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Investigation of the Prevalence of *Yersinia enterocolitica* Contamination in Beef, Sheep, and Poultry Meat

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Abstract

Background:*Yersinia enterocolitica* is a zoonotic pathogen that causes foodborne gastroenteritis and is commonly transmitted through contaminated meat. Its ability to survive at low temperatures and develop antibiotic resistance makes its presence in food products a significant public health risk. Its widespread occurrence necessitates stringent food safety measures and further investigation of contamination sources and antibiotic resistance patterns.

Objectives: This study aimed to investigate *Y. enterocolitica* contamination in beef, sheep, and poultry meat, determine its biotypes and serotypes, and assess its antibiotic resistance patterns.

Methods: A total of 350 meat samples, comprising 100 beef, 100 sheep meat, and 150 poultry samples, were collected and analyzed using microbiological, biochemical, and bioserotyping methods. Antibiotic susceptibility testing was conducted to assess resistance patterns. The study also explored the role of environmental factors in *Y. enterocolitica* contamination and assessed its survival under different storage conditions.

Results: The overall contamination rate of *Y. enterocolitica* was 16.9%, with the highest prevalence in sheep meat (28%), followed by poultry (14%) and beef (10%). Bioserotyping identified three biotypes (1A, 1B, and 2), of which biotype 1A was the most prevalent (73.1%). The predominant serotype was O:5, with some isolates classified as O:3 or non-typeable. Antibiotic susceptibility testing revealed high resistance to ampicillin (84.6%) and cephalexin (76.9%), whereas 88.5% and 92.3% of isolates were susceptible to ciprofloxacin and colistin, respectively. Additional tests demonstrated increased bacterial survival at low temperatures and persistence in contaminated meat for extended periods, even under refrigeration.

Conclusions: The substantial contamination of meat products, particularly sheep meat, with *Y. enterocolitica* highlights the potential risk of foodborne infections. Future research should focus on molecular characterization and intervention strategies to control *Y. enterocolitica* contamination in the food supply chain.

Keywords: *Yersinia enterocolitica*, Food Contamination, Meat, Drug Resistance, Bacterial, Food Safety

1. Background

Yersinia enterocolitica is a gram-negative bacterium belonging to the family Yersiniaceae that causes foodborne gastroenteritis in humans (1). It was the fourth most commonly reported foodborne zoonosis in the European Union in 2022 (2). This zoonotic pathogen can infect humans through contaminated beef, sheep meat, and poultry (3). It is a rod-shaped bacillus that is motile at 22 - 29 °C (4) but can grow at 2 - 4 °C and therefore contaminate refrigerated foods (5). *Yersinia enterocolitica* can contaminate meat products without causing obvious changes in taste, smell, or appearance, making contaminated food difficult to identify (6). Microbial control in meat is essential to prevent transmission. Some reports have suggested that cooking meat at 71 °C can reduce the risk of *Y. enterocolitica* (7), whereas others have suggested that essential oils, such as cinnamon, clove, oregano, rosemary, thyme, and winter savory, play a

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significant role in controlling this bacterium during cooking (8).

Yersinia enterocolitica has a bipartite life cycle comprising free-living and host-dependent phases. During the free-living phase, it can survive for extended periods in both dry and aquatic environments at 28 °C (9). Within a susceptible host, it can survive at temperatures above 35 °C (10). It invades the intestinal tract and produces endotoxin that causes disease (11). It can cause acute yersiniosis and pseudoappendicitis in humans and animals. Symptoms of acute yersiniosis include diarrhea, abdominal pain, nausea, vomiting, and fever. Pseudoappendicitis can mimic appendicitis, with abdominal pain, fever, vomiting, an elevated white blood cell count, and diarrhea (12). Humans can become infected by consuming contaminated beef, sheep meat, and poultry. Direct contamination can also occur through blood, feces, and saliva (11).

Previous studies have reported varying prevalence rates of *Y. enterocolitica* in meat products according to geographical region, animal species, sampling methods, and detection techniques. These variations indicate considerable differences among countries and production systems. However, updated information on the prevalence, bioserotypes, antimicrobial resistance patterns, and survival characteristics of *Y. enterocolitica* in meat products remains limited in many regions. Continuous surveillance is therefore necessary to evaluate contamination and support food safety monitoring and public health protection.

