Emerging nalidixic acid and ciprofloxacin resistance in non-typhoidal *Salmonella* isolated from patients having acute diarrhoeal disease

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Background: Non-typhoidal *Salmonella* are one of the key etiological agents of diarrhoeal disease. The appearance of multiple drug resistance along with resistance to quinolones in this bacterium poses a serious therapeutic problem. We determined the prevalence of nalidixic acid and ciprofloxacin resistance in non-typhoidal *Salmonella* isolated from faecal samples of patients with acute diarrhoeal disease attending the outpatient and inpatient departments of a hospital in Saudi Arabia during the years 1999 to 2002.

Methods: Non-typhoidal *Salmonella* were isolated from faecal samples. Antimicrobial susceptibility was tested by the disc diffusion test. MICs to nalidixic acid and ciprofloxacin were determined by the agar dilution method.

Results: During the study period, 524 strains of non-typhoidal *Salmonella* were isolated. Strains belonging to serogroup C1 were the commonest (41.4%) followed by serogroups B and D (15.6% and 14.5%, respectively). Resistance to ampicillin was observed in 22.9% and to trimethoprim/sulfamethoxazole in 18.5% of the strains. Nalidixic acid resistance was encountered in 9.9% and ciprofloxacin resistance in 2.3% of the strains. Resistance to nalidixic acid significantly increased from 0.1% in 1999 to 5.5% in 2002 (P=0.0007) and ciprofloxacin resistance increased significantly from 0.1% in 1999 to 0.9% in 2002 (P=0.0001). MICs to nalidixic acid and ciprofloxacin were determined among 29 nalidixic acid-resistant strains of non-typhoidal *Salmonella* isolated during 2002. The MIC was >256 µg/mL to nalidixic acid and 8 to 16 µg/mL to ciprofloxacin.

Conclusion: The increasing rates of antimicrobial resistance encountered among non-typhoidal *Salmonella* necessitate the judicious use of these drugs in humans. Moreover, these findings support the concern that the use of quinolones in animal feed may lead to an increase in resistance and should be restricted.

Key words: Non-typhoidal *Salmonella*, nalidixic acid, ciprofloxacin, fluoroquinolones, microbial drug resistance, Saudi Arabia

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Foodborne non-typhoidal *Salmonella* infections have become a major problem in the industrialized and developing countries.[1] They are one of the most important causative agents of acute diarrheal disease in children and adults. The World Health Organization has determined that the non-typhoidal *Salmonella* are emerging as one of the most important etiological agents of infectious diseases in the world.[2] The global emergence of multiple drug resistance in non-typhoidal *Salmonella* is a serious public health concern and is posing a severe problem in treatment of intestinal and extra-intestinal infections due to these organisms.[3-6] The rapid international dissemination of these multidrug-resistant strains of non-typhoidal *Salmonella* among humans suggests that the multidrug resistance is associated with enhanced virulence.[7] A study from Denmark has reported 4.8 times higher mortality in patients having infection due to multidrug-resistant *Salmonella* typhimurium in comparison to infection with sensitive strains.[8]

Quinolones remain the treatment of choice for non-typhoidal *Salmonella* infections, after the appearance of multidrug resistance to commonly used antibiotics like ampicillin, trimethoprim/sulfamethoxazole and chloramphenicol. The recent appearance of quinolone resistance in non-typhoidal *Salmonella* is a matter of great disquiet for treating physicians and microbiologists.[9] Resistance to nalidixic acid, which has been associated with reduced efficacy of fluoroquinolones, such as ciprofloxacin, has been reported recently from many countries.[10,11] Epidemiological studies have shown that the number of *Salmonella* isolated with ciprofloxacin resistance are increasing in Europe.[12-14] Multidrug-resistant *Salmonella typhimurium* DT104, also resistant to quinolones, has been responsible for extensive outbreaks worldwide, leading to high mortality.[15,16]

The present study describes the prevalence of nalidixic acid and ciprofloxacin resistance in strains of non-typhoidal *Salmonella* isolated from the Al-Hasa region of Saudi Arabia.

