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Antibiotic resistance profiles of *Salmonella* serotypes isolated from animals in Iran: a meta-analysis

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Abstract

Background: *Salmonella* infection (salmonellosis) is a zoonotic bacterial disease. Widespread use of antibiotics in livestock and poultry production for different purposes such as treatment and growth promotion has led to the emergence of antibiotic-resistant *Salmonella*, causing treatment of *Salmonella* infections more difficult with each passing year. **Aims:** To determine the antibiotic resistance prevalence of *Salmonella* serotypes isolated from animals in different provinces of Iran. **Methods:** To find eligible articles, we searched the international and national electronic databases using appropriate keywords in English and Persian. **Results:** After applying predefined criteria, 54 articles reporting antibiotic resistance profiles of *Salmonella* serotypes were included. *Salmonella* isolates were mostly resistant against nalidixic acid (67%), tetracycline (66.9%), and streptomycin (49.6%), followed by trimethoprim/sulfamethoxazole (41.6%) and kanamycin (23.6%). The highest sensitivity was observed against imipenem, meropenem, and cefepime with 1.7%, 1.4%, and 1.9% of all isolates being resistant, respectively. **Conclusion:** Results revealed that the prevalence of resistant isolates to nalidixic acid, tetracycline and streptomycin is high and their use must be restricted. In addition, resistance to other antibiotics such as chloramphenicol, ampicillin, cephalothin, cefixime, and enrofloxacin is at an alarming level that calls for attention in the future infection control and antibiotic stewardship programs.

Key words: Animals, Antibiotic resistance, *Salmonella*, Salmonellosis

Introduction

Salmonella are Gram-negative rod-shaped bacteria belonging to the Enterobacteriaceae. *Salmonella* genus comprises two species including *S. enterica* and *S. bongori*. *Salmonella enterica* is the type species and is further divided into six subspecies including *diarizonae*, *enterica*, *salamae*, *arizonae*, *indica*, and *houtenae* (Su and Chiu, 2007). Based on Kauffmann-White scheme, which uses combination of H, O, and Vi surface antigens for serotyping, more than 2,600 unique serotypes have already been reported. The majority of identified serotypes belong to *S. enterica* subsp. *enterica* (Su *et al.*, 2007; Gal-Mor *et al.*, 2014). It has been shown that gastrointestinal tract of animals such as pigs, birds and cattle is the main reservoir of *Salmonella* serotypes. These bacteria can survive out of animal body and humans are mostly infected by contaminated foods such as meat, fruit and vegetables (Paniel and Noguier, 2019). Based on the severity of disease, infections caused by *Salmonella* serotypes are categorized into typhoidal and non-typhoidal infections. Infections caused by non-typhoidal *Salmonella* (Typhimurium) are usually self-restricted diseases in different mammals and birds, whereas typhoidal *Salmonella* (Typhi and Paratyphi)

cause life-threatening diseases in human (Gal-Mor *et al.*, 2014; GutVasiljevic *et al.*, 2018; Paniel and Noguier, 2019). *Salmonella* infection (salmonellosis) is a zoonotic bacterial disease mainly transmitted to human by contaminated food and water. Poultry products and raw meat are known as the most important sources of *Salmonella* species transmitted to humans. In fact, the slaughtering process of food animals such as poultry and cattle is considered as one of the most important routes of *Salmonella* transmission to human. Salmonellosis affects intestine and the most important signs of infection are abdominal cramps, fever, vomiting and diarrhea (Gut *et al.*, 2018; Paniel and Noguier, 2019). Widespread use of antibiotics in livestock and poultry production for different purposes such as treatment, growth promotion and prophylaxis has led to the emergence of antibiotic-resistant serotypes, causing treatment of *Salmonella* infections more difficult with each passing year (Rahmani *et al.*, 2013). Owing to the importance of *Salmonella* serotypes in human public health and necessity of information about epidemiology of *Salmonella* antibiotic resistance patterns to launch infection control programs, the aim of present study was to determine antibiotic resistance profiles of *Salmonella* serotypes isolated from animals in Iran.

