Journal of Global Antimicrobial Resistance 19 (2019) 122–128

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Distribution of antimicrobial resistance genes and integrons among *Shigella* spp. isolated from water sources

Khashayar Shahin[a,b], Majid Bouzari[a,*], Ran Wang[b,*], Mohammad Rabbani Khorasgani[a]

[a] Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
[b] State Key Laboratory Cultivation Base of MOST, Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, Nanjing, PR China

ARTICLE INFO

Article history:
Received 22 January 2019
Received in revised form 28 March 2019
Accepted 30 April 2019
Available online 8 May 2019

Keywords:
Shigella
Antimicrobial resistance gene
Mobile genetic element
Water source

ABSTRACT

Objectives: *Shigella* spp. are an important group of waterborne pathogens worldwide. This study aimed to determine the frequency of *Shigella* spp. in a large collection of water samples and to uncover molecular aspects of antimicrobial resistance in the recovered isolates.
Methods: The antimicrobial resistance patterns, antimicrobial resistance genes (ARGs), including β-lactamases (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}, bla_{PER}, bla_{VEB}, bla_{GES} and bla_{CMY}), carbapenemases (bla_{KPC}, bla_{NDM} and bla_{IMP}), plasmid-mediated quinolone resistance (PMQR) genes [*qnrA*, *qnrB*, *qnrS* and *aac(6′)-Ib*] and tetracycline resistance genes [*tet*(A), *tet*(B), *tet*(C) and *tet*(D)], as well as class 1 and 2 integrons were analysed in *Shigella* spp. isolated from different water sources in Iran.
Results: Of 788 tested samples, *Shigella sonnei* and *Shigella flexneri* were detected in 9 (1.1%) and 6 (0.8%) samples, respectively. A multidrug-resistant (MDR) phenotype was observed in all of the isolates. Among the 15 *Shigella* isolates, 12 (80.0%), 5 (33.3%) and 7 (46.7%) were positive for genes encoding β-lactam resistance, PMQR and tetracycline resistance, respectively. Class 1 integrons were more frequently detected among the isolates (8/15; 53.3%), consisting of 7 isolates (87.5%) with *dfrA17–aadA5* and 1 isolate (12.5%) with *sat1–aadA1* gene cassettes. The class 2 integron was detected in 3 isolates (20.0%) with the classic gene cassette array *dfrA1–sat2–aadA1*.
Conclusions: Overall, this study showed that *Shigella* spp. are prevalent in water sources in Iran. Furthermore, the potential role of ARGs and integrons in the emergence of a MDR phenotype in *Shigella* isolates of water origin was demonstrated.

1. Introduction

Shigellosis is characterised as an enteric bacterial infection caused by *Shigella* spp. bacteria and is considered a serious health problem worldwide [1]. According to a report of the World Health Organization (WHO), *Shigella* spp. are responsible for approximately 165 million cases of shigellosis disease and 1 million deaths annually [2]. In Iran, *Shigella* spp. is one of the main causes of acute diarrhoea, especially in young children [3].

Shigella is a genus of rod-shaped, Gram-negative, facultative anaerobic, non-spore-forming, non-motile bacteria [4]. The genus consists of four known species, including *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*, which have also been classified serologically as subgroups A–D, respectively [5]. *Shigella sonnei* and *S. flexneri* have been identified as the predominant species and cause enteric infections in several developed and developing countries, respectively [6].

Shigella is generally known as a waterborne pathogen. It is also transmitted via contaminated food, fomites and direct person-to-person contact. Several shigellosis outbreaks associated with contaminated water sources have been reported throughout the world, e.g. China [7], Oman [8], Israel, Sierra Leone, Sudan and South Africa [9].

Anthropogenic pollution of the environment, in particular water sources, with antibiotics has been regularly reported owing to the irregular and extensive use of antibiotics in veterinary, industry, agriculture and medical treatment [10]. The selection pressure exerted by the presence of antibiotics in the environment alters microbial communities by elimination of susceptible strains and resulting in a higher likelihood of survival of strains harbouring antimicrobial resistance genes (ARGs) [11]. The sources for these ARGs are hospitals and urban wastewater facilities, untreated sewage, aquaculture discharges and agricultural runoff [12]. Horizontal gene transfer of ARGs, which is the main cause of rapid emergence of multidrug resistance in pathogenic and

* Corresponding authors.
E-mail addresses: bouzari@sci.ui.ac.ir (M. Bouzari), ranwang@jaas.ac.cn (R. Wang).

https://doi.org/10.1016/j.jgar.2019.04.020
2213-7165/

non-pathogenic bacteria in the environment [13], occurs frequently between bacterial cells or bacteria and the environment [12].

Moreover, the ability of integrons to acquire, integrate and express gene cassettes encoding ARGs accelerates the horizontal transfer of resistance gene clusters [14]. Fundamentally, integrons are not categorised as mobile elements, but close association between integrons and mobile genetic elements, e.g. transposons, insertion sequences and conjugative plasmids, facilitates their frequent intraspecies and interspecies transmission [15].

Although shigellosis is usually treated with fluid and nutrition support, antibiotics are recommended to reduce the duration of disease and the risk of bacterial excretion [16]. *Shigella* spp. are becoming resistant to various antibiotics, including first-line antibiotics [e.g. ampicillin, trimethoprim/sulfamethoxazole (SXT) and tetracycline] as well as broad-spectrum antibiotics (e.g. quinolones, fluoroquinolones, extended-spectrum cephalosporins and carbapenems) [17]. The emergence of multidrug-resistant (MDR) phenotypes has been reported among clinical, water and food isolates of *Shigella* in the last decade [3,5,16,18,19].