Methods

The study was carried out at the 500-bed King Fahad Hospital, Al-Hofuf, during the period 1999 to 2002. The non-typhoidal *Salmonella* were isolated from faecal samples of patients admitted to or attending the out-patient and inpatient departments of the hospital. Faecal samples were cultured directly on xylose lysine deoxycholate agar and inoculated in selenite F broth (Oxoid Ltd, UK), which was subcultured on xylose lysine deoxycholate agar after 24 hours of incubation. *Salmonella* strains were biochemically identified by the API-20E system (bioMerieux SA, France) and serogrouped using somatic group *Salmonella* A-G antisera (Murex Biotech Ltd, UK). Antibiotic susceptibility was performed by the disc diffusion technique according to the criteria of National Committee for Clinical Laboratory Standards (NCCLS) 17. Susceptibility tests were done on Mueller Hinton agar (Oxoid Ltd, UK) using the following concentrations (µg/disc) of antibiotics (Becton Dickinson Co, Maryland, USA): ampicillin-25, amoxicillin/clavulanic acid-20/10, cephalothin-30, cefoxitin-30, cefotaxime-30, ceftriaxone-30, chloramphenicol-30, trimethoprim/sulfamethoxazole-1.25/23.75, gentamicin-10, amikacin-30, imipenem-10, aztreonam-30, piperacillin-100, nalidixic acid-30, ciprofloxacin-5. The minimum inhibitory concentration (MIC) for nalidixic acid and ciprofloxacin of the 29 nalidixic acid-resistant strains isolated during 2002 was determined by the agar dilution method.[18] Statistical analysis for comparison of data on resistance between years was done by the Chi-square and Fisher's exact tests. Statistical significance was set at the 0.05 level.

Results

During the 4-year period, 524 strains of non-typhoidal *Salmonella* were isolated from the faecal samples of patients having diarrhoeal illness. *Salmonella* strains belonging to serogroup C1 were the commonest (41.4%) followed by serogroups B and D (15.6% and 14.5%, respectively). *Salmonella* strains belonging to serogroup G were least frequent (2.7%) (Table 1). Resistance to ampicillin was observed in most strains (22.9%) followed by resistance to trimethoprim/sulfamethoxazole (18.5%). Resistance to chloramphenicol and amoxicillin/clavulanic acid was observed in 8.6% and 5.3% of the strains, respectively. Resistance to nalidixic acid was noticed in 9.9% and to ciprofloxacin in 2.3% of the strains. All the isolated strains were sensitive to cephalothin, cefotaxime, ceftriaxone, gentamicin, amikacin, imipenem, aztreonam and piperacillin.

Overall antibiotic resistance among the non-typhoidal *Salmonella* strains increased from 8.2% in 1999 to 25.3% in 2002. There was a trend for increasing resistance to nalidixic acid and ciprofloxacin during the study period. Resistance to nalidixic acid significantly increased from 0.1% in 1999 to 5.5% in 2002 (P=0.0007) and ciprofloxacin resistance also significantly increased from 0.1% in 1999 to 0.9% in 2002 (P=0.0001) (Table 2). Multiple antibiotic resistance (resistance to two or more of the antibiotics) was observed in 123 (37.2%) of the strains. Resistance was observed most frequently (39.8%) among the strains belonging to serogroup C1, followed by serogroups C2 and B (18.9% and 16.9%, respectively) (Table 3).

Resistance to nalidixic acid was more common among the strains belonging to serogroup C1 than the strains from other serogroups. Of 52 (9.9%) strains resistant to nalidixic acid, 12(2.3%) were also resistant to ciprofloxacin. Isolated resistance to nalidixic acid and ciprofloxacin was observed more frequently. There was a 29 times increase in resistance to nalidixic acid during the study period, from 1 resistant strain in 1999 to 29 strains in 2002 (Table 4). The MIC of non-typhoidal *Salmonella* to nalidixic acid and ciprofloxacin was determined only for the 29 strains isolated during 2002 that were resistant to these antibiotics. The MIC to nalidixic acid was >256 µg/mL in all the strains. The MIC to ciprofloxacin ranged from 8 to 16 µg/mL among all the five strains that were resistant to this antibiotic. Two of the strains resistant to nalidixic acid had reduced susceptibility (MIC 0.20 µg/mL) to ciprofloxacin.

