Antimicrobial Resistance in Salmonella Strains from Fattening Pigs and Sows

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Abstract

The prevalence of antibiotic resistance and of different resistance patterns for Salmonella isolates collected from sows, from fattening pigs at different ages and at slaughter was determined. All 901 isolates were submitted to antimicrobial susceptibility testing for 14 compounds using the disk agar diffusion test.

Overall, 50.9% of the strains were resistant to at least 1 antimicrobial agent. The highest percentage of resistance was found to oxytetracycline (34.2%), streptomycin (32.5%), sulphamethizole (27.6%) and ampicillin (24.9%). Multiresistance (resistance to 2 or more antimicrobials) was observed in 33.2% of the strains. A significant lower proportion (p<0.01) of resistant strains was recovered in faecal samples from sows than from fattening pigs during the weaning, growing and finishing period. When designing antimicrobial resistance surveillance programmes for Salmonella in pigs, it is important to take multiple samples within each herd from fattening pigs at different time points.

Introduction

The major sources for human salmonellosis, one of the most occurring foodborne infections, are eggs, poultry meat and pork. In Belgium, 11,065, 10,075 and 12,894 human cases were reported in 2001, 2002 and 2003, respectively (NRSS, 2003). Further identification revealed that each year, S. Enteritidis (64.2, 63.5 and 71.4%, respectively) was the most common serotype isolated, followed by S. Typhimurium (21.4, 24.2 and 19.5%, respectively).

In addition to gastrointestinal disorders, there is the hazard of therapeutic failure due to antimicrobial resistance of the causative Salmonella strains. In Belgium, an antimicrobial drug surveillance programme has been launched by the National Reference Centre for Salmonella and Shigella since 2000 (Wybo et al., 2004). Recent data showed that approximately 40% of the human Salmonella Typhimurium isolates were resistant to four or more antimicrobial agents (Wybo et al., 2004).

Antimicrobial agents are frequently used in the pig industry and pork is the most frequently consumed meat species in Belgium (NIS, 2005). Therefore, Salmonella contaminated pork may be an important vector for transmitting microbial resistant Salmonella strains to humans. Currently, only limited data about antibiotic resistance in Salmonella isolates from pigs is available. The aims of the present study were to determine the occurrence of antibiotic resistance in Salmonella isolates collected from sows, nursery, growing and finishing pigs. The occurrence of different resistance patterns was additionally investigated.

Materials and Methods

All 901 samples originated from 2 field studies that were conducted during 2001-2002 (Nollet et al., 2004) and 2003-2004 (Nollet et al., 2005), respectively. During study 1, 1821 mesenteric lymph node samples were collected of which 1066 were Salmonella positive. From the 1066 isolates, 527 were selected at random (using random tables) to be tested for their antimicrobial susceptibility. In study 2, faecal samples were taken individually from sows during one production cycle and from pigs during the nursery (5-11 weeks of age), growing (12-18 weeks of age) and finishing period (19-26 weeks of age). The day before pigs were moved to another unit, environmental swabs (overshoes) of that particular unit were taken. In addition, feed samples were gathered at each herd visit. Fifty-six isolates were recovered from the sows; 30, 85 and 52 from the fattening pigs during the nursery, growing and finishing period, respectively, and 86, 34 and 20 isolates from small intestinal content, colon content and mesenteric lymph nodes, respectively. Isolates were serotyped following the Kauffman-White scheme (Popoff and Le Minor, 1992). Only the isolates of study 2 were characterised at strain-level using RAPD and PFGE (Nollet et al., 2005a).

To determine the antimicrobial resistance patterns, the disk agar diffusion test was used according to the guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). In total, 14 antimicrobial agents were tested. The antibiotic disks (Neo-Sensitab, Rosco, Taastrup, Denmark) used were: amoxycillin/clavulanate (30/15 µg), ampicillin (33
µg), ceftiofur (30 µg) chloramphenicol (60 µg), enrofloxacin (10 µg), florphenicol (30 µg), flumequine (30 µg) gentamycin (40 µg), nalidixic acid (130 µg), neomycin (120 µg) oxytetracycline (80 µg), streptomycin (100 µg), sulphamethizole (240 µg), and trimethoprim/sulphamethoxazole (5.2/240 µg). In this way, the most commonly used products in swine medicine (tetracyclines, penicillines) were included, as well as the antimicrobial agents used to treat human Salmonella infections (fluoroquinolones, cephalosporines).

