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Increase in Nalidixic Acid Resistance among *Salmonella enterica* Isolates Recovered from Patients in Tehran, Iran

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Abstract

Nalidixic acid resistance rate is increasing amongst *S. enterica* isolates worldwide. We described here an increase in nalidixic acid resistance among *S. enterica* isolates recovered in Tehran, in Iran. The study included all *Salmonella* isolates recovered from clinical cases in Tehran, Iran during 2006 and 2009. Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute. The statistical analysis was performed using SPSS.

One hundred thirty eight *S. enterica* isolates were collected; of those, 89 (64%) were

resistant to nalidixic acid. The percentage of isolates resistant to nalidixic acid was 59% (19/32) in 2006, 64% (34/53) in 2007, and 67% (36/53) in 2008. The most common multidrug resistance phenotype amongst nalidixic acid-resistant isolates was resistance to doxycycline, kanamycin, neomycin, streptomycin, sulfamethoxazole, and tetracycline. We found nalidixic acid resistance is increasing amongst nontyphoidal *S. enterica* isolates in our study. Over the past 3 years, there has been a significant increase in the proportion of nalidixic acid resistant *S. enterica*. Continued surveillance of antimicrobial resistance among *S. enterica* is needed to mitigate the increasing prevalence of nalidixic acid resistance among *Salmonella*.

Keywords: Nalidixic acid, *Salmonella*, fluoroquinolone.

Background

The strains of *Salmonella enterica* are amongst the most important causes of food borne disease worldwide (1,2). Although most *Salmonella* infections result in mild-to-moderate self-limiting gastroenteritis, severe infections, such as bacteremia and meningitis, may occur (3). Antimicrobial agents are not essential for the treatment of most *Salmonella* infections but may be lifesaving for patients with severe infections (4). The prevalence of resistance among *Salmonella* to several antimicrobial agents, including ampicillin and trimethoprim-sulfamethoxazole, has increased in recent decades (5).

Quinolones and fluoroquinolones are broad-spectrum antimicrobial agents that target DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV genes (*parE* and *parC*), and the resulting drug-enzyme-DNA complex inhibits DNA synthesis (6,7). Isolates with nalidixic acid resistance commonly have decreased susceptibility to ciprofloxacin (8).

Public health surveillance for resistance to nalidixic acid is useful in monitoring emerging fluoroquinolone resistance. Furthermore, fluoroquinolone treatment has failed for patients infected with nalidixic acid-resistant *Salmonella* (6,9,10). We described an increase in nalidixic acid resistance among nontyphoidal *S. enterica* isolates in Iran between 2006 and 2009.

Materials and Methods

The study included all *Salmonella* isolates recovered from clinical cases in Tehran, Iran during 2006-2009. The isolates were identified by the conventional biochemical methods and serotyped by slide agglutination with specific antisera (Staten Serum Institut, Copenhagen, Denmark).

Antimicrobial drug resistance was determined by using the disc diffusion method on Muller-Hinton agar (Merck, Darmstadt, Germany), according to the Clinical and Laboratory Standards Institute's (formerly NCCLS) recommendations (11) using disks (Oxoid, Basingstoke, Hampshire, United Kingdom) including amoxicillin-clavulanic acid (20+10µg), ampicillin (10µg), cefotaxime (30µg), ceftazidime (30µg), ceftizoxime (30µg), ceftriaxone (30µg), cephalothin (30µg), chloramphenicol (30 µg), doxycycline (30µg), gentamycin (10µg), kanamycin (30µg), nalidixic acid (30µg), neomycin (30µg), streptomycin (10µg), tetracycline (30µg), and trimethoprim-sulfamethoxazole (1.25+23.75µg). The organisms used for quality control were *Escherichia coli* (ATCC 25922; American Type Culture Collection) and *Staphylococcus aureus* (ATCC 25923). The statistical analysis was performed using SPSS, Version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Between 2006 and 2009, 138 nontyphoidal *S. enterica* isolates were collected; of those, 89 (64%) were resistant to nalidixic acid. The percentage of isolates resistant to nalidixic acid was 59% (19/32) in 2006, 64% (34/53) in 2007, and 67% (36/53) in 2008.

