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Original Article

Multidrug resistant *Salmonella enterica* isolated from conventional pig farms using antimicrobial agents in preventative medicine programmes

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ABSTRACT

A longitudinal study was conducted to investigate the presence of multidrug antimicrobial resistance (multi-AR) in *Salmonella enterica* in pigs reared under conventional preventative medicine programmes in Spain and the possible association of multi-AR with ceftiofur or tulathromycin treatment during the pre-weaning period. Groups of 7-day-old piglets were treated by intramuscular injection with ceftiofur on four farms ($n = 40$ piglets per farm) and with tulathromycin on another four farms ($n = 40$ piglets per farm). A control group of untreated piglets ($n = 30$ per farm) was present on each farm. Faecal swabs were collected for *S. enterica* culture prior to treatment, at 2, 7 and 180 days post-treatment, and at slaughter. Minimal inhibitory concentrations of 14 antimicrobial agents, pulsed-field gel electrophoresis and detection of resistance genes representing five families of antimicrobial agents were performed. Plasmids carrying cephalosporin resistant (CR) genes were characterised. Sixty-six *S. enterica* isolates were recovered from five of eight farms. Forty-seven isolates were multi-AR and four contained bla_{CTX-M} genes harboured in conjugative plasmids of the IncI1 family; three of these isolates were recovered before treatment with ceftiofur. The most frequent AR genes detected were *tet*(A) (51/66, 77%), *sul1* (17/66, 26%); *tet*(B) (15/66, 23%) and *qnrB* (10/66, 15%). A direct relation between the use of ceftiofur in these conditions and the occurrence of CR *S. enterica* was not established. However, multi-AR was common, especially for ampicillin, streptomycin, sulphonamides and tetracycline. These antibiotics are used frequently in veterinary medicine in Spain and, therefore, should be used sparingly to minimise the spread of multi-AR.

Introduction

Salmonella enterica is a major foodborne pathogen, causing infections in human beings and animals worldwide. In 2014, 89,873 confirmed human cases of salmonellosis were reported in the European Union (EU) (EFSA, 2017). Control programmes for *S. enterica* in eggs and poultry have been effective in reducing the number of salmonellosis cases in human beings (EFSA, 2014). However, this trend is levelling out, owing to the persistence of *S. enterica* in pigs and porcine products (Pires et al., 2011). On the basis of examination of mesenteric lymph nodes of fattening pigs, there is a wide range (0–36.4%) in the frequency of detection of *S. enterica* between countries in the EU (EFSA, 2015). In Spain, a major producer of pigs in the EU, *S. enterica* was detected in 30% of pigs (EFSA, 2015).

Vaccination of pigs against *S. enterica* serovars that are non-pathogenic for pigs is not recommended, since pigs can be asymptomatically infected and can transmit the bacteria to human beings (San Román et al., 2013). *S. enterica* control programmes in pigs are mainly based on hygiene/disinfection, biosecurity measures and farm management practices. Treatment with antimicrobial agents are necessary for control of clinical outbreaks involving bacteria as primary or secondary pathogens, and have been used as a metaphylactic and prophylactic tool when there is a high probability of an outbreak of unknown bacterial aetiology (Barton, 2014). The selective pressure exerted by antimicrobial agents may contribute to the emergence of bacteria with antimicrobial resistance (AR) (Garcia-Migura et al., 2014). Pigs

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Table 1
Salmonella enterica serovars isolated from faeces at different intervals and treatments administered in each farm during the rearing cycle.

