

# Frequency of Antimicrobial-Resistant Genes in *Salmonella enteritidis* Isolated from Traditional and Industrial Iranian White Cheeses

Abbas Doosti<sup>1</sup> · Elham Doosti<sup>1</sup> · Ebrahim Rahimi<sup>2</sup> · Payam Ghasemi-Dehkordi<sup>3</sup>

Received: 11 December 2014 / Revised: 21 April 2015 / Accepted: 30 May 2015  
© The National Academy of Sciences, India 2015

**Abstract** Iranian white cheese is one of the most important kinds of cheese produced in large scale with high consumption in the country. This dairy product transmits bacterial pathogens like *Salmonella* spp. Antibiotic resistant *Salmonella* are widespread in the world. This study was performed to evaluate the frequency of antimicrobial-resistant *Salmonella enteritidis* and related genes isolated from traditional and industrial Iranian white cheeses. A total of 200 traditional and industrial Iranian white cheeses were collected within Chaharmahal Va Bakhtiari province (southwest Iran). After culturing on specific media using standard bacterial tests the *Salmonella* sp. was isolated. For specific detection of *S. enteritidis* from other *Salmonella* strains *sefA* gene was studied. Finally, the antibiotic susceptibility patterns were investigated. Results showed that 17 % of cheese samples were contaminated by *Salmonella* and 5.5 % of specimens by *S. enteritidis*. The frequencies of resistance genes including *tetA*, *tetB*, *tetC*, *cat3*, and *floR* in isolated *S. enteritidis* were 36.4, 54.5, 81.8, 54.5, and 36.4 %, respectively. All isolated *S. enteritidis* were susceptible to ciprofloxacin, cefotaxime, and ceftazidime (100 %). In addition, most of them were resistance to

chloramphenicol (64 %) and susceptible to gentamicin (98 %). The *Salmonella* contamination was more frequent in traditional Iranian white cheeses (11.5 %) as compared to industrial (5.5 %) samples ( $p < 0.05$ ). As compared to industrial samples, high level of resistant genes in *Salmonella enteritidis* isolated from traditional Iranian white cheeses were observed ( $p < 0.05$ ). Therefore, traditional Iranian white cheeses are important source of *Salmonella* contamination in the country hence examination of dairy products for the presence of this pathogen is important.

**Keywords** *Salmonella enteritidis* · Iranian white cheese · Antibiotic susceptibility pattern

## Introduction

Cheese is a food product derived from clabber milk (sour) that is produced in a wide range of flavors, textures, and forms. This fermented product is usually made from cow's milk and sometimes from that of goat, sheep or buffalo [1]. At present, different kinds of cheeses especially Iranian white cheese are being produced in Iran. This product is a salt-water cheese with salty taste and low level of acidity. These cheeses are traditionally made by ranchers or in factories using dairy milk of cattle, sheep or goats [2].

Raw milk and its products (like cheeses and yogurts) may be contaminated during food processing by pathogenic bacteria including *Listeria*, *Brucella*, and *Salmonella* spp. *Salmonella*, a gram-negative facultative rod-shaped bacterium, is a member of family Enterobacteriaceae that can cause food poisoning, typhoid fever and inflammation of the brain [3, 4]. The symptoms of *Salmonella* contamination (Salmonellosis) are typically diarrhea, fever, vomiting, and abdominal cramps 12–72 h after infection [5].

✉ Abbas Doosti  
biologyshki@yahoo.com

<sup>1</sup> Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Rahmatieh, P.O. Box 166, Shahrekord, Iran

<sup>2</sup> Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

<sup>3</sup> Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

The genus *Salmonella* is a facultative intracellular pathogen consisting of two species including *Salmonella enterica* and *Salmonella bongori* that cause disease in humans. *S. enterica* serotype *enteritidis* (*S. enteritidis*) is facultative anaerobe with food-borne outbreaks and can be transmitted easily from animals to human beings [6]. While, poultry and egg products are important source of this bacterium, the consumption of contaminated dairy products like milk and cheese can also transmit *S. enteritidis* to human beings [7–9]. This bacterium is one of the important agents of gastroenteritis (food poisoning) caused by non-typhoid *Salmonella* and it depends on the dose of bacteria [8, 10]. Diarrhea caused by *S. enteritidis* is often mucopurulent (containing mucus or pus) and bloody. The illness period is between 4 and 7 days, and most people recover without treatment [11, 12].

The frequency of *Salmonella* contamination in dairy products is variable. In Japan, Greece, Turkey, and Iran (Isfahan city) 11.4, 5.1, 2.4, and 1.66 % of samples have been reported to be contaminated, respectively [13–15]. Therefore, preparing the safe dairy products decrease the food-borne pathogens and is important for public health.

In recent decades, antimicrobial-resistant strains of *Salmonella* have increased and it is one of the significant human hazards [16, 17]. Resistance to antibiotics such as chloramphenicol, florfenicol, and tetracycline are encoded by *cat3*, *floR*, *tetA*, *tetB*, or *tetC* genes [18].