Despite the fact that numerous studies have reported the prevalence of *Shigella* in clinical samples and food products as well as shigellosis outbreaks all around the world, there are only few studies addressing waterborne isolates of *Shigella* spp. [19]. To our knowledge, this is one of the first comprehensive studies on *Shigella* spp. isolated from water samples and their antimicrobial resistance phenotypes, associated ARGs and integrons.

2. Materials and methods

2.1. Sampling

Sampling was performed within a radius of 60 km from the city of Isfahan, Iran, between April–November 2015 (Fig. 1). A total of 788 water samples were collected from surface, ground and farm waters. Water temperature was measured using a handheld thermometer before each sampling. Samples were collected in sterile containers and were then transported immediately to the microbiology laboratory for further analysis.

2.2. Shigella *spp. isolation*

All water samples (200–250 mL) were centrifuged at 5000 × *g* for 5 min and the sediment was transferred to GN broth supplemented with novobiocin (0.2 mg/L) (Sigma-Aldrich, Taufkirchen, Germany) and was incubated anaerobically overnight at 37 °C. A loopful of the enriched sample was then streaked onto xylose–lysine–desoxycholate (XLD) agar (Condalab, Madrid, Spain) and Hektoen enteric agar (Condalab) to pre-select and isolate *Shigella* isolates. Suspected *Shigella* spp. colonies were identified by colony morphology (green to blue-green colonies on Hektoen enteric agar and red or colourless colonies on XLD agar), Gram staining, and conventional biochemical methods including triple sugar iron, urea, motility, citrate utilisation, oxidase, indole production, methyl red and Voges–Proskauer (Merck, Darmstadt, Germany) tests [20,21]. *Shigella sonnei* ATCC 9290 and *S. flexneri* ATCC 12022 were used as positive controls. Isolates were stored at −80 °C in tryptic soy broth containing 25% glycerol for further analyses.

2.3. Molecular identification and serogroup determination

Every biochemically identified isolate was confirmed as *Shigella* by PCR specific for the *invC* gene. For species identification, primers specific for the genes *wbgZ*, *rfc* and *rfpB* were used for detection of *S. sonnei*, *S. flexneri* and *S. dysenteriae*, respectively. Primer information is summarised in Supplementary Table S1. Agglutination on glass slides using polyclonal antisera (Baharafshan Institute of Research & Development, Tehran, Iran) was used for serogrouping [5].

2.4. Antimicrobial susceptibility testing

Susceptibility to a wide range of antibiotics was determined by the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [22]. In addition, the minimum inhibitory concentration (MIC) of imipenem was determined by Etest (Liofilchem, Roseto degli Abruzzi, Italy) according to CLSI guidelines [22]. Extended-spectrum β-lactamase (ESBL)-producing strains were identified by the double-disk synergy test using ceftazidime (30 μg) and ceftazidime (30 μg) alone and with clavulanic acid (10 μg) (Rosco Diagnostica A/S, Taastrup, Denmark) [22]. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Klebsiella pneumoniae* ATCC 700603 were used as controls.

2.5. Characterisation of antimicrobial resistance genes and class 1 and 2 integrons

DNA templates were prepared as previously described [23]. The presence of ARGs for β-lactamases (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}, bla_{PER}, bla_{VEB}, bla_{GES} and bla_{CMY}), carbapenems (bla_{KPC}, bla_{NDM} and bla_{IMP}), plasmid-mediated quinolone resistance (PMQR) [*qnrA*, *qnrB*, *qnrS* and *aac(6′)-Ib*] and tetracycline resistance [*tet*(A), *tet*(B), *tet*(C) and *tet*(D)] as well as class 1 and class 2 integrons was studied by PCR independently from the antimicrobial susceptibility pattern of isolates using specific primers (Supplementary Table S1). Amplification reactions were performed in a total volume of 25 μL of Green Master Mix (TsingKe, Beijing, China) according to the manufacturer's protocol. Amplification reactions were carried out in an Eppendorf thermal cycler (Eppendorf AG, Hamburg, Germany) and the reaction products were subjected to electrophoresis on a 1% agarose gel. The presence and size of amplicons were analysed by the Gel Doc system (Bio-Rad, Hercules, CA, USA).

2.6. DNA sequencing and nucleotide accession numbers

All PCR products were purified from the agarose gel using a QIAquick® Gel Extraction Kit (QIAGEN, Tokyo, Japan). The purified DNA fragments were sequenced using an ABI DNA Analyzer Model 3730xl (TsingKe) and were deposited in DDBJ/EMBL/GenBank under the accession nos. **LC310935**–**LC310976**.

3. Results

3.1. Bacterial strains

A total of 15 *Shigella* spp. (1.9%) were isolated from 788 water samples, all of which were confirmed as *Shigella* spp. by detection of the *invC* gene. From the 788 water samples, 9 (1.1%) and 6 (0.8%) were identified as *S. sonnei* and *S. flexneri*, respectively, using serological and molecular methods. The average number of water samples per month was 90–105. *Shigella* spp. detection was more common during July (2.9%; 3/105), August (4.9%; 5/103) and September (4.0%; 4/100), followed by June (2.0%; 2/100) and October (1.0%; 1/100); no *Shigella* spp. were isolated in April, May and November. The average water temperature ranged from 14 °C (April, May and November) to 22 °C (July, August and September). Thus, detection of *Shigella* throughout the sampling period suggests a seasonal presence, e.g. 4.9% of the samples taken in August were positive *for Shigella*, whilst no *Shigella* were detected in samples taken in April, May and November. *Shigella* was isolated from all three water sources, including surface water

