Extended spectrum beta lactamase producing animal enterobacteriaceae isolates as potential risk to public health – review

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ABSTRACT

The widespread use of extended-spectrum cephalosporins creates a reservoir of resistant bacteria. Moreover, multi-resistance frequently associated with strains carrying ESBLs, which could dramatically reduce the treatment options. Resistance against β-lactams is increasingly being reported and is one the rise in Enterobacteriaceae from both humans and animals. Resistance may be transferred directly from animal to human and may possibly be acquired indirectly, through the transfer of resistance genes from bacteria of animal origin to bacteria infecting humans. The presence of ESBLs among commensal Enterobacteriaceae in animals seem to be confined to specific individual countries. For instance CTX-M-15 is the most widely diffused enzyme among human Enterobacteriaceae in Europe, was only recently detected among E. coli from poultry, pigs and cattle. Also to date, only a few studies have been published reporting ESBLs producing Enterobacteriaceae isolated from diseased pigs, cattle and companion animals. Currently carbapenams are regarded as the drug of choice for treatment of infections caused by ESBL-producing microorganisms. Unfortunately, use of carbapenams has been associated with the emergence of carbapenem-resistant bacteria. The increasing number of Enterobacteriaceae with ESBLs that also contain MBLs or AmpCs and other new mechanisms of resistance to fluoroquinolones or aminoglycosides indicate that recent increase of ESBLs – producing bacteria in Europe constitutes a complex problem.

Keywords: ESBLs, Enterobacteriaceae spp., animals, risk for public health

RÉSUMÉ

Bêta-lactamase de spectre étendu chez les entérobactéries en production animale comme risque potentiel en santé humaine, une revue

L'utilisation généralisée des céphalosporines à large spectre crée un réservoir de bactéries résistantes. En outre, une multi-résistance est fréquemment observée sur certaines souches, ce qui pourrait réduire considérablement les options de traitement. La résistance contre les β-lactamines est de plus en plus signalée chez les entérobactéries isolées chez l’homme et l’animal. La résistance peut être transférée directement d’animal à l’homme et peut éventuellement être acquise indirectement, par le transfert de gènes de résistance des bactéries d’origine animale à des bactéries qui infectent les humains. La présence de bêta-lactamase de spectre étendu (BLSE) parmi les entérobactéries commensales chez les animaux semble être confinée à certains pays spécifiques. Par exemple, CTX-M-15 qui est l’enzyme de résistance la plus largement reproduite parmi les entérobactéries d’origine humaine en Europe, a été récemment détectée chez E. coli de la volaille, les porcs et les bovins. A ce jour, seules quelques études ont été publiées sur la présence de BLSE chez des entérobactéries isolées chez les porcs, le bétail et les animaux de compagnie malades. Actuellement les carbapénèmes sont considérés comme le médicament de choix pour le traitement des infections causées par des micro-organismes produisant des ESBLs. Malheureusement, l’utilisation des carbapénèmes a été associée à l’apparition de bactéries résistantes contre les carbapénèmes. Le nombre croissant d’entérobactéries productrices de BLSE qui contiennent également des mécanismes de résistance aux fluoroquinolones et aux aminoglycosides en Europe constitue un problème complexe.

Mots clés : Entérobactéries, Bêta-lactamase, productions animales, santé humaine

Introduction

Bacterial resistance to β-lactams can be due to at a few mechanisms. One of mechanism consists of mutations in genes encoding PBPS, the acquisition of alternative PBPS. These altered PBPS have a reduced affinity for β-lactams

by various Gram-positive and Gram-negative microbial species. Some of them are exocellular, i.e. are secreted out of the microbial cell (staphylococcal penicillinases) whereas others, especially in Gram-negative bacteria, are located in the periplasmic space and are available only after cell wall breakdown. In a number of bacterial species they are inducible, while in others they are constitutively synthesized. Enzyme inactivation is of special interest due to its clinical significance, ecological aspects and evolutionary development [3]. Genes determining beta-lactamase production are most commonly extra-chromosomal – on plasmids, but could be also found within the bacterial chromosome. Chromosomal-mediated inducible beta-lactamases in some representatives of family Enterobacteriaceae and genus Pseudomonas are of special concern and major clinical significance, as are the plasmid-encoded broad-spectrum beta-lactamases of Klebsiella genus and other Gram-negative bacteria, conferring resistance to
novel cephalosporins. It is acknowledged that plasmids play an essential role in the transmission of bacterial resistance and epidemic outbreaks caused by resistant bacterial species in both men and animals [129]. Since 2009, the number of unique protein sequences associated with beta-lactamases has exceeded 890 [24].

