AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

69 (2022) 4, 323–331

DOI:
10.1556/030.2022.01841

RESEARCH ARTICLE

Antimicrobial resistance patterns and virulence gene profiles of *Salmonella enteritidis* and *Salmonella typhimurium* recovered from patients with gastroenteritis in three cities of Iran

JAVAD YASBOLAGHI SHARAHI[1], ALI HASHEMI[2], SEYYED MOHAMMAD JAVAD MOUSAVI[2], ABAZAR POURNAJAF[3] and SOHEILA MORADI BIDHENDI[1]*

[1] Department of Microbiology, Razi Vaccine and Serum Research Institute (RVSRI), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

[2] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Department of Microbiology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran

Received: July 25, 2022 • Accepted: September 29, 2022
Published online: November 3, 2022

ABSTRACT

This study evaluated distribution of virulence factors and antibiotic resistance in clinical isolates of *Salmonella enteritidis* and *Salmonella typhimurium* in three cities of Iran. Altogether 48 *S. enteritidis* and *S. typhimurium* isolates were collected from patients at certain Iranian hospitals between May 2018 and September 2021. Antimicrobial susceptibility testing was performed by disk diffusion and broth microdilution methods. The presence of antibiotic-resistance genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{NDM}, *strA, strB, aadA1, tetA, tetB, floR, sul1, sul2, dfrA*), integrons (classe 1 and 2), and virulence-associated genes (*invA, stn, sopB, spvC, rck, phoPQ*) was investigated by PCR and sequencing. Antimicrobial agents like trimethoprim-sulfamethoxazole and imipenem represent highly efficient agents with 97% susceptibility. *S. enteritidis* and *S. typhimurium* exhibited high resistance to ciprofloxacin (n = 20, 71.43%) and ceftazidime (n = 9, 45%), respectively. Overall, 3 (6.25%), 13 (27.08%), and 6 (12.5%) isolates were divided into strong, moderate, and weak biofilm producers, respectively. Moreover, bla_{CTX-M}, bla_{TEM}, bla_{SHV}, *sul1, sul2, tetA, tetB, floR, strA,* and *strB* resistant genes were detected in 10 (20.8%), 5 (10.4%), 1 (2.08%), 7 (14.58%), 1 (2.08%), 3 (6.25%), 2 (4.1%), 1 (2.08%), 2 (4.1%), 2 (4.1%), respectively. Furthermore, 7 (14.58%) strains had classe 1 integron. All tested *S. enteritidis* strains had *invA* and *sopB*, and all *S. typhimurium* strains had *invA* and *phoPQ*. However, *spvC* remained undetected in all isolates. Extensive surveillance and efficient control measures against infection help to stop the upsurge of various antibiotic-resistant isolates.

KEYWORDS

gastroenteritis, *S. enteritidis*, *S. typhimurium*, antibiotic resistance genes, virulence factors

INTRODUCTION

Salmonella spp. are gram-negative, facultative anaerobic, flagellated bacteria [1]. Being among the major causative diarrhea agents, *Salmonella* induces a considerable illness burden. There are outbreaks of foodborne illness linked to *Salmonella* that result in approximately 230,000 deaths worldwide yearly [1, 2]. *Salmonella enteritidis* and *Salmonella typhimurium* isolates are the predominant cause of gastroenteritis and more than 2,500 serotypes have

*Corresponding author.
E-mail: smoradibidhendi@yahoo.com

been described. Different serotypes of *Salmonella* exhibit differences in pathogenicity, prevalence, and antibiotic resistance. Patients under 5 and above 60 years old, as well as immunocompromised patients, are exposed to the severe infections induced by *Salmonella* gastroenteritis [2, 3]. The frequency of *Salmonella* in gastroenteritis-induced children was found 40% in an Iranian investigation [4]. Patients with *Salmonella*-induced gastroenteritis do not require antibiotic treatment however, patients with invasive *Salmonella* infections were treated with ampicillin, cotrimoxazole (trimethoprim-sulfamethoxazole), and chloramphenicol. Another issue to consider is Multi-Drug-Resistant (MDR) *Salmonella*, exhibiting resistance to three or more types of antibiotics [4, 5]. The formation of MDR infections, passing from animals to humans through the food chain, is deemed to be responsible for the widespread applicability of antibiotics in livestock, poultry, and for the empirical salmonellosis treatment. The prevalence of MDR *Salmonella* among patients has significantly increased in Iran over recent years [5]. Common cases of antibiotic resistance in *Salmonella* spp. primarily result from a number of mechanisms, including chromosomal mutations, resistance spreading through mobile genetic elements (primarily plasmids), reduced antibiotic permeability of the cell wall, enzymatic inactivation of antibiotics, efflux pump, and altered antibiotic target positions [6, 7]. A large number of antibiotic-resistant genes found in gram-negative microbes are detected in integrons as a gene cassette. Integrons are genetic components that incorporate foreign genes, such as antibiotic resistance, into their structure. Several integron classes have been reported in recent years. In clinical isolates of Enterobacteriaceae, class 1 integrons predominate [8, 9]. Bacteria are responsible for the emergence of difficulties that arise in hostile environments. One of the most important survival strategies utilized by bacteria is the production of biofilms, which are frequently implicated in numerous microbial illnesses in the body and are thought to be the cause of more than 60% of all microbial infections in humans. Biofilms have an important role to play in antibiotic resistance [10, 11]. Salmonella's pathogenicity is attributed to a variety of chromosomal and plasmid factors [12]. Salmonella's Type-3 Secretion Systems (T3SSs), being part of pathogenicity islands, are important in the pathogenesis of the organism [13]. In the same way that other pathogenicity islands are necessary for bacterial virulence in a particular animal, *Salmonella* pathogenicity islands (SPIs) represent substantial gene cassettes in the *Salmonella* chromosome that encode determinants charged with setting up a particular communication with the host [14, 15]. 20 SPIs have been described. Different genes including *invA*, *spv*, and *stn* are important virulence factors in salmonellosis [5, 16]. The genome contains the *invA* gene, which produces a protein on the inner membrane that causes epithelial cell invasion and salmonellosis [5, 16]. A quick and trustworthy method that is applicable to diagnostic situations to identify Salmonella spp. is PCR for the *invA* gene. The most prevalent and clinically relevant genetic marker for the serovar causing salmonellosis around the world is the *invA* virulence gene [5, 16]. Both *S. typhimurium* and *S. enteritidis* contain this marker [5]. Non phagocytic cell invasion is aided by *SopB* [17]. It was discovered that *SopB* influenced cytoskeletal rearrangements, host cell invasion, phospholipase C activation, membrane fission, chloride homeostasis, and nuclear mRNA export [17, 18]. The rck open reading frame (ORF) on the *pefI-srgC* operon encodes an outer membrane protein that confers resistance to complement-mediated killing. Recent research has shown that Rck contributes to Salmonella's invasion of non phagocytic cell lines [19, 20]. PhoPQ is a global regulatory system of more than 200 genes and in Salmonella, this system is involved in adaptation to extra- and intracellular environments during infection. It also regulates environmental adaption to low Mg^{2+} conditions [21, 22]. The *Salmonella* pathogenicity island-2 type-three secretion system transports SpvC into the host cell. SpvC was demonstrated to suppress MAP kinase signaling and exhibited phosphothreonine lyase activities [23]. Recently, diarrhea and dysentery have increased dramatically in the central part of Iran, and *S. enteritidis* and *S. typhimurium* are considered one of its main causes. The major goal of this paper was to assess the distribution of virulence factors, antimicrobial resistance patterns, and molecular causes of antibiotic resistance among clinical isolates of *S. enteritidis* and *S. enteritidis* from patients who were admitted to hospitals in Tehran, Qom, and Babol. In addition, to confirm the relationship between biofilm formation and the rise of antibiotic resistance, the capacity of isolates to produce biofilm was investigated.