## Material and Methods

### Sampling

The present study was conducted from April, 2014 up to September, 2014 in Shahrekord city (Chaharmahal Va Bakhtiari province, southwest Iran). A total of 200 cheese samples including 100 industrial and 100 traditional were randomly collected and used for detection of *Salmonella* by biochemical and molecular techniques [19]. Sample size for frequency survey was determined using Cochran's formula at 5 % level of significance.

$$N = \frac{(Z_{\alpha/2})^2 \times P(1 - P)}{d^2}$$

N is the sample size,  $Z^2$  is the abscissa of the normal curve that cuts off an area  $\alpha$  at the tails, d is the acceptable sampling error, P is the estimated proportion of an attribute that is present in the population

### Isolation and Culture of *Salmonella*

The cheese samples were transferred to concentrated peptone water and were incubated at 37 °C for 24 h. Then,

samples were transferred to Rappaport–Vassiliadis (RV) and incubated again at 43 °C for 24 h. The isolated bacteria were cultured on *Salmonella–Shigella* (SS) agar. Black and gray colonies were selected as suspected *Salmonella*. Complementary assays and differential microbial tests including urease, IMViC [Methyl Red (MR), and Voges–Proskauer (VP) broth], as well as culturing in triple sugar iron agar (TSI) according to Bergey's manual were used to detect *Salmonella* strain [20]. Then, the lysine decarboxylation was performed on isolated bacteria by culturing in lysine decarboxylase broth to identify the *Salmonella* family. Sulfide indole motility (SIM) medium was used for direct detection of *Salmonella* spp. by sulfide production, indole formation, and motility. This test is positive for *Salmonella* family except *Salmonella paratyphi*.

### Genomic DNA Extraction

Bacterial genomic DNA was extracted using QIAamp mini kit (Qiagen, GmbH, Germany) according to the manufacturer's description. The concentration of extracted DNA was measured by NanoDrop ND-1000 (PecLab) spectrophotometer according to the method described by Sambrook and Russell [21].

### Gene Amplification

The polymerase chain reaction (PCR) using designed specific oligonucleotide primers for 16S rRNA gene was performed for direct detection of *Salmonella* family in the samples. The amplified 16S rRNA fragments were sequenced in an ABI 3730xl automated sequencer (Applied Biosystems) by Macrogen Inc. (Seoul, Korea) using Sanger sequencing method for the final confirmation of isolated *Salmonella*. In the present study, *sefA* gene was targeted for specific detection of *S. enteritidis* from other *Salmonella* strains [22, 23]. The primer sequences for *sefA* gene were obtained from Pan et al. study for gene amplification [22]. The specific oligonucleotide primers for gene amplification of antimicrobial resistance genes including *tetA*, *tetB*, *tetC*, *cat3*, and *floR* in isolated *S. enteritidis* were obtained from Ma et al. and Chuanchuen et al. studies [24, 25]. The sequences of primers were analyzed by basic local alignment search tool (BLAST) in GenBank data (Table 1).

PCR reaction was carried out in a final reaction of 25 µL in 0.5 mL micro-tubes containing 2 mM MgCl<sub>2</sub>, 200 µM dNTPs mix, 2.5 µL of 10X PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 1 unit of *Taq* DNA polymerase (all Fermentas, Germany), 1 µg of template DNA, and 1 µM of each primer. A 2 µL of sterile ultrapure deionized water instead of template DNA and extracted DNA from *S. enteritidis* ATCC 13076 obtained from Pasteur Institute

**Table 1** The sequence of primers used for gene amplification

Gene	Primers name	Primer sequence	Accession number	Products length (bp)
<i>sefA</i>	sefA-F	5'-TGCTATTTTGGCCCTGTACACTGC-3'	CP008927	214
	sefA-R	5'-TTCGGGGGAGACTATACCTACAG-3'		
<i>16S rRNA</i>	16S rRNA-F	5'-AACCGACTCACTCTGGCAG-3'	CP009084	214
	16S rRNA-R	5'-TAACGCGATAGCGCTTC-3'		
<i>tetA</i>	tetA-F	5'-GCTGTCGGATCGTTTCGG-3'	AB723628	658
	tetA-R	5'-CATTCGAGCATGAGTGCC-3'		
<i>tetB</i>	tetB-F	5'-CTGTCGCGGCATCGGTCAT-3'	HQ840942	615
	tetB-R	5'-CAGGTAAAGCGATCCCACC-3'		
<i>tetC</i>	tetC-F	5'-CTTGAGAGCCTTCAACCCAG-3'	J01749, JN990823	418
	tetC-R	5'-ATGGTCGTCATCTACCTGCC-3'		
<i>cat3</i>	cat3-F	5'-AACGGCATGATGAACCTGAA-3'	AJ401047	547
	cat3-R	5'-ATCCCAATGGCATCGTAAAG-3'		
<i>floR</i>	floR-F	5'-ATGACCACCACACGCCCG-3'	AF261825	1213
	floR-R	5'-AGACGACTGGCGACTTCTCG-3'		

