Research Article

Isolation and Identification of *Yersinia enterocolitica* from Yamuna River Water and Analyzing the Bacterium at Various Abiotic Stress Levels Further Performing Antibiotic Susceptibility

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Received: 09-01-2025; **Revised:** 30-03-2025; **Accepted:** 05-04-2025; **Published online:** 20-04-2025.

ABSTRACT

Yersinia enterocolitica is a gram-negative, anaerobic, motile bacterium capable of thriving in diverse environmental conditions. This study investigates its growth characteristics, antimicrobial susceptibility, and response to bio-preservatives to assess its potential as a foodborne pathogen. The bacterium demonstrated the ability to grow across a broad pH range (3 to 13) and temperatures from 15°C to 55°C. It exhibited significant proliferation on selective and differential culture media, including SS agar, EMB, NAM agar, and MacConkey agar. Antimicrobial susceptibility tests indicated a higher inhibition zone for cefixime (1.7 cm) compared to ciprofloxacin (1.5 cm), while erythromycin showed no inhibitory effect. Additionally, growth response to preservatives such as citric, acetic, and lactic acids revealed that *Y. enterocolitica* exhibited the fastest growth in acetic acid, followed by lactic acid and citric acid. These findings underscore the bacterium's adaptability, antimicrobial resistance, and persistence in food products, emphasizing the need for stringent food safety measures.

Keywords: *Y. enterocolitica*, Foodborne Pathogen, Antimicrobial Susceptibility, Bio-preservatives, Public Health, Food Safety.

INTRODUCTION

Y. enterocolitica, Y. pestis, and Y. pseudotuberculosis are three of the 11 species in the *Yersinia* genus that are particularly well known for their capacity to infect humans with diseases[1]. Yersinioses are zoonotic infections, with humans serving only as incidental hosts and not actively supporting the pathogen's life cycle.

Most often, an infection spreads via the faecal-oral pathway. Consuming pork, especially raw or undercooked pork products, is what causes yersiniosis[2]. Additionally, outbreaks caused by this bacterium in untreated drinking water have been observed in Norway and New Zealand. There are case reports of infections spreading through transfused blood products and from a domestic pet that is affected[3]. When *Yersinia* invades epithelial cells and breaks through the mucosa, lymphoid tissue (Peyer patches) gets colonized. The organism may then invade further organs from this point[4].

In a herd of pigs, the infection can spread from one pig to another. The insect can spread to other meat components while the pig is being slaughtered and can contaminate items like tongue, tonsils, and neck trimmings[5]. Animal excrement is the source of its entry into soil and water systems; it has also been isolated from the soil and the top layers of many different bodies of water, including lakes and streams[6]. Yersiniosis, the illness that causes acute gastroenteritis, is communicated to people by water or food. This infection results in diarrhoea and gastrointestinal (GI) issues like fever. Very rarely, yersiniosis can lead to further medical problems like a rash and joint pain[7].

Yersiniosis can affect anyone. However, youngsters experience it more frequently than adults[8]. Enterocolitis, pseudo appendicitis, reactive arthritis, sepsis, pharyngitis, myocarditis, mesenteric adenitis, and dermatitis are all possible symptoms of *Yersinia* infections. Two clinical manifestations of the infection are possible:

Yersiniosis acute

The symptoms of this illness include diarrhoea, stomach pain, nausea, vomiting, and fever. Diarrhoea anywhere between 12 and 22 days. Given the comparable appearance to other causes of severe diarrhoea, yersiniosis is challenging to distinguish from them. The location of the pain in the lower right quadrant may be a yersiniosis diagnostic indication. The occurrence of bloody diarrhoea is more common. Infants, patients with impaired immune systems, and people with iron excess have all been classified as having sepsis, which has a fatality rate of 50% overall. After an acute illness, germs may remain excreted in the stool for a median of 40 days (with a range of 17 to 116 days)[9].

Pseudo appendicitis

In addition to right lower quadrant stomach discomfort, fever, vomiting, an increased white blood cell count, and diarrhoea, acute yersiniosis might mimic appendicitis. The mesenteric lymph node and terminal ileum of patients who are taken for surgery are inflamed, but the appendix is healthy. The most frequent victims of pseudo appendicitis are small children, who frequently require an appendectomy[10].