MATERIALS AND METHODS

Ethical statement

For the sake of patients' confidentiality, anonymity was honored and no personal information was requested.

Bacterial isolates

From May 2018 to September 2021, the total number of 48 *Salmonella* isolates (*S. enteritidis*: 28, *S. typhimurium*: 20) were collected from patients in three cities of Iran (Tehran, Qom, and Babol). All samples were transferred to the Microbiology Department and were verified via conventional biochemical tests and maintained at −70 °C in a trypticase soy broth supplemented with 20% glycerol.

Antimicrobial susceptibility testing

In line with Clinical and Laboratory Standards Institute (CLSI) guidelines, antimicrobial susceptibility of each *S. enteritidis* and *S. typhimurium* isolate on Mueller Hinton agar (Merck, Germany) was specified through the Kirby-Bauer disk diffusion test (Mast Group, Merseyside, UK) [24]. Disks of ampicillin 10 mg (AMP), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), imipenem (IPM, 10 µg), gentamicin (GEN, 10 µg), amikacin (AK, 30 µg), tetracycline (TET, 30 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin

Table 1. Resistance phenotypes and incidence of integrons, resistance genes and virulence factors in *Salmonella* isolates

Isolate	Serovar	City	Gender	Resistance phenotype	Integrons/resistance genes	Virulence factor	Biofilm
SE1	*S. enteritidis*	Babol	female	CIP, NA	-	*invA, sopB, stn*	-
SE2	*S. enteritidis*	Babol	female	CIP, NA	-	*invA, sopB, stn*	-
SE3	*S. enteritidis*	Babol	female	AMP, CAZ, CTX, AK,GM, CIP, NA	bla_{CTX-M}, bla_{TEM}, *int1, sul1*	*invA, sopB, stn, rck*	moderate
SE4	*S. enteritidis*	Babol	female	-	-	*invA, sopB, stn*	-
SE5	*S. enteritidis*	Babol	male	AMP, AK,GM, CHL, CIP, NA	*strA*	*invA, sopB, stn*	moderate
SE6	*S. enteritidis*	Babol	male	CIP	-	*invA, sopB, stn*	-
SE7	*S. enteritidis*	Qom	female	AK, CIP, NA	-	*invA, sopB, stn, rck*	weak
SE8	*S. enteritidis*	Qom	female	CIP, NA	-	*invA, sopB, stn*	-
SE9	*S. enteritidis*	Qom	male	AMP, CTX, AK, CIP, NA	bla_{CTX-M}	*invA, sopB, stn*	moderate
SE10	*S. enteritidis*	Qom	male	CIP, NA	-	*invA, sopB, stn*	-
SE11	*S. enteritidis*	Qom	male	CIP, NA	-	*invA, sopB, stn*	-
SE12	*S. enteritidis*	Qom	male	-	-	*invA, sopB, stn*	-
SE13	*S. enteritidis*	Qom	male	-	-	*invA, sopB, stn*	-
SE14	*S. enteritidis*	Tehran	female		-	*invA, sopB, stn*	-
SE15	*S. enteritidis*	Tehran	female	AMP, CAZ, CTX, CIP, NA	bla_{CTX-M}	*invA, sopB, stn*	moderate
SE16	*S. enteritidis*	Tehran	female	CIP	-	*invA, sopB, stn*	-
SE17	*S. enteritidis*	Tehran	female	CIP, NA	-	*invA, sopB, stn, rck*	weak
SE18	*S. enteritidis*	Tehran	female	-	-	*invA, sopB, stn*	-
SE19	*S. enteritidis*	Tehran	female	CHL, CIP, NA	*floR*	*invA, sopB, stn*	moderate
SE20	*S. enteritidis*	Tehran	female	-	-	*invA, sopB, stn*	-
SE21	*S. enteritidis*	Tehran	male	AMP, CAZ, CTX, AK,GM, CHL, CIP, NA	bla_{CTX-M}, bla_{TEM},*int1, sul1, sul2*	*invA, sopB, stn, rck*	strong
SE22	*S. enteritidis*	Tehran	male	AMP, CAZ, AK,GM, CIP, NA	bla_{CTX-M}, *int1, strB, sul1*	*invA, sopB, stn, rck*	moderate
SE23	*S. enteritidis*	Tehran	male	CIP, NA	-	*invA, sopB, stn*	weak
SE24	*S. enteritidis*	Tehran	male	CIP, NA		*invA, sopB, stn*	-
SE25	*S. enteritidis*	Tehran	male	-	-	*invA, sopB, stn*	-
