ORIGINAL ARTICLE

Extended-spectrum beta-lactamas in urinary tract infections caused by Enterobacteria: Understanding and guidelines for action

A. García-Tello*a, H. Gimbernat a, C. Redondo a, D.M. Arana b, J. Cacho b, J.C. Angulo a

a Servicio de Urología, Hospital Universitario de Getafe, Departamento Clínico, Facultad de Ciencias Biomédicas, Universidad Europea de Madrid, Madrid, Spain
b Servicio de Microbiología, Hospital Universitario de Getafe, Departamento Clínico, Facultad de Ciencias Biomédicas, Universidad Europea de Madrid, Madrid, Spain

Received 9 May 2014; accepted 21 May 2014
Available online 11 November 2014

KEYWORDS
Extended-spectrum beta-lactamas; Urinary tract infection; Antibiotic resistance; Carbapenems

Abstract
Context: Beta-lactamas are bacterial enzymes that protect microorganisms from the lethal effects of β-lactam antibiotics. The production of beta-lactamas is the most important mechanism of resistance to these antibiotics, especially in Gram-negative bacteria.

Objective: Review the magnitude of the problem of extended-spectrum beta-lactamas (ESBLs) in the urological setting and present the fundamental action guidelines on the issue, the main risk factors and the prevention strategies.

Acquisition of evidence: A structured search strategy for patient, problem, intervention, comparison and result was conducted in the PubMed-Medline database to identify the most relevant studies related to the management of patients with urinary tract infection by ESBL-producing microorganisms. We also present a caseload analysis of our center on this issue.

Summary of the evidence: ESBLs are found in Enterobacteria, mainly Klebsiella sp. and Escherichia coli and are characterized by their hydrolytic ability compared with beta-lactam antibiotics, which entails resistance to penicillin, cephalosporin and aztreonam. They are also associated with resistance to other antibiotics. There is a high risk of infection and colonization by ESBL producers in patients with prolonged hospital stays or who required invasive devices. The prior use of antibiotics and stays in residential care are also risk factors. Prevention programs should focus on preventing nosocomial infection. It is essential that a restrictive policy on the use of antibiotics be implemented. The therapy of choice for severe infections is focused on carbapenems, although their indiscriminate use should be avoided. In uncomplicated lower urinary tract infections, fosfomycin and nitrofurantoin are the best treatment alternatives.


*a Corresponding author.
E-mail address: anagtello@yahoo.es (A. García-Tello).

© 2014 AEU. Published by Elsevier España, S.L.U. All rights reserved.

2173-5786
**Introduction**

Beta-lactamasas are bacterial enzymes encoded in chromosomes or plasmids that protect microorganisms from the lethal effects of beta-lactam antibiotics by hydrolyzing the beta-lactam ring. Its production is the most important mechanism of resistance to these antibiotics, especially in Gram-negative bacteria. The first plasmid-mediated beta-lactamases in gram-negative bacteria (TEM-1, SHV-1) were described in the 1960s.\(^1\) The extended-spectrum beta-lactamases (ESBLs) are a group of these enzymes, encoded on plasmids, which are characterized by having hydrolytic capacity against beta-lactam antibiotics of the oximino group. They confer, thus, resistance to penicillins, cephalosporins of first, second, and third generation and aztreonam. They are inhibited in vitro by beta-lactamase inhibitors such as clavulanic acid or tazobactam. They are often found in enterobacteria, mainly *Klebsiella* sp. and *Escherichia coli*. The presence of ESBL was also associated with a high proportion of resistance to other non-beta-lactam antibiotics, such as fluoroquinolones, aminoglycosides, or cotrimoxazole.

