Research Note

Surveillance and Antimicrobial Resistance of *Salmonella* Strains Isolated from Slaughtered Pigs in Spain

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**ABSTRACT**

The prevalence of and the antibiotic resistance shown by *Salmonella* isolated from pigs in Andalusia (southern Spain) is reported. *Salmonella enterica* was recovered from 40 (33%) of 121 sampled herds, and a total of 65 isolates were serotyped. The most common *Salmonella* serotypes were Typhimurium and Rissen (30.7% each); others included Derby (9.2%), Brandenburg (9.2%), Newport (7.7%), Bredeney (4.6%), Anatum (3.0%), Hadar (1.5%), and Goldcoast (1.5%). One strain (1.5%) belonging to the monophasic variant of the Typhimurium serotype (*Salmonella* 4,5,12:i−) was also detected. Definitive phage type (DT) 104b was the most common Typhimurium phage type isolated. These *Salmonella* strains were resistant to various antimicrobial agents, including tetracycline (84.6%), streptomycin (69.2%), neomycin (63.0%), sulfonamides (61.5%), ampicillin (53.8%), and amoxicillin (53.8%). All isolates were fully susceptible to ceftriaxone, ciprofloxacin, and colistin. Thirty-nine strains (64%) resistant to four or more antimicrobial agents were defined as multidrug resistant. Multidrug resistance profiles were observed in *Salmonella* serotypes Typhimurium, Rissen, Brandenburg, Bredeney, a monophasic variant, Goldcoast, Hadar, and Anatum, with serotypes Typhimurium and Brandenburg showing the most complicated resistance patterns (resistant to ≥11 drugs).

Salmonellosis is one of the most common causes of gastroenteritis and septicaemia in humans. Foods containing poultry, beef, and pork are an important source of *Salmonella*, but infection can also occur through contact with carrier animals on farms and in slaughterhouses (3, 19, 24, 28). The proportion of human salmonellosis caused by swine carriers has been estimated at 20% (30).

The proportion of pig herds infected with *Salmonella* is almost certainly very high. Possible routes of infection include parent animals, pigs introduced from other herds, carriers of other species (rodent pests and birds), and primary or secondary contaminated feedstuffs. Hence, the prevalence of *Salmonella* within a herd depends mainly on the conditions of hygiene and the manner in which the animals are kept and handled (30). Carrier pigs are presumed to be the major source of infection for both swine and humans (16). Asymptomatic pigs on farms are the most common source for *Salmonella* contamination in pork and pork products. These animals can also infect other pigs during transport to slaughterhouses or in abattoir holding pens (6).

Research into porcine salmonellosis can be performed either on farms or in slaughterhouses. The latter option is usually chosen, since it allows the testing of different types of samples (e.g., lymph nodes, feces, carcasses, serum) from animals originating from different farms. Moreover, farm sampling could overlook positive animals that are not shedding. Stress, high animal density, and recontamination during the transport and holding of pigs before slaughter have been linked to the increased prevalence of *Salmonella* at abattoirs (27). In slaughterhouses, the bacteriological examination of feces and intestinal lymph nodes, as well as the checking of meat juices for *Salmonella* antibodies, is important in evaluating the prevalence of infected animals within herds and for estimating the prevalence of infected herds (29).

The emergence of resistant and multidrug-resistant (MDR) bacterial strains associated with animal species has become an important worldwide health problem; these strains are becoming more difficult to kill with the antimicrobial agents currently available. In Spain, antibiotic resistance from animal sources seems to be related to the overuse of antibiotics on farms. Moreover, pig-related strains are reportedly more resistant than those from other sources; this can be explained by the more intense use of antibiotics in swine than in other species (33).

The aims of the present study were to estimate the prevalence of *Salmonella* in pigs from farms in Andalusia (southern Spain), to determine the resistance patterns of *Salmonella* strains isolated from slaughtered pigs, and to determine the relationship of these patterns with serotypes and phage types.

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MATERIALS AND METHODS

Samples. The study sample consisted of 121 Andalusian swine production units, predominantly individual finishers and multiple sites. These herds were representative of all Andalusian swine farms at the time of sampling (SIEGAN: data of the Agriculture and Fishery Service). Win Episcope Software version 2.0 was the means by which we calculated the necessary sample size and carrier percentages, using data reported in earlier studies (23) (accepted error, 8%; 95% confidence interval). At slaughter in the processing plant, paired samples of ileocecal lymph nodes and fresh feces were collected from 10 pigs at each of the 121 farms (total = 1,210 pigs). All samples were placed in sterile plastic bags (100 ml) and transported in a portable refrigerator to the laboratory, where they were processed on the same day.