Materials and Methods

Search strategy

This study was carried out based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines (Liberati *et al.*, 2009). To find potentially eligible articles, we searched international and national electronic databases including Google Scholar (<https://scholar.google.com>), Scopus (<https://www.scopus.com>), PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>), ISI Web of Knowledge (<https://www.webofknowledge.com>), Scientific Information Database (<https://www.sid.ir>), and Magiran (<https://www.magiran.com>). Based on MeSH terms, the following keywords and their combinations in Persian and English languages were used; *Salmonella*, antibiotic resistance, antimicrobial resistance, animal sources, drug resistance and Iran. To find missing articles, reference lists of eligible articles were manually searched. The last search was performed on September 2019.

Study selection, quality assessment and data extraction

Two independent researchers assessed the eligibility of articles. Joanna Briggs Institute (JBI) checklist was used to evaluate the quality of obtained papers (Munn *et al.*, 2015). The titles and abstracts of articles were evaluated to find more relevant studies. We included studies reporting antibiotic resistance patterns of *Salmonella* serotypes isolated from animals. For each eligible article, first author name, provinces of study, antibiotic resistance method, sample origin and time of study were extracted. Additionally, articles were excluded if materials and methods were unclear, samples were collected from non-animal samples, non-original study, and full text of articles was not available.

Statistical analysis

We used Comprehensive Meta-Analysis (CMA) software (Biostat, Englewood, NJ, USA) for statistical analysis. Also, based on random- or fixed-effects models, data analysis was performed and the results were expressed as percentage and 95% confidence intervals (CIs). Heterogeneity among studies was also assessed using the I^2 and Cochran Q test. In addition, funnel plots were generated to find potential publication bias (Smith *et al.*, 1997).

Results

The detailed steps of process of study selection are shown in Fig. 1. Briefly, the electronic search engine resulted in 13186 potentially relevant records. Based on the screening of titles and abstracts, 12,986 articles were removed because they were duplicate or non-relevant and 200 were selected for full text checking to find eligible studies. After applying inclusion and exclusion criteria, 146 articles were removed due to their poor materials and methods or insufficient data or low-quality

scores. Finally, 54 articles, reporting antibiotic resistance profiles of *Salmonella* serotypes were included in the study (Rajaeian *et al.*, 2003; Salehi *et al.*, 2005; Jafari *et al.*, 2007; Mehrabian *et al.*, 2007; Mirmomeni *et al.*, 2007; Emadi *et al.*, 2009; Morshed and Peighambari, 2010; Peighambari *et al.*, 2011; Akbarmehr *et al.*, 2012; Firoozeh *et al.*, 2012; Jadidi *et al.*, 2012; Tajbakhsh *et al.*, 2012; Ahmadi *et al.*, 2013; Ezatpanah *et al.*, 2013; Fallah *et al.*, 2013; Ghorbani-Ranjbari *et al.*, 2013; Malidareh *et al.*, 2013; Peighambari *et al.*, 2013; Rahmani *et al.*, 2013; Salehi *et al.*, 2013; Tajbakhsh and Tajbakhsh, 2013; Asadpour *et al.*, 2014; Asgharpour *et al.*, 2014; Halimi *et al.*, 2014; Jamali *et al.*, 2014; Zare *et al.*, 2014; Ghasemmahdi *et al.*, 2015; Ghodousi *et al.*, 2015; Jahantigh *et al.*, 2015; Oskouizadeh *et al.*, 2015; Peighambari *et al.*, 2015; Raeisi and Ghiamirad, 2015; Sodagari *et al.*, 2015; Amini, 2016; Doosti *et al.*, 2016a, b; Ghaderi *et al.*, 2016; Namroodi and Behine, 2016; Namroodi *et al.*, 2016; Zare *et al.*, 2016; Doulatyabi and Peyghambari, 2017; Fasaei *et al.*, 2017; Moradi *et al.*, 2017; Naghizadeh and Moradi, 2017; Ommi *et al.*, 2017; Azizpour, 2018a, b; Farahani *et al.*, 2018; Mahdavi *et al.*, 2018; Mir *et al.*, 2018; Mozajin *et al.*, 2018; Peighambari *et al.*, 2018; Ghazalibina *et al.*, 2019; Peighambari *et al.*, 2019). Most of the reports were from Tehran and Ardabil provinces with seven and four studies, respectively (Table 1), followed by Arak (three studies), Mazandaran (three studies), and Shahrekord (three studies) provinces. Disk diffusion was the most common method used for the determination of antibiotic resistance profiles of *Salmonella* serotypes isolated from animals in Iran. The majority of samples were collected from poultry, sheep and cow. *Salmonella* Typhimurium and Enteritidis were the most prevalent isolated serotypes (Table 1). The forest plot of resistance against tetracycline is shown in Fig. 2. As shown in Fig. 3, we observed some evidence of publication bias due to the asymmetric distribution of studies in funnel plots.

