tract, as the infection is located and antibiotics are excreted in large quantities via the urine. They have also found themselves a good way to treat uncomplicated infections from the community caused by ESBL producers, especially as they have the benefits of oral administration. The benefits of inhibiting ESBLs are that they seem to affect the development and spread of plasmids that carry *Klebsiella*. In addition, inhibitors can be administered in vitro to ESBLs, facilitate the reverse mutation of these inhibitors into less harmful enzymes.

In uncomplicated cases there is also concern that misuse of carbapenems will lead to resistance to carbapenem. Thus the therapeutic options for serious infections are restricted to carbapenems, colistin, polymyxin, temocillin, tigecycline. However, a range of antibiotics can manage uncomplicated infections like non-bacteremic urinary tract infections depending on their susceptibility. These include trimethoprim, nitrofurantoin, fosfomicin, co-amoxiclav and mecillinam or intravenous agents such as aminoglycosides and inhibitor combinations. These agents include oral antibiotics. The drugs of choice for serious infections of producers of ESBL include these carbapenems. In nosocomial infection, imipenem and meropenem are preferred, while in community infections.

Although no synergy, additivity and antagonism were found in combination therapeutics (carbapenem+aminoglycoside) in in vitro studies, imipenem bactericidal activity in combination with amikacin was higher than imipenem alone. This is because amikacin has been killed faster. In the first few days of treatment of life-threatening infections such as septicemia, hospital acquired pneumonia and intra-visceral abscesses, carbapenems can be combined with a second agent (ami-acin). Patients resistant to all other antibiotics, including carbapenems, are reserved for tigecycline, temocillin, collistine and polymyxin.

CONCLUSIONS

Including prolonged spectrum cephalosporins and are connected to high morbidity and death, ESBLs clinically limit the efficacy of β -lactam. In addition, the indiscriminate use of carbapenems can decide on resistance to these key medicines and sow seeds for major therapeutic problems in the future. There is no doubt that ESBLs are becoming more complex and diverse and that detection in clinical microbiology laboratories is becoming more and more difficult. Efficient infection-control practices for containing outbreaks are therefore required and intervention strategies, such as antibiotic rotation, are needed to reduce additional selection and spread of the growing resistance pathogens.

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