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التعليم العالي والبحث العلمي
جامعة ميسان
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مجلة ميسان للدراسات الاكاديمية

مجلة ميسان للدراسات الأكاديمية

للعلوم الانسانية والاجتماعية والتطبيقية

Misan Journal For Academic Studies Humanits, Social and applied Sciences

ISSN (Print) 1994-697X

(Online)-2706-722X

المجلد 24 العدد 55 ايلول 2025

24 vol 55 Issue 2025 Sep

Misan Journal for Academic Studies - Humanities, Social and Applied Sciences
College of Basic Education, University of Misan
ISSN –Print 1994-697X Online 2706-722X

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Vol24 Issue55 Sep 2025

رقم الايداع في المكتبة الوطنية العراقية 1326 لسنة 2009

journal.m.academy@uomisan.edu.iq
https://www.misan-jas.com/index.php/ojs

MISAN JOURNAL FOR ACADEMIC STUDIES
HUMANITS, SOCIAL AND APPLIED SCIENCES
VOLE 24 ISSUE 55 SEP,2025
MJAS

ISSN (Print) 1994-697X
ISSN (Online) 2706-722X

DOI:
https://doi.org/10.54633/2333-024-055-006

Received:15/June/2025
Accepted:28/June/2025
Published online:30/Sep/2025

Prevalence and detection of Yersinia enterocolitica isolated from different clinical cases

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ABSTRACT:

Yersinia enterocolitica (Y. enterocolitica) is Gram-negative pathogen causing Yersiniosis, which is characterized by diarrhea, ileitis, and mesenteric lymphadenitis types of infection. This bacterium starts its pathogenic pathway by colonizing within host's intestinal tract after ingestion of contaminated food. It constitutes a challenge for researchers and food handlers because its growth habits, low concentrations in samples, morphological similarity with other bacteria and absence the rapid, cost-effective, and precise detecting methods.

A total of 200 human's stool samples with diarrhea at different ages were collected from several hospitals in different regions at Babylon governorate. All tested strains were identified using the ordinary biochemical tests; in addition the present investigation carried out molecular detection for the virulence related *virF* gene, and tests the antibiotic resistance for the obtained clinical isolates of *Y.* enterocolitica.

The infection rate of female was 41% and 59% for males. The highest incidence rate (36.4%) is recorded in infants (<1 year). The age group (1-6 year) is second most affected group, represents 24.70%. In contrast, the incidence rate declines significantly in older children and adolescents (7-18 years), collectively represents 8.22%. Interestingly, young adults (19-24 year) reveal a moderate prevalence rate of 5.30%, which stands up slightly in individuals at age of 25-30 years to 7.60%. Regarding to middle-aged adults (31-42 years), the prevalence rate remaining relatively low, with both 31-36 and 37-42 year age categories account for 3% for each. However, an observable expand in cases appears in patients aged 49-54 years (5.90%) and ≥55 years (5.20%). The present study showed that the prevalence rate of *Y. enterocolitica* accounted 4%; It had a significantly ($p<0.05$) lower incidence as compared with other causes of diarrhea. In the context of molecular detection of virF gene, it is found that this gene was identified in 6 isolates with percent of 75%. Regarding to antibiotic resistance, *Y. enterocolitica* isolates showed a highly level of resistance (100%) to ampicillin, chloramphenicol, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, and tetracycline, which revealing potential challenges for therapeutic options. In contrast, moderate resistance (25%) was recorded for ceftriaxone, ciprofloxacin, gentamicin, and colistin.

Y. enterocolitica-though detected at a low rate reveal to harbor significant virulence potential and alarming antibiotic resistance. The high prevalence rate of *virF* gene and resistance to commonly secondhand antimicrobials emphasized its emergence clinical relevance.

Key words: *Yersinia* enterocolitica, virF gene, antibiotic resistance, diarrhea, virulence genes

INTRODUCTION:

Yersinia enterocolitica (*Y. enterocolitica*) is an important foodborne pathogen accountable for a wide range of gastrointestinal infections of humans; it is highly distributed in the nature and has been isolated from different sources, including foods, animals, and clinical specimens(Le Guern et al.,2024). Infection with this bacterium in young children and infants involves acute feverish diarrhea and may be go along with abdominal pains and the stool samples contain leukocytes, red blood cells, as well as mucus. In older children and adults, right-sided abdominal pain and fever are the predominant symptoms(Brachman&Evans,1998;Ray et al.,2004). The ability of *Y. enterocolitica* to grow at low temperatures makes it especially relevant in foodborne outbreaks corresponding with refrigerated products(Bottone,1997).

