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Original Article

Detection of Extended Spectrum Beta Lactamase Producing *Salmonella* spp. and Multidrug Resistance Pattern

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ABSTRACT

Background and Objectives: ***Salmonella*** **infections are endemic in many developing countries with poor sanitary conditions, but emerge sporadically as a serious public health threat in developed countries. Infections with multidrug resistant (MDR) strains of** ***Salmonella*** **have been associated with treatment failures.** ***Salmonella*** **spp. resistant to extended spectrum cephalosporins are increasing in prevalence worldwide. The aim of this study was to determine the antimicrobial susceptibility, multidrug resistance and extended spectrum beta lactamase (ESBL) production among clinical isolates of** ***Salmonella*** **spp. during 2007 in Tehran, Iran.**

Patients and Methods: **In this cross-sectional study, fifty** ***Salmonella*** **spp. were identified by API 20E system and serotyped by the slide agglutination test. Disk diffusion test was performed. Double disk synergy test was used as a screening test for ESBL production, using disks of cefotaxime and ceftazidime with and without clavulanic acid.**

Results: **From 50** ***Salmonella*** **spp. 12 (24%) were** ***S. enterica*** **serogroup paratyphi B, 24 (48%)** ***S. enterica*** **serogroup paratyphi C and 14 (28%) were** ***S. enterica*** **serogroup Typhi. The most susceptibility and resistance were observed to ceftazidime (98%) and amoxicillin-clavulanic acid (96%), respectively. 28(56%) were resistant to 5 or more antibiotics. ESBL production was detected by double disk synergy test in one isolate (2%).**

Conclusion: **Results showed increase in antibiotic and multidrug resistance pattern of** ***Salmonella*** **spp. comparing to previous studies in Iran and other countries. It seems that this is the first report of** ***Salmonella*** **spp. ESBL producing in Iran.**

Key words: ***Salmonella*****, antibiotic susceptibility, Multidrug Resistance, ESBL, Double disk synergy, Iran**

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Introduction

Salmonellosis ranges clinically from the Salmonella gastroenteritis to enteric fevers, which are life-threatening febrile systemic illness requiring prompt antibiotic therapy. It is endemic in many developing countries with poor sanitary conditions, but emerges sporadically as a serious public health threat in developed countries (1). Invasive complication such as meningitis, sepsis and bacteraemia are more common in infant, elderly people, and immunocompromised patients (2).

Antimicrobial resistance in enteric pathogens is of great importance in the developing counties, where the rate of diarrheal disease is high. The progressive increase in antimicrobial resistance among enteric pathogens in developing countries is becoming a critical area of concern. Among the bacterial causes of diarrheal illnesses, *Salmonella* spp. continues to be a major public health problem. Although most Salmonella infections are self-limiting, serious sequelae, including systemic infection and death, can occur (3). In potentially life-threatening cases the antibiotics of choice are fluroqunolones and extended spectrum cephalosporin (2).

Antibiotic resistance in *Salmonella* emerged in the 1970s initially as chloramphenicol resistance but later as multidrug resistance. The appearance of both plasmid mediated antibiotic resistant against conventional anti Salmonella drugs and chromosomal resistance to flouroquinolons has reduced therapeutic options to the more recently developed betalactams or macrolides (4).

Resistance to extended spectrum cephalosporins has been recognized since 1988, and is increasing worldwide. This is of particular concern for the treatment of salmonellosis in children, because fluoroquinolons should not be used in this age group (2).

Multidrug resistant strains of *Salmonella* are endemic to many Asian countries. Expanded spectrum cephalosporins and flouroquinolons are used for treating such infections. Infections with these isolates have been associated with treatment failures, particularly when very short duration has been used (5).

The aim of this study was to determine the antimicrobial susceptibility, multidrug resistance and extended spectrum beta lactamase (ESBL) production among clinical isolates of *Salmonella* spp. during 2007 in Tehran, Iran.

