

Occurrence of extended-spectrum beta-lactamases in *Salmonella enterica* strains isolated from broilers and food of animal origin in Portugal

3rd ASM Conference on AMR in Zoonotic Bacteria and Foodborne Pathogens
26 a 29 de Junho, 2012

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Salmonella enterica is a zoonotic bacteria transmitted through the food chain and isolates harbouring extended-spectrum beta-lactamases (ESBLs) have emerged worldwide during the last decade, with the CTX-M group being particularly important. The aim of the present study was to determine the antimicrobial susceptibility of *S. enterica* strains isolated from broilers and food of animal origin and to characterize ESBLs producers.

On the scope of the national antimicrobial resistance surveillance programme on *Salmonella*, a total of 283 strains isolated from broilers ($n=100$) and food of animal origin ($n=183$), were received at the National Laboratory of Veterinary Research in 2011. The minimum inhibitory concentration (MIC) of 11 antimicrobials (nalidixic acid, ciprofloxacin, ampicillin, cefotaxime, chloramphenicol, florfenicol, streptomycin, gentamicin, tetracycline, sulphamethoxazole and trimethoprim) for all isolates was determined by agar dilution method. Susceptibility towards ceftiofloxacin was determined through disk diffusion method. Breakpoints were interpreted accordingly to EUCAST epidemiological cut-off values. 'Non-wild type' ('NWT') isolates for cefotaxime (MIC>0.5mg/L) and ceftiofloxacin (<19mm) were screened for the presence of ESBL- (*bla*_{TEM}, *bla*_{OXA}, *bla*_{SHV}, *bla*_{CTX}) and PMA_-encoding genes, using PCR method. Sequencing was applied to fully identify beta-lactamases.

Among broilers, we identified 62% of 'NWT' isolates for ciprofloxacin, 57% for nalidixic acid and 28% for sulphamethoxazole, whereas in isolates from food of animal origin, 71%, 63% and 56% were 'NWT' isolates for tetracycline, sulphamethoxazole and ampicillin, respectively. Among all, 5/283 (1.8%) strains presented 'NWT' MICs for cefotaxime and were multidrug resistant: 2 *Salmonella* Havana isolated from broilers and 3 *Salmonella* S. 4,[5],12:i:- isolated from food of animal origin (swine); these isolates had one *bla*_{CTX-M-type} gene, and 2 from food of animal origin presented 1 *bla*_{TEM-type} gene and 1 *bla*_{SHV-type} gene, respectively; they were 'wild type' for ceftiofloxacin and no PMAB-encoding gene was detected. To our knowledge, this is the first time in Portugal that ESBL-encoding genes, particularly from *bla*_{CTXM} family, were detected in isolates of *Salmonella* Havana, a very common serotype isolated from our broiler population. It should also be emphasised that third generation cephalosporins are not allowed in the national poultry

production, contrary to the large animal production, which may explain the detection of ESBL-encoding genes in our strains from swine origin. Horizontal gene transfer may be responsible for the coresistance of strains to non-beta-lactam antibiotics. This study shows that national animal health monitoring systems play an important role and should be improved in an international level.