Comprehension the epidemiological patterns and genetic characteristics of *Y. enterocolitica* isolates is crucial for the clinical detection and public health surveillance(Fredriksson-Ahomaa et al.,2006;Shoaib et al.,2019). In Iraq, the incidence of *Y. enterocolitica* in clinical and environmental specimens still underexplored. Previous investigation had revealed that locally produced raw milk may serve as a reservoir for *Y. enterocolitica*, constituting a potential public health risk(Ali&Al-Samarai,2020).

Y. enterocolitica expresses various pathogenic mechanisms, primarily mediated by several virulence genes like *virF*, which has a pivotal role in regulating the expressions of other virulence agents(Zheng et al.,2008). Studies have revealed that the expression of *virF* is robustly related with *Y. enterocolitica* pathogenicity, thus it considered a reliable marker to identify the virulent strains(Delibato et al.,2023). The presence of virulence genes differs among isolates from different origins and geographical regions, highlights the significance of continual surveillance and molecular characterization. The current investigation aims to investigate the prevalence of *Y. enterocolitica* isolated from clinical specimens and to detection the presence of the *virF* gene.

MATERIALS AND METHODS:

Sampling and Preparation of Bacterial Inoculum:

The present study included collection stool samples from 200 patients with diarrhea at different ages ranging from <1year to >55 years, they were taken from different hospitals in different regions at Babylon governorate during at duration of October 2024 to January 2025. Bacterial isolates were isolated and identified by growing them on suitable culture media for *Y. enterocolitica*, studying the morphological characteristics of bacterial colonies, and conducting microscopic examination and biochemical tests. In a 4-5mL brain heart infusion, at least 3-5 well isolated colonies were suspended. The broth culture was incubated for 8 hours at 37°C.

The turbidity of the actively developing broth culture was corrected with sterile broth to achieve an optically similar turbidity to the 0.5 McFarland standards tube (growth corresponding to1.5108 cell/ml). The bacterial isolates under study were preserved as stated in(Cheesbrough,2005).

Antimicrobial Susceptibility test:

Antibiotic susceptibility testing was performed using Kirby–Bauer disk diffusion methods, according to(Yoon,2022).

Genotypic detection of virF gene:

Genomic DNA of *Y.* enterocolitica isolates had been was extracted according to the manufacturer's instructions of Genomic DNA Purification Kit (Geneaid/Turkey). The purity of the extracted genomic DNA was determined using a Nano-drop spectrophotometer, which measures DNA concentration (ng/µl) and reads the absorbance at (260/280 nm). Primers specific from (IDT, Canada) for *virF* is shown in [Table. 1]. The conventional polymerase chain reaction (PCR) has been utilized for amplifying this gene; it was achieved in 50 µl volumes containing 5µl of DNA template, 25µl of PCR Taq Master Mix (Abm/Korea), 3µl of each forward and reverse primer, and 14µl of Nuclease free water (Bioneer/Korea). The conventional PCR thermocycler apparatus (Techne/UK) has been second-hand for genetic detection. [Table. 2] displays the PCR thermocycler setting that had been applied for investigated gene. The PCR products have been analyzed in (1.5) Agarose that stained using (1%) ethidium bromide (Bio basic/Canada) and photo-documented under UV illumination (UVP/USA).

Table (1): Size and sequences of *Vir F* primers

Target gene		Primer sequence (5'- 3')	Product size (bp)
Vir F	**F**	GGCAGAACAGCAGTCAGACATA	591 bp
	R	GGTGAGCATAGAGAATACGTCG	

Table (2): PCR Thermo cycling Conditions of Virulence Factors

***virF* gene**			
PCR steps	**Cycle**	**Temp.**	**Time**
Initial denaturation	1	95C	2 min.
Denaturation	35	95C	30sec.
Annealing		56	30sec.
Extension		72C	45sec.
Final extension	1	72C	7min
Hold	-	4C	Forever

RESULTS AND DISCUSSIONS:

Distribution of diarrhea cases by sex:

Fig. 2 shows the distribution of study samples by gender, where 41% were females and 59% were males.