(Tehran, Iran) were used as negative and positive controls, respectively. The mixtures were placed onto thermocycler (Eppendorf AG 22331, Hamburg, Germany). The PCR temperature condition was an initial denaturation step at 94 °C for 5 min; followed by 32 cycles, each of a denaturation step at 94 °C for 1 min, a primer-annealing step at 58 °C (*16S rRNA* and *sefA*), 59 °C (*tetC*), 60 °C (*cat3*), 61 °C (*tetA* and *tetB*), and 63 °C (*floR*) for 1 min, elongation at 72 °C for 1 min, and a final elongation at 72 °C for 5 min. The PCR products were analyzed in 2 % agarose gel electrophoresis. The electrode buffer was TBE (10.8 g of Tris-base 89 mM, 5.5 g of boric acid 2 mM, EDTA (pH 8.0) 4 mL of 0.5 M EDTA (pH 8.0), combined all components in sufficient H<sub>2</sub>O and were stirred to dissolve). A 100 bp DNA ladder plus (Fermentas, Germany) was used as a molecular weight marker to determine the length of the amplified fragments. The PCR products (10 µL) were loaded on ethidium bromide-agarose gel and run at 110 V for 1 h. After electrophoresis, images were obtained using UVIdoc gel documentation system (Uvitec, UK).

### Antibiogram Test

After detection of positive *S. enteritidis* samples from other *Salmonella* strains (using *sefA* gene amplification) the colonies were cultured on Nutrient Agar (Merck, Darmstadt, Germany) at 37 °C for 24 h. Then, the colonies were sub-cultured into Nutrient Broth (Merck, Germany) and incubated at 37 °C in shaking incubator for 12–16 h. The bacterial turbidity was compared to McFarland 0.5 turbidity standards. After standardization, 100 µL of bacterial suspensions were cultured on Mueller–Hinton agar medium (Merck, Darmstadt, Germany). Disk diffusion test (also known as the Kirby–Bauer method) using standard

antibiotic discs were performed according to Clinical and Laboratory Standards Institute (CLSI) protocols published in January 2013 [26] for determination of antibiotic susceptibility patterns of *S. enteritidis* isolated from cheeses and the cultures were incubated at 37 °C for 24 h. The bacterial specimens were categorized in susceptible, resistant, or intermediate after determination of inhibitor zone around disks in standard minimal inhibitory concentrations (MICs) at 6.25 µg/mL. The antibiotic disks were used for determination of antibiotic susceptibility patterns of *S. enteritidis* isolated from chesses were Nalidixic acid, Ciprofloxacin, Tetracycline, Streptomycin, Gentamicin, Chloramphenicol, Ampicillin, Ceftazidime, and Cefotaxime.

### Statistical Analysis

Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. All data were collected in Statistics programs for the Social Sciences software, version 20 (SPSS, Inc., Chicago, IL, USA) and Chi square test was used for comparison of antimicrobial resistance genes in *S. enteritidis* isolated from traditional and industrial Iranian white cheeses. The *p* value less than 0.05 were considered statistically significant.

## Results and Discussion

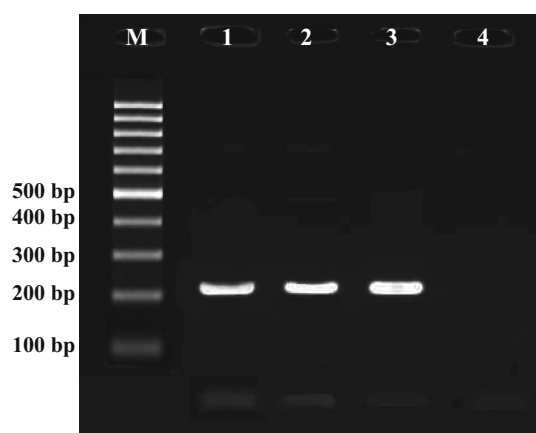
### Bacterial Tests

The cultures of isolated bacteria on *Salmonella*–*Shigella* (SS) showed black and gray colonies as suspected *Salmonella*. The presence and growth of isolated *Salmonella*

after culturing in TSI media were confirmed by glucose fermentation, producing hydrogen sulfide, and changing the color of media. The urease negative bacteria without changing the culture color to pink (negative) and culture in MR-VP were determined as samples contaminated by *Salmonella*. The indole-negative bacteria producing H<sub>2</sub>S with positive motility in SIM media were confirmed as *Salmonella* spp. Finally, the lysine decarboxylase test was used for screening of *Salmonella* strain. This test which makes the purple color by lysine decarboxylation was positive for *Salmonella*.

