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Short communication

Clinically relevant multidrug resistant *Salmonella enterica* in swine and meat handlers at the abattoir

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ABSTRACT

The presence of multidrug resistant (MDR) *Salmonella* serotypes in slaughtered swine, carcasses, meat and meat handlers is scarcely evaluated. Recently we demonstrated that diverse *Salmonella* serotypes are frequently present in swine, pork meat and carcasses, and meat handlers at Portuguese abattoirs. Here we have characterized their antibiotic resistance phenotypes and genotypes, helping elucidate the flow of MDR *Salmonella* in the food chain. Testing 60 *Salmonella* isolates from different serotypes, the highest frequencies of resistance were observed for tetracycline (T) [70% ($n = 42/60$), *tet*(A)/*tet*(B)/*tet*(G)], streptomycin (S) [63% ($n = 38/60$), *aadA2*/*strA*/*strB*], sulfamethoxazole (Sul) [62% ($n = 37/60$), *sul1*/*sul2*/*sul3*] and ampicillin (A) [57% ($n = 34/60$), $bla_{\text{PSE-1}}$/bla_{TEM}]. Thirty-seven percent ($n = 22/60$) carried class 1 integrons and multidrug resistance was frequently observed (63% $n = 38/60$), including those serotypes common to human infections [*S.* Typhimurium 78% $n = 25/32$; *S.* 4,[5],12:i:- 67% $n = 2/3$; *S.* Rissen 75% ($n = 3/4$); *S.* London 67% $n = 2/3$; *S.* Derby 55%; $n = 6/11$)]. The emergent *S.* 4,[5],12:i:- isolates were mostly characterized by ASSuT phenotype [bla_{TEM}/*strA-strB*/*sul2*/*tet*(B)], typical of the European clone, while for the first time the ST phenotype [*strA-strB-tet*(A)-*tet*(B)] was also observed. Moreover, we report a first finding of a MDR phenotype in *S.* London [ANSSuT; bla_{TEM}-*strA-strB-sul2-tet*(A)]. Our findings suggest that the abattoir environment and the slaughter operations seem not only to harbor MDR serotypes that originated in the pig reservoir, but also propagate them through cross-contamination processes, involving meat handlers. The present study suggests a probable relationship between swine and human salmonellosis throughout the food chain, which is of interest for epidemiological, animal health and public health purposes.

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1. Introduction

In the European Union, ten to twenty percent of human *Salmonella enterica* infections may be attributable to pig sources, as reported by EFSA (EFSA, 2010). In addition, an increasing global trend for antibiotic resistance, namely to ampicillin, tetracycline, streptomycin and sulfonamides, has been consistently observed in pigs and pork products (CIPARS, 2011; DANMAP, 2013; EFSA, 2013; NARMS, 2009, 2011), which could reach humans through the food chain (EFSA, 2010). Particular multidrug-resistant (MDR) *S. enterica* isolates with clinical relevance, such as *S.* Typhimurium monophasic variant (*S.* 4,[5],12:i:-), *S.* Typhimurium DT104 and *S.* Rissen have been detected in swine production holdings and in pigs/pork products (Antunes et al., 2006, 2011; Alcaine et al., 2007; Hauser et al., 2010). In spite of the evidence that the animal setting is a worldwide reservoir of MDR strains (Alcaine et al., 2007; EFSA, 2013) their characterization in swine at slaughter, as well as in meat handlers' samples, has been scarcely studied, a critical point in the link with swine as a source of human MDR *Salmonella* infection cases (Hauser et al., 2010; EFSA, 2010, 2013). Recently we demonstrated that diverse *Salmonella* serotypes, including clinically relevant clones (e.g. *S.* Typhimurium DT104 and *S.* 4,[5],12:i:-), are frequently present in swine, pork meat and carcasses, and meat handlers at Portuguese abattoirs (Gomes-Neves et al., 2012). In this work, we characterized their antibiotic resistance phenotypes and genotypes, helping elucidate pork products' contribution to MDR *Salmonella* human infections.

2. Materials and methods

2.1. Salmonella isolates, serotypes and PFGE types

The study included 60 *S. enterica* isolates (26 ileoceacal lymph node samples, 16 carcass swabs and 14 meat samples; 4 from meat handlers' hands), collected between July 2007 and August 2008 from eight swine abattoirs (A-H) (Table 1) (Gomes-Neves et al., 2012). Isolates belonged to nine serotypes and 17 PFGE types (Gomes-Neves et al., 2012), which included 32 *S.* Typhimurium (PFGE types T1–T9) and 3 *S.* 4,[5],12:i:- (T1 PFGE type), 11 *S.* Derby (D1), 4 *S.* Rissen (R1), 3 *S.* London (L1), 3 *S.* Mbandaka (M1, M2), 2 *S.* Give (G1), 1 *S.* Enteritidis (E1) and 1 *S.* Sandiego (S1) (Table 1).

