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Research Paper

Identification of *Yersinia* Species in Raw Chicken Meat in Tehran Retail Stores and Determination of Their Antibiotic Resistance Pattern

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ABSTRACT

Introduction: *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 antibiotic resistance patterns.

Materials & Methods: Between April and September 2023, a total of 220 chicken meat samples were systematically collected and analyzed for contamination by *Yersinia* species. Initial isolation was conducted through enrichment in saline phosphate at 4 °C for three weeks, followed by secondary enrichment utilizing 5.0% potassium hydroxide. The resultant samples were subsequently cultured on CIN agar medium. Following performing 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.

Results: From 220 chicken meat samples, 12(5.5%) suspect strains of *Yersinia* were successfully isolated and definitively identified as *Yersinia* through biochemical testing. Application of the API 20E kit revealed that the isolates comprised the following species: *Y. enterocolitica* (3 strains), *Y. intermedia* (5 strains), *Y. frederiksenii* (2 strains), and *Y. kristensenii* (2 strains). Notably, all isolated strains exhibited resistance to ampicillin, tetracycline, and cefixime, while remaining sensitive to other antibiotics tested.

Conclusion: The results of this study indicate the presence of various strains of *Yersinia* in chicken meat samples across Tehran. Given the emergence of microbial resistance to specific antibiotics, it is imperative that antibiotic usage be managed with judicious strategies.

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1. Introduction

Yersinia enterocolitica is a gram-negative, coccobacillus-shaped bacterium that is transmitted through the consumption of contaminated food and water. It is responsible for various conditions in humans, including gastrointestinal diseases, mesenteric lymphadenitis, and erythema nodosum [1-3]. This bacterium is psychrotrophic and is capable of growth at temperatures ranging from 2-45 ˚C. As a result, *Y. enterocolitica* can survive and reproduce in refrigerated environments, posing a significant threat to the safety of stored food products [4, 5]. The most important food items contaminated with *Y. 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 [3-9].

Globally, millions of people, especially in developing countries, are affected by this disease and even lose their lives. After Campylobacteriosis and Salmonellosis, Yersiniosis ranks third among significant bacterial zoonosis in the European Union and is a causative agent of diarrhea [10]. *Y. enterocolitica* causes approximately 117,000 infections, 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 [13-15]. 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 *Y. 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, *Y. enterocolitica* strains are divided into two groups: 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 [16-18]. Additionally, *Y. pseudotuberculosis* has 6 serotypes, and *Y. 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 *Y. enterocolitica* and the presence of antibiotic-resistant strains, implementing hygiene measures to reduce the contamination of chicken meat with *Y. enterocolitica*, along with the proper use of antibiotics in the poultry industry, is 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 patterns.

2. Materials and Methods

2.1. Sampling

In this study, a total of 220 samples of chicken meat were collected from April to September 2023 from chicken retailers in Tehran to isolate *Y. enterocolitica* and other atypical *Yersinia* species. The samples examined included 55 cases of wings and necks, 55 cases of hearts and livers, 55 cases of chicken legs, and 55 cases of chicken breast

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 5-gram quantity of chicken meat was finely sliced with a sterile scalpel into 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 enriched suspension was thoroughly mixed with 9 milliliters of 0.5% potassium using an electric mixer for 30 seconds. A loopful of this mixture was then cultured on CIN agar medium (Cefsulodin-Irgasan-Novobiocin) (Merck, Germany) and incubated at 30 ˚C for 24 hours. After this period, suspicious colonies were examined.

2.3. Identification using phenotypic methods

Suspicious of *Yersinia* colonies observed on CIN medium, characterized by a red center and transparent edges (referred to as bull's eye appearance), 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 ˚C, MR&VP, urease, hydrogen sulfide production, Kliger iron agar, ornithine decarboxylase, citrate, and ONPG tests. Finally, strains exhibiting biochemical characteristics of lactose and negative oxidase

