Identification of *Yersinia* species in raw chicken meat in Tehran retail stores and determination of their antibiotic resistance pattern

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Abstract

Yersinia constitutes one of the predominant bacterial agents implicated in foodborne illnesses. The objective of this investigation is to ascertain the presence of *Yersinia* species in raw chicken meat procured from retail establishments in Tehran, alongside an examination of their patterns of antibiotic resistance. Between April and September 2023, a total of 220 chicken meat samples were systematically collected and analyzed for contamination by *Yersinia* species. The initial isolation was conducted through enrichment in saline phosphate at a temperature of 4 degrees Celsius over a duration of three weeks, succeeded by secondary enrichment utilizing 5.0% potassium hydroxide, and the resultant samples were subsequently cultured on CIN agar medium. Following the execution of warm staining and the microscopic observation of Gram-negative cocci, biochemical assays were employed to differentiate the strains, and the findings were corroborated using the API 20E kit. Ultimately, antibiotic resistance profiles were established via the agar disk diffusion methodology encompassing seven different antibiotics. From the totality of 220 chicken meat samples, 12 (5.5%) suspect strains of *Yersinia* were successfully isolated and were definitively identified as *Yersinia* through biochemical testing. Application of the API 20E kit revealed that three of the strains were classified as *Enterococcus* species, five as Entermedia species, two as Fredericksen species, and two as Christensen species. Notably, all isolated strains exhibited resistance to ampicillin, tetracycline, and cefixime antibiotics, while remaining sensitive to other antibiotics. The results of this study indicate the presence of various strains of *Yersinia* in

chicken meat samples at the Tehran level. Given the emergence of microbial resistance to specific antibiotics, it is imperative that antibiotic usage is approached with judicious strategies.

Keywords: *Yersinia*, *Yersinia enterocolitica*, chicken meat; antibiotic resistance

1. Introduction

Yersinia enterocolitica is a gram-negative, coccobacillus-shaped bacterium that is transmitted through the consumption of contaminated food and water. It causes gastrointestinal diseases, mesenteric lymphadenitis, and erythema nodosum in humans (3-1). This bacterium is psychrotrophic and is capable of growth at temperatures between 2-45 degrees Celsius. As a result, *Yersinia enterocolitica* can survive and reproduce in refrigerated environments where food is stored. This ability poses a significant threat to the safety of food products (4,5). The most important food items contaminated with *Yersinia enterocolitica* bacteria, which have been studied so far, include various processed products such as different types of red meat, fish, chicken, milk, eggs, fruits, and vegetables (9-6). Every year, millions of people worldwide, especially in developing countries, are affected by this disease and even lose their lives. After Campylobacteriosis and Salmonellosis, *Yersiniosis* ranks third among significant bacterial zoonoses in the European Union and is a causative agent of diarrhea (10). *Yersinia enterocolitica* causes approximately 117,000 cases of infection, 640 hospitalizations, and 35 deaths annually in the United States (11). In the 1980s, two major outbreaks of *Yersinia* with over 500 reported cases were documented in China (12). According to studies conducted worldwide, most *Yersinia infections* occur in infants and young children (15-13). As a result, due to the high incidence of diarrhea caused by the consumption of food contaminated with *Yersinia* (especially in children) and the importance of determining the biotype and serotype in the pathogenicity of *Yersinia enterocolitica* strains, as well as the existence of a wide diversity of species (such as *Y. intermedia*, *Y. frederiksenii, Y. kristensenii, Y. pseudotuberculosis*) and the lack of sufficient information in this field in Iran, there is a need for investigation and research on this bacterium in the country. Generally, *Yersinia enterocolitica* strains are divided into two groups of American and European variants. American strains belong to biotype 1B and include serotypes O8, O13a, O13b, O20, and O21. European strains belong to biotypes 5-2 and include

serotypes O3, O5, O9, and O27 (18-16). Additionally, *Yersinia pseudotuberculosis* 6 serotypes and *Yersinia enterocolitica* has 27 serotypes (19).

To design effective strategies for controlling diarrheal diseases caused by this bacterium, it is important to have accurate information regarding the epidemiology of these strains in Iran (20). On the other hand, considering the high contamination of chicken meat and intestinal feed with *Yersinia enterocolitica* and the presence of antibiotic-resistant strains, implementing hygiene measures to reduce the contamination of chicken meat with *Yersinia enterocolitica* and proper use of antibiotics in the poultry industry can be essential to prevent the spread of antibiotic-resistant strains and their transmission to the human food chain (7,16). Therefore, the present study aimed to identify *Yersinia* species in raw chicken meat in Tehran's retail stores and determine their antibiotic resistance pattern.

