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Determination of frequency, multiple antibiotic resistance index and resistotype of *Salmonella* spp. in chicken meat collected from southeast of Iran

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Abstract

Background: Zoonotic food-borne pathogens such as *Salmonella* spp., which can be hosted by some raw foods, play a crucial role in ranking the public health of a country

Objectives: The present study was conducted to assess the frequency, antibiotic resistance pattern and index of multiple antibiotic resistance (MAR) of *Salmonella* spp. in chicken meat

Methods: A cross-sectional survey was conducted from October 2017 to March 2018. One-hundred and fifty chicken meat samples were collected from meat stores in Zahedan, southeast of Iran and screened for contamination with *Salmonella* spp. using the polymerase chain reaction assay targeting the *inv-A* gene. Antimicrobial susceptibility testing was performed against 11 commonly prescribed antimicrobial agents in the veterinary treatment to calculate the MAR index

Results: The contamination rate was 2.7% (4/150). The antimicrobial resistance rate was 100% (n = 4) against penicillin, tylosin, tetracycline, erythromycin and tiamulin, 50% (n = 2) against trimethoprim/sulfamethoxazole, difloxacin and lincomycin/spectinomycin and 25% (n = 1) against flumequine and florfenicol. All isolates were sensitive to fosfomycin. Interestingly, all isolates (n = 4) exhibited different MAR patterns. Furthermore, the MAR index ranged from 0.45 to 0.81

Conclusions: In addition to the MAR index, which indicated that the isolate originated from a source where antibiotics were used to a great degree and/or in large amounts, the results showed that the chicken meat hosted resistant strains of *Salmonella* spp. in the study area. Overall, the findings indicated an important public health problem. To reduce this alarming signal, the poultry industry should implement control measures in the study area.

KEYWORDS
antibiograms, chickens, Iran, meat, public health, *Salmonella*

1 | Introduction

As a global public health concern, *Salmonella* spp. is a remarkable food-borne pathogen (Antunes et al., 2018; Jalali et al., 2008; Sodagari et al., 2015). *Salmonella* spp. is the causative agent of salmonellosis. *Salmonella* spp. can cause gastroenteritis, particularly in the children, elderly and immunocompromised individuals (Engberg et al., 2004; Jamali et al., 2014). Intestinal salmonellosis usually eliminates within 5 to 7 days without antibiotic treatment. However, immunocompromised or elderly patients may develop bacteremia (Mehrabian & Jaberi, 2007). Resistance of *Salmonella* spp. to commonly used antimicrobial agents is an important threat to public health. The patterns of resistance in the *Salmonella* spp. are constantly changing. The treatment of patients infected with multidrug-resistant isolates is difficult and may lead to treatment failure (Parry & Threlfall, 2008).

Nowadays, chicken meat is one of the widely consumed protein-rich foods in Iran (Faghihi et al., 2017). Several reports have shown that consumption of contaminated foods of animal origin is the main route of infection with *Salmonella* spp. (i.e., salmonellosis) and/or antimicrobial-resistant *Salmonella* spp. (Khaltabadi et al., 2019; Mehrabian & Jaberi, 2007; Mthembu et al., 2019).

The use of antimicrobial agents in veterinary health is an ordinary practice to prevent and treat diseases and to promote growth (Mehrabian & Jaberi, 2007; Phillips et al., 2004). A member of β-lactam antibiotics (penicillin), a member of tetracycline antibiotics (tetracycline), a member of aminoglycoside antibiotics (erythromycin), a member of phosphonic acid antibiotics (fosfomycin) and a member of folate pathway inhibitor antibiotics (trimethoprim/sulfamethoxazole or co-trimoxazole) are frequently used in both human and veterinary medicine (Faghihi et al., 2017). On the other hand, a member of the macrolide antibiotics (tylosin), a member of pleuromutilin antibiotics (tiamulin), a member of combination antibiotics (lincomycin/spectinomycin or lincospectin), a member of the second generation of quinolone antibiotics (difloxacin), a member of the first generation of quinolone antibiotics (flumequine) and a member of phenol antibiotics (florfenicol) are frequently used only in veterinary medicine (Faghihi et al., 2017).