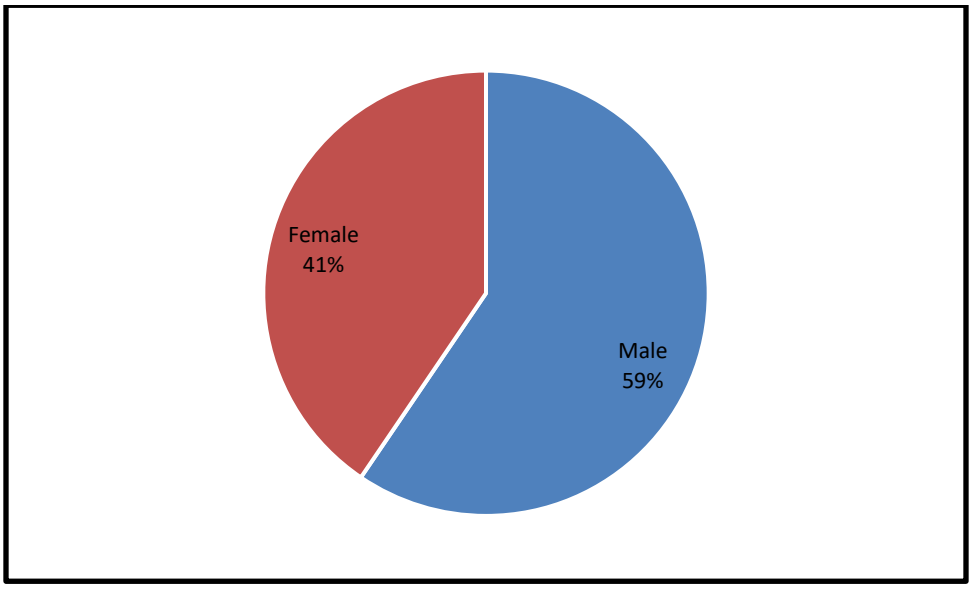

Figure (1): Distribution of diarrhea cases by sex

The present study investigated 200 diarrhea cases and showed that 41% of these cases were females, while 59% were males. This difference, although not fundamental and very large, but suggests that biological, behavioral, as well as social factors may affect the prevalence of bacterial diarrhea among different sexes.

From a biological context, some investigations indicated that males may be more susceptible to diarrhea as a result of differences in immunological response. Jarman et al. reported that in low- and middle-income countries, diarrhea incidence tends to be higher among males than females(Jarman et al.,2018). One potential explanation is that hormonal factors contributing to a stronger immunological response in females, makes them less vulnerable to bacterial infection. With regard be to havioral factors, Arun et al. showed that males more engaged in outdoor activities, which mounting exposure to contaminated environments(Arun et al.,2017). As well, the current study agrees with a previous study in Iraqi population, which revealed that children were more likely to vulnerable the bacterial diarrhea, where males are affected more frequently; this investigation identified E. coli as one of major bacterial pathogens contributing causing to this trend(Hasan et al.,2021).

Distribution of diarrhea cases according to age:

The distribution of diarrheal cases according to age groups is presented in [Fig. 2], which reveals significant trends in diarrhea incidence. The highest incidence rate (36.4%) is recorded in infants (<1 year). The age group (1-6 year) is the second most affected group, represents 24.70% of cases. In contrast, the incidence rate declines

significantly in older children and adolescents (7-18 years), collectively represents only 8.22% of studied cases. Interestingly, young adults (19-24 year) reveal a moderate prevalence rate of 5.30%, which stands up slightly in individuals at age of 25-30 years to 7.60%.

Regarding to middle-aged adults (31-42 years), the prevalence rate remaining relatively low, with both 31-36 and 37-42 year age categories account for 3% for each. However, an observable expand in cases appears in pateints aged 49-54 years (5.90%) and ≥55 years (5.20%).