Reactive arthritis, which typically affects several joints, can also develop following *Yersinia.* Usually, the major joints are affected, and the symptoms can persist for up to 120 days. The symptoms of the joints typically manifest 7–14 days

after those of the gastrointestinal system. Erythema nodosum lesions that emerge 2–14 days following gastrointestinal pain are another symptom of *Yersinia*. Adult females are more likely to get the lesions, which typically go away on their own[11].

About 117,000 persons in the US are affected by yersiniosis each year. The intake of chocolate milk contaminated with *Y. enterocolitis* in 1976 was blamed for the first Yersiniosis outbreak in New York. Other regions of the United States, Europe, Australia, Sweden, and India have also recorded epidemics of Yersiniosis that is milk-borne. Following this, other Yersiniosis outbreaks brought on by consuming tainted pig products were recorded in Hungary, the United States, Norway, China, and other European nations[12].

Yersiniosis has been recorded on numerous occasions during the past few years, although in the majority of these instances, the source of the illness is still unknown[12]. The danger of Yersiniosis has increased as a result of the most recent trend of increased processed food intake[13]. Other *Yersinia* organisms, such as *Y. intermedia, Y. frederiksenii, and Y. ruckeri*, have been discovered. Enteric red mouth disease, which is caused by *Y. ruckeri* in these species, is characterized by haemorrhaging of the subcutaneous tissues beneath the fins as well as around the eyes and mouth[14].

The purpose of this study was to examine the isolation and identification of bacteria from Yamuna River water, as well as their pathogenicity about bio preservatives that may be used in food products, as well as their susceptibility to antibiotics. It also included observation of the bacteria under various abiotic stress conditions, including those involving temperature and various pH levels.

MATERIALS AND METHODS

The Yamuna River water sample was collected from the Delhi industrial area and used for further investigations. To obtain the number of colonies that can be counted, dilute the sample significantly. Put a few diluted samples on the appropriate media and let them grow there. By using serial dilution technique, the concentration of an organism is gradually lowered by resuspended in predetermined amounts of liquid diluent usually a multiple of 10. Take 6 glass test tubes and label them as 10^{-1},10^{-2},10^{-3},10^{-4},10^{-5} and 10^{-6}. Add 9 ml saline water 0.85% NaCl solution in each test tube. Take 1 ml of suspended solution from the beaker and add it to a test tube labelled as 10^{-1}. Resuspend it with the help of a micropipette. Now take 1 ml of solution from the test tube labelled as 10^{-1} add it to the test tube labelled as 10^{-2} and resuspend it again. Repeat the steps till 10^{-6} test tubes are diluted[15].

Pour Plate Method:

For obligate and anaerobic microorganisms, pour plate plating is a typical plating technique. This method is used to isolate microbial colonies by serial dilution and counting the colony-forming units. This technique involves pouring the liquid sample into the petri dish before the agar medium solidifies. After the medium has solidified, colonies appear on the medium's surface as well as inside. On the other hand, the colonies developing within the media are confluent; those on the surface are employed for viable counts. After that NAM (Nutrient Agar Media) spreading, NAM streaking and EMB (Eosin methylene blue) spreading were done. We also observe the growth of *Y. enterocolitica* on different culture media such as MacConkey agar and Salmonella Shigella agar by the Streak Plate method. After that nutrient broth was also prepared for studying the growth of bacteria in broth samples[16].

MORPHOLOGICAL IDENTIFICATION OF BACTERIA

Gram Staining:

Gram-staining is used to differentiate between two groups of bacteria based on the differences in their cell wall contents. By colouring cells violet or red, Gram-staining distinguishes between Gram-positive and Gram-negative groupings[17,18].

Motility test:

Motility is the ability to move through the use of flagella. The flagella, which are thread-like locomotor appendages that extend from the plasma membrane and cell wall, allow motile bacteria to move and non-motile bacteria show Brownian movement. It was done by using the hanging-drop method[19].

BIOCHEMICAL TESTS

Numerous bacterial species have similar traits including size, shape, and other properties, and can only be distinguished by looking for specific traits that can be found via biochemical assays. There was a total of 14 biochemical tests performed for the identification of bacterial species[20,21].

