Antimicrobial Resistance of *Salmonella* Serotypes Isolated from Slaughter-Age Pigs and Environmental Samples

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ABSTRACT

The aim of this study was to determine the antimicrobial resistance patterns of *Salmonella* strains isolated from slaughter-age pigs and environmental samples collected at modern swine raising facilities in Brazil. Seventeen isolates of six serotypes of *Salmonella enterica* subsp. *enterica* were isolated out of 1,026 collected samples: *Salmonella* Typhimurium (1), *Salmonella* Agona (5), *Salmonella* Sandiego (5), *Salmonella* Rissen (1), *Salmonella* Senftenberg (4), and *Salmonella* Javiana (1). Resistance patterns were determined to extended-spectrum penicillin (ampicillin), broad-spectrum cephalosporins (cefotaxime and ceftriaxone), aminoglycosides (streptomycin, neomycin, gentamicin, amikacin, and tobramycin), narrow-spectrum quinolone (nalidixic acid), broad-spectrum quinolone (ciprofloxacin and norfloxacin), tetracycline, trimethoprim, and chloramphenicol. Antimicrobial resistance patterns varied among serotypes, but isolates from a single serotype consistently showed the same resistance profile. All isolates were resistant to tetracycline, streptomycin, and nalidixic acid. One isolate, *Salmonella* Rissen, was also resistant to cefotaxime and tobramycin. All serotypes were susceptible to ceftriaxone, norfloxacin, ciprofloxacin, ampicillin, gentamicin, and chloramphenicol. The high resistance to tetracycline and streptomycin may be linked to their common use as therapeutic drugs on the tested farms. No relation was seen between nalidixic acid and fluoroquinolone resistance.

INTRODUCTION

NONTYPHOIDAL SALMONELLOSIS has become an important cause of enteric infections in many countries,[17,23,30] and several human salmonellosis outbreaks linked to the consumption of contaminated pork have been reported during the last decade.[3,20,22,24,29] Furthermore, antibiotic-resistant strains have emerged worldwide and have led to increasing consumer concern about animal-derived food consumption. Infections caused by resistant strains have shown a higher fatality rate compared to those caused by susceptible organisms.[15]

The extensive use of antibiotics on farms for both therapeutic and growth promotion purposes have been considered to be an important trigger for the emergence of antibiotic-resistant pathogens,[9,14,33] and their real impact on public health contin-

ues to be a matter of debate. High numbers of tetracycline-resistant *Salmonella* strains have been isolated from pig farms.[7,34] However, resistance to first-choice antibiotics used for treatment of human infections causes even more concern. An increasing number of reports on *Salmonella* isolates from animals and humans show reduced susceptibility to fluoroquinolones.[2,31,35] Also, resistance to third-generation cephalosporins has been recently published.[4,5]

Considering the marked importance of *Salmonella* organisms as food-borne pathogens and the world-wide emergence of resistant or multidrug-resistant *Salmonella* strains,[6,33] in the present paper we describe the antimicrobial resistance patterns of *Salmonella* serotypes isolated from pigs and environmental samples collected from modern swine-raising systems in Brazil.

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MATERIALS AND METHODS

Antibiotic resistance patterns were studied in 17 *Salmonella* strains previously isolated from four pig farms located in São Paulo State, Brazil (Oliveira *et al*., manuscript submitted). Sixteen positive samples were isolated out of 1,026 collected samples and the serotypes of *Salmonella enterica* subsp. *enterica* comprised *Salmonella* Typhimurium (1), *Salmonella* Agona (5), *Salmonella* Sandiego (5), *Salmonella* Rissen (1), *Salmonella* Senftenberg (4), and *Salmonella* Javiana (1). Sixteen positive samples were identified out of all samples collected, including feces, feed, floors, feeders, and water. Two different serotypes (*S*. Agona and *S*. Sandiego) were cultured from a single positive fecal sample. Bacteriological procedures for feces and water samples were followed as previously described.[1,12] Floor and feeder samplings were performed after the animals were removed and the pens disinfected, using drag swab sets.[21] The isolated *Salmonella* strains were serotyped according to Popoff and Le Minor.[27] Resistance to each antibiotic was determined by the Kirby-Bauer disk susceptibility test[28] using commercially prepared filter paper disks (Oxoid Ltd., Hampshire, UK), each impregnated with a specified amount of an antibiotic. The antibiotics tested and respective breakpoints were as follows: extended spectrum penicillin (ampicillin, ≥ 32 μg/ml); broad-spectrum cephalosporins (cefotaxime, ≥ 64 μg/ml; ceftriaxone, ≥ 64 μg/ml); aminoglycosides (streptomycin, $\geq 2,000$ μg/ml; neomycin, ≥ 16 μg/ml; gentamicin, ≥ 16 μg/ml; amikacin, ≥ 64 μg/ml; tobramycin, ≥ 16 μg/ml); narrow-spectrum quinolone (nalidixic acid, ≥ 32 μg/ml); broad-spectrum quinolones (ciprofloxacin, ≥ 4 μg/ml; norfloxacin, ≥ 16 μg/ml; tetracycline, ≥ 16 μg/ml; trimethoprim, ≥ 16 μg/ml; chloramphenicol, ≥ 32 μg/ml). The results were analyzed and interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS).[25] Briefly, after pre-inoculation in tryptone soy broth and adjustment of turbidity to a 0.5 McFarland turbidity standard, bacterial suspensions were streaked onto freshly prepared Mueller-Hinton agar plates (Oxoid Ltd., Hampshire, UK) using sterile swabs. The reference strains used were *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Seven disks were placed on each 150-mm plate after 5 min to allow any excess surface moisture to be absorbed, and plates were aerobically incubated for 16–18 hr at 35°C. The strains were finally interpreted as resistant, intermediate or susceptible to each antibiotic according to the criteria currently recommended by the NCCLS, based on the diameters of the zones of complete inhibition to the nearest whole millimeter.

