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Reduced Susceptibility to Quinolones among *Salmonella* Serotypes Isolated from Poultry at Slaughter in Venezuela†

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ABSTRACT

Today there are recognized global ''hot spots'' that are areas in which nontyphoid *Salmonella* serotypes have been reported to have a high prevalence of quinolone resistance. There is concern that resistant strains can be disseminated from these localized geographical areas by travelers or via commercial food products. The objective of this article is to report a high frequency of reduced susceptibility to first- and second-generation quinolones among nontyphoid *Salmonella* isolates from poultry at slaughter in two processing plants belonging to the largest poultry integration companies in Zulia State, Venezuela. Nearly all (74 of 77; 96.1%) of the isolated strains were resistant to nalidixic acid, and 3.7% were resistant to ciprofloxacin; most (45 of 77; 58%) exhibited reduced susceptibility to ciprofloxacin and norfloxacin (15 of 77; 19.5%). In contrast, all of the isolates were susceptible to β-lactamic antimicrobial drugs. Ninety-three percent (72 of 77) of the isolates were either *Salmonella* Parathyphi B or *Salmonella* Heidelberg, which have been reported as invasive *Salmonella*. The predominant serotypes in each slaughter plant showed different antimicrobial susceptibilities, only having in common their high resistance to nalidixic acid, suggesting that different clones disseminated in each commercial integration. The detection of high frequency of reduced susceptibility to first- and second-generation quinolones among nontyphoid *Salmonella* isolates from fresh poultry during processing is noteworthy. Resistance to quinolone drugs will not only make antimicrobial therapy more complicated if foodborne disease results, but also these quinolone-resistant strains can disseminate from this local hot spot to other geographical areas, spreading the resistance against this important antimicrobial drug.

The prevalence of quinolone resistance, specifically to nalidixic acid with reduced susceptibility to ciprofloxacin, among *Salmonella* has been reported to range from 0.5 to 6.3% in North America and up to 14.1 to 16% in Latin America, the European Union, and the Asia-Pacific region *(1, 8).* However, global ''hot spots'' have been described that are geographic regions in which nontyphoid *Salmonella* serotypes are reported to have a higher-than-expected prevalence of quinolone resistance *(4, 6, 7, 10, 13).* This situation is a public health concern because fluoroquinolones, which are synthetic compounds of recent development, are used as the drug of first choice for the treatment of invasive gram-negative and -positive bacterial infections including salmonellosis *(1, 17).* The high levels of quinolone resistance in these global hot spots are likely due to clonal spread of a single strain. This assumption is based on the fact that resistance to quinolones among *Salmonella* serotypes is principally mediated by chromosomal mutations in genes encoding the subunits of the drugs' target enzymes (DNA gyrase and topoisomerase IV), and in genes that affect the expression of diffusion channels in the outer membrane and multidrug-resistance efflux systems *(9).* Once the quinolone-resistant strain has emerged in a geographical region, additional epidemiologic factors such as drug use in animals and animal-to-human, human-to-human, and human-to-animal transmission appears to contribute to the spread of such strains, which can become endemic in these areas *(9).*

An important concern with these global hot spots is that resistant strains can be rapidly disseminated to other geographical regions by travelers or via commercial food products. One recent example describing this situation was reported in 2003, when imported poultry from The Netherlands was identified as the source of an outbreak of quinolone-resistant *Salmonella* Java infections in humans in Scotland *(3).*

In Venezuela, fluoroquinolones have been used in animals and humans for more than 20 years for therapeutic purposes. Fluoroquinolones (e.g., enrofloxacin) are commonly used in the poultry industry in Venezuela for treatment of a variety of conditions, and their use in food animals does not require a prescription. However, the extent of quinolone use in poultry in Venezuela is unknown, because manufacturers and farmers are not required to report this information. Antimicrobial resistance patterns of *Salmonella* isolated from poultry in Zulia State have not been reported. Therefore, the objective of this study was to determine the antimicrobial

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susceptibility among nontyphoid *Salmonella* serotypes isolated from chicken gut in two slaughter plants belonging to the two largest poultry integration companies in the region. This study was initiated due to an increased number of human cases presenting quinolone-resistant *Salmonella* infections in this area *(12, 14).*

MATERIAL AND METHODS

Location of slaughter plants. The two slaughter plants, identified as plants 1 and 2, are located in Zulia State, Maracaibo Municipality, Venezuela. Each plant has the capacity to slaughter approximately 45,000 broilers a day. The selected plants are the largest of seven poultry slaughter plants in Zulia State, processing over 50% of the poultry and poultry products consumed in Zulia and neighboring states.