To determine the abiotic stress at different temperatures on *Y. enterocolitica:*

The range of temperatures at which microbes can grow can be used to classify them roughly. At the temperature where the organism will develop the fastest, growth rates are at their highest. *Y. enterocolitica* was grown at different temperatures from low to high (15℃ to 55℃) in nutrient broth inoculated with bacteria and kept in an incubator at 37℃ for 24 hrs and observed the readings by using a spectrophotometer[22].

Screening of *Y. enterocolitica* at various pH levels:

pH is a measurement of how much free hydrogen and hydroxyl ions are present in water. By carefully controlling the passage of cations across the membrane, bacteria primarily manage the cytoplasmic pH. Growth of *Y. enterocolitica* was observed in different nutrient broth media at different pH (pH 3 to pH 13)[23,24].

To determine the effect of preservatives on *Y. enterocolitica:*

Bio preservatives are described as substances derived from natural sources or generated in food that can stop or delay spoiling caused by chemical or biological degradation. For studying the pathogenic nature of *Y. enterocolitica* there were three different bio-preservatives were used Acetic acid, Lactic acid and Citric acid were added to a nutrient broth medium at different concentrations (20µl, 40µl, 80µl, 160µl) and inoculated with a bacterial sample then put in an incubator at 37℃ for 24 hrs. And observe the readings by using a spectrophotometer at 600 nm[25].

Screening of *Y. enterocolitica* growth in the presence of various antibiotics at various concentrations:

Bacterial Sample: Use freshly prepared sample (18-24 hours)

Antibiotics used: Erythromycin, Ciprofloxacin and Cefixime

Erythromycin: The antibiotic class known as macrolide antibiotics includes erythromycin. By decreasing the production of critical proteins required for the survival of the bacterium, macrolide antibiotics inhibit the growth of sensitive bacteria or even cause their death. The antibiotic erythromycin is used to treat or stop a wide range of bacterial illnesses.

Ciprofloxacin: Various bacterial infections can be treated with the fluoroquinolone antibiotic ciprofloxacin. Among other things, this includes infections of the bones and joints, the belly, particular types of infectious diarrhoea, the respiratory and skin tracts, typhoid fever, and urinary tract infections. For some diseases, it is used in combination with other antibiotics.

Cefixime: A multitude of bacterial infections are treated with cefixime, an antibiotic drug. Otitis media, strep throat, pneumonia, urinary tract infections, gonorrhea, and Lyme disease are a few of these infections. Typically, just one dose is needed to treat gonorrhea[26].

CONCENTRATION OF ANTIBIOTICS:

Table 1: Antibiotic concentrations of different antibiotics

Antibiotic	T1 conc.	T2 conc.	T3 conc.	T4 conc.	T5 conc.	T6 conc.	T7 conc.	T8 conc.
Erythromycin	2 mg/ml	1.75 mg/ml	1.5 mg/ml	1.25 mg/ml	1 mg/ml	0.75 mg/ml	0.50 mg/ml	0.25 mg/ml
Cefixime	2 mg/ml	1.75 mg/ml	1.5 mg/ml	1.25 mg/ml	1 mg/ml	0.75 mg/ml	0.50 mg/ml	0.25 mg/ml
Ciprofloxacin	2 mg/ml	1.75 mg/ml	1.5 mg/ml	1.25 mg/ml	1 mg/ml	0.75 mg/ml	0.50 mg/ml	0.25 mg/ml

Technique Used: Well-diffusion method (well diameter used 6 mm)

RESULTS

Table 2: Morphological and Biochemical Identification.

Media used	Form	Surface	Thermogenesis	Gram staining	Shape	Motility Test
EMB	Circular	Smooth	Pink	-ve	Coccus	+ve
NAM	Circular	Smooth	Yellow	-ve	Baccilo-cocci	+ve
MacConkey Agar	Irregular	Smooth	Bright Pink	-ve	Rod	+ve
SS Agar	Irregular	Smooth	Cream	-ve	Rod	+ve