Materials and Methods

This study was followed according to Helsinki declaration on ethical Principles for medical research involving human subjects.

Bacterial isolates:

A total of 50 *Salmonella* spp. isolated from stool samples of patients admitted in different hospitals in Tehran, Iran, during 2007 were investigated. Only one isolate per patient was included in the study. Samples were cultured on Eosin Methylene Blue (EMB) agar and Xylose Lysine Deoxycholate (XLD) agar (Merck Ltd, Darmstadt, Germany) plates and incubated in 37^0 C for 24 h. Lactose negative colonies were identified by API 20E system (BioMerieux Ltd, Marcy, France). All *Salmonella* spp. isolates were serotyped with *Salmonella* specific O and H antigens by the slide agglutination test (6).

Antibiotic susceptibility testing:

Disks diffusion test was performed according to Clinical and Laboratory Standards Institute recommendations (7). The disks were ampicillin(AM) 10mg, ceftazidime (CAZ) 30mg, ciprofloxacin (CIP) 5mg, chloramphenicol (C) 30mg, kanamycin (K) 10mg, gentamycin (GM) 10mg, nalidixic acid (NA) 30mg, tetracycline (TET) 30mg, trimetoprim-sulfamethoxazole (SXT) 1.25/23.75 mg, amoxicillin-clavulanic acid (AMC) 20/10 mg, cefotaxime (CTX) 30mg (Mast Group Ltd., Merseyside, UK). *Escherichia coli* ATCC 25922 and *Staphylococcus* aureus ATCC 25923(Mast Group Ltd., Merseyside, UK) were used for quality control.

Double disk synergy test (DDST):

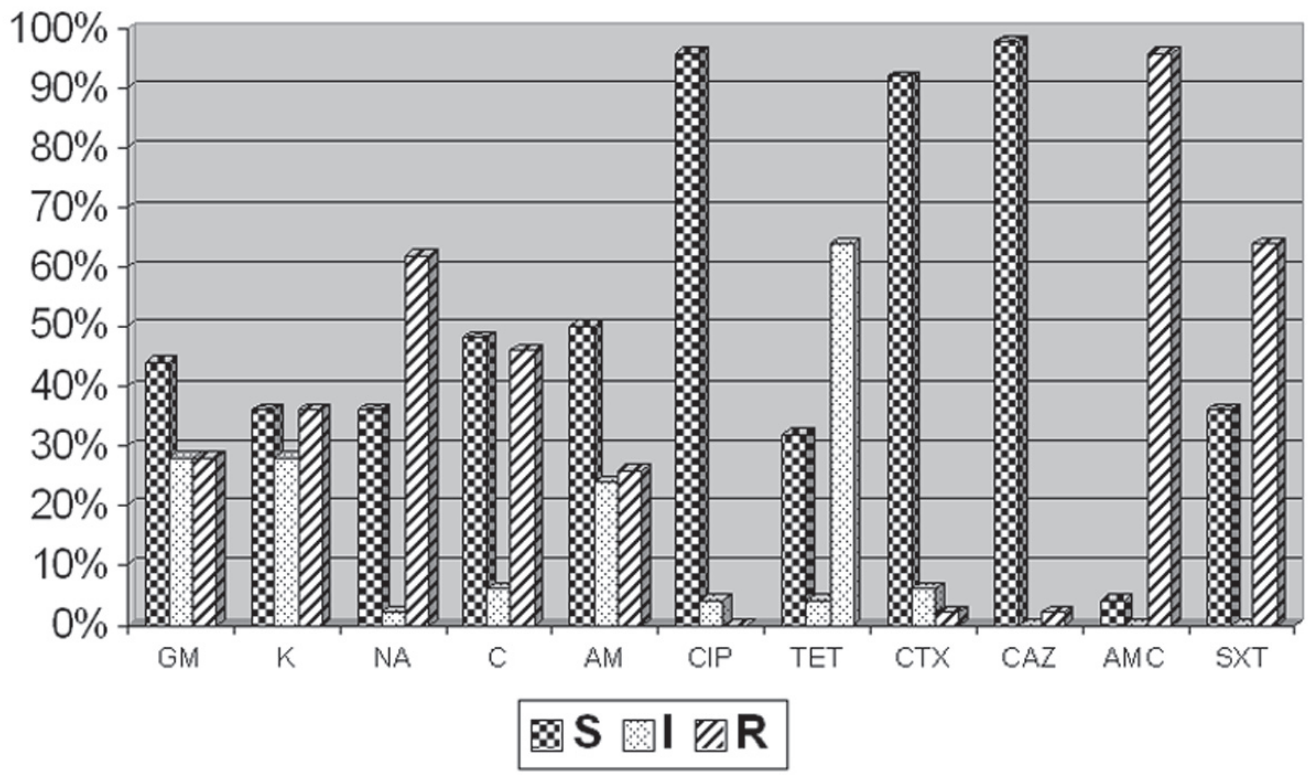
Disk approximation test was used in this study as a screening test for ESBL production, using disks of cefotaxime 30mg and ceftazidime 30 mg with and without clavulanic acid 10 mg (Mast Group Ltd., Merseyside, UK). The 0.5 McFarland turbidity of bacteria were spread on the surface of Muller Hinton agar (Merck Ltd, Darmstadt, Germany). Double disk synergy test (DDST) was performed by comparing the inhibition zone of disks containing cefotaxime or