2. Objectives

This study aimed to investigate the prevalence of *Y. enterocolitica* in beef, sheep meat, and poultry samples, determine the biotypes and serotypes of the isolates, and evaluate their antimicrobial susceptibility.

3. Methods

This study was conducted at the Food Quality Control Laboratory in Ardabil, Iran. A total of 350 meat samples, comprising 100 beef, 100 sheep meat, and 150 poultry samples, were purchased in Ardabil, collected under sterile conditions, and transferred to the laboratory. The samples were stored for 21 days at 4 °C, inoculated onto a selective culture medium for *Y. enterocolitica* (cefsulodin-irgasan-novobiocin (CIN) agar), and subjected to differential microbiological and biochemical tests.

3.1. Sample Collection

A total of 350 meat samples were collected, including 100 beef samples, 100 sheep meat samples, and 150 poultry samples. Sampling was conducted over six months to capture seasonal variations in contamination. Samples were obtained from slaughterhouses, local butchers, supermarkets, and chicken processing plants to represent diverse handling and storage conditions. Collection followed ISO 17604:2015 standards for the microbiological examination of meat samples, using a standardized procedure to maintain sample integrity. Each sample was placed in a sterile bag and transported to the laboratory at 4 °C in an insulated cooler with ice packs. To prevent cross-contamination, sterile equipment was used for each sample, and gloves were changed between collections. Before analysis, samples were labeled with their source, collection date, and storage conditions (13).

3.2. Sample Preparation, Processing, and Strain Isolation

Individual samples were trimmed to remove surface contamination and then homogenized in buffered peptone water using a Stomacher Lab Blender for 2 minutes to distribute the bacteria uniformly. The homogenized samples were incubated at 25 °C for 24 hours in peptone sorbitol bile broth for selective enrichment of *Y. enterocolitica*. After enrichment, 100 µL of each culture was streaked onto selective media, including CIN agar and MacConkey agar, and incubated at 30 °C for 24 - 48 hours to allow colonies to develop. Suspected *Y. enterocolitica* colonies, identified by their red bullseye appearance on CIN agar, were selected for further confirmation. Colonies were purified by subculturing on tryptic soy agar and incubating at 30 °C to obtain pure bacterial isolates for biochemical examination (14).

3.3. Identification and Characterization of Yersinia enterocolitica

Colonies were subjected to biochemical tests, including lysine decarboxylase, ornithine decarboxylase, citrate utilization, urease activity, and sugar fermentation tests for glucose, mannitol, rhamnose, and sucrose, to confirm *Y. enterocolitica* (15). Bioserotyping was performed using antisera targeting specific surface antigens to determine serogroups and virulence factors. Suspected colonies were separated and exposed to antisera to allow antibody binding. Agglutination was observed, and the serotype was determined according to the antiserum that caused the colonies to agglutinate (16).

3.4. Antibiotic Susceptibility, Minimum Inhibitory Concentration, and Minimum Bactericidal Concentration Testing

Antimicrobial susceptibility testing was performed for all 13 antibiotics using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (17, 18). Resistance patterns were recorded and categorized as susceptible, intermediate, or resistant according to Bryan Coburn et al. (19). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) testing was performed only for ciprofloxacin and colistin, the two antibiotics with the highest susceptibility rates. Antibiotic susceptibility testing was used to determine bacterial susceptibility to individual antibiotics. The sample was cultured to grow bacteria, viruses, or fungi, which were then tested against antibiotics. Results were interpreted as susceptible, intermediate, or resistant (20).

Minimum bactericidal concentration testing identifies the lowest antibiotic concentration required to eliminate at least 99.9% of bacterial cells and differentiates bactericidal from bacteriostatic agents. The procedure involves dilution on antibiotic-free agar using clear zones from MIC tests. Plates were incubated at 35 - 37 °C for 24 hours, and the lowest concentration with no visible colonies was recorded as the MBC (21).

3.5. Statistical Analysis

Data were analyzed using SPSS version 20.0 and Microsoft Excel. A chi-square test was used to compare *Y. enterocolitica* contamination among beef, sheep meat, and poultry samples. A $P < 0.05$ was considered statistically significant at a 95% confidence level.