Discussion

Antimicrobial resistance in non-typhoidal *Salmonella* has increased worldwide as a consequence of excessive use of antimicrobial agents.[19] Antimicrobial susceptibility to ampicillin, trimethoprim/sulfamethoxazole and quinolones for the *Salmonella* isolated from faecal samples should be routinely tested and reported by clinical laboratories as per the recommendations of NCCLS. Susceptibility to chloramphenicol and cephalosporins is recommended for *Salmonella* strains isolated from extra-intestinal sources, as the clinical efficacy of other drugs in intestinal infections has not been proven though they may appear susceptible in in-vitro tests. However, susceptibility to other antimicrobial agents in intestinal isolates of *Salmonella* has epidemiological importance.[17] Ampicillin, trimethoprim/sulfamethoxazole and the fluoroquinolones are established as standard first-line therapy for Salmonellosis. The appearance of resistance to these antibiotics in non-typhoidal *Salmonella* is posing a serious problem in the treatment of infections due to these organisms.[9] Resistant *Salmonella* has a selective advantage in the environment where excessive antibiotics are used and the antibiotic treatment itself is a major risk factor for infection with the resistant bacteria.[20,21] The emergence of quinolone resistance in non-ty-

References

1. Threlfall EJ,Frost JA,Ward LR,Rowe B. Increasing spectrum of resistance in multiresistant *Salmonella typhimurium*. **Lancet.**1996;347:1053-1054.

2. World Health Organisation. Emerging food borne diseases. Fact Sheet 2002; No.124.

3. Panhotra BR, Agarwal KC. Drug resistance in Salmonella typhimurium. **Trop Geogr Med.** 1982;34: 277-279.

4. Agarwal KC, Panhotra BR, Garg RK,Verma AD, Ayyagri A, Mahanta J. Drug resistance in *Salmonella* isolated at Chandigarh. **Antoine Van Lauwenhoek**.1980;46: 383-390.

5. Threlfall JE, Fisher IST, Berghold C, Gerner-Smidt P, Tschape H, Cormican M. Antimicrobial drug resistance in isolates of *Salmonella enterica* from cases of Salmonellosis in humans in Europe in 2000: Results of international surveillance. **Eurosurveillance.** 2003; 8:41-45.

6. Threlfall EJ,Ward LR,Skinner JA,Rowe B. Increase in multiple antibiotic resistance in non-typhoidal *Salmonella* from human in England & Wales: Comparison of data of 1994 and 1996. **Microb Drug Resist.**1997;3:263-266.

7. Chruchaga S, Echeita A, Aladuena A, Garcia-Pena J, Frias N, Usera MA. Antimicrobial resistance in Salmonella from humans, food and animals in Spain **Antimicrob Chemother.** 2001; 47:315-321.

8. Helms M,Vastrup P,Gerner-Smidt P, Mølbak K. Excess mortality associated with antimicrobial drug resistant *Salmonella typhimurium*. **Emerg Infect Dis.** 2002;8: 490-496.

9. Nakaya H, Yasuhara A, Yoshimura K, Oshihoi Y, Izumiya H, Watanabe H. Life-threatening infantile diarrhea from fluoroquinolone resistant *Salmonella enterica typhimurium* with mutation in both gyrA and parC. **Emerg Infect Dis.** 2003;9:255-257.

10. Aspergilla MO, Smego RA, Scott LK. Quinolone antibiotics in treatment of *Salmonella* infections. **Rev Infect Dis.**1990; 12:873-889.

11. Mølbak K,Gerner-Smidt P, Wegener HC. Increasing quinolone resistance in *Salmonella enterica* serotype enteritidis. **Emerg Infect Dis.** 2002;8:514-515.

12. Mølbak K, Baggesen DL, Aarestrup FM, Ebbesens JM, Engberg J, Frydendahl K. An outbreak of multi drug resistant quinolone resistant *Salmonella typhimurium*. **N Engl J Med.** 1999;341:1420-1425.

13. Frost JA, Kellehr A, Rowe B. Increasing resistance in *Salmonella* in England & Wales 1991-1994. **J Antimicrob Chemother.** 1996; 37:85-96.

14. Hakanen A, Slitonen A, Kotlilanen P, Huovinen P. Increasing fluoroquinoline resistance in *Salmonella* serotypes in Finland during 1995-1997. **J Antimicrob Chemother.**1999;43:145-148.