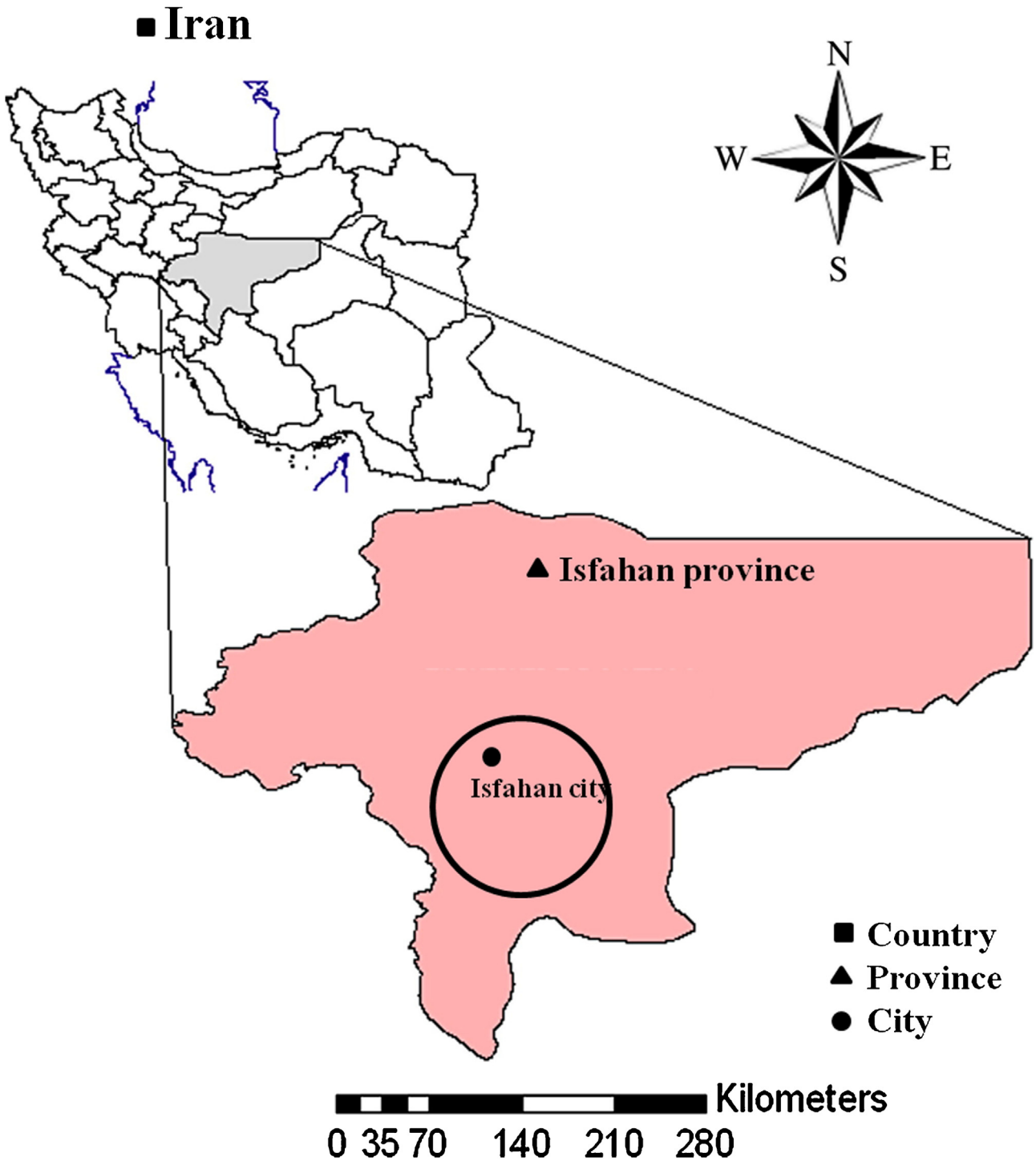

Fig. 1. Sampling locations in Isfahan Province, Iran.

(4 isolates; w45, w81, w2 and w7), ground water (4 isolates; w8, w46, w47 and w19) and farm water (7 isolates; w9, w43, w44, w16, w18, w27 and w90). No *S. dysenteriae* or *S. boydii* were recovered from the water samples.

3.2. Antimicrobial susceptibility and analysis of extended-spectrum β-lactamase production

Antimicrobial susceptibility testing showed that the prevalent resistance phenotypes were against streptomycin (15 isolates; 100%), cefalotin (14; 93.3%), ampicillin (14; 93.3%), nalidixic acid (14; 93.3%) and SXT (9; 60.0%). On the other hand, the lowest resistance rates were observed for chloramphenicol (3 isolates; 20.0%), tobramycin (4; 26.7%), cefepime (5; 33.3%), amikacin (5; 33.3%) and ceftazidime (8; 53.3%). As shown in Table 1, *S. flexneri* isolates were resistant to more antimicrobial agents than *S. sonnei* isolates. Notably, all 15 *Shigella* isolates were resistant to at least three antimicrobial agents and were considered MDR. Except for isolate w44, minor variations in the MIC of imipenem were observed for *Shigella* spp. (Table 2).

Table 1
Frequency of antimicrobial resistance, antimicrobial resistance genes (ARGs) and integrons in water isolates of *Shigella* spp. in Iran.