Test	Observations	Results
Dextrose Test	Positive results were obtained as there was a colour change from orange to yellow after 24 hours.	+ve
Sucrose Test	Positive results were obtained as there was a colour change from orange to yellow after 24 hours.	+ve
D-Mannitol Test	Positive results were obtained as there was a colour change from orange to yellow after 24 hours.	+ve
Maltose Test	Positive results were obtained as there was a colour change from orange to yellow after 24 hours.	+ve
Lactose Test	Negative results were obtained as there was no colour changed after 24 hrs .	-ve
Nitrate Reduction Test	Positive results were obtained for the bacteria as there was no colour change occurred with the addition of Zn-dust in the culture	+ve
Indole Test.	Negative results were obtained as there was no cherry-red ring formation on the interface after Kovac's reagent was poured into a test tube	-ve
Citrate Test	Negative results were obtained as there was no blue-coloured slant appeared after 24 hrs of incubation.	-ve

H_2S Test	Negative results were observed for the H_2S test as there was no black colour observed.	-ve
Urease Test	Positive results were obtained for the Urease test because the colour turned pink after 24 hours of incubation.	+ve
Methyl Red Test	The formation of a cherry red ring was observed while testing thus confirming a positive result	+ve
Voges Proskauer Test	Positive results were obtained as there was red colour change after adding alpha-naphthol and the KOH solution prepared.	+ve
Catalase Test	Positive results for bacteria were obtained as there is a visible bubble formation	+ve
Casein Hydrolysis	Negative results were obtained for Casein hydrolysis as there was no zone of inhibition observed	-ve

The biotype was determined using biochemical testing. We performed total 14 biochemical tests to identify the bacteria and then compare the findings with previous published biochemical results for different bacteria, either positive or negative, and shows perfect match with the bacterium *Y. enterocolitica*.

Screening of *Y. enterocolitica* at Various Abiotic Stress Parameters

To determine the abiotic stress at different temperatures of *Y. enterocolitica*:

Table 3: Bacterial growth at different temperatures

Temp.	Absorbance at 600 nm
15	0.035
30	0.03
37	0.068
45	0.271
48	0.412
55	0.237

It was observed from **Table 3** that *Y. enterocolitica* exhibited variable growth at different temperatures i.e., at 15˚C the growth of bacteria is 0.035 in an inoculated broth, at various temperatures the values mentioned in the table above. *Y. enterocolitica* has been observed this bacterium show the highest growth at 48˚C. We also observed that *Y. enterocolitica* shows the least growth at 30˚C.

Screening of *Y. enterocolitica* at various pH levels

Table 4: Bacterial growth at different pH

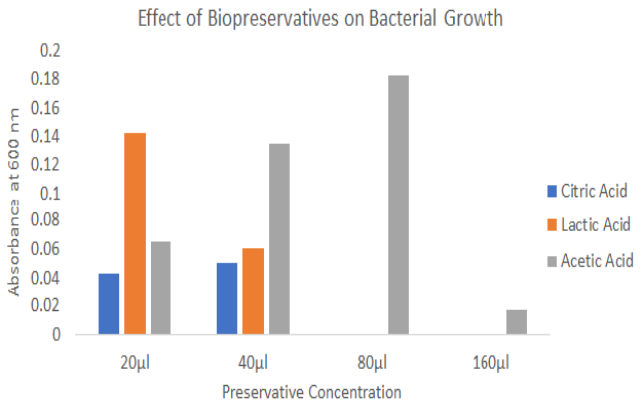
pH	Absorbance at 600 nm
pH 3	0
pH 4	0.235
pH 5	1.823
pH 6	0.423
pH 7	0.261
pH 8	0.263
pH 9	0.211
pH 10	0.032
pH 11	0.007
pH 12	0.058
pH 13	0.113

From **Table 4**, it was apparent that different growth patterns of *Y. enterocolitica* were observed at various pH levels. At pH 3, no growth was observed. However, as the pH increased to 4, the absorbance of the broth was recorded at 0.235. A significant increase in growth was observed at pH 5, with an absorbance value of 1.823. At pH 6, the absorbance decreased to 0.423. For pH levels 7, 8, and 9, the absorbance was approximately half of that at pH 6. Beyond pH 9, the absorbance values declined further, remaining below 0.03 from pH 10 to pH 13, indicating minimal to no bacterial growth in highly alkaline conditions.

