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Frequency of Typhoidal and Non-Typhoidal Salmonella Species and Detection of Their Drugs Resistance Patterns

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Abstract

Background: To determine the prevalence of typhoidal and non-typhoidal *Salmonella* species in Hamadan City, west of Iarn and detection of antibiotic susceptibility patterns of isolates.

Methods: In a cross-sectional descriptive study, 296 *Salmonella* species including 192 strains of typhoidal *Salmonella* and 104 strains of non- typhoidal *Salmonella* were examined for serotyping and determining of antibiotic susceptibility. The strains were collected from patients referred to cilinical centers in Hamadan during 2001 to 2004. They were serotyped and then tested for their antibiotic susceptibility patterns, using Stokes disc diffusion method for 8 antibiotics.

Results: Among 296 samples, 64.8% were typhoidal and 35.2% were non-typhoidal *Salmonella* species. Typhoidal *Salmonella* species were as follows: *S. typhi* 45.6%, *S. paratyphi B* 8.1%, *S. paratyphi C* 7.1% and *S. paratyphi a* 4.7%. Non-typhoidal *Salmonella* species were as follows: *S.typhimurium* 21.2%, *S.enteritidis* 4.4%, S. species 2.1%, *S. cholerasuis* 1.7%, *S. arizona* 1.3%, *S. agona* 1.1%, *S. thompson* 0.7%, *S.muenchen, S.lexington* and *S. hirschfeldii* 0.35%. A proportion of strains (>60%) were resistance to cefotaxime and ampicillin. Resistance to ciprofloxacin and amikacin was very low (<15%). *S.typhimurim* (100%), *S. typhi* (95.7%) *S. paratyphi B* (89.2%) and *S. enteitidis* (60%) showed multi-drug resistance.

Conclusion: *S. typhi* and *S. typhimurium* were the most predominant serotypes in this area. Most of the *Salmonella* species isolated from patients were resistant to beta-lactam antibiotics and co-trimoxazole, whereas, most of them were sensitive to ciprofloxacin, gentamicin and amikacin. As the prevalence of multidrug-resistant serovar Typhi increases, newer, more expensive, and less readily available antimicrobial agents will be required for the treatment of typhoid.

Keywords: Antibiotics, *Salmonella typhi*, *prevalence, Iran*

Introduction

The epidemiological studies indicate that the incidence of salmonellosis is increasing throughout the world (1-3). *Salmonella enterica* serovar Typhi (*S.enterica* ST) is the etiological agent of typhoid fever, of which there are an estimated 16 million cases with 600,000 related deaths worldwide (4). This gram-negative rod-shaped bacterium is pathogenic only in humans, where it can be cultured from blood and stools. Infection occurs when water or food contaminated with *S. enterica* ST is consumed. Most patients who recover from the infection are able to eliminate the bacterium completely from their bodies. However, some of them may remain as healthy carriers, continuously shedding *S. enterica* ST in their stools (5). Typhoid fever manifests as a prolonged illness, and it is a sometimes-fatal infection in both adults and children if it results in inflammatory destruction of intestines and other organs, including the bone marrow (1, 6).

S. typhi and *S. para-typhi* are causes of typhoidal and para-typhoidal infections, whereas non-typhoidal *Salmonella* with 2300 serotypes (for example *S. typhimurium, S. thompson*,

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S. muenchen, S. lexington and *S. hirschfeldii*) are causes of non-typhoidal infections such as gastroenteritis and septicemia (1-2). *S. typhi* and *S. paratyphi A, B and C* are the predominant types of *Salmonella* responsible for enteric fever; particularly in the summer (2). Non-typhoidal *Salmonella* strains are usually isolated from animal sources (1, 2).

The resistance of to *Salmonella* species commonly prescribed antibiotics is increasing both in developing as well as in developed countries (7). Resistance has emerged even to newer, more potent antimicrobial agents. The development of resistance to multiple drugs is a major problem in the treatment of salmonellosis. *Salmonella* species have the ability to become resistant to multiple different drugs. Strains of *Salmonella* that are resistant to more than two antibiotics, assigned as multi-drug resistance (MDR) (7, 8). They have done this via several mechanisms: increased mutation rate as a stress respons, transfer copies of DNA that codes for a mechanism of resistance to other bacteria by plasmids, decreased cell wall permeability to antibiotics, enzymatic deactivation of antibiotics, efflux mechanisms to remove antibiotics and altered target sites of antibiotic (6-8).