Antimicrobial agent/ARG/integron	*n* (%)		
	S. sonnei (*n* = 9)	*S. flexneri* (*n* = 6)	Total (*n* = 15)
Antimicrobial agent			
Ampicillin	8 (88.9)	6 (100)	14 (93.3)
Chloramphenicol	1 (11.1)	2 (33.3)	3 (20.0)
Streptomycin	9 (100)	6 (100)	15 (100)
Tobramycin	3 (33.3)	1 (16.7)	4 (26.7)
Amikacin	3 (33.3)	2 (33.3)	5 (33.3)
SXT	5 (55.6)	4 (66.7)	9 (60.0)
Tetracycline	5 (55.6)	5 (83.3)	10 (66.7)
Nalidixic acid	8 (88.9)	6 (100)	14 (93.3)
Ciprofloxacin	2 (22.2)	4 (66.7)	6 (40.0)
Cefalotin	8 (88.9)	6 (100)	14 (93.3)
Cefuroxime	4 (44.4)	6 (100)	10 (66.7)
Ceftazidime	4 (44.4)	4 (66.7)	8 (53.3)
Cefotaxime	4 (44.4)	5 (83.3)	9 (60.0)
Cefepime	2 (22.2)	3 (50.0)	5 (33.3)
ARG/integron			
bla_{TEM-1}	5 (55.6)	–	5 (33.3)
bla_{SHV-2}	–	2 (33.3)	2 (13.3)
$bla_{CTX-M-15}$	4 (44.4)	3 (50.0)	7 (46.7)
bla_{CMY-2}	1 (11.1)	–	1 (6.7)
qnrB2	–	1 (16.7)	1 (6.7)
qnrS1	–	1 (16.7)	1 (6.7)
aac(6′)-Ib-cr	3 (33.3)	1 (16.7)	4 (26.7)
tet(A)	4 (44.4)	3 (50.0)	7 (46.7)
tet(B)	1 (11.1)	1 (16.7)	2 (13.3)
tet(C)	1 (11.1)	–	1 (6.7)
dfrA17–aadA5	5 (55.6)	2 (33.3)	7 (46.7)
sat1–aadA1	1 (11.1)	–	1 (6.7)
dfrA1–sat2–aadA1	2 (22.2)	1 (16.7)	3 (20.0)

SXT, trimethoprim/sulfamethoxazole.

Moreover, a total of nine *Shigella* isolates (four *S. sonnei* and five *S. flexneri*) were identified as ESBL-producers by the double-disk synergy method (Table 2).

3.3. Detection and identification of antimicrobial resistance genes

As shown in Tables 1 and 2, 80.0% (12/15), 33.3% (5/15) and 46.6% (7/15) of the recovered isolates contained at least one of the β-lactamase-encoding, PMQR and tetracycline resistance genes, respectively. DNA sequencing revealed that all of the TEM-, SHV-, CTX-M- and CMY-encoding genes were bla_{TEM-1}, bla_{SHV-2}, $bla_{CTX-M-15}$ and bla_{CMY-2}, respectively. Moreover, DNA sequencing showed that the PMQR *qnr* genes were present in two isolates (one *qnrB2* and one *qnrS1*) and the fluoroquinolone-modifying acetyltransferase gene *aac(6′)-Ib*-cr was present in four isolates (Tables 1 and 2). Among the *tet* genes, *tet*(A) was the most prevalent type and was detected in 7 isolates (46.7%), followed by *tet*(B) (2 isolates; 13.3%) and *tet*(C) (1 isolate; 6.7%), whilst *tet*(D) was not detected in any of the isolates (Tables 1 and 2).

3.4. Detection and identification of class 1 and class 2 integrons

Among the 15 *Shigella* spp. isolates, 8 (53.3%) were positive for a class 1 integron. DNA sequencing of the inserted gene cassette fragments revealed two types of class 1 integrons with four different ARG cassettes (Tables 1 and 2), including dihydrofolate reductase (*dfrA17*), aminoglycoside adenyltransferase (*aadA5*), aminoglycoside adenylyltransferase (*aadA1*) and acetyltransferase (*sat1*). Class 2 integrons were detected in three isolates (20.0%). DNA sequencing results showed the inserted gene cassettes were composed only of the classical type of three conserved resistance gene including *dfrA1*, *sat2* and *aadA1* (Table 1).

4. Discussion

Since the early 1960s, *Shigella* spp., as the most important waterborne and foodborne pathogen worldwide, has been reported as acquiring multidrug resistance [5,16,18]. A reason for the emergence of MDR *Shigella* spp. is the natural ability of the pathogen to acquire resistance factors (resistance genes, plasmids, transposons and integrons) from other Enterobacteriaceae and from the environment [24].

As mentioned previously, several waterborne outbreaks of shigellosis have been reported worldwide. Furthermore, many water sources have been analysed for the presence of *Shigella* spp. For example, *Shigella* was isolated from ponds, lakes, wells and rivers in Bangladesh, small water systems in mountainous areas in Taiwan, and the Narmada River in India [19]. In the current study, *Shigella* spp. were detected in only 1.9% of tested water samples. A high rate of *Shigella* spp. isolation from surface water samples of Narmada River (22.2%) was reported by Sharma et al. [19]. Poor sanitation, low public-health levels, and lack of effective wastewater treatment systems in the research location of Sharma et al. may explain the considerable difference in *Shigella* spp. isolation between the two studies.

In the present study, the highest isolation rate was observed during July–September, indicating a correlation between temperature and the occurrence of *Shigella* spp., consistent with a previous report from Iran [5].

