

Diversity of beta-lactamase-encoding genes in *Escherichia coli* strains isolated from food-producing, companion and zoo animals in Portugal

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A rapid development of plasmid-mediated resistance to extended-spectrum cephalosporins has been observed in Enterobacteriaceae worldwide, predominantly due to the dissemination of extended-spectrum beta-lactamases (ESBL) and plasmid-mediated AmpC beta-lactamases (PMAB). The aim of the present study was to evaluate the extension of ESBL- and PMAB-producing *E. coli* strains isolated from different animal origins in Portugal.

For surveillance purposes, 376 *E. coli* isolates identified at National Laboratory of Veterinary Research (2009-2011) were submitted to antimicrobial susceptibility testing: 123, 51 and 202 were isolated from food-producing, companion and zoo animals, respectively. Minimum Inhibitory Concentrations (MIC) of 11 antimicrobials for all isolates was determined through agar dilution method. Susceptibility towards cefoxitina was determined through disk diffusion method. Breakpoints were interpreted accordingly to EUCAST epidemiological cut-off values. 'Non-wild type' (NWT) isolates for cefotaxime (MIC>0.25mg/L) and/or cefoxitina (<19mm) were screened for the presence of ESBL (blaTEM, blaOXA, blaSHV, blaCTX) and PMAB encoding genes, using PCR method. Sequencing was applied to fully identify beta-lactamases.

Seventeen isolates (4.5%) were 'NWT' strains for cefotaxime, being 5 (29.4%) from companion animals, 4 (23.5%) from food-producing animals and 8 (47.1%) from zoo animals. We identified blaCTX-M-14 (n=1) in a dog and blaCTX-M-15-type genes (n=9) in 6 zoo animals and 3 in food-producing animals. We also identified blaCMY-type genes (n=3) in 'NWT' isolates for cefoxitin, one from each animal category. Other beta-lactamase encoding genes were identified: blaOXA in 5 strains (29.4%) isolated from dolphins, blaTEM in 7 strains (41.2%) isolated from 3 companion animals, 2 food-producing and 2 zoo animals, and blaSHV identified in one isolate (5.9%) from a zoo animal; 13 beta-lactamase-producing isolates (76.5%) were multidrug resistant.

Among 'NWT' *E. coli* isolates for cefotaxime, we identified an important diversity of ESBL encoding genes, belonging to different families, being blaCTX-M-15-type gene the predominant. The spread of ESBL-producing bacteria among species from different origins, such as food-producing, companion and zoo animals, is a concern at public health level. Thus, it should be a priority to monitor and identify the reservoirs of antimicrobial resistance, contributing to a single health for all.