SE26	*S. enteritidis*	Tehran	male	NA	-	*invA, sopB, stn*	-
SE27	*S. enteritidis*	Tehran	male	CIP, NA	-	*invA, sopB, stn*	weak
SE28	*S. enteritidis*	Tehran	male	CIP, NA	-	*invA, sopB, stn*	-
ST1	*S. typhimurium*	Babol	female	AMP, CAZ,CTX,TET,AK,GM, CHL, CIP	bla_{CTX-M}, bla_{TEM}, *int1, sul1*	*invA*, *phoPQ*, *stn*, *rck*	strong
ST2	*S. typhimurium*	Babol	male	AMP, CAZ,CTX,TET, AK,GM	bla_{CTX-M}, *tetB, strB*	*invA*, *phoPQ*, *stn*, *rck*	moderate
ST3	*S. typhimurium*	Babol	male	-	-	*invA*, *phoPQ*, *stn*	-
ST4	*S. typhimurium*	Babol	male	-	-	*invA*, *phoPQ*, *stn*, *rck*	-
ST5	*S. typhimurium*	Qom	female	-	-	*invA*, *phoPQ*, *stn*, *rck*	-
ST6	*S. typhimurium*	Qom	male	-	-	*invA*, *phoPQ*, *stn*, *rck*	-
ST7	*S. typhimurium*	Qom	male	AMP, CAZ,TET, AK, CHL, CIP	-	*invA*, *phoPQ*, *stn*, *rck*	moderate
ST8	*S. typhimurium*	Qom	male	AMP, CAZ,CTX,TET, CHL	bla_{CTX-M}	*invA*, *phoPQ*, *stn*, *rck*	moderate
ST9	*S. typhimurium*	Tehran	female	AMP, CAZ,CTX, TET, CHL, CIP	bla_{TEM},*int1, sul1*	*invA*, *phoPQ*, *stn*, *rck*	moderate
ST10	*S. typhimurium*	Tehran	female	CIP	-	*invA*, *phoPQ*, *stn*	weak
ST11	*S. typhimurium*	Tehran	female	-	-	*invA*, *phoPQ*, *stn*	-
ST12	*S. typhimurium*	Tehran	male	-	-	*invA*, *phoPQ*, *stn*, *rck*	-
ST13	*S. typhimurium*	Tehran	male	AMP, CAZ,CTX,TET, CHL	*tetA*	*invA*, *phoPQ*, *stn*, *rck*	moderate
ST14	*S. typhimurium*	Tehran	male	-	-	*invA*, *phoPQ*	-

(continued)

Table 1. Continued

Isolate	Serovar	City	Gender	Resistance phenotype	Integrons/resistance genes	Virulence factor	Biofilm
ST15	*S. typhimurium*	Tehran	male	AMP, CAZ,CTX,TET, AK,GM, CHLTS,IMI	bla_{CTX-M}, bla_{TEM}, bla_{SHV}, *int1, sul1*	*invA*, *phoPQ*, *stn, rck, sopB*	strong
ST16	*S. typhimurium*	Tehran	male	AMP	-	*invA*, *phoPQ*, *stn, rck*	-
ST17	*S. typhimurium*	Tehran	male	-	-	*invA*, *phoPQ*, *stn*	-
ST18	*S. typhimurium*	Tehran	male	CAZ,CTX, AK	-	*invA*, *phoPQ*, *stn*	moderate
ST19	*S. typhimurium*	Tehran	male	-	-	*invA*, *phoPQ*, *stn, rck*	weak
ST20	*S. typhimurium*	Tehran	male	CAZ.CTX, AK,GM, CHL, CIP	bla_{CTX-M}, *int1, strA, sul1*	*invA*, *phoPQ*, *stn, rck*	moderate

(CIP, 5 µg), trimethoprim-sulfamethoxazole (TS, 2.5 µg), and nalidixic acid were used. Minimum Inhibitory Concentrations (MICs) of 5 antibiotics such as imipenem, ceftazidime, cefotaxime, ciprofloxacin, and tetracyclines were obtained by broth microdilution method, and the result was analyzed in line with the CLSI guidelines [24]. The antibiotic powders were bought from Sigma-Aldrich. In the case of disk diffusion and MIC results, a quality control strain namely, *Escherichia coli* ATCC 25922 was applied. MDR *Salmonella* represents any isolate exhibiting resistance to three or more antibiotic classes.

Phenotypic detection of β-lactamases

The phenotypic confirmatory disc diffusion method is used in this section to study extended-spectrum beta-lactamase (ESBL) phenotype. Discs containing ceftazidime (30 µg), cefotaxime (30 µg), ceftazidime/clavulanic acid (30 µg/10 µg), and cefotaxime/clavulanic acid (30 µg/10 µg) were applied, as previously reported [25, 26]. *Klebsiella pneumoniae* ATCC700603 and *E. coli* ATCC 25922 were considered ESBL positive and negative controls, respectively.

