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Prevalence and Antimicrobial Resistance of *Salmonella Enterica* Serovar Infantis Isolates from Poultry: a review

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Abstract

Salmonella Infantis (*S.* Infantis) is one of the most important zoonotic bacteria, which has become one of the leading public health problems in the world, especially in developing countries. The prevalence of multi-drug resistant (MDR) *S.* Infantis strains has increased worldwide and can be prevented by controlling the use of antibiotics in poultry. The purpose of this review article is to discuss the status of *S.* Infantis antibiotic resistance, especially, its prevalence, detection methods and resistance mechanisms in isolates from poultry samples using search engines such as Web of Science, Scopus, and PubMed. Based on our review, *S.* Infantis was the most prevalent serovar in poultry accompanied by an enhancing number of resistance genes in these strains. The use of different genotypic and genetic methods can rapidly detect the presence of *Salmonella* in suspicious specimens to prevent disease and epidemics. Genes such as *invA*, *hilA* and *fliC* were most commonly used genes in the detection of *Salmonella*, and other genes were *viaB*, *spv*, *flijB*, *rfbJ* and *16Sr* RNA. The results of studies emphasize that poultry could act as reservoirs of MDR with a high tendency for dissemination. Resistance to the beta-lactam family is an important issue, because antibiotics such as beta-lactams are the best candidates for the treatment of salmonellosis, and this has raised concerns in the treatment of invasive *Salmonella*. These findings highlight the need to find ways to manage and reduce the impact of antibiotic use in poultry and prevent the transmission of antibiotic-resistant *S.* Infantis to the human food chain and to find potential alternatives to antibiotics.

Introduction

Salmonella enterica serovar Infantis (*S.* Infantis) is a gram-negative rod-shaped bacterium that can infect humans and poultry, especially chickens. Six subspecies and more than 2700 serotypes have been reported for *Salmonella enterica* (Wajid *et al*., 2019). This bacterium is a zoonotic pathogen, so its serotypes can circulate between poultry, humans and livestock via direct contact with vegetables or by consuming animal-sourced foods such as meat, milk, and eggs (Moradi Bidhendi, 2016; Libera *et al*., 2022; Ferrari *et al*., 2019). It is one of the most common pathogenic bacteria in food animals, the sixth most common bacterium in the United States and the third one in Europe after *S.* Enteritidis and *S.* Typhimurium (Aviv *et al*., 2016). *S.* Infantis is mainly present in the poultry industry worldwide and is a major cause of salmonellosis in poultry. This bacterium can cause a variety of infections in humans and a number of animal species and therefore is associated with significant economic losses (Rajagopal and Mini, 2013). Salmonellosis is caused by the bacteria *salmonella*, which can cause diarrhea, fever and stomach cramps in humans. Although serovar Infantis often affects children, it also causes disease in adults and those with a compromised immune system (Ghoddusi *et al*., 2019). According to the recent reports, approximately 1.3 billion patients are diagnosed with nontyphoidal salmonellosis worldwide each year (Acar *et al*., 2019). The prevalence of *Salmonella* in humans is mostly related to the consumption of contaminated animal products

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(Ghoddusi *et al.*, 2019). Poultry and poultry-related products are well-known reservoirs for transmitting bacteria resistant to antimicrobial agents and antimicrobial resistance genes. Due to the presence of *Salmonella* in poultry as a risk factor for meat and egg contamination, national programs in the European Union (EU) have been set to reduce the prevalence of *Salmonella* serovars. The program led to a significant decline in human salmonellosis cases between 2008 and 2013. However, *Salmonella* is still the leading cause of food-borne outbreaks in the EU (Pate *et al*., 2019). Also, *S*. Infantis has become an emerging nontyphoidal *Salmonella* and an important worldwide health concern due to the transmission of its resistant strains to humans (European Food Safety Authority, 2017). In recent years, the multi-drug resistant (MDR) strains of *S*. Infantis have increased significantly in the world (Pate *et al*., 2019; European Food Safety Authority, 2018). Although complete removal of *Salmonella* from poultry is difficult, and clearance and disinfection methods may often be ineffective, the spread of antibiotic resistance from poultry to humans can be prevented by controlling the antibiotics usage (Pate *et al*., 2019). In many countries, *S*. Enteritidis and *S*. Typhimurium have been identified as the most prevalent serovars in poultry, followed by *S*. Infantis (Moradi Bidhendi *et al*., 2015).

The purpose of this review article is to investigate the prevalence, detection methods and resistance mechanisms in *S*. Infantis, as an important strain in the zoonotic pathogen, isolated from poultry samples using databases such as Web of Science, Scopus, and PubMed.

