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CIPROFLOXACIN SUSCEPTIBILITY REDUCTION OF *SALMONELLA* STRAINS ISOLATED FROM OUTBREAKS

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ABSTRACT

The antimicrobial susceptibility of 212 *Salmonella* strains isolated from patients and foods was evaluated and 45% were found to be resistant to nalidixic acid. Nalidixic acid resistant strains showed a higher minimal inhibitory concentration for ciprofloxacin than sensitive strains. During the study an increase of strains with reduced susceptibility to ciprofloxacin was also observed.

Key words: antimicrobial resistance; Minimal Inhibitory Concentration (MIC); quinolones; fluoroquinolones.

Salmonella spp. is recognized as a frequent cause of foodborne disease (22). In Parana State, Brazil, 55 out of 399 districts notified salmonellosis outbreaks between 1999 and 2006. The Enteritidis serovar was responsible for 82% of the outbreaks. Due to its self-limiting nature, gastroenteritis caused by *Salmonella* usually does not require antimicrobial therapy. However, the use of antimicrobials is necessary for immunocompromised patients, older persons and children or in cases of severe or systemic salmonellosis (3, 18).

The more commonly used drugs for salmonellosis treatment during the last decades were ampicillin, chloramphenicol and sulphametoxazole-trimethoprim. Growing resistance to these antimicrobials significantly reduced their use in clinical medicine and they were gradually substituted by fluoroquinolones (3, 14).

The non-therapeutic use of fluoroquinolones in veterinary medicine, as in prophylactic supplements or growth-promoting agents, can facilitate the selection of resistant bacteria or reduce susceptibility to these antimicrobials. Thus, the use of antimicrobial agents in animals raised for human consumption is, probably, the main cause for the increase and spread of resistant *Salmonella* strains (1, 9, 12).

The high prevalence of strains resistant to nalidixic acid, a 1st generation quinolone, constitutes a public health concern since most of these strains show reduced susceptibility to fluoroquinolones, and have been associated with treatment failures (8, 15, 18).

The present study was carried out to evaluate the antimicrobial susceptibility profile of 212 *Salmonella* spp. strains isolated from patients and foods related to outbreaks which occurred between 1999 and 2006 in Parana State, Brazil in order to alert about the more careful use of antibiotics in veterinary and human clinical treatment, especially fluoroquinolones.

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All *Salmonella* strains were obtained from the Laboratório Central do Paraná (LACEN, Curitiba, Parana, Brazil) and serotyped and phagotyped at the Laboratório de Enterobactérias, Departamento de Bacteriologia da Fundação Oswaldo Cruz (FIOCRUZ, Rio de Janeiro, Brazil).

Sixteen different serovars were analysed, including 174 (82%) strains of S. Enteritidis, seven (3.3%) *S*. Infantis, six (2.9%) *S*. Typhimurium, six (2.9%) *S*. Derby, four (1.9%) *S*. Newport, three (1.4%) *S*. Johannesburg, two (0.9%) *S*. London, two (0.9%) *S*. Pomona and one strain of the following serovars: Anatum, Mbandaka, Oranienburg, Agona, Saintpaul, Heidelberg, 09:12 and Albany. The strains were maintained in Brain-Heart Infusion broth (BHI) (DIFCO®) containing 15% glycerol and stored at -15°C until testing.

Antimicrobial susceptibility was evaluated according to the Kirby-Bauer disk diffusion method as recommended by the *Clinical and Laboratory Standards Institute* (CLSI) (5). Antimicrobial agents tested were (OXOID®): ampicillin (10 µg); cefotaxime (30 µg), chloramphenicol (30 µg), sulphametoxazole-trimethoprim (25 µg), nalidixic acid (30 µg) and ciprofloxacin (5 µg). The minimal inhibitory concentration for ciprofloxacin was determined with the microdilution method using Müller-Hinton broth (DIFCO®) according to CLSI (5).

All *Salmonella* strains tested in the present study were susceptible to cefotaxime, chloramphenicol and ciprofloxacin. Ninety five (45%) strains were resistant to nalidixic acid; one (0.5%) to ampicillin and one (0.5%) to sulphametoxazole-trimethoprim. An increase in nalidixic acid resistance was observed from 15% in 1999 to 62.5% in 2006 (Figure 1). Similar results were previously found by other researchers (6, 17).