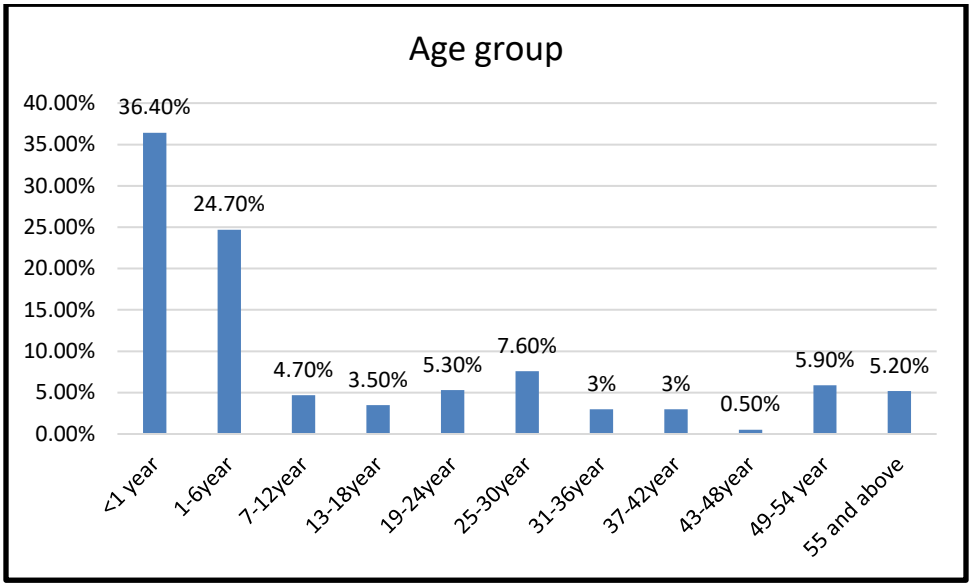

Figure (2): Distribution of diarrhea cases according to age

The distribution of diarrheal cases according to different age groups in the current study shows distinct epidemiological pattern. The highest incidence rate (36.4%) is observed in infants at <1 of age, followed by children at age of 1-6 years (24.70%), indicated that early childhood is a critical interval for diarrheal infection. This trend agrees with findings of Kyu et al., which revealed that children <5 years old endure the highest burden of diarrheal diseases worldwide, this is because their immune systems are not fully mature as well as escalated exposition to pathogens via contaminated food, water, along with poor sanitation Likewise, Fischer Walker et al. demonstrated that enterotoxigenic *E. coli* and *Vibrio cholerae* were the major causes of diarrhea hospitalizations of young children, consolidating the vulnerability at this age group .

However, our investigation also shows a secondary peak in diarrhea prevalence among adults aged 25-30 years (7.60%) and those >49 years (5.90%), which unlike some global trends. While Kyu et al. reported a decline in diarrheal burden among older population(Kyu et al.,2025), Fischer Walker et al. identified that *Salmonella* species and *Shigella* species were more frequently isolated in the outpatient setting among older children and adults(Fischer Walker et al.,2010), suggested that

occupational exposure, dietary habits, along with underlying health states can engaged with sustained infections rates. The difference among studies and our findings may be due to regional variations in sanitation infrastructures, healthcare access, and dietary habits, which can affect the persistence of diarrheal infections in certain age groups.

Moreover, the relatively low incidence among adolescents and middle-age adults in the current study agrees with global observations that boosted hygiene practices and vigorous immune defenses lessen the susceptibility at these age groups. On the other hand, the resurgence of cases in older adults may be due to age-related immune decline and expanded antibiotics resistance, as reported by Behera and Mishra, who revealed that *Campylobacter* was a major bacterial cause in diarrheal infection in older Indian populations(Behera&Mishra,2022). These observations suggest that age-related physiological alterations and chronic health status may share in increasing vulnerability in older patients, thus justifying the observed trends in current study.

Etiological Landscape of Studied Diarrheal cases: A Multifactorial analysis:

The analysis of diarrheal cases in the current study highlights the prevalence rate of *Y. enterocolitica* as well as the other etiological agents that observed in current investigation as illustrated in [Table. 3] and [Fig.3]. The present study showed that the prevalence rate of *Y. enterocolitica* accounted 4%; It had a significantly ($p<0.05$) lower incidence as compared with other causes of diarrhea.