For the direct detection of Salmonella, swabs taken from the lymph nodes were streaked onto xylose lysine deoxycholate agar (Oxoid, Madrid, Spain). To detect Salmonella in feces, a 10-g sample was added to 100 ml of buffered peptone water (Oxoid) and incubated at 37°C for 18 to 24 h. Aliquots (0.1 ml) were then enriched in 10 ml of Rappaport-Vassiliadis broth (Oxoid) at 42°C for 24 to 48 h. Finally, swabs from the Rappaport-Vassiliadis enrichment were subcultured on xylose lysine deoxycholate agar. Presumptive Salmonella isolates (lactose−, SH2+) were biochemically confirmed with lysine iron agar (Difco, Becton Dickinson, Barcelona, Spain), Kliger iron agar (Oxoid), and motility indole ornithine agar (Difco, Becton Dickinson). These isolates were then stored at −80°C on Microbank beads (Pro-Lab Diagnostics, Quimigrenal S.A., Spain) for later typing.

Strain characterization. All Salmonella isolates were serotyped by the slide agglutination method with commercial antisera (Bio-Rad, Madrid, Spain; Statens Serum Institut, Izasa, Spain). Phage typing of Typhimurium and Hadar Salmonella serotypes was performed at the National Reference Laboratory for Salmonella and Shigella (Majadahonda, Spain). Phages were provided by the International Phage Typing Reference Laboratory (Colindale, London, UK).

Antimicrobial susceptibility tests. By the disk diffusion method, all strains were screened on Mueller-Hinton agar (Oxoid) for resistance to 18 antimicrobial agents: ampicillin (10 µg per disk), amoxicillin (30 µg per disk), cefotaxime (30 µg per disk), ceftriaxone (30 µg per disk), nalidixic acid (30 µg per disk), fluo- mequine (30 µg per disk), enrofloxacin (5 µg per disk), ciprofloxacin (5 µg per disk), gentamicin (10 µg per disk), streptomycin (10 µg per disk), apramycin (15 µg per disk), neomycin (30 µg per disk), sulfonamide compounds (200 µg per disk), trimethoprim-sulfamethoxazole (1.25 and 23.75 µg per disk), tetracycline (30 µg per disk), chloramphenicol (30 µg per disk), colistin (50 µg per disk), and nitrofurantoin (300 µg per disk).

Escherichia coli ATCC 25922 was included as a quality control. The measurement and interpretation of growth inhibition diameters were assessed following the NCCLS guidelines for veterinary antimicrobial susceptibility tests (5); the strains were therefore classified as susceptible, resistant, or intermediate resistant. Those resistant to four or more antimicrobial agents were considered MDR strains.

Statistical analysis. Associations between the presence of Salmonella and the herd size and production system were analyzed by the odds ratio and V Cramer tests, respectively. All calculations were performed by SPSS version 11.0 software for Windows (SPSS Inc., Chicago, Ill.).

RESULTS

Prevalence and percentage of carriers. Overall, 40 (33.05%) of the 121 farms were Salmonella positive. In total, 65 (5.4%) of the 1,210 isolates—22 from ileocecal lymph nodes and 43 from feces—were confirmed as Salmonella. Herd size was significantly related to the presence of Salmonella (odds ratio, 0.29; confidence interval, 0.12 to 0.68), with herds of more than 1,000 pigs determined to be a risk factor. No significant association was seen between Salmonella infection and the production system (finishing pig herds, closed farms, or farrow-to-finish herds) (V Cramer, 0.109; P = 0.533).

Serotypes and phage types. Ten serotypes were identified. The two most common were Salmonella Typhimurium and Rissen, with 20 strains each (30.7%), followed by Brandenburg (6 strains [9.2%]), Derby (6 strains [9.2%]), Newport (5 strains [7.7%]), Bredeney (3 strains [4.6%]), Anatum (2 strains [3.0%]), Hadar (1 strain [1.5%]), and Goldcoast (1 strain [1.5%]), with 1 strain (1.5%) of Salmonella enterica serotype 4,5,12:i− (Salmonella 4,5,12:i−) also detected. The latter is thought to be a monophasic variant of Typhimurium (10).