Prevalence of *Salmonella* Infantis

According to poultry studies, *S*. Enteritidis is the most prevalent serovar in Asia, Latin America, Europe, and Africa. Also, S. Kentucky and S. Sofia are the most prevalent serovars in North America and Oceania, respectively (Ferrari *et al*., 2019). In recent years, *S*. Infantis has been the most common salmonellosis-causing serotype in the poultry industry due to MDR (Azizpour, 2021). Mori *et al*. (2018) showed that poultry meats and poultry-processing plants were infected with *Salmonella* in Japan. Among the isolates, *S*. Infantis (131/311, 42.2%) was the most common detected serotype. In the study of Mori *et al*. (2018), the prevalence of *Salmonella* in poultry meats was similar to their previous study in 2012. In another study conducted in Japan, among 243 *Salmonella* strains isolated in four consecutive years belonging to three serovars, *S*. Infantis was the most frequent serovar. In the study of Duc *et al*. (2019) in Japan, out of 3071 samples collected from broiler chickens from 2009 to 2011, the proportion of *S*. Infantis isolates decreased from 66% to 50% but increased again to 57.6% in 2012. A declining trend of *Salmonella* in poultry was also observed in Spain between 1993 and 2006 (55.0% in 1993 and 12.4% in 2006) (Álvarez-Fernández *et al*., 2012). The declining prevalence of *Salmonella* indicates that EU mandatory measures to reduce the incidence of *Salmonella* in poultry were apparently successful at the time (Álvarez-Fernández *et al*., 2012). In a study in Korea, of the samples collected from chickens to determine *Salmonella* serotypes, 5 samples of 16 serotypes were *S*. Infantis, and *Salmonella enterica* serovars Montevideo and Virchow were the most common serotypes (*Lee et al*., 2019). However, previous studies have demonstrated that *S*. Enteritidis, *S*. Typhimurium, and *S*. Infantis are the most common serotypes causing clinical symptoms of salmonellosis in Korea (Park *et al*., 2019, Choi *et al*., 2015). In Pakistan, out of 149 *Salmonella* strains, 54 isolates (36.2%) were confirmed as *S*. Infantis (Wajid *et al*., 2019). Whereas, of the 787 suspected *Salmonella* specimens isolated from poultry origin from different parts of India (2011- 2016), *S*. Gallinarum, *S*. Enteritidis and *S*. Typhimurium had the highest frequency, followed by *S*. Infantis (2.7%) (Kumar *et al*. 2019). These results show differences in the prevalence of serovar Infantis in the two neighboring countries. In Iran, studies conducted on the prevalence of *Salmonella* in poultry showed that *S*. Infantis was the most prevalent serovar, followed by *S*. Enteritidis and *S*. Typhimurium (Ghoddusi *et al*. 2019). However, these three *Salmonella* serotypes also appear to be the predominant strains isolated from poultry in many countries (Cosby *et al*., 2015, Kagambèga *et al*., 2013). A high prevalence of S. Infantis in chickens (52%- 90%) has also been reported in Iran (Fallah *et al*., 2013, Ghoddusi *et al*., 2019, Rahmani *et al*., 2013).

In one study in Egypt, Ammar *et al*. (2019) examined broiler samples and showed that 15.6% of the samples were infected with *Salmonella*, among which the most *Salmonella* serovars belonged to *S*. Enteritidis with a prevalence of 43.3%, and only 16.6% of the samples were *S*. Infantis. The results of this study were consistent with their previous study (Ammar *et al*., 2010) and another study conducted in Egypt (Ibrahim *et al*., 2013). Of the 239 Salmonella isolates, the prevalence of *S*. Infantis in Brazilian broilers was 22.6% (Mendonça *et al*., 2019). In other studies conducted in Brazil, Medeiros *et al*. (2011) reported a much lower prevalence (7.6%) of *S*. Infantis, while Cunha-Neto *et al*. (2018) showed a higher prevalence (35.4%; 11 out of 31 *Salmonella* strains). Also, the prevalence of *S*. Infantis in Srpska was 26.8% (Kalaba *et al*, 2017), while the prevalence was 94% in the study of Vinueza-Burgos *et al*. (2019). Of all *Salmonella* serovars, the proportion of *S*. Infantis isolated from poultry sources in Italy increased from 2.3% in 2008 to 22.7% in 2018 (Di Marcantonio *et al*., 2022). In Slovenia, the number of

S. Infantis isolates from broiler flocks continuously enhanced from 0.7% in 2010 to 11.5% in 2017. Also, it was found that *Salmonella* spp. had the highest incidence rate in broiler meat (26.7%- 28.4%) and *S*. Infantis was the predominant serovar (92%- 100%) (Pate *et al*., 2019).

In the United States, *S*. Infantis is consistently isolated from chickens and is relatively rare in other animal sources. In addition, the prevalence of *S*. Infantis in poultry meat in the United States was less than 0.4% from 2002 to 2012, while the incidence of human salmonellosis had increased during these years (Ferrari *et al*., 2019). Although Shah *et al*. (2017) found no significant relationship between an increase in human disease caused by *S*. Infantis and the prevalence of *S*. Infantis in chicken meat.