To determine the effect of bio preservatives on *Y. enterocolitica*:

Table 5: Comparison of preservatives

Preservatives Used	Absorbance at conc. 20µl	Absorbance at conc. 40µl	Absorbance at conc. 80µl	Absorbance at conc. 160µl
C. acid	0.043	0.05	0	0
L. acid	0.142	0.061	0	0
A. acid	0.065	0.135	0.183	0.017



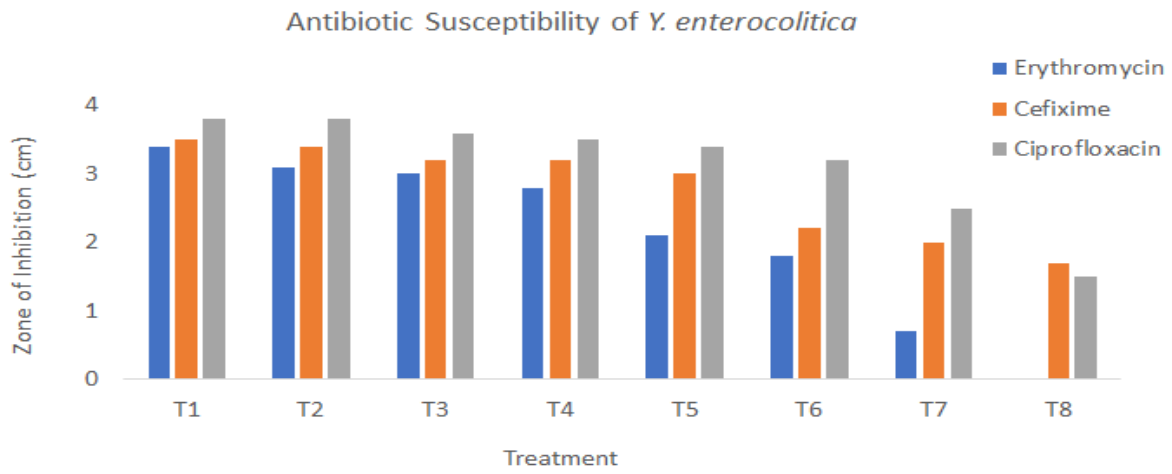
Graph 1: Comparison of preservatives with different concentrations at 600 nm

The data in **Table 5 and Graph 1** illustrate the growth response of *Y. enterocolitica* to varying concentrations of different bio-preservatives, as determined by absorbance measurements at 600 nm. Among the three bio-preservatives used, citric acid showed the least growth. In contrast, lactic acid exhibited significant growth at the lowest concentration (20 µl) but showed a rapid decline after 40 µl. However, acetic acid demonstrated the highest growth rate up to 80 µl and continued growing until 160 µl.

Screening of *Y. enterocolitica* growth in the presence of various antibiotics at various concentrations:

Table 6: Diameter of zone of clearance of antibiotics against bacteria (in cm)

Antibiotic	T1 conc.	T2 conc.	T3 conc.	T4 conc.	T5 conc.	T6 conc.	T7 conc.	T8 conc.
Erythromycin	3.4 cm	3.1 cm	3 cm	2.8 cm	2.1 cm	1.8 cm	0.7 cm	0 cm
Cefixime	3.5 cm	3.4 cm	3.2 cm	3.2 cm	3 cm	2.2 cm	2 cm	1.7 cm
Ciprofloxacin	3.8 cm	3.8 cm	3.6 cm	3.5 cm	3.4 cm	3.2 cm	2.5 cm	1.5 cm



Graph 2: Antibiotic susceptibility on bacterial growth with different concentrations

Table 6 and Graph 2 show that there are different zones of inhibition of *Y. enterocolitica* by using various antibiotics (Erythromycin, Cefixime, and Ciprofloxacin) at various concentrations i.e., T1, T2, T3, T4, T5, T6, T7, and T8 **(Table 1)** to study the most effective antibiotic against bacteria. Cefixime antibiotic was found to be the most effective antibiotic against *Y. enterocolitica* showing the best results followed by Ciprofloxacin and Erythromycin which came out to be less effective in comparison to those two. A zone of inhibition of diameter (1.7 cm) was observed at T8 concentration of Cefixime followed by Ciprofloxacin (1.5 cm) and Erythromycin (no inhibition zone) respectively.

DISCUSSION

This study aims to analyse *Y. enterocolitica* under various abiotic stress circumstances, such as various pH levels, and temperature settings. At different concentrations of preservatives, *Y. enterocolitica* can develop and its antibiotic susceptibility against different antibiotics. Latent acid bacteria's ability to produce active metabolites like bacteriocins, organic acids, hydrogen peroxide, and other toxins is attributed to their role in food preservation[25]. The good diffusion technique was then utilized to examine *Y. enterocolitica* susceptibility to various antibiotic concentrations **(Table 1)**.