Of the Typhimurium serotypes, 11 belonged to the definitive phage type (DT) 104b. Two strains, including the monophasic variant Salmonella 4,5,12:i−, belonged to U302. One strain belonged to phage type 29. Finally, four strains did not conform to any published phage type and were recorded as RDNC (reacted, but did not conform); three could not be typed (NT strains). One strain belonging to the Hadar serotype was identified under the Hadar phage type system as phage type 2.

Antimicrobial resistance patterns (MDR strains). The prevalence of resistance to the different antimicrobial agents among the isolated salmonellae was as follows: tetracycline, 84.6%; streptomycin, 69.2%; neomycin, 63.0%; sulfonamide compounds, 61.5%; ampicillin, 53.8%; and amoxicillin, 53.8% (Table 1). Resistances to neomycin and tetracycline were the most common and involved all serotypes. All 20 Typhimurium strains were susceptible to colistin, apramycin, ceftriaxone, and ciprofloxacin. Within Salmonella Typhimurium, the RDNC phage type strains were not resistant to multiple antibiotics, unlike the DT 104b, DT U302, DT 29, and NT phage types.

Twenty-seven different patterns of resistance were observed. Thirty-nine strains (64%) were resistant to multiple antibiotics, and nine strains belonging to the Salmonella Brandenburg, Bredeney, and Typhimurium serotypes (including the monophasic variant strain) were resistant to ≥10 antibiotics (Table 2). The classic penta-resistant pattern R-ACSSuT, in conjunction with other antimicrobial resistance patterns, was detected in 26 strains belonging to the Typhimurium, monophasic, Rissen, Brandenburg, and Bredeney serotypes. All strains were susceptible to ceftri- axone, ciprofloxacin, and colistin; four were susceptible to all of the antimicrobial agents tested. Tables 1 and 2 summarize the susceptibility results obtained and the multiple resistance profiles observed.
TABLE 1. Resistance rates of Salmonella strains isolated from slaughtered pigs

<table>
<thead>
<tr>
<th>Antimicrobial agents (grouped by families)</th>
<th>% susceptible</th>
<th>% intermediate</th>
<th>% resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin (Aml)</td>
<td>46.2 (±17)</td>
<td>0 (14–16)</td>
<td>53.8 (±13)</td>
</tr>
<tr>
<td>Ceftriaxone (Cef)</td>
<td>78.5 (±21)</td>
<td>21.5 (18–20)</td>
<td>0 (±17)</td>
</tr>
<tr>
<td>Cefluroxone (Cefl)</td>
<td>100 (±17)</td>
<td>0 (14–16)</td>
<td>0 (±13)</td>
</tr>
<tr>
<td>Callidixic acid (Cll)</td>
<td>81.5 (±20)</td>
<td>6.2 (17–20)</td>
<td>12.3 (±16)</td>
</tr>
<tr>
<td>Enrofloxacin (Enr)</td>
<td>98.5 (±21)</td>
<td>1.5 (17–20)</td>
<td>0 (±16)</td>
</tr>
<tr>
<td>Flumequine (Flu)</td>
<td>96.9 (±19)</td>
<td>3.0 (14–18)</td>
<td>0 (±13)</td>
</tr>
<tr>
<td>Ciprofloxacine (Cip)</td>
<td>100 (±21)</td>
<td>0 (17–20)</td>
<td>0 (±16)</td>
</tr>
<tr>
<td>Gentamicin (Gen)</td>
<td>83.0 (±15)</td>
<td>4.6 (13–14)</td>
<td>12.3 (±12)</td>
</tr>
<tr>
<td>Neomycin (N)</td>
<td>9.2 (±15)</td>
<td>21.5 (13–14)</td>
<td>69.2 (±12)</td>
</tr>
<tr>
<td>Neomycin (N)</td>
<td>6.2 (±17)</td>
<td>30.8 (15–16)</td>
<td>63.0 (±14)</td>
</tr>
<tr>
<td>Apramycin (Apr)</td>
<td>76.9 (±15)</td>
<td>9.2 (13–14)</td>
<td>13.8 (±12)</td>
</tr>
<tr>
<td>Sulfonamide compounds (Su)</td>
<td>38.5 (±17)</td>
<td>0 (13–16)</td>
<td>61.5 (±12)</td>
</tr>
<tr>
<td>Trimethoprim-sulfa-methoxazole (Stx)</td>
<td>67.7 (±16)</td>
<td>1.5 (11–15)</td>
<td>30.8 (±10)</td>
</tr>
<tr>
<td>Chloramphenicol (C)</td>
<td>58.5 (±18)</td>
<td>0 (13–17)</td>
<td>41.5 (±12)</td>
</tr>
<tr>
<td>Tetracycline (T)</td>
<td>12.3 (±19)</td>
<td>3.0 (15–18)</td>
<td>84.6 (±14)</td>
</tr>
<tr>
<td>Colistin (Col)</td>
<td>100 (±15)</td>
<td>0 (±14)</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin (Nit)</td>
<td>52.3 (±17)</td>
<td>13.8 (14–16)</td>
<td>33.8 (±13)</td>
</tr>
</tbody>
</table>

* Breakpoint (expressed in millimeters): growth inhibition diameters that define each category.