Biofilm formation assay

Basically, plate microtiter method has been used to identify biofilms in agreement with other studies [25, 27]. The OD of each well was then measured at 492 nm for analysis. Categorization of isolates was performed in previous studies based on such criteria as strong (4 × ODc < OD), moderate (2 × ODc < OD _ 4 × ODc), weak biofilm producer (ODc < OD _2 × ODc), and no biofilm producer (OD _ ODc).

Detection of multidrug-resistant and virulence factor genes

The DNA extraction kit (GeNet Bio Company, Daejeon, Korea; Cat. No, K-3000) was applied to extract DNA based on the instructions given by manufacturers. Resistant genes were detected via PCR and their identities were verified through sequencing. Isolates were screened via PCR amplification using specific primers to detect virulence genes (*invA*, *stn*, *sopB*, *spvC*, *rck* and *phoPQ*), ESBL-encoding genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}), carbapenemase gene (bla_{NDM}), aminoglycoside-resistance genes (*strA, strB*, *aadA1*), tetracycline-resistance genes (*tetA*, *tetB*), amphenicols-resistant genes (*floR*), sulfonamides-resistant genes (*sul1*, *sul2*), trimethoprim-sulfamethoxazole-resistant gene (*dfrA*), and integrons (classes 1 and 2) [5, 28–31]. Forward and reverse primers were used for DNA sequencing at Macrogen Company (Korea).

Statistical analysis

SPSS software, 21.0 (SPSS Inc., Chicago, IL, USA) was employed for Chi-squared test to investigate possible considerable differences between datasets. $P = 0.05$ was assumed to be statistically significant.

RESULTS

Bacterial isolates

In this study, 48 non-duplicate non-consecutive isolates of *S. enteritidis* and *S. typhimurium* were gathered from 30 (62.5%) male and 18 (37.5%) female population among six hospitals in Iran during May 2018 to September 2021. The patients ranged between 1 and 67 years in age. All isolates were collected from fecal samples taken from those who had been referred to medical centers and hospitals in Qom, Tehran, and Babol cities (Tehran: 27, Qom: 11, Babol: 10) (Table 1).

Antimicrobial susceptibility testing

Antibiotic-resistance patterns of 48 isolates of *S. enteritidis* and *S. typhimurium* are shown in Table 2. All *S. enteritidis* isolates exhibited sensitivity to tetracycline, imipenem, and trimethoprim-sulfamethoxazole, while the lowest rate of resistance was observed among *S. typhimurium* isolates for imipenem and trimethoprim-sulfamethoxazole 1 (5%). Table 3 shows the MIC ranges, MIC50, MIC90, and percentages of isolates resistant, intermediate, or susceptible to seven antimicrobial agents. From the antibiotic susceptibility tests, the number of MDR isolates was 16 (*S. enteritidis*: 8, *S. typhimurium*: 8) (Table 1).

Phenotypic detection of β-lactamases

Herein, the recurrent ESBL-producing *S. enteritidis* and *S. typhimurium* were 7 (25%) and 9 (45%) in number, respectively. The prevalence percentage of ESBL-producing *S. enteritidis* and *S. typhimurium* that were resistant to cephalosporin and other antibiotics was considerably higher than that of non-ESBL-generating strains ($P < 0.05$).

Biofilm formation

The results of biofilm formation assay on polystyrene were strong in 3 (6.25%) isolates (*S. enteritidis*: 1, *S. typhimurium*: 2) and moderate in 13 (27%) isolates (*S. enteritidis*: 6, *S. typhimurium*: 7), while 6 weak isolates (12.5%) (*S. enteritidis*: 4, *S. typhimurium*: 2) and 26 isolates (54.16%) (*S. enteritidis*: 17, *S. typhimurium*: 9) did not form biofilm. Among 7 ESBL-producing *S. enteritidis*, 1 (3.57%) and 6 (21.43%) isolates were strong and moderate biofilm producers, respectively. Moreover, among 9 ESBL-producing *S. typhimurium*, 2 (10%) and 7 (35%) isolates were strong and moderate biofilm producers, respectively, and all isolates enjoyed the biofilm formation capability (Table 1).

PCR screening for genes of virulence factor

As for virulence genes in *S. enteritidis*, all isolates contained *invA*, *sopB*, *and stn* genes. Among the virulence genes, *rck* was found in 5 (17.85%) isolates. The *phoPQ* gene was not detected in *S. enteritidis*. In the case of *S. typhimurium*, all isolates carried *invA* and *phoPQ* genes and *stn*, *rck*, and *sopB* were found in 19 (95%), 14 (70%), and 1 (5%), respectively. The *spvC* gene was not detected in any *S. typhimurium* and *S. enteritidis* strain (Tables 1 and 4).

PCR screening for genes of antimicrobial resistance

There were 16 (33.3%) ESBL-producing isolates detected, and $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} were found in 10 (20.8%), 5 (10.4%), and 1 (2.08%) isolates, respectively. Among the 48 isolates, none of the isolates were MBL-producing strain, no bla_{NDM} positive strain was found in this study. Out of 48 isolates, 7 (14.58%) had classe 1 integron while classe 2 integron was not detected in our isolates. Out of 28 *S. enteritidis* isolates, *sul1*, *floR, strA, and strB* genes were detected in 3 (10.7%), 1 (3.57%), 1 (3.57%), and 1 (3.57%) isolates, respectively. Among 20 *S. typhimurium* isolates, the frequencies of *sul1*, *sul2*, *tetA*, *tetB*, *strA,* and *strB* genes were 4 (20%), 1 (5%), 3 (15%), 2 (10%), 1 (5%), and 1 (5%), respectively. Based on the results, none of *tetA, tetB, aadA1, and dfrA* genes were identified in *S. enteritidis* isolates, and none of *floR*, *aadA1*, *and dfrA* genes were seen in *S. typhimurium* isolates (Tables 1 and 5).