The intricacy of therapeutic approaches using third- and fourth-generation cephalosporins in infections caused by Enterobacteriaceae could be also attributed to the multi-resistance of strains. In some bacteria producing extended spectrum beta lactamases (ESBL), such as AmpC beta-lactamase producers, the presence of plasmids could determine the co-transfer of resistance to aminoglycosides. Unfortunately, the alternative therapy including application of carbapenems in ESBL producers during the last years is also becoming inefficient due to the increasing spread of carbapenem-resistant Stenotrophomonas spp., Pseudomonas spp. strains.

Another important epidemiological issue of ESBL producing bacteria spread is the existence of similar ESBL phenotypes in human and animal isolates, with particular significance of representatives of resident intestinal microflora in animals.

Epidemiology of ESBL- producing bacteria is a complex with resources between hospitals and the community. E. coli producing CTX-M – beta- lactamases are mainly the community ESBL producers which are differ from the Klebsiella spp. producing TEM- and SHV types of ESBL [Table I].

### Beta lactam antibiotics structural classification, mechanism of action and use in veterinary practice

Beta-lactams include several groups of compounds with a β-lactam ring. According to their structure they could be classified as follows, penams, carbapenams, oxapenams or clavams, carbapenams, penems, cephs (cephalosporins), carbacephems, oxacephems (moxalactam), monobactams (aztreonam) [50], [Table II].

The similarity in the mode of action of beta-lactams is their ability to inhibit peptidoglycane complex of bacterial cell wall via action on penicillin-binding proteins (PBPs). PBPs are transpeptidases and carboxypeptidases involved in the last stage of peptidoglycane synthesis. The effect of beta-lactams on them consists in cell integrity loss. Interpeptide bonds are formed under the influence of specialised acetyltransferases, which are immobilised by penicillins [50]. Penicillins impede the regulation of cell autolysis control, resulting in autolysis of bacterial cell.

Data about the use of beta-lactam antibiotics in animals from numerous monitoring programmes carried in different parts of the world (NARMS, 2004, CIPARS, 2006, DANMAP,
Classification of beta-lactamases

The first beta-lactamases have been discovered in soil bacteria, natural producers of penicillins several years before their introduction in clinical practice [85]. The first plasmid-determined beta-lactamase detected in Gram-negative bacteria is TEM-1. The nomenclature is associated with the name of a patient – Temoniera, from whose blood culture was isolated the E. coli producing strain [137]. SHV-1 is another type of plasmid-determined beta-lactamase in E. coli and Klebsiella pneumoniae.

Later, several different enzyme classes were established – penicillinases, cephalosporinases, carbapenemases etc. They are distinguished by their physicochemical properties, hydrolytic activity, genetic determination etc. but all are capable to hydrolyze the beta-lactam ring of the antibiotic and hence, to inactivate it.

ESBL possess a broad range of point mutations altering the active sites configuration of original beta-lactamases such as TEM-1, TEM-2, SHV-1. ESBL-producing bacteria are often multi-resistant as the genes determining the resistant to other classes of chemotherapeutics are located in the same plasmids where ESBL genes are situated. As mentioned above with respect to this statement, some of ESBL-producing bacteria are resistant to aminoglycosides, 4-quinolones etc.

Extended spectrum beta-lactamases are active against most beta-lactam antibiotics, including oxyimino-beta-lactams, ceftazidime, ceftiofur, aztreonam, could be inactivated by clavulanic acid, but some of them are not active against cephamycins, cefoxitin and carbapenems. Their extended spectrum could be regarded as a consequence of the increased sensitivity of ESBL to beta-lactamase inhibitors [16].

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**Table II: Structural classification of beta-lactams**

<table>
<thead>
<tr>
<th>Structure of beta-lactams</th>
<th>Representatives</th>
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<tbody>
<tr>
<td><strong>Beta-lactams fused to saturated five-membered ring</strong></td>
<td>Beta-lactams with thiazolidine ring – penams, natural and semi-synthetic penicillins; Beta-lactams with pyridoline ring – carbapenems</td>
</tr>
<tr>
<td><strong>Beta-lactams fused to unsaturated five-membered ring</strong></td>
<td>Beta-lactams containing 2,3-dihydro-1H-pyrrrole ring – carbapenems (imipenem); Beta-lactams containing 2,3-dihydrothiazole ring – penems</td>
</tr>
<tr>
<td><strong>Beta-lactams fused to unsaturated six-membered ring</strong></td>
<td>Beta-lactams containing 3,6-dihydro-2H-1,3-thiazine ring – cephems (cephalosporins); Beta-lactams containing 1,2,3,4-tetrahydroprydine ring – carbacephems</td>
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Monobactams (aztreonam)
According to JACOBY et al. (2004), ESBL are detected with especially high frequency in Klebsiella spp. and E. coli resistant to cefotaxime, ceftazidime, ceftriaxone, aztreonam and other oxyimino beta-lactams [67]. One of the most commonly encountered ESBL profiles are associated with point mutations, in which lysine substitutes glutamine, serine substitutes arginine and lysine – glycine [16]. Amino acid substitutions with respect to ESBL result in altered active site configuration which permits the access to oxyimino beta-lactams and expands the spectrum of beta-lactam antibiotics, which could be hydrolyzed [68]. As a result of enzymatic active site modification and the variety of beta-lactam substrates prone to hydrolysis, an increased sensitivity of isolates to beta-lactam inhibitors, clavulanic acid, sulbactam and tazobactam is observed [68].