While the fatty drop-related conditions accounted for the highest percentage represented 26.5%; whereas 19.1% of the examined cases contains different bacterial types; followed by Monilia at percent of 15%. The relatively comparable prevalence rates including: *Helicobacter pylori* (*H. pylori*) reached to 10.5% of cases, the parasitic infections (8.5%), other digestive disorders (8.51%), and undigested food (8.1%). The P-value of 0.0001 indicates statistically significant differences in the distribution of these causative agents across the studied population.

Table (3): Etiological agents of Studied Diarrheal cases

Causative	Number	%
Yersinia enterocolitica	8	4%
Undigestng food	16	8%
Digestive disorders	17	8.50%
Parasites	17	8.50%
Helicobacter pylori	21	10.50%
Monilia	30	15%
Others bacteria	38	19%
Fatty drop	53	26.50%
Total	200	100%
P value	0.0001*	
***Statistical deference under p value 0.05 by chi-square test**		

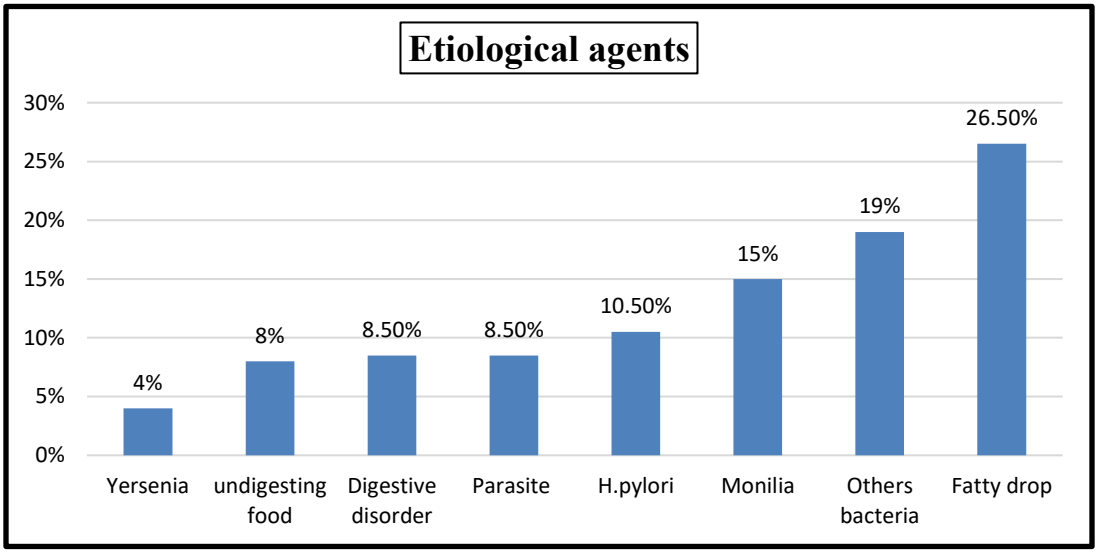

Figure (3): Etiological agents of Studied Diarrheal cases

The prevalence of *Y. enterocolitica* in diarrheal cases, as observed in the current study (4%), emphasizes its role as a bacterial pathogen, despite it represents a less incidence rate as compared with other causative factors like as fatty drop-related conditions (26.5%) and other types bacterial infections (19%). While investigations on incidence rate of *Y. enterocolitica* infections among humans in Iraq are nearly absent, but the previous veterinary studies had reported the prevalence rates in livestock and food sources that closely agree with our findings, which suggest a potential zoonotic transmission patterns that required further investigation.

Where the relatively low prevalence rate of *Y. enterocolitica* that recorded in the present study consistent with the previous findings of Khalid and Abbas, which reported that the prevalence rates of *Y. enterocolitica* in raw milk specimens in Al-Basrah were 8% in cow's and buffalo's milk, and 4% sheep's milk(Khalid&Abbas,2021); this finding proposed that while *Y. enterocolitica* is present in Iraq, its transmission via food sources may be a contributing factor to its prevalence in the present human infections. Also, Saleh and Zenad conducted an investigation on sheeps in southern of Iraq and revealed that the total isolation rate of *Y. enterocolitica* was 5.16%, with higher rate found in Al-Muthana (6.01%) while the lower rates in Al-Basrah (3.8%)(Saleh&Zenad,2018). On the other hand, Almashhadany et al. examined contamination rate of *Y. enterocolitica* in raw sheeps and goats milk in Erbil and reported a contamination rate of 9.7%,

with non-significant differences between sheeps and goats milk sources(Almashhadany et al.,2025). The discrepancy between this investigation and our findings may be return to the difference in specimen sources, diagnostic approaches, along with environmental conditions, emphasizes the necessity for further studied on human infections with of *Y. enterocolitica* in Iraq.

