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Molecular characterization and antimicrobial resistance of *Yersinia enterocolitica* isolated from raw chicken meat in Iran: polymerase chain reaction ribotyping insights

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Abstract

Yersinia enterocolitica is a well-known foodborne pathogen that is widely distributed among domestic and wild animals. This study aimed to determine the prevalence of Y. enterocolitica in raw chicken meat and to characterize the isolates in terms of bio/serotypes, virulence factors, antimicrobial sensitivity, and genetic diversity. In 2021-2023, a total of 622 raw chicken meat samples were collected, from which *Y. enterocolitica* strains were isolated and confirmed by *16S rRNA* detection. Biotype and serotype were identified using biochemical assays and agglutination methods, respectively. Six virulence-associated genes were examined using polymerase chain reaction (PCR). Antimicrobial susceptibility was assessed via the disk diffusion, and molecular typing was conducted by PCR ribotyping. The overall prevalence of *Y. enterocolitica* was 9.3%. A significant relationship was found between seasonal variation and prevalence (p=0.0001). Bio/serotypes 1A/O:8, 1A/O:5, 1A/O:NI, 1B/O:8, and 1B/O:NI were identified, with most isolates belonging to 1A/O:NI and 1A/O:8. A significant relationship was also observed between seasons and biotype distribution (p=0.031). In biotype 1B, the most frequent virulence genes were *ystA, myfA, virF, ail,* and *inv,* whereas biotype 1A predominantly harbored *ystB* and *inv*. All isolates were susceptible to 14 antibiotics. Ceftazidime and ampicillin resistance, however, was 100%. Notably, there were also notable levels of resistance to trimethoprim-sulfamethoxazole and nitrofurantoin. Pathogenic strains (IRT1 and IRT2) were grouped independently from non-pathogenic strains (IRT3) using PCR ribotyping. The detection of highly pathogenic *Y. enterocolitica* (1B/O:8) in Iran may pose a serious public health concern.

Key words: *Yersinia enterocolitica,* chicken, PCR, ribotyping, virulence factors.

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Introduction

One of the main causes of food contamination and a major health risk to consumers is pathogenic microorganisms. Therefore, enhancing food hygiene requires constant food product monitoring and control (Silva and Ribeiro, 2024). *Yersinia enterocolitica* is one of the major foodborne bacteria (Hanifian and Khani, 2012). Unlike other enteropathogens such as *Campylobacter*, *Y. enterocolitica* strains cannot be easily categorized into human and animal species. Various microorganisms are responsible for zoonotic diseases, but in 2023, *Y. enterocolitica* was reported as the fourth most common zoonotic pathogen globally, following *Campylobacter*, *Salmonella*, and Shiga Toxin-producing *Escherichia coli* (EFSA and ECDC, 2024). The incidence of infections caused by *Y. enterocolitica* has been rising in recent years. Since the late 1970s, this bacterium has been frequently isolated from patients with gastrointestinal disorders (Rahimi *et al*., 2014). The first peculiar feature of *Y. enterocolitica* is that it can live and even multiply at refrigerated temperatures (Zadernowska *et al*., 2014), which is especially significant in terms of food safety. Since the majority of foodborne *Y. enterocolitica* are found in chilled surroundings, the bacterium has to adapt to the change in temperatures before taking root in the intestinal tract of man. This adaptation includes the changes in surface antigenic structures and the use of temperature-controlled chromosomally and plasmid-encoded virulence factors, including *yad*, *yop*, *inv*, *ail*, and *yst*. The reversal of this effect is the so-called warm-cold transition cycle (Bottone, 1999). The virulence of *Y. enterocolitica* is caused by several genes. The *ail* gene relates to the resistance to host defenses. The *yst* gene contains an enterotoxin, and *virF* has a fundamental role in enabling the transcription of the *yadA* and other transcripts on the plasmid that carries the virulence genes (Fazlara *et al*., 2016). Mucoid yersinia factor (*myfA*) is important in the process of initiating infection. Adherence to enterocytes implicates MyfA as well (Pakharukova *et al*., 2016). One of the major determinants that enables penetration of bacteria into the epithelial cells is the invasin. Of them, *ail* is the only factor that is highly correlated with virulence since it is only observed in virulent *Yersinia* strains, with *inv* being highly conserved in all isolates. Attachment-invasion locus (*ail*) is implicated in adhesion, invasion of particular tissue culture cells, and the surviving bactericidal effect of serum (Fàbrega and Vila, 2012).