Two primary approaches to the classification of beta-lactamases are known, with more than 530 enzymes [4].

The classification of Bush is based upon the substrate and inhibition profile of enzymes together with their molecular structure. Thus, four groups were defined, termed Bush-Jacoby – Medeiros groups [20, 21]. In some of them, several subgroups were formed [21]. Later, in 2010, Bush and Jacoby have published an updated classification of beta-lactamases on the basis of molecular structure in which group 1 (class C) includes cephalosporinases, group 2 (classes A and D) – broad-spectrum, inhibitor-resistant, and extended-spectrum beta-lactamases and serine carbapenemases [24]. Group 3 comprises metallo-beta-lactamases (Table III).

**Group 1.** Includes cephalosporinases which are not inhibited by clavulanic acid and belong to the molecular class C.

**Group 2.** Includes penicillinases and cephalosporinases, inhibited by clavulanic acid, belonging to molecular classes A and D, expressed by original TEM and SHV genes. As their number is progressively increased, they are divided in two subgroups – 2a and 2b. The first includes only penicillinases belonging to class A, and the second – broad-spectrum beta-lactamases, inactivating both penicillins and cephalosporins. This subgroup is also heterogeneous and comprises the following varieties:

- **2be** – beta-lactamases belonging to ESBL – penicillinases and cephalosporinases conferring resistance to penicillins, cephalosporins (with the exception of cephemycins), monobactams but not to beta-lactam inhibitors such as the clavulanic acid [22, 52].

- **2br** – broad-spectrum beta-lactamases conferring acquired resistance to clavulanic acid and other inhibitors.

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Subgroup</th>
<th>Molecular class</th>
<th>Main substrate</th>
<th>Representatives of beta-lactamases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>C</td>
<td>all groups of beta-lactam antibiotics except carbapenems</td>
<td>chromosome-encoded AmpC beta-lactamases, some plasmid-encoded AmpC beta-lactamases- are not inhibited by clavulanic acid</td>
</tr>
<tr>
<td>2</td>
<td>2a</td>
<td>A</td>
<td>penicillins</td>
<td>penicillinases of Gram-positive bacteria-are inhibited by clavulanic acid</td>
</tr>
<tr>
<td></td>
<td>2b</td>
<td>A</td>
<td>penicillins, cephalosporins</td>
<td>broad-spectrum beta-lactamases (TEM-1, TEM-2, SHV-1)- are inhibited by clavulanic acid</td>
</tr>
<tr>
<td></td>
<td>2be</td>
<td>A</td>
<td>penicillins, cephalosporins, monobactams</td>
<td>extended-spectrum beta-lactamases (ESBL) –are inhibited by clavulanic acid</td>
</tr>
<tr>
<td></td>
<td>2br</td>
<td>A</td>
<td>penicillins</td>
<td>inhibitor-resistant beta-lactamases of TEM and SHV types</td>
</tr>
<tr>
<td></td>
<td>2c</td>
<td>A</td>
<td>penicillins, carbenicillin</td>
<td>carbenicillin- hydrolyzing PSE type beta-lactamases</td>
</tr>
<tr>
<td></td>
<td>2e</td>
<td>A</td>
<td>cephalosporins</td>
<td>inducible cephalosporinases from Proteus spp.- are inhibited by clavulanic acid</td>
</tr>
<tr>
<td></td>
<td>2f</td>
<td>A</td>
<td>penicillins, cephalosporins, carbapenems</td>
<td>serine- carbapenemases –are inhibited by clavulanic acid</td>
</tr>
<tr>
<td></td>
<td>2d</td>
<td>D</td>
<td>penicillins, oxacillin</td>
<td>OXA-Type beta-lactamases hydrolyzing oxacillin-are poorly inhibited by clavulanic acid</td>
</tr>
<tr>
<td>3</td>
<td>3a, 3b, 3c</td>
<td>B</td>
<td>most beta-lactams, including carbapenems</td>
<td>metallo-beta-lactamases-are not inhibited by clavulanic acid but are inhibited by EDTA</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>penicillins</td>
<td>penicillinases not belonging to other groups</td>
</tr>
</tbody>
</table>

Table III: Classification of beta-lactamases (Rubutsova et al., 2010)
At present, 36 out of the described 150 TEM enzymes possess such properties, TEM-30, TEM-31 (IRT-2, IRT-1) and 5 out of the known about 111 SHV enzymes.