DISCUSSION

The prevalence of Salmonella in pigs can be predicted from preslaughter or on-farm sampling (2). At the herd level, Salmonella prevalence varies greatly from country to country (4, 7, 21, 34); in the present study, the prevalence at the herd level was 33% (40 of 121 farms). The prevalence of Salmonella in infected pigs (5.4%) was similar to that observed in a study performed at five European slaughterhouses (18). The prevalence of Salmonella carriers reported in other studies has varied, depending on the sampling and culture methods used as well as on the production and handling systems (26, 34), thus making comparisons among countries difficult.

In the present study, Typhimurium and Rissen were the most common Salmonella serotypes found in Andalusian pigs, which agrees with several other reports from Spain. In one such study conducted in Catalonia (northeastern Spain), the second most common serotype was Salmonella Typhimurium in infected pigs (5.4%) was similar to that observed in a study performed at five European slaughterhouses (18). The prevalence of Salmonella carriers reported in other studies has varied, depending on the sampling and culture methods used as well as on the production and handling systems (26, 34), thus making comparisons among countries difficult.

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The monophasic variant Salmonella Typhimurium DT 4,5,12:i− showed an R-AAmCnSnAPrSuStxCTN1 multiresistance pattern. Monophasic Salmonella Typhimurium strains lack the second-phase flagellar antigen, H2, encoded by the fljB gene—

A total number of isolates resistant to four or more antimicrobial agents: 39 (64%) of 61. S, streptomycin; N, neomycin; Su, sulfonamide compounds; T, tetracycline; N, nalidixic acid; A, ampicillin; Aml, amoxicillin; C, chloramphenicol; Nit, nitrofurantoin; Sxt, trimethoprim-sulfamethoxazole; Cn, gentamicin; Apr, apramycin.

MDR profile profiles of Salmonella strains isolated from slaughtered pigs

<table>
<thead>
<tr>
<th>MDR</th>
<th>Salmonella serotypes</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNSuT</td>
<td>Rissen</td>
<td>2</td>
</tr>
<tr>
<td>NsSNT</td>
<td>Typhimurium</td>
<td>1</td>
</tr>
<tr>
<td>AAmlSCTN1</td>
<td>Hadar</td>
<td>1</td>
</tr>
<tr>
<td>AAmlSUSxtT</td>
<td>Rissen</td>
<td>2</td>
</tr>
<tr>
<td>AAmlNSSxtT</td>
<td>Anatum</td>
<td>1</td>
</tr>
<tr>
<td>AAmlSUSTN1</td>
<td>Typhimurium</td>
<td>6</td>
</tr>
<tr>
<td>AAmlNSSxxT</td>
<td>Rissen</td>
<td>1</td>
</tr>
<tr>
<td>AAmlNSSxxT</td>
<td>Typhimurium</td>
<td>7</td>
</tr>
<tr>
<td>AAmlSSxCTN1</td>
<td>Typhimurium</td>
<td>1</td>
</tr>
<tr>
<td>AAmlNSSxCTN1</td>
<td>Brandenburg</td>
<td>1</td>
</tr>
<tr>
<td>AAmlNSSxCTN1</td>
<td>Brandenburg</td>
<td>2</td>
</tr>
<tr>
<td>AAmlNSSxCTN1</td>
<td>Typhimurium</td>
<td>1</td>
</tr>
<tr>
<td>AAmlNSSxCTN1</td>
<td>Brandenburg</td>
<td>1</td>
</tr>
</tbody>
</table>

Salmonella Hadar, a poultry-related serotype with important human health implications, has been displacing Salmonella Virchow in Spain since 1995 (15) and has now been recognized by the European Commission as being of public health significance (Commission Regulation 2160/2003) (11). In the present study, the detected strain of this serotype showed an R-NsSNT MDR profile.

