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Evaluation of Antibiotic Resistance to Fluoroquinolones and Third Generation Cephalosporines in Iranian Clinical Isolates of *Salmonella* spp.

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ABSTRACT

Salmonella enterica serotypes are one of the most important food borne pathogens and significant public health concerns around the world in humans and other animal species. A total of eighty three epidemiologically unrelated clinical isolates of *Salmonella enterica* serovars were subjected to antimicrobial susceptibility testing. Eleven isolates (13.1%) which were resistant to at least 4 groups of antimicrobial agents considered as multidrug resistant (MDR) *Salmonella* serovars. Emergence of MDR *Salmonella* serovars demonstrates that antimicrobial selection pressure is widespread in our clinical settings. According to the results of antimicrobial susceptibility testing, *Salmonella* clinical isolates are more susceptible to fluoroquinolones and third generation cephalosporins and these drugs may be used as drugs of choice to treat *Salmonella* infections.

1. Introduction

Salmonella enterica serotypes are one of the most important food borne pathogens and significant public health concerns around the world in humans and other animal species (Hopkins et al., 2005; Yildirim et al., 2011). Invasive *Salmonella* spp. infections can be fatal and antimicrobial treatment is essential in this situation (Yan et al., 2005). In the past the antibiotics used for the treatment of salmonellosis were chloramphenicol,

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sulfamethoxsazole-trimetoprim and ampicillin (Ling et al., 2003). The last two decades have witnessed the emergence and spread of multidrug resistance against conventional drugs among infections caused by *Salmonella* spp. especially in south and south-east Asia (Gaind et al., 2006; Dimitrov et al., 2007). Following the worldwide prevalence of multidrug-resistant strains, the use of conventional antibiotics has been discontinued and fluoroquinolones and third-generation cephalosporins as alternative drugs were used in the treatment of salmonellosis (Gaind et al., 2006). Unfortunately, since 1991, reports on the prevalence of resistant serotypes to fluoroquinolones and cephalosporins have been released in different countries (Su et al., 2004). In this study; the antibiotic resistance profile to fluoroquinolones and cephalosporins, the two important drug choices, in clinical isolates of *Salmonella* spp. was assessed.

2. Material and methods

2.1. Isolation and identification of bacteria

Salmonella spp. clinical samples were collected from different health centers through the years 2008-2010. A total of 83 *Salmonella* isolates were recovered from stool, blood, urine and articular liquid. All isolates were identified by standard microbiological techniques as previously described (Ahmed et al., 2009). The serogroup was checked with specific antisera by the slide agglutination method (Difco Laboratories, Detroit, MI).

2.2. Antimicrobial susceptibility test

Antibiotic susceptibility was determined by Kirby Bauer disk diffusion method on Muller Hinton agar and Minimum Inhibitory Concentrations (MICs) by broth micro dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2009).

Antibiotic disks prepared by MAST company (Mast Co, Merseyside, UK), included: ampicillin (10 µg), sulfamethoxazole-trimethoprim (30 µg), chloramphenicol (30µg), nalidixic acid (30µg), ciprofloxacin (5µg), norfloxacin (10µg), ofloxacin (5µg), levofloxcacin (5µg), gatifloxacin (5µg), enrofloxacin (5µg), moxifloxacin (5µg) and cephalosporins such as cefotaxime (30µg), ceftazidime (30µg), cefixime (5µg), ceftriaxone (30µg) and cefepime (30µg). The control strain used for all susceptibility tests was *Escherichia coli* ATTC 25922.

The minimum inhibitory concentrations (MICs) Was carried out for the ampicillin, chloramphenicol, trimetoprim, nalidixic acid, ciprofloxacin, ofloxacin and ceftazidim. To perform the tests, a series of tubes was prepared with Mueller Hinton broth medium to which various concentrations (1024-0.01µg/ml) of the antimicrobial agents were added. The tubes were then inoculated with a standardized suspension (1.5×10^8 cfu/ml) of the test organism. After incubation at 35± 2ºC, they were examined and the MIC was determined. The MIC was defined as the lowest concentration of the drug that completely visible growth after incubation.

Interpretive criteria for sensitive and resistant strains for nalidixic acid were MIC>32 µg/ml and MIC<16 µg/ml, respectively. For the ciprofloxacin and ofloxacin antibiotics, isolates were considered sensitive if the MIC was <0.5 µg/ml and resistant if the MIC was >1 µg/ml. Isolates were considered sensitive to ceftazidim if the MIC was <4 µg/ml and resistant if the MIC was >16 µg/ml. The breakpoins for ampicillin and chloramphenicol were MIC<8 µg/ml (susceptible) and MIC>32 µg/ml (resistant) and for trimetoprim MIC<2 µg/ml (susceptible) and MIC>4 µg/ml (resistant).