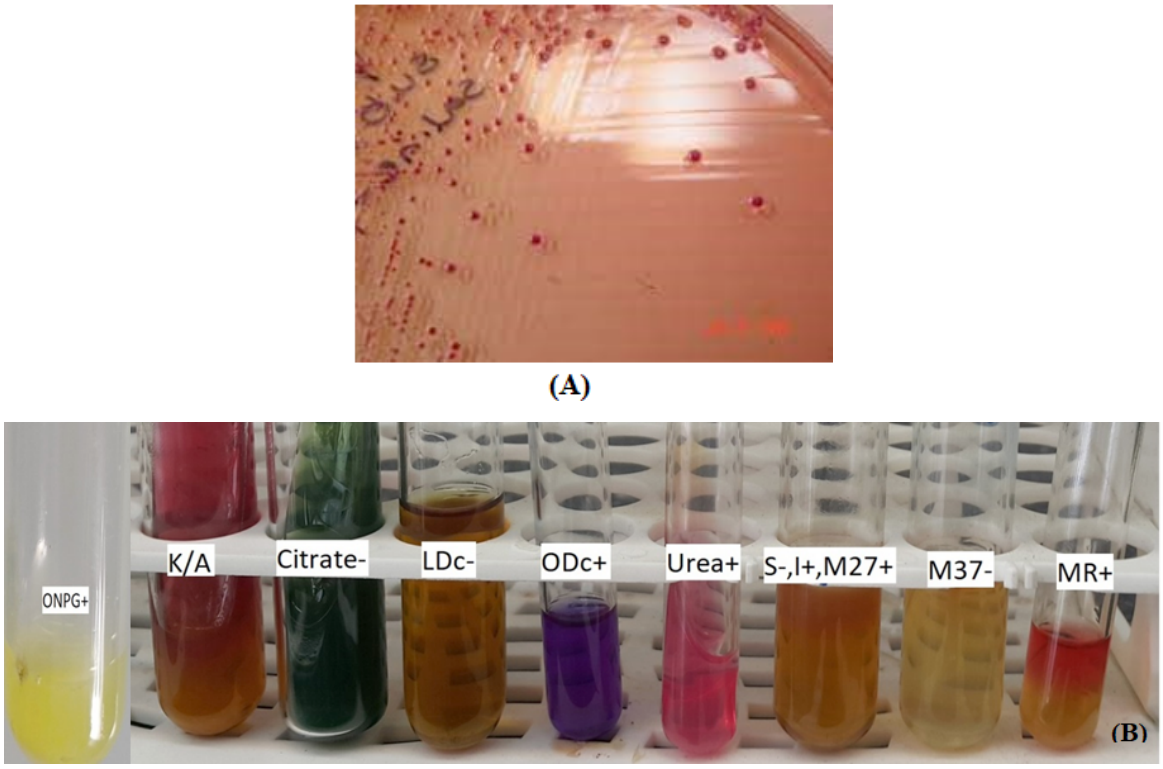

Figure 1. Identification of *Yersinia* spp. from chicken meat

A) Suspicious 'bull's eye' colonies of *Yersinia* on CIN agar; B) Biochemical test results: from left to right – positive urease (urea agar), acid/alkaline reaction (Kligler's iron agar), negative citrate (Simmon's citrate), positive ornithine decarboxylase, positive indole (SIM medium), and positive urease (urea broth)

and indole, urease, ONPG, and ornithine decarboxylase positive, and motility at 25 ˚C 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* through phenotypic methods, antibiotic susceptibility testing was performed using the disk diffusion method [22]. Initially, a pure colony from a fresh culture was added to a Mueller-Hinton broth (Charlo, Spain) to achieve a turbidity equivalent to the 0.5 McFarland standard. A microbial suspension obtained with sterile water was cultured in Muller-Hinton agar medium (Charlo, Spain). Commercial antibiotic disks, including chloramphenicol (30 µg), ampicillin (10 µg), tetracycline (5 µg), ceftriaxone (30 µg), trimethoprim-sulfamethoxazole (25 µg), gentamicin (10 µg), and cefixime (30 µg) (MAST, England), were placed on the plates. Microbial resistance was determined after 24-18 hours based on the standard method provided by the Clinical and Laboratory Standards Institute (CLSI) 2018 [23].

3. Results

3.1. Results of identification of separated strains' identity

Among the 220 collected and cultured chicken meat samples, 12 samples (5.5%) exhibited 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. The biochemical test results for suspicious *Y. enterocolitica* strains were performed using API 20E kits (Figure 2). Out of the 12 identified strains as *Yersinia*, 3 belonged to the species *Enteroclitica*, 5 to *Intermedia*, 2 to *Fredericksenii*, and 2 to *Kristensenii*.

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

The antibiotic susceptibility 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, all species demonstrated 100% sensitivity to the antibiotics gentamicin,

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

cefoxitin, trimethoprim-sulfamethoxazole, and chloramphenicol, while the highest level of resistance (100%) in all species was observed for the antibiotics ampicillin, tetracycline, and cefixime.

4. Discussion

Foodborne diseases are considered a major challenge for the development of the food industry and a significant concern for countries worldwide. The most important bacterial agents involved in these diseases include *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *Y. enterocolitica*. 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 patterns 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 breasts. In total, 12 samples (5.5%) were 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 Soltan Dallal et al. (2016) [7] and Momtaz et al. (2013) [24], which aimed to determine the prevalence of *Yersinia* species in chicken meat samples collected from retail centers in western regions of Iran, reported the prevalence of *Y. enterocolitica* as 15.5% and 18.33%, respectively, and the prevalence of *Yersinia intermedia* as 7%. These figures are higher than those reported in our study. This issue may be due to the differences in the study populations [7, 24]. The study by Soltan Dallal et al., which 450 collected samples (226 chicken meat and 224 beef samples), was conducted in supermarkets across Tehran [7]. Additionally, Momtaz et al. conducted their study using 720 samples [24]. Furthermore, in our previous study in 2003, 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 *Y. enterocolitica* and 47 strains (30.3%) were *Y. 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, and improved packaging of meat and poultry. In other countries, numerous studies have been conducted on the identification and isolation of *Y. enterocolitica* and *Y. intermedia*; comparing the results with the present study can help in understanding the epidemiology of *Yersinia*. For example, Wang et al. (2021) investigated the prevalence of *Y. enterocolitica* in food samples in China. Their study

