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Short Communication

Emergence of Nalidixic Acid Resistance in *Shigella sonnei* Isolated from Patients Having Acute Diarrheal Disease: Report from Eastern Province of Saudi Arabia

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SUMMARY: During the 5 years of the study period (October 1999 - October 2003), 110 strains of *Shigella* were isolated from fecal samples of patients having acute diarrheal diseases. *Shigella sonnei* phase 1 was the most prevalent (88/110, 80.0%) serotype. Resistance to nalidixic acid was not encountered from 1999 - 2002. Nalidixic acid resistance was observed in 6/13 (46.1%) of the *S. sonnei* phase 1 strains isolated from April - August 2003. Minimum inhibitory concentration to nalidixic acid among these strains was 48 - 96 μg/ml. All the six nalidixic acid resistant strains of *S. sonnei* phase 1 had reduced susceptibility (MIC 0.25 μg/ml) to ciprofloxacin.

Shigellosis is one of the significant causes of diarrheal disease in the developing countries. Worldwide, approximately 165 million cases of shigellosis occur annually, the majority in developing countries, accounting for 1.1 million deaths (1). Appropriate antibiotic therapy for shigellosis reduces the severity, duration of symptoms, complications, and excretion of organisms (2). Emergence of multiple drug resistance to cost effective antibiotics against *Shigella* is a matter of concern for the health authorities in developing countries. Over the last three decades, multidrug resistant *Shigella dysenteriae* 1 was responsible for widespread epidemics of shigellosis on the Indian subcontinent and in other developing countries, leading to very high mortality (3-5). On the African continent, such epidemics were related to political upheaval in the refugee camps (6). Nalidixic acid remained the drug of choice for its highly proven efficacy for treatment of *Shigella dysenteriae* 1 infections, in the wake of the appearance of resistance to commonly used antibiotics such as ampicillin and trimethoprim/sulphamethoxazole (4). *Shigella flexneri* remains the predominant serotype endemic in developing countries, while the *Shigella sonnei* serotype is predominant and endemic in industrialized countries (1). *S. sonnei*, previously known to cause milder self limiting disease and to be less resistant to antimicrobial agents than other serotypes, has developed multidrug resistance and is responsible for outbreaks of clinically severe disease (1). The appearance of resistance to nalidixic acid in *S. sonnei* is a matter of disquiet for the treating physician and the microbiologists (7,8).

The present study endeavors to describe the prevalence of serotypes of *Shigella* and the sudden emergence of nalidixic acid resistance in *S. sonnei* during 2003 in the Al-Hasa region of Eastern Province of Saudi Arabia.

The study was carried out at the King Fahad Hospital and Tertiary Care Center (550 beds), Al-Hofuf, Al-Hasa, during the period from October 1999 - October 2003. Al-Hasa region is an oasis in the Eastern Province of Saudi Arabia, with a native population of approximately 1 million. The *Shigella* strains were isolated from the fecal samples of admitted patients or those attending the out patient department of the hospital, all of whom showed signs and symptoms of bacillary dysentery. Fecal samples were collected in transport medium (Amies Ltd., Aulabor, Barcelona, Spain) and cultured within 2 h of collection. These samples were cultured directly on Xylose Lysine Deoxycholate agar and MacConkey's agar (Oxoid Ltd., Basingstoke, Hampshire, UK). *Shigella* strains were biochemically identified by API20E system (Bio Merieux, Marcy Etoile Sa, France) and were serotyped using *Shigella* antisera (Murex Biotech Ltd., Dartford, UK). Antibiotic susceptibility was determined by disc diffusion technique according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) (9). Susceptibility testing of all the *Shigella* strains isolated during the study period from 1999 - 2003 was performed on Mueller Hinton agar (Oxoid) using the following concentrations (μg/disc) of antibiotics (Becton Dickinson Co., Cockeysville, Md., USA): ampicillin-25, amoxicillin/clavulanic acid-20/10, cephalothin-30, cefoxitin-30, cefotaxime-30, ceftriaxone-30, chloramphenicol-30, trimethoprim/sulphamethoxazole-1.25/23.75, gentamicin-10, amikacin-30, imipenem-10, aztreonam-30, piperacillin-100, nalidixic acid-30, and ciprofloxacin-5. Minimum inhibitory concentration (MIC) of *S. sonnei* strains for nalidixic acid and ciprofloxacin was determined by agar dilution method (9).

