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*Brief Original Article*

# Clonal relatedness and resistance patterns of *Salmonella* Corvallis from poultry carcasses in a Brazilian slaughterhouse

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## Abstract

Introduction: *Salmonella* is a major cause of foodborne disease, and poultry products are important contributors to the transmission of this zoonotic pathogen. Although considered to be rare in most countries, *Salmonella* Corvallis has been reported in specific geographic areas isolated from both human and non-human sources. The aim of this study was to report the occurrence, the antimicrobial resistance profiles including the extended-spectrum beta-lactamase (ESBL) production, and the clonal relatedness of *S.* Corvallis strains.

Methodology: A total of 132 fragments of poultry carcasses from a slaughterhouse in São Paulo State, Brazil, were collected at different stages of the manufacturing process (post-bleeding, post-plucking, and post-chilling) and analyzed for the presence of *Salmonella*. Antimicrobial resistance was determined by disc diffusion method and Etest. Clonal relatedness was determined by pulsed-field gel electrophoresis (PFGE).

Results: Among the 272 Salmonella strains recovered, fourteen were *S.* Corvallis. Ten (71.4%) showed ESBL production and resistance to at least three antimicrobial agents. Nalidixic acid resistance and reduced ciprofloxacin susceptibility was verified in four (28.6%) strains. PFGE analyses showed that all the *S.* Corvallis strains belonged to the same pulsotype.

Conclusion: This study identified genetically related *S.* Corvallis strains exhibiting ESBL production and reduced susceptibility to quinolone. The results suggest the need to improve the sanitary conditions in the slaughterhouse. Moreover, from a public health perspective, continuous surveillance on *Salmonella* is needed to control the dissemination of this important zoonotic pathogen and its resistance determinants.

**Key words:** Poultry; Corvallis; *Salmonella*; ESBL; PFGE; fluoroquinolones.

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## Introduction

Foodborne diseases have serious economic and health implications. In the United States alone, more than 300,000 hospitalizations and 3,000 deaths were estimated to be attributed to foodborne infections in 2011 [1]. *Salmonella* is a major agent of foodborne illness [1] and contaminated eggs, poultry meat, and by-products are considered the main sources of non-typhoidal salmonellosis [2]. Although 2,610 serovars of *Salmonella* have been identified [3,4], the majority of human infections are caused by a limited number of serovars. *S. enterica* Typhimurium and *S. enterica* Enteritidis are the most prevalent serovars isolated from human infections, although other serovars have been recognized in specific regions [5].

*S.* Corvallis is considered to be rare in most countries; however, in recent years, a higher incidence has been reported in specific geographic areas such as Bulgaria, Denmark, Tunisia and Japan [6-8]. This serovar has been isolated from poultry, food products of animal origin, human infections, and even in outbreaks of foodborne diseases [6-8].

Cephalosporins and fluoroquinolones are the drugs of choice for treating patients with complicated *Salmonella* infections [9]. However, the emergence of extended-spectrum β-lactamase enzymes and decreased susceptibility to ciprofloxacin in *Salmonella* strains has drastically reduced the treatment options, which may lead to unfavorable outcomes [9,10]. In veterinary medicine, the use of antimicrobial drugs for therapeutic purposes and as growth promoters may

stimulate the selection of antimicrobial-resistant *Salmonella* strains [11,12], causing a major public health concern.

Extended-spectrum β-lactamase (ESBL) production in *Salmonella* is not common. However, the reports of ESBL-producing *Salmonella* serovars are increasing worldwide over the last decades [13,14]. ESBL-producing *Salmonella* strains have been detected in poultry and poultry products in different countries, including Brazil [14- 16].

Limited information is available regarding the occurrence of ESBL-producing strains among the *Salmonella* strains recovered from the Brazilian poultry industry. In this study, we report the occurrence, antimicrobial resistance, and genetic relatedness of *S.* Corvallis strains isolated from poultry carcasses in the state of São Paulo, Brazil.

## Methodology

*Salmonella isolation and identification*

A total of 132 poultry fragments (breast, right and left legs quarter) from carcasses were collected in a slaughterhouse located in the Central Western area of the State of São Paulo, Brazil during 2011-2012. Samples were collected at three different manufacturing stages in the industrial plant: post-bleeding, post-plucking, and post-chilling. *Salmonella* isolates were recovered using standard procedures consisting of a pre-enrichment with buffered peptone water 1%, enrichment with Rappaport Vassiliadis broth and Tetrathionate broth, and isolation onto Xylose Lysine Desoxycholate agar and Bismuth Sulfite agar plates [17]. The presumptive isolates were confirmed to belong to the *Salmonella* genus based on a subset of conventional biochemical tests [18]. *Salmonella* isolates were serotyped by agglutination tests using the somatic O, phase 1 and phase 2 of the H flagellar antisera according to the White-Kauffmann-Le Minor scheme [3].