There are limited reports regarding *S*. Infantis isolated from poultry in different countries; however, this serotype is increasing as a pansensitive MDR phenotype and has been reported in countries such as Germany, Hungary, Italy and Japan, with an increase in human factors in these countries (Shah *et al*., 2017). In general, the ecology and epidemiology of serovar Infantis have not yet been extensively studied. The results obtained from these reports may depend on the study area, the sample collection method, especially the isolation season, and the isolation method. Also, the prevalence of serovars may change over time and be replaced by another serovar (Ghoddusi *et al*., 2019).

Detection of *Salmonella* and *Salmonella* serovar

Due to the high prevalence of salmonellosis, the identification and control of this disease is of great importance in terms of public health. The detection procedures of *Salmonella* serotype are time-consuming and complex and are sometimes associated with erroneous negative results. Usually, microbiological methods based on culture and examination of microscopic, macroscopic and biochemical properties, along with serological tests, are used to identify *Salmonella* and other bacteria (Shi *et al*., 2015). In the conventional method for determining *Salmonella* serovars by the phenotype-based White-Kauffmann-Le minor scheme method, bacterial cell surface antigens are detected using antiserum. According to this serological method, a serovar is determined based on the O (somatic), H (flagella) and Vi (capsular) antigens (Brenner *et al*., 2000). Although the culture method has the ability to study live bacteria and allows further evaluation with biochemical and serological methods to achieve a definite positive result, but this method is time consuming. The use of different genotypic and genetic methods can rapidly detect the presence of *Salmonella* in suspicious specimens to prevent disease and epidemics (Rementeria *et al*., 2009). Molecular techniques commonly used to describe bacterial macromolecules have been developed to overcome challenges associated with culture methods. Pulsed field gel electrophoresis (PFGE) has high typing, reproducibility and distinguishes between unrelated strains. Restriction fragment length polymorphism (RFLP) analysis is a rapid, simple and repeatable method for detecting bacteria. In sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), largely eliminates the effect of structure and charge, and proteins are separated only based on the length of the polypeptide chain. Random amplified polymorphic DNA analysis (RAPD-PCR) has the potential to detect polymorphisms throughout the genome. Random amplified polymorphic DNA analysis (RAPD-PCR) has the potential to detect polymorphisms throughout the genome. Other techniques such as plasmid profiling, DNA amplification fingerprinting (DAF) and RAPD-PCR are also used to determine *Salmonella* and *Salmonella* serovars (Adebowale *et al*., 2020; Moradi Bidhendi *et al*., 2015).

One method is the use of molecular techniques such as polymerase chain reaction (PCR), which leads to better identification of *Salmonella* serovars due to its high sensitivity, specificity and speed (Hong *et al*., 2008). This method is based on the normal phenomenon of DNA replication in cells, converting a transcript of a gene into more than one billion transcripts in a matter of hours, allowing even a single bacterial cell to be detected in a sample. The PCR is a rapid and reliable technique using different genes that play a role in the development of pathogenesis in *Salmonella* (Shi *et al*., 2015; Wei *et al*., 2019). The genes used in the molecular detection of *Salmonella* include *viaB, rfbJ*, *fljB, invA*, *fliC*, *spv* and *sefA*. The *16S r*RNA gene is also a preferred phylogenetic marker for the identification of bacteria (Stavnsbjerg *et al*., 2017). This bacterial gene is similar in length but contains highly conserved regions that vary depending on the species, genus, and family. Out of 149 isolates, Wajid *et al*. (2019) identified 54 isolates as *S*. Infantis with an amplified product of serovar Infantis-specific *fliB* gene fragments by PCR. Also, sequence analysis of *16S r*RNA and *fljB* gene amplicons reaffirmed the isolates with 99% similarity as *S*. Infantis. Another gene is *hilA*, which encodes transcriptional regulatory proteins to express invasive genes and facilitate the penetration of *Salmonella* into intestinal epithelial cells. Using this gene, the variability of the *Salmonella* population has been shown in different parts of the poultry gastrointestinal tract and their relationship with the function, tasks and physical and chemical environment of these parts (Moradi Bidhendi, 2016). The *InvA* gene, an important virulence gene, has a specific sequence for the genus *Salmonella* that makes it a suitable PCR target for the detection of this bacterium (Salehi *et al*., 2007). The

presence of this gene in *Salmonella* proves the ability of bacteria to penetrate host epithelial cells and eventually become infected (Lin *et al.* 2007). Another gene to identify *S.* Typhi using PCR is the *viaB* gene. Identification of this gene by the above method for the detection of *S.* Typhi in clinical specimens is a rapid, inexpensive, specific and highly sensitive method (Saadati *et al*., 2008). On the other hand, this gene is present in all *Salmonella* and can be recruited to detect the genus *Salmonella*. Other genes that can be employed to identify *S.* Typhimurium are *rfbJ*, *fljB*, *invA* and *filC* and *spv* and *sefA* genes to identify *S.* Enteritidis (Chashni *et al*, 2009; Mirzaie *et al*., 2010).