Beyond Iraq, global research had reported varied prevalence rates of *Y. enterocolitica* in human diarrheal cases. The European Centre for Disease Prevention and Control revealed that yersiniosis is a third most commonly reported gastrointestinal infection in EU/EEA, despite its notification rate still lower than that of *Salmonella* and *Campylobacter*(ECDC,2022); this suggested that while *Y. enterocolitica* is a recognized pathogen, but its prevalence may be affected by regional differences in food consumption, diagnostic approaches, as well as environmental conditions. Similarly, Riahi et al. reported higher incidence rates of *Y. enterocolitica* in gastroenteritis patients in the Americas, especially in regions with recurrent pork consumption, revealing the crucial roles of dietary habits and food safety measures in transmission dynamics(Riahi et al.,2021).

The Molecular detection of *VirF* gene:

Fig. (4) displays the PCR outcomes for (8) isolates of *Y. enterocolitica*, which tested for *VirF* gene at the product size of 591bp, which was identified in 6 isolates (75%) of the total clinical (8) isolates of *Y. enterocolitica*.

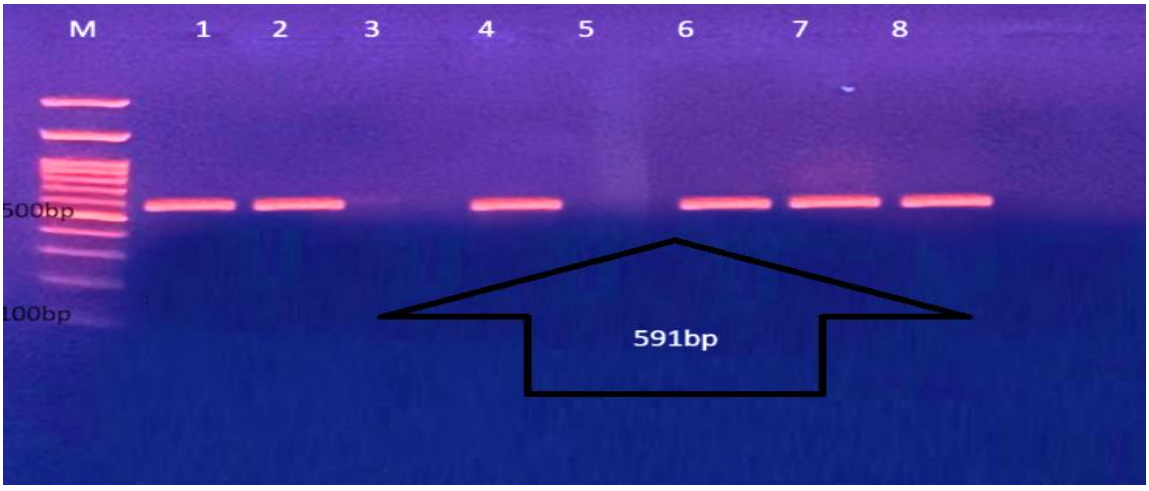

Figure (4): The *virF* gene (591 bp) from *Y. enterocolitica* isolates was amplified by PCR in gel electrophoresis. Every lane has a certain area of 591 base pairs, showing that the test amplified the virulence gene indicated by *virF*.

The molecular detection of *virF* gene in the clinical *Y. enterocolitica* isolates in the current study demonstrated a prevalence at 75% (6 out of 8 isolates), this result suggests a vigorous association between *virF* gene and pathogenicity of *Y. enterocolitica*. It is a plasmid-borne virulence factor that has a key role in regulating expressions of other virulence genes, particularly those engeged with ability of this bacteria to invad host and immunological evasion. The high prevalence found in the current investigation agrees with the privous study conducted by Thoerner et al., which demonstrated that plasmid-borne genes, including *virF*, were detected with variable rates due to the heterogeneity across bacterial populations; they revealed that while most pathogenic *Y. enterocolitica* isolates carried *virF* gene, yet some strains lacked this gene due to plasmid instability or loss(Thoerner et al.,2003).

