

First CTX-M type β -lactamase-Producing and Ciprofloxacin Resistant *Salmonella* Infection Acquired by a Child in IRAN

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The frequency of multidrug-resistance (MDR) among *Salmonella* isolates has increased dramatically. Here we report the first case of CTX-M type β -lactamase producing ciprofloxacin-resistant *Salmonella* infection as a child in Iran. Antimicrobial resistance profile was determined according to the Clinical and Laboratory Standards Institute. A double disk synergy test was used in the production of extended-spectrum β -lactamases (ESBLs). PCR assays were carried out to detect blaTEM, blaSHV and blaCTX-M beta lactamase genes. The presence of class 1 integrons was investigated by PCR assays. The *Salmonella Enteritidis* isolate from the child was ciprofloxacin and extended-spectrum cephalosporins resistant and found to carry the blaCTX-M beta-lactamase gene and class 1 integrons, suggests that antimicrobial resistance determinants may be located on the same genetic unit of the class 1 integrons identified here. Resistance to ciprofloxacin and extended-spectrum cephalosporins, the drugs of choice for treating invasive salmonellosis, especially in children is a public health problem.

Keywords: Ciprofloxacin; blaCTX-M; Extended Spectrum β -lactamase; Multidrug Resistance; *Salmonella Enteritidis*

1. Introduction

Salmonella enterica is a common cause of the food borne gastroenteritis. Though non-typhoidal salmonellosis is self-limiting, but antibiotic therapy can be life saving in children, elderly, and immune compromised personals with systemic infections (1). Fluoroquinolones and third-generation cephalosporins are major antibiotics used for treatment of invasive salmonellosis (2). Increasing occurrence of antimicrobial resistant *Salmonella* strains have complicated the treatment process. In particular, detection of fluoroquinolon and expanded spectrum cephalosporin's resistant *Salmonella* strains is a newly emerging threat in many countries (3). In this study, we report a ciprofloxacin and expanded spectrum cephalosporin's resistant *Salmonella Enteritidis* isolated from a child with severe gastroenteritis. Resistance to the drugs of choice for severe salmonellosis, especially in children is of major clinical concern. This is the first report of emerging CTX-M type β -lactamase-producing ciprofloxacin resistant *Salmonella* isolated from a human clinical specimens.

2. Case Report

In April 2010, a one-year-old boy with acute abdominal

pain, fulminant diarrhea, nausea, vomiting and fever admitted to the pediatric infection diseases' ward due to gastroenteritis. The stool and blood specimens for culture were sent to the Microbiology Laboratory in Pasteur Institute of Iran. Direct microscopic examination of the stool specimens showed leukocytes. Culture of the stool was performed, and on the 3rd day of hospitalization identified as *Salmonella* species, was isolated from the two consecutive stool cultures. The identification of the isolated as *Salmonella enterica* was confirmed by conventional standard biochemical and serological tests (4). The isolate from the child was serotyped by multiplex PCR assays and confirmed using commercial antisera as described previously and found to be *Salmonella enterica* serotype Enteritidis (5). The antimicrobial resistance of *Salmonella Enteritidis* isolate to twenty-one antimicrobial agents was determined by agar disk diffusion, and the minimum inhibitory concentrations (MICs) of ciprofloxacin and ceftriaxone were evaluated by the broth-microdilution methods' accordance with the standards of the Clinical and Laboratory Standards Institute. The strain was found to be resistant to ampicillin, streptomycin, oxytetracycline, doxycycline, trimethoprim-sulfamethoxazole, chloramphenicol, cephalothin, ceftazidime, ceftriaxone,