Researchers use different methods such as culture, ELISA and PCR to identify *Salmonella* in their studies (Nair *et al.* 2019). The ELISA is another technique capable of overcoming many of the limitations and disadvantages of other methods. In 1989, researchers used the ELISA method to identify *Salmonella* in food samples (Prusak-Sochaczewski and Luong, 1989; Wu *et al*., 2014; Hosseinpour *et al*., 2013). The ELISA method provides acceptable sensitivity and specificity for the detection of *Salmonella* in food, and so commercial kits are a valid screening method to control the production line of food production factories by health organizations (Moradi Bidhendi, 2016; Ardestani *et al*., 2007).

Pulsed field gel electrophoresis (PFGE) is a molecular technique; the investigation of genetic relationship between isolates under study is a feature of molecular typing methods. The PFGE has high typing, reproducibility and differentiation capabilities, which is one of the best methods in which the typing is performed based on DNA due to the size and number of bands created on the gel, high reproducibility, usability for all human pathogens and the ability to differentiate between unrelated strains. This method can be used to differentiate, detect, classify, and examine the phylogenetic relationship between isolated strains and epidemiological studies (Shi *et al*., 2015; Moradi Bidhendi, *et al*., 2015). Rahmani *et al*. (2013) applied PFGE for comparison of genetic relatedness. Among 27 isolates of *S.* Infantis from three Northern provinces in Iran, two distinct PFGE patterns were observed. The PFGE patterns of the isolates were very similar, indicating clonal correlation at different geographical locations. Ahmadi *et al*. (2013) used PFGE method (XbaI restriction enzyme) and genetic band similarity, among 40 strains of *Salmonella enterica* serotype Enteritidis, two genetic patterns were obtained which were classified into cluster A and B. The high recognition in this method indicates that different subtypes can exist in a serotype isolated from different geographical areas (Khaki *et al*., 2013). Pate *et al*. (2019) using the PFGE method divided the *S.* Infantis isolates into five clusters (A- E) with >90% profile similarity. In the study of Vinueza-Burgos *et a*l. (2016) 70 isolates by PFGE method belonged to 11 genotypes, among which eight genotypes (I to VIII) were in the group of S. Infantis isolates.

Other methods include RFLP and ERIC-PCR, which use restriction endonuclease and amplification of genetic material in extra-genomic regions and the space between two genes, respectively. In the first method, various enzymes can be used and in the second method, specific primers are used for ERIC regions (Moradi Bidhendi, 2016; Khaki *et al*., 2013). The RFLP is a rapid, simple and repeatable method. Jong *et al.* (2010) examined 47 isolates of *Salmonella* from 20 different serovars derived from poultry samples in Thailand by *fliC* / *fljB* PCR- RFLP assay using restriction endonucleases of *Mbo* I and *HhaI*. They showed that a combination of *fliC* and *fljB* profiles could differentiate over 80% of the serovars from each other. But, in a study by Khaki *et al.* (2013) HhaI for gene *fliC* had a similar band pattern to *S.* Typhimurium and *S.* Infantis, and was unable to differentiate. According to research, the PCR-RFLP method cannot replace serotyping (Wang *et al*., 2018). In the second method, specific primers are used for ERIC regions. This method has more interspecies and intraspecies differentiation ability compared to RFLP method. Using this method, a large number of suspicious specimens can be fingerprinted in a short time and at a low cost. Examination of genetic diversity of 30 *S.* Enteritidis samples isolated from food and human cases using ERIC-PCR method showed that 29 isolates were in 4 main groups with 95% similarity and 1 isolate in a group with a different pattern. This study showed that there is not much genetic diversity between *S.* Enteritidis strains and the origin of these strains from a single clone is very likely (Salehi *et al*., 2008).

Other methods of molecular identification include the loop-mediated isothermal amplification (LAMP) method, which has become more popular due to its speed, sensitivity, specificity and low cost. In this method, DNA is amplified using 4 to 6 specific primers capable of binding completely to 6 to 8 regions of the target sequence and through a sequential process by forming hairpin loop regions and using the DNA polymerase *Bst* enzyme over a period of 60 minutes and isothermal conditions. Due to the mentioned advantages, the application of this method using a specific gene is unique to *S.* Typhimurium serovar (Yang *et al*., 2016; Moradi *et al*., 2009).

Molecular methods performed to identify *Salmonella* in different cases in the research showed that *invA, hilA* and *fliC* genes were the most commonly used genes in the detection of *Salmonella*, and other genes were *viaB, spv*, *flijB*, *rfbJ* and *16Sr* RNA. Due to the complexity and diversity of

Salmonella serovars, effective methods to identify the most common salmonellosis-related serovars and emerging rare serovars, or the outbreaks of unusual serovars are needed. However, the use of the molecular methods as high sensitivity, low cost and short time approaches mentioned above provides better clarity than traditional serotyping and is, a valuable method for grouping foodborne pathogens.