Harnett et al. applied a multiplex PCR technique to identify the presence of *ail*, *yst*, and *virF* genes in *Y. enterocolitica*, and they confirmed that *virF* gene was found in pathogenic isolates while it absent in non-pathogenic isolates; where these findings support the concept that *virF* gene is a deem as reliable marker to identify the virulent strains. However, their study also observed that some strains of *Y. pseudotuberculosis* have *virF* gene, which indicated a potential cross-species genetic correspondences(Harnett et al.,1996).

Otherwise, Shabana et al. reported a lower prevalence for *virF* (37.5%) in *Y. enterocolitica* isolates from chicken meat specimens, suggested that foodborne isolates may show diminish virulence compared to clinical isolates(Shabana et al.,2015). The lower prevalence rate in food specimens may reveal that some strains have lost the virulence plasmid or may possess a substitute pathogenicity mechanisms.

Overall, our study reinforces the importance of *virF* as a crucial virulence determinant in *Y. enterocolitica* at a prevalence rate aligns to findings from clinical isolates. The variations in prevelance rates among studies highlights the impact of bacterial strain diversity, stability of plasmid, along with environmental factors on presence of *virF*.

The antibiotic resistance profile of Y. enterocolitica:

The results in [Table. 4] and [Fig. 5] exhibit the antibiotic resistance profile for *Y. enterocolitica*, where the present study demonstrates significant resistance patterns. The *Y. enterocolitica* isolates showed a highly level of resistance (100%) to several commonly secondhand antibiotics, including ampicillin (AM), chloramphenicol (C), amoxicillin-clavulanic acid (AMC), trimethoprim-sulfamethoxazole (SXT), and tetracycline (TE), which revealing potential challenges for therapeutic options. In contrast, moderate resistance (25%) was recorded for ceftriaxone (CRO), ciprofloxacin (CIP), gentamicin (CN), and colistin (HLS), suggested that while these antibiotics may still give some efficacy, but the resistance rate appears to be increasing.

Table (4): Patterns of antibiotic resistance among *Y. enterocolitica*

Antibiotics	No. of R	No. of I	No. of S	Resistant (%)
CRO (10)	1	0	3	25%
CIP (10)	1	2	1	25%
CN (10)	1	0	3	25%
AM (25)	4	0	0	100%
C (10)	4	0	0	100%
AMC (30)	4	0	0	100%
SXT (25)	4	0	0	100%
TE (10)	4	0	0	100%
HLS (300)	1	0	3	25%