DISCUSSION

The most significant cause of gastrointestinal infections includes non-typhoid *Salmonella*. Typhimurium and Enteritidis serovars of *Salmonella* are particularly significant.

Table 2. Antibiotic resistance patterns of 48 isolates of *S. enteritidis* and *S. typhimurium*

Species (no (%) of isolates)	Antibiotic resistance patterns	AMP	CAZ	CTX	TET	AK	GEN	CHL	CIP	NA	TS	IMI
S. enteritidis (N:28)	Susceptible	20 (71.43%)	21 (75%)	20 (71.43%)	28 (100%)	20 (71.43%)	22 (78.57%)	25 (89.28%)	5 (17.8%)	9 (32.1%)	28 (100%)	28 (100%)
	Intermediate	2 (7.1%)	3 (10.7%)	4 (14.28%)	-	2 (7.1%)	2 (7.1%)	-	3 (10.7%)	-	-	-
	Resistant	6 (21.43%)	4 (14.28%)	4 (14.28%)	-	6 (21.43)	4 (14.28%)	3 (10.7%)	20 (71.43%)	19, (67.86%)	-	-
S. typhimurium (N:20)	Susceptible	11 (55%)	9 (45%)	9 (45%)	12 (60%)	11 (55%)	14 (70%)	13 (65%)	10 (50%)	13 (65%)	19 (95%)	19 (95%)
	Intermediate	1 (5%)	2 (10%)	3 (15%)	1 (5%)	3 (15%)	2 (10%)	-	5 (25%)	-	-	-
	Resistant	8 (40%)	9 (45%)	8 (40%)	7 (35%)	6 (30%)	4 (20%)	7 (35%)	5 (25%)	7 (35%)	1 (5%)	1 (5%)

AMP ampicillin, CAZ ceftazidime, CTX cefotaxime, TET tetracyclines, GM gentamicin, AK Amikacin, CHL chloramphenicol, CIP ciprofloxacin, NA nalidixic acid, TS trimethoprim-sulfamethoxazole, IMI imipenem.

Table 3. MIC of the *S. enteritidis and S. typhimurium* clinical isolates (n = 48)

Antibiotic	MIC(μg ml^{-1})			NO (%)		
	Range	50%	90%	sensitive	intermediate	resistant
ceftazidime	≤1- ≥64	4	32	28 (58.3%)	6 (12.5%)	14 (29%)
cefotaxime	≤1- ≥64	1	8	28 (58.3%)	7 (14.58%)	13 (27%)
tetracycline	≤1- ≥32	4	32	36 (75%)	5 (10.4%)	7 (14.58%)
ciprofloxacin	≤1- ≥64	8	32	14 (29%)	10 (20.8%)	24 (50%)
imipenem	≤1- ≥8	1	2	43 (89.5%)	4 (8.3%)	1 (2.08%)

Table 4. The features related to virulence genes in isolates

	invA	*stn*	*sopB*	*phoPQ*	*rck*	*spvC*
S. enteritidis (N:28)	28 (100%)	28 (100%)	28 (100%)	-	5 (17.85%)	-
S. typhimurium (N:20)	20 (100%)	19 (95%)	1 (5%)	20 (100%)	14 (70%)	-

According to a study from Iran, Enteritidis and Typhimurium serovars are among the most prevalent serovars in humans that cause salmonellosis. According to the nation and serovar, the mortality rate from non-typhoid salmonellosis ranges from 1 to 4 percent. The prevalence of this illness is up to 63 percent. Particularly in underdeveloped nations and in instances of antibiotic resistance, this rate is higher in the elderly, children, and individuals with compromised immune systems. In the current research, antibiotic resistance was higher and the symptoms were more severe in three patients with diabetes. Of the incorporated isolates, the highest resistance in *S. enteritidis* was related to ciprofloxacin (n = 20, 71.43%) and nalidixic acid (n: 19, 67.86%) and in *S. typhimurium* to ceftazidime (n = 9, 45%). In a study performed on 26 isolates of *S. typhimurium*, Jafari et al. reported the highest resistance of isolates to ceftazidime, cefotaxime, amikacin, and tetracycline (100%) and the lowest resistance to imipenem (3.85%) and nalidixic acid (7.69%), being significantly greater than the values obtained in our study [6]. Fardsanei et al. pointed to the high prevalence of resistance to cefuroxime (n = 20, 74.1%) and ciprofloxacin (n = 13, 48.2%) [5]. Ghaznavi et al. found the greatest antibiotic resistance to nalidixic acid (71.4%) and tetracycline (8.42%) [32]. Biofilms appear to be linked to various microbial infections, which is 80% of all infections by one estimate. Based on our results of antibiotic sensitivity tests, 16 isolates were MDR. Recently, MDR strains are increasing in number throughout various parts of the world, including Iran [5, 6, 33]. Antibiotic resistance in a biofilm remains much higher than that in non-biofilm bacteria and might be greater by as much as 5,000 times [10]. According to the results of our study, 3 (6.25%), 13 (27.08%), and 6 (12.5%) isolates were respectively considered strong, moderate, and weak biofilm generators. All the MDR and ESBL-generating strains, formed biofilms, and the three isolates that formed strong biofilms were all ESBL positive. Many studies have confirmed biofilm production in clinical isolates of Salmonella. Ethan et al. investigated the ability of 71 strains of *Salmonella enterica* derived from products, meat, or clinical sources to form biofilms. According to their results, all *Salmonella* isolates produced a considerable amount of biofilm upon their cultivation in a proper medium [34]. Based on previous findings, compared to other species, *S. typhimurium* strains are stronger producers in biofilm formation in different environmental conditions [35]. Plasmids and integrons represent easily spreadable elements. Counteracting the spread of antibiotic-resistant genes and nosocomial infections remains the most problematic challenge. There have been numerous reports about the link between the presence of plasmid-dependent antibiotic-resistant genes such as ESBL and an increase in multiple resistance strains, which can be very dangerous. In our paper, ESBL-generating *S. enteritidis* and *S. typhimurium* had frequency rates of 25% and 45%, respectively. So far, the ESBL-generating enzymes are detected around the world, especially many countries in Africa, Asia, Australia, the Europe, and Americas. It is concerning that ESBL and MBL are so prevalent and have spread so widely over the world [36]. In our study, ESBL-generating strains had a higher percentage of resistance than other isolates, being in agreement with the results of previous studies. Jafari et al. examined 26 isolates of *S. typhimurium* and found that a total of 15 isolates (57.69%) tested positive for broad-spectrum beta-lactamases, with the bla_{TEM} accounting for the majority of these cases (34.61%). [6]. Yang et al. in Korea focused on *S. enteritidis* and *S. typhimurium* which were isolated from animal samples. Only five out of the 40 *S. enteritidis* isolates and 22 *S. typhimurium* isolates had bla_{TEM} and bla_{SPE} genes found [6]. Also, there are several reports on the relationship between the presence of ESBL genes and plasmid-dependent quinolone resistance genes, which can be very dangerous. In our study, the prevalence of integron class I was 7 (14.58%). In our study, as in previous studies, the prevalence of integron class I was higher; therefore, Jin et al. reported the prevalence of integron class I at 13% while Abdel Aziz et al. determined its prevalence at 83% [28, 37]. Fortunately, the prevalence of MBL genes among *Salmonella* strains is not widespread. In the current study, bla_{NDM} gene remained undetected in all the isolates. One of the most widely used antimicrobials is sulfonamide.