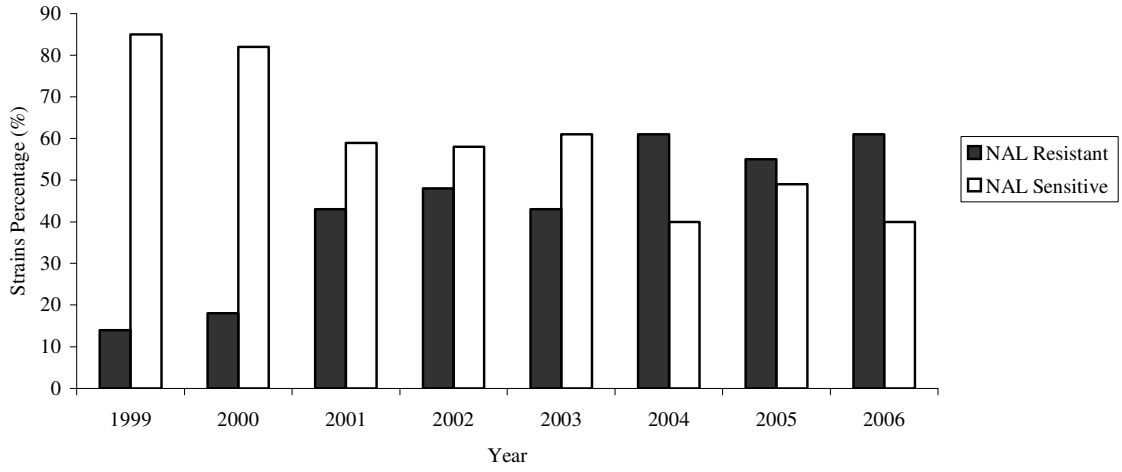

Figure 1. Nalidixic acid (NAL) susceptibility profile for strains associated with salmonellosis outbreaks occurred between 1999 and 2006 in Parana State, Brazil.

Quinolones have been increasingly used to treat human and animal infectious diseases since the introduction of nalidixic acid in the 1960s. The consequence of this widespread use has been an increase in bacterial resistance (11). An increase in nalidixic acid resistance is an indicator for the decrease in ciprofloxacin susceptibility, an antibiotic commonly used in the treatment of *Salmonella* infections (10, 11, 13).

Although no fluoroquinolone resistant strains were observed in the present study, a significant increase in minimal inhibitory concentration for ciprofloxacin (CipMIC) was verified for the strains resistant to nalidixic acid (Figure 2). All nalidixic acid resistant strains had higher CipMIC than the sensitive ones. Most of them had CipMIC's eight times higher, demonstrating the correlation between nalidixic acid resistance increase and fluoroquinolone susceptibility decrease.

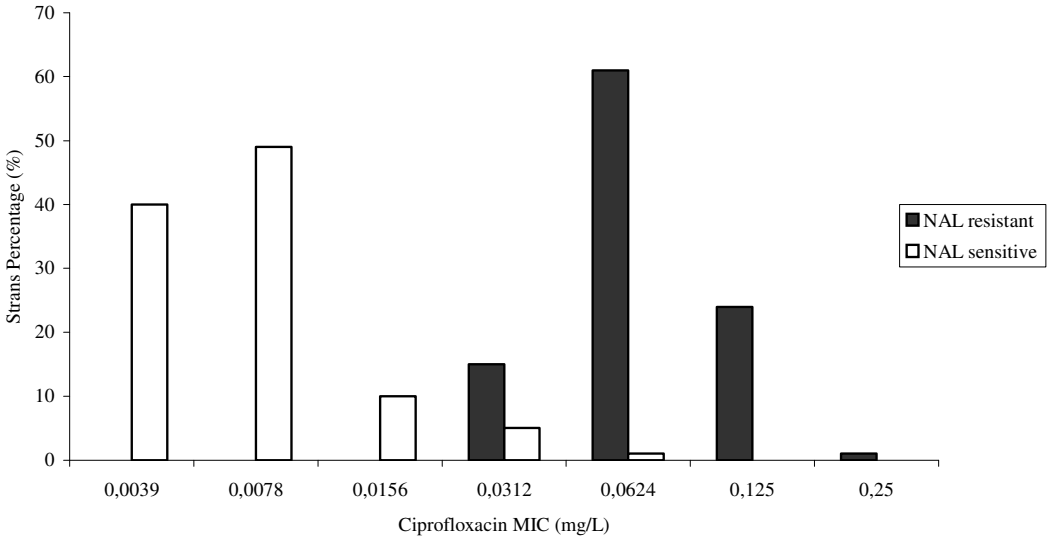

Figure 2. Ciprofloxacin MIC distribution according to nalidixic acid (NAL) susceptibility for *Salmonella* strains associated with outbreaks occurred between 1999 and 2006 in Parana State, Brazil