Samples. Samples were collected from each plant every 15 days for a period of 3 months (five total visits per plant). At each visit, 33 or 34 samples were collected, for a total of 166 samples per plant. A sample consisted of the liver, spleen, and cecum of a single chicken. Three hundred twenty-two samples were collected by hand, using individual examination gloves for each sample. The samples were systematically collected every 200 to 300 chickens in order to ensure that chickens from multiple source farms were included each sampling day. Individual samples were placed inside of a prelabeled, sterile, hermetic plastic bag, and stored inside a thermal box with ice for transportation. The samples were processed at the Infectious Diseases Diagnostic Laboratory, College of Veterinary Sciences, the University of Zulia.

Processing. Once at the laboratory, the liver, spleen, and colic ceca from each individual sample were aseptically cut into small pieces to make a pool to be used for the isolation of *Salmonella*. The basic *Salmonella* culture procedure has been previously described *(2),* with minor modifications. The visceral pool was incubated in tetrathionate broth with iodine (2%), brilliant green, and Tergitol NP-7 at 37°C for 24 h. A 100-μl aliquot of tetrathionate broth was transferred to Rappaport-Vassiliadis R10 broth and incubated at 37°C for 24 h. Then, 100 μl of Rappaport-Vassiliadis R10 broth were plated onto xylose–lysine–Tergitol 4 agar plates for isolation. Typical black colonies were selected after incubation (37°C for 24 h) and streaked onto MacConkey agar plates for isolation. Following incubation (37°C for 24 h), lactose-negative colonies were inoculated to triple sugar iron agar and urea broth for biochemical presumptive identification. All confirmed isolates were tested with polyvalent *Salmonella* antiserum ''O'' (Poly A-I and Vi), and if positive they were preserved in nutrient broth.

Serotyping and antimicrobial susceptibility test. All of the identified *Salmonella* isolates were sent to the Venezuelan National Institute of Health (Instituto Nacional de Higiene ''Rafael Rangel''; INHRR) in Caracas, Venezuela, for serotyping and antimicrobial susceptibility testing.

All presumed *Salmonella* isolates were confirmed as *Salmonella* at the INHRR, with standard biochemical tests including Kligler (glucose-lactose) agar, lysine-iron agar, motility-indol-ornithine agar, urea, orthonitrophenyl-galactosidase, citrate, malonate, lysine, and motility *Salmonella* media. The isolates were further identified to the serotype level according to the Kauffman and White scheme using somatic (O) and flagellar (H) antigens (Denka Seiken Co., Ltd., Tokyo, Japan).

The Kirby-Bauer Disk diffusion antibiotic susceptibility tests were performed at INHRR. The *Salmonella* isolates were categorized as susceptible, intermediate, or resistant to the antimicrobial agents tested on the basis of the guidelines provided by the Clinical and Laboratory Standards Institute (formerly NCCLS) *(5).* Isolates were tested for susceptibility to 13 antimicrobials of public health interest including ampicillin, ampicillin-sulbactam, amoxicillin–clavulanic acid, nalidixic acid, ciprofloxacin, norfloxacin, ceftazidime, cefotaxime, tobramycin, gentamicin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole.