Drug resistance is of considerable importance to microbiologists and is posing a major therapeutic problem for the public and for public health authorities (8, 9). Resistance to commonly used antibiotics, such as chloramphenicol, ampicillin, and co-trimoxazole has been reported from different parts of world in the last two decades (10). In the recent past, fluoroquinolones and cephalosporins have gained importance for the treatment of salmonellosis (11-13). The antibiotics of choice for treatment infections caused by most *Salmonella* species are chloramphenicol, amoxicillin, ciprofloxacin, co-trimoxazole and third-generation cephalosporin. There are many reports from many parts of the world especially in developing countries indicate that different types of *Salmonella* species have increasingly become resistant to the common antibiotics used in therapy, including chloramphenicol, amoxicillin and co-trimoxazole (11-15).

The objectives of this study were determination of prevalence of typhoidal and non-typhoidal *Salmonella* species in Hamadan City, west of Iran and detection of antibiotic resistance patterns of isolates.

Materials and Methods

In a descriptive cross-sectional study, 296 *Salmonella* species including 192 strains of typhoidal *Salmonella* (T.S) and 104 strains of non- typhoidal *Salmonella* (N.T.S) were examined for serotyping and determining of their antibiotic susceptibilities. The strains were collected from patients referred to clinical centers (Sina and Ekbatan hospitals) in Hamadan City, during 2001 to 2004. They were serotyped and then tested for their antibiotic resistance patterns, for 8 antibiotics. The required data including sex, age, type of specimens and type of isolates were gathered through questionnaire and analyzed using SPSS statistical software.

The samples including stool, urine and blood were collected and transferred to Medical Microbiology Laboratory of Hamadan University of Medical Sciences, immediately. The faecal samples were collected in Cary-Blair transport medium and were then inoculated into an enrichment medium (Selenite F broth) and incubated at 37^{o} C for 24 h. The specimens were cultured again onto selective medium *Salmonella-Shigella* agar (S-S agar) and incubated for 24-48 h. The sediment of 10 ml of urine of patients was also cultured directly onto S-S agar. The 10 ml of blood of the patients suspected to enteric fever (typhoid or paratyphoid) was drawn and inoculated into commercially broth media (Triptycase Soy Broth) and incubated at 37^{o} C for 48 h. Blood cultures suspected of being positive (turbitidy of broth media) were subcultured onto S-S agar. Black colonies on S-S agar with urease negative reaction were isolated and eventu-

ally were identified by biochemical tests and antisera method (16-17). Biochemical tests were as follows: Indole production, methyl red reaction, urease production, H_2S production, TSI reactions, VP reaction, motility test and phenylalanine deaminase production.

Serotyping of strains was performed by Biomerieux polyvalent and monovalent antisera commercially available (made in France). The samples were serotyped by anti O, anti H and anti Vi antisera. Serotyping of organisms was performed on slid glass (16). Single colonies of isolates were tested serologically by slide ag-glutination with specific antisera against either typhoid or non-typhoid *Salmonella* strains. A suspension of the organisms in physiologic saline was tested for autoagglutination before antiserum was added.

To detection of the susceptibility of isolates to routine antimicrobial drugs, all isolates were tested by disk diffusion method using guidelines established by the NCCLS (18, 19). A total of 8 selected antibiotic disks (Mast Group LTD, UK) including cefotaxime (CX, 20 μg), chloramphenicol (CH, 30 μg), ciprofloxacin (CP, 5 μg), amikacin (AN, 30 μg), ceftizoxime (CT, 20 μg), gentamicin (GM, 30 μg), ampicillin (AM, 10 μg) and cotrimoxazole (COT, 25 μg) were applied in the test. Isolates were cultured onto Mueller Hinton Agar medium with an inoculum of 10^5 organisms/ml, followed by 24 h incubation and interpreted using a standard protocol (19). The or-ganisms used for quality control were *E. coli* (ATCC 25922; American Type Culture Collection) and *Staphylococcus aureus* (ATCC 25923).

Those strains of *Salmonella* showed resistance to two or more antibiotics, assigned as MDR.

Results

Among 296 persons who were positive for *Salmonella* species, 60.4% (178) were male. The range of age groups of participants who had positive culture was between 1 to 68 yr old. Among 296 samples, 192 cases (64.8%) were typhoidal and 104 cases (35.2%) were non-typhoidal *Salmonella* species. Typhoidal *Salmonella* species were as follows: *S.typhi* 45.6%, *S. paratyphi B* 8.1%, *S. paratyphi C* 7.1% and *S. paratyphi A* 4.7%. Non-typhoidal *Salmonella* species were as follows: *S. typhimurium* 21.2%, *S.enteritidis* 4.4%, S. species 2.1%, *S. cholerasuis* 1.7%, *S. arizona* 1.3%, *S. agona* 1.1%, *S. thompson* 0.7%, *S. muenchen, S. lexington* and *S. hirschfeldii* 0.35%.