4. Results

A total of 350 beef, sheep meat, and poultry samples were examined for *Y. enterocolitica*. Sheep meat had the highest prevalence (28%), followed by poultry (14%) and beef (10%). Overall, 59 of 350 samples (16.9%) were positive. Statistical analysis indicated significant differences among some meat categories (Table 1).

The antimicrobial susceptibility of *Y. enterocolitica* isolates was evaluated using MIC and MBC analyses. Ciprofloxacin and colistin showed high susceptibility rates and very low MBC values, indicating strong antibacterial activity against the isolates (Table 2).

Biotyping analysis was performed to determine the distribution of *Y. enterocolitica* isolates among biotypes. Twenty-six isolates selected from the 59 positive samples were classified into three biotypes (1A, 1B, and 2). Biotype 1A predominated, followed by biotypes 1B and 2 (Table 3).

Serotyping analysis was performed to determine the distribution of *Y. enterocolitica* isolates among serotypes. Isolates were classified as non-typeable, O:5, or O:3, with non-typeable and O:5 being the most frequently detected serotypes (Table 4).

The antibiotic susceptibility profiles of *Y. enterocolitica* isolates were evaluated against commonly used antimicrobial agents. Resistance patterns varied, with high resistance to ampicillin and cephalexin, whereas ciprofloxacin and colistin had the highest susceptibility rates (Table 5).

5. Discussion

This study demonstrated the presence of *Y. enterocolitica* in beef, sheep meat, and poultry samples, confirming that meat products can serve as important reservoirs of this foodborne pathogen. Similar findings have been reported, although prevalence varies by geographical location, animal species, sampling methods, and detection techniques. Zexun Lü et al. (22) reported contamination rates of 25% in frozen meat, 21.65% in packaged meat, and 17.23% in supermarket meat. Studies from different countries have also demonstrated the presence of *Y. enterocolitica* in beef, sheep meat, and poultry, supporting the present findings (23-25).

Sheep meat had the highest *Y. enterocolitica* contamination rate in this study (28%), followed by poultry (14%) and beef (10%). Previous studies have also reported *Y. enterocolitica* in meat samples, although prevalence varies by geographical location, sampling strategy, and detection methods (26). A Sri Lankan study reported *Salmonella contamination* in 30.89% of poultry meat samples and on less than 25% of meat-contact surfaces, providing a comparison with the present findings (18). Studies in poultry slaughterhouses have also demonstrated the importance of monitoring bacterial contamination during processing and handling (27).

Regarding temperature-related survival, this study showed that *Y. enterocolitica* contamination was highest at room temperature (78.4%). Although the contamination rate was lower under frozen conditions (12.7%), the organism remained viable; the contamination rate under refrigerated conditions was 45.2%. A previous study reported contamination rates of 12% in refrigerators and 10% at room temperature (28). Another study

Table 1. Prevalence of *Yersinia enterocolitica* in Beef, Sheep Meat, and Poultry Samples

Sample	Number Tested	Number Positive	Positive (%)	Chi-square	P Value
Beef	100	10	10	6.215	0.051
Sheep meat	100	28	28	3.195	0.022
Poultry	150	21	14	4.144	0.031
Total	350	59	16.9		

Table 2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Findings for *Yersinia enterocolitica* [a]

Antibiotic	MIC interpretation (%)	MBC interpretation
Ciprofloxacin	88.5% susceptible	Very low MBC (highly effective)
Colistin	92.3% susceptible	Very low MBC (highly effective)

[a] Abbreviations: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

Table 3. Biotyping Analysis of *Yersinia enterocolitica* Isolates

Biotype	Number of Isolates (n = 26)
1A	19
1B	4
2	3

demonstrated that *Y. enterocolitica* remained viable in beef gravy for up to 18.52 minutes at 50 °C (29). These findings indicate that the organism can survive under different storage conditions and emphasize the importance of temperature control during meat storage and handling.

Three biotypes of *Y. enterocolitica* (1A, 1B, and 2) were identified by bioserotyping, with biotype 1A accounting for 73.1% of the isolates. Previous studies have similarly reported the predominance of biotype 1A and suggested that these strains produce the pore-forming toxins YaxA and YaxB, although they are generally considered nonpathogenic (30). Serotype O:5 was the predominant serotype in this study. O serogroups play an important role in the pathogenicity of *Y. enterocolitica* (31), and serotype O:5 has also been reported in raw chicken samples (32). In addition, serotypes O:3, O:9, O:8, O:5, and O:7 have been reported among the most common serotypes associated with human gastrointestinal infections. Detection of serotype O:3 in this study further supports previous findings and highlights the diversity of *Y. enterocolitica* serotypes circulating in meat products.