Recently, another 4 subgroups of group 2 were defined. Subgroup 2c includes carbenicillinases belonging to class A, inhibiting mainly carbenicillin and at a lesser extent β-lactamases and oxacillin. Subgroup 2d comprises clavuloxacinases, inactivating clavuloxacin rather than β-lactamases. They are slightly inhibited by clavulanic acid and some of them are characterised as ESBLs. Subgroup 2e includes cephalosporinases which cleave monobactams, inhibited by clavulanic acid. Subgroup 2f contains carbapenemases, other than zinc-containing carbapenemase from group 3.

Group 3 gathers zinc-containing metallo-lactamases from molecular class B, which hydrolyze penicillins, cephalosporins and carbapenems and are not inhibited by clavulanic acid.

Group 4, although formed but still disputable, consists of penicillinases resistant to clavulanic acid that could not be referred to any molecular class.

The classification of Ambler divides β-lactamases on the basis of amino acid sequences in classes from A to D [3]. Their active site contains serine and from the point of view of their architectonics and function, they are similar to protein-binding penicillins of the bacterial cell wall. Ambler classes A, C and D include evolutionary distant serine β-lactamases, while class B harbors metallo β-lactamases [22, 84].

Class A is the largest one and includes β-lactamase enzymes representing part of group 2 as per Bush's classification. These are penicillinases produced by staphylococci [22]. β-Lactamases from subgroup 2b hydrolyze penicillins and the first cephalosporins, cephaloridine, cefotin, but are inhibited by clavulanic acid and tazobactam. The group encompasses the most commonly encountered β-lactamases of the 1970s TEM-1, TEM-2, SHV-1 [85, 113].

Beta-lactamases from the TEM group: They are among the β-lactamases most frequently produced by Gram-negative bacteria. According to LIVERMORE (1995) almost 90% of ampicillin-resistant E. coli strains produce TEM-1 [84]. This enzyme is capable to hydrolyze penicillins as well as first-generation cephalosporins. Its first derivative is TEM-2, and the difference between both enzymes is one substituted amino acid resulting in altered isoelectric point of the enzyme, but not in a change in its substrate profile [16]. TEM-3 however is the first representative of the TEM-type belonging to ESBLs, reported in 1989 [136].

Amino acid substitutions of TEM enzymes are observed in limited number of positions. The combinations of substitutions result in a number of complex changes in the ESBLs phenotype – substrate profile, isoelectric point etc. [16]. According to BUSH AND JACOBY (2010) the described diversity of about 150 TEM enzymes allows for investigation of the prevalence if individual genetic determinants conferring resistance to β-lactams [24]. It is therefore clear that TEM-enzymatic profile is transformed into ESBLs profile – for instance after replacement of arginine at position 163 with histidine and serine, or glutamine at position 238 with serine and alanine, glycine at position 240 with lysine.

SHV – β-lactamases: The first reported member of this group is SHV-1 [23]. It is chromosomally determined and detected in a number of Klebsiella pneumoniae strains. In coli bacteria, it is plasmid-determined [57]. Derivatives of SHV-1 are SHV extended spectrum β-lactamases, in which glycine at position 238 is replaced by serine. According to AL-JASSER (2006), the SHV-type ESBL is the most encountered among clinical isolates [2]. At present, more than 114 SHV enzymes are described (JACOBY AND BUSH, 2008); they are also the most frequently seen in K. pneumoniae [63]. BRADFORD (2001) affirms that they determine about 20% of plasmid-mediated resistance to ampicillin in this microbial species [16]. The presence of serine at position 238 is critical for the efficient hydrolysis of cefazidime while lysine at position 240 – for the efficient hydrolysis of cefotaxime. One SHV-10 variant possesses inhibitor-resistant phenotype profile. This enzyme is derivative of SHV-5 as it has an additional serine substitution for glycine at position 130. An interesting fact is that inhibitor-resistant phenotype profile conferred to the presence of serine-140/glycine mutation makes ineffective the strict ESBL profile if enzymes with glycine-238/serine and glutamate-240-lysine substitutions. Apart in Klebsiella pneumoniae, SHV enzymes are also observed in other microbial species – Citrobacter diversus, E.coli, P. aeruginosa [16].

