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Antimicrobial susceptibility and oxymino-β-lactam resistance mechanisms in *Salmonella enterica* and *Escherichia coli* isolates from different animal sources

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Abstract

The impact of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases (PMAβs) of animal origin has been a public health concern. In this study, 562 *Salmonella enterica* and 598 *Escherichia coli* isolates recovered from different animal species and food products were tested for antimicrobial resistance. Detection of ESBL-, PMAβ-, plasmid-mediated quinolone resistance (PMQR)-encoding genes and integrons was performed in isolates showing non-wild-type phenotypes.

Susceptibility profiles of *Salmonella* spp. isolates differed according to serotype and origin of the isolates. The occurrence of cefotaxime non-wild-type isolates was higher in pets than in other groups. In nine *Salmonella* isolates, $bla_{\text{CTX-M}}$ ($n = 4$), $bla_{\text{SHV-12}}$ ($n = 1$), $bla_{\text{TEM-1}}$ ($n = 2$) and $bla_{\text{CMY-2}}$ ($n = 2$) were identified. No PMQR-encoding genes were found. In 47 *E. coli* isolates, $bla_{\text{CTX-M}}$ ($n = 15$), $bla_{\text{SHV-12}}$ ($n = 2$), $bla_{\text{CMY-2}}$ ($n = 6$), $bla_{\text{TEM-type}}$ ($n = 28$) and PMQR-encoding genes *qnrB* ($n = 2$), *qnrS* ($n = 1$) and *aac(6′)-Ib-cr* ($n = 6$) were detected. To the best of our knowledge, this study is the first to describe the presence of $bla_{\text{CMY-2}}$ ($n = 2$) and $bla_{\text{SHV-12}}$ ($n = 1$) genes among *S. enterica* from broilers in Portugal.

This study highlights the fact that animals may act as important reservoirs of isolates carrying ESBL-, PMAβ- and PMQR-encoding genes that might be transferred to humans through direct contact or via the food chain.

Keywords: Antimicrobial resistance; *Salmonella enterica*; *Escherichia coli*; ESBL; PMAβ; PMQR

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1. Introduction

Salmonella is a widely distributed foodborne pathogen and one of the most common causes of bacterial foodborne illnesses, with tens of millions of human cases occurring worldwide every year (http://www.who.int). In the European Union it is the second most reported zoonotic disease in humans, with a total of 92,916 cases; most infections are caused by serovars Enteritidis, Typhimurium and Typhimurium monophasic 1,4,[5], 12:i:- [1].

Escherichia coli is the most prevalent commensal of the human and animal gastrointestinal tract and remains one of the most frequent causes of several bacterial infections in both humans and animals [2].

Antimicrobial resistance in Enterobacteriaceae, namely in non-typhoidal *Salmonella* serotypes and *E. coli*, is an expanding problem [3,4]. Wide uncontrolled use of antimicrobial compounds in human and veterinary practices, animal production, agriculture and industrial technology, and an increase in population mobility and in the circulation of food and raw materials for food production across different countries are factors responsible for the emergence and dissemination of resistant and multiresistant bacterial strains that constitute a risk for human and animal health due to an increase in morbidity, mortality and the cost associated with treatment of infections [4,5].

β-lactams and fluoroquinolones are two important classes of antimicrobials used to treat severe infections in humans and in animals [4]. Resistance to third generation cephalosporins is generally due to production of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases (PMAβs). Various β-lactamase-encoding genes have been detected in diverse serotypes located in plasmids or in integrons, facilitating its transmission between serotypes and other bacteria [6].

Animals have the potential to act as reservoirs for a number of zoonotic infections, including those caused by pathogenic and commensal *E. coli* ESBL producers, which might be transmitted to humans through direct contact or via the food chain [5,6].

In Europe, a wide range of ESBL genotypes have been reported from animals [6,7], some of which are also found in humans [8]. Companion animals, including horses, dogs and cats, also constitute a potential reservoir of ESBL-encoding genes, as they often live in close contact with their owners, facilitating occurrence of transmission [9–11]. Wild animals living in the wilderness or in captivity may also represent a source of ESBL-producing *E. coli* isolates to the ecosystems [12,13], stressing the importance of the environment on dissemination of resistance genes and potential zoonotic transmission due to contact between zoo animals, zookeepers and visitors [14].

In this study, we present updated data on antimicrobial resistance in *Salmonella* recovered from animals, with particular emphasis on food-producing animals, poultry feed and food of animal origin, as well as in *E. coli* isolates collected from food-producing animals, pets and zoo animals. The presence of antimicrobial resistance mechanisms in isolates with reduced susceptibility to third-generation cephalosporins and/or cephamycins was also evaluated.

2. Materials and methods

2.1. Bacterial isolates

This study included 562 *Salmonella* spp. isolates representing 50 different serotypes (Table 1), recovered from breeders ($n = 23$), broilers ($n = 193$), layers ($n = 73$), turkeys ($n = 17$), animal feed ($n = 52$), other animal species ($n = 22$) and food products of animal origin ($n = 182$), over the period of 2012–2013.

In poultry farms, samples were collected from feces and environment using sterile boots/sock swabs. Food products consisted of: uncooked fresh products like minced meat, hamburgers, meat cuts, sausages and table eggs, randomly collected at various retail stores. Samples from other animal species (pigeons, partridges, ducks, pets and exotic animals) consisted of hemoculture and organs (lung, liver, spleen, kidneys and intestine) collected during post-mortem examination for bacteriological control.

All samples were examined according to ISO norm 6579:2002 applied to *Salmonella* detection in food and animal feeding stuffs [15]. After biochemical confirmation, *Salmonella* isolates were sent to the *Salmonella* National Reference Laboratory (INIAV-Lisbon) in triple sugar iron slopes or SMID plates for serotype characterization.

Also included in this study were 598 *E. coli* isolates (Table 1) collected over the period of 2009–2013 from food-producing animals [(bovine, swine and poultry) ($n = 215$), pets (dogs, cats, horses and cage birds) ($n = 113$), and zoo animals (terrestrial and aquatic mammals, birds and reptiles), ($n = 270$)]. Samples consisted of swabs from organic fluids and cavities, fecal samples, urine samples, hemocultures and organs collected during post-mortem examination and submitted for bacteriological analysis. Suspected *E. coli* colonies obtained in MacConkey agar were confirmed by means of API 20E strips.

Salmonella spp. and *E. coli* isolates were preserved in cryovials containing tryptose soya broth and glycerol at −70 °C for further antimicrobial susceptibility tests and molecular assays.

2.2. Serotyping of Salmonella isolates

Salmonella isolates were serotyped by the slide agglutination method for their O and H antigens using the method of Kauffmann–White scheme [16].

2.3. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined by agar dilution following standard recommendations [17], using a panel of nine antimicrobial compounds: ampicillin (A), cefotaxime (Ct), nalidixic acid (Na), ciprofloxacin (Cp), gentamicin (G), chloramphenicol (C), tetracycline (T), sulfamethoxazole (Su) and trimethoprim (Tm) (Table 2).

To assess non-wild-type strains, interpretation of results was done according to the epidemiological cut-off values recommended by the European Committee on Antimicrobial