Drug resistance in *Salmonella* Infantis

The emergence of resistance among *Salmonella* serotypes isolated from human, animal and poultry samples has been considered in recent years. The gradual increase in isolated *Salmonella* strains from humans and their resistance to various drugs and antibiotics may be due to the widespread use of these drugs in poultry for food production (Mendonça *et al*., 2019). Although *Salmonella* infections are often asymptomatic in poultry, eating meat contaminated with microorganisms that are resistant to antibiotics endangers human health. Thus, resistant *Salmonella* can be transmitted to humans through food chains such as poultry and poultry eggs (VT Nair *et al*., 2018). Unfortunately, the indiscriminate use of these antibiotics on the one hand and the ability of bacteria to transmit drug resistance genes on the other hand have led to an increasing prevalence of resistant strains (Li and Webster, 2018). Because poultry farms use antibiotics extensively, the number of multidrug-resistant bacteria in humans has also increased (Marchello *et al*., 2020). Therefore, a high percentage of isolates resistant to these drugs, as a warning, can make treatment more difficult. The use of antibiotics to increase the growth, prevention and treatment of animal products also increases the prevalence of resistance among human pathogens such as some *Salmonella* serovars (Mendonça *et al*., 2019; Singer *et al*., 2003). The use of antibiotics is the best candidate for the treatment of salmonellosis. Therefore, the emergence of resistance to antibiotics, especially beta-lactams, against invading *Salmonella* has raised concerns in this area. Veterinary drugs can cause antibiotic resistance in human consumers. Hence, reducing the use of antibiotics in veterinary medicine can help reduce therapeutic problems in humans (Mendonça *et al*., 2019; (VT Nair *et al*., 2018).

The emergence of resistance in *S.* Infantis, a common serotype isolated from poultry specimens, has raised concerns about the transmission of this resistance to humans; 100% resistance to some antibiotics has also been observed in this serotype (Asgharpour *et al*., 2014). Wajid *et al*. (2019) reported a high prevalence of antimicrobial resistance genes in *S.* Infantis. In this study, all 54 *S.* Infantis strains were resistant to at least three antibiotics. Moreover, 12 of the strains were MDR, 31 were XDR (extensively drug-resistant) and 11 were PDR (pandrug-resistant). The highest resistance (94.4%) was against Pefloxacin, followed by chloramphenicol (83.3%) and imipenem (77.7%). In contrast, *S.* Infantis isolates were most sensitive to ertapenem, cefotaxime and cefixime. Also, the most common resistance genes were *aadA* for aminoglycosides (42.3%), *parE* for quinolones (62.5%), *Int1* for penicillin, (62.9%) *cat3* for chloramphenicol (66.1%) and *blaTEM-1* for beta-lactam (44.4%). As the results showed, the highest prevalence of chloramphenicol-related gene resistance was in *cat3*. All *S.* infantis isolates in the study of Ghoddusi *et al.* (2015) were resistant to *floR* and *catI* (phenicols), but none of the isolates was resistant to *tetA* or *tetG* (tetracycline). And other studies, the highest antibiotic resistance of *Salmonella* in samples was related to sulfonamide (42.5%) (Mendonça *et al*., 2019), nalidixic acid (Lee *et al*., 2019) tetracycline (Ghoddusi *et al*., 2019), nalidixic acid and Ampicillin (Ahmed *et al*., 2014; Kalaba *et al*, 2017; Lee *et al*., 2019). In the meantime, some isolates were resistant to at least four or more antibiotics (Kalaba *et al*, 2017; Vinueza-Burgos *et al*., 2019). In a study, 11 cases of *S.* Infantis had MDR, and the most common resistance was to trimethoprim, trimethoprim/ sulfamethoxazole and sulfonamide antibiotics (Cunha-Neto *et al*., 2018). Fallah *et al*. (2013) examined 34 *Salmonella* samples isolated from poultry (25/34 *S.* Infantis samples), and reported that all isolates were resistant to nalidixic acid, tetracycline and streptomycin, and only 10% of the samples were resistant to ampicillin. The most common pattern of resistance (34.1%) was resistant to six antibiotics and 6.8% of the strains were resistant to at least three antibiotics. In previous studies, we examined the integron resistance gene in MDR strains of *S.* Infantis and the results showed that 36% of *S.* Infantis isolates carried the *Int1* gene, 42% *Int2* and 4% *Int3*. In addition, 11 strains contained both *Int1* and *Int2* integrons. All tetracycline-resistant strains carried the *tetA* gene and 5 strains carried the *tetB* gene, and all chloramphenicol-resistant isolates contained the *floR* and *cat1* genes. Moreover, 18% of streptomycin-resistant *S.* Infantis isolates carried the *strA* gene (Asgharpour *et al*., 2018; Asgharpour *et al*., 2014). The results of this study are similar in terms of resistance to the results of Rahmani *et al*. (2013) which isolated 27 samples of *S.* Infantis from three Northern provinces of Iran (Mazandaran, Gilan and Golestan). However, resistance to the isolates was observed in at least six or more antibiotics. In the study of gene resistance, the *Int1* gene resistance was confirmed in all 27 *S.* Infantis specimens. This resistance to *Int1* gene and the number of strains resistant to both *Int1* and Int2 integrons were higher than the samples isolated from Mazandaran province in the report of Asgharpour *et al*., (2018), but they were similar in terms of *tetA* gene resistance to determine tetracycline resistance. Resistance to *floR*

(chloramphenicol), *aadA1* (aminoglycosides), *dfrA14* (trimethoprim) and *sulI* (sulfonamides) was observed in some strains of this study (Rahmani *et al*., 2013). Although the *Int2* gene in a study by Ahmed *et al*. (2014) was not found in four isolated *S*. Infantis and the resistance of these four isolates to Class 1 (*aadA1*), *blaTEM-1*, *floR*, *qnrB* genes was reported.