According to the pathogenicity, *Y. enterocolitica* is divided into

two types of biotypes: non-pathogenic (1A) and pathogenic (1B, 2, 3, 4, and 5) (Mastrodonato *et al*., 2018). Both plasmid-encoded and chromosomal factors are needed to specify the virulence of *Y. enterocolitica* (Peruzy *et al*., 2017). The environmental sources of biotype 1A strains include water, wastewater, soil, and food (Gupta *et al*., 2015). However, biotype 1A is non-virulent, not in possession of the virulence plasmid pYV, yet it is isolated more regularly than all other biotypes, which in many cases possess chromosome-based genes crucial to their virulence, including *ail*, *myfA* and *ystA* (Gupta *et al*., 2015; Mastrodonato *et al*., 2018). Biotype 1B is regarded as highly pathogenic compared to other biotypes (Fàbrega and Vila, 2012). In recent years, molecular techniques such as polymerase chain reaction (PCR) ribotyping, pulsed gel electrophoresis (PFGE), and chromosomal restriction fragment length polymorphism analysis have been employed to identify and classify *Y. enterocolitica*, as well as to study its genetic diversity and geographical distribution. These techniques give resolution information regarding the diversity and the epidemiological association of the strains. Of all these, ribotyping could be thought of as the best method of strain typing since strains could be well typed, and it has high discrimination power (Virdi and Sachdeva, 2005). This study was conducted to achieve these goals: i) investigation of the prevalence of *Y. enterocolitica* and the relationship between the prevalence of this bacterium and season, biotype, serotype, and virulence factors; ii) determination of antibiotic sensitivity and resistance; iii) determination of ribotypes and heterogeneity of strains of *Y. enterocolitica* by the PCR ribotyping method, and determining the relationship between ribotypes and pathogenic factors in isolated strains of *Y. enterocolitica.*

Materials and Methods

Sample size and collection

A total of 622 raw chicken samples were collected from poultry shops across various regions of Zanjan province, Iran, between July 2021 and July 2023. The samples were transported to the laboratory of the Faculty of Medicine at Zanjan University of Medical Sciences under a cold chain to ensure integrity.

Sample enrichment, identification, and confirmation of isolates

25 g of chicken meat were aseptically sliced into thin pieces using a sterile scalpel and added to 225 mL of phosphate-buffered saline plus 1% sorbitol and 0.15% bile salts (PBSSB, pH 7.2). The mixture was homogenized using a stomacher (400 circulator, Seward, Worthing, England). The samples were then stored at 4°C for 3 weeks. On days 7, 14, and 21, 1 mL of the enriched suspension was added to 9 mL of 25% KOH and mixed thoroughly with an electric stirrer for 31 seconds. The suspension was treated, and a loopful of it was spread on the plate of Cefsulodin Irgasan Novobiocin (CIN) agar (Merck, Darmstadt, Germany) and incubated at a temperature of 30°C for a period of 48 hours. Presumptive *Y. enterocolitica* colonies Colony characteristic characteristics of colonies Colonies that were colonies with a red center and colorless margin on CIN agar were noted as presumptive *Y. enterocolitica* colonies. There was colony exclusion by the oxidase test. Specific biochemical reactions, such as urease, triple sugar iron, SIM, ONPG disk, Voges-Proskauer, methyl red, Simmons citrate, ornithine decarboxylase, lysine decarboxylase, and broth were then done. All the biochemical tests are listed in *Supplementary Table 1*. To molecularly confirm *Y. enterocolitica*, traditional PCR amplification was used of the *16S rRNA* gene. PCR reaction (25 µL total volume) contained sterile deionized water, 1X reaction buffer (Sinaclon, Tehran, Iran), $MgCl_2$ (Sinaclon, Tehran, Iran) at a concentration of 1.5 mM, dNTPs (Sinaclon, Tehran, Iran) at a concentration of 0.2 mM, forward and reverse primers (Metabion, Planegg, Germany) each 0.2 µL, 0.5 units of Taq DNA polymerase (Fermentase, St. Leon-Rot, Germany), and 2 µL of DNA template. DNA was extracted using a commercial kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. The thermal steps for performing PCR in a thermocycler (Analytik, Jena, Germany) were as follows: 4 minutes at 94°C for initial denaturation, 30 cycles of 60 seconds at 94°C for denaturation, 40 seconds at 54°C for annealing, 60 seconds at 72°C for extension, and finally a final extension of 10 minutes at 72°C. The amplification primer sequences of the *16S rRNA* gene are shown in *Supplementary Table 2*.