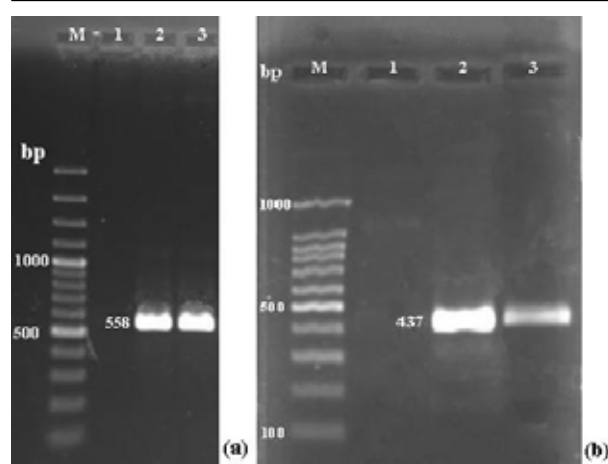
Implication for health policy/practice/research/medical education:

In the present study, we described the first report of ciprofloxacin resistant *Salmonella* infection acquired by a child in Iran.

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cefixime, cefotaxime, aztreonam, ciprofloxacin, and norfloxacin. Ciprofloxacin and ceftriaxone minimum inhibitory concentrations (MICs) values were 4 µg/mL and 128 µg/mL, respectively. The isolate was investigated for ESBL production using the double disk synergy test according to published methods (3). The genes responsible for the ESBL phenotype (bla_{TEM}, bla_{SHV}, bla_{CTX-M}) were identified by PCR assay using primers and conditions reported previously (3). By PCR assays the *Salmonella Enteritidis* isolate was found to carry class 1 integrons (Figure 1). PCR amplification conditions and primers described previously (2, 5).

Figure 1. PCR amplification of Class 1 Integrons *intI* and *SulI* Genes in *Salmonella Enteritidis* Isolated From an one-year-old Boy.



(A) Lane M: 1 kbp DNA ladder as the molecular size marker; lane 1: PCR mix with no template; lane 2: positive control; lane 3: the *intI* gene was detected in *Salmonella* isolate from child. (B) Lane M: 1 kbp DNA ladder as the molecular size marker; lane 1: PCR mix with no template; lane 2: positive control; lane 3: the *sulI* gene was detected in *Salmonella* isolate from child.

3. Discussion

Fluoroquinolones and extended-spectrum cephalosporins are commonly used to treat *Salmonella* infections. Bacterial resistance to these important drugs has dramatically increased. Animals and animal products are significant sources of resistant bacteria for the human population (6).

In this study, we have isolated a ciprofloxacin-resistant *Salmonella Enteritidis* from clinical specimens of a child with invasive salmonellosis. Previous study has reported ciprofloxacin-resistant in *Salmonella* strains isolated from poultry in our country (7). The enrofloxacin is main prescribed fluoroquinolone in treatment of bacterial infections in poultry in Iran (2). It is probable that the widely use of fluoroquinolones such as enrofloxacin in poultry, led to cross resistance against ciprofloxacin and

selection of ciprofloxacin resistance genes that transferred into the human. It is probable that ciprofloxacin and extended spectrum β-lactam resistant *Salmonella* strain identified here are transmitted to a child via contaminated egg.

Quinolone resistance in gram-negative bacteria is commonly due to mutations in quinolone target genes, DNA gyrase (*gyrA*) and topoisomerase IV (*parC*) and/or regulatory genes of outer membrane proteins or efflux pumps (8). Plasmid mediated quinolone/fluoroquinolone resistance determinants *qnrA*, *qnrB1-8*, *qnrS1-2* have since been described (9). In most studies was shown that *qnr* genes were accompanied by genes encoding extended-spectrum β-lactamases and often embedded in complex *sulI* type integrons (ESBLs). Here, the ciprofloxacin-resistant *Salmonella Enteritidis* strain, found to carry *sulI*-associated class-1 integrons and the bla_{CTX-M} beta-lactamase gene. Thus, the fluoroquinolone and extended-spectrum cephalosporins resistance determinants may be located on the same genetic unit of the class 1 integrons described here.

It is obvious that the use of antimicrobials will result in the selection of resistance strains. Accordingly, efforts must be made to reduce the use of certain antibiotics in animals, to delay the transmission of antibiotic-resistant *Salmonella* strains to human via food products.

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Authors' Contribution

All authors have participated equally in the study.

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