Globally, despite arising from a public health agency, the critically important antimicrobial (CIA) list developed by the World Health Organisation (WHO) and World Organisation for Animal Health (OIE) with a considerable overlap serves as a benchmark for food animal producers around the world and provides important guidance to global retail companies (OIE, 2015; Scott et al., 2019). Apart from the WHO CIA list, the OIE CIA list is important in veterinary medicine (OIE, 2015; Scott et al., 2019). The OIE has defined beta-lactams, tetracycline, aminoglycoside, second generation quinolones, macrolide and phenicol antibiotics as veterinary CIA agents. Moreover, the OIE has defined first generation quinolones, phosphonic acid and pleuromutilin antibiotics as veterinary highly important antimicrobial agents (OIE, 2015).

Although several studies have reported the resistance rate of *Salmonella* spp. against antimicrobial agents such as ampicillin, tetracycline, trimethoprim/sulfamethoxazole, gentamicin, chloramphenicol, kanamycin, ciprofloxacin, ceftriaxone, streptomycin, nalidixic acid, cephotaxime, amikacin, cefalexin, erythromycin, furazolidone, nitrofurantoin, norfloxacin, amoxicillin/clavulanic acid, ceftazidime, colistin, imipenem and trimethoprim (Afshari et al., 2018; Diaz-Lopez et al., 2011; Jalali et al., 2008; Mehrabian & Jaberi, 2007; Nikbakht & Sani 2016; Sodagari et al., 2015; Soltan Dallal et al., 2014; Zare Bidaki et al., 2013), no study has described the resistance level of meat-origin *Salmonella* spp. against antimicrobial agents used in veterinary medicine in Iran (i.e., tylosin, tiamulin, lincomycin/spectinomycin, difloxacin, flumequine and florfenicol), in particular, according to the OIE CIA list (OIE, 2015). Tylosin is a feed additive. The WHO listed macrolides as the 'highest priority' in the list of CIAs for human medicine (Scott et al., 2019). The use of tylosin as a growth promoter is banned in European countries where there have been reports of a decrease in resistance to tylosin, while the rate of resistance against tylosin has increased in countries where this antibiotic has been administrated as a growth promoter (Mthembu et al., 2019). The bird's body relatively removes tiamulin after a withdrawal time of 72 h if a therapeutic level of the drug is administered. Then, meat products can be safely consumed (Islam et al., 2009). Prophylactic application of lincomycin/spectinomycin during the first 3–5 days after hatching decreases the mortality rate in growing chicken (Tavakkoli et al., 2014). Difloxacin and flumequine are fluoroquinolones. Fluoroquinolone antibiotics are listed as the 'highest priority' in the WHO list of CIAs for human medicine. Fluoroquinolone antibiotics may promote the evolution of fluoroquinolone-resistant strains of the bacterium *Campylobacter*, a human pathogen (Sproston et al., 2018). Moreover, the prevalence of *S.* Enteritidis, which is quinolone-resistant, increased by almost 10-fold from 1995 to 2000 (Engberg et al., 2004; Mølbak et al., 2002). Florfenicol is derived from chloramphenicol. Resistance against florfenicol can be disseminated via horizontal gene transfer among different and same species or genera of bacteria (Lu et al., 2018).

The multiple antibiotic resistance (MAR) index is described as a cost-effective and valid method to track the source of bacteria (Adzitey, 2015; Davis & Brown, 2016; Krumperman 1983; Parveen et al., 1997; Paul et al., 1997). It is a rapid and easy method to perform (Khan et al., 2015). High-risk sources of faecal contamination of foods can be identified via the MAR indexing of bacteria, such as *Escherichia coli* and *Salmonella* spp. (Khan et al., 2015; Krumperman 1983; Parveen et al., 1997; Paul et al., 1997). Indices are larger than 0.2 if an isolate originates from a source where antibiotics are used to a great degree and/or in large amounts (Krumperman 1983; Mthembu et al., 2019). No study has investigated the MAR index of meat-origin *Salmonella* spp. in Iran.