The Salmonella isolates were first cultured on Columbia agar with 5% sheep blood and incubated overnight at 37°C. Colonies of each isolate were suspended into 4-5 mL 0.9% NaCl solution until the turbidity was approximately 0.5 McFarland standard (Densimat, Biomerieux). A sterile cotton swab was dipped into the adjusted suspension and streaked over the entire surface of Mueller-Hinton agar plates. Consequently, neo-sensitabs were added to the plates using a dispenser (Rosco, Taastrup, Denmark) followed by incubation at 37°C for 18-24 hours. The diameters of the inhibition zones (tablets included) were measured in millimetres using a ruler. Results were scored as susceptible, intermediate susceptible and resistant following the manufacturers criteria (Rosco, Taastrup, Denmark).

Results In total, 901 Salmonella isolates were tested of which the 10 most occurring serotypes were S. Typhimurium (42.3%), S. Derby (25.1%), S. Goldcoast (73%), S. Infantis (4.8%), S. Panama (4.3%), S. Livingstone (4.0%), S. London (2.4%), S. Brandenburg (2.3%), S. Rissen (2.0%) and S. Anatum (1.1%). The number of resistant strains per serotype and per antimicrobial agent is shown in Table 1. Overall, 459 of the 901 isolates (50.9%) were resistant to at least 1 antimicrobial agent tested. The highest number of resistant strains was found for oxytetracycline (34.2%), streptomycin (32.5%), sulphamethizole (27.6%) and ampicillin (24.9%). None of the strains were resistant to enrofloxacin or flumequine. Seven isolates showed resistance to ceftiofur. The proportion of resistant strains was higher for S. Typhimurium than for S. Derby, S. Goldcoast, S. Infantis, S. Panama, S. London and S. Brandenburg. (Table 1).

Multiresistance was observed in 299 of the strains (33.2%) and 43 different resistance patterns were observed. S. Typhimurium showed the highest proportion of multiresistant isolates (58.0%). One of the isolates was resistant to 8, and 20 isolates to 7 of the antimicrobial agents tested. The ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracycline) resistance pattern was found in 16 S. Typhimurium and 3 S. Bovismorbificans isolates. One hundred and one isolates were, besides the penta-resistance pattern also resistant to florphenicol. More than one resistance pattern was observed within each serotype. Significant lower proportions of resistance (p<0.01) were found in the strains recovered from faecal samples from the sows than from nursery, growing and finishing pigs. No significant differences were found between the proportion of resistant strains found in faecal samples from finishing pigs and those found during the nursery (p=0.12) and growing period (p=0.22).

The feed isolate and 8 of the isolates recovered from overshoes were also resistant to at least one antimicrobial agent (data not shown).

Discussion The high number of strains resistant to oxytetracycline, streptomycin, sulphamethizole and ampicillin observed in the present study were similar to those in antimicrobial resistance surveys in other European countries (van der Wolf et al., 1999; Mateu et al., 2002; van Duijkeren et al., 2003; Bywater et al., 2004). Based on the present results and on data obtained by Timmerman et al. (2005), tetracycline and amoxicillin are the most commonly used antimicrobial agents in the Belgian pig industry. As reported in the literature, the frequent occurrence of resistance to tetracycline, streptomycin, sulphonamide and ampicillin is generally attributed to the frequent use of
those molecules in veterinary medicine (van der Wolf et al., 1999; van Duijkeren et al., 2003).

Resistance to newer antimicrobial agents such as second generation fluoroquinolones was not observed in the present study. Only 6 isolates were resistant to nalidixic acid. The incidence of reduced susceptibility to fluoroquinolones was thus very low, which is a favourable situation with regard to public health. The prevalence of human nalidixic acid resistant isolates in Belgium was 29.5% in 2003 but was most often observed in S. Virchow and S. Hadar (NRSS, 2003) which were not isolated in the present study. Based on the present results, pigs do not seem to be an important source for human nalidixic acid resistant Salmonella isolates.

About half of the isolates (50.9%) tested were resistant to at least one antimicrobial agent, of which 65% showed multi-resistance. The most frequently occurring pattern in the present study was resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide, oxytetracycline (ACSSuT) and florphenicol. Salmonella isolates showing this resistance pattern were isolated from 8 different herds and were all serotyped as S. Typhimurium. Multidrug resistance has been frequently described for Salmonella and is often associated with the occurrence of Salmonella Typhimurium phage type DT104 (Threlfall, 2000).