The results of these studies show that resistance genes in *S.* Infantis strains isolated from poultry are increasing and most of the reported resistance was to the antibiotics nalidixic acid, tetracycline, ampicillin, streptomycin and trimethoprim (Fallah *et al*., 2013). A review article in Iran from 2010 to 2015 showed that resistance to nalidixic acid increased in human samples and the rate of this resistance was reported to be very high. Observation of resistance to tetracycline and nalidixic acid in humans and poultry indicates the development of resistance genes to these two antibiotics in both cases (Moradi Bidhendi *et al*., 2015). In addition, the emergence of resistance to beta-lactam antibiotics has raised concerns in the treatment of invasive *Salmonella*, which may be due to the presence of beta-lactamase-producing plasmid genes that inactivate the beta-lactam ring and inactivate the drug (Nair *et al*., 2019).

Due to the increase in antibiotic-resistant *Salmonella* serotypes, especially *S*. Infantis, in food animals, and increased rates of death due to the lack of efficacy of current antibiotics, new and safe antibacterial drugs, as well as rapid detection for the prevention and effective control of antibiotic-resistant pathogens, are required (Di Marcantonio *et al*., 2022). The use of bacteriophages (phages) is one of the best options to quickly diagnose and reduce the incidence of *Salmonella* and ensure food safety. Although several diagnostic methods have been reported to target *Salmonella* in combination with bacteriophages, the use of new phages combined with antimicrobial technology to detect synergistic effects against pathogens is of interest (Wei *et al*., 2019).

Mechanism of resistance in *Salmonella* Infantis

The emergence of antimicrobial resistance among bacteria has been raised as one of the major public health concerns. One of the causes of bacterial resistance can be the irrational use of antimicrobial drugs or antibiotics (Ahmed *et al*., 2014). The plasmids that carry drug-resistant genes are easily transported through interspecies gene exchange processes and even different bacterial genera. Aminoglycoside antibiotics such as streptomycin, neomycin, and kanamycin, as well as various beta-lactamases, are examples of genetic mechanisms of plasmid-mediated antibiotic resistance. *S*. Infantis, like other *Salmonella* serovars, is resistant to various antimicrobial agents, as reported in several studies (Wajid *et al*., 2019), Such as the use of antimicrobial drugs against beta-lactams in the treatment of human and animal infections that has led to the development of resistance to them. According to reports, resistance to various beta-lactams may be due to the production of beta-lactamase enzymes in *Salmonella* serovars. (Souza *et al*., 2020). The beta-lactamase enzymes can analyze broad - spectrum third - generation cephalosporins, such as ceftazidime, cefotaxime, ceftriaxone and monobactams (aztreonam), and are ineffective on cephamycins (cefoxitin and cefotetan) and carbapenems (imipenem and meropenem). Their activity is inhibited by clavulanic acid, sulbactam and tazobactam. These new and broad-spectrum enzymes are known as Extended Spectrum Beta Lactamases (ESBLs) (Naderi Mozajin *et al*., 2018). *Salmonella* resistance to the beta-lactam family is an important issue because antibiotics such as beta-lactams are the best candidates for the treatment of salmonellosis, and this has raised concerns in the treatment of invasive *Salmonella*. Excessive use of this type of antibiotic on the one hand and the ability of bacteria to transmit resistant genes on the other hand can cause problems in the treatment process (Tate *et al*., 2017). A study in the United States showed that the pESI-like plasmid was present in most *S*. infantis isolates and its transport rate increased from 2017 to 2018. Also, the prevalence of *S*. infantis carrying the extended-spectrum β-lactamase gene (blaCTX-M-65) in raw chicken was reported in the year 2018 in the United States (Mc Millan *et al*., 2020). The spread of resistance to beta-lactam antibiotics such as third-generation cephalosporin is very serious as the first choice against invading *Salmonella*. In such cases, the plasmid genes of *Salmonella*, which provide the enzyme beta-lactamase and inactivate the central core of cephalosporins, make this antibiotic unusable (Yang *et al*., 2017). The presence of different genes or the development of different mutations in specific genes also leads to the emergence of antimicrobial resistance in different bacteria, for example, aminoglycoside-modifying enzymes (AME) are the most common type of aminoglycoside resistance. AME-genes with encode the aminoglycosides acetyltransferase, aminoglycoside nucleotideyltransferase and adenyltransferase enzymes cause resistance to aminoglycoside drugs, whereas *Salmonella* resistance to quinolones is associated with different types of gene mutations (Matayoshi *et al*., 2015; Acheampong *et al*., 2019).