The first ESBL-producing strain appeared in 1983 in Germany.\(^2,3\) Since then, various outbreaks have been published in Europe, the USA, and Asia. The incidence of strains producing these enzymes has possibly been undervalued in many countries. In Spain, the first ESBL-producing microorganisms were described in 1988.\(^4\) As in the rest of Europe, the incidence of infections caused by these pathogens has increased in recent decades and its epidemiology is changing. Until recently, these were considered an exclusively nosocomial problem, with disease outbreaks, but in recent clinical studies, a high percentage of gram-negative
bacteremia of the community was caused by ESBL-producing enterobacteriaceae. \(^5\)

Urinary tract infections are a major cause of bacterial infection both in hospitalized patients and in the community, and beta-lactam antibiotics have been widely used to treat these infections. The emergence of gram-negative bacteria producing extended-spectrum beta-lactamases complicates therapy by severely limiting the treatment options. In addition, antibiotics are usually empirically indicated before obtaining laboratory results. There are studies that show that inadequate antibiotic treatment is a predictor of mortality in patients with bacteremia of urinary origin. \(^6-8\) For this reason, it is extremely important to start an appropriate therapy as soon as possible. It is essential to understand the microorganisms that more often cause these infections in our environment and their patterns of sensitivity and antimicrobial resistance, to carry out proper treatment.

**Definition of extended-spectrum beta-lactamases**

The continuous description of new beta-lactamases has created problems in their classification and nomenclature. Currently, more than 890 enzymes are known. They are usually classified according to 2 general schemes. The Ambler molecular classification, proposed in 1980, \(^2\) divides beta-lactamases into 4 main groups (A, B, C, and D) based on their proteic homology. \(^10\) The functional classification was proposed by Bush in 1989 based on the affinity of the enzymes through different substrates and their sensitivity to the inhibitory action through clavulanic acid. This classification was revised in 1995 by Bush et al. \(^11\) and updated again in 2010. It describes 4 major groups and numerous subgroups. Due to their interest and clinical implications, it is worth stressing the following betalactamases:

- **Extended-spectrum beta-lactamases (groups 2be, 2ber, and 2de of the classification by Bush and Jacoby):** TEM, SHV, CTX-M, and OXA-type enzymes.
- **Beta-lactamases resistant to the inhibitors (group 2br):** TEM and SHV-type enzymes.
- **AmpC-type beta-lactamases (group 1):** LAT, MIR, CMY, and FOX-type enzymes.
- **Carbapenemases (2f, 2df, and 3 groups) VIM, IMP, IMI, KPC, NDM, and OXA-type.

Extended-spectrum betalactamases are defined as beta-lactamases capable of conferring bacterial resistance to penicillins, cephalosporins (including extended-spectrum), and monobactams (aztreonam) by means of hydrolysis of these antibiotics and that are inhibited by beta-lactamase inhibitors such as clavulanic acid or tazobactam. They cannot hydrolyze cephamycins (cefoxitin) or carbapenems (imipenem, meropenem, ertapenem). The genes that encode them are found in mobile elements that facilitate its spread and often show co-resistance with other antibacterials such as aminoglycosides, cotrimoxazole, and quinolones.

Most belong to the Ambler molecular class A. These include TEM and SHV, derived from beta-lactamases with a lower spectrum of hydrolysis, the CTX-M family, from chromosomal beta-lactamases from the Kluyvera gender, and other less prevalent such as PER, VEB, BES, GES, TLA, and SFO, all of them included in the functional group 2be by Bush and Jacoby. They also belong to class A, subgroup 2ber, TEM complex mutant beta-lactamases (CMT). Some enzymes of the OXA family (class D Ambler and functional group 2de) are also extended-spectrum beta-lactamases. Since its initial description, over 300 different ESBLs have been identified, most of them belonging to the TEM, SHV, and CTX-M families. \(^12\) The predominant ESBLs in Europe were initially those of SHV type, but from 2000, the CTX-M type has become the most prevalent in most of the world, especially in certain countries of Europe and South America. \(^13\) The enzymes of this group confer a high activity against cefotaxime and ceftazidime. They are not limited to nosocomial infection, but they have a significant potential to expand beyond the hospital environment, and thus they represent a real public health problem. In Spain, the most common types are CTX-M9 and M14. \(^14\)

