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 department 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 department 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-10, 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/

Table 1. Prevalence of *Salmonella* serogroups isolated during 1999 to 2002.

Serogroup	Year of isolation (n, %)				
	1999	2000	2001	2002	Total
B	16 (3.0)	15 (2.8)	22 (4.1)	29 (5.5)	82 (15.6)
C1	26 (4.9)	94 (17.9)	56 (10.6)	41 (7.8)	217 (41.4)
C2	14 (2.6)	16 (3.0)	26 (4.9)	16 (3.0)	72 (13.7)
D	22 (4.1)	14 (2.6)	29 (5.5)	11 (2.1)	76 (14.5)
E	16 (3.0)	25 (4.7)	17 (3.2)	5 (0.9)	63 (12.0)
G	–	4 (0.7)	3 (0.5)	7 (1.2)	14 (2.7)
Total	94 (17.9)	168 (32.1)	153 (29.2)	109 (20.8)	524

Table 2. Antibiotic resistance in *Salmonella* isolated during 1999 to 2002.

Antibiotics	Antibiotic resistance (n,%)					Odds ratio (95% CI), *P* value*
	1999	2000	2001	2002	Total	
TMP/SMX	15 (2.8)	20 (3.8)	35 (6.6)	27 (5.1)	97 (18.5)	0.475 (0.209-1.084), 0.080
AMP	16 (3.0)	24 (4.6)	32 (6.1)	48 (9.1)	120 (22.9)	0.953 (0.442-2.066), 1.000
AMC	1 (0.1)	2 (0.3)	7 (1.3)	18 (3.4)	28 (5.3)	6.574 (0.880-136.086), 0.070
CHL	9 (1.7)	14 (2.6)	16 (3.0)	6 (1.1)	45 (8.6)	0.178 (0.052-0.598), 0.060
NAL	1 (0.1)	3 (0.5)	19 (3.6)	29 (5.5)	52 (9.9)	11.125 (4.913-25.790), 0.0007†
CIP	1 (0.1)	3 (0.5)	3 (0.5)	5 (0.9)	12 (2.3)	14.143 (6.865-29.946), 0.0001†
Total	43 (8.2)	66 (12.5)	112 (21.3)	133 (25.3)	354 (67.5)	

TMP/SMX, trimethoprim-sulfamethoxazole; AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CHL, chloramphenicol; NAL, nalidixic acid; CIP, ciprofloxacin.
* Resistance in 1999 vs. 2002; † Statistically significant.

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

Table 3. Overall antimicrobial resistance in *Salmonella* by serogroup during 1999 to 2002.

Serogroup	Antibiotic resistance (n)				
	1999	2000	2001	2002	Total (n, %)
B	2	7	14	37	60 (16.9)
C1	21	28	44	48	141 (39.8)
C2	14	10	20	23	67 (18.9)
D	–	7	26	10	43 (12.1)
E	6	8	8	7	29 (8.1)
G	–	6	–	8	14 (3.9)
Total	43	66	112	133	354

Table 4. Resistance to nalidixic acid and ciprofloxacin in *Salmonella* by serogroup during 1999 to 2002.

Serogroup	Antibiotic resistance (type and number of strains)				
	1999	2000	2001	2002	Total (n)
B	–	nc-1	–	n-4, nc-1	6
C1	nc-1	nc-2	n-1, nc-3	n-11, nc-4	22
C2	–	–	n-3	n-6	9
D	–	–	n-8	n-2	10
E	–	–	n-4	n-1	5
G	–	–	–	–	–
Total	1	3	19	29	52

n, nalidixic acid; c, ciprofloxacin

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-typhoidal *Salmonella* is a matter of great concern as these are the first-line drugs for treatment of Salmonellosis.[11,22] Apart from clear resistance to fluoroquinolones, there are reports of reduced susceptibility to these drugs, leading to treatment failure. Epidemiological studies have shown that the number of *Salmonella* isolates with a reduced susceptibility to fluoroquinolines has increased in Europe.[23, 24] Resistance to ciprofloxacin increased in Denmark from 0.8% in 1995 to 8.5% in 2000 in non-typhoidal *Salmonella* isolated from humans.[11] Nalidixic acid and ciprofloxacin resistance in human isolates of non-typhoidal *Salmonella* in Finland increased from 3.9% in 1995 to 23.5% in 1999 and this rise was greater (5.6% to 50%) among persons who traveled to Thailand.[25] The Enter-net surveillance report of 2000 from 10 European countries documented resistance to nalidixic acid in 14% and to ciprofloxacin in 0.5% of all isolates of non-typhoidal *Salmonella*.[5] Studies from the USA have reported resistance to nalidixic acid in 0.5% and to ciprofloxacin in 0.02% of strains of non-typhoidal *Salmonella*.[22]

In the present study, overall resistance to nalidixic acid was observed in 52(9.9%) of the non-typhoidal *Salmonella* strains during the 4 years of study, and it significantly increased from 0.1% in 1999 to 5.5% in 2002 (P=0.0007). Resistance to ciprofloxacin was encountered in 12 (2.3%) of the strains during the 4 years, and this also significantly increased from 0.1% in 1999 to 0.9% in 2002 (P=0.0001). Resistance to these drugs was more frequently observed among the strains belonging to serogroup C1 followed by strains from serogroup D. Although the resistance to nalidixic acid and ciprofloxacin has started emerging in this region of Saudi Arabia, it is presently less than that has been reported from the European countries.[5,11,25]

Emergence of resistance to these drugs in non-typhoidal *Salmonella* is a serious problem for the future as the choice of drugs for peroral treatment will become limited. The emergence of resistance can also lead to cross-resistance to other antibiotics.[9] Resistance to quinolones appears by point mutation leading to an amino acid change at the gyr gene. This type of mutational resistance is promoted by the selective pressure caused by the use of these drugs in humans or animals and agriculture. Lately, an alarming increase in quinolone resistance has been observed among foodborne pathogens like *Campylobacter spp*, which suggests that quinolone resistance might be the effect of using these drugs in animals, as use of fluoroquinolones has been approved for animals in Asia.[26,27] Recent studies have shown that enrofloxacin (fluoroquinolone used in animal feed) can select *Salmonella* mutants resistant to nalidixic acid and ciprofloxacin.[28] Extensive use of nalidixic acid in humans can also lead to the emergence of resistance in the bacteria belonging to family *Enterobacteriaceae*, which could explain the fluoroquinolone resistance as well in non-typhoidal *Salmonella*.[28] Nalidixic acid is frequently used for treatment of bacillary dysentery in many Asian countries and subsequently this led to emergence of nalidixic acid resistance in Shigellae.[30-33] The extensive use of nalidixic acid in treatment of dysentery in developing countries might have exerted selective pressure for emergence of nalidixic acid resistance in non-typhoidal *Salmonella*, which then spread worldwide through international travel. However, there is no information available on the quantity of nalidixic acid used in this region for treatment of various human illnesses. Ciprofloxacin was introduced in this hospital only in early 2002 but resistance to this drug started appearing earlier, from 1999 onwards.

There is a significant increase in the trend of resistance to nalidixic acid and ciprofloxacin among non-typhoidal *Salmonella*. To reduce the selective pressure for development of resistance to quinolone and fluoroquinolone, these drugs should be used with some restraint and a continuous vigil should be maintained for the appearance of resistance to these drugs in Saudi Arabia.

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