RESULTS

Seventeen *Salmonella* strains were isolated out of 1,026 samples collected. The *Salmonella* serotypes identified and the types of samples from which they were detected are shown in Table 1. The antimicrobial resistance patterns are shown in Table 2. Susceptibility varied among serotypes, but isolates from a single serotype showed the same resistance pattern. All isolates were resistant to at least three antimicrobial agents, tetracycline, streptomycin, and nalidixic acid. One isolate, *Salmonella* Rissen, was also resistant to cefotaxime and tobramycin. All serotypes were susceptible to ceftriaxone, norfloxacin, ciprofloxacin, ampicillin, gentamicin, and chloramphenicol. Fifteen isolates showed intermediate resistance to neomycin. Intermediate resistance to amikacin (3), trimethoprim (3), nalidixic acid (1), tobramycin (1), and cefotaxime (1) was detected as well. The serotypes from farm A were isolated from different types of samples; however, a very similar antimicrobial resistance profile was seen for these strains (Table 2).

DISCUSSION

Considering the marked importance of *Salmonella enterica* serotypes as food-borne pathogens[19] and the world-wide emergence of resistant or multidrug-resistant *Salmonella* strains,[16,33] our goal was to report the antibiotic resistance profiles of *Salmonella* strains isolated from pigs and environmental samples collected from modern swine raising systems in Brazil. Despite the marked limitation of this study due to the small number of isolates (16 out of 1,026 samples), some results may be discussed.

All *Salmonella* isolates collected from four pig farms showed resistance to tetracycline and streptomycin. Although these antibiotics are not allowed for use as growth promoters by Brazilian legislation, they have been used by veterinarians for clini-

TABLE 1. NUMBER OF *SALMONELLA* SEROTYPES CULTURED FROM SWINE FARMS A, B, C, AND D, LOCATED IN SÃO PAULO STATE (BRAZIL), CONSIDERING THE TYPES OF SAMPLES FROM WHICH THEY WERE ISOLATED

Test farm	Serotype (number of isolates)	Samples (number of positive samples)
	Salmonella Agona (3)	Rectal feces (1), dunging gutter water (2)
	Salmonella Agona (1)	Rectal feces (1)
A	*Salmonella* Sandiego (4)	Pool of feces (2), feed (1) drinking water (1)
	Salmonella Javiana (1)	Rectal feces (1)
	Salmonella Agona (1) + *Salmonella* Sandiego (1)[a]	Rectal feces (1)
B	*Salmonella* Rissen (1)	Rectal feces
C	*Salmonella* Senftenberg (2)	Rectal feces (2)
	Salmonella Senftenberg (2)	Rectal feces (2), pool of feces (2)
D	*Salmonella* Typhimurium (1)	Clean feeder (1)

[a]Isolation of two serotypes from one fecal sample.

TABLE 2. ANTIMICROBIAL RESISTANCE PATTERNS FROM 17 *SALMONELLA* STRAINS ISOLATED FROM DIFFERENT TYPES OF SAMPLES COLLECTED ON INTENSIVE PIG FARMS LOCATED IN SÃO PAULO, BRAZIL

Sample	Serotype (number of isolates)	Total resistance pattern	Intermediate resistance pattern
Rectal feces	*Salmonella* Agona (3)	Str/Tet/Nal	Tm/Neo
	Salmonella Rissen (1)	Ctx/Str/Tet/Tob	Tm/Nal/Neo
	Salmonella Senftenberg (2)	Str/Tet/Nal	Ami/Neo/Tob
	Salmonella Javiana (1)	Str/Tet/Nal	Neo
	Salmonella Sandiego (1)	Str/Tet/Nal	Neo
Feed	*Salmonella* Sandiego (1)	Str/Tet/Nal	Neo
Pool of feces	*Salmonella* Senftenberg (2)	Str/Tet/Nal	Ami/Neo/Tob
	Salmonella Sandiego (2)	Str/Tet/Nal	Neo
Drinking water	*Salmonella* Sandiego (1)	Str/Tet/Nal	Neo
Clean feeder	*Salmonella* Typhimurium (1)	Str/Tet/Nal	Ctx/Ami
Dunging gutter water	*Salmonella* Agona (2)	Str/Tet/Nal	Tm/Neo

