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Short communication

Prevalence and fluoroquinolones resistance of *Campylobacter* and *Salmonella* isolates from poultry carcasses in Rio de Janeiro, Brazil

Pedro Henrique Nunes Panzenhagen [a, *], Waldemir Silva Aguiar [a], Beatriz da Silva Frasão [a], Virginia Léo de Almeida Pereira [a], Dayse Lima da Costa Abreu [a], Dalia dos Prazeres Rodrigues [b], Elmiro Rosendo do Nascimento [a], Maria Helena Cosendey de Aquino [a]

[a] Department of Veterinary Public Health and Public Health, Federal Fluminense University (UFF), 24230-340, 64 Vital Brazil Filho Street, Niterói, RJ, Brazil
[b] National Reference Laboratory Diagnosis of Enteric Bacteria, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (FIOCRUZ), 21045-900, 4365 Brazil Avenue, Rio de Janeiro, RJ, Brazil

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ABSTRACT

To investigate the prevalence of *Campylobacter* and *Salmonella* in poultry carcasses in state of Rio de Janeiro, Brazil, 60 samples from 6 slaughterhouses were collected over a period of 6 months. A total of 82 *Campylobacter* isolates were obtained from twenty seven (45%) positive chicken carcasses, including 44 isolates (53.66%) of *Campylobacter jejuni* and 38 (46.34%) of *Campylobacter coli*. The identification of all strains was confirmed by PCR. *Salmonella* was isolated from 4 (6.67%) carcasses by conventional method and was detected in 5 (8.33%) of 60 chicken carcasses by PCR. Two *Salmonella* Albany and two *Salmonella* Typhimurium were identified. Antimicrobial susceptibility testing was primarily done by the disk diffusion method and later by assessing minimum inhibitory concentrations (MICs) against all the isolates. All the *Campylobacter* isolates were resistant to ciprofloxacin and enrofloxacin. It was observed high MIC values for enrofloxacin (64 μg/mL) in one *C. jejuni* and two *C. coli* strains, and for ciprofloxacin (≥128 μg/mL) in one *C. jejuni* and three *C. coli* strains. No *Salmonella* isolate was resistant to these antibiotics by both methods. These findings reveal a broad extent of fluoroquinolone resistance in *Campylobacter* isolates from chicken carcasses in Brazil and underline the need for prudent use of these antibiotics in poultry production to minimize the spread of fluoroquinolone resistant *Campylobacter*.

1. Introduction

Salmonellosis and Campylobacteriosis are among the most frequently reported foodborne diseases worldwide. While numerous potential vehicles of transmission exist, commercial chicken meat has been identified as one of the most important food vehicles for these organisms (FAO/WHO, 2009). Although The Center for Disease Control and Prevention (CDC) reports *Salmonella* as leading causes of hospitalization by foodborne illness in United States (Scallan et al., 2011), interestingly, *Campylobacter* remains the most commonly reported gastrointestinal bacterial pathogen in humans since 2005 within European Union population (EFSA., 2013).

In developing countries, outbreak information is frequently incomplete because health authorities lack the capabilities or resources for detection, or presumably, because diarrheal diseases are highly endemic and outbreaks may be less common or obvious than in industrialized countries (Zaidi et al., 2008). Despite this incomplete outbreak information in Brazil there are several reports of *Salmonella* prevalence in chicken carcasses ranging from 5.9% to 86.7% (Cardoso & Tessari, 2008; Duarte et al., 2009; Fuzihara, Fernandes, & Franco, 2000; Matheus, Rudge, & Gomes, 2003; Oliveira et al., 2006).

Campylobacter prevalence around the world is very variable and range from 0.29% to 96.7% in chicken carcass (Aquino, Pacheco, Ferreira, & Tibana, 2002; Garin et al., 2012; Wang, Guo, & Li, 2013). In Brazil, the *Campylobacter* presence is not investigated in most cases of bacterial gastroenteritis because the methodologies of isolation and characterization are different from those used in the research of common enteropathogenic bacteria, such as

* Corresponding author. Analytical Laboratory Center, Department of Food Technology, Faculty of Veterinary, Fluminense Federal University, 64, Vital Brazil Filho, Niterói, 24230-340, Rio de Janeiro, Brazil.
E-mail address: pedronepl@yahoo.com.br (P.H.N. Panzenhagen).

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Salmonella, *Shigella* and the *Escherichia coli* group. However, some authors such as Aquino et al. (2002) and Hungaro et al. (2015) found 60% and 16.8% of *Campylobacter* prevalence in chicken carcasses respectively.

Fluoroquinolones, such as ciprofloxacin and enrofloxacin, have an extensive application both in human and veterinary medicine with spectrum of action over Gram-negative and Gram-positive bacteria (Ruiz, 2003). Enrofloxacin, a quinolone developed exclusively for use in animals, has a wide antibacterial activity and is commonly used in poultry production in Brazil. Ciprofloxacin, a metabolite of enrofloxacin (Idowu, Peggins, Cullison, & Bredow, 2010), besides its use in poultry production, is also used for the treatment of human Salmonellosis and Campylobacteriosis (Agunos et al., 2013). Once fluoroquinolones residues could persists in the animal body and may result in the development of bacteria resistant strains, several studies have linked the therapeutically and prophylactically use with the emergence and spread of resistance from these pathogens (Cheng et al., 2012; Finley et al., 2013; Yan, Wang, Qin, Liu, & Du, 2011).