Prevalence of resistance against cephalosporins

Due to the presence of a high degree of heterogeneity among included studies, we used random-effects models for the meta-analysis of data on the prevalence of antibiotic resistance of *Salmonella* serotypes to cephalosporins except for ceftizoxime. The highest resistance rate was observed against cephalothin 13.5% (95% CI: 7.1-24.1; $I^2=88.8\%$; $Q=153$; $P=0.00$) and cefixime 9% (95% CI: 5.6-14; $I^2=63.6\%$; $Q=52.2$; $P=0.00$), followed by ceftazidime 5.5% (95% CI: 3-9.9; $I^2=82.2\%$; $Q=118.6$; $P=0.00$), ceftriaxone 5.2% (95% CI: 3.1-8.6; $I^2=68.2\%$; $Q=81.9$; $P=0.00$), cefotaxime 3.7% (95% CI: 1.6-8.6; $I^2=85.2\%$; $Q=108.8$; $P=0.00$), ceftizoxime 3.4% (95% CI: 1.2-9.2; $I^2=18.7\%$; $Q=1.2$; $P=0.26$), and cefepime 1.9% (95% CI: 0.7-5.3; $I^2=33.5\%$; $Q=7.5$; $P=0.18$).

Prevalence of resistance against quinolones

Similar to cephalosporins, a random-effects model was used for the meta-analysis of data on the prevalence