During the 5-year study period, 110 strains of *Shigella* were isolated, and out of these 88 (80.0%) belonged to serotype *S. sonnei* phase 1 and 22 (20.0%) were *S. flexneri*. *S. dysenteriae* and *Shigella boydii* serotypes were not encountered in this region. *S. sonnei* was the most prevalent serotype isolated throughout the study period (Table 1). Resistance to more than two antibiotics was observed in 8/88 (9.1%), and resistance to two antibiotics in 17/88 (19.3%) of the *S. sonnei* strains. Nalidixic acid resistance in *S. sonnei* was not encountered from 1999 - 2002; it appeared suddenly in 2003, when 6/13 (46.1%) of the isolated strains were detected to be resistant to nalidixic acid (Table 2). All these nalidixic acid

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Table 1. Serotypes of *Shigella* isolated from 1999-2003

Shigella	1999	2000	2001	2002	2003	Total (%)
Shigella sonnei	20	19	19	17	13	88 (80.0)
Shigella flexneri	8	4	4	4	2	22 (20.0)
Total	28	23	23	21	15	110

Table 2. Nalidixic acid resistance in *Shigella sonnei* phase 1 strains isolated from 1999-2003

Antibiotic resistance	1999	2000	2001	2002	2003	Total (%)
Sxt, A, NA	0	0	0	0	6	6 (6.8)
Sxt, A, C	1	0	1	0	0	2 (2.2)
Sxt, A	9	1	2	3	2	17 (19.3)
Sxt	5	14	5	4	1	29 (32.9)
Sensitive to all	5	4	11	10	4	34 (38.6)
Total	20	19	19	17	13	88

Sxt: trimethoprim/sulphamethoxazole, A: ampicillin,
C: chloramphenicol, NA: nalidixic acid.
0: no resistance encountered.

resistant strains of *S. sonnei* phase 1 were isolated from April-August 2003. Two of the patients from whom these strains were isolated were children (<5 years), and the other four were adults.

Resistance to ampicillin and to trimethoprim/sulphamethoxazole was observed among seven strains of *S. flexneri*, whereas six strains of *S. flexneri* were observed to be resistant only to trimethoprim/sulphamethoxazole and no nalidixic acid resistance was encountered. All the isolated *Shigella* strains were sensitive to cephalothin, cefoxitin, cefotaxime, ceftriaxone, gentamicin, amikacin, imipenem, aztreonam, ciprofloxacin, piperacillin, and amoxicillin/clavulanic acid. MIC to nalidixic acid of *S. sonnei* strains was 48 μg/ml in two strains and 96 μg/ml in four strains (NCCLS breakpoint <8 μg/ml). All six of the nalidixic acid-resistant strains of *S. sonnei* had reduced susceptibility (MIC 0.25 μg/ml) to ciprofloxacin (MIC of *Escherichia coli* ATCC 25922-0.01 μg/ml, NCCLS breakpoint <1 μg/ml), although these strains were susceptible to ciprofloxacin by disc diffusion test (zone diameter >21 mm). *Shigella* are pathogenic to humans only, thus the appearance of antimicrobial resistance such as that in non-typhoidal *Salmonellae* cannot be blamed on the overuse of antibiotics in animals. Resistance to first line drugs in *Shigella* has increased worldwide as a consequence of excessive use of these agents. The emergence of nalidixic acid resistance in *S. dysenteriae* 1 was disquieting, given that this agent was being used as first line drug treatment for shigellosis; this resistance was related to excessive use of this drug in widespread epidemics (4,10). Recently, nalidixic acid resistance has started appearing in *S. sonnei*. Although it is still uncommon, limited reports are available from developing countries. Nalidixic acid resistance in *S. sonnei* was reported in 94-100% of the strains isolated from 2001-2002 from India (8,11). However, it is very low (0-0.5%) among strains isolated from the USA, UK, Canada, and Germany (12). In the present study, no resistance to nalidixic acid in *S. sonnei* phase 1 strains was encountered from 1999-2002, then resistance suddenly appeared among the strains isolated in 2003. All the patients from whom these nalidixic acid-resistant strains were isolated were native Saudi, and they had no history of recent travel to countries in which such strains are prevalent. All 6/13 (46.1%) of these nalidixic acid-resistant strains of *S. sonnei* phase 1 were isolated during the summer months of April-August. Patients from whom these strains were isolated belonged to different areas of this region, and there was no concentration of these patients in one area. The drinking water supply in Saudi Arabia is safe and is procured by the consumer from reverse osmosis units which desalinate the underground water after chlorination. The climate in this region is dry and hot during the majority of the year and there are no flies. Excellent standards of food hygiene is maintained in the public eating places by law, and there is a well planned sewage system in the region. Despite these factors, shigellosis due to *S. sonnei* is endemic in the region, which could reflect direct human-to-human transmission. It appears that the sensitive strain of *S. sonnei* is being replaced by the nalidixic acid-resistant strain in this region under the selective pressure of this drug being used for treatment of diarrhea and other ailments such as urinary tract infections. The wide distribution and sudden appearance of nalidixic acid resistance and reduced susceptibility to ciprofloxacin in *S. sonnei* in this region gives rise to a growing quandary, which calls for vigilant and guarded use of nalidixic acid and ciprofloxacin in the treatment of shigellosis. Because *S. sonnei* is the most prevalent serotype in developed and industrialized countries, continuous surveillance should also be maintained in those regions on appearance of nalidixic acid resistance among these strains.