2.2. Antimicrobial susceptibility testing

All *S. enterica* isolates were tested for antimicrobial susceptibility by the disk diffusion method, following CLSI standards (CLSI, 2009). Ten antimicrobial agents were tested: ampicillin (A), gentamicin (G), kanamycin (K), streptomycin (S), ciprofloxacin (Ci), nalidixic acid (N), chloramphenicol (C), tetracycline (T), sulfamethoxazole (Su) and trimethoprim (W), using *Escherichia coli* ATCC 25922 as the control strain. Ampicillin resistant isolates were further tested for susceptibility to extended-spectrum β-lactams (ceftazidime, ceftriaxone, cefotaxime, cefepime, cefoxitin, aztreonam and imipenem) and the double disk synergy test for ESBL detection was also performed (CLSI, 2009). Multidrug resistance (MDR) was considered when the isolates were resistant to three or more classes of antibiotics.

2.3. Characterization of antimicrobial resistance genes and class 1 integrons

Genes coding for resistance to ampicillin (bla_{TEM}, $bla_{\mathrm{PSE\text{-}1}}$), streptomycin (*aadA, strA-strB*), tetracycline [*tet*(A), *tet*(B), *tet*(G)], sulfamethoxazole (*sul1*, *sul2*, *sul3*), chloramphenicol (*floR*, *cmlA, catA*) and trimethoprim (*dfrA1*, *dfrA12*) were screened by PCR, using primers and conditions previously described (Antunes et al., 2006, 2011). The detection and characterization of class 1 integrons was performed by PCR and sequencing, as reported (Antunes et al., 2006). Positive and negative controls were included in all PCR assays.

The 95% confidence intervals (95%CI) of the proportion of resistance were estimated according to the Wilson procedure with a correction for continuity (Wilson, 1927; Newcombe and Robert, 1998).

3. Results and discussion

3.1. Antibiotic resistance and resistance phenotypes

Antibiotic resistance was found in 75% (95%CI: 61.88–84.89; *n* = 45/60) of all *Salmonella* isolates, with 63% (95%CI: 49.85–75.1; *n* = 38/60) MDR. Interestingly, the lowest incidence of resistance was observed in isolates from lymph nodes (58%; 95%CI: 37.19–76.02; *n* = 15/26). This compares to resistance found in samples of subsequent slaughter operations, with 94% (95%CI: 67.71–99.67; *n* = 15/16) from carcasses and 86% (95%CI: 56.15–97.48; *n* = 12/14) from meat and 75% (95%CI: 21.4–98.68; *n* = 3/4) from meat handlers. Resistance to tetracycline (70%; 95%CI: 56.63–80.8; *n* = 42/60;), streptomycin (63%; 95%CI: 49.85–75.1; *n* = 38/60), sulfamethoxazole (62%; 95%CI: 48.19–73.65; *n* = 37/60;) and ampicillin (57%; 95%CI: 43.3–69.19; *n* = 34/60) was detected more frequently than for chloramphenicol (15%; 95%CI: 7.5–27.8; *n* = 9/60), trimethoprim (8%; 95%CI: 3.11–19.11; *n* = 5/60) and nalidixic acid (5%; 95%CI: 1.3–14.82; *n* = 3/60). In fact, the most frequent resistance phenotypes (R-type) among our isolates were ASSuT (38%; 95%CI: 24.16–53.46); *n* = 17/45), ACSSuT (16%; 95%CI: 7.0–30.07; *n* = 7/45) and SSuT (13%; 95%CI: 5.54–27.48; *n* = 6/45), which may provide a selective advantage in the intensive animal production setting, where those antibiotics are significantly used in pig production (Grave et al., 2010) These resistance phenotypes were also the most frequently reported in *Salmonella* isolates from humans, pigs and pork meat in the EU (EFSA, 2013), although overall they were found in lower percentages than in our study. No ESBL-producing strains were detected.

3.2. Dissemination of resistance determinants and serotypes

A variety of genes encoding antibiotic resistance were detected among isolates of different serotypes/clones,