CTX-M family β-lactamases: These are the most commonly encountered enzymes with ESBL profile according to JACOBY AND MINOZ-PRICE (2005) [62]. The investigation of CANTON AND COQUE (2006) demonstrates that in the beginning of the new millennium, CTX-M cephalaxinases are the most prevalent ESBL in Europe and South America [25]. They exhibit 40% identity with TEM and SHV enzymes [16]. A high degree of homology with chromosomal AmpC enzymes of Klyuyvera ascorbata is determined. The group is most closely related to chromosomally determined cephalosporinases produced by K. oxytoca, C. diversus, Proteus vulgaris, Serratia fonticola. Their prevalence is not limited to nosocomial infections caused by Klebsiella spp. only, but they are also found in E. coli, and what’s more, in an out hospital environment [100]. Unlike TEM and SHV type emerging following point mutations, it is believed that CTX-M enzymes with ESBL profile appear consequently to combinations of chromosomal ESBL of Klyuyvera spp. into a mobile plasmid [11].

CTX-M genes could be classified on the basis of amino acid sequences. BONNET; 2004 [11], PITOUT et al., 2007,
Plasmid-determined beta-lactamases from Ambler class C. Plasmids carrying genes encoding AmpC beta-lactamase production often possess other resistant genes conferring resistant to aminoglycosides, chloramphenicol, sulfonamides, tetracyclines, trimethoprim and mercury ions [109]. Clinical strains producing AmpC beta-lactamases and other beta-lactamases, whose genes are in the same or another plasmid, are isolated [99]. HANSON (2003) affirms that the phenotype features of plasmid-determined AmpC beta-lactamases is difficult for determination [59].

Plasmid-determined Amp C beta-lactamases are classified in 5 families (C1-C5) according to amino acid sequences [6]. The most commonly encountered member of this group is CMY-2 beta-lactamase.

Amblader's class D also includes ESBL from group 2 of Bush classification. The difference is that class D enzymes could hydrolyze cloxacillin [52]. The primary place in this class is occupied by OXA- beta-lactamase family which, at present, comprises 11 enzymes with ESBL profile are known to belong to this family. They originate from OXA-10, OXA-1 and OXA-2 after amino acid substitutions (24). According to BRADFORD (2001) they confer resistance to ampicillin and cephaholin, as well as possess a high hydrolytic ability against oxacillin and cloxacillin. These enzymes are poorly inhibited by clavulanic acid [16]. OXA-enzymes with ESBL phenotype are encountered mainly in P. aeruginosa [4]. They differ from TEM and SHV-types in that they belong to Ambler's class D and functional group 2d [21]. Many P. aeruginosa and A. baumannii strains producing OXA beta-lactamases are isolated in Turkey and France [4]. ESBLs associate with OXA-10 have underwent one or two substitutions of asparagine for serine at position 73 or glycine at position 157.

ESBL from human Enterobacteriaceae spp. isolates, some essential views for their distribution and multi-resistance

ESBL-producing bacteria from the Enterobacteriaceae family were isolated for the first time from in-hospital patients during the 1980s. After 2000, their spread in out-hospital environment is increasingly discussed [4, 23, 86, 106]. Most commonly isolated intestinal bacterial ESBL producers are E. coli and Klebsiella pneumoniae. The report of TALBOT et al. presented before the Infectious Diseases Society of America in 2006 sets ESBL-producing bacterial species as priority bacterial species which necessitate immediate novel therapeutic approaches [138].

Numerous researchers have concluded that the presence of ESBL-producing strains depends on various factors including the nature of bacterial infections: nosocomial or
not, apart the species and geographic location specific traits [16, 28, 86, 87]. In humans, the spectrum of affected cohorts is various, with the first place being held by patients from intensive care units, pediatric wards and clinics, including neonatology sectors. Often, such strains are isolated also from cancer and burn patients, in rehabilitation and geriatric care units [128].

During the last two decades, apart E. coli, a higher prevalence of Salmonella spp. strains possessing plasmid-mediated ESBL from the CTX-M family is observed, in particular S. enterica serovar Typhimurium. Reports indicate that some Enterobacteriaceae spp. strains were most commonly responsible for focal outbreaks of infections in East Europe (Latvia, Poland, Russia), South America and Japan [46, 47]. These strains have been preferentially determined to be involved in the etiology of human out-hospital urological tract infections and bloodstream infections unlike TEM- and SHV-producing bacteria which are traditionally associated with nosocomial infections.

Since 2003, the major part of important etiological agents of both in-hospital and out-hospital urological infections and bacteremia reported to be spread in various geographic locations are E. coli strains producing CTX-M enzymes (CTX-15 in particular) [125]. From molecular aspect, epidemiological analyses indicate that the increased spread of such CTX-M-15 producing coli-bacterial strains in European countries as Spain, France, Portugal, Switzerland and some countries in Asia and the Near East was frequently associated with a ST131 clone [25, 110]. IncF plasmids carrying the bla<sub>CTX-M-15</sub> gene are not specific only to ST131, they were determined in other E. coli sequence types (ST405, ST354, ST28, and ST695) in a Shigella sonnei strain, in Salmonella enterica serovar Enteritidis and in a Klebsiella pneumoniae strain [34, 41, 64]. Information concerns existences of bla<sub>TEM-1</sub> gene, mobilized by the Tn3 transposon, with the bla<sub>CTX-M-15</sub> gene on the same plasmid was discussed by BOYD et al. [14].