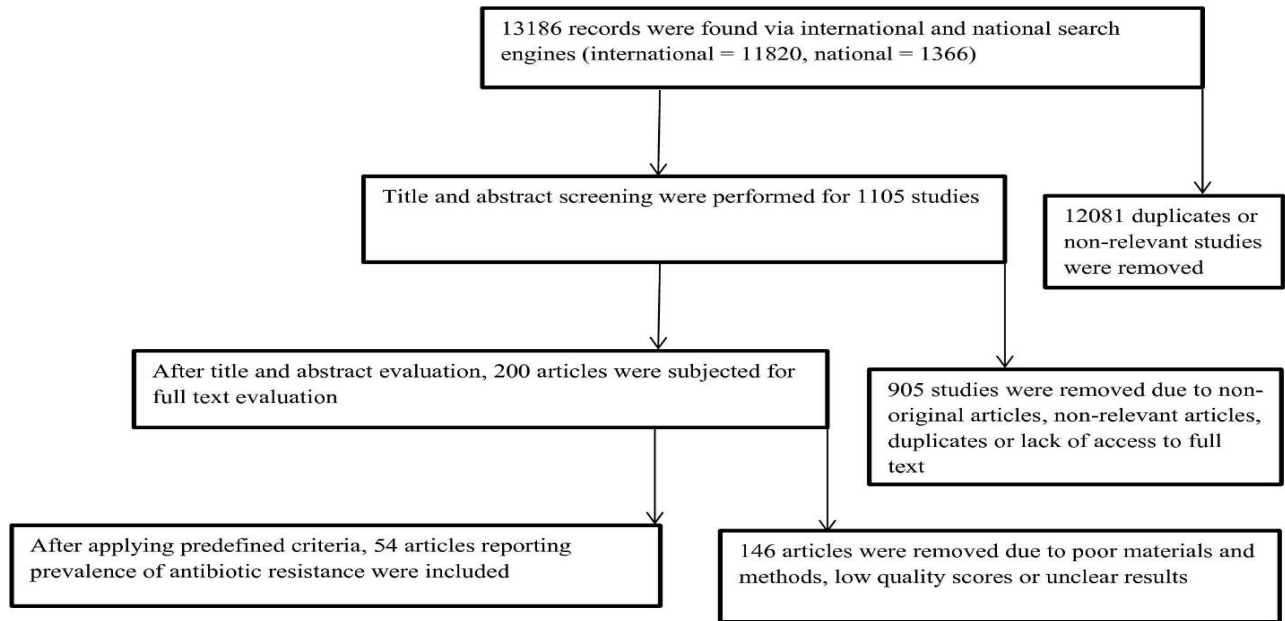


Fig. 1: Study selection progress

Meta-analysis

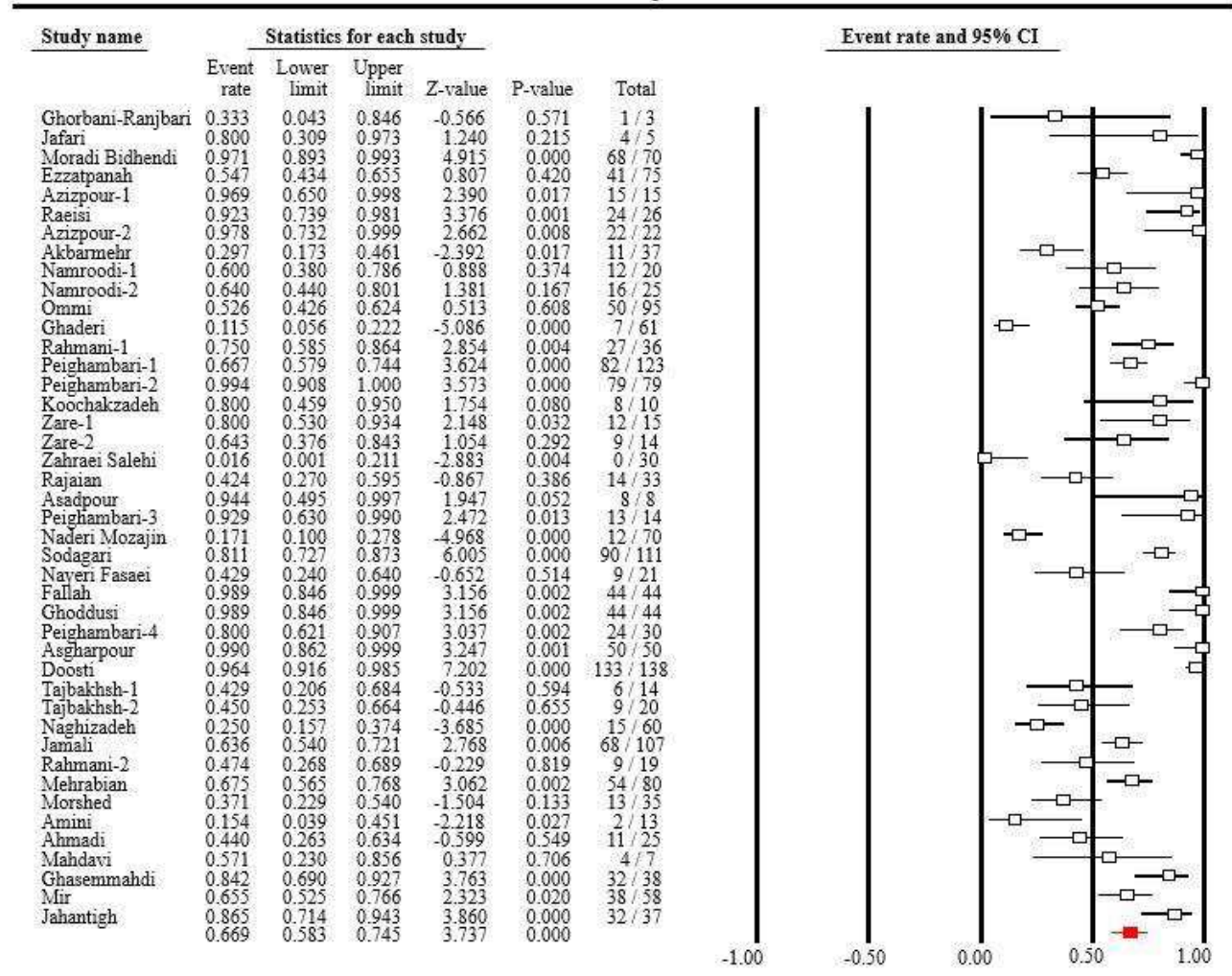


Fig. 2: The forest plot examining the overall prevalence of resistance to tetracycline on studies performed in Iran

