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Antimicrobial Resistance of Salmonella enterica Strains Isolated from Diarrheal Children in Tehran

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ABSTRACT

Background: Salmonellosis is a common bacterial disease that affects the intestinal tract. *Salmonella Enteritidis* (SE) is the predominant cause of the food-borne salmonellosis in humans. The aim of this study was to investigate antimicrobial resistance of *Salmonella enterica* isolates detected in stool samples taken from infected children in Tehran, Iran.

Methods: Stool samples of patients with diarrhea in pediatric hospital in Tehran were collected, from June 2017 to May 2018. Isolation and identification carried out by conventional methods and susceptibility testing were performed according to the standard procedure of the Clinical and Laboratory Standards Institute (CLSI). The ERIC-PCR was used to study the genetic relatedness of the isolates.

Results: Of 800 samples, 24 were identified as *Salmonella* species with 14 different antibiotypes. The dominant strain was *S. enteritidis* 12 (50%).

***Conclusion*:** Our findings showed that *S. enteritidis* was the most frequent isolate among salmonellosis in Tehran.

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Introduction

Non-typhoidal *Salmonella* serovars are pathogens of both human and animals (1). According to the WHO reports, there are about 17 million cases annually of acute gastroenteritis or diarrhea due to non-typhoidal salmonellosis with 3 million deaths (2). Both meat and eggs are known to be a source of human pathogens such as *Salmonella*. (3). Due to the importance of *Salmonella* in food borne diseases, several typing methods have been used for surveillance of foodborne in trace-back of infections to food sources (4). Gastroenteritis due to *Salmonella* is typically self-limited and usually does not require antibiotic therapy among immunocompetent persons, but some immunocompromised patients such as the HIV-positive, diabetics or rheumatoid arthritis have an increased risk of developing bacteremia, this population may benefit from antibiotics to minimize the occurrence of complications (5,6). A number of different DNA-based typing methods such as plasmid profiling, biotyping, Ribotyping, IS200 profile, Pulsed field gel electrophoresis (PFGE), and Multilocus enzyme analysis have been used to identify *Salmonella* serovars. Overall, these approaches have provided useful insights into the evolutionary and epidemiological relationships of strains of several *Salmonella* serovars (7, 8). However, each method has its own advantages and limitations (9).

The basis of REP-PCR is on primers which are complementary to the short repetitive sequence elements along the target sequence. In case of bacteria, the amplification of these elements generate DNA fragments that may produce fingerprints with high discrimination degree between bacterial species or even strains (2, 10, 11). The current study was undertaken to investigate the molecular characterization and antimicrobial resistance pattern of *Salmonella enterica* strains isolated from diarrheal patients in Tehran, Iran.

Materials and Methods

Samples

Total of 800 rectal swabs samples from children under 5 years of age admitted to the Children's Medical Centre Hospital in Tehran, were taken from June 2017 to May 2018. After overnight incubation of inoculated Hektoen enteric agar (HEK) and Xylose Lysine Deoxycholate (XLD) plates (Himedia Laboratories Corporate Office, Mumbai, India) at 37°C, suspected colonies were taken and identified by their biochemical tests (11,12). In the present study, 10cc blood sample was drawn from patients with AML voluntary (n=10) as well as ten healthy people as control group from Imam Khomeini Hospital (Tehran, Iran).

Phenotypic detection

There was no pre-designed primer for *Salmonella paratyphi* A, B, C, and senftenberg, so serotyping for these serovars carried out by classical methods and, for other serovars, PCR was performed. Serotyping of the isolated *Salmonella* strains was performed by commercially reliable antisera (Difco, Detroit, USA), and the results were interpreted according to the Kaufmann-White scheme (Popoff and Le Minor, 1992) (13). The *Salmonella* isolates were first biochemically identified and then serotyping of strains was performed using the available antisera: O (B, D, E, C) and H according to the slide and tube agglutination tests (14). Antimicrobial susceptibility was determined by disc diffusion method according to CLSI guidelines and *Salmonella enteritidis* ATCC 23564 was used as a control (15). The following antibiotics, amoxicillin (20 µg), cefotaxime (30 µg), ceftazidim (30 µg), ceftriaxone (30 µg), cephalexin (30 µg), gentamicin (10 µg), streptomycin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), colistin sulfate (25 µg), chloramphenicol (30 µg), nitrofurantoin (300 µg), co-trimoxazole (25 µg), tetracycline (30 µg), imipenem (10 µg) and

meropenem (10 µg) (Bootle, Mersey side, Mast UK) were used.

Molecular detection

Each bacterial colony was grown on Tripticase soy agar overnight, and template DNA was purified using a commercial kit (Bioneer, Seoul, Korea). PCR was performed by commercial kit (Qiagen Mississauga, Ontario, Canada). Primer pairs for enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), as a well-known Repetitive element PCR fingerprinting (REP-PCR) protocol for genotyping of enterobacteria, were: ERIC-1R (5'-ATGTAAGCTCCTGGGGAT TCAC-3,) and ERIC-2 (5'-AAGTAAGTGACTG GGGTGAGCG-3'). PCR mixture contained hot start Taq plus Master Mix 12.5 µl, 20 pmol of above primers, 3 µL of template DNA, 2.5 µL buffer and 5 µl ultra-pure water (final volume 25 µL). The DNA fragments were amplified as follows: one cycle at 94°C for 5 min, 30 cycles of 94°C for 30 sec, 53°C for 1 min and at 72°C for 2 min and the final extension at 72°C for 7 min (16, 17). After reactions, 10 µl of PCR products were separated on 1.5% agarose gel, electrophoresed at 70 V for 2 hours and stained with ethidium bromide.