Determination of the biotype and serotype of isolated

The biotype of the isolates was established on the principles referred to by Wang *et al.* (2010). To perform serotyping, commercial antisera (Denka Seiken, Tokyo, Japan) against serogroups O:3, O:5, O:8 and O:9 were employed. A pure colony was put in Physiological Saline, then a drop of antiserum was added, and its agglutination was seen.

Antibiotic susceptibility testing

The isolates were tested on Mueller-Hinton agar (Merck, Darmstadt, Germany) by the Kirby-Bauer disc diffusion procedure according to the Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute, 2023).

Molecular detection of virulence factors

The virulence-associated genes *ail*, *inv*, *virF*, *myfA*, *ystA* and *ystB* were also detected by PCR with the extracted DNA template using a commercial kit (QIAGEN, Hilden, Germany). The primers and the anticipated product sizes are illustrated in *Supplementary Table 2*. The positive control DNA was prepared using *Y. enterocolitica* ATCC 23715. The experiments were done in a 25 µL PCR reaction using the same conditions as for the *16S rRNA* gene PCR mentioned above. Annealing temperatures were *ail* (57°C), *inv* (54°C), *virF* (57°C), *myfA* (55°C), *ystA* (51°C), and *ystB* (54°C).

16S-23SrDNA spacer region length polymorphism or polymerase chain reaction ribotyping

The PCR ribotyping was conducted according to the instructions of Estrada *et al*. (2011). Amplification was performed using the same conditions as for the *16S rRNA* gene PCR mentioned above, and a 25 µL reaction volume was set. Primer sequences and product sizes are listed in *Supplementary Table 2*. The annealing temperature was 57°C.

Statistical analysis

Chi-squared tests have been conducted to determine the relationship between contamination and various variables. The statistical tests, such as chi-square, were conducted using SPSS 18.0 (SPSS Inc., Chicago, IL), as well as GraphPad Prism version 8.0.2 (https://www.graphpad.com/updates). The NTsys software version 2.02 was used in generating dendrograms. The Jaccard similarity coefficient was used in the analysis of binary presence/absence data (0/1), whereas clustering was carried out *via* the Unweighted Pair Group Method with Arithmetic Mean. Simpson diversity index (DI) was used to determine the DI of PCR ribotyping, as shown by Estrada *et al.* (2011).

Results

Prevalence of *Yersinia enterocolitica* and its relationship with months and seasons

The number of strains of *Y. enterocolitica* isolated during the testing of 622 samples of raw chicken meat was 58, which is equal to the prevalence rate of 9.3%. Biochemical reaction profiles of the isolated are shown in *Supplementary Table 1*. The distribution of isolates by month was 10.3% in October, 13.8% in November, 13.8% in December, 25.9% in January, 10.3% in February, 6.9% in March, and 19% in April (Figure 1A). A significant relationship was also observed between prevalence and months of the year (p=0.0001). The prevalence of *Y. enterocolitica* was in winter (50%), spring (25.86%), and fall (24.14%) (Figure 1B). A significant relationship was also observed between prevalence and seasons (p=0.0001).