Earlier studies showed that strains with MIC 0.125 mg/L had reduced ciprofloxacin susceptibility and were associated with treatment failures (15, 16). In this study, 25% of the strains resistant to nalidixic acid had CipMIC ranging from 0.125 mg/L to 0.250 mg/L.

MIC_{50} values are important indicators for *in vitro* antimicrobial activity. Although these values do not necessarily predict *in vivo* activity, an increase in $CipMIC_{50}$ from 0.0039 mg/L to 0.0625 mg/L observed during this study indicates a possible reduction in ciprofloxacin potency.

Strains with high MIC for ciprofloxacin have been associated with single point mutations in the quinolone resistant determining region (QRDR) of the *gyr*A gene that codifies DNA-gyrase enzyme, which is the primary target of fluoroquinolone action (9, 15). Single point mutations may be sufficient to generate high levels of resistance to such non-fluorated quinolones as nalidixic acid. However, apparently additional mutations are necessary to achieve resistance to fluoroquinolones such as ciprofloxacin (7, 12). It is also known that the antimicrobial concentration reduction within the cell due to hyperexpression of the efflux pump system contributes to a heightened quinolone MIC in *Salmonella* spp. but is insufficient to generate high fluoroquinolone resistance levels (2, 19).

The emergence of *Salmonella* strains with fluoroquinolones reduced susceptibility is a potential public health risk and may compromise effective antibiotic therapy restricting therapeutic options against infectious diseases (1, 9, 12).

The serovar and phagotype can also influence the antimicrobial resistance levels in *Salmonella* spp. It has been reported that serovars Hadar, Virchow, Blockley and Typhimurium showed higher resistance fluoroquinolone levels than Enteritidis, the most prevalent serovar in the present study (4, 21).

Threfall (30) found that Enteritidis PT1 isolated in England and Wales exhibited higher reduction in ciprofloxacin susceptibility than other phagotypes. The Enteritidis phagotype PT1 analysed in this study showed higher reduction in ciprofloxacin susceptibility than other phagotypes, such as PT4 (13, 20). An increased incidence of *S.* Enteritidis PT1 from 52.5% in 1999 to 72.9% in 2006 probably explains the increase of NAL-resistant strains prevalence during this period (Figure 1).

Regarding the importance of fluoroquinolones for salmonellosis treatment, the results obtained in the present study alert to a more careful use of these antimicrobials in order to maintain their clinical efficacy and broad spectrum.