Since various distributions of food contamination with *Salmonella* spp. are expected in different countries and in different parts of a country (Afshari et al., 2018; Diaz-Lopez et al., 2011; Jalali et al., 2008; Mehrabian & Jaberi, 2007; Nikbakht & Sani 2016; Sodagari et al., 2015; Soltan Dallal et al., 2014; Zare Bidaki et al., 2013), country- and/or regional-wise investigation of the prevalence of *Salmonella* spp. is indispensable. An accurate estimation of the prevalence of food-borne zoonotic pathogens, as the first step, especially in various meat products, is proposed as an approach to improve the public health concerning food-borne zoonotic diseases (Jalali et al., 2008). Moreover, it is logical to track the presence of the bacterium in food media by rapid

and sensitive methods to control the pathogen effectively (Barrow & Freitas Neto, 2011; Gordon, 2008). In addition, the use of antimicrobial agents in veterinary health plays a crucial role in the emergence of antibiotic-resistant bacteria in domestic livestock, which may be subsequently transferred to humans through the food chain (Mehrabian & Jaberi, 2007; Phillips et al., 2004).

For these reasons, the present study was conducted to determine the frequency, antibiotic resistance pattern and MAR index of *Salmonella* spp. in the chicken meat collected from the southeast of Iran.

2 | Materials and Methods

2.1 | Study design, sample size and area

This study was a cross-sectional survey. To increase the generalisability of this study, it was calculated where 75 subjects (n) were required using the formula explained by Rodríguez Del Águila & González-Ramírez (2014; Equation 1). The 95% confidence interval (t_α) and accepted margin of error (e) were considered as 1.96 (≅ 2) and 9%, respectively (Equation 1). Assuming a priori values in different investigations (Afshari et al., 2018; Jalali et al., 2008; Mehrabian & Jaberi, 2007; Mojaddar Langroodi et al., 2016; Nikbakht & Sani 2016; Sodagari et al., 2015; Soltan Dallal et al., 2014; Zare Bidaki et al., 2013), the percentage of the frequency of *Salmonella* spp. originating from chicken meat samples (p) averaged 18% (Equation 1). The calculated sample size (n) was doubled to decrease the margin of error (Taddese et al., 2019).

$$n = \frac{t_\alpha^2 \times p \times (1-p)}{e^2} \cong \frac{2^2 \times 0.18 \times 0.82}{0.09^2} \cong 75. \quad (1)$$

We used the simplest and oldest sampling method, that is, the 'simple random sampling' method, in this study. The staff of Sistan and Baluchistan Branch of Iran Veterinary Organisation was consulted to find and list the stores of the study area. In total, 76 meat stores were listed empirically according to the high capacity of supply and demand. Every 10 days from October 2017 to March 2018, one store was selected using a random number table. The selected store was visited, and one sample was collected per store randomly.

One hundred and fifty chicken meat samples (i.e., breast muscle) were collected from different local meat stores in Zahedan, Iran (Nikbakht & Sani, 2016). Zahedan (latitude: 29°30′N; longitude: 60°50′E), an important Iranian city and the capital of the largest province of Iran, that is, Sistan and Baluchistan, is located in the southeast of Iran and borders two countries, Afghanistan (Das et al., 2018) and Pakistan (Lozano 2018). It, as a corridor, may play an important role in connecting commercial and/or economic affairs between the southeast and centre of Iran and neighbouring countries. Using sterile gloves, each breast muscle was separately coded, packaged and transferred to the laboratory under cold conditions (i.e., using a cold box). Sample preparation was started on the same day of sample collection.

2.2 | Sample preparation

Homogenisation of each breast muscle (25 g) was performed with 225 ml of buffered peptone water (BPW; Himedia) for 2 min, and the homogenate was incubated at 37°C for 24 h (Sodagari et al., 2015).