Prevalence of biotypes, serotypes, and bio/serotypes

Out of the 58 *Y. enterocolitica* isolated, 65.5% were of biotype 1A and 34.5% of biotype 1B (Figure 2A). The isolated counts of biotype 1A were higher in the fall, and those of biotype 1B in winter. There was a significant relationship between the distribution of biotypes and the months and seasons (p=0.031).

In terms of serotype, the prevalence rates were O:NI (non-agglutinating with antisera O:3, O:5, O:8, and O:9) at 51.7%, O:8 at 43.1%, and O:5 at 5.2%. The bio/serotype distribution was as follows: 1A/O:NI (35.1%), 1A/O:8 (28.1%), 1B/O:NI (14%), 1B/O:8 (17.5%), and 1A/O:5 (5.3%) (Figure 2B). The 1B/O:8 was mostly observed in the winter season. Seasonal distribution revealed that 1A/O:NI and 1A/O:5 were most prevalent in the fall, while 1A/O:8, 1B/O:NI, and 1B/O:8 were most common in winter. No statistically significant relationship was observed between seasons and bio/serotype (p=0.37).

Antibiotic sensitivity and resistance

All *Y. enterocolitica* isolates were 100% susceptible to all antibiotics listed in Table 1, except ampicillin, ceftazidime, trimethoprim-sulfamethoxazole, and nitrofurantoin. Complete resistance (100%) was observed to both ampicillin and cef-

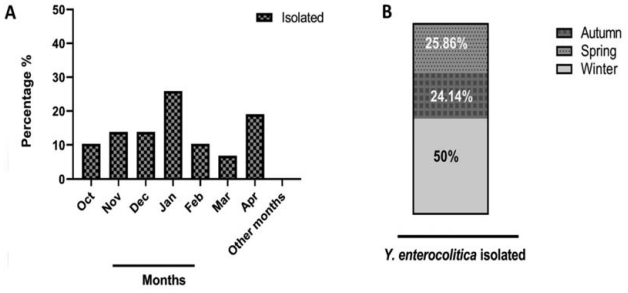

Figure 1. Frequency of *Yersinia enterocolitica* isolates in relation to (**A**) months and (**B**) seasons.

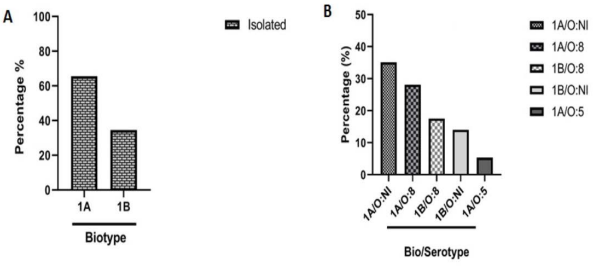

Figure 2. Frequency of *Yersinia enterocolitica* isolates in relation to (**A**) biotype and (**B**) serobiotype

Table 1. Antimicrobial resistance rates of 58 *Y. enterocolitica* strains isolated from raw chicken meat.