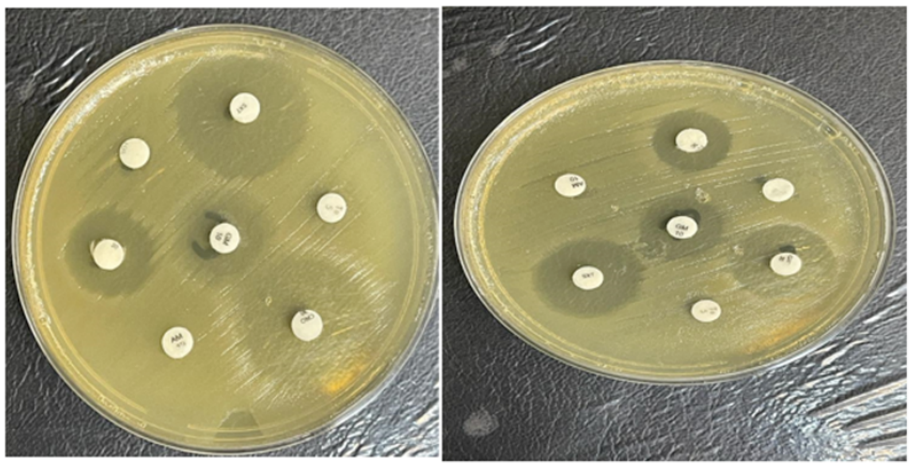

Figure 3. Antibiotic sensitivity testing of *Y. entermedia* and *Y. enterocolytica* species (resistant to ampicillin, tetracycline, and cefixime; sensitive to other antibiotics used)

Table 1. Sensitivity of *Yersinia* strains to antibiotics

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

focused 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%) were 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 meat [25]. This study differs in 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 *Y. enterocolitica* was reported to be 58%, which is significantly higher than the percentage in our study [26].

In a study conducted in Egypt in 2019, a significant prevalence of 8.15% of *Yersinia* was reported among 120 chicken meat samples, which is consistent with other studies indicating a high percentage of *Yersinia* prevalence compared to our study [27]. Generally, variations in 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 methodologies, bacterial isolation methods from food substances(particularly during the enrichment stage), season, year,and geographical conditions. On the other hand, the decreasing sensitivity of bacteria to various types of antibiotics has made selecting appropriate treatments for challenging infections difficult. Chloramphenicol is considered a drug for treating gastrointestinal infections. Using appropriate antibiotics can reduce the duration and severity of the disease, which is why multiple antibiotics have been introduced for controlling diarrhea [24]. 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 our results, the highest antibiotic sensitivity was observed towards chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole, and ceftriaxone (100%), while the highest resistance was observed toward ampicillin, tetracycline, and cefixime (100%).

In this regard, Peng et al.(2018) that 8.4% of chicken meat samples in China were contaminated with ampicillin-resistant *Y. enterocolitic*a. 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 et al. identified *Y. enterocolitica* as one of the important causative agents of gastroenteritis, with a contamination rate of 5.32% in chicken meat. All strains were sensitive to ciprofloxacin, chloramphenicol, nalidixic acid, trimethoprim-sulfamethoxazole, tetracycline, and gentamicin [28]. In another study conducted in Iran, Soltan Dallal et al. (2010) reported a 16% prevalence of *Yersinia* in beef and chicken meat with 98% resistance to cefalotin (a first-generation cephalosporin), and 52% resistance to ampicillin [29]. 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 are likely to be suitable treatment options for *Yersinia* infections in Tehran. However, it should be noted that the emergence of multidrug-resistant strains, driven by the indiscriminate use of antibiotics in poultry farms, poses a significant threat to both animal and human health [25, 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 *Y. enterocolitica* has not been previously considered in assessing the sanitary quality of food in Iran, and the investigation of contamination with this bacterium in various food products has only recently 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.

5. Conclusion

This study confirms the presence of multiple *Yersinia* species, including *Y. enterocolitica*, *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii*, in raw chicken meat sold in Tehran retail stores. The overall contamination rate (5.5%) is lower than previously reported in Iran, suggesting possible improvements in hygiene practices. However, the high level of resistance to ampicillin, tetracycline, and cefixime among all isolates is concerning. Judicious use of antibiotics in poultry production and continuous surveillance are essential to prevent the spread of resistant strains through the food chain.

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Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (Code: IR.TUMS.SPH.REC.1399.046).

Data availability

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

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Authors' contributions

Conceptualization, study design, review and editing: Mohammad Reza Mohammadi; Methodology, data acquisition and analysis: All authors; Investigation: Mohammad Reza Mohammadi and Zahra Rajabi; Writing the original draft: Mohammad Reza Mohammadi and Mohammad mehdi Soltan dalla.

Conflict of interest

The authors declared no conflict of interest.

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