2. Materials and Methods

2.1. Sampling

In this study, a total of 220 samples of chicken meat were collected from April to September 2024 from chicken meat retailers in Tehran to isolate *Yersinia enterocolitica* and other atypical *Yersinia* species. The samples included 55 cases of wings and necks, 55 cases of heart and liver, 55 cases of chicken legs, and 55 cases of chicken breast for examination.

2.2. Cultivation and separation of bacteria

The collected samples were examined for the detection of the *Yersinia* genus based on the method provided by the Food and Drug Administration (FDA) (21). A quantity of 5 grams of chicken meat was finely sliced with a sterile scalpel and added to very thin layers. Then, 45 milliliters of saline phosphate buffer with a pH of 7.2 were added and the mixture was refrigerated at a cold temperature for three weeks. On the twenty-second day, one milliliter of suspension enriched with 9 milliliters of 0.5% potassium was thoroughly mixed using an electric mixer for 30 seconds, and then a loop of this mixture was cultured on CIN agar medium (Cefsulodin-Irgasan-Novobiocin) (Merck, Germany) and incubated at 30 degrees Celsius for 24 hours. After this period, suspicious colonies were examined.

2.3. Identification using phenotypic methods

Suspicious colonies of *Yersinia* were observed on CIN medium, which consisted of colonies with a red center and transparent edges (referred to as bull's eye appearance) and were selected for microscopic examination. After performing Gram staining and observing Gram-negative cocci

under the microscope, the isolates were subjected to differential biochemical tests such as catalase, indole, nitrate reduction, motility at 25 degrees Celsius, MR&VP, urease, hydrogen sulfide production, Kliger iron agar, ornithine decarboxylase, citrate, and ONPG tests. Finally, strains with biochemical characteristics of lactose and negative oxidase and indole, urease, ONPG, and ornithine decarboxylase positive, and motility at 25 degrees Celsius were identified as a strain belonging to the genus *Yersinia*. Subsequently, the isolated *Yersinia* bacterial strains were confirmed and identified using the API 20 E kit (BioMérieux, France) (16).

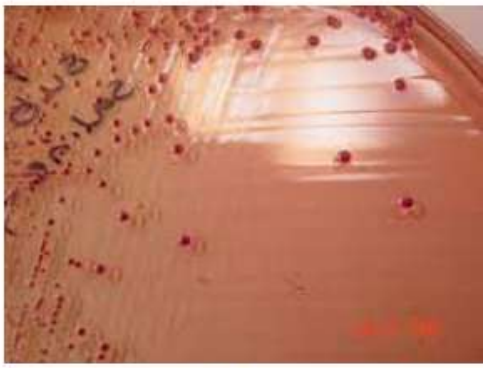
2.4. Determination of antibiotic sensitivity pattern

For all strains identified as *Yersinia* by phenotypic methods, antibiotic susceptibility testing was performed using the disk diffusion method (22). Initially, a pure colony from fresh culture was added to a Mueller-Hinton broth (Charlo, Spain) to achieve a turbidity equivalent to 0.5 McFarland standard. A microbial suspension obtained with sterile water was cultured in Muller-Hinton agar medium (Charlo, Spain). Commercial antibiotic disks of chloramphenicol (30 micrograms), ampicillin (10 micrograms), tetracycline (5 micrograms), ceftriaxone (30 micrograms), trimethoprim-sulfamethoxazole (25 micrograms), gentamicin (10 micrograms), and cefixime (30 micrograms) (MAST, England) were placed on them, and microbial resistance was determined after 24-18 hours based on the standard method provided by the Clinical and Laboratory Standards Institute 2018 (CLSI).

3. Results

3.1. Results of identification of separated strains' identity

Among the 220 collected and cultured samples of chicken meat, 12 samples (5.5%) were found to have ox-eye lesions (Figure 1) and negative oxidase reaction. Finally, they were examined using a gallery containing Kliger's medium, SIM, urea, Simon citrate, and ornithine decarboxylase. Confirmation of the results of biochemical tests on some suspicious strains of *Yersinia enterocolitica* was performed using API 20E kits (Figure 2). Out of the 12 identified strains as *Yersinia*, 3 strains belonged to the species *Enteroclitica*, 5 strains were Intermedia, 2 strains were *Fredericksenii*, and 2 strains were *Kristensenii*.