RESULTS

Salmonella was recovered from 77 (23%) of the 332 collected samples, of which 54 were from plant 1 (33% prevalence) and 23 were from plant 2 (14% prevalence). Five different serotypes were detected among the 77 isolates including *Salmonella* Parathyphi B (62%), *Salmonella* Heidelberg (31%), *Salmonella* Amager (3%), *Salmonella* Javiana (3%), and *Salmonella* Idikan (1%). Ninety-three percent (72 of 77) of the isolates were either *Salmonella* Parathyphi B or *Salmonella* Heidelberg, both of which have been reported to be pathogenic with invasive properties and capable of producing extraintestinal infections and septicemia *(16).* A description of the serotype distribution of isolates in each plant has been presented *(2).*

The susceptibility patterns of the 77 *Salmonella* isolates are summarized in Table 1. The most commonly observed resistance was against nalidixic acid (74 of 77; 96.1% of isolates), trimethoprim-sulfamethoxazole (47 of 77; 61%), and tetracycline (38 of 77; 49.4%). In addition, these strains exhibited intermediate resistance to the antimicrobials chloramphenicol (61%; 46 of 77), ciprofloxacin (58.4%; 45 of 77), and norfloxacin (19.5%; 15 of 77). In contrast, greater than 90% of the isolates were susceptible to ampicillin and ampicillin plus sulbactam, and 100% were susceptible to amoxicillin plus clavulanic acid, ceftazidime, cefotaxime, and tobramycin.

When the results were analyzed by slaughter plant, slightly different resistance patterns were observed. In plant 1, antimicrobial resistance was primarily against nalidixic acid (94%; 51 of 54) and trimethoprim-sulfamethoxazole (85.2%; 46 of 54), with intermediate susceptibility to ciprofloxacin (72.2%; 39 of 54) and norfloxacin (27.8%; 15 of 54) also observed. In plant 2, all isolates were resistant to nalidixic acid (23 of 23), and many were resistant to tetracycline (87%; 20 of 23). Intermediate resistance to ciprofloxacin (26.1%; 6 of 23) was also observed among isolates from plant 2.

The antimicrobial susceptibility patterns of the identified serotypes for each individual plant and overall are summarized in Tables 2, 3, and 4. In slaughter plant 1, the most common serotype was *Salmonella* Paratyphi B (47 of 54), which was highly resistant to nalidixic acid (95.7%; 45 of 47) and trimethoprim-sulfamethoxazole (89.4%; 42 of 47); intermediate resistance to chloramphenicol (93.6%; 44 of 47) and ciprofloxacin (78.7%; 37 of 47) was also observed. In contrast, in slaughter plant 2, the most common serotype was *Salmonella* Heidelberg (20 of 23), with 100% of isolates (20 of 20) resistant to nalidixic acid and tetracycline, and intermediate resistance to ciprofloxacin in 30% (6 of 20) of the isolates. It should be noted that the predominant

TABLE 1. *Resistance and susceptibility patterns to 13 antimicrobials of 77 strains of* Salmonella *isolated from poultry in two slaughter plants belonging to the largest poultry integration companies in Zulia State, Venezuela*[a]

	Plant 1			Plant 2			Both		
Antimicrobial	R	I	S	R	I	S	R	I	S
Ampicillin	9 (5/54)	0	91 (49/54)	0	4 (1/23)	96 (22/23)	7 (5/77)	1 (1/77)	92 (71/77)
Ampicillin-sulbactam	0	2 (1/54)	98 (53/54)	0	0	100 (23/23)	0	1 (1/77)	99 (76/77)
Amoxicillin–clavulanic acid	0	0	100 (54/54)	0	0	100 (23/23)	0	0	100 (77/77)
Nalidixic acid	94 (51/54)	2 (1/54)	4 (2/54)	100 (23/23)	0	0	95 (74/77)	1 (1/77)	3 (2/77)
Ciprofloxacin	4 (2/54)	72 (39/54)	24 (13/54)	0	26 (6/23)	74 (17/23)	3 (2/77)	58 (45/77)	39 (30/77)
Norfloxacin	0	28 (15/54)	72 (39/54)	4 (1/23)	0	96 (22/23)	1 (1/77)	20 (15/77)	79 (61/77)
Ceftazidime	0	0	100 (54/54)	0	0	100 (23/23)	0	0	100 (77/77)
Cefotaxime	0	0	100 (54/54)	0	0	100 (23/23)	0	0	100 (77/77)
Tobramycin	0	0	100 (54/54)	0	0	100 (54/54)	0	0	100 (54/54)
Gentamicin[b]	2 (1/43)	2 (1/43)	96 (41/43)	—	—	—	2 (1/43)	2 (1/43)	96 (41/43)
Tetracycline	33 (18/54)	0	67 (36/54)	87 (20/23)	4 (1/23)	9 (2/23)	49 (38/77)	1 (1/77)	49 (38/77)
Chloramphenicol	9 (5/54)	83 (45/54)	8 (4/54)	4 (1/23)	9 (2/23)	87 (20/23)	8 (6/77)	61 (47/77)	31 (24/77)
Trimethoprim-sulfamethoxazole	85 (46/54)	6 (3/54)	9 (5/54)	4 (1/23)	0	96 (22/23)	61 (47/77)	4 (3/77)	35 (27/77)