2.3 | Bacterial examination

According to the Iranian National Standards Organisation protocol No. 2394 (Institute of Standards and Industrial Research of Iran, 2019), briefly, 1 ml of incubated BPW was transferred to 10 ml of Rappaport Vassiliadis (RV) broth (Merck) and incubated at 42°C for 24 h. One millilitre of incubated RV was added to 10 ml selenite cysteine (SC) broth (Merck) and incubated at 37°C for 24 h. Then, one loopful from each of the enriched broths was streaked onto Xylose Lysine Desoxycholate (Merck) and Brilliant-Green Phenol-Red Lactose Sucrose (Merck) agar plates and incubated at 37°C for 18 to 24 h (Nikbakht & Sani, 2016). Finally, suspected colonies were purified by MacConkey's Lactose Agar (Merck). *Salmonella* spp. was biochemically confirmed using TSI, urea, Simmons' citrate agars, Methyl Red/Voges-Proskauer and SH2/Indole/Motility media and oxidase test (all from Merck). Biochemically identified *Salmonella* spp. isolates were stored at −80°C. Nutrient broth (Merck) containing 30% glycerol (NBG) was used to store the isolates at −80°C. For preservation, the biochemically identified isolates were first streaked on nutrient agar plates (Merck) and incubated at 37°C for 18 h to obtain a single colony of *Salmonella* spp. Second, the single colony was inoculated into 5 ml of NBG and incubated at 37°C for 2 h with shaking. Finally, the inoculated NBG was aliquoted and stored at −80°C.

2.4 | Polymerase chain reaction condition (PCR)

For the PCR assay, the isolates were revived by streaking on nutrient agar plates and incubation at 37°C for 18 h. DNA was extracted from revived isolates according to the boiling method as described before (Afshari et al., 2018). The supernatants were directly collected and used for PCR or stored at −20°C.

The *invA* gene with the was targeted by inv-A(F): 5′-AAA CGT TGA AAA ACT GAG GA-3′ and inv-A(R): 5′- TCG TCA TTC CAT TAC CTA CC-3′ primers (Nikbakht & Sani, 2016). The PCR reactions were performed in a total volume reaction of 50 μl consisting of 25-μl Master Mix (Pishgam), 3 μl (10 μM) of each primer (Pishgam), 4 μl of DNA and deionised distilled nuclease-free water (15 μl). The DNA template was replaced with water as the negative control. A reference strain, *S. enterica* subsp. *enterica* serovar Typhimurium (ATCC® 14028™; PTCC® 1709™) was considered as the positive control.

An initial denaturation at 95°C for 5 min and a final extension at 72°C for 5 min followed by thermocycling of the reaction mixture for 35 times, including 95°C for 45 s (denaturation), 50°C for 45 s (annealing) and 72°C for 1 min, were performed using a programmable

gradient Eppendorf's Master cycler® pro (Eppendorf). The expected PCR product size for the target gene (i. e., *invA*) was 199 bp.

Electrophoresis of the PCR products was conducted on a 1.5% agarose gel (Sinaclon) for 1 h at 100 V stained with 2-μg ml^{-1} ethidium bromide (Sinaclon) for 15 min. UV Gel Documentation (Syngene) was used to visualise the fluorescent bands (Jamshidi et al., 2010).

2.5 | Antimicrobial susceptibility testing (resistotyping)

Eleven antimicrobial agents, commonly prescribed in the veterinary treatment of the study area, namely, penicillin (10 μg), tylosin (30 μg), tetracycline (30 μg), erythromycin (15 μg), tiamulin (30 μg), fosfomycin (200 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), difloxacin (10 μg), lincomycin/spectinomycin (15/200 μg), flumequine (30 μg) and florfenicol (30 μg) were included (PadtanTeb). The antibiotic resistance of all isolates was determined using the disc diffusion method as described previously (Bauer et al., 1966; Taddese et al., 2019) with Mueller–Hinton agar (Himedia) plates. The control strain was *E. coli* (ATCC 25922). The guidelines of Clinical and Laboratory Standards Institute (2018) were applied to interpret the data obtained from antimicrobial susceptibility testing. Intermediate susceptible and sensitive isolates were marked as not resistant.

2.6 | Identification of multi-drug resistance (MDR)

MDR was defined as resistance to more than two classes of antibiotics among all tested antibiotics (Khan et al., 2015; Magiorakos et al., 2012).

2.7 | MAR index calculation

The MAR index was calculated and interpreted according to Krumperman (1983) using the formula: *a*/*b*, where '*a*' represents the number of antibiotics to which an isolate was resistant, and '*b*' represents the total number of antibiotics tested.

3 | RESULTS

The results of the present study indicated that among 150 chicken meat samples, 2.7% (four samples) were contaminated with *Salmonella* spp., and all isolates (4/4) contained the *inv*-A gene.