15. Threlfall EJ, Ward LR, Rowe B. Multiresistant *Salmonella typhimurium* DT104 and *Salmonella bacteremia*. **Lancet.** 1998; 352:287-288.

16. Wall PG, Morgan D, Landen K, Ryan M, Griffin M, Threlfall EJ. A case control study of infection with an epidemic strain of multiresistant *Salmonella typhimurium* DT 104 in England & Wales. **Commun Dis Rep CDR Rev.**1994;4:130-135.

17. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. 6th edition. **NCCLS document M2-A6**. Wyne, PA, USA;1997.

18. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. 12th informational supplement. **NCCLS document M100-S12.** Wyne, PA, USA; 2002.

19. Cohen ML, Tauxe RV. Drug resistant *Salmonella* in United States: An epidemiologic perspective. **Science.**1986; 234:964-969.

20. Ryan CA, Nickels MK, Hargrett-Bean NT, Potter ME, Endo T, Mayer L. Massive outbreak of antimicrobial Salmonellosis traced to pasteurized milk. **JAMA.**1987;258:3269-3274.

21. Holmberg SD, Osterholm MT, Senger KA, Cohen ML. Drug resistant *Salmonella* from animals fed antimicrobials. **N Engl J Med.**1984; 311:617-622.

22. Heriksted H, Hayes P, Mokhtar M, Fracaro ML, Threlfall EJ, Angulo FJ. Emergence of quinolone resistant Salmonella in United States. **Emerg Infect Dis.**1997; 3:371-372.

23. Frost JA, Kelleher A, Rowe B. Increasing ciprofloxacin resistance in *Salmonella* in England & Wales 1991-1994. **J Antimicrob Chemother.**1996;37:85-91.

24. Piddock LJ, Ricci V, McLaren I, Griggs DJ. Role of mutation in the gyrA and parC genes of Nalidixic acid resistant *Salmonella* serotypes isolated from animals in the United Kingdom. **Antimicrob Chemother.** 1998; 41:635-641.

25. Hakanen A, Kotlainin P, Huovinen P, Helenius H, Siitonen A. Reduced fluoroquinolone susceptibility in *Salmonella enterica* serotypes in travelers returning from South East Asia. **Emerg Infect Dis.** 2001;7: 996-1003.

26. Smith KE, Berser JM, Hedberg CW, Leano FT, Bender JB, Wicklund JH. Quinolone resistant *Campylobacter jejunum* infection in Minnesota 1992-1998. **N Engl J Med.**1999;340:1525-1532.

27. Saenz Y, Zarazaga M, Lantero M, Gastaures MJ, Baquero F, Torres C. Antibiotic resistance in *Campylobacter* strains isolated from animals, food & humans in Spain 1997-1998. **Antimicrob Agent Chemother.** 2000;44:267-271.

28. Giraud E, Brisabois A, Martel JL, Chaslus-Dancla E. Comparative studies of mutations in animal isolates and experimental in-vitro and in-vivo selected mutants of *Salmonella spp* suggests a counter selection of highly fluoroquinolone resistant strains in the field. **Antimicrob Agent Chemother.** 1999;43:2131-2137.

29. Ronald AR, Turck M, Petersdrof RC. A critical evaluation of Nalidixic acid in urinary tract infections. **N Engl J Med.**1966; 275:1081-1089.

30. Hoge CW, Bodhidatta L, Tungtaem C, Echeverria P. Emergence of Nalidixic acid resistant Shigella dysenteriae 1 in Thailand: Outbreak associated with consumption of a coconut milk dessert. **Int J Epidemiol.**1995; 24; 1228-1236.

31. Panhotra BR, Bhavana Desai. Resistant *Shigella dysenteriae1*.**Lancet.**1983; ii: 1420.

32. Panhotra BR, Bhavana Desai, Sharma PL. Nalidixic acid resistant *Shigella dysenteriae1*. **Lancet.** 1985;i: 763.

33. Gosh AR, Sugunan AP, Seghal SC, Bhardwaj AP. Emergence of nalidixic acid resistance *Shigella sonnei* in acute diarrhea patients on Andaman and Nicobar Island, India. **Antimicrob Agents Chemother.** 2003;47;1483-1484.