Antibiogram disc	Sensitivity	Intermediate Number (percentage)	Resistance
Ampicillin (Am_{10})	0 (0)	0 (0)	25 (100)
Tetracycline (TE_{30})	58 (100)	0 (0)	0 (0)
Gentamicin (GM_{10})	58 (100)	0 (0)	0 (0)
Ciprofloxacin (CP_5)	58 (100)	0 (0)	0 (0)
Amikacin (AN_{30})	58 (100)	0 (0)	0 (0)
Nalidixic acid (NA_{30})	58 (100)	0 (0)	0 (0)
Chloramphenicol(C_{30})	58 (100)	0 (0)	0 (0)
Streptomycin (S_{10})	58 (100)	0 (0)	0 (0)
Trimethoprim-Sulfamethoxazole ($SXT_{1.25/23.75}$)	0 (0)	27 (46.55)	31 (53.45)
Ceftazidime (CAZ_{30})	0 (0)	0 (0)	58 (100)
Tobramycin (TOB_{10})	58 (100)	0 (0)	0 (0)
Imipenem (IPM_{10})	58 (100)	0 (0)	0 (0)
Meropenem (MEN_{10})	58 (100)	0 (0)	0 (0)
Piperacillin-Tazobactam ($PTZ_{100/10}$)	58 (100)	0 (0)	0 (0)
Aztreonam (AZT_{30})	58 (100)	0 (0)	0 (0)
Nitrofurantoin (FM_{300})	0 (0)	9 (15.52)	49 (84.48)
Levofloxacin (LEV_5)	58 (100)	0 (0)	0 (0)
Cefepime (FEP_{30})	58 (100)	0 (0)	0 (0)

tazidime. It is noteworthy that resistance to trimethoprim-sulfamethoxazole and nitrofurantoin was also observed in this study.

Frequency of virulence genes

Virulence genes in 1B/O:8 and 1B/O:NI bio/serotypes were in the form of *ystA*+, *ystB*-, *virF*+, *myfA*+, *ail*+, and *inv*+; in 1A/O:8 and 1A/O:NI bio/serotypes were *ystA*-, *ystB*+, *virF*-, *myfA*-, *ail*-, and *inv*+; and in 1A/O:5 bio/serotype were *ystA*-, *ystB*-, *virF*-, *myfA*-, *ail*-, and *inv*+. An agarose gel image of the amplified virulence genes is provided in *Supplementary Figure 1*. The distribution of virulence genes across bio/serotypes is summarized in Table 2.

16S-23S rDNA spacer region length polymorphism or polymerase chain reaction ribotyping

PCR ribotyping identified a total of five different band patterns observed on the agarose gel, which were utilized in the differentiation of the strains (Figure 3A). Three ribotypes were identified by

