Characterisation of the Resistance Patterns to Non Beta-Lactam Antimicrobials in Esbl-Producing Enterobacteriaceae Isolated from Dogs and Their Owners

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Abstract

Enterobacteriaceae producing extended-spectrum beta-lactamase (ESBL) enzymes are resistant to beta-lactam agents and are also commonly multidrug resistant being associated with the resistance to other classes of antibiotics.

The aim of our study was to characterise resistance patterns in non-beta-lactam antibiotics of ESBL-producing Enterobacteriaceae strains isolated from faecal matter of pets and owners.

The study was carried out on 63 samples of faecal matter (42 from pets and 21 from owners). The ESBL screening was carried out using the Brilliance ESBL Oxoid chromogenic medium. The isolated strains that generated characteristic presumptive ESBL-producing colonies were cultivated on 5% sheep blood medium for the extraction of bacterial DNA using the boiled preps technique. The confirmation of *E. coli* species was performed molecularly based on the detection of *bla*_{uidA} and *bla*_{uspA} genes. Other Enterobacteriaceae species were identified based on the minimum biochemical characteristics using the MIU and TSI medium. The phenotypical confirmation of presumptive ESBL-producing strains was carried out using the Double Disc Synergy Test (DDST) using a combination of 3rd generation cephalosporins and beta-lactamase inhibitor agents. The determination of the resistance degree in other classes of antibiotics was carried out through the Kirby-Bauer diffusimetric method, and the results were interpreted according to the CLSI standard.

Following the species investigation of isolates, 60/63 (95.28%) belonged to the *E. coli* species and 3/63 (4.72%) to the *K. pneumoniae* species. Animal isolates were resistant to sulphonamides (54.76% resistance to SXT), fluoroquinolones (45.23% resistance to ENR) and tetracyclines (54.75% resistance to TE). In addition to strains of animal origin for isolates of human origin, an increased resistance has been noticed to phenicols and aminoglycosides.

This study has identified a high prevalence of ESBL-producing Enterobacteriaceae strains and associated with multidrug resistance for pets and their owners.

Keywords: dogs, owners, ESBL, Enterobacteriaceae, antibioresistance

Introduction

The emergence of infections with extended-spectrum beta-lactamase enzyme-producing bacteria is continuously rising, and the resistance mechanism determined by these enzymes influen-

ces the first-line treatment with beta-lactams in anti-infectious therapy both of human and of animal origin (Nóbrega, 2014). The resistance determined by the ESBL enzymes is commonly associated with resistance to other antimicrobial

classes, the phenomenon of in-cross resistance and multidrug resistance being often reported in extended-spectrum beta-lactamase enzyme-producing Enterobacteriaceae strains (Ewers, 2012). In Romania, the studies carried out for the characterisation of resistance associated to ESBL-producing Enterobacteriaceae strains are rare and carried out particularly on strains of human origin.

The aim of the study was to characterise the resistance phenotype in non-beta-lactam antibiotics of ESBL-producing Enterobacteriaceae strains isolated from faecal matter of pets and owners.

Materials and methods

The study was carried out on 63 ESBL-producing Enterobacteriaceae strains (42 of animal origin and 21 of human origin) and isolated from faecal matter samples collected from animals with the sterile swab from the rectum, while the owners used stool culture containers.

After collection, the faecal matter samples were impregnated on Oxoid Brilliance ESBL Agar medium (Oxoid, Basingstoke, UK), a medium specific for isolating ESBL-producing Enterobacteriaceae (Huang, 2010). The isolated strains that produced characteristic colonies on the medium used for screening were considered presumptive ESBL and cultivated on blood agar medium (Blood Agar Oxoid) for the extraction of bacterial DNA using the boiled preps technique in order to achieve taxonomic classification of isolated strains.

The *E. coli* strains were taxonomically classified through the PCR identifying the *bla_{uidA}* and *bla_{uspA}* genes keeping the protocol recommended by Anastasi *et al.* (2010), and McDaniels *et al.* (1996), respectively. The other Enterobacteriaceae were taxonomically classified based on some minimum biochemical characteristics using the MIU and TSI media. The phenotypical confirmation of presumptive ESBL-producing strains was carried out through the combination disc test, a method recommended by the CLSI standard (CLSI, 2014).

The characterisation of the resistance phenotype in other non-beta-lactam antibiotics of analysed strains was carried out through the dif-fusimetric method (Kirby Bauer) using antibiotics from the following classes: fluoroquinolones, aminoglycosides, phenicols, sulphonamides, tetracyclines. The chosen antibiotics were selected according to the principle of classification of the

isolated strains in the MDR (multidrug resistance) category (Magiorakos, 2012). The interpretation of the results was carried out according to the CLSI standard (2).

Results and discussion

Following processing of faecal matter samples, 63 presumptive ESBL-producing Enterobacteriaceae strains have been isolated (42 of animal origin and 21 of human origin).

In regard to the spectrum of isolated strains, of the 63 Enterobacteriaceae strains, 60 (95.28%) belonged to the *E. coli* species and 3 (4.72%) to the *K. pneumoniae* species. All Enterobacteriaceae strains isolated following ESBL screening were phenotypically confirmed through the combination disc test as being extended-spectrum beta-lactamase enzyme-producing Enterobacteriaceae.

The investigations carried out to characterise the resistance degree to other classes of non-beta-lactam antibiotics revealed, for the strains of animal origin, a high degree of resistance for the antibiotics from the following classes: sulphonamides (23/42; 54.76% resistance to sulfamethoxazole/trimethoprim), fluoroquinolones (19/42; 45.23% resistance to enrofloxacin), and tetracyclines (23/42; 54.76% resistance to tetracycline) (figure 1). The isolated strains of human origin were characterised by a high degree of resistance to the antibiotics from the following classes: tetracyclines (15/21; 71.43% resistance to tetracycline), sulphonamides (12/21; 57.14% resistance to sulfamethoxazole/trimethoprim), phenicols (10/21; 47.62% resistance to chloramphenicol) and aminoglycosides (10/21; 47.62% resistance to gentamicin) (figure 2).

The results obtained following the conducted investigations are in accordance with the specialised literature. Moreover, they are supported, if we associate the results with the statistics regarding the consumption of antibiotics in Romania, both in human medicine, and in veterinary medicine. In 2015, the reports of the ECDC (European Center for Diseases Prevention and Control) and CDDEP (Center for Disease Dynamics, Economics and Policy) presented a high consumption of antibiotics from the classes: beta-lactams, quinolones, sulphonamides and aminoglycosides (Gelband, 2015; ECDC, 2015). In regard to the consumption of antibiotics in veterinary medicine, according to the report of

Figure 1. Resistance of ESBL-producing bacterial strains to other classes of antibiotics

Figure 2. Resistance of ESBL-producing bacterial strains, isolated from owners, to other classes of antibiotics

the European Medicines Agency from 2016, the antibiotics from the classes: tetracyclines, beta-lactams, sulphonamides and aminoglycosides are the most used in the treatment of infectious diseases of animal origin (European Medicine Agency, 2016).

A bacterial strain resistant to antibiotics from at least 3 different classes of antibiotics is associated with multidrug resistance and classified in the MDR category (8). According to the previously presented results, this study has identified a high prevalence of ESBL-producing bacterial strains and associated with multidrug resistance. Thus, from the total of 42 strains of animal origin, 39 (92.85%) have been classified in the MDR category. For the isolated strains of human origin, 19/21 (90.47%) were MDR.

Conclusion

This study has identified a high prevalence of ESBL-producing Enterobacteriaceae strains and

associated with multidrug resistance for pets and their owners.

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