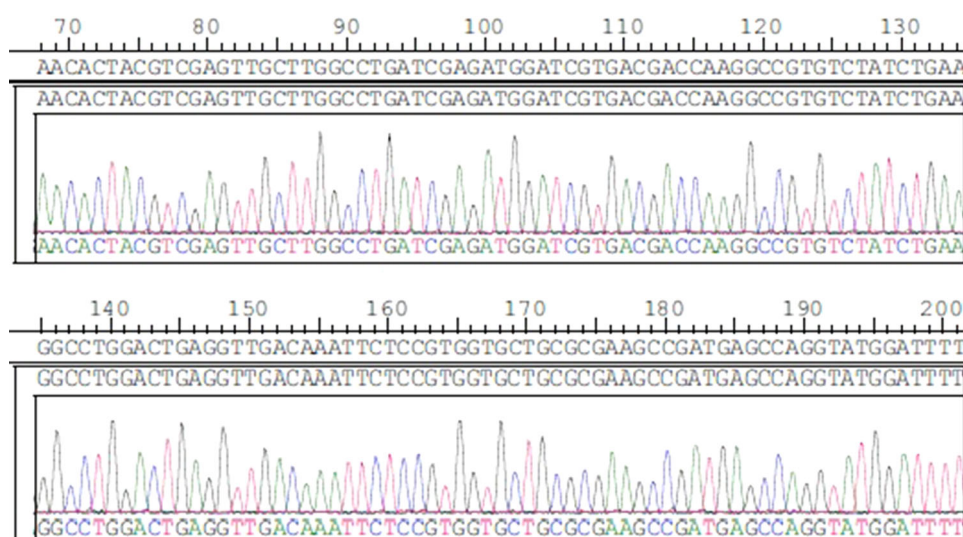
### Molecular Identification

For direct identification of *S. enteritidis* in cheese samples, *16S rRNA* gene was amplified revealing fragments with the length of 214 bp after electrophoresis (Fig. 1).



**Fig. 1** The agarose gel electrophoresis of representative PCR products for amplification of *16S rRNA* gene of isolated *Salmonella* (lane M is a 100 bp DNA ladder (Fermentas, Germany), lanes 1 and 2 are amplified fragments, lane 3 is positive control (*S. enteritidis* ATCC 13076), and lane 4 is negative control (without DNA))

**Fig. 2** A diagram of the DNA sequencing of amplified *16S rRNA* gene for confirmation of the isolated *Salmonella*



The amplified *16S rRNA* gene was sequenced as shown in Fig. 2 and the identity of *Salmonella* sp. isolated from cheese sample was confirmed.

For differentiation of *S. enteritidis* from other *Salmonella* strains, *sefA* gene was used and after amplification the amplified fragments were separated on agarose gel electrophoresis (Fig. 3).

### The Rate of *Salmonella* Contamination in Cheese Samples

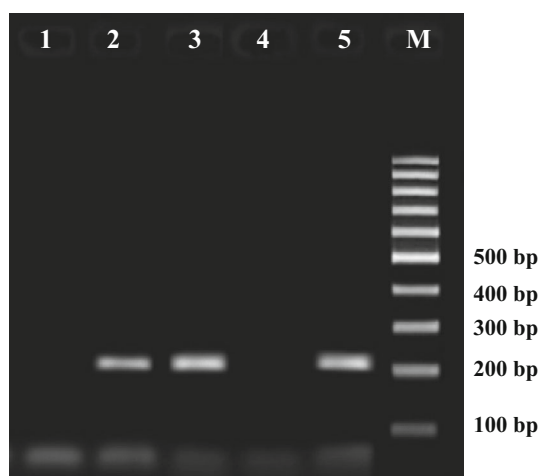
The positively isolated *Salmonella* by bacterial tests were subjected to *16S rRNA* and *sefA* gene amplification. In a total of 200 collected traditional and industrial Iranian white cheeses, 34 (17 %) specimens were contaminated by *Salmonella* and 11 (5.5 %) samples were confirmed by *S. enteritidis* as observed by molecular investigation (Table 2). The rate of *S. enteritidis* contamination in Iranian traditional white cheese (4.5 %) was significantly ( $p < 0.05$ ) higher than its rate in the industrial cheese samples (1 %).

### Antibiotic Susceptibility Patterns

The disk diffusion test was used for investigating the antibiotic susceptibility patterns of isolated *S. enteritidis* cultured on Mueller–Hinton agar and standard MIC. The antibiotic disks were selected by their effect on *Salmonella*. The antibiotic susceptibility patterns of *S. enteritidis* isolated from traditional and industrial Iranian white cheeses are shown in Table 3.