*Antimicrobial susceptibility test*

Antibiotic susceptibility was determined by the Bauer-Kirby disk diffusion method according to the guidelines recommended by the Clinical Laboratory Standards Institute (CLSI, 2013) for *S.* Corvallis strains. The following antimicrobial agents were tested: ampicillin (10 µg), cefepime (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), meropenem (10 µg), aztreonam (30 µg), amikacin (30 µg), gentamicin (10 µg), streptomycin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), sulfonamides (300 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), and tetracycline (30 µg) (all Oxoid, Basingstoke, Uniteds Kingdom) The minimum inhibitory concentrations (MIC) were determined for nalidixic acid and ciprofloxacin by the Etest (BioMérieux, Marcy l'Etoile, France) according to the manufacturer's recommendations. The inhibition halo diameter and the MIC values were categorized as susceptible, intermediate, or resistant according to the CLSI recommendations [19].

ESBL production was confirmed by the double-disk diffusion test when the key-hole effect was observed between the ceftazidime, cefepime, cefotaxime, or ceftriaxone and amoxicillin/clavulanate disks, indicating the partial or total restoration of the activity of cephalosporin by clavulanic acid [20]. *Escherichia coli* ATCC25922, *E. coli* ATCC35218, and *Pseudomonas aeruginosa* ATCC27853 were used as reference strains for the antimicrobial susceptibility testing.

*Pulsed field gel electrophoresis (PFGE)*

PFGE was performed according to the PulseNet protocol [21] developed by the Centers for Disease Control and Prevention (CDC). Briefly, cell lysis was followed by proteinase K treatment and DNA restriction with XbaI (New England Biolabs, Ipswich, MA). Electrophoresis was performed in a CHEF-DR III system (Biorad Laboratories Inc., Hercules, CA) with the following running parameters: 6 V/cm; temperature, 14°C; initial switch, 2.2 s; final switch, 63.8 s; and length, 18 h. The Lambda Ladder PFG Marker (New England Biolabs, Ipswich, USA) was employed for gel normalization. TIFF images were analyzed with BioNumerics 5.0 software (Applied Maths), and a dendrogram was generated by the unweighted pair group method with arithmetic mean (UPGMA) and the Dice coefficient for band matching with 1.5% tolerance and 1.5% optimization. A *S.* Typhimurium strain was included as an experimental control.

## Results

A total of 272 *Salmonella* isolates were recovered from poultry fragments, of which 177 (65.1%) were serotyped. *S.* Corvallis was the fifth most prevalent serotype (7.9%), after Mbandaka (21.5%), Senftenberg (17.5%), Enteritidis (14.1%), *S. enterica* subsp. *enterica* O:3,10 (8.5%). Eighteen other serotypes were also detected (30.5%). Fourteen *S.* Corvallis strains were recovered from the poultry plant (nine at post-bleeding stage, three at the post plucking stage, and

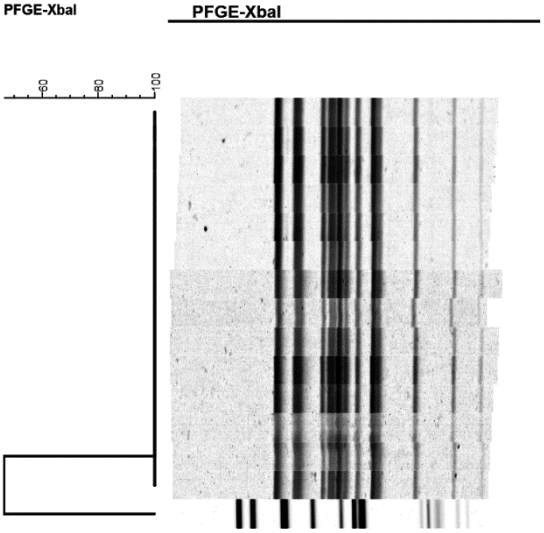
two at the post-chiller stage), of which 71.4% (10/14) were ESBL-producing. This unexpected drug resistance pattern led us to further investigate the *S.* Corvallis strains. ESBL-producing *S.* Corvallis displayed resistance to ampicillin, ceftriaxone, and cefotaxime. Nalidixic acid resistance (MIC ≥ 32 µg/mL) and reduced susceptibility to ciprofloxacin (MIC range: 0.25 - 0.5 µg/mL) were detected in four *S.* Corvallis strains (28.6%) (Figure 1). Intermediate susceptibility to aztreonam (diameter inhibition halos between 18 and 20 mm) and cefepime (diameter inhibition halos between 15 and 17 mm) were observed in another four (28.6%) strains. All *S.* Corvallis strains were susceptible to amikacin, ceftazidime, chloramphenicol, cefoxitin, streptomycin, gentamicin, meropenem, sulfonamides, trimethoprim-sulfamethoxazole, and tetracycline. The dendrogram generated from the PFGE analysis showed that all the *S.* Corvallis strains belonged to the same pulsotype (100% similarity) despite the different antimicrobial resistance patterns observed (Figure 1).