Moreover, all isolates (n = 4; 100%) were resistant to penicillin, tylosin, tetracycline, erythromycin and tiamulin and were sensitive to fosfomycin. In addition, half of the isolates (n = 2; 50%) were resistant to trimethoprim/sulfamethoxazole, difloxacin and lincomycin/spectinomycin, while approximately one-third (n = 1; 25%) of the isolates was resistant to flumequine and florfenicol. According to Table 1, all isolates in the present study exhibited the MDR pattern.

TABLE 1 Multiple antibiotic resistance pattern of different isolates against antibiotics tested*

Isolate No.	Antimicrobial resistance pattern
S1	Penicillin, tylosin, tetracycline, erythromycin, tiamulin
S2	Penicillin, tylosin, difloxacin, tetracycline, erythromycin, tiamulin
S3	Penicillin, trimethoprim/sulfamethoxazole, flumequine, tylosin, difloxacin, tetracycline, lincomycin/spectinomycin, erythromycin, tiamulin
S4	Penicillin, trimethoprim/sulfamethoxazole, tylosin, florfenicol, tetracycline, lincomycin/spectinomycin, erythromycin, tiamulin

Note: *Even two members of quinolone antibiotics, including difloxacin which is a member of quinolone second generation and flumequine, which is a member of quinolone first generation have been classified as one antibiotic (i.e., quinolone antibiotic) in the study design, all isolates (n = 4) are multi-drug resistance and exhibited different multiple antibiotic resistance (MAR) pattern, individually.

TABLE 2 MAR index of *Salmonella* spp. isolates

Isolate No.	No. of antibiotics to which isolate was resistant (*a*)	MAR index = *a*/*b*
S1	5	0.45
S2	6	0.54
S3	9	0.81
S4	8	0.72

Note: b, the number of antibiotics to which the isolate was exposed (n = 11).

Interestingly, the different MDR patterns were distinctively observed among isolates (Table 1).

The MAR index ranged from 0.45 to 0.81 with the average MAR index being 0.63 in four isolates (Table 2). Interestingly, the MAR index was completely different for all isolates.

4 | DISCUSSION

The present study addressed an interesting question regarding the food-borne *Salmonella* infection rate and the antibiogram of the contaminants in a particular geographical location. However, further country-level studies including a larger number of isolates tested for antibiotic susceptibility/resistance would provide a more concrete conclusion. In the present investigation, the sample size was calculated, and the 'viable' isolates were detected. All four isolates harboured the *InvA* gene. As a result, the *InvA* gene is suggested for PCR as an indicator for rapid and reliable detection-confirmation method for *Salmonella* spp. isolates obtained from chicken meat samples, which is consistent with previous reports (Afshari et al., 2018; Alzwghaibi et al., 2018; Beaubrun et al., 2017; Mehrabian & Jaberi, 2007; Khaltabadi et al., 2019; Löfström et al., 2004; Mthembu et al., 2019; Rahn et al., 1992).

The findings revealed that the contamination rate of chicken meat (2.7%; 4/150) was considerably harmful in the study area according

to the standards (Iran Veterinary Organization, 2009) set by the Iran Veterinary Organisation (Executive Protocol for Control and Hygienic Monitoring of Raw Food Products).

The prevalence of *Salmonella* spp. in the present study (2.7%; 4/150) was lower than reports from other parts of the world (5%–25%, chicken meat, Mojaddar Langroodi et al., 2016; 67.5%, chicken meat, Lertworapreecha et al. 2013) and provinces of Iran (20%, chicken, beef, veal and mutton, roast beef and sausage fermentative meat, Mehrabian & Jaberi, 2007; 17.9%, chicken meat, Jalali et al. 2008; 45.3%, chicken meat, Soltan Dallal et al. 2014; 19.8%, retail chicken meat, giblets livers, gizzards and hearts, Sodagari et al. 2015; 14%, poultry carcasses, Afshari et al. 2018). However, it was higher, compared to a study by Zare Bidaki et al. (2013; 1.6%, poultry carcasses). Interestingly, our findings, compared to the results of a study by Nikbakht & Sani (2016) who conducted a similar study in the study area (5.6%, poultry meats), demonstrated a decrease in the level of contamination of *Salmonella* spp. in the chicken meat during successive years, which may be due to proper policies implemented to control and treat salmonellosis, particularly in the study area. However, we believe that studies with larger sample sizes are necessary to draw such a conclusion.