The study utilized Eosin-methylene blue Agar (EMB Agar), a selective medium for gram-negative bacteria, to enhance its specificity. The growth of pathogenic bacteria was monitored on Nutrient Agar Media, which allowed quick growth within 18-24 hours, and Salmonella Shigella Agar (SS-Agar), which required a 24-36-hour incubation period for slower development and other details are mentioned in **(Table 2)**. Research studies on *Yersinia* have utilized prepared media such as MacConkey agar, Hektoen enteric agar, XLD, CIN agar, and VYE agar to isolate enterocolitica, revealing distinct colonies[27].

The Yamuna River water was used to isolate *Y. enterocolitica*, which was then diluted and spread over Eosin-methylene blue Agar (EMB Agar) media plate. Gram staining confirmed the presence of gram-negative colonies, while biochemical tests were conducted to identify the bacteria. Gram staining showed that the bacteria were rod-shaped, with positive results from catalase, urease, nitrate reduction, methyl red, Voges-Proskauer, glucose, maltose, sucrose, mannitol tests, while indole, citrate, hydrogen sulphide, lactose and casein hydrolysis tests results were found negative as mentioned in **(Table 2)**.

The biotype was identified using biochemical tests on the activity of the enzyme's lipase, salicin, esculin hydrolysis,

xylose, trehalose, indole synthesis, ornithine decarboxylase, Voges-Proskauer test, pyrazinamide, sorbose, inositol, and nitrate reduction[20]. The Gram stain colony morphological characteristics and the following biochemical tests were used for the initial identification of *Y. enterocolitica* isolates: catalase, oxidase, urease synthesis, Simmons citrate, triple sugar iron, and sugar fermentation[28].

Y. enterocolitica can grow at a high temperature of 55°C as on examination **(Table 3)** The reported growth range of *Y. enterocolitica* is −2 to 42°C[22]. While Discussing observations at various abiotic stress levels and antibiotic susceptibility *Y. enterocolitica* can grow in a wide range of pH levels ranging from pH 3 to 13 and very clear growth was observed in **(Table 4)**.

Acetic acid is the most bactericidal substance, followed by lactic acid, citric acid, and sulfuric acid. However, formic acid, acetic acid, propionic acid, and lactic acid had the greatest suppressive effects at pH values of 5.8 and 5.4 for aerobic conditions, and only pH 5.8 for anaerobic conditions. The sequence of inhibitory effect shifted to formic acid > lactic acid > acetic acid > propionic acid at lower pH values below pH 5.4 anaerobically and pH 5 aerobically[29]. We experiment by varying the growth patterns of *Y. enterocolitica* by adding different preservatives at different concentrations. By using the three bio preservatives, citric acid grows the least while lactic acid grows the most at the lowest concentration (20µl) and demonstrates a rapid fall after that. Acetic acid still rises at the highest rate among all other compounds, reaching its peak at 80µl and continuing to increase until 160µl as explained in **(Table 5 and Graph 1)**.

Heading to antibiotic susceptibility studies Cefixime was shown to be the antibiotic that worked best against *Y. enterocolitica*, followed by Ciprofloxacin, while Erythromycin was found to be less effective than those two drugs. At the T8 concentration of Cefixime, a zone of inhibition with a diameter of (1.7 cm) was seen, followed by Ciprofloxacin (1.5 cm), and Erythromycin with no zone of inhibition **(Table 6 and Graph 2)**.

CONCLUSION

This study confirms that *Y. enterocolitica* is a highly adaptable and potentially pathogenic bacterium capable of surviving under extreme environmental conditions. Its ability to proliferate in a wide pH range, and various growth media confirms its resilience as a foodborne contaminant. The antimicrobial susceptibility analysis revealed significant resistance to commonly used antibiotics, raising concerns about its potential role in antibiotic-resistant infections. Furthermore, the bacterium's ability to thrive in organic acid-based preservatives highlights its resistance mechanisms, which could impact food preservation strategies.