Antimicrobial resistance is an increasing worldwide concern and has been developed over the past 30 years regarding the emergence of multidrug-resistant phenotypes among *Salmonella* and *Campylobacter* (Hur, Jawale, & Lee, 2012). Alarmed by the rise in multidrug-resistant *Salmonella* in the 1960s, the United Kingdom's *Swann Report* of 1969 recognized the possibility that AGPs (Antimicrobial Growth Promoters) were contributing largely to the problem of drug-resistant infections. These reports concluded that animal growth promotion with antibiotics used for human therapy should be banned. However, this practice has continued in many countries although with antibiotics that are not used therapeutically in humans (Marshall & Levy, 2011).

We notice that little is known with regards to the simultaneous occurrence of *Campylobacter* and *Salmonella* on chicken carcasses in Brazil, and their resistance to fluoroquinolones. Therefore, the aim of this study was to investigate their prevalence and pattern of enrofloxacin and ciprofloxacin resistance in carcasses of slaughtered chicken in Rio de Janeiro State, Brazil.

2. Materials and methods

2.1. Sample collection

During 6 months in 2013, 60 chicken carcasses were collected from 6 slaughterhouses in state of Rio de Janeiro, Brazil. From each slaughterhouse, 10 chicken carcasses were randomly collected from the chiller tank and transported on ice in sterilized plastic bags to the laboratory. Microbiological analyses were carried out within at least 3 h after collection. The slaughterhouses were also randomly selected and its identification were preserved changing their names by the letters A to F.

2.2. Salmonella examination method

Skin samples of neck, breast and cloacal (25 g) were homogenized in a stomacher (Stomacher 80 Laboratory Blender Seward) for 2 min and pre enriched with 225 mL of buffered peptone water (BPW) at 37 °C for 24 h. After incubation, isolation of *Salmonella* was performed in general accordance with U.S.FDA Bacteriological Analytical Manual (Hammack, Andrews, & Jacobson, 2014). Isolates were subjected to *Salmonella* Poly O and Poly H antibody assays (Probac do Brazil®).

At the same time, *Salmonella* detection was performed by PCR. The analyses were carried out using 1.0 mL of 24 h pre-enrichment incubated buffered peptone water (BPW) at 37 °C. DNA extraction and amplifications were performed in accordance with Myint, Johnson, Tablante, and Heckert (2006). The primer set of ST 11 (AGC CAA CCA TTG CTA AAT TGG CGC A) and ST 15 (GGT AGA AAT TCC CAG CGG GTA CTG), originally designed by Aabo, Rasmussen, Roseen, Sørensen, and Olsen (1993), is highly specific for *Salmonella* species and defines an amplified fragment of 429 bp. *Salmonella* isolates were sent to the National Reference Center, Institute Oswaldo Cruz, Rio de Janeiro, Brazil for serotyping.

2.3. Campylobacter examination method

The chicken carcass were rinsed with 250 mL of 0.1% buffered peptone water and massaged in sterile plastic bag. Loopfuls were used for *Campylobacter* isolation according to Stern, Patton, Doyle, Park, and Mccardell (1992) and 3 to 5 suspected colonies per each plate were picked and identificated by PCR in accordance with Harmon, Ransom, and Wesley (1997). DNA was extracted with the commercial extraction kit 'Wizard® Genomic DNA Purification Kit' (PROMEGA®). Primers used were pg3/pg50 that amplify a conserved region in the two species (*Campylobacter jejuni* and *Campylobacter coli*) related to flagellin gene and primers C-1/C-4 which amplify a specific region of the species *C. jejuni* strains. The amplification reaction was performed with a final volume of 50 mL containing 5 μL of the sample DNA, 1X PCR Buffer 500 mM KCl, 100 mM Tris−HCl (pH 8.5), 4 μL (200 μM each) dATP, dCTP, dGTP and dTTP, 0.4 uM of each primer pg3 and pg50, 0.2 μM of each primer C1 and C4, 2.5 U Taq polymerase and 5.5 mM/L $MgCl_2$. The initial denaturation was performed at 94 °C for 4 min followed by 25 amplification cycles consisting of 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C and extension at 72 °C for 7 min. Verification of amplicons was performed in a horizontal electrophoresis tank 'Electrophoresis Cell (BioAmérica) with 0.5x TBE, with Pac Power source 300 (Bio-Rad) in 1.5% agarose gel stained with GelRed.

2.4. Antimicrobial susceptibility test

Salmonella and *Campylobacter* isolates were tested for enrofloxacin (5 μg) and ciprofloxacin (5 μg) (Cefar Brazil) susceptibilities. The susceptibility testing was primarily done by the disk diffusion method and later by assessing minimum inhibitory concentrations (MICs) against all the resistant isolates detected by disk diffusion method. The minimal inhibitory concentration (MIC) was determined by the agar dilution method containing ciprofloxacin and enrofloxacin (Sigma−Aldrich®) on the following concentrations: 128 μg/mL, 64 μg/mL, 32 μg/mL, 16 μg/mL, 8 μg/mL, 4 μg/mL, 2 μg/mL, 1 μg/mL, 0.5 μg/mL (NCCLS., 2003). The MIC were decided based on visible growth and breakpoint of ≥4 μg/mL for ciprofloxacin (CLSI., 2008) and enrofloxacin (Chen et al., 2010) were used. *C. jejuni* ATCC 33560 and *C. Coli* ATCC 33559 were included on every plate as a quality control.

2.5. Statistical analysis

Statistical analyses for *Salmonella* detection methods followed procedures described previously (Thrusfield, 2007). The program InStat, version 3.1 (GraphPad, 2009) was used for the calculations. The Chi-square test and Fisher's exact two-tailed test were used for statistical analysis. A P value <0.05 was used for statistical significance.

3. Results and discussion

Salmonella was isolated from 4 (6.67%) of 60 samples. Two *S.* Albany and one *S.* Typhimurium were isolated from the slaughterhouse “B” and one *S.* Typhimurium was isolated from the slaughterhouse “F” (Table 1). *Salmonella* was detected in 5 (8.33%)