According to the disk diffusion test, 100 % of isolated *S. enteritidis* were resistant to nalidixic acid, tetracycline, and streptomycin antibiotics, 100 % of them were susceptible to ciprofloxacin, cefotaxime, and ceftazidime. Furthermore, most isolated *S. enteritidis* were susceptible to





**Fig. 3** Analysis of the amplified fragments (214 bp) of *sefA* gene on agarose gel electrophoresis for direct identification of *S. enteritidis* from other *Salmonella* strains (lane M is a 100 bp DNA ladder (Fermentas, Germany), lane 1 is negative control (without DNA), lanes 2, 3 and 5 are amplified fragments of *sefA* gene, and lane 4 is negative sample)

**Table 2** The frequency of *Salmonella* contamination and *S. enteritidis* samples in collected traditional and industrial Iranian white cheeses

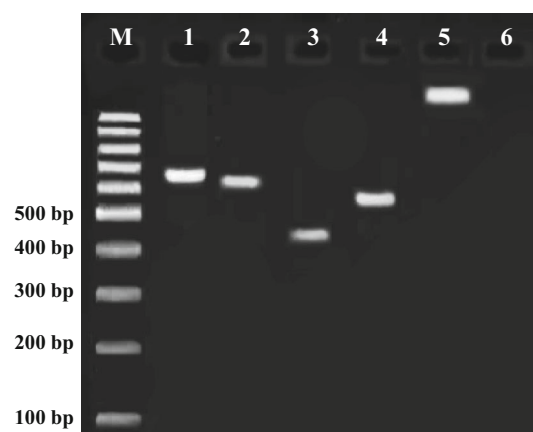
Cheese sample	<i>Salmonella</i> contamination No. (%)	<i>S. enteritidis</i> positive No. (%)
Traditional cheese	23 (11.5)	9 (4.5)
Industrial cheese	11 (5.5)	2 (1)
Total	34 (17)	11 (5.5)

**Table 3** The antibiotic susceptibility patterns of *S. enteritidis* (11 samples) isolated from Iranian white cheeses

Antibiotics	Resistant No. (%)	Intermediate No. (%)	Susceptible No. (%)
Nalidixic acid	11 (100)	0 (0)	0 (0)
Ciprofloxacin	0 (0)	0 (0)	11 (100)
Tetracycline	11 (100)	0 (0)	0 (0)
Streptomycin	11 (100)	0 (0)	0 (0)
Ampicillin	8 (72.7)	0 (0)	3 (27.3)
Gentamicin	1 (9.1)	0 (0)	10 (90.9)
Chloramphenicol	7 (63.6)	1 (9.1)	3 (27.3)
Cefotaxime	0 (0)	0 (0)	11 (100)
Ceftazidime	0 (0)	0 (0)	11 (100)

gentamicin and some of them were resistant to chloramphenicol (Table 3).

Detection of antibiotic resistance genes in isolated *S. enteritidis* using PCR technique revealed fragments with



**Fig. 4** The agarose gel electrophoresis for detection of resistant genes in *S. enteritidis* isolated from traditional and industrial Iranian white cheeses (lane M is a 100 bp DNA marker (Fermentas, Germany), lanes 1–5 are amplified fragments for *tetA*, *tetB*, *tetC*, *cat3*, and *floR* genes, respectively, and lane 6 is negative control)

**Table 4** The frequency of resistance genes (*tetA*, *tetB*, *tetC*, *cat3*, and *floR*) in *S. enteritidis* isolated from industrial and traditional Iranian white cheeses

Specimens	<i>tetA</i> No. (%)	<i>tetB</i> No. (%)	<i>tetC</i> No. (%)	<i>cat3</i> No. (%)	<i>floR</i> No. (%)
Traditional cheeses	3 (27.3)	5 (45.4)	8 (72.7)	5 (45.4)	3 (27.3)
Industrial cheeses	1 (9.1)	1 (9.1)	1 (9.1)	1 (9.1)	1 (9.1)
Total	4 (36.4)	6 (54.5)	9 (81.8)	6 (54.5)	4 (36.4)

the length size of 658, 615, 418, 547, and 1213 bp for *tetA*, *tetB*, *tetC*, *cat3*, and *floR* genes, respectively (Fig. 4).

According to Table 4 the analysis of PCR products showed that the frequency of resistance genes in *S. enteritidis* isolated from traditional Iranian white cheeses was higher ( $p < 0.05$ ) as compared to industrial cheeses.

Comparison of antibiotic susceptibility patterns of *S. enteritidis* isolated from cheese samples by disk diffusion test showed that 63.6 and 11 % were resistant to chloramphenicol and tetracycline. Moreover, PCR analysis revealed that 36.4, 54.5, 81.8, and 54.5 % of bacteria respectively contained resistant genes *tetA*, *tetB*, *tetC*, and *cat3*. These findings indicate that the disk diffusion test is not as sensitive and accurate as molecular techniques for detection of antibiotic resistant bacteria.