Given the high prevalence of virulence genes and resistance patterns observed in this study, further molecular and epidemiological research is warranted to develop effective mitigation strategies. Implementing stringent monitoring and control measures in the food industry is essential to limit the spread of *Y. enterocolitica* and prevent potential public health risks.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Odyniec M, Bancerz-Kisiel A. Assessment of the role of free-living and farmed fallow deer (*Dama dama*) as a potential source of human infection with multiple-drug-resistant strains of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *Pathogens*. 2022;11(11). doi:10.3390/pathogens11111266.
2. Sihvonen LM, Jalkanen K, Huovinen E, Toivonen S, Corander J, Kuusi M, Skurnik M, Siitonen A, Haukka K. Clinical isolates of *Yersinia enterocolitica* Biotype 1A represent two phylogenetic lineages with differing pathogenicity-related properties. *BMC Microbial*. 2012;12. doi:10.1186/1471-2180-12-208.
3. Rivas L, Strydom H, Paine S, Wang J, Wright J. Yersiniosis in New Zealand. *Pathogens*. 2021;10(2):1-28. doi:10.3390/pathogens10020191.
4. Ribeiro M, Sousa CA, Simões M. Harnessing microbial iron chelators to develop innovative therapeutic agents. *J Adv Res.* 2022; 39:89–101. doi: 10.1016/j.jare.2021.10.010.
5. Zdolec N, Kiš M, Jankuloski D, Blagoevska K, Kazazić S, Pavlak M, Blagojević B, Antić D, Fredriksson-Ahomaa M, Valeij P. Prevalence and persistence of multidrug-resistant *Yersinia enterocolitica* 4/O:3 in tonsils of slaughter pigs from different housing systems in Croatia. *Foods*. 2022;11(10). doi:10.3390/foods11101459.
6. Le Guern AS, Martin L, Savin C, Carniel E. Yersiniosis in France: overview and potential sources of infection. *Int J Infect Dis.* 2016; 46:1-7. doi: 10.1016/j.ijid.2016.03.008.
7. Platt-Samoraj A. Toxigenic properties of *Yersinia enterocolitica* biotype 1A. *Toxins* (Basel). 2022;14(2). doi:10.3390/toxins14020118.
8. Bottone EJ. *Yersinia enterocolitica*: revisitation of an enduring human pathogen. *Clin Microbiol Newsl.* 2015; *37*(1): 1–8. doi:10.1016/j. clinmicnews.2014.12.003.
9. Triantafillidis JK, Thomaidis T, Papalois A, Papalois A. Terminal ileitis due to Yersinia infection: an underdiagnosed situation. *BioMed Res Int*. *2020*; 2020:1240626. doi:10.1155/2020/1240626.
10. Fernandes S, Vasconcelos-Castro S, Teixeira C, Soares-Oliveira M. *Yersinia* enterocolitis may mimic appendicitis: 12 years of experience in a single tertiary center. *GE Port J Gastroenterol*. 2020;28(1):26–31. doi:10.1159/000507555.
11. Alnabwani D, Durrani M, Prasad A, Pandya S, Ghodasara K, Hasan BI, Greenberg A, Cheriyath P. *Yersinia enterocolitica* sepsis in an elderly male with no iron overload: a case report from the Northeastern United States. *Cureus*. 2022. doi:10.7759/cureus.26431.

12. Shoaib M, Shehzad A, Raza H, Niazi S, Khan IM, Akhtar W, Safdar W, Wang Z. A comprehensive review of the prevalence, pathogenesis and detection of *Yersinia enterocolitica*. *RSC Adv*. 2019;9(70):41010–21. doi:10.1039/c9ra06988g.

13. Le Guern AS, Savin C, Brémont S, Payro G, Bon D, Carniel E, Pizarro-Cerdá J. First isolation of *Yersinia entomophaga* in the human urinary tract. *New Microbes New Infect*. 2018; 26:3–7. doi: 10.1016/j.nmni.2018.08.002.

14. Kumar G, Menanteau-Ledouble S, Saleh M, El-Matbouli M. *Yersinia ruckeri*, the causative agent of enteric red mouth disease in fish. *Vet Res*. 2015;46(1):50-56. doi:10.1186/s13567-015-0238-4.

15. Haile S, Masi C, Tafesse M. Isolation and characterization of pectinase-producing bacteria (*Serratia marcescens*) from avocado peel waste for juice clarification. *BMC Microbiol*. 2022;22(1). doi:10.1186/s12866-022-02536-8.