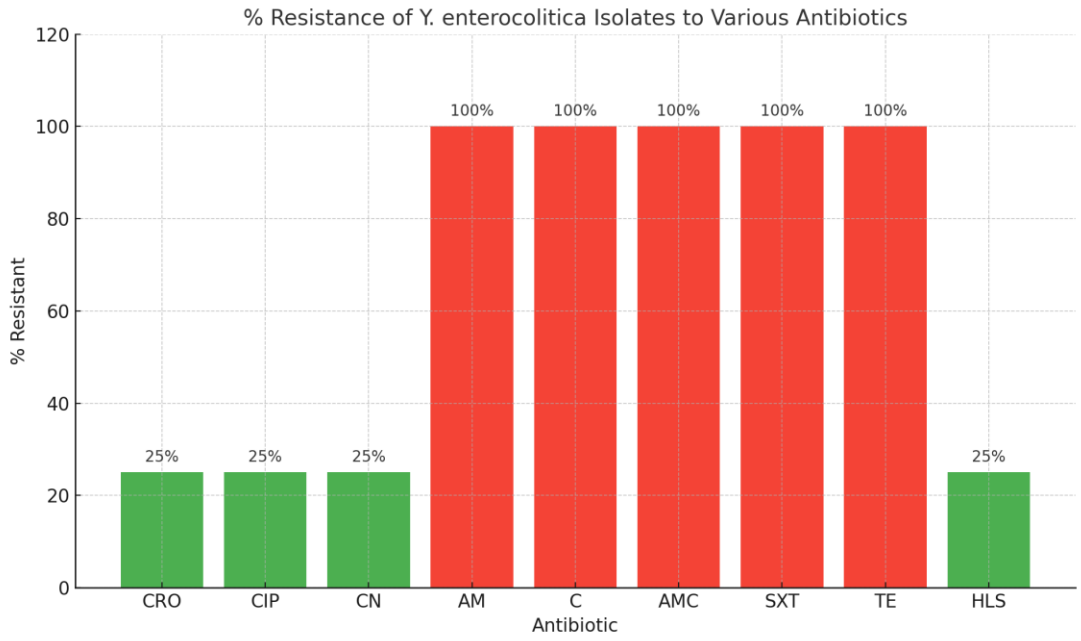

Figure (5): Bar chart showing percentage of resistance among *Y. enterocolitica* isolates for each tested antibiotic. Red bars represent antibiotics with 100% resistance, while green bars indicate lower resistance (25%).

The antibiotic resistance profile of *Y. enterocolitica* in the present investigation demonstrates a concerning trend, particularly with high resistance (100%) observed against AM, C, AMC, SXT, and TE. This finding indicates that these antibiotics may no longer be available to treat infections caused by *Y. enterocolitica*, highlights the necessity for alternative therapeutic approaches and prompts antimicrobial stewardship. The moderate resistance (25%) observed for CRO, CIP, CN, and HLS reveals that while these antibiotics may still give some efficacy, but the resistance rate appears to be increasing, requiring careful selection of antibiotics depend on susceptibility testing.

The current results consistent with global principle of *Y. enterocolitica* having adaptive resistance mechanisms, in particular against β-lactam antibiotics family. The present study aligns with previous investigation conducted by Seakamela et al., who reported that *Y. enterocolitica* isolated from meat specimens in South Africa showed high resistance to AM and TE(Seakamela et al.,2022). Likewise, Salimi et al. observed high resistance rates in *Y. enterocolitica* strains isolated from raw milk and traditional cheeses(Salimi et al.,2024), further enhancing the notion that foodborne transmission playing a role in dissemination of resistant strains. However, the present findings contrast the finding of Almashhadany et al., who examined *Y. enterocolitica* that isolated from sheep and goat milk in Iraq and found a lower resistance to CIP and CN(Almashhadany et al.,2025). This disparity may be assigned to differences in samples sources, bacterial strains diversity, and regional antibiotic utilization patterns. In addition, differences in laboratory methodologies like difference in susceptibility testing protocols, may contribute to these differences in the resistance rates.

Overall, these high resistance rates observed in the current study highlights the urgent demand for antimicrobial surveillance and prudent antibiotic utilization to alleviate the spread of resistant *Y. enterocolitica* strains.

Conclusion:

The present study provides valuable insights into the epidemiological and microbiological profile of *Y. enterocolitica* among diarrheal cases in studied population. Although it isn't the most dominant causative agent among the examined cases, but its prevalence at 4% reinforces its significance as an emerging pathogen associated with enteric infections. The genetic detection of *virF* virulence gene in 75% of isolates boosts the pathogenic potential for identified strains, highlighting the clinical importance of *Y. enterocolitica* in gastrointestinal infections.

Alarmingly, antibiotic susceptibility test demonstrated ahigh resistance rate (100%) against ordinary secondhand antibiotics, emphasizing the emerging challenge of antimicrobial resistance in *Y. enterocolitica*. The present findings not only mirror global trends but also point out to the regional misuse or overuse for antibiotics that could accelerating resistance.

Declaration of Competing 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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Conflicts of Interest Statement……

Manuscript title:

Prevalence and detection of Yersinia enterocolitica isolated from different clinical cases

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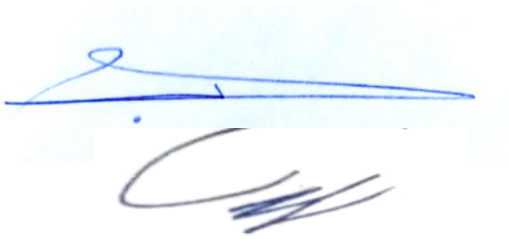
Baneen Maan Kareem		2025/6/28
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