[a] Values are percentages with number/total number presented in parentheses. R, resistant; I, intermediate resistance; S, susceptible.
[b] Because of technical problems, not all of the strains were tested against gentamicin (only 43 of 54 in plant 1).

serotype present in each slaughter plant expressed a different antimicrobial susceptibility pattern, with their only similarity being resistance to nalidixic acid and other quinolones. This suggests that different *Salmonella* clones were widely disseminated within each commercial integration company.

Of the 77 *Salmonella* isolates, 18 (23.4%) were resistant to three or more antimicrobials. The seven multiresistance patterns that were observed are summarized in Table 5. The antimicrobials most frequently involved in the multiresistance patterns were nalidixic acid, tetracycline, and trimethoprim-sulfamethoxazole. Three *Salmonella* Heidelberg isolates from slaughter plant 1 were resistant to four or five antimicrobials. Resistance to both nalidixic acid and tetracycline was common to all observed multiresistance patterns.

DISCUSSION

As indicated by Hakanen et al. *(6),* ''the emergence of antimicrobial resistance in any part of the world may have global implications and is, therefore, of universal concern.'' The emergence of resistance to quinolone drugs is especially concerning because of the importance of this drug in treating invasive gram-negative and -positive infections. The detection of a high prevalence of *Salmonella* with reduced susceptibility to first- and second-generation quinolones contaminating food animals at slaughter is noteworthy regardless of location. This resistance to quinolones will potentially make antimicrobial therapy more complicated for any consumers in Zulia or neighboring states who may become ill. In addition, these quinolone-resistant strains can disseminate from this hot spot to other geographical areas via international travelers or the commercial products as has been previously reported *(3, 6, 11, 13).*

It is interesting to note that in The Netherlands, *Salmonella* Paratyphi B variant Java isolations have increased alarmingly among chickens and in chicken products from less than 2% of all isolates before 1996 to 60% in 2002. In addition, up to 50% of human patients with *Salmonella* Paratyphi B variant Java infection had pulsed-field gel electrophoresis profiles indistinguishable from those recovered from poultry *(15).* The same report observed that reduced susceptibility to ciprofloxacin (39% in 2002) was developing faster in *Salmonella* Paratyphi B variant Java than it was in other serotypes *(15).* This had international public health implications when imported poultry from The Netherlands was recognized as a vehicle for quinolone-resistant *Salmonella* Paratyphi B variant Java infections in Scotland, with pulsed-field gel electrophoresis patterns indistinguishable from those in The Netherlands.

In the report of *Salmonella* Paratyphi B variant Java in The Netherlands *(15),* the authors speculated that ''the easy

TABLE 2. *Resistance patterns to 13 antimicrobials of* Salmonella *serotypes isolated from poultry at slaughter plant 1 in Zulia State, Venezuela*[a]

