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Antimicrobial susceptibility of *Salmonella* isolates from healthy pigs and chickens (2008–2011)

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ABSTRACT

Using the agar dilution method, antimicrobial susceptibility to human-use antibiotics was determined among Belgian faecal *Salmonella* isolates from healthy pigs and broiler chickens. Both epidemiological cut-off values and clinical breakpoints were applied for interpretation of the results. Cephalosporin-resistant isolates were examined for the presence of genes encoding CTX-M, SHV, TEM and CMY β-lactamases. All isolates with decreased quinolone susceptibility were screened for plasmid-borne genes *qnr*, *qepA* and *aac(6′)-Ib-cr*. In all, 368 *Salmonella* isolates were recovered from pigs and 452 from chickens. Clinical resistance to ciprofloxacin was absent in isolates of both host species, and was 1.9 and 13.1% to cefotaxime in pig and poultry isolates, respectively. Decreased susceptibility to cefotaxime amounted to 2.2 and 0.7%, whereas for ciprofloxacin this was 3.0 and 23.0% in pig and poultry isolates, respectively. Ciprofloxacin decreased susceptibility was limited to few serovars, mainly Paratyphi B. Multidrug resistance was markedly higher for pig isolates (39.7%) than for chicken isolates (17.3%). Sixty-six cefotaxime-resistant isolates, 59 from chickens and 7 from pigs, were phenotypically determined as ESBL/AmpC producers; predominantly Paratyphi B and Typhimurium serovars. $bla_{CTX\text{-}M}$ (mostly $bla_{CTXM\text{-}1}$, but also $bla_{CTXM\text{-}2}$ and $bla_{CTXM\text{-}9}$) and $bla_{TEM\text{-}52}$ were the predominant ESBL genes. Only few isolates expressed SHV-12 or an AmpC enzyme (CMY-2). Isolates of four serovars carried *qnr* genes: Brandenburg and Llandof from pigs, both *qnrS*; Indiana and Paratyphi B from chickens with *qnrB* and *qnrA*. The latter isolate carried $bla_{CTX\text{-}M\text{-}9}$ and was the only strain with a plasmid-borne quinolone resistance gene among the ESBL/AmpC producers. This *Salmonella* survey confirms that the ESBL/AmpC producers are particularly prevalent in chickens (12.8%), and much less in pigs (1.9%). A link between plasmid-borne quinolone resistance genes and ESBLs/AmpC was uncommon.

1. Introduction

Salmonella enterica is a major global food-borne pathogen, causing life-threatening infections mostly among elderly and immunocompromised patients. Various animal species, such as poultry, pigs, cattle, and reptiles, are reservoirs for *S. enterica*, and humans generally become infected by eating undercooked or contaminated food. Strains of *S. enterica* that are resistant to antimicrobial agents have become a worldwide concern since they may compromise the effective treatment of infections in humans. In the 1980s resistance began to emerge to conventional antimicrobial agents such as ampicillin, chloramphenicol and trimethoprim/sulfonamides, and since then, for invasive salmonellosis in man fluoroquinolones and

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extended-spectrum cephalosporins are antimicrobials of choice for severe infections (Hohmann, 2001; Harrois et al., 2014). Cephalosporin resistance among *Enterobacteriaceae* is commonly due to the production of broad-spectrum β-lactamases, such as extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases. Resistance to fluoroquinolones is mainly caused by point mutations in the quinolone resistance-determining region of gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) genes. In addition, efflux pumps and decreased permeability of the outer membrane can also contribute to the level of quinolone resistance.

Over the last decade, broad-spectrum β-lactamases have been frequently demonstrated in the microbiota of food-producing animals (Smet et al., 2010; Ewers et al., 2012). In those reports, CTX-M-1 and CMY-2 were the most predominant ESBL and AmpC enzymes. This may have an impact on human health as genes encoding these enzymes may be present in zoonotic bacteria causing a direct problem or present in commensals which may act as a reservoir of resistance genes for pathogens (Hasman et al., 2005; Smet et al., 2009; Riaño et al., 2009). In Belgium, the emergence of resistance to extended-spectrum cephalosporins has so far only been reported in serovar Virchow and Infantis isolates from poultry. Resistance was due to the ESBL encoding genes $bla_{\text{CTX-M-2}}$ and $bla_{\text{TEM-52}}$ (Bertrand et al., 2006; Cloeckaert et al., 2007).