ceftazidime with and without clavulanic acid. When zones were enlarged more than 5 mm around the disk containing clavulanic acid the isolate were considered as ESBL positive. Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 7006039(Mast Group Ltd., Merseyside, UK) were used as negative and positive control respectively (7).

Results

From 50 *Salmonella* spp. isolates, 12 cases (24%) were *S. enterica* serogroup paratyphi B, 24 cases (48%) *S. enterica* serogroup paratyphi C and 14 cases (28%) were *S. enterica* serogroup typhi. The most susceptibility and resistance were observed to ceftazidime (98%) and amoxicillin-clavulanic acid (96%), respectively. The results of antibiotic susceptibility test for 11 antimicrobial agents are shown in Fig. 1. From the 50 *Salmonella* spp., 8 isolates (16%) were resistance to one antibiotic, 7 isolates (14%) resistant to two antibiotics, 2 isolates (4%) resistant to three antibiotics, 5 isolates (10%) resistant to four antibiotics, 11 isolates (22%) resistant to five antibiotics, 9 isolates (18%) resistant to six antibiotics, 6 isolates (12%) resistant to seven antibiotics and 2 isolates (4%) resistant to eight antibiotics. Resistance profiles for the isolated *Salmonella* spp. are shown in Table 1. ESBL production was detected by double disk synergy test (DDST) in one isolate (2%). *S. enterica* serogroup typhi was resistant to amoxicillin-clavulanic acid, nalidixic acid, ceftazidime, cefotaxime and trimethoprim-sulfametoxazole and susceptible to ciprofloxacin.

Fig. 1: Results of antibiotic susceptibility test for isolated *Salmonella* spp.



AM: Ampicillin
CAZ: Ceftazidime
CIP: Ciprofloxacin
C: Chloramphenicol
K: Kanamycin
GM: Gentamycin
NA: Nalidixic acid
TET: Tetracycline
SXT: Trimetoprim- sulfamethoxazole
AMC: Amoxicillin- clavulanic acid
CTX: Cefotaxime

Table 1: Resistance profiles for isolated *Salmonella* spp.