Results

Prevalence of serovars

The diversity of *Salmonella* species was as follows: *S. enteritidis* 5 (21%), *S. paratyphi* C 3 (13%), *S. paratyphi* B and 2 (8 %) *S. arizonae* 2 (8 %) and *S. paratyphi* A respectively.

Antimicrobial assay

About 75% of isolates were resistant to at least one antibiotic. The resistance rate of nitrofurantoin and nalidixic acid were 100% and 70.8%, respectively. The resistance rate of clinical samples to amoxicillin and tetracycline were 12.5% and 25%, respectively. All the isolates were susceptible to cephalexin, ceftriaxone, ceftazidim, cefotaxim, ciprofloxacin, gentamycin, imipenem and meropenem (Table 1). The most common anti-biotype was AB1 (33.3%), which was indicative of resistance to nitrofurantoin. The second antibiotype was AB2 (25%) which showed a nitrofurantoin-nalidixic acid resistance phenotype.

Discussion

In recent years, *Salmonella enterica* subsp. *enterica* serovar *enteritidis* has been a major cause of gastroenteritis and food poisoning, worldwide (18, 19). Recent reports from India and Spain showed *Salmonella enterica* as the most frequent serotype with incidence of 9.86% and 15.15 %, respectively (20,21).

Antibiotics are among the most important components related to animal feed production. Generally, the use of antibiotics in animals is for the treatment which may also cause various side effects such as transfer of antibiotic resistant bacteria to humans (22, 23). Most of the antimicrobials use in livestock can lead to development of antimicrobial residues in food products leads to drug resistance of bacteria. It is known that raw meat and poultry are vehicles for *Salmonella* transmission which may be a serious risk of salmonellosis outbreaks (24, 25). The use of animal waste for enhancing soil fertility is a potential danger in the spread of *Salmonella*. It has been shown that they can survive in the soil at least 14 days after the application of the slurry (2, 26). Genotyping of the bacterial isolates are essential for epidemiological surveillance and outbreak investigation. (11, 21). We observed that ERIC-PCR was useful enough in differentiation of the isolates, in accordance with the report by Suh and Song (16). Some of other investigators reported the acceptable discriminative ability of such REP-PCR protocols for genotyping of *Salmonella* subspecies (7, 27).

Table 1. Frequency distribution of common *Salmonella enterica* serovars by clinical symptoms.

	Species prevalence Number (%)						
Clinical symptom	*S. enteritidis*	*S. paratyphi A*	*S. paratyphi B*	*S. paratyphi C*	*S. arizonae*	Total	P value
dysentery	2(%100)[a]	0(%0)	0(%0)	0(%0)	0(%0)	2(%100)	
Non-bloody diarrhoea	10(%45.5)[b]	2(%9.1)	3(%13.6)	5(%22.7)	2(%9.1)	22(%100)	0/183
Vomiting	10(%50)[c]	2(%10)	2(%10)	4(%20)	2 (%10)	20(%100)	0/002
Nausea	12(%50)[d]	2(%8.3)	3(%12.5)	5(%20.8)	2(%8.3)	24(%100)	0/031
Fever	8(%44.4)[e]	2(%11.1)	3(%16.7)	4(%22.2)	1(%5.6)	18(%100)	0/003
Headache	8(%47)[f]	2(%11.8)	2(%11.8)	4(%23.5)	1(%5.9)	17(%100)	0/003
Abdominal cramps	11(%50)[g]	2(%9.1)	3(%13.6)	5(%22.7)	1(%4.6)	22(%100)	Pv<0.001

[a] represents the number and the percentage of patients involved with dysentery. [b] represents the number and the percentage of patients involved with Non-bloody diarrhoea.[c] represents the number and the percentage of patients involved with vomiting.[d] represents the number and the percentage of patients involved with nausea.[e] represents the number and the percentage of patients involved with fever.[f] represents the number and the percentage of patients involved with headache.[g] represents the number and the percentage of patients involved with abdominal cramps.

Table 2. Different antibiotypes in clinical sources isolates.

Antibiotype (AB)	CTX	A	T	NI	TS	S	C	CRO	GM	CO	CAZ	CIP	CFX	NA	MEM	IMI	Resistance (%)
AB_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	33.3%
AB_2	S	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	25%
AB_3	S	S	S	R	S	S	S	S	S	S	S	S	S	I	S	S	8.33%
AB_4	S	R	R	R	R	S	S	S	S	S	S	S	S	R	S	S	8.33%
AB_5	S	S	R	R	R	R	S	S	S	S	S	S	S	R	S	S	4.2%
AB_6	S	S	R	R	S	I	S	S	S	S	S	S	S	S	S	S	4.2%
AB_7	S	S	I	R	S	S	S	S	S	S	S	S	S	R	S	S	4.2%
AB_8	S	S	I	R	S	S	I	S	S	S	S	S	S	R	S	S	4.2%
AB_9	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	4.2%

CTX:Cefotaxime, A:Amoxycillin, T:Tetracyclin, NI: Nitrofurantion TS: Co-trimoxazole, S: Streptomycine, C: Chloromphenicol, CRO: Ceftriaxone ,GM: Gentamicine, Co: Colistin sulfate, CAZ: Ceftazidime, CIP: Ciprofloxacin, CFX: Cephalexin, NA: Nalidixic acid, MEM: Meropenem , IMI: Imipenem.

Conclusion

Salmonella enteritidis showed various rate of susceptibility for different antibiotics. Finding of infection origin and source is a one of the important applications of subtract of strains in epidemiological studies that we can use to improve infection control strategies.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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