The results of different studies investigating the prevalence of *Salmonella* spp. may be affected by differences in the sampling technique, season and size or may arise from the tendency of isolates to grow considering the special geographic areas of the world and/or the country (Sodagari et al., 2015), food given to domestic animals in the course of animal husbandry (Löfström et al., 2004) and contamination of un-hatched eggs and day-old chicks of broiler breeder flocks delivered to broiler farms (Jalali et al., 2008), which in turn would lead to contamination of broiler flocks (Taheri et al., 2016). Moreover, the level of sanitation in the slaughterhouse and the possible contamination of the market and tools are included in this query (Afshari et al., 2018; Jalali et al., 2008; Mikolajczyk & Radkowski, 2002; Sodagari et al., 2015). A pitfall of our research was the identification of major species and/or serotypes of *Salmonella* spp. in chicken meat products, including Typhimurium and Enteritidis (Afshari et al., 2018); hence, monitoring of the prevalence, determinant virulence factors and antibiotic-resistant profile, even for other meat products, are proposed in the study area (Antunes et al., 2016; Mthembu et al., 2019).

Several studies reported the phenotypic resistance pattern of *Salmonella* spp. isolated from poultry meat or poultry meat production in Iran from 2006 to 2018 in which some antimicrobial agents were fairly different from the present study (Afshari et al., 2018; Jalali et al., 2008; Mehrabian & Jaberi, 2007; Nikbakht & Sani, 2016; Sodagari et al., 2015; Soltan Dallal et al., 2014; Zare Bidaki et al., 2013). There are several guidelines to select antibiotics for antibiotic-resistant profiling (Magiorakos et al., 2012; OIE, 2015; Scott et al., 2019); however, we preferred to select 11 commonly prescribed antibiotics in human and/or veterinary health according to the criteria including (i) OIE recommendation (2015), (ii) lack of previous testing for antimicrobial susceptibility in the study area (Afshari et al., 2018; Diaz-Lopez et al., 2011; Jalali et al., 2008; Mehrabian & Jaberi, 2007; Nikbakht & Sani 2016; Sodagari et al., 2015; Soltan Dallal et al., 2014; Zare Bidaki et al., 2013), (iii) frequent prescription in animal farms in the study area and (iv) availability in the study area.

This is the first report documenting the presence of penicillin–, tylosin–, tiamulin–, difloxacin–, lincomycin/spectinomycin–, flumequine– and florfenicol– resistant *Salmonella* spp. isolated from chicken meat. It is not possible to compare the results of this study with other observations since we found no study that evaluated the resistance of meat-origin *Salmonella* spp. against these antibiotics (i.e., antimicrobial agents used in veterinary medicine; Faghihi et al., 2017) and fosfomycin in Iran (Faghihi et al., 2017). Thus, we explain probable reasons for the results of these antimicrobial agents, hoping that it will convince the readers of the novelty of the work and the clinical implication of the study regarding veterinary science.

In our study, the proportion of the *Salmonella* spp. isolate resistant to tetracycline in chicken meat (100%) was higher than those found in Phatthalung Province, Thailand (60%; Lertworapreecha et al., 2013), Tehran Province, Iran (93%; Mehrabian & Jaberi 2007) and Alborz Province, Iran (81%; Sodagari et al., 2015). Moreover, the proportion of the *Salmonella* spp. isolate resistant to erythromycin in chicken meat (100%) was higher than the proportion found in Tehran Province, Iran (97%; Mehrabian & Jaberi 2007). In the present study, the proportion of the *Salmonella* spp. isolate resistant to trimethoprim/sulfamethoxazole in chicken meat (50%) was higher than Phatthalung Province, Thailand (5%; Lertworapreecha et al., 2013), while it was lower than Tehran Province, Iran (77%; Mehrabian & Jaberi 2007) and Alborz Province, Iran (61.2%; Sodagari et al., 2015).