3. Results

3.1. Bacterial isolates

Out of 83 *Salmonella* spp. clinical isolates, 66 isolates (79.5%) of the stool, 8 isolates (9.63%) of the blood, 3 isolates (61/3%) of the bone marrow, 3 isolates (61/3%) of synovial fluid, 1 isolate (20/1%) of the abscess, 1 isolate (20/1%) of ascites and an isolated (20/1%) of urine were obtained. They consisted of 50 female (62/3%) and 33(37/6%) male. A total of 83 *Salmonella* isolates including *S.typhi* (n =39), *S.paratyphi* A (n=2), *S.paratyphi* B (n=14), *S.paratyphi* C (n=18) and *S.entritidis* (n=10)

were studied. Out of 39 serotype Typhi, 26 isolates from the stool, 7 isolates from the blood, 2 isolates from the bone marrow, 2 isolates from the synovial fluid, 1 isolate from the urine and one was isolated from the abscess. 11 serotypes of *S.paratyphi* B were obtained from stool, 1 isolate of ascites, 1 isolates of bone marrow and 1 isolates of the synovial fluid. Of total *S.entritidis* serotypes; 9 strains were isolated from stool and one was obtained from the blood. All isolates of serotype Paratyphi A and Paratyphi C were isolated from stool.

3.2. Disk diffusion and Minimum Inhibitory Concentration (MIC)

According to the disk diffusion results and minimum inhibitory concentration (MIC), antimicrobial resistance patterns were: 21.6% of isolates were resistant to sulfamethoxazole-trimethoprim, 14.4% to chloramphenicol, 79.5% to ampicillin, 56.6% to nalidixic acid, 4.8% to ciprofloxacin, 7.2% to cefotaxime, 9.6% to cefexime, 7.2% to ceftriaxon, 10.8% to ceftazidime. All the isolates were sensitive to ofloxacin, levofloxacin, norfloxacin, gatifloxacin, moxifloxacin. Of 83 isolates only 4 isolates (4.8%) were sensitive to all of the tested antimicrobial agents.

3.3. Identification of Multidrug Resistant (MDR) Isolates

Multidrug resistance (MDR) was defined as resistance to at least 4 groups of antimicrobial agents. Of the 83 isolates, 10.8 % of isolates were considered as MDR *Salmonella* serovars. These MDR isolates were *S. typhi* (n=4), *S.paratyphi* C (n=3) and *S. paratyphi* A (n=1). Two of the MDR isolates were recovered from the bone marrow and seven MDR isolates were originated from stool. The results of antibiotic resistance among serotypes are shown in Table 1.

Table 1. Antibiotic resistance among *Salmonella* spp. clinical isolates based on MIC determination

	No. of resistant isolates (%)										
	AMP a	CHL	SXT	CTX	CAZ	CFM	CRO	FEP	NAL	CP	AMP a
S.typhi	31(37%)	5(6%)	5(6%)	2(2%)	4(5%)	4(5%)	2(2%)	2(2%)	26(31%)	3(4%)	31(37%)
S.paratyphi A	2(2%)	1(1%)	0	1(1%)	0	0	1(1%)	1(1%)	1(1%)	0	2(2%)
S.paratyphi B	10(12%)	3(4%)	4(5%)	0	2(2%)	0	1(1%)	1(1%)	4(5%)	1(1%)	10(12%)
S.paratyphi C	16(19%)	9(11%)	1(1%)	3(4%)	2(2%)	4(5%)	2(2%)	2(2%)	11(13%)	0	16(19%)
S.entritidis	7(8%)	0	2(2%)	0	1(1%)	0	0	0	5(6%)	0	7(8%)
All Serotypes	66(79%)	18(21%)	12(14%)	6(7%)	9(10%)	8(9%)	6(7%)	6(7%)	47(56%)	4(4%)	66(79%)

a Abbrevation of mentioned antibiotics: AMP, ampicillin; CHL, chloramphenicol; SXT, sulfamethoxazole-trimethoprim; CTX, cefotaxime; CAZ, ceftazidime; CFM, cefepime; CRO, cefteriaxone; FEP, cefepime; NAL, nalidixic acid; CP, ciprofloxaci

4. Discussion

The increased level of antimicrobial resistance observed in *Salmonella* spp. has become a public health issue (Hur et al., 2011). Surveillance data demonstrated an obvious increase in overall antimicrobial resistance among *Salmonella* from 20%–30% in the early 1990s to as high as 70% in some countries at the turn of the century (Su et al., 2004). Due to the increased resistance to conventional antibiotics, extended-spectrum cephalosporins and fluoroquinolones have become the drugs of choice for the treatment of infections caused by multidrug-resistant *Salmonella* serotypes (Lynne et al., 2009). In 2000, a multiple–health care center survey in 10 European countries identified a cefotaxime resistance rate of 0.6% in *Salmonella* isolates recovered from human sources. Such a trend of increase in drug-resistant *Salmonella* was also noted in some Asian countries, including Taiwan. In the United States, a national survey conducted in 1994–1995 found that, of the 4008 *Salmonella* isolates tested, 21 (0.5%) were resistant to nalidixic acid and 1 (0.02%) was resistant to ciprofloxacin (MIC, 4 mg/ml); by 2000, the rate of nalidixic acid resistance had increased 5-fold, to 2.5% (Su et al., 2004). In this study, of 83 isolates only 4 isolates (4.8%) were sensitive to all of the tested antimicrobial agents. Resistance to conventional