Farm	Antimicrobial agents used at different post-birth intervals			Sampling at different post-birth intervals			
				Number of isolates/number of samples analysed (%) Number of serovars			
	Intramuscular (day 7)	Pre-starter/starter 1 (days 21–49)	Starter 2 (days 50–79)	Day 7	Day 9	Day 14	Day 187
1	Untreated	Amoxicillin Colistin sulphate	NA	2/30 (7%) 2 Rissen	3/30 (10%) 3 Rissen	3/28 (11%) 3 Rissen	2/28 (7%) 2 Rissen
	Tulathromycin			0/40	0/40	0/39	2/39 (5%) 2 Derby
	Sows			0/7			
3	Untreated	Amoxicillin Apramycin	Tiamulin Oxytetracycline	1/30 (3%) 1 Panama	3/30 (10%) 3 Typhimurium	0/30	2/24 (8.3%) 2 Panama
	Tulathromycin	Tiamulin Oxytetracycline		2/40 (5%) 2 Panama	1/40 (2.5%) 1 Panama	0/40	3/36 (8.3%) 1 Panama 2 Typhimurium
	Sows			1/7 (14.2%) 1 Typhimurium			
6	Untreated			0/30	0/30	0/30	0/24
	Tulathromycin			0/40	0/40	0/40	2/34 (5.8%) 1 Rissen 1 Typhimurium
	Sows			0/7			
7	Untreated			0/30	0/30	0/30	0/16
	Tulathromycin			0/40	0/40	0/40	0/30
	Sows			0/7			
2	Untreated	Amoxicillin Apramycin Tiamulin	Tiamulin Oxytetracycline	13/30 (43%) 2 Brandenburg 11 Rissen[a]	4/30 (13%) 4 Rissen[a]	1/30 (3.3%) 1 Rissen	1/22 (4.5%) 1 Typhimurium
	Ceftiofur	Oxytetracycline		7/40 (18%) 3 Anatum[a] 3 Rissen[a] 1 Brandenburg	5/40 (13%) 3 Brandenburg 2 Rissen	1/38 1 Rissen	1/38 (2.6%) 1 Typhimurium
	Sows			1/7 (14.2%) 1 Brandenburg			
4	Untreated			0/30	0/30	0/30	0/18
	Ceftiofur			0/40	0/40	0/40	0/35
	Sows			0/7			
5	Untreated			0/30	0/30	0/26	4/17 (24%) 4 Rissen
	Ceftiofur			0/40	0/40	0/37	1/33 (3%) 1 Rissen
	Sows			0/7			
8	Untreated			0/29	0/29	0/21	0/21
	Ceftiofur			0/37	0/36	0/34	0/0
	Sows			0/7			
Total strains 66				27/614 (4.4%)	16/555 (0.9%)	5/533 (0.9%)	18/415 (4.3%)

[a] A total of four cephalosporin resistant (CR) *Salmonella enterica* serovars were found. One *S.* Anatum isolates were obtained at day 7 in the cephalosporin treated group. Three *S.* Rissen isolates were obtained in the untreated group (2 strains at day 7 and 1 strain at day 9). NA, not applicable.

carrying AR bacteria may have implications for public health, since there is a risk of foodborne transmission to consumers through the food chain.

Third and fourth generation cephalosporins, such as ceftiofur and cefquinome, are licensed for the treatment of systemic bacterial infections in pigs (Cameron-Veas et al., 2015). These β-lactam antimicrobial agents are some of the most important compounds used in human medicine, constituting the main therapeutic choice for treatment of infections caused by *Enterobacteriaceae* (Collignon et al., 2009). The possible selection of cephalosporin resistant (CR) *S. enterica*, together with concerns relating to their entry into the food chain, has raised questions regarding the use of these antimicrobial agents in pigs. In Spain, multi-AR was detected in 55% of *S. enterica* isolates in 2015 (EFSA, 2017).

This longitudinal study was undertaken to evaluate the presence of multi-AR *S. enterica* on conventional pig farms in Spain that use antimicrobial agents in their preventative medicine programmes. β-Lactam antimicrobial agents (penicillins and cephalosporins) and macrolides (tulathromycin and tildipirosin) are the most commonly prescribed drugs in pigs during the suckling period in Spain (Moreno, 2014). The aim of this study was to assess the effect of ceftiofur and tulathromycin treatment on the emergence of CR *S. enterica* during the pre-weaning period, and in the *S. enterica* population in pigs from day 7 until slaughter. The genotypic and phenotypic diversity of the *S. enterica* serovars obtained from each of the farms were analysed. Since