Antimicrobial susceptibility of *Salmonella enterica* isolated from food-producing animals, animal feed and food products of animal origin, in Portugal - Genetic analysis of isolates with reduced susceptibility/resistance to third generation cephalosporins and cephamycins

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Abstract

Salmonella is a widely distributed foodborne pathogen and one of the most common causes of bacterial foodborne illnesses in humans.

An epidemiologic study was conducted on 1600 Salmonella spp isolates recovered from poultry, swine, other animal species, animal feed and food products of animal origin, over the period of 2009-2013, to determine their serotype and antimicrobial susceptibility to a panel of ten antimicrobials (ampicillin, cefotaxime, ciprofloxacin, nalidixic acid, trimethoprim, sulfamethoxazole, chloramphenicol, streptomycine, gentamicine and tetracycline), through the determination of Minimum Inhibitory Concentration (MIC), using the agar dilution technique.

Molecular characterization of isolates showing a non-wild type MIC to cefotaxime was performed, to determine extended spectrum β-lactamases (ESBL), plasmid mediated AmpC (PMAβ), plasmid mediated quinolone (PMQR) resistance determinants and mobile genetic elements involved in the dissemination of resistance genes.

In live poultry (breeders, broilers, layers) of the 843 isolates recovered, 27.9% comprised *S*. Enteritidis, 23,5% *Salmonella* Havana and 14.1% *Salmonella* Mbandaka; in turkeys, *Salmonella* Derby was the most common serovar isolated (44%), followed by *Salmonella* I 4,[5],12:i:- (16%). In swine, of 101 isolates 21.8% comprised *Salmonella* Rissen and *Salmonella* Typhimurium, 10.9% *Salmonella* Derby and *Salmonella* London. In other animal species, *Salmonella* Typhimurium was the prevalent serovar with 65.6% of the isolates, followed by *Salmonella* I 4,[5],12:i:- (9.8%). Overall, *S*. 4,[5],12:i:- was the most common serotype recovered from food products (25.8%), followed by *S.* Typhimurium (19.2%) and *Salmonella* Rissen (18.4%). *S*. Enteritidis was the most frequent serotype in poultry products (36.3%).

Susceptibility profiles differed according with the serotype and the origin of the isolates.

A higher frequency of multidrug resistant isolates was recovered from food of swine and bovine origin, with 62.6% and 59.4%, respectively.

Polymerase chain reaction and sequencing of the amplicons confirmed the presence of $bla_{CTX-M-type}$ (n=8), $bla_{SHV-type}$ (n=2), $bla_{TEM-type}$ (n=2) and plasmid-mediated AmpC β -lactamases (PMA β) genes (n=2). No plasmid mediated quinolone resistance-encoding genes were detected. Six isolates(three *S*. | 4,[5],12:i:-, two *S*. Havana and one *S*. Enteritidis) carried class 1 integrons and one *S*. | 4,[5],12:i:- isolate harboured a class 2 integron.

In conclusion, the growing concern of the emergence of bacterial strains bearing ESBL in food-producing animals highlights the importance of continuous monitoring.