**Detection of extended-spectrum beta-lactamases**

The clinical microbiology laboratory is the first step in the health system that should alert about the presence of bacterial resistance mechanisms of clinical relevance. ESBL detection methods are divided into 2 groups: phenotypic methods that use non-molecular techniques and detect the ability of ESBLs enzymes to hydrolyze different cephalosporins and genotypic methods that use molecular techniques to detect the genes responsible for the production of the above mentioned ESBLs. Phenotypic methods are more widely used because they are simpler and possibly more cost-effective, while genotypic ones are usually performed in specialized or reference laboratories. The former are crucial for proper treatment of patients, but the latter provide essential information in order to prevent and control infectious diseases.

The United States Clinical and Laboratory Standards Institute (CLSI) and the United Kingdom Health Protection Agency (HPA) have published guidelines for detection of ESBL production in enterobacteriaceae, particularly E. coli, Klebsiella sp., and Proteus sp.; the UK guideline includes other species such as Salmonella sp. \(^15,16\) Both recommend performing a screening test for the potential ESBL production and a second confirmatory test if the first proved positive. When the microdilution technique is used, the screening is performed with 8 mg/l (CLSI) or 1 mg/l (HPA) of ceftodoxime or 1 mg/l cefotaxime, ceftazidime, ceftrixione, or aztreonam. The presence of ESBL is suspected when the minimum inhibitory concentration (MIC) is increased compared to the expectations. When the disk diffusion technique is used, it is suspected when the inhibition halos decrease. The confirmatory test is done with ceftotaxime and ceftriaxone in combination with clavulanic at concentrations of 4 mg/l. In samples with ESBL, the MIC of ceftriaxone or ceftotaxime should be reduced in the presence of clavulanate. A decrease greater than or equal to 8 times the MIC of one or 2 antimicrobials in the presence of the inhibitor indicates the presence of ESBL. In the Centers that use the technique of double-disk synergy greater than or equal to 5 mm in the diameter of the inhibition zone between the discs of
Phenotypic

include

from

interpreted

identified

and

increase

intention

sensitivity

of

quite

eendotracheal

colonization

extended-spectrum

always

respectively.

mentation

We

have

present

for

Therefore,

the

patients

jejunostomy,

infection

analysis

to

emerged

recent

areas.

Figure

1

 Technique of positive double disk synergy to detect ESBL producing germs: difference equal to or greater than 5 mm in the diameter of the inhibition zone between the discs of each antimicrobial agent (ceftaxime and ceftazidime in different plate) with or without clavulanate (10 μg).

each antimicrobial agent, with or without clavulanate, it is interpreted as positive test (Fig. 1). With a correct implementation of the recommendations of these guidelines, the sensitivity and specificity to detect ESBL in E. coli, Klebsiella sp., and Proteus sp. are very high, above 94 and 98%, respectively. Phenotypic detection in other bacteria is controversial, because the effect of the clavulanic acid is not always present in other species such as Enterobacter or Citrobacter. Detection by means of genotypic techniques is quite complex, and it is further complicated due to the increase in the number of subtypes within each family of ESBL. Therefore, it is often limited to reference laboratories and in the context of studies of molecular follow-up.

Risk factors for colonization and infection by extended-spectrum beta-lactamase producers

We have conducted numerous case-control studies with the intention of determining the risk factors associated with colonization and infection by these pathogens. Although the analysis of the results of these studies is sometimes controversial because of differences in study populations, selection of cases, selection of controls, and sample size, some general conclusions can be drawn. There is high risk of infection or colonization by ESBL-producing pathogens in patients with prolonged hospital stay or that required invasive devices for long period of time (urinary catheter, endotracheal tube, central pathway). The hospital stay until the appearance of an isolated ESBL producer range from 11 to 67 depending on the series. Other factors that have been postulated in some studies in isolation include the presence of nasogastric tube, gastrostomy, or jejunostomy, arterial pathways, administration of parenteral nutrition, recent surgery (especially if it is urgent abdominal surgery), hemodialysis, bedsores, or poor nutritional status. Advanced age and diagnosis of diabetes mellitus have also emerged as potential factors, like traveling to endemic areas.