Shigella sonnei was identified as the most prevalent isolate throughout the study (9/15; 60.0%), followed by *S. flexneri* (6/15; 40.0%). Although *S. flexneri* had been reported to be the predominant species both in clinical and environmental sources in developing countries [19], in the last decades several reports have revealed that *S. sonnei* is becoming the most frequent species of *Shigella* in Iran [3,5,18,25]. This may indicate a correlation between the frequency of *Shigella* spp. isolated from human patients and from water sources in Iran. Moreover, improvements in personal and public hygiene levels as well as industrialisation during recent years may explain the predominance of *S. sonnei* versus *S. flexneri* in Iran [5]. Although no *S. boydii* or *S. dysenteriae* isolates were detected in the current study, they were reported as the predominant species in river catchments of South Africa and were responsible for shigellosis outbreaks in Sudan and Sierra Leone, respectively [9]. Moreover, *S. dysenteriae* was detected in 17.5% of samples collected from Narmada River in India [19].

In the current study, a high resistance rate to most of the tested antibiotics was observed. Therefore, it is clear that these isolates are MDR and require serious attention to a prevention strategy to avoid probable casualties. From a regional point of view, the results are in accordance with previous studies in Iran that also reported different high resistance rates to antibiotics agents among clinical *Shigella* spp. isolates [18,26]. Various resistance levels to nalidixic acid and ciprofloxacin have been reported among *Shigella* spp. in Asia–Africa regions (high frequency) and Europe–America regions (low frequency) [27]. In addition, in the USA, [28] reported 87% resistance to ciprofloxacin in *S. sonnei* infections. On the other hand, Ahmed and Shimamoto [16] reported high resistance to tetracycline (95.8%), nalidixic acid (95.8%), SXT and ampicillin (87.5%), chloramphenicol (58.5%) and ciprofloxacin, ceftriaxone and cefotaxime (37.5%) in food isolates of *Shigella* spp. in Egypt. Thus, comparing the results obtained in the current study with earlier clinical and food studies suggests that there has been a noticeable increase in resistance to common antibiotics [3,18].

The current results also demonstrated that all 15 *Shigella* isolates exhibited a MDR phenotype. In India, 52.5% of surface water (Narmada River) isolates of *Shigella* spp. were identified as MDR [19]. Moreover, in Egypt the incidence of MDR strains of

Table 2
Antimicrobial resistance phenotype, antimicrobial resistance genes (ARGs) and integrons in water isolates of *Shigella* spp. in Iran.

Isolate	Antimicrobial resistance profile	IPM MIC (μg/mL)	ESBL	Integron			ARGs
				Class	Size (bp)	Content	
S. sonnei- w8	STR, TOB, NAL	0.094	–	1	1472	*sat1*, *aadA1*	–
S. sonnei- w9	AMP, TET, STR, CHL, CEF	0.125	–	2	2162	*dfrA1*, *sat2*, *aadA1*	bla_{TEM-1}, *tet*(A), *tet*(C)
S. sonnei- w43	AMP, SXT, TET, STR, AMK, NAL, CEF	0.125	–	2	1805	*dfrA1*, *sat2*, *aadA1*	bla_{TEM-1}
S. sonnei- w44	AMP, TET, STR, NAL, CIP, CEF, CXM, CAZ, CTX, FEP	0.75	+	1	1663	*dfrA17*, *aadA5*	bla_{TEM-1}, $bla_{CTX-M-15}$, bla_{CMY-2}, *aac(6′)-Ib*-cr, *tet*(A), *tet*(B)
S. sonnei- w45	AMP, SXT, TET, STR, AMK, NAL, CEF, CXM, CAZ, CTX	0.19	+	1	1612	*dfrA17*, *aadA5*	$bla_{CTX-M-15}$, *aac(6′)-Ib*-cr, *tet*(A)
S. sonnei- w46	AMP, SXT, STR, TOB, NAL, CIP, CEF, CXM, CAZ, CTX	0.094	+	1	1608	*dfrA17*, *aadA5*	$bla_{CTX-M-15}$, *aac(6′)-Ib*-cr
S. sonnei- w47	AMP, STR, NAL, CEF	0.125	–	1	1609	*dfrA17*, *aadA5*	bla_{TEM-1}
S. sonnei- w81	AMP, SXT, TET, STR, TOB, AMK, NAL, CEF, CXM, CAZ, CTX, FEP	0.19	+	1	1611	*dfrA17*, *aadA5*	bla_{TEM-1}, $bla_{CTX-M-15}$, *tet*(A)
S. sonnei- w90	AMP, SXT, STR, NAL, CEF	0.125	–	–	–	–	–
S. flexneri- w2	AMP, SXT, TET, STR, NAL, CIP, CEF, CXM, CAZ, CTX, FEP	0.094	+	2	2149	*dfrA1*, *sat2*, *aadA1*	bla_{SHV-2}, *qnrB2*, *aac(6′)-Ib*-cr, *tet*(A)
S. flexneri- w7	AMP, SXT, TET, STR, NAL, CIP, CEF, CXM, CAZ, CTX, FEP	0.19	+	–	–	–	$bla_{CTX-M-15}$, *tet*(A)
S. flexneri-w16	AMP, STR, NAL, CEF, CXM, CAZ, CTX	0.094	+	–	–	–	bla_{SHV-2}
S. flexneri-w18	AMP, SXT, TET, STR, AMK, CHL, NAL, CIP, CEF, CXM, CTX	0.125	+	1	1609	*dfrA17*, *aadA5*	$bla_{CTX-M-15}$, *qnrS1*
S. flexneri-w19	AMP, SXT, TET, STR, TOB, AMK, CHL, NAL, CIP, CEF, CXM, CAZ, CTX, FEP	0.125	+	1	1612	*dfrA17*, *aadA5*	$bla_{CTX-M-15}$, *tet*(A), *tet*(B)
S. flexneri-w27	AMP, TET, STR, NAL, CEF, CXM	0.094	–	–	–	–	–

AMK, amikacin; AMP, ampicillin; CAZ, ceftazidime; CEF, cefalotin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; CXM, cefuroxime; ESBL, extended-spectrum β-lactamase; FEP, cefepime; IPM, imipenem; MIC, minimum inhibitory concentration; NAL, nalidixic acid, STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TOB, tobramycin.