The relationship between antimicrobial consumption and resistance is well-known (Morfin-Otero et al., 2015). Continuous administration of antibiotics in the study area (Alabi et al., 2013) or considering prevalent clinical symptoms, which may lead to the use of particular antibiotics, may result in increased resistance (Boireau et al., 2018; Nhung et al., 2017). In this regard, over-the-counter dispensing of antimicrobials is common in Iran, and it is recommended that existing laws be enforced to reduce their consumption (Faghihi et al., 2017). Furthermore, our finding can be explained by a high rate of contamination by gram-negative bacteria that are resistant to penicillin, tylosin, tetracycline, erythromycin and tiamulin in the environment of chicken meat. These issues require that apt strategies be implemented to observe and track resistance to these antimicrobials (for both pathogenic and commensal bacteria) in Iran, particularly in the study area. Furthermore, all isolates were sensitive to fosfomycin, which could be due to the rational use of fosfomycin together with proper education of the workers on the use of this antibiotic (Alabi et al., 2013) and the tendency of the farmers or vets to administer an effective synergic antibiotic to eliminate the resistant isolates at a specific time (Boireau et al., 2018; Nhung et al., 2017).

The antibiotype of four isolates was different in the present study (Table 1), indicating an alarming signal for human consumption, public health and microbial drug resistance. Furthermore, compared to another study reporting that 43% (n = 46 out of 106) of the isolates showed resistance to three or more antibiotics (Mthembu et al., 2019), all isolates were resistant to at least five antibiotics (n = 4 out

of 4; 100%) in the present investigation, which definitely poses a public health concern.

The main novelty of the present research was to calculate and report the MAR index for *Salmonella* spp. isolated from chicken meat from Iran for the first time. The MAR index of the present study was notably unique for each isolate (Table 2). All isolates tested in the present study showed an MAR index of higher than 0.2 indicating a high-risk source of contamination where antibiotics are often used (Khan et al., 2015). Compared to a study by Khan et al. (2015) who reported indexes ranging from 0.06 to 0.56 with the predominant MAR index being 0.37 in eight isolates and Mthembu et al. (2019) who reported indexes ranging from 0 to 0.875 with the predominant MAR index being 0.31 in the 361 faecal and environmental samples originating from various animal hosts, including sheep, cattle, pigs, goats, chickens and ducks, our findings (range: 0.45–0.81, predominant MAR index: 0.63 in four isolates) demonstrated a higher MAR index for *Salmonella* spp. in the study area. The dissemination of such resistant clones, despite their low frequency (2.7%), can pose a serious public health problem since the isolate originated from a source where antibiotics are used to a great degree and/or in large amounts. Therefore, we recommend that the frequency and status of antibacterial drug consumption be assessed in the farms of the study area and/or the province (Faghihi et al., 2017, 2019).

Efforts are needed to identify the critical points in the meat production and distribution process in order to improve, equip and industrialise the slaughterhouses and markets to decrease the spread of the pathogen in the study area. In addition, it is recommended that the regulations in the study area be observed and promoted.

In conclusion, the chicken meat (i.e., breast muscle) of retail meat stores hosted MDR *Salmonella* spp. in the study area. The results showed that chicken meat was harmful to human consumption according to the Iranian National Standards Organisation protocol No. 2394. Moreover, the MAR index revealed an important public health problem, indicating the isolate originated from a source where antibiotics are used to a great degree and/or in large amounts. The poultry industry should focus on implementing control measures to reduce the spread of the pathogen in the study area.

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ETHICS STATEMENT

Ethical approval and/or consent form have not been obtained because this research does not involve animals/human participants.

AUTHOR CONTRIBUTION

Saeed Salari designed the study and drafted the article. Reza Mir performed the experiments and acquired the data. Saeed Salari and Ahmad Rashki analysed and interpreted the data. Saeed Salari, Ahmad Rashki and Mohsen Najimi revised it critically for important intellectual content. Saeed Salari, Ahmad Rashki, Mohsen Najimi and Reza Mir confirmed the final approval of the version to be submitted.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

We have made our research data available in *Mendeley Data*, V1. https://doi.org/10.17632/p297gwpsrc.1

PEER REVIEW

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