A lower proportion of resistant strains was detected in sows in comparison with fattening pigs during the nursery, growing and finishing period. To the authors’ knowledge, no current data are available from antimicrobial resistance of Salmonella isolates recovered from sows. The lower prevalence might be due to the less frequent use of antimicrobial agents in sows. Another explanation is that, as it has been described in many species (Catry et al., 2003; Khachatryan et al., 2004), older animals might harbour less resistant strains because of differences in intestinal physiology. A selective advantage for resistant strains in non-adult pigs (< 6 months of age) might be related to the diet, but further research is necessary to confirm this hypothesis.

**Conclusion** The present study indicates that antimicrobial resistance frequently occurs in Salmonella isolates from pigs, with the highest proportion of resistant strains observed for oxytetracycline, streptomycin, sulphonamide and ampicilline. Pigs do not seem to be an important vector for fluoroquinolones resistant strains to human. Sampling strategies in antimicrobial resistance programmes should include multiple samples per herd with pigs of different ages included.

**References**


Serotype | Non-resistant | Resistant (%) | Antimicrobial compounds
---|---|---|---
S. Bovismorb. | 1 | 7 (87.5)<sup>a</sup> | Ac A Ce C E Ff F G Nd N S Su T Stp
S. Rissen | 3 | 15 (83.3)<sup>a</sup> | - 1 - -  - -  - - - - 1 - 15
S. Typhimurium | 87 | 294 (77.2)<sup>a</sup> | 5 202 6 48 - 120 - 4 - 30 203 213 236 64
S. Anatum | 4 | 6 (60.0)<sup>a</sup> | - 1 - 3 - 4 - - - - - 4 3 4
S. Panama | 18 | 21 (53.8)<sup>a</sup> | - - - - - - - - - - 12 14 - 10
S. Infantis | 23 | 20 (46.5)<sup>a</sup> | - 2 - - - - - - - - 1 6 17 - 3
S. Derby | 157 | 69 (30.5)<sup>a</sup> | 1 14 1 3 - - - - - 9 43 2 40 3
S. Brandenburg | 16 | 5 (23.8)<sup>a</sup> | - - - - - - - - 4 - - - 1 - 1
S. London | 18 | 4 (18.2)<sup>a</sup> | - - - - - - - - - - 1 - 1 2 - -
S. Goldcoast | 55 | 11 (16.7)<sup>a</sup> | - - - - - - - - - - 2 6 5 1 1
S. Livingstone | 30 | 6 (16.7)| - - - - - - - - - - - - - - -
Non-typhable | 5 | 1 (16.7)| - - - - - - - - - - 1 1 - 1
S. Urbana | 9 | - | - - - - - - - - - - - - - - -
S. Mbandaka | 3 | - | - - - - - - - - - - - - - - -
S. O4:1- | 3 | - | - - - - - - - - - - - - - - -
S. Agona | 2 | - | - - - - - - - - - - - - - - -
S. Wien | 1 | - | - - - - - - - - - - - - - - -
S. Enteritidis | 1 | - | - - - - - - - - - - - - - - -
S. Kingston | 1 | - | - - - - - - - - - - - - - - -
S. Muenster | 1 | - | - - - - - - - - - - - - - - -
S. Nagoya | 1 | - | - - - - - - - - - - - - - - -
S. Oranienburg | 1 | - | - - - - - - - - - - - - - - -
S. Rubislaw | 1 | - | - - - - - - - - - - - - - - -
S. Sundsvall | 1 | - | - - - - - - - - - - - - - - -
TOTAL(%) | 442 | (49.1)| 459 | (50.9)| 6 | (0.7)| 224 | (24.9)| 7 | (0.8)| 157 | (17.4)| 124 | (13.8)| 0 | (0.0)| 4 | (0.4)| 123 | (13.6)| 293 | (32.5)| 249 | (27.6)| 308 | (33.2)| 48 | (5.1)| 84 | (9.3)

Table 1: Overall number (%) of resistant *Salmonella* strains. All samples were collected from Belgian fattening pigs and sows during 2001-2004.

<table>
<thead>
<tr>
<th>Antimicrobial compounds</th>
<th>Ac</th>
<th>A</th>
<th>Ce</th>
<th>C</th>
<th>E</th>
<th>Ff</th>
<th>F</th>
<th>G</th>
<th>Nd</th>
<th>N</th>
<th>S</th>
<th>Su</th>
<th>T</th>
<th>Stp</th>
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</table>
| amoxicillin/clavulanate (Ac), ampicillin (A), ceftiofur (Ce), chloramphenicol (C), enrofloxacin (E), flumequine (F), gentamicin (G), nalidixic acid (Nd), neomycin (N), streptomycin (St), sulphonamethizole (S), oxytetracycline (T), trimethoprim/sulpha (Stp) and florfenicol (Ff)