On the other side IncI/M plasmids carrying the bla<sub>CTX-M-1</sub> gene were reported mainly in Eastern European countries and also in France, Belgium, and the aminoglycoside resistance gene armA has been located on the same IncI/M plasmid as the bla<sub>CTX-M-1</sub> gene [102, 105].

Resistant Klebsiella pneumoniae isolates are increased with emergence of carbapenemases such as OXA-48, which was first found in Turkey [120]. From these point of view data commented by Harris et al. (2015) concerns the possibilities in treatment management of bloodstream infections caused by resistant K. pneumoniae isolates to carbapenems had an important perspective [60]. Most VIM-producing isolates are sporadic, but clonal epidemics have been described in Greece [27, 120]. K. pneumoniae isolates carrying the bla<sub>VIM-1</sub> gene and E. coli isolates owning bla<sub>VIM-1</sub> and bla<sub>CMY-13</sub> genes collected in Greece were determined to belong to epidemic plasmids IncN group [27].

ESBL-producing bacteria have an important clinical significance also from the point of view of bacterial MDR. Some authors presume the spread of MDR Enterobacteriaceae spp. and MDR-encoding genes from farm animals to humans [31, 38]. Plasmid-mediated quinolone resistance (PMQR) has been reported by the acquisition of the qnr, qepA, and aac(6)-Ib-cr genes [116]. PMQR is also associated with ESBLs and aminoglycoside resistance genes on the same plasmid. The circulation of these multidrug resistance plasmids among Enterobacteriaceae strains has an important influence on the empirical treatment of urological infections in human patients [107].

**Distribution of ESBL-producing Enterobacteriaceae spp. isolates in farm animals, wild animals and pets, some support points for the public health risk**

An essential question in modern epidemiological research on ESBL producing Enterobacteriaceae spp. is the existence of similar ESBL phenotypes in human and animal isolates (from farm animals and pets) [26, 35, 133, 150]. There are different literature sources which presented information about the bacterial ESBL producers isolated from diseased and healthy animals as well as farmers [18, 80, 88].

So far, CTXM-1 and CTX-M-15 E. coli beta-lactamase producers are mostly isolated from poultry, swine and cattle [28, 42, 95, 147].

Numerous reports have witnessed various ESBL types among Enterobacteriaceae spp. isolated from poultry reared in intensive production systems. BRINAS et al. (2003) were the first to publish data about the occurrence of ESBL-producing E. coli in the foci of healthy birds in Spain [17]. Thus, the authors reported 1.6% ESBL coli strains, producing CTX-M-14 and SHV-12 in particular. Two years later, BRINAS et al. (2005) already reports a higher percentage (5%) E. coli ESBL producers of subtypes CTX-M-9, CTX-M-14, SHV-12 [18].

Also in 2005, AARESTRUP et al. reported about a Salmonella enterica serovar Virchow strain producing CTX-M-9, isolated from quail meat imported from France [1]. In Italy BORTOLAIA et al. (2010) detected bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-3</sub> and bla<sub>shv-12</sub> genes in poultry E. coli isolates [13]. Poultry TEM-52 and SHV-12 producing E. coli and other Enterobacteriaceae spp. strains are reported only in European countries: Spain [17], Italy [31], Belgium [32], Portugal [37]; The Netherlands [61], The Czech Republic [74].

Evidence for the presence of CTX-M producers from farm animals in France was provided in MEUNIER et al. in 2006, [95] and later by MADEC et al., 2008, [89]. With regard to their prevalence among coli bacteria and Salmonella spp. isolated from poultry, cattle and swine in Portugal, Belgium, the Netherlands, France, Spain and Italy, researchers discuss...
an especially frequent spread of types as TEM-52, TEM-106, CTX-M-1, CTX-M-2, CTX-M-9, CTX-M-14, CTX-M-15, SHV-2, SHV-12, CMY-2 [65, 135, 146]. Authors from the Netherlands, Ireland and Germany emphasize on the presence of animal isolates producing TEM-20, although less frequently [8, 15, 18, 39, 61, 63, 81, 98].

Apart poultry strains, WU et al. (2008) analyzed data from the implementation of the DANMAP project dedicated to antimicrobial resistance surveillance, including the spread of ESBL, and reported increased prevalence of CTX-M-1 enzymes in swine E. coli isolates [152]. They discussed the possibility for a selective pressure of ceftiofur use in pigs on the spread of ESBL-producing E. coli strains. Again, in a study on the prevalence of ESBL in Portugal with regard to the widespread utilisation of beta-lactams in intensive pig production systems, GONÇALVAS et al. (2010) have established that CTX-M-1 was the most frequently encountered ESBL type in E. coli pig isolates and that pigs are an essential reservoir of ESBL-producing coli bacteria [55]. At the same time in Spain, ESCUDERO et al. (2010) published comparable results from a survey on 80 pig farms in 13 Spanish provinces and reported ESBL prevalence rates of 41% SHV-12, 10% CTX-M-1, 10% CTX-M-9, 10% CTX-M-14 among coli bacterial isolates [45].