In recent decades, the food safety and quality of milk and other dairy products are becoming more and more important in public health. Dairy products especially cheese can transmit the pathogenic bacteria like *Salmonella* to human beings. Antimicrobial-resistant genes have been reported in *S. enterica* serovar *enteritidis* [25, 27]. In the present work, the bacterial tests including culture of specimens on specific media (SS agar and SIM), urease and

MR–VP assays were used for detection of *Salmonella* isolated from industrial and traditional Iranian white cheeses. The motile and indole-negative bacteria by producing  $H_2S$  in SIM media with the ability of lysine decarboxylation were selected as *Salmonella* family. The 16S rRNA gene amplification and sequencing was used for confirmation of isolated *Salmonella* from samples. In this work, *sefA* gene was used as specific candidate for differentiation of *S. enteritidis* from other *Salmonella* strains. As shown by molecular assays, in a total of 200 collected Iranian white cheeses, 17 % were contaminated by *Salmonella* among which 5.5 % were positive for *S. enteritidis*. In traditional and industrial cheeses, 4.5 and 1 % were contaminated with *Salmonella* respectively. After disk diffusion test all *S. enteritidis* isolated from cheese samples were resistant to nalidixic acid, tetracycline, and streptomycin antibiotics, while all of them were susceptible to ciprofloxacin, cefotaxime, and ceftazidime. In addition, high level of resistance to chloramphenicol (63.6 %) and susceptibility to gentamicin (90.9 %) in isolated *S. enteritidis* were observed. The frequency of resistance genes including *tetA*, *tetB*, *tetC*, *cat3*, and *floR* in *S. enteritidis* isolated from traditional Iranian white cheeses was significantly higher as compared to industrial samples ( $p < 0.05$ ). Also, the findings showed the presence of at least one resistance gene in *S. enteritidis* isolated from industrial Iranian white cheeses.

There are many studies performed to evaluate the antibacterial susceptibility patterns of *Salmonella* strains isolated from food products. In a study performed by White et al. the multidrug-resistant strains of *Salmonella*, including ceftriaxone-resistant isolates, are more frequent in retail ground meats in the greater Washington, D.C., area [28]. In the present study, the high level of antibiotic resistant *S. enteritidis* were observed in collected traditional Iranian white cheeses from Shahrekord city (Iran). The study on 250 Tulum cheese samples by Colak and co-workers in Istanbul (Turkey) showed 6 (2.4 %) samples contaminated with *Salmonella* spp. [13]. In 50 collected Turkish Van otlu (Herb) cheeses *Salmonella* spp. contamination was observed in 3 (6 %) specimens [29]. Their culture methods were similar to the present work with a minor exception where the authors used *Salmonella* specialized cultures. Moreover, in the present study 5.5 % of traditional and industrial Iranian white cheeses were contaminated whereas 6 % of Turkish Van otlu cheeses were positive for *Salmonella* spp. They reported that the antimicrobial resistance genes including *bla* (TEM), *cmlA*, *tetA*, *dfrA12*, *sul3*, and *aadA1* were detected in the majority strains of *Salmonella enteric* resistant to ampicillin (87 %), chloramphenicol (63 %), tetracycline (60 %), trimethoprim (42 %), sulphonamides (42 %), and streptomycin/spectinomycin (61 %), respectively [25]. In their study a low

*Salmonella* contamination rate was observed as compared to the present work.

As in present work, most of the isolated *S. enteritidis* were resistant to tetracycline. In a study by Hleba and colleagues in Slovakia a total of 67 samples including milk, cheese and other dairy products were examined for the presence of *Salmonella* contamination and 4 (5.97 %) samples were found positive for *Salmonella* spp., *S. enterica* ser. *typhimurium* and *enteritidis*. In Japan and Greece, 11.4 and 5.1 % of cheese samples contained *S. enteritidis*, respectively [14, 15]. In the present study, the frequency of contaminated Iranian white cheeses by this bacterium was similar to the frequency of this bacterium in the above-mentioned countries. The ampicillin, streptomycin and tetracycline- resistance strains observed in 57.14, 14.28 and 9.52 % of isolated bacteria from milk samples, respectively. In isolated bacteria from cheese samples 84.0 and 24 % were resistant to ampicillin and tetracycline, respectively and among all samples, resistance to ampicillin (69.26 %), tetracycline (30.22 %), streptomycin (9.52 %), and chloramphenicol (3.17 %) was clearly observed whilst resistance to other antibiotics was not detected [30]. In another study conducted by Mazurek and colleagues on multidrug resistant *Escherichia coli* (*E. coli*) resistance to streptomycin (88.3 %), cotrimoxazole (78.8 %), tetracycline (57.3 %), ampicillin (49.3 %), and doxycycline (44.9 %) was observed. The frequency of resistant genes among isolates from cattle include *bla* (SHV) (3.4 %), *tetA* (1.29 %), *bla* (TEM) (0.43 %), and *tetC* (0.43 %) [31]. Their study confirmed the high level of antibiotic resistance rates observed in family Enterobacteriaceae like *E. coli* and *Salmonella* spp. In another study on 49 tetracycline-resistant *Salmonella* isolates obtained from clinical and food samples 32, 13, 2 and one specimens were positive for *tetA*, *tetB*, *tetC* and for both *tetA* and *tetB* genes, respectively [32]. The study of Shaigan Nia in Iran (Isfahan province) on 360 milk and dairy products showed that 1.66 % of samples were *Salmonella* positive and raw sheep milk had the highest prevalence of *Salmonella* spp. contamination (7.5 %). Based on the results proportions of 0.27 % and 0.83 % of samples were contaminated by *S. enteritidis* and *S. typhimurium*, respectively [9]. In a recent study in Egypt, the florphenicol resistance gene (*floR*) was identified in 18.8 % of isolates, the  $\beta$ -lactamase-encoding genes were identified in 75.4 % and plasmid-mediated quinolone resistance genes detected in 27.5 % of isolates from meat and dairy products [33]. This is not contrary to the results of the present work where 30 % of *S. enteritidis* isolated from industrial and traditional Iranian white cheeses contained this gene. In another study in Wisconsin (USA) 51 % of *Salmonella enterica* serovars isolated from dairy cattle were resistant to at least one antimicrobial agent, and 29 % were resistant to 8–10 drugs. Also,