Of 89 nalidixic acid-resistant *Salmonella enterica* isolates serotyped, 41 (46%) were serotype Enteritidis, 37 (41%) serotype Infantis, 4 (4%) serotype Albany, 2 (2%) serotype Typhimurium, 2 (2%) serotype Muenchen, 2 (2%) serotype Hadar, and 1 (1%) serotype Haifa. The serotypes with the highest proportion of isolates that were nalidixic acid resistant were Infantis (92%; 37/ 40) and Enteritidis (71%; 41/57).

Discussion

Nalidixic acid resistance is increasing amongst nontyphoidal *S. enterica* isolates in Iran. Over the past 3 years, there has been a significant increase in the proportion of nontyphoidal *S. enterica* isolates that are resistant to nalidixic acid. Nalidixic acid resistance is noteworthy because fluoroquinolones are the most commonly used antimicrobial agent for the treatment of invasive *Salmonella* infections in adults in Iran, and fluoroquinolone treatment has failed for patients with nalidixic acid-resistant *Salmonella* infections (9,10,12). Resistance to nalidixic acid could be a good predictor for the emergence of fluoroquinolone resistance (13). Several studies have suggested that the use of fluoroquinolones in veterinary medicine contributes to the emergence and dissemination of nalidixic acid resistance in *Salmonella* amongst food animals, which may be transmitted to humans (10,14).

Nalidixic acid resistance in *Salmonella* has been previously reported from Iran and different parts of the world. Amir Mozafari N et al. reported that 24.4% of 45 *Salmonella* strains isolated resistant to nalidixic acid during 2005 and 2006 (15). This is in accordance with Hamidian M et al. (2009), who tested 129 *Salmonella* isolates in Iran and found that 45.7% of them were resistant to nalidixic acid (16) and Irajian G (2009) showed that 62% of 50 *Salmonella* strains isolated resistant to this antibiotic (17).

Stevenson et al. studied 12,252 nontyphoidal *Salmonella enterica* isolates in the United States during 1996 and 2003 and found that 1.6% were resistant to nalidixic acid. The percentage of isolates resistant to nalidixic acid was 0.4% in 1996, 0.9% in 1997, 1.4% in 1998, 1.1% in 1999, 2.5% in 2000, 2.6% in 2001, 1.8% in 2002, and 2.3% in 2003 (4). Akinyemi et al. showed that 51% of *Salmonella* isolates in Lagos, Nigeria were resistant to nalidixic acid during 1997 to 2004. The percentage of isolates resistant to nalidixic acid was 42% in 1997, 43% in 1998, 42% in 1999, 47% in 2000, 51% in 2001, 57% in 2002, 56% in 2003, and 59% in 2004 (18). Prats, et al. (2000) who were studying *Salmonellae*, found an increase in the rates of resistance to nalidixic acid from 0.1 to 11%, throughout their 7-year study (19).

Our findings and previous studies showed that the resistance rate of nontyphoidal *Salmonella enterica* isolates is increasing in Iran. Use of fluoroquinolones and other antimicrobial agents for humans also may contribute to transmission of nalidixic acid-resistant *Salmonella* to humans (20). The increasing prevalence of nalidixic acid resistance amongst *Salmonella* isolates should be considered in light of a modest concurrent decline in the incidence of *Salmonella* infections in Iran. Continued surveillance of antimicrobial resistance among nontyphoidal *S. enterica* are needed to mitigate the increasing prevalence of nalidixic acid resistance amongst *Salmonella*. Such an exercise also helps in identifying outbreaks of resistant organisms and the data generated can help in advocating appropriate antibiotic regimens.

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