REFERENCES

1. Kotloff, K. L., Winickoff, J. P. and Ivanoff, B. (1999): Global burden of *Shigella* infections: Implications for vaccine development and implementation. Bull. WHO, 77,651-668.
2. Salam, M. A. and Bennish, M. L. (1991): Antimicrobial therapy for shigellosis. Rev. Infect. Dis., 13 (Suppl. 4), S332-S341.
3. Panhotra, B. R. and Bhavana, D. (1983): Resistant *Shigella dysenteriae* 1. Lancet, ii, 1420.
4. Bose, R., Nashipuri, J. N., Sen, P. K., Dutta, P., Bhattacharya, S. K., Dutta, D., Sen, D. and Bhattacharya, M. K. (1984): Epidemic dysentery in West Bengal: clinician enigma. Lancet, ii, 1160.
5. Dutta, S., Dutta, D., Dutta, P., Matsushita, S., Bhattacharya, S. K. and Yoshida, S. (2003): *Shigella dysenteriae* 1, Kolkata, India. Emerg. Infect. Dis., 9, 1471-1474.
6. Goma Epidemiology Group (1995): Public health impact of Rwandan refugee crisis: What happened in Goma, Zaire in July 1994. Lancet, 345, 339-344.
7. Dagan, D., Orr, N., Yavzori, M., Yuhas, Y., Meron, D., Ashkenazi, S. and Cohen, D. (2002): Retrospective analysis of the clonal outbreak of nalidixic acid-resistant *Shigella sonnei* shigellosis in Israel. Eur. J. Clin. Microbiol. Infect. Dis., 21, 887-889.
8. Gosh, A. R., Sugunan, A. P., Seghal, S. C. and Bharadwaj, A. P. (2003): Emergence of nalidixic acid-resistant *Shigella sonnei* in acute diarrhea patients on Andaman and Nicobar Island, India. Antimicrob. Agent. Chemother., 47, 1483.
9. National Committee for Clinical Laboratory (1997): Performance standard for antimicrobial disc susceptibility test: Approved standard. M2-A6, 6th ed. Naional Committee for Clinical Laboratory Standards, Wayne, Pa, USA.

10. Panhotra, B. R., Bhavana, D. and Sharma, P. L. (1985). Nalidixic acid resistant *Shigella dysenteriae* type 1. Lancet, i, 763.
11. Jesudason, M. V. (2002): *Shigella* isolation in Vellore, South India (1997-2001). Indian J. Med. Res., 115, 11-13.
12. Replogle, M. L., Fleming, D. W. and Cieslak, R. (2000): Emergence of antimicrobial resistant shigellosis in Oregon. Clin. Infect. Dis., 30, 515-519.