A proportion of strains (>60%) were resistance to cefotaxime and ampicillin. More than 40% of typhoidal *Salmonella* were resistant to chloramphenicol and co-trimoxazole. Resistance to ciprofloxacin and amikacin were very low (<15%). Table 1 shows the antibiotic resistance of the typhoidal *Salmonella* strains isolated from patients. Most isolates were resistant to ampicillin, co-trimoxazole and cefotaxime, and were sensitive to amikacin, gentamycin and ciprofloxacin. Table 2 shows the antibiotic resistance of the non-typhoidal *Salmonella* strains isolated from patients. Most isolates were also resistant to ampicillin, ceftizoxim and cefotaxime, and were sensitive to amikacin and ciprofloxacin. Table 3 shows antibiotic resistance patterns of typhoidal and non-typhoidal *Salmonella* species to various antibiotics.

Table 1: Frequency of antibiotic resistance Typhoidal *Salmonella* species

Antibiotics* / Isolates	CH %	CX %	CP %	AN %	CT %	GM %	AM %	COT %
S. typhi	27	64	2	6	39	7	72	17
S. para-typhi B	60	68	3	9	32	14	86	25
S. para-typhi C	43	33	0	1	2	2	52	13
S. para-typhi A	21	28	0	14	36	7	43	7

* :CH= Chloramphenicol**,** CX= Cefotaxime, CP= Ciprofeloxacin, AN= Amikacin, CT=Ceftizoxim, GM= Gentamycin, AM= Ampicillin, COT= Co-trimoxazole

Table 2: Frequency of antibiotic resistance in Non-typhoidal *Salmonella* species

Antibiotics* / Isolates	CH %	CX %	CP %	AK %	CT %	GM %	AM %	COT %
S. typhimurium	54	86	5	11	75	36	91	78
S. enteritidis	20	26	0	0	20	6	53	47
S. cholerasuis	11	22	11	0	33	0	55	44
S. agona	14	14	0	0	14	0	28	28
S. arizona	33	0	0	0	17	17	50	17
Other *Salmonella*	20	40	0	20	40	0	60	0

*: CH= Chloramphenicol, CX= Cefotaxime, CP= Ciprofeloxacin, AN= Amikacin, CT=Ceftizoxim, GM= Gentamycin, AM= Ampicillin, COT= Co-trimoxazole

Table 3: Antibiotic resistance patterns of Typhoidal and Non-typhoidal *Salmonella* species to various antibiotics

Drug Resistance Patterns	*S. typhi* (135)	*S.para-B* (24)	*S.typhimurium* (57)	*S.enteritidis* (13)	Total
AM, CX, CT, COT, AN, CH, GM	-	1	1	-	2
AM, CX,COT,CH, GM,CP, CT	-	-	1	-	1
AM, CX, CT, COT, CH, AN	-	1	1	-	2
AM, CX, CT, GM, COT, CP	2	-	1	-	3
AM, CX, GM, CT, AN, CH	1	-	1	-	2
AM, CH, CT, CX, COT	3	-	18	-	21
AM, CX, COT, AN, CP	1	1	1	-	3
AM, CT, CX, GM, COT	-	2	16	1	19
AM, CX, CH, COT	15	2	2	2	21
AM, CX, CT, GM	4	1	-	-	5
AM, CX, AN, COT	1	-	1	-	2
AM, CX, CH	5	3	2	1	12
AM, CX, COT	2	0	1	0	3
AM, CT, CX	7	2	1	1	11
AM, CX, AN	6	-	2	-	8
AM, CX	20	-	1	-	21
CX, CH	5	2	2	-	9
AM, COT	0	2	1	1	4
AM, CT	22	1	1	-	25
AM, GM	4	-	-	-	4
AM	3	1	1	-	5
CX	15	-	-	-	15
CH	2	1	2	-	5
COT	-	-	-	1	1
CT	11	-	-	-	11
Total	129	20	57	7	213
Percent	95.5	83.3	100	53.8	93/1

Discussion

Several reports have been indicated that *S. typhi* from typhoidal *Salmonella,* and *S. typhimurium* and *S. enteritidis* from non-typhoidal *Salmonella* are the most prevalent *Salmonella* species isolated from human and animal sources (2, 3, 20-24). Our results also showed that *S. typhi* (45.6%) and *S. typhimurium* (21.2%) were the most species isolated from patients referred to clinical centers. In similar study that was performed in Turkey, (24) among the 620 *Salmonella* isolates, strains belonging to the serotypes *S. enteritidis* (47.7%), *S. typhimurium* (34.7%), *S. paratyphi B* (6.0%), *S. typhi* (2.9%), *S. paratyphi A* (0.2%) and serogroup C (8.5%) were found.