Resistant profile	Number
AMC, SXT, TET, NA, C, K, GM, AM	2
AMC, SXT, TET, NA, C, K, GM	2
AMC, SXT, TET, NA, C, K, AM	2
AMC, SXT, TET, C, K, GM, AM	2
AMC, SXT, TET, NA, C, K	6
AMC, SXT, TET, NA, K, GM	2
AMC, SXT, NA, AM, CTX, CAZ	1
AMC, SXT, TET, NA, C	5
AMC, SXT, TET, NA, AM	1
AMC, SXT, TET, GM, AM	1
AMC, SXT, TET, C, GM	1
AMC, SXT, TET, C, AM	2
AMC, SXT, TET, GM, AM	1
AMC, SXT, TET, NA	3
AMC, SXT, K, GM	1
AMC, TET, NA, C	1
AMC, NA, C	1
AMC, C, GM	1
AMC, TET	2
AMC, NA	1
AMC, GM	1
AMC, NA	2
NA, GM	1
NA	1
AMC	7

AM: Ampicillin, CAZ: Ceftazidime, CIP: Ciprofloxacin, C: Chloramphenicol, K: Kanamycin,
GM: Gentamycin, NA: Nalidixic acid, TET: Tetracycline, SXT: Trimetoprim- sulfamethoxazole,
AMC: Amoxicillin- clavulanic acid, CTX: Cefotaxime

Discussion

Among 50 *Salmonella* spp. isolated from patients admitted in different hospitals in Tehran during 2007, we found that 62% (CI: 48.55%, 75.45%) are resistant to nalidixic acid, this result is significantly higher than some studies (3, 8, 9) which do not found any resistance to this drug. Resistance to gentamycin was 28% (CI: 15.56%, 40.44%). In some studies (9, 10) resistance to this antibiotic was not reported, in another one (8) only 6% resistance was reported, our result is significantly higher than those are. Resistance to trimethoprim-sulfamethoxazole was 64% (CI: 50.7%, 77.3%), significantly higher than 3% (8), 29.7% (10) and 48% (11). Resistance to chloramphenicol was 46% (CI: 32.19%, 59.81%), significantly higher than 29.7% (10) and 33% (11). Resistance to ampicillin was 26% (CI: 13.93%, 38.07%) which was rather similar to 29.7% (10) and 38% l (11).

All of 50 *Salmonella* spp. in this study were susceptible to ciprofloxacin; this is similar to other reports, which do not report any resistance to this antibiotic (3, 8, 9, 10, 12). Susceptibility to ceftazidime was 98% (CI: 94.12%, 100%), which was similar to 100% (9).

Resistance to tetracycline and chloramphenicol was 46% (CI: 32.19%, 59.81%), significantly higher than 11.9% (9). Resistance to trimethoprim-sulfametoxazole, tetracycline and chloramphenicol was 44% (CI: 30.24%, 57.76%) significantly higher than 1.9% (9).

Resistant to two or more antibiotics were seen in 84% (CI: 73.84%, 94.16%), which was significantly higher than 5% (13).

The frequency of ESBL producing *Salmonella* spp. in this study is 2% (CI: 0, 5.9%), which do not have significant difference with frequency of ESBL producing *Salmonella* in Turkey (3.3%) (1), Nepal (0.6%) (13), South Africa (2.4%) (14), India (5.3%) (15) and Poland (0.4%) (16).

The results of this study show significant difference with frequency of ESBL producing *S. infantis* (91.4%) in Brazil (12), also with another study (17) which reported 67.2% of ESBL producing *E.coli* in Tehran, Iran. We did not find any report about ESBL producing *Salmonella* spp. in Iran; it seems that this is the first report in Iran.

Multiple antibiotic resistant in bacterial pathogens is now a common phenomenon in developing countries. This circumstance is most likely related to the frequent use of over the counter drug without proper or no medical supervision. It seems likely that the use of antibiotics for non-medical purposes also helps the increase of the reservoir of R factor and multiple antibiotic resistances. Results of this study show a significant increase in antibiotic and multidrug resistance in *Salmonella* spp. comparing to previous study in Iran and other countries.

Conclusion

In the recent years, ESBL producing *Salmonella* spp. has been reported from several countries. These enzymes show resistance to cefotaxime, ceftazidime and aztreonam. As ESBLs are encoded on conjugative plasmids, transposons or integrons, they can spread readily.

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