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REFERENCES

1. Angulo, F.; Johnson, K.; Tauxe, R.; Cohen, M. (2000). Significance and sources of antimicrobial-resistant nontyphoidal *Salmonella* infections in the United States. *Microb. Drug Resist.* , 1, 77-83.
2. Biedenbach, D.J.; Toleman, M.; Walsh, T.R.; Jones, R.N. (2006). Analysis of *Salmonella* spp. with resistance to extended-spectrum cephalosporin and fluoroquinolones isolated in North America and Latin America: report from the SENTRY Antimicrobial Surveillance Program (1997–2004). *Diagn. Microbiol. Infect. Dis.* , 54, 13-21.
3. Butaye P.; Michael, G.B.; Schwarz, S.; Barrett, T.J.; Brisabois, A.; White, D.G. (2006). The clonal spread of multidrug-resistant non-typhi *Salmonella* serotypes. *Microbes Infect.,* 8, 1891-1897.
4. Cebrian, L.; Sirvent, E.R.; Díaz, J.C. (2003). Characterization of *Salmonella* spp. mutants produced by exposure to various fluoroquinolones. *Int. J. Antimicrob. Agents,* 22, 134-139.
5. Clinical and Laboratory Standards Institute (2006). *Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement M100-S16*. CLSI, Wayne, PA, USA.
6. Duarte, D.A.M.; , Ribeiro, A.R.;, Vasconcelos, A.M.M.; Santos, S.B.; Silva, J.V.D.; Patrícia Andrade, P.L.A.; Falcão. L.S.P.C.A. (2009). Occurrence of *Salmonella* spp. in broiler chicken carcasses and their susceptibility to antimicrobial agents. *Braz. J. Microbiol.*, 40: 569-573.
7. Eaves, D.J.; Randall, L.; Gray, D.T.; Buckley, A.; Woodward, M.J.; White, A.P.; Piddock, L.J.V. (2004). Prevalence of Mutations within the Quinolone Resistance-Determining Region of *gyrA*, *gyrB*, *parC* e *parE* and Association with Antibiotic Resistance in Quinolone-Resistant *Salmonella enterica*. *Antimicrob. Agents Chemother.* , 48 (10), 4012-4015.
8. Escribano, I.; Rodríguez, J. C.; Cebrian, L.; Royo, G. (2004). The importance of active efflux systems in the quinolone resistance of clinical isolates of *Salmonella* spp.. *Int. J. Antimicrob. Agents,* 24, 428-432.
9. Giraud, E.; Baucheron, S.; Cloeckaert, A. (2006). Resistance to fluoroquinolones in *Salmonella*: emerging mechanisms and resistance prevention strategies. *Microb. Infect.* , 8, 1937-1944.
10. Hakanen, A.; Kotilainen, P.; Huovinen, P. (2001). Reduced fluoroquinolone susceptibility in *Salmonella enterica* serotypes in travelers returning from Southeast Asia. *Emerging Infect. Dis.,* 7, 996-1003.
11. Hooper, D.C. (2001) Emerging Mechanism of Fluoroquinolone Resistance. *Emerging Infect. Dis.,* 7, 337-341.
12. Hopkins, K.L.; Davies, R.H.; Threlfall, E.J. (2005). Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: Recent developments. *Int. J. Antimicrob. Agents,* 25, 358-73.
13. Murray, A.; Coia, J.E.; Mather, H.; Brown, D.J. (2005). Ciprofloxacin resistance in non-typhoidal *Salmonella* serotypes in Scotland, 1993-2003. *J. Antimicrob. Chemother.*, 56, 110-114.
14. Piddock, L.J.V. (1998). Fluoroquinolone resistance: Overuse of fluoroquinolones in human and veterinary medicine can breed resistance. *BMJ*, 317, 1029-1030.
15. Piddock, L.V.J. (2002). Fluoroquinolone resistance in *Salmonella* serovars isolated from humans and food animals. *FEMS Microbiol.Rew.* , 26, 3-16.
16. Reller, M.; McClellan, J.; Joyce, K.; Polyak, C.; Mintz, E.; Angulo, F., and NARMS WORKING GROUP (2002). Emerging resistance to quinolones among *Salmonella* Typhi isolates in the United States, 1999-2001. *Infect. Dis. Soc. Am.*
17. Ribeiro, A.R.; Kellermann, A.; Santos, L.R.; Fittél, A. P.; Nascimento, V.P. (2007). *Salmonella* spp. in Raw Broiler Parts: Occurrence, Antimicrobial Resistance Profile and Phage Typing of The *Salmonella* Enteritidis Isolates. *Braz. J. Microbiol*., 38, 296-299.
18. San-Martín, B.; Lapierre, L.; Toro, C.; Bravo, V.; Cornejo, J.; Hormazabal, J. C.; Borie C. (2005). Isolation and molecular characterization of quinolone resistant *Salmonella* spp. from poultry farms. *Vet. Microbiol.* , 110, 239-244.
19. Soto, S.M.; Ruíz, J.; Mendoza, M.C.; Vila, J. (2003). In vitro fluoroquinolone-resistant mutants of *Salmonella enterica* serotype Enteritidis: analysis of mechanisms involved in resistance. *Int. J. Antimicrob. Agents,* 22, 537-540.
20. Threlfall, E.J. (2002) Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. *FEMS Microbiol. Rev.* , 26, 141-148.
21. Threlfall, E.J.; Fisher, I.S.T.; Berghold, C. (2003). Antimicrobial drug resistance in isolates of *Salmonella enterica* from cases of salmonellosis in humans in Europe in 2000: results of international multicentre surveillance. *Eurosurveillance - European Communicable Disease Bolletin: Salmonella,* 82(2):41-45.
22. WHO Global Salm-Surv. (2006) Progress report (2000-2005): building capacity for laboratory-based foodborne disease surveillance and outbreak detection and response.