Shigella spp. in food samples was reported to be approximately 90% [16]. It is noticeable that in recent studies in Iran on clinical isolates of *Shigella* spp., the prevalence of multidrug resistance ranged from 45% [25] to 98.5% [5] and 100% [18]. This may indicate a direct correlation between clinical cases and contamination of water sources. It is important to note that the progressive increase in antimicrobial resistance among bacterial pathogens is becoming a global issue and a threat to public health.

The detection and characterisation of different antimicrobial resistance determinants in water-source bacteria is not new [11]. In this study, bla_{TEM-1}, bla_{SHV-2}, $bla_{CTX-M-15}$ and bla_{CMY-2} were detected among the *Shigella* spp. isolates. Furthermore, among the nine *Shigella* spp. isolates with an ESBL phenotype, two harboured the bla_{SHV-2} gene, one harboured bla_{TEM-1} and $bla_{CTX-M-15}$, one harboured bla_{TEM-1}, $bla_{CTX-M-15}$ and bla_{CMY-2}, and five harboured $bla_{CTX-M-15}$.

The $bla_{CTX-M-15}$ gene, conferring resistance to cefotaxime, was the predominant β-lactamase gene in isolated strains, in agreement with earlier reports in which $bla_{CTX-M-15}$ was identified as the most common type of cefotaxime resistance gene among clinical *Shigella* isolates in Iran [18] and the USA [29] as well as among food isolates of *Shigella* in Egypt [16]. Furthermore, bla_{SHV-2} and bla_{CMY-2} were rarely detected among *Shigella* isolates in Iran and Egypt [16,18].

Ciprofloxacin, a fluoroquinolone, is recommended by the WHO as the first-line antimicrobial for treatment of shigellosis [6]. However, the appearance of fluoroquinolone resistance has resulted in a lower efficacy of fluoroquinolones in shigellosis treatment. In the present study, *qnrB*, *qnrS* and *aac(6′)-Ib* genes were detected in the isolates. Furthermore, among the six isolates resistant to ciprofloxacin, one harboured *qnrS*, two harboured *aac(6′)-Ib*, and one harboured *qnrB* and *aac(6′)-Ib*, whereas two harboured no PMQR genes. In Egypt, *qnrB* (7.4%), *qnrS* (25.9%) and *aac(6′)-Ib* (11.1%) were detected among food isolates of *Shigella* spp. [16]. In the USA, *qnrB*, *qnrS* and *aac(6′)-Ib* were detected in 30% of *Shigella* spp. [29]. The *qnrS* gene was detected for the first time in a clinical strain of *S. flexneri* isolated in Japan [30]. In Iran, *qnrS* was identified as the only PMQR gene among clinical isolates of *Shigella* spp. [3,18]. Thus, in the present study, *qnrB* was detected for the first time in waterborne *S. flexneri* in Iran.

PCR results for tetracycline resistance genes revealed that 46.7%, 13.3% and 6.7% of the isolates were positive for *tet*(A), *tet*(B) and *tet*(C), respectively. Moreover, among the 10 *Shigella* spp. isolates resistant to tetracycline, 4 harboured *tet*(A), 2 harboured *tet*(A) and *tet*(B), and 1 harboured *tet*(A) and *tet*(C), whereas 3 harboured no tetracycline resistance gene. These results demonstrated that *tet*(A) was the dominant tetracycline resistance-related gene in these water isolates of *Shigella* spp. In the case of clinical *Shigella* isolates, Alizadeh-Hesar et al. reported that *tet*(A) was the main tetracycline resistance-related gene in clinical isolates in Iran [5]. The *tet*(B) gene was identified as the major tetracycline resistance-related gene in other countries (e.g. Brazil, USA, Canada, Thailand and Bangladesh) [31]. On the other hand, the *tet*(C) gene, which has not been previously reported in *Shigella* spp. [5,31], was detected in the current study. The presence of *tet*(C) in other Gram-negative bacteria has been reported recently [32]. Therefore, the transmissibility of resistance determinants in the bacterial community by horizontal transfer (e.g. plasmids and transposons) [13] may explain the acquisition of new resistance genes by *Shigella* spp.