(A)

Figure 1. (A) Suspicious colonies of *Yersinia* with a morphology resembling cow's eye in CIN medium. (B) Biochemical tests for *Yersinia* diagnosis; from left to right: positive urea test in urea agar medium, acid-alcohol reaction in Kligler's medium, negative citrate utilization in Simon's citrate medium, positive ornithine decarboxylase reaction, SIM medium, positive indole reaction, and positive urea test in urea broth medium.

Figure 2. API20E system for identification of *Yersinia enterocolitica*

3.2. Results of determining the antibiotic sensitivity pattern of *Yersinia* strains

The antibiotic sensitivity pattern for 12 strains of *Yersinia* isolated from chicken meat (5.5%) was determined using the agar disk diffusion method for 7 antibiotics (Table 1). As shown in Figure 3, both species demonstrated 100% sensitivity to the antibiotics gentamicin, cefoxitin, trimethoprim-sulfamethoxazole, and chloramphenicol, while the highest level of resistance (100%) in both species was observed for the antibiotics ampicillin, tetracycline, and cefixime.

Table 1. Sensitivity of *Yersinia* strains to antibiotics

Disc **Bacteria**	**Ampicillin**	**Trimethoprim-sulfamethoxazole**	**Tetracycline**	**Cefoxitin**	**Chloramphenicol**	**Gentamicin**	**Cefixime**
Yersinia enterocolitica	R	S	R	S	S	S	R
Yersinia intermedia	R	S	R	S	S	S	R
Yersinia frederiksenii	R	S	R	S	S	S	R
Yersinia kristensenii	R	S	R	S	S	S	R

Figure 3. Antibiotic sensitivity testing of *Y.entermedia* and *Y.enterocolytica* species (resistant to ampicillin, tetracycline, and cefixime; sensitive to other antibiotics used).

4. Discussion

Foodborne diseases are considered a major challenge for the development of the food industry and one of the most concerning factors for any country worldwide. The most important bacterial agents involved in these types of diseases include Escherichia coli, *Salmonella*, *Shigella*, Campylobacter, and *Yersinia enterocolitica*, which numerous studies conducted in our country have demonstrated their high prevalence in food-related illnesses and gastroenteritis (21).

In this study, an attempt was made to determine the prevalence of *Yersinia* species and their antibiotic resistance pattern in chicken meat in Tehran. A total of 220 chicken meat samples were collected, including 55 wings and necks, 55 hearts and livers, 55 chicken legs, and 55 chicken breasts. In total, 12 samples (5.5%) were found to be positive for *Yersinia* species, belonging to 4 species: Enteroclitica (25%), Intermedia (66.7%), Frederiksenii (16.7%), and Kristensenii (16.7%). Among the studies conducted in Iran, the study by Mr. Mohammad and colleagues in 95 and Mr. Momtaz and colleagues in 92, which aimed to determine the prevalence of *Yersinia* species on chicken meat samples collected from retail centers in western regions of Iran, reported the prevalence of *Yersinia enterocolitica* as 15.5% and 18.33%, respectively, and the prevalence of *Yersinia intermedia* as 7%. This is higher than the prevalence reported in our study.

This issue may be due to the difference in the study population in these studies (23,24). Mr. Mohammad study, using 450 collected samples (226 samples of chicken meat and 224 samples

of beef), was conducted in supermarkets in Tehran. Additionally, Momtaz et al. conducted their study using 720 samples. Furthermore, in our previous study in 82, the prevalence of *Yersinia* in chicken meat samples was found to be 4.44%.

Based on the biochemical tests conducted, out of 155 isolated strains of *Yersinia*, 53 strains (34.2%) were *Yersinia enterocolitica* and 47 strains (30.3%) were *Yersinia intermedia* (7). Our findings, along with those of others, indicate that the prevalence of *Yersinia* has been decreasing over the years. The downward trend in the levels of *Yersinia* in meat and poultry can be attributed to the implementation of hygiene education and adherence to sanitary principles by suppliers and consumers, as well as the packaging of meat and poultry. In other countries, numerous studies have been conducted on the identification and separation of *Yersinia enterocolitica* and *Yersinia intermedia*, and comparing the results obtained from them with the present study can help in understanding the epidemiology of *Yersinia*. For example, Juan Wang and colleagues in 2021 investigated the prevalence of *Yersinia enterocolitica* in

food samples in China. A study was conducted on frozen food samples and packaged chicken meats, while our study was conducted on fresh raw meat samples. They reported 37 out of 1588 samples (2.5%) contaminated with *Yersinia*. Among these, 19 samples (3.51%) were from frozen foods, 11 cases (7.29%) were from various types of meats, and 2 samples (4.5%) were from packaged chicken meats (25). This study differs in terms of the prevalence of *Yersinia* compared to the present study, and the geographical conditions may play a prominent role in the higher prevalence of *Yersinia*. In another study conducted in China (2019) from July 2011 to May 2014, a total of 2363 food samples were collected from 24 cities, and the prevalence of *Yersinia enterocolitica* was reported to be 58%, which is higher than the percentage in our study (26).