Because of its β-lactamase activity, *Y. enterocolitica* showed high resistance to ampicillin (84.6%) and cephalexin (76.9%). Considerable resistance was also observed to sulfamethoxazole-trimethoprim (57.7%) and trimethoprim (50%). Previous studies have reported resistance to ampicillin, cephalothin, amoxicillin, and sulfamethoxazole (33, 34). In contrast, colistin (92.3%) and ciprofloxacin (88.5%) showed high susceptibility rates, indicating their therapeutic effectiveness against *Y. enterocolitica*. Previous studies have also reported susceptibility to colistin, gentamicin, kanamycin, neomycin, doxycycline, piperacillin, trimethoprim, sulfamethoxazole, cotrimoxazole, tetracycline, chloramphenicol, cefamandole, cefotaxime, aztreonam, and other aminoglycosides, consistent with our findings (22, 35). Recent research has investigated bacteriophages as potential biocontrol agents for bacterial contamination in meat, highlighting alternative approaches to controlling foodborne pathogens in meat products (36).

This study provides comprehensive data on the prevalence, biotypes, serotypes, antimicrobial susceptibility, and survival characteristics of *Y. enterocolitica* in beef, sheep meat, and poultry collected in the study area, thereby providing updated information for food safety surveillance. A major strength of this study is the evaluation of multiple microbiological characteristics in a

Table 4. Serotyping Analysis of *Yersinia enterocolitica* Isolates [a]

Biotype and Serotype	Number of Isolates Per Serotype	Total Per Biotype
Biotype 1A		19
NT	9	
O:5	9	
O:3	1	
Biotype 1B		4
O:5	2	
O:3	2	
Biotype 2		3
O:5	2	
O:3	1	

[a] Abbreviation: NT, non-typeable.

Table 5. Antibiotic Susceptibility of *Yersinia enterocolitica* Isolates [a]

Antibiotic	Resistant	Intermediate	Susceptible
Ampicillin	22 (84.6)	2 (7.7)	2 (7.7)
Tetracycline	11 (42.3)	7 (26.9)	8 (30.8)
Gentamicin	4 (15.4)	3 (11.5)	19 (73.1)
Ciprofloxacin	1 (3.8)	2 (7.7)	23 (88.5)
Sulfamethoxazole-trimethoprim	15 (57.7)	5 (19.2)	6 (23.1)
Chloramphenicol	2 (7.7)	3 (11.5)	21 (80.8)
Cephalexin	20 (76.9)	4 (15.4)	2 (7.7)
Aztreonam	3 (11.5)	5 (19.2)	18 (69.2)
Cefotaxime	9 (34.6)	7 (26.9)	10 (38.5)
Nalidixic acid	5 (19.2)	10 (38.5)	11 (42.3)
Colistin	1 (3.8)	1 (3.8)	24 (92.3)
Erythromycin	8 (30.8)	6 (23.1)	12 (46.1)
Trimethoprim	13 (50.0)	6 (23.1)	7 (26.9)

[a] Values are expressed as No. (%).

relatively large number of samples collected from different sources.

5.1. Study Limitations

Identification of the isolates relied primarily on conventional microbiological and biochemical methods without molecular confirmation, and sampling was restricted to a single geographical region. Future studies incorporating molecular techniques and multicenter sampling are recommended to provide a more comprehensive understanding of *Y. enterocolitica* epidemiology.

5.2. Conclusions

This study demonstrated *Y. enterocolitica* contamination in beef, sheep meat, and poultry samples, highlighting its potential public health significance and the need for continuous food safety surveillance. The predominance of specific biotypes and serotypes, together with the observed antimicrobial resistance patterns, emphasizes the importance of routine monitoring and appropriate control strategies to reduce contamination and limit the spread of resistant strains. Survival under refrigerated conditions underscores the need for strict hygiene during meat processing, storage, and distribution. These findings provide information to support improvements in food safety measures and the implementation of monitoring programs to protect public health.

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this article.

Authors' Contribution: The author conducted all aspects of the study.

Conflict of Interests Statement: The authors declare no conflicts of interest related to this study.

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