As to the widely spread among Enterobacteriaceae ESBL type CTX-M-15 in human strains, there are data for its occurrence in strains obtained from healthy birds in Belgium and the United Kingdom, as well as in E. coli isolated from diseased French cattle [89, 135, 121] and strains of the same species, detected in Chinese pigs [140]. Animal Salmonella isolates producing CTX-M-15 are reported by RODRIGUEZ et al. (2009), in particular Salmonella enterica serovar Typhimurium, isolated from a horse in Germany [124].

As pets are concerned, most data about ESBL-producing microbial pathogens, primarily E. coli are reported in dogs, between 2001 and 2006. For some EC countries as Portugal and Italy, the prevalence ranges from 7% to 20% and the types TEM-52, CTX-M-1, CMY-2 were most commonly reported. Almost at the same time, FERIA et al. (2002), as well as POMBA et al. (2009) in Portugal, communicated that there were ESBL-producing members of the family Enterobacteriaceae spp. isolated from dogs with chronic urological infections [48, 118]. CTX-M-1 produced by commensal coli bacterial isolates from dogs and cats are reported in Latin America [97].

From epidemiological point of view, reports for ESBL-producing Enterobacteriaceae strains isolated from wild animals are also of interest. For example in Portugal, COSTA et al. (2006) and POETA et al. (2008) described the prevalence of fecal E. coli isolates from wild birds, producing CTX-M-1, CTX-M-14, TEM-52 [36, 114].

So far, according to available information about ESBL production by Salmonella spp., strains from farm animals and respective foodstuffs, it is far less frequent than that observed in E. coli strains. Data of the Spanish Veterinary Antimicrobial-Resistance Surveillance Network demonstrate prevalence rates of 0.2% and 2.5% in Salmonella spp. isolates from pigs and poultry, respectively [123]. According to a study by CHIARETTO et al. (2008), their prevalence ranges between 0.5% and 0.6% [31].

While CTX-M-1 domination has been demonstrated in Europe, the spectrum of CTX-M bacterial producers in Asian countries as China, Japan and Korea is distinguished with a remarkable variety including subtypes CTX-M-3, -13, -14, -15, -12, -24, -55, -64, -65 [44, 73, 77, 78, 82, 91, 122, 133]. ESBL-producing Enterobacteriaceae spp., ESBL especially TEM- and SHV producers isolated from diseased cattle and swine were also detected in Korea by RAYAMAJHI et al., in 2008, [122].

The spread of ESBL genes among animals could be mediated by plasmids consequently to the use of third-generation cephalosporins. This is the commonest explanation for the presence of AmpC types. In particular CMY-2 among cattle in the USA after the introduction of ceftiofur for treatment of bovine respiratory infections [151]. DONALDSON et al. (2006) also determined for the high incidence of the same beta-lactamase (88.5%) among coli bacterial isolates in dairy cattle farms as per is the extensive use of ceftiofur for respiratory infections therapy [43]. SMET et al. (2009) specified CMY-2 as the most commonly encountered enzymes among the isolates from farm isolates. According to the same research team, the spread of genes encoding the production of beta-lactamases could occur via indirect plasmid transfer (Inc II), for example between different Salmonella serotypes [135]. BLANCO et al. in Italy also discussed the presence of coli bacteria producing AmpC enzymes, isolated from poultry, pig and rabbit farms [9]. In a survey on 26 Dutch broiler chicken farms performed in 2012, DIERIX et al. communicated that 80% of studied farms were positive for E. coli possessing ESBL/AmpC genes [40].

The co-selection between beta-lactams and other groups of chemotherapeutics is determined by the fact that resistant genes are often plasmid-determined, and this is a hazard assisting for ESBL gene spread. Thus, combinations of ESBL class A and AmpC enzymes confer resistance not only to oximino-cephalosporins, but also to beta-lactamase inhibitors, cephemycins, even to carbenemems and aztreonam [14]. Apart this phenomenon, there is a statement about associated resistance to beta-lactams and to ciprofloxacin [106].