antibiotics such as ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim were 79.5%, 14.4% and 21.6%, respectively. Therefore these antibiotics are not the drugs of choice in the treatment of *Salmonella* infections anymore.

In the survey conducted in 2008 in China, all isolates were susceptible to cefotaxime and ceftazidime; 5 isolates obtained in 2004 were intermediately susceptible to cefepime (MIC 16 μg/ml) (Cui et al., 2008). In this study, the resistance pattern of isolates to cephalosporin antibiotics was as follow: 7.2% to cefotaxime, 9.6% to cefexime, 7.2% to ceftriaxon, and 10.8% to ceftazidime.

With the large-scale use, and often mistreatment, of antimicrobials over the time, fluoroquinolone and extended-spectrum cephalosporin resistant *Salmonella* isolates have been recognized in numerous locations with variable frequency including the USA, Taiwan and Hong Kong. High prevalence of fluoroquinolone-resistant *Salmonella* has been seen in the outpatient. In a study conducted in China was a high prevalence of fluoroquinolone-resistant *Salmonella* has been seen in the outpatient (Cui et al., 2009).

Our finding showed that among 47 resistant isolates to nalidixic acid, 25 isolates have decreased susceptibility to ciprofloxacin (MIC≥ 0.125μg/ml). Nalidixic acid resistance has to be considered as a marker for screening of strains with reduced susceptibility to ciprofloxacin (Whichard et al., 2007). It can be concluded that the strains with reduced susceptibility to ciprofloxacin are prone to be resistant to these antibiotics. Of 83 isolates, 3 isolates were resistant to ciprofloxacin. Susceptibility was high in other fluoroquinolones. The susceptibility of levofloxacin, norfloxacin and gatifloxacin was reported in more than 95% and there were no resistant isolates for these antibiotics. Dimitrov et al. showed that of the 135 *Salmonella enterica* serotypes Typhi and Paratyphi A isolated from patients, 50 (37%) were MDR and 94 (69.6%) isolates of both serotypes were nalidixic acid resistant (NAR). Between 90 and 100% of MDR and NAR strains had decreased susceptibility to ciprofloxacin (0.125–1μg/ml). Low-level resistance to ciprofloxacin (MIC 0.125-1μg/ml) was also detected in 13.8 and 33.3% of nalidixic acid-susceptible isolates of *S.typhi* and *S.paratyphi* A, respectively (Dimitrov et al., 2007). In India, Nalidixic-acid resistant strains were observed in 51% of the isolates, of which 98.9% was shown to have decreased susceptibility (MIC≥0.125-1 μg/ml) to ciprofloxacin (Capoor et al., 2007).

Decreased susceptibility to quinolones (nalidixic acid MIC >32 μg/ml or ciprofloxacin MIC >0.12 μg/ml) and extended-spectrum cephalosporins (ceftiofur or ceftriaxone MIC>2 μg/ml) was identified in the United States during 1996–2004 (Dimitrov et al., 2007).

Data of a 5-year survey, from 2005 to 2009, of antimicrobial drug resistance among 858 clinical isolates of *Salmonella enterica* serovar typhimurium in Slovakia, revealed an increasing trend of resistance to nalidixic acid from 0% in 2005 to 11% in 2009 (Majtan et al., 2010). Ashtiani, et al. found an increase in the rates of resistance to nalidixic acid (from 9.2 to 42.3%) and ceftazidime (from 3 to 23.4%) among *Salmonella* spp. between 2001-2005 (Ashtiani et al., 2010).

One of the reasons for the emergence of antibiotic resistance in *Salmonella* might be attributed to uncontrolled use of antimicrobial agents as growth promoters or in treatment of bacterial infections of livestock and poultry. The use of antibiotics for growth promotion has been banned in European Union but permitted in USA and Canada and most of the rest of the world (Yildirim et al., 2011). Since high costs spending on treating infections, establishment of standardized monitoring systems for determining the occurrence of resistance among food of animal origin is also needed.

Ultimately, a better understanding of the genetics and distribution of antimicrobial resistance will allow for better measures to minimize the burden of antimicrobial resistance on health and well-being. To design new antimicrobial agents that provide effective therapy for infections caused by organism resistant to older agents, we have to understand the mechanism responsible for drug resistance in older agents.

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