Table 5. The features related to resistance genes in isolates

	sul1	*sul2*	*tetA*	*tetB*	*floR*	*strA*	*strB*	*aadA1*	*dfrA*	$bla_{\text{CTX-M}}$	bla_{TEM}	bla_{SHV}	bla_{NDM}
S. enteritidis (N:28)	3 (10.7%)	-	-	-	1 (3.57%)	1 (3.57%)	1 (3.57%)	-	-	5	2	-	-
S. typhimurium (N:20)	4 (20%)	1 (5%)	3 (15%)	2 (10%)	-	1 (5%)	1 (5%)	-	-	5	3	1	-

Pathogens that are food-borne are more resistant when antimicrobials are used in animals. Resistance to sulfonamides among *Salmonella* spp. is linked to the presence of *sul* genes [30]. Maka et al. tested sulfonamide-resistant isolates for the existence of *sul1*, *sul2*, *sul3*, and *int1* genes using multiplex PCR. In sum, 44.0% (37/84) isolates contained the *sul1* gene and 46.4% (39/84) were sul2 positive; however, the *sul3* gene was not identified in all the studied sulfonamide-resistant isolates [30]. Antunes et al. studied the relation between the existence of *sul* genes and integron classes. According to the results, all isolates carrying *sul* genes had at least one integron class. This relationship was also true in our study [38]. Tetracyclines were employed to enhance feed efficiency and growth in cattle, pigs, and poultry. The most often seen active efflux systems in *Salmonella* are tet(A), tet(B), tet(C), tet(D), tet(G), and tet (H) [39]. In our study, the prevalence of tet genes was lower than that in other studies and was observed only in 10.4% of the isolates. Adesij et al. found that 66% of the isolates exhibited resistance to tetracycline, of which all of them carried *tetA* gene, 30% *tetB*, and 35% *tetC* [40]. *floR*, *cmlA*, and *cat1* genes frequently operate as mediators of fenicol resistance in *Salmonella* and other bacteria. We observed the *floR* gene in simply one of the samples resistant to chloramphenicol [39]. In the study of Asgharpour et al., the *floR* gene was seen in all chloramphenicol-resistant isolates, while in our study, this gene was identified in only one of the chloramphenicol-resistant isolates [41]. In previous studies, there was a correlation between the presence of *aadA* gene and integron classes, while in our study, this gene was not observed even in integron-positive isolates. Today, streptomycin is rarely used due to toxic side effects. *strA* and *strB* genes encode resistance to streptomycin. Pezzella et al. identified *strA* and *strB* genes in 84% of streptomycin-resistant isolates [42]. Li Long et al. concluded that most (83.9%) *Salmonella* isolates carried four antimicrobial-resistant genes, especially $bla_{\text{TEM-1}}$, *strA*, *strB*, *sul2*, and *tetB* genes [43].

CONCLUSION

The dominant rise of MDR isolates affecting patients with severe infections hospitalized at critical wards and immunocompromised individuals is quite concerning. It should be noted that non-essential antimicrobial drug prescription corresponds to normal flora removal, hence significant spread of MDR isolates. In the near future, the appearance and expansion of integrons, ESBL, and other antibiotic-resistant genes in *S. typhimurium* and *S. enteritidis* will restrict the available options of treatment and put the public health in danger. As demonstrated by our findings, MDR strains represent a new hazard to various units and must be handled through opportune identification and solid isolation methods, which facilitate attenuating the horrendous consequences and decreasing mortality rate in critically ill patients. This paper illustrated the fast-paced appearance of substantially drug-resistant *S. typhimurium* and *S. enteritidis* isolates in hospitalized subjects. It was found that the

adopted molecular mechanisms played a substantial role in enhancing resistance to antimicrobials, namely cephalosporins. Additional factors namely other virulence factors, efflux pumps, and porins are the mechanisms that remain to be explored further in the future.