Many of the genes responsible for resistance to gram-negative bacteria are part of the gene cassette in integrons. Integron is a gene of the λ integrase family that performs recombination between two distinct target sites. The integrons are encoded based on their amino acid sequences of integrase and are classified by the *intI* gene. According to recent studies, more than four types of integron classes have been identified.

Table 1. Identification and antimicrobial resistance of *Salmonella enterica* serovar Infantis

Authors	Country	Sample	Detection of *S.* Infantis	Isolate of *S.* Infantis	Resistance phenotype	Antimicrobial-resistant genes
Wajid *et al*. 2019	Pakistan	Poultry	Sequencing of *16S* RNA and *fljB* genes	54	Ofloxacin, norfloxacin, ciprofloxacin, imipenem, levofloxacin, Doripenem, meropenem, ertapenem, aztreonam, ceftazidime, cefepime, cefotaxime, cefixime, piperacillin, ampicillin, piperacillin/tazobactam,ticarcillin, tobramycin, gentamicin, amikacin,	*parE, gyrB, parC, gyrA, Int1, Class 1 intron, blaTEM-1, blaOXA-1, blaSHV, blaPSE-1, blaTEM, Cls1, intB, IntA, blaCMY-2, blaSHV-12, blaDHA-1, blaSHV-1, blaCTX-M1, blaCTX-M2, blaCTM-M14,blaOXA-2, blaCMY, ampC, blaCTX-M-15, aadA, aadA1, strB, aadB, strA, aphAI-IAB, aacC2, aadA2, aacC, aac (3)-Ia,aac(3)-IIa, aac(3)-Iva*
Medeiros *et a*l. 2011	Brazil	Broiler chicken	Serotypes	19	Ampicillin, aztreonam, ceftiofur, florfenico, ST, gentamicin, nalidixic acid , sulfonamide, trimethoprim, trimethoprim-sulfamethoxazole	-
Mendonça *et al*. 2019	Brazil	Broiler chicken	Serotypes	54	Sulfonamide, tetracycline, amoxicillin, neomycin, trimethoprim, ceftazidime, gentamicin	-
Rahmani *et al*. 2013	Iran	Broiler chicken	Serotypes	36	Ciprofloxacin, florfenicol,, nalidixic acid, spectinomycin, streptomycin, sulfamethoxazole, tetracycline, trimethoprim	*tetA, tetB, tetC, tetD, tetG, aadA, strA, strB, dfrA14, florR, sullI, sul1/qacEΔ1I, sulI, qacEΔ1, IntI1, IntI2, IntI2 variable, gyrA, parC*
Fallah *et al*. 2013	Iran	Broiler chicken	Serotypes	34	Gentamicin, ampicillin, colistin, ceftazidime, amoxi/clavulanic, trimetoprim, chloramphenicol, amoxicillin, streptomycin, tetracyclines, nalidixic acid	-
Asgharpour *et al*. 2014	Iran	Broiler chicken	Serotypes	50	Nalidixic acid, tetracycline, streptomycin, chloramphenicol, trimethoprim, ceftazidime	class 1 integron

The rest of Table 1 follows:

Authors	country	sample	Detection of *S.* Infantis	Isolate of *S.* Infantis	Resistance phenotype	Antimicrobial-resistant genes
Asgharpour. *et al*. 2018	Iran	Broiler chicken	Serotypes	48	Ceftazidime, nalidixicacid, chloramphenicol, trimethoprim-sulfametoxasol, streptomycin, tetracycline	*tetA, cat1, flor, Int1, Int2, Int3, tetB, strA*
Ghoddusi *et al*. 2019	Iran	Broiler chicken	Serotypes	38	Ampicillin, ceftriaxone, ceftazidime, cefepime, chloramphenicol, florfenicol, tetracycline , streptomycin , spectinomycin , kanamycin , trimethoprim , sulfonamides ,	-
Ammar *et al*. 2010	Egypt	Broiler chicken	Serotypes	5	Nalidixic acid, cefuroxime, amoxicillin/clavulanic acid , cefepime, streptomycin, gentamicin ,doxycycline, sulphamethaxole/trimethoprim, ampicillin , ceftriaxone	*blaTEM, blaCTX, qnrA, qnrS*
Ahmed *et al*. 2014	Egypt	Broiler chicken	Culture and PCR	4	Ampicillin , aztreonam , cefotetan, cefotaxime , cefoxitin, gentamicin, kanamycin, oxacillin , spectinomycin, sulfamethoxazole/trimethoprim, tetracycline, chloramphenicol, nalidixic acid, streptomycin	Class 1 *(*aadA1*), blaTEM-1, floR, qnrB,*
Acar *et al*. 2019	USA	Broiler chicken	serotyping, MLST, and PFGE	23	-	*aadA, sulI, tetA, tetR, str, tetA, dhfrV, aphA1,*
Mori *et al*. 2018	Japan	Poultry	Serotypes	113	Ampicillin , cefazolin, streptomycin , tetracycline , cefotaxime, kanamycin, nalidixic acid ,	-
Duc *et al*. 2019	Japan	Broiler chickens	Serotypes	140	Streptomycin, oxytetracycline, sulfamethoxazole, ampicillin , cefotaxime, ceftiofur , kanamycin, cefoxitin, ofloxacin, chloramphenico	-
Carfora *et al*. 2018	Denmark	Broilers chicken	Serotypes	4	Ampicillin, ceftazidime, cefotaxime, ciprofloxacin, colistin, nalidixic acid, sulfamethoxazole, tetracycline , trimethoprim , chloramphenicol;	*aph(3'), blaCTX−M−1, mcr-1.1, sul1, tet(A), dfrA1, dfrA14, aadA1, aadA2, blaTEM−1B, cmlA1, sul3*

