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# Characterization of quinolone resistance in *Salmonella* spp. isolates from food products and human samples in Brazil

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ABSTRACT

Non-typhoidal salmonellosis is an important zoonotic disease caused by Salmonella enterica. The aim of this study was to investigate the prevalence of plasmid-mediated quinolone resistance in Salmonella spp. and its association with fluoroquinolone susceptibility in Brazil. A total of 129 NTS isolates (samples from human origin, food from animal origin, environmental, and animal) grouped as from animal ($n = 62$) and human ($n = 67$) food were evaluated between 2009 and 2013. These isolates were investigated through serotyping, antimicrobial susceptibility testing, and the presence of plasmid-mediated quinolone resistance (PMQR) genes (qnr, aac(6′)-Ib) and associated integron genes (integrase, and conserved integron region). Resistance to quinolones and/or fluoroquinolones, from first to third generations, was observed. Fifteen isolates were positive for the presence of qnr genes (8 qnrS, 6 qnrB, and 1 qnrD) and twenty three of aac(6′)-Ib. The conserved integron region was detected in 67 isolates as variable regions, from ±600 to >1000 pb. The spread of NTS involving PMQR carriers is of serious concern and should be carefully monitored.



## Introduction

Foodborne diseases caused by non-typhoid *Salmonella* represent not only an important public health problem, but also an economic burden in many parts of the world. It has been estimated that the global incidence of gastroenteritis caused by non-typhoid *Salmonella* is almost 93.8 million cases per year with 155,000 deaths.[1] Non-typhoidal *Salmonella* spp. are zoonotic agents that have been linked to a variety of food

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sources, particularly foods of animal origin, e.g., beef, poultry, eggs, and dairy products as well as raw fruits and vegetables.[2]

The emergence and spread of antimicrobial-resistant *Salmonella* spp. originating from food of animal origin has become a serious health hazard worldwide, especially in developing countries.[3,4] Antimicrobial-resistant bacteria can be selected through the therapeutic treatment of infections caused by susceptible bacterial populations, both in humans and animals; many mechanisms involved in resistance to quinolones have been studied.[5]

Quinolones, particularly fluoroquinolones, are among the most widely used antibiotics for treating salmonellosis in both human and veterinary infections because of their broad spectrum in antimicrobial activity.[6]

Quinolone resistance in *Enterobacteriaceae* is mostly mediated by point mutations in the quinolone resistance-determining regions (QRDR) of the DNA gyrase and topoisomerase IV genes, leading to target modification. Plasmid Mediated Quinolone Resistance (PMQR) has emerged in *Salmonella* spp. and in other *Enterobacteriaceae* with increasing prevalence.[7] This resistance involves efflux pump mechanisms and the more recently discovered target protection mechanisms controlled by the *qnr* genes. Enzymatic modifications encoded by the *aac(6′)Ib-cr* gene have also been found to contribute to the drug resistance of this antimicrobial class.[8]

The aim of this study was to identify the occurrence of some PMQR in *Salmonella* spp. isolated from animal and human origin sin Brazil between 2009 and 2013.

## Materials and methods

A total of 129 *Salmonella* spp. isolates with resistance to quinolone and/or fluoroquinolone were evaluated. Of this total, 51.9% (67/129) were from human clinical isolates, 30.2% (39/129) from food products for human consumption (beef, eggs, and milk), 7.1% (9/129) from food of animal origin for human consumption (poultry, swine, and cattle), and 10.8% (14/129) from environmental samples (water, drag swabs). The strains identified were stored in phosphate-buffered agar and sent to the National Reference Laboratory of Enteric Diseases (LRNEB/IOC/RJ) between 2009 and 2013.

## Antigenic characterization

*Salmonella* serotypes were determined by slide agglutination according to the Kauffmann–White scheme using O and H antisera. All antisera used for serological determination were prepared in the LRNEB/IOC/RJ.

## Antimicrobial susceptibility

The resistance profiles obtained were confirmed by the disk diffusion test according to CLSI (2013/2014) using representatives of the quinolone class (OXOID) for human and veterinary therapeutic use such as Nalidixic Acid 30 μg, Ciprofloxacin 5 μg, Enrofloxacin 5 μg, Ofloxacin 5 μg, and Levofloxacin 5 μg; bacterial suspensions (0.5 Mac Farland scale) were distributed throughout the surface of plates containing Mueller Hinton agar (OXOID). Discs were deposited on the surface of the culture medium, which already contained the inoculum. After incubation for 24 h at 35 °C, the diameters of inhibition zones formed around the discs were observed and measured in millimeters. The interpretation of results for assignment of the categories of susceptible, intermediate, and resistant was according to CLSI (2013). Quality control was performed in parallel by testing *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853.

MIC determinations were performed in 96-well microplates for Nalidixic Acid (SIGMA), Ciprofloxacin (SIGMA), Enrofloxacin (SIGMA), Levofloxacin (SIGMA), and Ofloxacin (SIGMA) according to the CLSI (2013) broth microdilution assay. MIC was defined as the lowest concentration of drug that inhibits visible growth after 24 h of incubation at 37 °C. Bacterial suspensions grown at 37 °C in BHI broth (OXOID) up to in the concentration at 0.5 of the MacFarland scale that were transferred to BHI broth and plates containing different concentrations of antimicrobials were incubated at 37 °C for 24 h. Quality control was performed for every determination by testing *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853.

## Detection of PMQR

Total DNA was extracted using the DNEASY Tissue Qiagen® kit and its concentration was measured using the Nanodrop spectrophotometer (ND-1000 Uniscience). The studied genes were detected by PCR amplification using the primer sequences presented in Table 1. The *qnrA*, *qnrB*, and *qnrS* genes were amplified through multiplex PCR reactions; the *rrs* gene was used as the reaction control. The *qnrC, qnrD, aac(6′)-Ib, integrase*, and variable *integron* region genes were amplified by simplex PCR.

Positive and negative controls were included in each PCR reaction. Amplified products were identified by their molecular weights after electrophoresis on 1.0% agarose gels at 180 V for 90 min and staining with ethidium bromide.

## Results

Altogether, 26 different *Salmonella* serovars were identified. The predominant serovar was *Salmonella* Typhimurium (48.8%, 63/129) followed by *Salmonella* Enteritidis (19.4%, 25/129). The prevalent serovars associated with resistance to quinolones are presented in Table 2.

The highest incidence of resistant isolates was observed in 2012 (88/129), followed by 2011 (16/129). Most isolates were isolated in 2012.

Among these 129 isolates that were previously resistant to Nalidixic Acid, 5 were sensitive to all tested quinolones (including Nalidixic Acid), 55 (42.6%) were resistant to Ciprofloxacin, 63 (48.8%) to Enrofloxacin, 51 (39.53%) to Ofloxacin, and 48 (37.2%) to Levofloxacin through the disc diffusion test.

The broth microdilution test identified 47 (36.4%) isolates with decreased susceptibility to Ciprofloxacin (MICs between 0.125 mg/mL and 0.5 mg/mL), 26 (20.1%) to Enrofloxacin, 8