# PHD THESIS- COZMA ANDREEA PAULA

## ABSTRACT

**Key words: *Enterobacteriaceae*, *E.coli*, ESBL, antibioresistance, pets, owners, RAPD**

The doctoral thesis entitled *"Determining the risk of carriage and direction of transmission of ESBL-producing enterobacteriacee in pets and their owners"* has 174 pages and is structured according to the usage, in two parts: the first part which includes *"The current stage of knowledge"* and the second part which includes our own researches on the proposed subject.

The first part has 36 pages and it synthetically presents in two main chapters the reference data necessary for a good understanding of the proposed subject, and information in regard to the main characteristics of extended spectrum beta-lactamase enzymes and the implications of these enzymes on public health are described. The second chapter from the first part includes information about the phenotypic and genotypic methods to detect positive ESBL bacterial strains.

The second part of the thesis has 138 pages and includes information about our own researches and it is structured in 5 main chapters which present the purpose and objectives of the proposed subject, the results obtained following the phenotypic and molecular investigations carried out on the ESBL *enterobacteriaceae* strains isolated from fecal matter of pets and owners or veterinary sanitary personnel. The results regarding the diversity and degree of genetic homology of the analyzed bacterial strains are also presented in the second part of the thesis. Each chapter also describes the materials and work method for each conducted investigation and the related partial conclusions.

The paper has 30 figures, 27 tables, and was carried out based on 287 reference titles.

Chapter II includes the *"Purpose and objectives of the thesis"*. The main hypothesis to be tested is whether pets can represent a reservoir of ESBL *enterobacteriaceae* for the owners or the veterinary sanitary personnel.

The main objectives of this research were:

- isolating and identifying the positive ESBL *enterobacteriaceae* strains isolated from the two categories of analyzed subjects;

- phenotypically confirming the ESBL isolated strains through the combination disc test;

- characterizing the resistance phenotype in other classes of antibiotics of phenotypically confirmed strains as being ESBL;

- molecularly analyzing the positive samples in the ESBL screening for the characterization of genes present in the analyzed animal and human strains;

- molecularly characterizing the isolated ESBL strains in order to identify the genes that determine the resistance to fluoroquinolones;

- identifying the types of plasmids on which genes that codify the ESBL enzymes are found;

- analyzing the genetic diversity of *E. coli* ESBL strains isolated from pets and those isolated from the owners using the RAPD technique;

- conducting a conjugation experiment in order to confirm the transmission of the ESBL genes to the identified plasmids.

Chapter IV *"Phenotypic screening for the detection of positive ESBL enterobacteriaceae strains"*. 279 samples of fecal matter, which were sterilely collected, were collected and processed (228 of animal origin and 51 of human origin). Following the ESBL screening, 117 presumptive ESBL *enterobacteriaceae* strains were isolated (81 of animal origin and 36 of human origin). The taxonomic categorization of the isolated strains was carried out by identifying the $bla_{uidA}$ and $bla_{uspA}$ genes for *E. coli* and based on some minimum biochemical characteristics for the species of *Klebsiella pneumoniae* and *Proteus spp*. The investigations showed that of the 117 analyzed strains, 88.89% belong to the *E. coli* species, 5.98% to the *Klebsiella pneumoniae* species and 5.13% to the *Proteus spp*.

In this chapter, the investigations for the phenotypic confirmation of isolated strains, obtained following the ESBL screening, were also carried out. Of the 117 strains, 63 (53.84%) were confirmed as being ESBL through the combination disc test. The rest of the phenotypically non-confirmed strains may also simultaneously present another resistance mechanism which could conceal the ESBL phenotype and lead to false negative results. Based on these reasons, all 117 presumptive ESBL isolated strains were tested in order to characterize the molecular resistance substrate to antibiotics.

The last subchapter from chapter IV included investigations carried out in order to determine the resistance phenotype to other classes of

antibiotics for the phenotypically confirmed strains as being ESBL. The testing of sensitivity was carried out through the diffusimetric method, and sensitivity was determined in 9 antibiotics from 7 different classes: beta-lactams, fluoroquinolones, aminoglycosides, tetracyclines, sulfonamides, carbapenems, amphenicols. The investigations revealed a high degree of multiple resistance to antibiotics (92.85% for the animal strains and 90.47% for the human isolated strains). Unlike the animal strains, the human strains presented a high level of sensitivity to the action of amoxicillin/clavulanic acid. Also, all of the 63 presumptive ESBL strains for which the resistance profile was determined were sensitive to the action of imipenem.

Chapter V *"Identification of the main genes involved in the appearance and transmission of resistance to beta-lactam antibiotics"* describes the molecular investigations carried out in order to identify the main genes that codify the extended spectrum beta-lactamase enzymes, the genes that codify the AmpC beta-lactamase enzymes, and the genes that determine the resistance to fluoroquinolones and colistin.