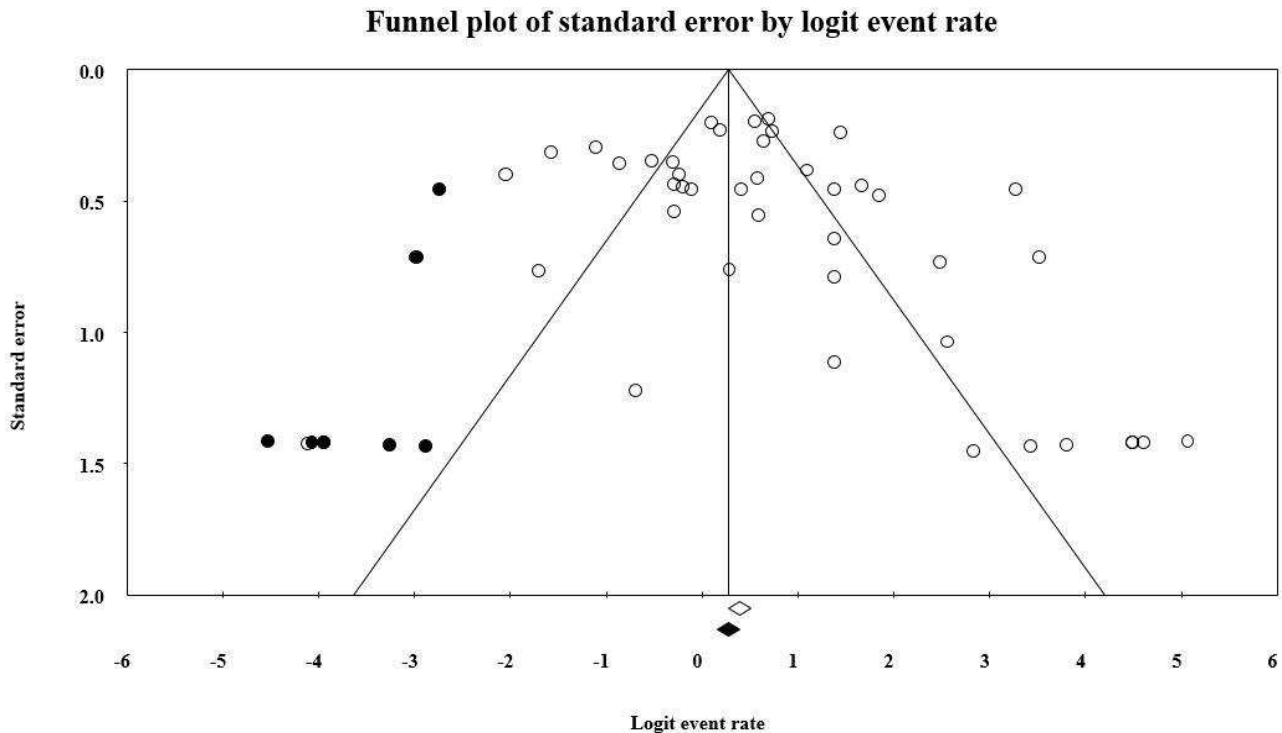


Fig. 3: Funnel plot with pseudo 95% confidence intervals demonstrating the effect sizes derived from each study

of antibiotic resistance of *Salmonella* serotypes to quinolones. Antibiotic resistance patterns of *Salmonella* serotypes against quinolones were as follows; nalidixic acid 67% (95% CI: 56.1-76.3; $I^2=90.7\%$; $Q=378.2$; $P=0.00$), enrofloxacin 10.7% (95% CI: 6.8-16.6; $I^2=75.3\%$; $Q=101.3$; $P=0.00$), ciprofloxacin 7% (95% CI: 4-11.8; $I^2=87.6\%$; $Q=267.8$; $P=0.00$), norfloxacin 4.3% (95% CI: 1.8-9.9; $I^2=78.9\%$; $Q=66.3$; $P=0.00$), and ofloxacin 3.5% (95% CI: 1.4-8.6; $I^2=55.3\%$; $Q=15.6$; $P=0.02$).