The rest of Table 1 follows:

Authors	country	sample	Detection of *S.* Infantis	Isolate of *S.* Infantis	Resistance phenotype	Antimicrobial-resistant genes
Vinueza-Burgos *et al,* 2019	Ecuador	Broiler chicken	Serotyping, ERIC PCR, PFGE pattern	66	Nalidixic acid, ciprofloxacin, cefotaxime, ampicillin, tetracycline, sulfamethoxazole+trimethoprim, chloramphenicol, kanamycin, gentamicin, ceftazidime	-
Mejía *et al*. 2020	Ecuador	Broiler chicken	Serotyping and PCR	182	Nitrofurantoin, tetracycline, Sulfamethoxazole/trimethoprim, streptomycin, gentamicin, cefotaxime, chloramphenicol fosfomycin, ciprofloxacin, Azithromycin, cefoxitin, Amoxicillin + clavulanic acid	-
Sanchez-Salazar *et al*. 2020	Ecuador	Poultry	PCR	31	Families, aminoglycosides, cephalosporins, phenicol, , nitrofurans, thrimethoprim/sulphamethoxazole, tetracycline.	*blaTEM , blaCTX-M, blaCTX-M group 1, blaCTX-M group 9, sul1, tetA*
Lee *et al*. 2019	Korea	Broiler chicken	Serotyping and PCR	5	Ampicillin, amoxicillin-clavulanic acid, cefoxitin, ceftiofur, cephalothin, chloramphenicol, ciprofloxacin, colistin,florfenicol, gentamicin, nalidixic acid, neomycin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole	-
Choi *et al*. 2015	Korea	Broiler chicken	Serotyping, MLST	8	Ampicillin, ceftazidime, cefotaxime, cephalothin, cefazolin, streptomycin, tetracycline, nalidixic acid	*blaCTX-M-1 , blaCTX-M-9*
Pate *et al*. 2019	Slovenia	Broiler chicken	PFGE pattern	87	Ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid, streptomycin, tetracycline	-
Kalaba *et al.* 2017	Srpska	poultry	Serotyping	29	Streptomycin, cefotaxime, cefaclor, cephalexin, ceftazidime, pipemidic acid, amoxicillin, nalidixic acid	-

The integrons enable the release of resistance genes across bacteria through transposons and plasmids (Zhang *et al*., 2018; Rowe-Magnus *et al*., 2001). Investigation of Class 1 (*int1*), class 2 (*intI2*), class 3 (*intI3*) integron gene in *S.* Infantis are shown in Table 1.

Also, efflux pumps in bacteria, especially gram-negative bacteria, cause antibiotic resistance by controlling and regulating important proteins that are involved in the removal of toxins, including antimicrobial agents. In a study using the phenotypic method of Ethidium Bromide-Agar Cartwheel (EtBrCW) to identify efflux pump activity, 14 out of 45 isolates of *S*. Infantis (5MDR, 5XDR and 4 PDR) had an active efflux system, and the highest prevalence of genotype from efflux pump belonged to *armA* gene (74.2%) followed by *qnrS* (42.6%) (Wajid *et al*., 2019; Martins *et al*., 2013).

Another factor for bacterial infection is swimming motility, a bacterial movement associated with chemotaxis that allows bacteria to track nutrients or prevent the excretion of unwanted substances, which ultimately helps them retain the desired material for colonialization. In a study on resistant isolates of *S*. Infantis, there was a significant difference in swimming mobility ($p = 0.043$) between PDR and MDR isolates (Wajid *et al*., 2019).

Conclusion

Based on our review, the use of antibiotics in poultry diets not only causes the emergence of antibiotic-resistant strains and their transmission to humans, but also is not economically affordable, and imposes irreparable damage on nutritional health and public health. These results also emphasize that poultry may act as reservoirs of MDR. Additionally, the emergence of resistance to beta-lactam antibiotics, as the best candidates for the treatment of salmonellosis, has raised concerns in the treatment of invasive *Salmonella*. Due to the prevalence of MDR strains in this serovar at the international level, further research is needed to monitor and track the transmission and sources of *S.*Infantis domestically as well as internationally. The best way to prevent salmonellosis is to take measures, such as thoroughly cooking animal products, hand washing after handling raw meat or unwashed vegetables and avoiding unpasteurized foods.

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