Salmonella Typhimurium DT 104b was the most common phage type detected. DT 104, DT 104b, and DT U302 are the most common phage types reported by other authors who have studied Salmonella isolates from humans, pigs, and other sources (8, 32, 33, 36).

The observed resistance to tetracycline (85% of the strains), streptomycin (69%), neomycin (63%), sulfonamide compounds (61%), and trimethoprim-sulfamethoxazole (31%) may be linked to the continuous use of these drugs as preventive or therapeutic agents on the farms tested. Similar percentages of antimicrobial resistance in Salmo-
**RESISTANCE OF SALMONELLA FROM SLAUGHTERED PIGS**

Salmonella isolates from swine have been reported from the United States and Denmark (1, 20). A comparison with data from the year 2000 has shown that animal strains are, in general, more resistant than human strains in Spain, particularly to aminoglycosides, sulfonamides, and trimethoprim-sulfamethoxazole (25).

Ceftriaxone is the first drug of choice for invasive Salmonella infections, particularly in children for whom quinolones are contraindicated. A ceftriaxone-resistant (plasmid-mediated) Salmonella Typhimurium derived from cattle has, however, been reported (13). In the present study, all tested strains were susceptible to ceftriaxone. Twenty-one percent of the present isolates had intermediate resistance to cefotiofur; it is possible that these will transform into resistant strains in the future.

Quinolones are one of the drugs of choice for treating gram-negative sepsis in humans and animals. However, a widespread reduction in susceptibility to quinolones has been reported (22, 24, 31, 35). In the present study, resistance to nalidixic acid (12.3%) was the only quinolone resistance seen. In Catalonia, however, some porcine strains are also reported to show reduced susceptibility (intermediate strains) or resistance to enrofloxacin (12 and 4%, respectively) (22). The generalized use of old quinolones, such as flumequine, or the introduction of enrofloxacin in cattle and pigs, authorized in Spain since 1986, may explain this increase in resistance.

Marked resistance was noted against chloramphenicol and nitrofurantoin, the use of which has been banned in the pig industry for years. This may indicate the persistence of previously acquired resistances.

Colistin is widely used in piglet feed in Spain to prevent porcine diarrhea (23, 33); however, no resistance to this agent was detected. The present results agree with those of studies conducted in Catalonia (22), which report colistin to be the compound to which susceptibility levels are highest (99.3%). Similar findings have been reported from The Netherlands (34).

Sixty-four percent of the strains detected were MDR strains. Such strains were found for all serotypes, except for Derby and Newport, indicating that the problem of multidrug resistance is not exclusive to Salmonella Typhimurium. Multidrug resistance in Catalonia (49.7%) appears to be less prevalent (22). The present data agree with the results of other authors who indicated that 64.8% of the porcine strains in Spain (216 isolates tested) were MDR strains (33). The latter study showed that porcine strains were more resistant than those from other species and sources, except with respect to nalidixic acid in poultry and streptomycin in sheep and goats. In addition, Salmonella resistance was more widespread in 2000 (81.5%) than in 1996 (61.7%), which suggests the increased use of antibiotics during that period or gene movement. In the United States, multidrug resistance reportedly increased from 11 to 20% between 1997 and 2003 (12).

The following conclusions can be drawn. (i) Porcine Salmonella serotype diversity is wide; Salmonella Typhimurium and Salmonella Rissen were the main serotypes detected in the studied area. (ii) The prevalence of infected herds is moderate (33%). (iii) Antimicrobial resistance is widespread among porcine Salmonella strains in southern Spain, and there is a large percentage of MDR strains (64%). (iv) Strains with MDR profiles can be found among Typhimurium (16), Rissen (11), Brandenburg (6), Bredeney (2), a monophasic variant (1), Goldcoast (1), Hadar (1), and Anatum (1) serotypes. (v) The Typhimurium and Brandenburg serotypes show the most complicated resistance patterns (resistance to ≥11 drugs). (vi) Salmonella strains exist that are resistant to tetracycline (84.6%), streptomycin (69.2%), neomycin (63%), sulfonamides (61.5%), ampicillin (53.8%), and amoxicillin (53.8%); this indicates rational use should be made of antimicrobial agents in porcine preventive and therapeutic treatments in southern Spain.

The surveillance of Salmonella serotypes and phage types from animal isolates is important for identifying sources of infection and for implementing prevention and control measures. The control of Salmonella risk factors at pig farms, the rational use of antimicrobial agents, and the monitoring of resistant strains may help safeguard both animal and public health.

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**REFERENCES**