In a study conducted in Egypt in 2019, among 120 samples of chicken meat, a significant prevalence of 8.15% of Yersinia was reported, which is consistent with other studies indicating a high percentage of *Yersinia* prevalence compared to our study (27). Generally, the variation in the level of contamination of different food substances in different regions of the world can be attributed to various factors, including the type and quantity of samples, study methods, and bacterial isolation methods from food substances, particularly during the enrichment stage, season, year, geographical conditions, and other factors.

On the other hand, due to the decreased sensitivity of bacteria to various types of antibiotics, selecting an appropriate antibiotic for the treatment of challenging infections is difficult. Chloramphenicol is considered a drug of choice for treating gastrointestinal infections. The use of appropriate antibiotics can reduce the duration and severity of the disease, which is why multiple antibiotics have been introduced for controlling diarrhea (28). In this study, the disk diffusion method was used to determine the antibiotic sensitivity of strains to chloramphenicol,

ampicillin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, ceftriaxone, and cefixime. According to the results of this study, the highest antibiotic sensitivity was observed towards chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole, and ceftriaxone (100%), while the highest resistance was observed towards ampicillin, tetracycline, and cefixime (100%).

In this regard, Zixin Peng and colleagues reported in 2018 that 8.4% of chicken meat samples in China were found to be contaminated with Ampicillin-resistant *Yersinia enterocolitica*. However, 80% of these strains were sensitive to Gentamicin and 91% were sensitive to trimethoprim sulfamethoxazole (26). Furthermore, in another study conducted in Italy in 2010 by Bonardi and colleagues, *Yersinia enterocolitica* was identified as one of the important causative agents of gastroenteritis. This study indicated that the contamination of chicken meat with this organism was 5.32%. All strains were sensitive to ciprofloxacin, chloramphenicol, nalidixic acid, trimethoprim-sulfamethoxazole, tetracycline, and gentamicin (27). In another study conducted in Iran by Soltan Dallal et al. (2011), the prevalence of *Yersinia* in beef and chicken meat was reported to be 16%, with 98% resistance to cefalotin, a first-generation cephalosporin antibiotic, and 52% resistance to ampicillin (28). By comparing the results of previous studies with the present study, a similar antibiotic pattern was observed in this study. Therefore, gentamicin and trimethoprim-sulfamethoxazole antibiotics are likely to be suitable treatment options for *Yersinia* infections in Tehran. However, it should be noted that the emergence of multidrug-resistant strains, due to the indiscriminate use of antibiotics in poultry farms, can potentially be perceived as a threat to animals and consequently to humans (29, 30).

In the research conducted by Zahran et al., it is evident that the prevalence, virulence characteristics, and antibiotic resistance of Yersinia spp., particularly *Y. enterocolitica*, in poultry meat products have been identified as significant factors concerning potential foodborne pathogens. This study is noteworthy for its comprehensive reporting on these aspects. The findings revealed that all strains of *Y. enterocolitica* examined belonged to biotype 1A, which is generally considered non-pathogenic to humans. Nevertheless, these strains may act as opportunistic pathogens, potentially contributing to the spread of other intestinal diseases and causing diarrhea. Therefore, the presence of virulence-associated genes in *Y. enterocolitica* biotype 1 strains found in meat products raises concerns regarding their potential harmful effects (31).

In general, the prevalence of *Yersinia* in meat products in the country is decreasing compared to previous years. Considering the above explanations and the fact that *Yersinia enterocolitica* has not been previously considered in assessing the sanitary quality of food in Iran and recently the investigation of contamination with this bacterium in various food products has begun, the lack of information on the prevalence and distribution of *Yersinia* in various food products, especially raw animal-derived products, can be helpful and effective in controlling and preventing sudden outbreaks of this bacterium.

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Authors' Contribution

Conception and design: MMSD. Methodology: MMSD, MRM, ZR. Investigation: MRM, ZR. Acquisition and analysis of data: MMSD, MRM, ZR. Writing the original draft: MMSD, MRM. Critical revision of the manuscript for important intellectual content: MMSD

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Ethics approval statement

This study has the ethics code IR.TUMS.SPH.REC.1399.046.

Consent for publication

The authors declare that they consent for publication of this study.

Data availability statement

Data that support the findings of this study are available in the manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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