The antimicrobial agents tested were: cefotaxime (Ctx), ceftriaxone (Cro), amikacin (Ami), norfloxacin (Nor), streptomycin (Str), trimethoprim (Tm), neomycin (Neo), tobramycin (Tob), ciprofloxacin (Cip), tetracycline (Tet), ampicillin (Amp), gentamicin (Gen), nalidixic acid (Nal), and chloramphenicol (Chl).

cal therapy on the tested farms. Furthermore, high frequencies of isolates resistant to tetracycline have been also detected in *Salmonella* isolates of swine origin collected in other countries.[7,34] In the United States, 84.2% of *Salmonella* strains isolated from swine were found to be resistant to tetracycline.[7] It is likely that a link might exist between the long-term therapeutic use of certain antibiotics and the high number of isolates showing resistance patterns to them. Furthermore, the use of antimicrobials has been implicated in the emergence and maintenance of resistant determinants in various ecosystems.[9,22,26] However, the real impact of the therapeutic use of these antibiotics in farm animals on the spread of resistance remains to be clarified.

We detected *Salmonella* Rissen resistant to cefotaxime. Some recent reports[4,5,8] also showed resistance of other *Salmonella* strains to third-generation cephalosporins. Despite the importance of expanded-spectrum cephalosporins in the treatment of invasive salmonellosis in children, the incidence of *Salmonella* resistant to them remains very low. No resistance to cefotaxime was detected in 1,257 *Salmonella* isolates from the United States.[7] Furthermore, *Salmonella* Rissen has been rarely isolated from human sources in Brazil. In a retrospective study on *Salmonella* isolates from human sources in Brazil from 1991 to 1995,[17] only 4 *Salmonella* Rissen strains were isolated from human sources compared to 200 strains of *Salmonella* Typhimurium. For these reasons, we believe that the isolation of a cefotaxime-resistant *Salmonella* Rissen strain may not be a direct concern to human health but could be an important observation because such resistance could be transferred to other important pathogenic serotypes.

Treatments failed when fluoroquinolones were used to treat *Salmonella* infections caused by nalidixic acid-resistant strains.[13,24] Indeed, *Salmonella* isolates resistant to nalidixic acid have been shown to be less susceptible to fluoroquinolones.[35,36] Strains showing decreased susceptibility to ciprofloxacin, ofloxacin, and norfloxacin have been reported to be uniformly resistant to nalidixic acid, a fact attributed to a mutation in the *gyrA* gene.[10,11] Conversely, our results showed that all strains were nalidixic acid resistant but norfloxacin and ciprofloxacin susceptible, corroborating data previously reported in The Netherlands.[18] Therefore, no link between nalidixic acid and fluoroquinolone resistance was detected. We believe this is an important point because fluoroquinolone resistance has received special attention from the scientific community. Many reports have indicated a possible link between the increased recovery of *Salmonella* organisms showing reduced susceptibility to ciprofloxacin and the importance of using quinolones in farm animals, with the consequent emergence of fluoroquinolones resistance.[10,32]

Curiously, all *Salmonella* serotypes from farm A showed a very similar resistance profile, despite the fact that the serotypes isolated may develop antibiotic resistance through different mechanisms.[35] This result may indicate that horizontal gene transfer between bacteria is important to resistant genes spreading in a given environment, supporting previous reports.[15] However, conclusions should be reached with caution because all serotypes were theoretically submitted to the same antimicrobial selective pressure on such farm. Further work looking at the genetic characterization of resistance determinants is underway to determine the identity and homology of resistance genes.

The isolation of a multiresistant *Salmonella* Typhimurium strain from a clean and supposedly disinfected feeder may indicate failures of biosecurity procedures that play an important role in the introduction and maintenance of resistant serotypes on farms. Nevertheless, further studies of the risk factors that might contribute to the maintenance of resistant *Salmonella* strains are needed.

There is a one-way trend worldwide to implement changes in pig production toward health-oriented systems. This is a crucial attitude for the reduction of antibiotic use on farms, since the benefits of such drugs are emphasized in systems with low hygienic standards.[9] Several antibiotics earlier used as growth promoters have been banned in Brazil and at this time only 14 antibiotics are allowed for use on pig farms for this purpose. Furthermore, special attention has been paid to the therapeutic use of antibiotics for farm animals in this country.

Finally, we also assume that the implementation and im-

provement of biosecurity procedures on pig farms have a great potential to prevent the maintenance of resistant strains through internal cycles of contamination. To test this hypothesis, further studies focusing on the dynamics of resistance transfer in the environment and among animals are needed.

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