	Paratyphi B (n = 47)		Heidelberg (n = 4)		Amager (n = 1)		Idikan (n = 1)		Javiana (n = 1)	
Antimicrobial	R	I	R	I	R	I	R	I	R	I
Ampicillin	4 (2/47)	0	50 (2/4)	0	100 (1/1)	0	0	0	0	0
Ampicillin-sulbactam	0	0	0	25 (1/4)	0	0	0	0	0	0
Amoxicillin–clavulanic acid	0	0	0	0	0	0	0	0	0	0
Nalidixic acid	96 (45/47)	0	100 (2/4)	0	100 (1/1)	0	100 (1/1)	0	100 (1/1)	0
Ciprofloxacin	2 (1/47)	79 (37/47)	25 (1/4)	50 (2/4)	0	0	0	0	0	0
Norfloxacin	0	28 (13/47)	0	50 (2/4)	0	0	0	0	0	0
Ceftazidime	0	0	0	0	0	0	0	0	0	0
Cefotaxime	0	0	0	0	0	0	0	0	0	0
Tobramycin	0	0	0	0	0	0	0	0	0	0
Gentamicin[b]	0	0	25 (1/4)	25 (1/4)	0	0	0	0	0	0
Tetracycline	28 (13/47)	0	100 (4/4)	0	0	0	100 (1/1)	0	0	0
Chloramphenicol	2 (1/47)	94 (44/47)	100 (4/4)	0	0	0	0	100 (1/1)	0	0
Trimethoprim-sulfamethoxazole	89 (42/47)	4 (2/47)	50 (2/4)	25 (1/4)	0	0	0	0	0	0

[a] Values are percentages with number/total number presented in parentheses. R, resistant; I, intermediate resistance.
[b] Because of technical problems, not all of the strains were tested against gentamicin (only 43 of 54 in plant 1).

spread of this resistant clone in chickens, and the persistence in the environment once a farm is infected are the likely reasons for the accelerated development of resistance.'' A similar situation may be represented in our results. Because multiple serotypes with different antimicrobial resistance patterns were observed in the two poultry slaughter plants in our study, it is likely that unique *Salmonella* clones were disseminated within each poultry integration company. However, similar antimicrobial use practices may have led to the similarities in antimicrobial resistance in these unique strains.

The detection of quinolone-resistant *Salmonella* in food animal products at slaughter is a serious public health threat. Fortunately, the isolates we recovered were susceptible to other important antimicrobial drugs used to treat invasive gram-negative and -positive infections. Therefore, viable alternatives for therapeutic treatment of patients who may become infected are available.

The restricted use of quinolones in poultry production and active surveillance for the development of quinolone resistance may contribute to reduced selection pressure and clonal spread of quinolone-resistant *Salmonella.* When increasing resistance to fluoroquinolones was observed among *Campylobacter* isolates from human cases in the United States, the use of fluoroquinolones in poultry production was discontinued. Fluoroquinolones remain available for use in food animal production in Venezuela and in many other countries. Thus, international surveillance may be an important tool for identifying global hot spots. It should be noted that when our results were presented to the poultry integration companies involved in our study, they immediately reduced their use of fluoroquinolones in an attempt to reduce the development of quinolone resistance. Approximately 1 year following the completion of this study, *Salmonella* isolates recovered from both plants 1 and 2 were still resistant to nalidixic acid (73.3%, or 107 of 146; data not shown).

We did not attempt to measure the potential exposure of consumers to the quinolone-resistant *Salmonella* we observed in this study. However, it is likely that at least some consumers were exposed and may have become infected if proper cooking and handling procedures were not followed. In Venezuela, there is no routine surveillance for antimicrobial susceptibility in food animals or fresh meat products, so national trends in antimicrobial resistance of *Salmonella* cannot be identified. Human cases of invasive, quinolone-resistant *Salmonella* infections have been reported in Zulia State *(14).* Our data suggest that these cases could be present but unrecognized and underreported.

In summary, we recovered quinolone-resistant *Salmonella* isolates from poultry at slaughter in Zulia State, Venezuela. The presence of a high prevalence of quinolone-resistant *Salmonella* in fresh food products may be an important public health concern. The possibility that interna-

TABLE 3. *Resistance patterns to 13 antimicrobials of* Salmonella *serotypes isolated from poultry at slaughter plant 2 in Zulia State, Venezuela*[a]