Carbapenems and aminoglycosides-resistant *Salmonella*

Antibiotic resistance profiles of *Salmonella* serotypes to carbapenems and aminoglycosides were assessed using either random- or fixed-effects models. The prevalence of resistance against imipenem and meropenem were 1.7% (95% CI: 0.2-11.3; $I^2=0.0\%$; $Q=0.0$; $P=0.00$), and 1.4% (95% CI: 0.4-4.6; $I^2=80.8\%$; $Q=89$; $P=0.00$), respectively. The highest resistance rate was seen against streptomycin and kanamycin with 49.6% (95% CI: 40.6-58.7; $I^2=87.7\%$; $Q=278.1$; $P=0.00$), and 23.6% (95% CI: 17.1-31.6; $I^2=85.7\%$; $Q=217.7$; $P=0.00$) of isolates being resistant, respectively. The lowest resistance rate was against gentamicin 6.3% (95% CI: 3.8-10.3; $I^2=82.8\%$; $Q=232.9$; $P=0.00$).

Prevalence of resistance against other antibiotics

The prevalence of resistant isolates to tetracycline, trimethoprim/sulfamethoxazole, ampicillin, trimethoprim, and chloramphenicol were as follows: 66.9% (95% CI: 58.3-74.5; $I^2=87.2\%$; $Q=330.4$; $P=0.00$), 41.6% (95% CI: 31.4-52.5; $I^2=89.6\%$; $Q=250.5$; $P=0.00$), 21.9% (95% CI: 15.1-30.6; $I^2=89.7\%$; $Q=390.6$; $P=0.00$), 19.7% (95%

CI: 9.8-35.8; $I^2=91.1\%$; $Q=135.8$; $P=0.00$), and 18.9% (95% CI: 14.1-24.9; $I^2=85.6\%$; $Q=300.5$; $P=0.00$), respectively. Meta-analysis of data on the prevalence of antibiotic-resistant *Salmonella* serotypes to these drugs was done using a random-effects model.

Discussion

Antimicrobial resistance is one of the most important public health concerns worldwide and contributes to the economic burden of both developed and developing countries through increasing the costs associated with treatment, hospitalization and infection control procedures (Antonelli *et al.*, 2019). *Salmonella* isolates are among the most important food-borne pathogens that are predominantly transmitted to humans *via* consumption of eggs, dairy products and fresh fruits contaminated with animal faeces (Eng *et al.*, 2015). In Iran, no comprehensive study is available on the prevalence of antibiotic-resistant *Salmonella* strains isolated from animals. Therefore, we conducted this study to investigate the antibiotic resistance profile of *Salmonella* serotypes isolated from animals in different provinces of Iran. In fact, resistance profiles of *Salmonella* serotypes against 22 antibiotics belonging to different classes such as beta-lactams, aminoglycosides and quinolones were investigated. Our results revealed that the majority of *Salmonella* strains were resistant to nalidixic acid, tetracycline and streptomycin, with 67%, 66.9% and 49.6% of all isolates being resistant, respectively. These resistance rates are in agreement with the results reported from United States, reporting 63% and 26% resistance to tetracycline and streptomycin, respectively (Velasquez *et al.*, 2018). In Iran, tetracycline