Among the 15 *Shigella* spp. isolates, 8 (6 *S. sonnei* and 2 *S. flexneri*) were positive for a class 1 integron and 3 were positive for a class 2 integron. A high prevalence of integrons had been observed among *Shigella* isolated from faeces in Iran [5,25]. Similar studies on *Shigella* isolates from patients and food in Iran and other countries reported a higher prevalence of class 2 compared with class 1 integrons. In Iran, class 1 and class 2 integrons were identified in 78.12% and 40.6% of clinical isolates of *Shigella* spp., respectively [25]. In a more recent study in Iran, class 1 and class 2 integrons were detected in 22.85% and 87.71% of clinical isolates of *Shigella* spp., respectively [5]. Previous studies reported the prevalence of class 1 and class 2 integrons in clinical isolates of *Shigella* spp. as 3.8% and 80% in Japan [33] and 9.4% and 84.3% in

China, respectively [34]. In contrast, many studies have reported a higher prevalence of class 1 compared with class 2 integrons in other Gram-negative bacteria, e.g. *E. coli* [35], *E. coli* O157: H7 [36], *Salmonella* spp. [37] and *K. pneumoniae* [38]. Comparison of the presence of class 1 and 2 integrons in different bacteria supports the fact that Enterobacteriaceae act as a reservoir of integrons that can be transferred easily by different mobile genetic elements, e.g. plasmids, to other species within or outside the family and alter the incidence of class 1 and 2 integrons in different environments.

5. Conclusions

This study describes the distribution of *Shigella* spp. isolated from water in Iran for the first time. Although *Shigella* spp. were isolated in approximately 2% of the water samples, there are no reports regarding waterborne shigellosis in the study region. This indicates the acceptable performance of drinking water treatment systems in Iran. The risk of contamination still exists in cases of inaccessibility to treated drinking water, such as camping near rivers and ponds, swimming, washing or drinking contaminated water, and using private wells. Moreover, the presence of *Shigella* in water sources act as a bioindicator, meaning that other waterborne pathogens such as *Salmonella* spp., *E. coli*, *Klebsiella* etc. may also be present. Thus, from a public-health perspective, it is important to study the sampling areas further for isolation and characterisation of other important pathogens. This study also characterised the genetic basis of antimicrobial resistance in *Shigella* spp. isolated from water sources. To the best of our knowledge, these data could reveal new aspects of the molecular basis of antimicrobial resistance in *Shigella* spp. owing to the presence of *tet*(C) as well as a high rate of class 1 integrons that was reported for the first time in the current study.

Funding

This study was supported by the University of Isfahan (Isfahan, Iran) [No. A/94/32650] and the Jiangsu Agricultural Science and Technology Foundation (Nanjing, China) [No. CX (16) 1060].

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgment

The authors would like to give special thanks and appreciation to the all persons who made this research possible.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.04.020.