	Paratyphi B (n = 1)		Heidelberg (n = 20)		Amager (n = 1)		Javiana (n = 1)	
Antimicrobial	R	I	R	I	R	I	R	I
Ampicillin	0	0	0	5 (1/20)	0	0	0	0
Ampicillin-sulbactam	0	0	0	0	0	0	0	0
Amoxicillin–clavulanic acid	0	0	0	0	0	0	0	0
Nalidixic acid	100 (1/1)	0	100 (20/20)	0	100 (1/1)	0	100 (1/1)	0
Ciprofloxacin	0	0	0	30 (6/20)	0	0	0	0
Norfloxacin	0	0	5 (1/20)	0	0	0	0	0
Ceftazidime	0	0	0	0	0	0	0	0
Cefotaxime	0	0	0	0	0	0	0	0
Tobramycin	0	0	0	0	0	0	0	0
Gentamicin[b]	—	—	—	—	—	—	—	—
Tetracycline	0	0	100 (20/20)	0	0	100 (1/1)	0	0
Chloramphenicol	0	0	5 (1/20)	10 (2/20)	0	0	0	0
Trimethoprim-sulfamethoxazole	100 (1/1)	0	0	0	0	0	0	0

[a] Values are percentages with number/total number presented in parentheses. R, resistant; I, intermediate resistance.
[b] Because of technical problems, not all of the strains were tested against gentamicin.

TABLE 4. *Antimicrobial resistance patterns of* Salmonella *serotypes isolated from poultry at two slaughter plants belonging to the largest poultry integration companies in Zulia State, Venezuela*[a]

	Paratyphi B (n = 47)		Heidelberg (n = 24)		Amager (n = 2)		Idikan (n = 1)		Javiana (n = 2)	
Antimicrobial	R	I	R	I	R	I	R	I	R	I
Ampicillin	4 (2/48)	0	8 (2/24)	4 (1/24)	50 (1/2)	0	0	0	0	0
Ampicillin-sulbactam	0	0	0	4 (1/24)	0	0	0	0	0	0
Amoxicillin–clavulanic acid	0	0	0	0	0	0	0	0	0	0
Nalidixic acid	96 (45/48)	0	100 (24/24)	0	100 (2/2)	0	100 (1/1)	0	100 (2/2)	0
Ciprofloxacin	2 (1/48)	73 (35/48)	4 (1/24)	33 (8/24)	0	0	0	0	0	0
Norfloxacin	0	27 (13/48)	4 (1/24)	8 (2/24)	0	0	0	0	0	0
Ceftazidime	0	0	0	0	0	0	0	0	0	0
Cefotaxime	0	0	0	0	0	0	0	0	0	0
Tobramycin	0	0	0	0	0	0	0	0	0	0
Tetracycline	27 (13/48)	0	100 (24/24)	0	0	50 (1/2)	100 (1/1)	0	0	0
Chloramphenicol	2 (1/48)	92 (44/48)	21 (5/24)	8 (2/24)	0	0	0	100 (1/1)	0	0
Trimethoprim-sulphamethoxazole	90 (43/48)	4 (2/48)	8 (2/24)	4 (1/24)	0	0	0	0	0	0

[a] Values are percentages with number/total number presented in parentheses. R, resistant; I, intermediate resistance.

TABLE 5. *Multiple antibiotic resistance patterns of 18 serotypes of* Salmonella *isolated from poultry at two slaughter plants belonging to the largest poultry integration companies in Zulia State, Venezuela*

Multiple antibiotic resistance patterns[a]	Isolates	%	Serotypes	Plant 1	Plant 2[b]
TMP/SMX-TET-NAL	10	12.3	Paratyphi B	10	0
CHL-TET-NAL	2	2.6	Heidelberg	1	1
NOR-TET-NAL	1	1.3	Heidelberg	0	1
TMP/SMX-AMP-TET-NAL	2	2.6	Paratyphi B	2	0
GEN-AMP-CHL-TET-NAL	1	1.3	Heidelberg	1	0
TMP/SMX-AMP-CHL-TET-NAL	1	1.3	Heidelberg	1	0
TMP/SMX-CIP-CHL-TET-NAL	1	1.3	Heidelberg	1	0
Total	18	23.4		16	2

[a] AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; NOR, norfloxacin; TET, tetracycline; TMP/SMX, trimethoprim-sulfamethoxazole.

[b] Because of technical problems, the strains from this plant were not tested against gentamicin.

tional travelers may become infected has important global implications. An effective surveillance system to detect, track, manage, and monitor these global hot spots may help to prevent the widespread dissemination of antimicrobial resistance genes or resistant pathogens among animals and humans.

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