and quinolones are generally used in the livestock and poultry industries; therefore, this high frequency of resistance was predictable. In fact, antibiotic resistance profile of food-borne pathogens such as *Salmonella* serotypes can reflect the antibiotics used for animal treatment (Dallal *et al.*, 2010). Tetracyclines, as protein synthesis inhibitors, bind to the small ribosomal subunit and prevent attachment of aminoacyl-tRNA to protein synthesis complex. Several mechanisms can confer resistance against tetracycline, including efflux pumps, mutations and enzymatic inactivation (Hao *et al.*, 2016). Genes conferring resistance against tetracycline are often located in transferable genetic elements such as integrons and plasmids. Hence, it can be concluded that overuse of tetracycline might have resulted in the elimination of sensitive isolates and dissemination of resistant isolates harboring resistance genes (Hao *et al.*, 2016). For several years, as the traditional first-line treatments, antimicrobial agents such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole were used for *Salmonella* infection treatment. However, with the emergence of resistant isolates, the traditional antibiotics were replaced with cephalosporins (Eng *et al.*, 2015). Based on the results of the present study, prevalence of isolates resistant to chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole were 18.9%, 21.9% and 41.6%, respectively. These findings are in agreement with studies from Pakistan, India and Nepal, but in contrast to a previous study from Egypt (Ochiai *et al.*, 2008; El-Sharkawy *et al.*, 2017). Cephalosporins belong to the beta-lactam antibiotic family and inhibit bacterial growth by disruption of peptidoglycan. Cephalosporins have been extensively used in food animals, resulting in the development of antibiotic resistance in food-borne pathogens. Similar to the findings of a previous studies performed in different provinces of China, including Henan, Shaanxi, Fujian, Sichuan, Guangdong and Guangxi (Wang *et al.*, 2015), our results revealed a relatively high susceptibility of *Salmonella* strains to cephalosporins, with prevalence of resistance against cephalothin 13.5%, cefixime 9%, ceftriaxone 5.2%, ceftizoxime 3.4% and cefotaxime 3.7%. Resistance to cephalosporins and carbapenems is often caused by beta-lactamase enzymes. Extended-spectrum beta-lactamases (ESBLs) are powerful group of enzymes that can hydrolyze the structure of beta-lactam antibiotics, especially new generation of cephalosporins and carbapenems (imipenem and meropenem). Based on their structure, these enzymes are divided into four groups: A, B, C and D (Bush and Jacoby, 2010). Because limited antibiotics are available for the treatment of infections caused by ceftazidime-, cefepime- and imipenem-resistant *Salmonella*, the prevalence of ESBLs-positive isolates is of critical importance. Although the prevalence of ESBLs-positive *Salmonella* is still rare, there has been an increasing trend in recent years that is alarming (Hur and Lee, 2012). In this study, we also investigated antibiotic resistance profile of *Salmonella* strains against aminoglycoside antibiotics. Unfortunately, based on our findings, resistance rates to

streptomycin and kanamycin were high, with 49.6% and 23.6% of isolates being resistant, respectively. Aminoglycoside-modifying enzymes including aminoglycoside acetyltransferases, expressed by *acc* genes, and aminoglycoside phosphotransferases (*strA*, *strB*, *aph(3)-Ib* and *aph(6)-Id*) are the most important genes located in transferable genetic elements (integrons and plasmids), and are responsible for resistance against kanamycin and streptomycin (Vtnair *et al.*, 2018). Unfortunately, the majority of resistance determinants, including those that confer resistance against β -lactam antibiotics, aminoglycosides, fluoroquinolones, chloramphenicol and tetracyclines, have been identified in various *Salmonella* serovars isolated from the food animals (Vtnair *et al.*, 2018). The most important point is that these resistance genes are often located in the transferable genetic elements; therefore, misuse of antibiotics may lead to the elimination of susceptible isolates and increase the resistant population and then facilitate spread of multi-drug resistant *Salmonella* infections.

Similar to findings of this study, several independent studies in Iran showed that *Salmonella* strains isolated from human infections were mostly resistant against fluoroquinolones, tetracycline, streptomycin, chloramphenicol and ceftizoxime (Eshraghi *et al.*, 2010; Ranjbar *et al.*, 2011; Khademi *et al.*, 2020). Owing to the fact that *Salmonella* is able to establish zoonotic infections and also is able to acquire resistance genes from other enteric pathogens through mobile genetic elements such as integrons, plasmids and transposons, the misuse of antibiotics in food animal industries can lead to the spread of drug-resistant *Salmonella* infections (Ranjbar *et al.*, 2010; Khademi *et al.*, 2020).

To the best of the authors' knowledge, this is the first comprehensive study on the prevalence of antibiotic resistance profile of *Salmonella* species isolated from animals. Our results revealed that resistance to nalidixic acid, tetracycline, streptomycin and kanamycin are high and their use must be restricted. *Salmonella* is one of the most important food-borne pathogens responsible for life-treating infections in humans. The routine practice of giving antibiotics to animal as a means of promoting growth and preventing disease creates selection pressure that results in the survival of antibiotic-resistant pathogens. Subsequently, these antibiotic-resistant pathogens can be transmitted to humans *via* direct contact with animals or even consumption of contaminated foods of animal origin. Therefore, appropriate use of antibiotics in veterinary medicine and animal feeding will reduce the emergence of multi-drug resistant infections.

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