The prevalence of similar ESBL phenotypes in animal strains poses the question about the risk factors that possibly influence the transformation of multi-resistance strains from animal to human ones. The strong impulse provided by molecular and epidemiological research in this field methodology was the prerequisite of genetic analysis. It was established that the specifics of enzymes by which resistance was mediated, their expression level and the presence of
other possible mechanisms reflected on the variety of types among ESBL-positive isolates [21, 53, 86]. It became evident that the relationship between multi-resistance and ESBL expression was complex and dependent on the localization of genes determining resistance in several different integrons possessing resistant gene cassettes [115]. The increasing spread of bacterial strains carrying genes for ESBL among farm animals, horses, pets, some wild animal species and men, probably confirmed the thesis for the more and more efficient mobilization of these genes at a global scale [110].

The potential transfer of extended spectrum beta-lactamases from animal pathogens to strains that could pose a risk for human health is among the most important challenges issuing from the global problem with antimicrobial resistance. The Scientific Opinion of the EFSA Panel on Biological Hazards from (2011) stipulates that in the future, the impart of frozen poultry meat from Latin America should be considered a potential source for spread of coli bacteria, producing ESBL from the AmpC type [7, 12, 38].

In 2001, VAN DEN BOGAARD et al., and later PRICE et al. (2007) commented on the possibility for transmission and colonization of Enterobacteriaceae spp. between domestic poultry and farmers [144, 119]. CLOECKAER et al. (2007) discussed the possibility for direct transfer and thus, spread of TEM-52 producing Salmonella spp strains in poultry and humans [32]. MARCADE et al. (2009) discussed that bla \text{CTX-M-1} gene was associated with IncI1 in E. coli isolated from human in France and in poultry fecal samples, that’s why these situation determined a risk for dissemination of the gene in country between animals and humans (90). LEVERSTEIN VAN HALL et al. (2011) established a similarity with respect to 35% of resistance genes determining ESBL production in human and poultry E. coli isolates [75]. According to authors, 39% of ESBL producing coli bacteria from poultry were from a genotype identical to that of human strains – MLST. The authors argued about the possibility of spread of genetic determinants via the food chain.

Others (HOPKINS et al., 2006; NASEER et al., 2010; WINOKUR et al., 2000) have outlined that the transmission of CMY-2 producing E. coli from cattle and swine to humans was based on the association of resistance genes with ISEcpI [63, 101, 151]. MOODLEY et al. (2009) have also asserted the likelihood for spread of CTX-M-1 producing coli bacteria from pigs to farmers [96]. Comparable facts about the similarity of genetic determinants in humans and animals are reported by PLATTEEL et al (2013) by establishing CTX-M-1 and TEM-52 positive coli bacteria isolated from nosocomial urological infections in humans as well as from birds [113].

Conclusion remarks

The use of chemotherapeutics in animal husbandry and veterinary medicine confers increased resistance not only of bacterial pathogens, but also in resident bacteria. Resistant bacterial microflora in animals and zoonotic intestinal bacteria could infect more frequently humans through direct contact but also, through animal foodstuffs. Resistant bacterial species could colonize humans and transfer genes of resistance to other members of the bacterial resident microflora. Resistant Enterobacteriaceae commensals could provoke infections in immunocompromised subjects, but could be also regarded as a main reservoir of resistance genes. With this regard, the low resistance level of resident intestinal microflora is an important argument in favor of public health [104, 143, 149]. On the other hand, the low level of resistance could protect productive animals allowing for a more efficient use of chemotherapeutics in veterinary medicine (29, 142). Both animal and human resident coli bacteria are specified as indicators appropriate for monitoring of the prevalence of resistance profiles [130, 148]. Resident coli bacteria carrying ESBL genes could transfer resistance genes to other Enterobacteriaceae spp., Klebsiella spp., which cause severe infections in men [76]. The rapid dissemination of resistance genes via mobile gene elements increases the risk and creates prerequisites for more complications from a therapeutic point of view, with special emphasis on professionals groups associated with animal care, farmers, veterinarians at farms, slaughterhouse workers and other people engaged in animal foodstuff processing. Numerous questions as outlined by EWERS et al. (2012) would find their detailed answers with respect to the fact that the existing variety of plasmid-determined ESBL/AmpC human and animal isolates is associated with the presence of identical sequence types (STs), secondary both to gene transfer and micro-evolutionary development of genetic determinants [47].

Some action of the European Union has already taken aimed at reducing the veterinary use the modern cephalosporins [33]. On the other hand, application of biosecurity and hygiene programs in intensive sector of livestock breeding would be a favorable effect on the restriction transfer of antibiotic resistance.

Improved surveillance of antibiotic use and antibiotic-resistant bacteria in farm animals is another part of the plan of European Commission. There are some important initiatives already taken in this regard, in relation to antibiotic sales data and to the surveillance of antibiotic resistance. All Member States routinely monitor levels of antibiotic resistance in farm animals and on retail meat for Salmonella spp. and most of them represents data from monitoring programs for antimicrobial resistance in farm animals for resident bacteria like E. coli, Enterococcus faecium and Enterococcus faecalis.

References

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