The use of antibiotics in previous months has also been identified as a possible risk factor, with a wide range of variations depending on the studies: third-generation cephalosporins, aztreonam, quinolones, trimethoprim-sulfamethoxazole, aminoglycosides, and metronidazole. There is evidence at the same time of the association between infections due to isolated ESBL producers and the previous stay in care residences. In these centers, the frequent use of antibiotic treatments is usual and patients may have other associated risk factors such as ulcers, urinary catheters, or recent hospitalization. Many of them also have urinary or fecal incontinence, thereby exposing other residents to risk of contamination.

Prevention strategies

The main target that prevention programs should be focused on is nosocomial infection, which can occur in an epidemic or endemic manner. When it comes to clonal proliferation of a single strain, the transmission has occurred horizontally, but if it is not the same clone, it may mean that there has been a selection due to antibiotic use.

The environment and potential vectors for infection must be disinfected, because contamination was found in gels used for ultrasounds, bronchoscopes, pressure cuffs, glass thermometers, and even in soaps, wedges, and baby baths. Several studies reveal that the hands of the healthcare personnel are an important transfer factor in horizontal contamination, so that proper hand hygiene (washing with chlorhexidine or alcohol solutions) is a simple but very effective measure to reduce the time transmission.

Many patients may act as a reservoir, especially those with colonization by ESBL-producing organisms without clinical manifestation of infection. In certain cases, especially when an outbreak occurs in a hospital or unit, identification of these patients and decolonization may be necessary. Oropharyngeal and rectal sampling with swab is required to perform isolation of the carrier patients. There have also been reports of colonization in wounds or ulcers. Finally, to control endemic infection it is important to implement a restrictive policy on the use of antibiotics, based primarily on avoiding the use of cephalosporins and quinolones.
Table 1  Patterns of antibiotic resistance in patients hospitalized or seen in the emergency room from January to December 2013 at the University Hospital of Getafe, Madrid, Spain.

<table>
<thead>
<tr>
<th></th>
<th>Ciprofloxacin</th>
<th>Gentamicin</th>
<th>Amoxicillin-clavulanate</th>
<th>Fosfomycin</th>
<th>Nitrofurantoin</th>
<th>Cotrimazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>8.5%</td>
<td>37.7%</td>
<td>56.5%</td>
<td>23.2%</td>
<td>1.4%</td>
<td>73.9%</td>
</tr>
<tr>
<td><em>Klebsiella sp.</em></td>
<td>90%</td>
<td>55%</td>
<td>85%</td>
<td>50%</td>
<td>40%</td>
<td>80%</td>
</tr>
</tbody>
</table>

Treatment recommendations

The choice of appropriate treatment becomes complicated when we are facing ESBL-producing pathogens, among other factors, because many of these bacteria associate resistance to different antibiotics, as with quinolone resistance in CTXM-producing strains. In addition, the plasmids that encode beta-lactam resistance often carry resistance genes to cotrimoxazole and aminoglycosides. In most cases, treatment should be started empirically and there are 48 h until the results of the cultures and the antibiogram are received. The delay or failure in starting appropriate therapy affects seriously increased morbidity and even mortality in severe infection. It is therefore essential to individualize the treatment of choice for each individual case based on a knowledge of the susceptibility patterns of each country, each city, and each hospital.

There is another peculiarity which makes treatment of these infections difficult: clinical efficacy of a particular antibiotic does not always correspond to expectations for its in vitro activity. This fact is known as inoculum effect, which means that the MICs of antimicrobials can increase from 10 to 100 times simply because the bacterial load is large, and it was described for cephalosporins, piperacillin–tazobactam, and less in quinolones. Other drugs such as cephamycins are not recommended because of the risk of developing resistance during treatment, due to the modification of membrane proteins (porins) which results in decreased permeability.