The above-mentioned investigations were carried out on the 117 isolated strains following the ESBL screening and the results can be synthesized in the following manner:

- for the 81 animal isolated strains, following the molecular investigations, genes the codify the enzymes from the groups: CTX-M-1 (70,59%), CTX-M-9 (26,5%), TEM (27,16%), SHV (16,04%), OXA (1,23%) and CIT-M (1,23%) were identified;
- for the 36 human bacterial strains isolated following the ESBL screening, the molecular investigations revealed the presence of genes that codify the enzymes from the groups: CTX-M-1 (64,28%), CTX-M-9 (28,57%), TEM (41,67%), SHV (13,89%) and OXA (8,33%). For the human isolated strains, no genes that codify the AmpC beta-lactamase enzymes were identified;
- for the animal isolated strains, the results of sequencing indicated that the genes that codify the CTX-M-14, CTX-M-15 and CTX-M-1 enzymes were dominant;
- following sequencing of human isolated strains, the genes that codify the enzymes from the group CTX-M-14 and CTX-M-15 were dominant;
- for all the animal isolated strains obtained following the ESBL screening, the percentage of percentage of qnr and aac-(6')-Ib-cr plasmid coded genes was determined, which determines the resistance to quinolone. The dominant gene group was qnrS (13.58%), followed by qnrB and aac-(6')-Ib-cr by a ratio of 3.7% and qnrA (1.23%);

- for the 36 human isolated strains, the investigations for the isolation of the qnr and aac-(6')-Ib-cr genes showed a high prevalence for the qnrS gene (13.89%), followed by aac-(6')-Ib-cr (5.56%) and qnrB (2.78%). The qnrA gene was not identified in the human isolated strains.

Also in chapter V, researches in regard to the plasmid profile of strains, in which the resistance markers were identified, were carried out. The investigations were carried out through the PBRT (PCR based replicon typing) technique and revealed the presence of 12 plasmids of the 18 that were searched, and the IncF, IncI1, IncFIB and IncHi2 replicons were isolated at a high frequency both for the animal strains and the human strains. Plasmid IncN was identified in strains of *Escherichia coli* isolated from pets; in the specialized literature, the presence of this replicon was reported in pets only in the species of *Klebsiella pneumoniae*.

In order to demonstrate that the plasmids isolated by us are conjugative, a conjugation experiment was carried out. On this line, 16 positive CTX-M strains sensitive to nalidixic acid (12 of animal origin and 4 of human origin) were selected. Following the conjugation, 15 transconjugates were obtained, and all of them were tested through PCR for the isolation of $bla_{\text{CTX-M}}$ genes. The gene that codifies the CTX-M enzymes was highlighted for all transconjugates, and the replicons involved in the gene transfer process were Hi2 and I1.

Chapter VI entitled *"Determination of the degree of genetic homology between the animal and human positive ESBL strains"* describes the researches carried out for the purpose of classifying the identified *E. coli* strains into phylogenetic groups, isolating the strains that belong to the ST131 O25 clonal group and also the investigations carried out through the RAPD technique to establish the diversity and degree of genetic homology of strains that made the collection taken into study. The results of the research for this chapter can be synthesized in the following manner:

- the predominant phylogenetic group for the *E. coli* strains isolated from animals was B1 (35.71%);
- for the *E. coli* strains isolated from owners or the veterinary sanitary personnel, the predominant phylogenetic group was B2 (35.30%). The fecal carriage with strains from this group represents a public health problem, these isolated strains having a high pathogenicity character which results particularly from the association of several virulence characters;
- most CTX-M positive *E. coli* strains belonged to the phylogenetic group A;
- three strains of *E. coli* ST131-O25 were isolated: one animal strain, molecularly characterized by the presence of the $bla_{\text{TEM}}$ gene, and two human

strains whose molecular substrate was represented by the *bla*$_{OXA}$ , *bla*$_{qnrS}$, *bla*$_{aac-(6')-Ib-cr}$ and *bla*$_{CTX-M-9}$ genes, respectively;

- the RAPD investigations revealed a high genetic diversity among the animal strains, human strains and animal strains, and the human strains;
- animal strains isolated from dogs from paddocks or practices were identified, characterized by identical or similar phenotypic and molecular substrate, which indicates a clonal relationship between these strains and also that the common environment of the animals from which they were isolated play an important role in the transmission of these strains;
- strains of *E. coli* were identified, isolated in pairs from dog and owners, characterized by identical phenotypic and genotypic profiles (ESBL genes, identical phylogenetic group, the same RAPD profile), which proves that pets can represent a reservoir of ESBL bacteria for humans, the resistance genes being easily transmitted between animals and owners through direct contact.

Chapter VII *"Final conclusions, study limits and research perspectives"* presents the main aspects of the research conducted on the proposed subject, aspects which are synthesized under the form of 28 conclusions.