resistance phenotypes to streptomycin (44 %), tetracycline (42 %), sulfisoxazole (40 %), chloramphenicol (35 %), ampicillin (33 %), and cefoxitin (33 %) were reported [34]. These findings are in concordance to the high level of resistant genes and resistance to chloramphenicol that was observed in the present work.

## Conclusions

In Iran, white cheeses have high popularity and are produced in large scale. The findings of the present work showed higher level of *S. enteritidis* contamination in traditional Iranian white cheeses as compared to industrial cheese products. In addition, the frequency of resistance genes in *S. enteritidis* isolated from traditional white cheeses as compared to industrial cheeses was higher ( $p < 0.05$ ). Therefore, this pathogen can easily be transferred to human beings by consumption of contaminated dairy products especially cheese and it can be a serious threat to the public health. Therefore, more researches on transmission of pathogenic bacteria from cheese products could be helpful in future.

**Acknowledgments** This study was carried out in Biotechnology Research Center of Islamic Azad University of Shahrekord Branch in Iran (Grant no. 93/12/14). The authors would like to thank all the staff members of this department for their sincere support.

## References

- Wolfe BE, Button JE, Santarelli M, Dutton RJ (2014) Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. *Cell* 158(2):422–433
- Leitner G, Merin U, Silanikove N (2011) Effects of glandular bacterial infection and stage of lactation on milk clotting parameters: comparison among cows, goats and sheep. *Int Dairy J* 21(4):279–285
- Marcus SL, Brumell JH, Pfeifer CG, Finlay BB (2000) *Salmonella* pathogenicity islands: big virulence in small packages. *Microb Infect* 2(2):145–156
- Zhang XL, Jeza VT, Pan Q (2008) *Salmonella typhi*: from a human pathogen to a vaccine vector. *Cell Mol Immunol* 5(2):91–97
- Touch V, Hayakawa S, Yamada S, Kaneko S (2004) Effects of a lactoperoxidase–thiocyanate–hydrogen peroxide system on *Salmonella enteritidis* in animal or vegetable foods. *Int J Food Microbiol* 93(2):175–183
- Guerin MT, Martin SW, Darlington GA, Rajic A (2005) A temporal study of *Salmonella* serovars in animals in Alberta between 1990 and 2001. *Can J Vet Res* 69(2):88–99
- Thorns CJ (2000) Bacterial food-borne zoonoses. *Rev Sci Tech* 19:226–239
- Malorny B, Bunge C, Helmuth R (2007) A real-time PCR for the detection of *Salmonella enteritidis* in poultry meat and consumption eggs. *J Microbiol Meth* 70(20):245–251
- Shaigan Nia S, Rostami F, Safarpour Dehkordi F, Rahimi E, Yahaghi E, Khodaverdi Darian E, Bagheri Moghadam M (2014) Isolation and evaluation virulence factors of *Salmonella typhimurium* and *Salmonella enteritidis* in milk and dairy products. *Iran J Med Microbiol* 8(1):54–61
- Huang TM, Chang YF, Chang CF (2004) Detection of mutations in the *gyrA* gene and class I integron from quinolone-resistant *Salmonella enterica* serovar *Choleraesuis* isolates in Taiwan. *Vet Microbiol* 100:247–254
- Rodulfo H, Donato MD, Luiggi J, Michelli E, Millán A, Michelli M (2012) Molecular characterization of *Salmonella* strains in individuals with acute diarrhea syndrome in the State of Sucre, Venezuela. *Rev Soc Bras Med Trop* 45(3):329–333
- Fierer J, Okamoto S, Banerjee A, Guiney DG (2012) Diarrhea and colitis in mice require the *Salmonella* pathogenicity island 2-encoded secretion function but not SifA or Spv effectors. *Infect Immun* 80(10):3360–3370
- Colak H, Hampikyan H, Bingol EB, Ulusoy B (2007) Prevalence of *L. monocytogenes* and *Salmonella* spp. in Tulum cheese. *Food Control* 18(5):576–579
- Angelidis AS, Chronis EN, Papageorgiou DK, Kazakis II, Arsenoglou KC, Stathopoulos GA (2006) Non-lactic acid, contaminating microbial Xora in ready-to-eat foods: a potential food-quality index. *Food Microbiol* 23(1):95–100
- Kawasaki S, Fratamico P (2011) Development of the multiplex PCR detection kit for *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7. *Jpn Agric Res Q* 45(1):77–81
- Hadjinicolaou AV, Demetriou VL, Emmanuel MA, Kakoyiannis CK, Kostrikis LG (2009) Molecular beacon-based real-time PCR detection of primary isolates of *Salmonella Typhimurium* and *Salmonella Enteritidis* in environmental and clinical samples. *BMC Microbiol* 9(1):97
- Addis Z, Kebede N, Sisay Z, Alemayehu H, Wubetie A, Kassa T (2011) Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study. *BMC Infect Dis* 11:222
- Faldynova M, Pravcova M, Sisak F, Havlickova H, Kolackova I, Cizek A, Karpiskova R, Rychlik I (2003) Evolution of antibiotic resistance in *Salmonella enterica* Serovar *typhimurium* strains isolated in the Czech Republic between 1984 and 2002. *Antimicrob Agents Chemother* 47(6):2002–2005
- Ramsey MH, Ellison SLR, Czichos H, Hässelbarth W, Ischi H, Wegscheider W, Brookman B, Zschunke A, Frenz H, Golze M (2011) Quality in measurement and testing. Springer Handbook of Metrology and Testing, pp 39–141
- Garrity G, Brenner DJ, Krieg NR, Staley JR (2005) *Bergey's Manual of Systematic Bacteriology*. Vol 2, Part B, 2nd Edition, US, Springer
- Sambrook J, Russell DW (2001) *Molecular cloning: a laboratory manual*, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 148–190
- Pan TM, Liu YJ (2002) Identification of *Salmonella Enteritidis* isolate by polymerase chain reaction and multiplex polymerase chain reaction. *J Microbiol Immunol Infect* 35:147–151
- Woodward MJ, Kirwan SE (1996) Detection of *Salmonella Enteritidis* in eggs by the polymerase chain reaction. *Vet Rec* 138:411–413
- Ma M, Wang H, Yu Y, Zhang D, Liu S (2007) Detection of antimicrobial resistance genes of pathogenic *Salmonella* from swine with DNA microarray. *J Vet Diagn Investig* 19(2):161–167
- Chuanchuen R, Padungtod P (2009) Antimicrobial resistance genes in *Salmonella enterica* isolates from poultry and swine in Thailand. *J Vet Med Sci* 71(10):1349–1355
- Clinical and Laboratory Standards Institute (CLSI) (2013) *Clinical and Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing: 23rd informational supplement (M100-S23)*. CLSI, Wayne