Conflicts of interest: The authors declare that they have no conflict of interests.

ACKNOWLEDGMENTS

This study was supported by Razi Vaccine & Serum Research Institute *(RVSRI)*, Karaj, Iran.

REFERENCE

1. Shen H, Chen H, Ou Y, Huang T, Chen S, Zhou L, et al. Prevalence, serotypes, and antimicrobial resistance of Salmonella isolates from patients with diarrhea in Shenzhen, China. BMC Microbiol 2020; 20(1): 1–10.
2. Khademi F, Vaez H, Ghanbari F, Arzanlou M, Mohammadshahi J, Sahebkar A. Prevalence of fluoroquinolone-resistant Salmonella serotypes in Iran: a meta-analysis. Pathog Glob Health 2020; 114(1): 16–29.
3. Wang M, Qazi IH, Wang L, Zhou G, Han H. Salmonella virulence and immune escape. Microorganisms 2020; 8(3): 407.
4. Mahmoudi S, Pourakbari B, Moradzadeh M, Eshaghi H, Ramezani A, Ashtiani MTH, et al. Prevalence and antimicrobial susceptibility of Salmonella and Shigella spp. among children with gastroenteritis in an Iranian referral hospital. Microb pathogenesis 2017; 109: 45–8.
5. Fardsanei F, Dallal MMS, Salehi TZ, Douraghi M, Memariani M, Memariani H. Antimicrobial resistance patterns, virulence gene profiles, and genetic diversity of Salmonella enterica serotype Enteritidis isolated from patients with gastroenteritis in various Iranian cities. Iranian J Basic Med Sci 2021; 24(7): 914.
6. Sales AJ, Naebi S, Nasiri R, Bannazadeh-Baghi H. The antibiotic resistance pattern and prevalence of blaTEM, blaSHV, blaCTX-M, blaPSE-1, sipB/C, and cmlA/tetR genes in *Salmonella typhimurium* isolated from children with diarrhea in Tabriz, Iran. Int J Health Life Sci 2021; 7(4).
7. Bidhendi M. A Review of studies on isolation, diagnosis and antimicrobial resistance of Salmonella in Iran. Vet Researches Biol Prod 2015; 28(4): 21–30.
8. White PA, McIver CJ, Rawlinson WD. Integrons and gene cassettes in the Enterobacteriaceae. Antimicrob Agents Chemother 2001; 45(9): 2658–61.
9. Kaushik M, Kumar S, Kapoor RK, Virdi JS, Gulati P. Integrons in Enterobacteriaceae: diversity, distribution and epidemiology. Int J Antimicrob Agents 2018; 51(2): 167–76.
10. Sharahi JY, Azimi T, Shariati A, Safari H, Tehrani MK, Hashemi A. Advanced strategies for combating bacterial biofilms. J Cell Physiol 2019; 234(9): 14689–708.
11. Riahi Rad Z, Riahi Rad Z, Goudarzi H, Goudarzi M, Mahmoudi M, Yasbolaghi Sharahi J, et al. MicroRNAs in the interaction between host–bacterial pathogens: a new perspective. J Cell Physiol 2021; 236(9): 6249–70.
12. Gulig PA. Virulence plasmids of *Salmonella typhimurium* and other salmonellae. Microb pathogenesis 1990; 8(1): 3–11.
13. Kim K, Palmer AD, Vanderpool CK, Slauch JM. The small RNA PinT contributes to PhoP-mediated regulation of the Salmonella pathogenicity island 1 type III secretion system in Salmonella enterica serovar Typhimurium. J Bacteriol 2019; 201(19): e00312–9.
14. Kombade S, Kaur N. Pathogenicity island in Salmonella. Salmonella spp-A global challenge: IntechOpen; 2021.
15. Singh Y, Saxena A, Kumar R, Kumar Saxena M. Virulence system of Salmonella with special reference to Salmonella enterica. Salmonella-A Re-emerging pathogen; 2018.
16. Lan TT, Gaucher M-L, Nhan NT, Letellier A, Quessy S. Distribution of virulence genes among Salmonella serotypes isolated from pigs in Southern Vietnam. J Food Prot 2018; 81(9): 1459–66.
17. Lou L, Zhang P, Piao R, Wang Y. Salmonella pathogenicity island 1 (SPI-1) and its complex regulatory network. Front Cell Infect Microbiol 2019; 9: 270.
18. Walpole GF, Pacheco J, Chauhan N, Clark J, Anderson KE, Abbas YM, et al. Kinase-independent synthesis of 3-phosphorylated phosphoinositides by a phosphotransferase. Nat Cell Biol 2022; 24(5): 708–22.
19. Koczerka M, Douarre P-E, Kempf F, Holbert S, Mistou M-Y, Grépinet O, et al. The invasin and complement-resistance protein Rck of Salmonella is more widely distributed than previously expected. Microbiol Spectr 2021; 9(2): e01457–21.
20. Mambu J, Barilleau E, Fragnet-Trapp L, Le Vern Y, Olivier M, Sadrin G, et al. Rck of *Salmonella Typhimurium* delays the host cell cycle to facilitate bacterial invasion. Front Cell Infect Microbiol 2020; 10: 586934.
21. Kohli N, Crisp Z, Riordan R, Li M, Alaniz RC, Jayaraman A. The microbiota metabolite indole inhibits Salmonella virulence: involvement of the PhoPQ two-component system. PLoS One 2018; 13(1): e0190613.
22. Dehinwal R, Cooley D, Rakov AV, Alugupalli AS, Harmon J, Cunrath O, et al. Increased production of outer membrane vesicles by Salmonella interferes with complement-mediated innate immune attack. Mbio 2021; 12(3): e00869–21.
23. Gopinath A, Allen TA, Bridgwater CJ, Young CM, Worley MJ. The Salmonella type III effector SpvC triggers the reverse transmigration of infected cells into the bloodstream. PloS one 2019; 14(12): e0226126.
24. Weinstein MP. Performance standards for antimicrobial susceptibility testing: Clinical and Laboratory Standards Institute; 2021.
25. Sharahi JY, Hashemi A, Ardebili A, Davoudabadi S. Molecular characteristics of antibiotic-resistant Escherichia coli and Klebsiella pneumoniae strains isolated from hospitalized patients in Tehran, Iran. Ann Clin Microbiol antimicrobials 2021; 20(1): 1–14.
26. Rad ZR, Rad ZR, Goudarzi H, Goudarzi M, Alizade H, Mazraeh FN, et al. Detection of NDM-1 producing Klebsiella pneumoniae ST15 and ST147 in Iran during 2019–2020. Acta Microbiol Immunol Hung 2021; 68(3): 177–82.
27. Davoudabadi S, Goudarzi H, Goudarzi M, Ardebili A, Faghihloo E, Sharahi JY, et al. Detection of extensively drug-resistant and hypervirulent Klebsiella pneumoniae ST15, ST147, ST377 and ST442 in Iran. Acta Microbiol Immunol Hung 2022; 69(1): 77–86.