## Discussion

We report the occurrence of genetic related *S.* Corvallis presenting ESBL enzymes and reduced susceptibility to ciprofloxacin. *S.* Corvallis was originally described in 1949 [22] after being isolated from poultry, and its importance has been increasing in recent years due to its potential for causing local or global outbreaks [6,7,23,24]. Detection of ESBL-producing *Salmonella* strains has emerged over the years [13, 14]. One of the largest studies on *S.* Corvallis strains showed that different types of ESBL were occasionally simultaneously present in strains recovered from human sources and food products in Bulgaria, Thailand, and Denmark [6]. The emergence of ESBL in *Salmonella* isolated from animal production may be, at least in part, attributed to the selective pressure in this reservoir when antimicrobial agents are widely applied to promote growth [13,15,25]. Evidence of similar cassette arrays found in pork and human *Salmonella* isolates suggests the potential transmission of multidrug resistance between food of animal origin and human isolates [26].

In addition to the beta-lactam resistance, the presence of *S.* Corvallis strains presenting resistance to nalidixic acid and reduced susceptibility to ciprofloxacin was noteworthy. Nalidixic acid resistance is predictive of fluoroquinolone resistance, as observed in this study: all of the nalidixic acid-resistant strains presented reduced ciprofloxacin susceptibility. Fluoroquinolone resistance is an important concern that is less frequently reported in *Salmonella* than in other food-borne pathogens [27]; however, *Salmonella* with reduced susceptibility to fluoroquinolone has been reported worldwide [28,29] and in Brazil [30,31]. In fact, to the best of our knowledge, this study is the first to report ESBL-producing strains of *S.* Corvallis with reduced ciprofloxacin susceptibility in Brazil.

**Figure 1.** Pulsed field gel electrophoresis profiles, extended-spectrum beta-lactamase (ESBL) status, isolation source, and antimicrobial resistance patterns of *Salmonella* Corvallis isolated from a poultry slaughterhouse in Brazil.




| Strain Number | Source | Resistance Pattern | ESBL Status |
|---|---|---|---|
| 01 | Post bleeding | AMP, CRO, CTX | Positive |
| 02 | Post bleeding | NAL, AMP, CRO, CTX, CIP* | Positive |
| 03 | Post chiller | NAL, AMP, CRO, CTX, CIP* | Positive |
| 04 | Post chiller | AMP, CRO, CTX | Positive |
| 05 | Post bleeding | NAL, AMP, CRO, CTX, CIP* | Positive |
| 06 | Post plucking | NAL, AMP, CRO, CTX, CIP* | Positive |
| 07 | Post bleeding | AMP, ATM*, CRO, CTX, FEP* | Positive |
| 08 | Post plucking | Susceptible | Negative |
| 09 | Post plucking | AMP, ATM*, CRO, CTX, FEP* | Positive |
| 10 | Post bleeding | Susceptible | Negative |
| 11 | Post bleeding | AMP, ATM*, CRO, CTX, FEP* | Positive |
| 12 | Post bleeding | AMP, ATM*, CRO, CTX, FEP* | Positive |
| 13 | Post bleeding | Susceptible | Negative |
| 14 | Post bleeding | Susceptible | Negative |
| S. Typhimurium | Unrelated Strain | | |

AMP = ampicillin; ATM = aztreonam; FEP = cefepime; CRO = ceftriaxone; CTX = cefotaxime; CIP = ciprofloxacin; NAL = nalidixic acid. Asterisks indicate intermediate susceptibility.

Detection of *S.* Corvallis strains resistant to both cephalosporins and quinolones antimicrobials in this poultry slaughterhouse may have an impact on public health, since animals can represent important reservoirs and potential vehicles for antimicrobial resistance dissemination through the food chain [16,26].

PFGE has been demonstrated to be sufficiently discriminatory for subtyping different *Salmonella* serovars, including *S.* Corvallis [6,32,33]. Our finding that the genetic patterns of *S.* Corvallis recovered from different areas in the poultry processing plant were indistinguishable suggests that this strain is well adapted to the conditions at this plant. This adaptation may be attributed to the use of inadequate hygienic techniques [34] and/or to the ability of the bacteria to adhere to the materials used in the poultry industry [35]. In addition, strains of *S.* Corvallis with the same pulsotype presented different resistance patterns and ESBL production, suggesting that the mechanisms involved in these phenotypes are associated with mobile genetic elements, such as plasmids.

## Conclusion

This study identified genetically related *S.* Corvallis strains presenting reduced susceptibility to quinolone. To our knowledge, this is the first report of *S.* Corvallis, ESBL-producing isolates from slaughter plants in Brazil. Isolation of genetically related strains on different days of collection indicates the ability of this bacterium to survive in the environment. The well-recognized role of *Salmonella* as a zoonotic agent highlights the need for continuous surveillance to control the dissemination of this important pathogen.

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