References

[1] Shahin K, Bouzari M. Bacteriophage application for biocontrolling *Shigella flexneri* in contaminated foods. J Food Sci Technol 2018;55:550–9.
[2] World Health Organization (WHO). Antimicrobial resistance: global report on surveillance 2014. Geneva, Switzerland: WHO; 2014.
[3] Ranjbar R, Behnood V, Memariani H, Najafi A, Moghbeli M, Mammina C. Molecular characterisation of quinolone-resistant *Shigella* strains isolated in Tehran, Iran. J Glob Antimicrob Resist 2016;5:26–30.
[4] Shahin K, Bouzari M, Wang R. Isolation, characterization and genomic analysis of a novel lytic bacteriophage vB_SsoS-ISF002 infecting *Shigella sonnei* and *Shigella flexneri*. J Med Microbiol 2018;67:376–86.
[5] Alizadeh-Hesar M, Bakhshi B, Najar-Peerayeh S. Clonal dissemination of a single *Shigella sonnei* strain among Iranian children during Fall 2012 in Tehran, I.R. Iran. Infect Genet Evol 2015;34:260–6.
[6] Shigellosis: disease burden, epidemiology and case management. Wkly Epidemiol Rec 2005;80:94–9.
[7] He F, Han K, Liu L, Sun W, Zhang L, Zhu B, et al. Shigellosis outbreak associated with contaminated well water in a rural elementary school: Sichuan Province, China, June 7–16, 2009. PLoS One 2012;7:e47239.
[8] Abaidani I, Raju PA, Al-Shualli I, Al-Sa'di K, Al-Shaqsi N, Al-Khatri A. Shigellosis outbreak in Al Batinah South Governorate, Oman: case–control study. Sultan Qaboos Univ Med J 2015;15:382–9.
[9] Wose Kinge C, Mbewe M. Characterisation of *Shigella* species isolated from river catchments in the North West Province of South Africa. S Afr J Sci 2010;106:1–4.
[10] Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, et al. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. Lancet Infect Dis 2013;13:155–65.
[11] Winkworth-Lawrence C, Lange K. Antibiotic resistance genes in freshwater biofilms may reflect influences from high-intensity agriculture. Microb Ecol 2016;72:763–72.
[12] Garner E, Benitez R, von Wagoner E, Sawyer R, Schaberg E, Hession WC, et al. Stormwater loadings of antibiotic resistance genes in an urban stream. Water Res 2017;123:144–52.
[13] Yu Z, He P, Shao L, Zhang H, Lü F. Co-occurrence of mobile genetic elements and antibiotic resistance genes in municipal solid waste landfill leachates: a preliminary insight into the role of landfill age. Water Res 2016;106:583–92.
[14] Basu S, Mukherjee M. Incidence and risk of co-transmission of plasmid-mediated quinolone resistance and extended-spectrum β-lactamase genes in fluoroquinolone-resistant uropathogenic *Escherichia coli:* a first study from Kolkata, India. J Glob Antimicrob Resist 2018;14:217–23.
[15] Kaushik M, Kumar S, Kapoor RK, Virdi JS, Gulati P. Integrons in *Enterobacteriaceae*: diversity, distribution and epidemiology. Int J Antimicrob Agents 2018;51:167–76.
[16] Ahmed AM, Shimamoto T. Molecular characterization of multidrug-resistant *Shigella* spp. of food origin. Int J Food Microbiol 2015;194:78–82.
[17] Parvez S, Khan AU. Hospital sewage water: a reservoir for variants of New Delhi metallo-β-lactamase (NDM)- and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Int J Antimicrob Agents 2018;51:82–8.
[18] Tajbakhsh M, Migura LG, Rahbar M, Svendsen CA, Mohammadzadeh M, Zali MR, et al. Antimicrobial-resistant *Shigella* infections from Iran: an overlooked problem? J Antimicrob Chemother 2012;67:1128–33.
[19] Sharma A, Singh SK, Bajpai D. Phenotypic and genotypic characterization of *Shigella* spp. with reference to its virulence genes and antibiogram analysis from river Narmada. Microbiol Res 2010;165:33–42.
[20] Ahmed AM, Shimamoto T. Isolation and molecular characterization of *Salmonella enterica*, *Escherichia coli* O157: H7 and *Shigella* spp. from meat and dairy products in Egypt. Int J Food Microbiol 2014;168:57–62.
[21] Mikoleit M. Laboratory protocol: biochemical identification of *Salmonella and Shigella* using an abbreviated panel of tests. Geneva, Switzerland: WHO Global Foodborne Infections Network; 2010.
[22] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement CLSI document M100-S25. Wayne, PA: CLSI; 2015.
[23] Komijani M, Shahin K, Barazandeh M, Sajadi M. Prevalence of extended-spectrum β-lactamases genes in clinical isolates of *Pseudomonas aeruginosa*. Med Lab J 2018;12:34–41.
[24] Azmi IJ, Khajanchi BK, Akter F, Hasan TN, Shahnaij M, Akter M, et al. Fluoroquinolone resistance mechanisms of *Shigella flexneri* isolated in Bangladesh. PLoS One 2014;9:e102533.
[25] Eftekhari N, Bakhshi B, Pourshafie MR, Zarbakhsh B, Rahbar M, Hajia M, et al. Genetic diversity of *Shigella* spp. and their integron content. Foodborne Pathog Dis 2013;10:237–42.
[26] Jafari F, Hamidian M, Rezadehbashi M, Doyle M, Salmanzadeh-Ahrabi S, Derakhshan F, et al. Prevalence and antimicrobial resistance of diarrheagenic *Escherichia coli* and *Shigella* species associated with acute diarrhea in Tehran, Iran. Can J Infect Dis Med Microbiol 2009;20:e56–62.
[27] Gu B, Cao Y, Pan S, Zhuang L, Yu R, Peng Z, et al. Comparison of the prevalence and changing resistance to nalidixic acid and ciprofloxacin of *Shigella* between Europe–America and Asia–Africa from 1998 to 2009. Int J Antimicrob Agents 2012;40:9–17.
[28] Bowen A, Hurd J, Hoover C, Khachadourian Y, Traphagen E, Harvey E, et al. Importation and domestic transmission of *Shigella sonnei* resistant to ciprofloxacin—United States, May 2014–February 2015. MMWR Morb Mortal Wkly Rep 2015;64:318–20.
[29] Folster JP, Pecic G, Krueger A, Rickert R, Burger K, Carattoli A, et al. Identification and characterization of CTX-M-producing *Shigella* isolates in the United States. Antimicrob Agents Chemother 2010;54:2269–70.
[30] Hata M, Suzuki M, Matsumoto M, Takahashi M, Sato K, Ibe S, et al. Cloning of a novel gene for quinolone resistance from a transferable plasmid in *Shigella flexneri* 2b. Antimicrob Agents Chemother 2005;49:801–3.
[31] Hartman AB, Essiet II, Isenbarger DW, Lindler LE. Epidemiology of tetracycline resistance determinants in *Shigella* spp. and enteroinvasive *Escherichia coli*: characterization and dissemination of *tet*(A)-*1*. J Clin Microbiol 2003;41:1023–32.

[32] Seifi S, Khoshbakht R. Prevalence of tetracycline resistance determinants in broiler isolated *Escherichia coli* in Iran. Br Poult Sci 2016;57:729–33.
[33] Ahmed AM, Furuta K, Shimomura K, Kasama Y, Shimamoto T. Genetic characterization of multidrug resistance in *Shigella* spp. from Japan. J Med Microbiol 2006;55:1685–91.
[34] Pan J-C, Ye R, Meng D-M, Zhang W, Wang H-Q, Liu K-Z. Molecular characteristics of class 1 and class 2 integrons and their relationships to antibiotic resistance in clinical isolates of *Shigella sonnei* and *Shigella flexneri*. J Antimicrob Chemother 2006;58:288–96.
[35] Rezaee MA, Sheikhalizadeh V, Hasani A. Detection of integrons among multidrug resistant (MDR) *Escherichia coli* strains isolated from clinical specimens in northern west of Iran. Braz J Microbiol 2011;42:1308–13.
[36] Ahmed AM, Shimamoto T. Molecular analysis of multidrug resistance in Shiga toxin-producing *Escherichia coli* O157: H7 isolated from meat and dairy products. Int J Food Microbiol 2015;193:68–73.
[37] Ranjbar R, Giammanco GM, Farshad S, Owlia P, Aleo A, Mammina C. Serotypes, antibiotic resistance, and class 1 integrons in *Salmonella* isolates from pediatric cases of enteritis in Tehran, Iran. Foodborne Pathog Dis 2011;8:547–53.
[38] Rezaee MA, Langarizadeh N, Aghazadeh M. First report of class 1 and class 2 integrons in multidrug-resistant *Klebsiella pneumoniae* isolates from northwest Iran. Jpn J Infect Dis 2012;65:256–9.