28. Aziz SAA, Abdel-Latef GK, Shany SA, Rouby SR. Molecular detection of integron and antimicrobial resistance genes in multi-drug resistant Salmonella isolated from poultry, calves and human in Beni-Suef governorate, Egypt. Beni-Suef Univ J Basic Appl Sci 2018; 7(4): 535–42.
29. Thung TY, Radu S, Mahyudin NA, Rukayadi Y, Zakaria Z, Mazlan N, et al. Prevalence, virulence genes and antimicrobial resistance profiles of Salmonella serovars from retail beef in Selangor, Malaysia. Front Microbiol 2018; 8: 2697.
30. Mąka Ł, Maćkiw E, Ścieżyńska H, Modzelewska M, Popowska M. Resistance to sulfonamides and dissemination of sul genes among Salmonella spp. isolated from food in Poland. Foodborne Pathog Dis 2015; 12(5): 383–9.
31. Abbasi E, Goudarzi H, Hashemi A, Chirani AS, Ardebili A, Goudarzi M, et al. Decreased carO gene expression and OXA-type carbapenemases among extensively drug-resistant Acinetobacter baumannii strains isolated from burn patients in Tehran, Iran. Acta Microbiol Immunol Hung 2021; 68(1): 48–54.
32. Abbasi E, Mondanizadeh M, van Belkum A, Ghaznavi-Rad E. Low frequency of adenovirus, rotavirus, and norovirus in pediatric diarrheal samples from Central Iran. Arch Pediatr Infect Dis 2021 (In Press).
33. Fardsanei F, Dallal MMS, Douraghi M, Memariani H, Bakhshi B, Salehi TZ, et al. Antimicrobial resistance, virulence genes and genetic relatedness of Salmonella enterica serotype Enteritidis isolates recovered from human gastroenteritis in Tehran, Iran. J Glob Antimicrob Resist 2018; 12: 220–6.
34. Solomon EB, Niemira BA, Sapers GM, Annous BA. Biofilm formation, cellulose production, and curli biosynthesis by Salmonella originating from produce, animal, and clinical sources. J Food Prot 2005; 68(5): 906–12.
35. Beshiru A, Igbinosa IH, Igbinosa EO. Biofilm formation and potential virulence factors of Salmonella strains isolated from ready-to-eat shrimps. PloS one 2018; 13(9): e0204345.
36. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. Am J Infect Control 2006; 34(5): S20–8.
37. Jin Y, Ling J. Prevalence of integrons in antibiotic-resistant Salmonella spp. in Hong Kong. Jpn J Infect Dis 2009; 62(6): 432–9.
38. Antunes P, Machado J, Sousa JC, Peixe L. Dissemination of sulfonamide resistance genes (sul1, sul2, and sul3) in Portuguese Salmonella enterica strains and relation with integrons. Antimicrob Agents Chemother 2005; 49(2): 836–9.
39. Frye JG, Jackson CR. Genetic mechanisms of antimicrobial resistance identified in Salmonella enterica, Escherichia coli, and Enteroccocus spp. isolated from US food animals. Front Microbiol 2013; 4: 135.
40. Adesiji YO, Deekshit VK, Karunasagar I. Antimicrobial-resistant genes associated with Salmonella spp. isolated from human, poultry, and seafood sources. Food Sci Nutr 2014; 2(4): 436–42.
41. Asgharpour F, Mahmoud S, Marashi A, Moulana Z. Molecular detection of class 1, 2 and 3 integrons and some antimicrobial resistance genes in Salmonella Infantis isolates. Iranian J Microbiol 2018; 10(2): 104.
42. Pezzella C, Ricci A, DiGiannatale E, Luzzi I, Carattoli A. Tetracycline and streptomycin resistance genes, transposons, and plasmids in Salmonella enterica isolates from animals in Italy. Antimicrob Agents Chemother 2004; 48(3): 903–8.
43. Long L, You L, Wang D, Wang M, Wang J, Bai G, et al. Highly prevalent MDR, frequently carrying virulence genes and antimicrobial resistance genes in Salmonella enterica serovar 4,[5], 12: i:-isolates from Guizhou Province, China. Plos one 2022; 17(5): e0266443.