Plasmid-mediated quinolone resistance (PMQR) has been increasingly reported in *Enterobacteriaceae*, including several European countries (Cattoir and Nordmann, 2009; Veldman et al., 2011). However, studies reporting the prevalence of PMQR genes are rare. To date, three different transferable fluoroquinolones resistance mechanisms have been described: (i) five different *qnr* families (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*), each with different numbers of alleles, (ii) a modified aminoglycoside acetyl transferase gene (*aac*(6′)-*Ib-cr*), and (iii) active efflux pumps such as a specific quinolone pump (*qepA*) and multidrug resistance pumps like *oqxAB*. In general, PMQR-positive isolates display a distinctive phenotypic quinolone resistance pattern resulting in a decreased susceptibility to quinolones (Sjölund-Karlsson et al., 2010). Since PMQR mechanisms have frequently been associated with ESBL/AmpC-producing isolates of human origin (Robicsek et al., 2006; Strahilevitz et al., 2009), screening of ESBL- and AmpC-producing *Salmonella* isolates with decreased susceptibility to fluoroquinolones for the presence of PMQR may shed light whether the same relationship applies for ESBL/AmpC-producing isolates of veterinary origin.

As pigs and poultry are main reservoirs of human foodborne non-typhoidal *Salmonella* infections, a susceptibility survey with focus on fluoroquinolones and cephalosporins was conducted among Belgian *S. enterica* isolates from healthy pigs and chickens. Isolates were collected, by using a standard sampling and isolation procedure, together with MIC determination of antimicrobials commonly used in human medicine. Since cephalosporin-resistant *Salmonella* isolates are considered as potential ESBL or AmpC producers, a second purpose was to assess the prevalence of β-lactamases and to genotypically identify the phenotypically ESBL/AmpC-positive isolates. Finally, all isolates of our collection with decreased susceptibility to fluoroquinolones were examined for the presence of PMQR genes.

2. Materials and methods

2.1. Salmonella collection

Non-repetitive faecal sampling of pigs and chickens was randomly conducted at various farms and abattoirs across Flanders from 2008 to 2011. Sampling was evenly distributed throughout the year and it was assured that the samples were representative of animal production of Flanders. Whereas the large majority of the pig samples were collected at various individual farms, the focus for poultry was on several slaughterhouses. The isolates are epidemiologically unrelated, i.e., a single bird or animal was selected as being representative of a flock or herd. The dates of collection were always recorded and the origins (area codes) of the isolates investigated were always recorded for pigs and for part of the poultry isolates. Isolation and identification of *S. enterica* were performed by the Animal Health Care diagnostic laboratory in Flanders according to the international standard ISO 6579:2002/Amd 1:2007. Isolates were serotyped according to the Kauffmann–White typing scheme.

2.2. Antimicrobial susceptibility testing

Susceptibility testing to ciprofloxacin, cefotaxime, nine other human-use molecules (ampicillin, amoxicillin/clavulanic acid, ceftazidime (for phenotypic ESBL/AmpC tests only), chloramphenicol, colistin, gentamicin, meropenem (for phenotypic ESBL/AmpC tests only), tetracycline, trimethoprim/sulfamethoxazole) and two marker antimicrobial agents (nalidixic acid, streptomycin) was performed by agar dilution according to Clinical and Laboratory Standards Institute (CLSI) standards (VET01-A3; formerly M31-A3). Test ranges of the antibiotics were 0.125–128 mg/L, except for ciprofloxacin (0.004–64 mg/L) and cefotaxime and trimethoprim/sulfamethoxazole (0.015–128 mg/L). As quality controls, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were tested in each run. Both epidemiological cut-off values (ECOFFs) and clinical breakpoints were applied to categorize antimicrobial susceptibility. Clinical resistance was based on CLSI breakpoints (M100-S22, 2012). In the absence of CLSI interpretive criteria (colistin, streptomycin), the resistance breakpoints recommended by EUCAST (colistin: $\geq$4 mg/L) and adopted by the US National Antimicrobial Resistance Monitoring System (NARMS) (streptomycin: $\geq$64 mg/L) for *Enterobacteriaceae* were applied. If applicable, decreased susceptibility (percentage isolates with MICs > ECOFFs and < clinical breakpoints) was determined based on ECOFFs as defined by the European Food Safety Authority (EFSA, 2012). Multiple drug resistance was defined as simultaneous resistance to clinically relevant drugs of at least three different classes, i.e., ampicillin, cefotaxime, ciprofloxacin, chloramphenicol, colistin, gentamicin, tetracycline and trimethoprim of the trimethoprim/sulfamethoxazole combination. All cefotaxime non-wild type