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Resistance to colistin and production of extended-spectrum β-lactamases and/or AmpC enzymes in Salmonella isolates collected from pigs in NW Spain between 2008 and 2009

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Introduction

In the pig industry, the nursery is a critical production period as piglets are susceptible to a variety of enteric infections after weaning, and antimicrobials are commonly used as prophylactics to control Gram-negative (GN) infections. Colistin has been traditionally used to prevent post-weaning diarrhoea. Until recently, prevalence of colistin resistance (CR) was considered low and associated with chromosomal mutations of *pmrA* and *pmrB* genes (Adams et al., 2009). The recent detection and spread of new plasmid-mediated CR-associated genes (Lima et al., 2019), prompted the WHO in 2017 to declare colistin as a ´reserve´ drug against multidrug resistant (MDR) infections in human. In 2015, its use as prophylactic had been banned in Europe. In Spain, the use of colistin remained high (31.4 mg/PCU) until 2015. In that year, a voluntary plan to reduce colistin use in pigs resulted in a significant drop in colistin use (9 mg/PCU)[1].

β-lactam antibiotics have become some of the most used in pig production against GN bacteria (van Rennings et al., 2015). Resistance to these antibiotics is mediated by a wide range of genes coding for β-lactamase enzymes, which are associated with mobile genetic elements (Michael and Schwarz, 2016). The emergence of resistance to these antimicrobials in *Salmonella enterica* has been reported worldwide (Michael and Schwarz, 2016).

We estimate and characterize the prevalence of CR on a collection of *Salmonella* strains isolated from slaughtered pigs in Spain between 2008–2009, that is, much before the official policies on colistin reduction in animals. We also tested a subset of these strains for the detection of extended-spectrum β-lactamases (ESBLs) or AmpC enzyme production.

Methods

A total of 625 *Salmonella* isolates from mesenteric lymph nodes (MLN) from slaughtered pigs were tested for CR by the broth microdilution method (ISO 20776-1:2006), and the epidemiological cut-off (ECOFF) value of >2 mg/L was considered[2]. To assess the possible chromosomal origin of CR, *pmrA* and *pmrB* genes from resistant strains were sequenced and compared to the reference *Salmonella* strain LT2 using BLAST. The presence of the plasmid-mediated CR genes *mcr-1* to *mcr-4* was tested by PCR (García et al., 2018) on all strains with MIC>1mg/L.

A subset of 271 isolates were analysed for ESBL/AmpC production (Total ESBL+AmpC Confirm kit; Rosco Diagnostica, Denmark). At least one isolate of each serotype found in each *Salmonella*-positive herd was selected. Genetic characterization of ESBL/AmpC production was further assessed by PCR (Dallenne et al., 2010).

Results

Six (0.96%) *Salmonella* isolates from 4 different pig farms located far apart showed CR (4 *S.* 4,5,12:i:-, one *S.* Enteritidis, and one *S.* 9,12:-:-). The *mcr-1* gene was detected in all *S.* 4,5,12:i:-, 3 belonging to the same herd. In one strain (*S.* 9,12:-:-) polymorphisms producing protein variants in *pmrAB* were observed. The resistance detected in *S.* Enteritidis is still under characterization. Only one (0.37%) *Salmonella* (*S.* Bredeney) showed AmpC production, which was associated with the bla_{CMY-2} gene.

Discussion and Conclusion

The *mcr-1* gene was identified in *Salmonella* strains isolated one year earlier than the first *Salmonella* and *E. coli* strains reported to bear this gene in Spain (Quesada et al., 2016). Despite its presence, the prevalence of CR in *Salmonella* isolates from pigs exposed to colistin was low. Three of the *mcr-1* positive *Salmonella* isolates belonged to the same farm, suggesting a clonal spread, but the transmission of the *mcr-1* gene among *Salmonella* isolates might not be so frequent. *mcr-1* was detected only in *S.* 4,5,12:i:-, supporting the idea that *S.* Typhimurium and *S.* 4,5,12:i:- are the most common serotypes harbouring *mcr* genes (Lima et al., 2019). Most of resistant strains belonged to zoonotic serotypes, thus a potential transmission of CR to humans is possible. All *Salmonella* isolates harbouring the *mcr-1* displayed MDR (i.e. to aminopenicillins, phenicols, aminoglycosides, sulphonamides and tetracyclines), which may contribute to the co-selection of CR (Lima et al., 2019).

Resistance to 3rd generation cephalosporins was lower (0.37%), and within that observed in Europe for those years (Seiffert et al., 2013), likely because cephalosporin use in food animals was limited at that time (Hornish and Kotarskias, 2002). AmpC production was found in a S. Bredeney and related to the presence of the bla_{CMY-2} gene. This gene was first detected in Spain in 1999 (Navarro et al., 2001) and, although is usually associated with mobile genetic elements (Seiffert et al., 2013), has been scarcely found in *Enterobacteriaceae* from pigs in Spain (Dandachi et al., 2018). Indeed, to the author´s knowledge, this is the first time this gene is detected in a S. Bredeney isolated from pigs in the country. However it has been previously detected in S. Bredeney isolates associated to human cases (González-Sanz et al., 2009; de Toro et al., 2013) indicating its zoonotic potential. This isolate also displayed a MDR pattern, supporting the idea that the emergence/maintenance of resistance to 3rd generation cephalosporins in animals may be related to the co-selective pressure applied by the over usage of non-beta-lactams (Dandachi et al., 2018).

In conclusion, between 2008 and 2009 the prevalence of chromosomal and plasmid-based CR in *Salmonella* from pigs was low in Spain. ESBL/AmpC production was low as well. Both resistances were coded by genes associated with mobile genetic elements and involved zoonotic serotypes.

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