In summary, the use of cephalosporins must be avoided. In the case of the fourth-generation ones, their use is not recommended in severe infections, as they are very sensitive to the inoculum effect. In the case of using them, they should be administered at high doses and associated with another antimicrobial agent. Piperacillin-tazobactam is not recommended because of the high rate of resistance in many geographic areas and because it is also very sensitive to the inoculum effect. Cephamycins are a bad option due to the above mentioned, and aminoglycosides, cotrimoxazole, and quinolones should be restricted due to the high rate of co-resistance. On other agents such as temocillin and tigecycline, there are still not enough data available.

Thus, pending further evidence from randomized clinical trials, the treatment of choice for severe infections by gram-negative bacteria producing ESBL is carbapenems. However, its indiscriminate use should be avoided, since it is almost the only effective therapy against this type of microorganisms. In cases that they cannot be used because...
of intolerance or resistance, there is no specific treatment recommendation, although it is advisable to associate multiple antimicrobials. In the uncomplicated lower urinary tract infection, fosfomycin and nitrofurantoin seem to be the best therapeutic alternatives, because they both have good activity against ESBL. The use of nitrofurantoin is controversial because it requires longer treatment cycles, which makes therapeutic adherence difficult and may have toxic effects.

The failure of empirical treatment involves high morbidity and mortality and increased hospital costs, so we believe that therapeutic decisions should be based on knowledge of the local distribution of pathogens and their resistance patterns. In our environment, we proposed to define the patterns of susceptibility and antimicrobial resistance for this type of infections, in order to develop a treatment protocol. Positive urine cultures for ESBL-producing enterobacteria were analyzed in hospitalized patients or in those who had come to the emergency department from January to December 2013. Only one episode per patient was included, and in the case of having several urine cultures, the first one was selected. 92 patients were detected with a urine culture positive for ESBL-producing enterobacteraceae, 69 (75%) E. coli, 20 (21.7%) Klebsiella sp., one (1.1%) Enterobacter, and 2 (2.2%) other pathogens. A total of 84.8% were resistant to ciprofloxacina, 69.7% to cefotaximoxazol, 62% to amoxicillin-clavulanate, 37% to gentamicin, 31.5% to fosfomycin, and 10.9% and to nitrofurantoin. Table 1 shows the distribution of resistance depending on the pathogen. Only in 33% of the cases empirical treatment was adequate, and considered the administration of at least one antimicrobial with in vitro activity against the organism. No patient died of the infection. Taking these data into account, a protocol to facilitate the choice of treatment in patients with suspected infection with ESBL-producing microorganisms has been developed (Fig. 2).

Conclusions

ESBLs are enzymes with hydrolytic activity against beta-lactam antibiotics, which confers resistance to penicillins, cephalosporins, and aztreonam. In vitro are inhibited by beta-lactamase inhibitors. They are often found in enterobacteraceae, mainly in Klebsiella sp. and Escherichia coli, and are associated to a high proportion of resistance to other antibiotics such as fluoroquinolones, aminoglycosides, or cefotaximoxazol. Phenotypic methods are the most widely used for its detection, with high sensitivity and specificity if the expert recommendations for application are followed. Genotypic methods are carried out in specialized laboratories and provide essential information in order to prevent and control. Prevention strategies should focus on nosocomial infection, but it should not be forgotten that the epidemiology of these infections is changing and occurrence of these pathogens in community-acquired infections is becoming more and more frequent. The failure of empirical treatment involves high morbidity and mortality, so that therapeutic decisions should be based on knowledge of the local distribution of pathogens and their resistance patterns. The treatment of choice for severe infections due to ESBL-producing gram-negative bacteria is the carbapenems, so its indiscriminate use should be avoided.

Conflict of interest

The authors declare that they have no conflict of interest.

References