16. Díaz AM, Terrones-Fernandez I, Gamez-Montero PJ, Castilla R. Helical static mixer simulations for its integration in the pour plate method: mixing agar and a nutrient solution. *Energies*. 2023;16(15). doi:10.3390/en16155816.

17. Gram H. The differential staining of *Schizomycetes* in tissue sections and dried preparations. *Fortschr Med*. 1884; 2:185–9.

18. Yoshimura J, Ogura H, Oda J. Can Gram staining be a guiding tool for optimizing initial antimicrobial agents in bacterial infections? *Acute Med Surg*. 2023;10(1):18-23. doi:10.1002/ams2.862.

19. Sardar N, Aziz MW, Mukhtar N, Yaqub T, Anjum AA, Javed M, Ashraf MA, Tanvir R, Wolfe AJ, Schabaker DS, Forrester S, Khemmani M, Aqel AA, Warraich MA, Shabbir MZ. One health assessment of *Bacillus anthracis* incidence and detection in anthrax-endemic areas of Pakistan. *Microorganisms*. 2023;11(10):2462. doi:10.3390/microorganisms11102462.

20. Yue Y, Zheng J, Sheng M, Liu X, Hao Q, Zhang S, Xu S, Liu Z, Hou X, Jing H, Liu Y, Zhou X, Li Z. Public health implications of *Yersinia enterocolitica* investigation: an ecological modelling and molecular epidemiology study. *Infect Dis Poverty*. 2023;12(1). doi:10.1186/s40249-023-01063-6.

21. Pal M, Paulos Gutama K, Girmay G, Tewari A, da Silva Ruiz L. Cultural and molecular techniques for the detection of *Yersinia enterocolitica* in food: an update. *J Adv Microbiol Res*. 2023;4(1):36–40.

22. Bari ML, Hossain MA, Isshiki K, Ukuku D. The behaviour of *Yersinia enterocolitica* in foods. *J Pathog*. 2011; 2011:1–13. doi:10.4061/2011/420732.

23. Sutherland JP, Bayliss AJ. Predictive modelling of growth of *Yersinia enterocolitica*: the effects of temperature, pH and sodium chloride. *Int J Food Microbiol*. 1994;21:11-19.

24. Stern NJ, Pierson MD, Kotula AW. Effects of pH and sodium chloride on *Yersinia enterocolitica* growth at room and refrigeration temperatures. 2023.

25. Šimat V, Čagalj M, Skroza D, Gardini F, Tabanelli G, Montanari C, Hassoun A, Ozogul F. Sustainable sources for antioxidant and antimicrobial compounds used in meat and seafood products. *Adv Food Nutr Res*. 2021;97:55–118. doi: 10.1016/bs.afnr.2021.03.001.

26. Gkouletsos T, Patas K, Lambrinidis G, Neubauer H, Sprague LD, Ioannidis A, Chatzipanagiotou S. Antimicrobial resistance of *Yersinia enterocolitica* and presence of plasmid pYV virulence genes in human and animal isolates. *New Microbes New Infect*. 2019; 32:100604. doi: 10.1016/j.nmni.2019.100604.

27. Filik K, Szermer-Olearnik B, Niedziółka-Jönson J, Roźniecka E, Ciekot J, Pyra A, Matyjaszczyk I, Skurnik M, Brzozowska E. ϕYeO3-12 phage tail fibre Gp17 as a promising high-specific tool for recognition of *Yersinia enterocolitica* pathogenic serotype O:3. *AMB Express*. 2022;12(1):13. doi: 10.1186/s13568-021-01341-2.

28. Bonardi S, Bruini I, D'Incau M, Van Damme I, Carniel E, Brémont S, Cavallini P, Tagliabue S, Brindani F. Detection, seroprevalence and antimicrobial resistance of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in pig tonsils in Northern Italy. *Int J Food Microbiol*. 2016; 235:125–32. doi: 10.1016/j.ijfoodmicro.2016.07.033.

29. El-Ziney MG, De Meyer B'l H, Debevere JM. Growth and survival kinetics of *Yersinia enterocolitica* IP 383 0:9 as affected by equimolar concentrations of undissociated short-chain organic acids. *Int J Food Microbiol*. 1997; 34:259–73.