27. Chuanchuen R, Ajariyakhajorn K, Koowatananukul C, Wannaprasat W, Khemtong S, Samngamnim S (2010) Antimicrobial resistance and virulence genes in *Salmonella enterica* isolates from dairy cows. *Foodborne Pathog Dis* 7(1):63–69
28. White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, McDermott PF, McDermott S, Wagner DD, Meng J (2001) The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *N Engl J Med* 345(16):1147–1154
29. Kaan Tekinşen K, Özdemir Z (2006) Prevalence of foodborne pathogens in Turkish Van otlu (Herb) cheese. *Food Control* 17(9):707–711
30. Hleba L, Kačániová M, Pochop J, Lejková J, Čuboň J, Kunová S (2011) Antibiotic resistance of *Enterobacteriaceae* genera and *Salmonella* spp., *Salmonella enterica* ser. *typhimurium* and *enteritidis* isolated from milk, cheese and other dairy products from conventional farm in Slovakia. *J Microbiol Biotechnol Food Sci* 1(1):1–20
31. Mazurek J, Pusz P, Bok E, Stosik M, Baldy-Chudzik K (2013) The phenotypic and genotypic characteristics of antibiotic resistance in *Escherichia coli* populations isolated from farm animals with different exposure to antimicrobial agents. *Pol J Microbiol* 62(2):173–179
32. Akiyama T, Presedo J, Khan AA (2013) The *tetA* gene decreases tigecycline sensitivity of *Salmonella enterica* isolates. *Int J Antimicrob Agents* 42(2):133–140
33. Ahmed AM, Shimamoto T, Shimamoto T (2014) Characterization of integrons and resistance genes in multidrug-resistant *Salmonella enterica* isolated from meat and dairy products in Egypt. *Int J Food Microbiol* 189:39–44
34. Marrero-Ortiz R, Han J, Lynne AM, David DE, Stemper ME, Farmer D, Burkhardt W, Nayak R, Foley SL (2012) Genetic characterization of antimicrobial resistance in *Salmonella enterica* serovars isolated from dairy cattle in Wisconsin. *Food Res Int* 45(2):962–967