At the beginning of the antibiotic era, clinical isolates of Salmonella species exhibited a uniformly high sensitivity to antibiotics including chloramphenicol and co-trimoxazole. Therefore, these antibiotics were generally recommended as the antibiotic of choice in suspected *Salmonella* infections (7, 10, 15). The emergence and spread MDR among *Salmonella* species has become a major concern worldwide and is seriously challenging current treatment strategies. For more than 40 yr since its discovery, chloramphenicol was the drug of choice for the treatment of typhoid, while ampicillin/co-trimoxazole are other cost-effective and well-tried primary drugs of choice (14). Drug resistance to chloramphenicol in *S. typhi* first emerged in the United Kingdom in the 1950s and subsequently in Greece and Israel followed by the epidemics of MDR *Salmonella* in Mexico, India and other regions (7). However, the emergence in the late 1980s of MDR serovar Typhi (isolates resistant to ampicillin, chloramphenicol, and cotrimoxazole) in outbreaks reported in the Indian subcontinent, (8, 25, 26) Arabian country, the Philippines,(14) and South Africa (27) has led to the use of the fluoroquinolones as alternative drugs (28). In India, *S. typhi* drug resistance has been reported since 1960; followed by the first outbreak of multidrug resistant *S. typhi* in Calicut (29-30). Among the first reports of clinical treatment failure due to serovar Typhi resistant to amikacin and showing then an increased ciprofloxacin MIC (0.125 μg/ml) was in 1991 in a patient who had recently returned to the United Kingdom from India (31). Thereafter, several cases of MDR serovar Typhi also resistant to amikacin and the fluoroquinolones have been reported in Bangladesh, (32) India (9) Thailand, Vietnam, (13) and Tajikistan, (33) raising concerns about further spread to other regions where typhoid fever is endemic. In addition, molecular characterization of the serovar Typhi outbreak strains revealed that resistance to commonly used antimicrobial agents, including chloramphenicol, ampicillin, and trimethoprim, was encoded by plasmids of the HI incompatibility group (8, 14, 34). In another study in Turkey, (24) resistance to multiple antimicrobial agents was particularly high among *S. typhimurium* isolates (76.7%), and resistance or decreased susceptibility to ciprofloxacin (MIC> or =0.125 mg/l) was demonstrated in *S. aratyphi B, S. typhimurium* and *S. enteritidis* strains. All of the *S. typhi* isolates were susceptible to ciprofloxacin.

There are a few studies on prevalence of *Salmonella* and their drug resistance patterns in Iran. Our results showed that 40% of typhoidal *Salmonella* were resistant to chloramphenicol, whereas this antibiotic has been known as a choice drug for treatment of typhoid fever. Most strains were also resistant to co-trimoxazole and cefotaxime. These findings are consistent with other report from other part of Iran (Tabriz, Ahvaz) (35-36). In an epidemic-ological study of MDR in *S. typhi* in Tabriz, most strains showed more than 60% resistace to ampicillin, co-trimoxazole, chloramphenicol and amoxicillin (35). In another study, performed in Ahvaz most strains of *S. typhi* were sensitive to third-generation of cephalosporine antibiotics (36).

MDR transmitted genetically by plasmids among

enteric bacteria is a problem in *Salmonella* infections (7, 8). Our results also showed that most of *Salmonella* isolates were resistant to beta-lactam antibiotics (such as ampicillin and cefotaxime), whereas, most of them were sensitive to fluoroquinolones antibiotic (ciprofloxacin) and aminoglycosides (such as gentamicin and amikacin). As the prevalence of MDR Typhi increases, newer, more expensive, and less readily available antimicrobial agents will be required for the treatment of typhoid in our country.

In conclusion, we observe that outbreaks caused by MDR strains will require more expensive and not so readily available drugs for effective treatment, and this will be an added burden to the healthcare sector. Improvements in sanitation, prompt diagnosis, and the rational use of available effective drugs in treatment will be important in managing typhoid and in arresting any further escalation in outbreaks. We also suggest a further clinical study on usage of some newer antibiotics such as new beta-lactams (carbapenem and imipenem), fluoroquinolones, aztreonam, cefixime and ceftriaxone as drugs of choice and effective therapy against *Salmonella* species on patients in this region.

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