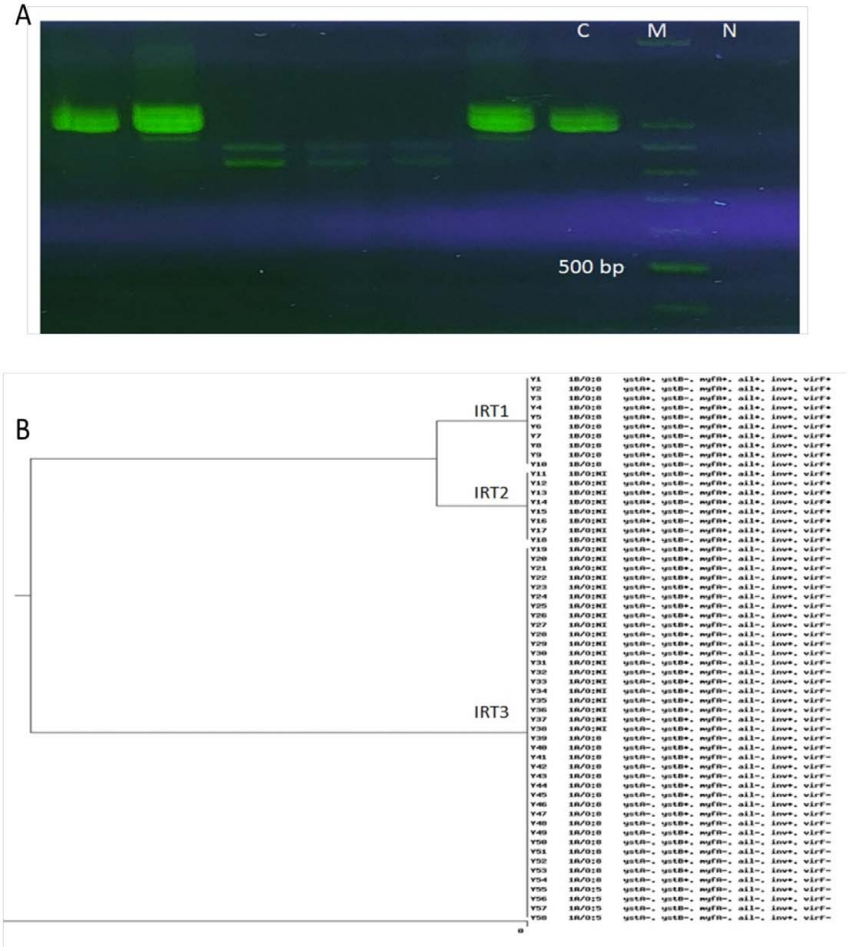

Figure 3. Polymerase chain reaction (PCR) ribotyping of 58 *Yersinia enterocolitica* strains isolated from chicken meat. **A**) 16S rRNA-23S rRNA gene amplification of *Y. enterocolitica* strains. M, 100 bp marker; C, positive control (*Y. enteroclitica* standard strain ATCC 23715); N, *E. coli* ATCC 2592; remaining wells strains studied; **B**) Unweighted Pair Group Method with Arithmetic Mean dendrogram based on similarity of PCR-ribotyping profiles.

Table 2. Frequency of virulence factors of 58 strains of *Y. enterocolitica* isolated in relation to bio/serotypes.

Strains isolated (No)	SR	Bio/Serotype	*ail* CHR	*inv* CHR	*myfA* CHR	*ystA* CHR	*ystB* CHR	*virF* Plasmid
Y_1-Y_{10} (10)	IRT_1	1B/O:8	10	10	10	10	0	10
Y_{11}-Y_{18} (8)	IRT_2	1B/O:NI	8	8	8	8	0	8
Y_{19}-Y_{38} (20)	IRT_3	1A/O:NI	0	20	0	0	20	0
Y_{39}-Y_{54} (16)	IRT_3	1A/O:8	0	16	0	0	16	0
Y_{55}-Y_{58} (4)	IRT_3	1A/O:5	0	4	0	0	0	0

No, Number; Chr, Chromosomal; Y, Numeral of *Y. enterocolitica* species isolated; O:NI, A serotype that is not agglutinated with any of the antisera O:3, O:5, O:8, O:9; SR, Intergenic Spacer Region; IRT, Iranian Ribotype.

these different band patterns, which were named Iranian ribotypes (IRT1, IRT2, IRT3). Each ribotype contained two or three bands with sizes ranging from 800 to 1200 bp. Biotype-based classification confirmed that there were two major clusters of the isolates. Cluster I consisted of biotype 1B strains, which were named IRT1 and IRT2. All biotype 1A strains were placed in cluster II, which was named IRT3. No specific serotype matched any specific ribotype (Figure 3B). The DI of PCR ribotyping was obtained as 0.48.

Discussion

Discussion on frequency and seasonal patterns

In this study, *Y. enterocolitica* was isolated from 9.3% of 622 cultured raw chicken meat samples, with a significant association between its prevalence and seasons (p=0.0001). Findings of the previous Iranian research of the 2006-2023 period also have a wide range of prevalence rates between 13.3 and 50% (Soltan Dallal *et al*., 2006; Mahdavi *et al*., 2012; Momtaz *et al*., 2013; Aghamohammad *et al*., 2015; Sirghani *et al.*, 2018; Soltan Dallal *et al*., 2022). Comparatively, it can be seen that over time, there has been a negative trend in the prevalence of *Y. enterocolitica* in chicken meat. This decrease could be a result of improved public health infrastructure, better water and sewage management (Fewtrell *et al*., 2005), improvements in food production and storage, particularly in the poultry industry (Aladhadh, 2023), modernized slaughterhouses (Arsić *et al*., 2023), and climatic factors like less rainfall and temperature fluctuations (Park *et al*., 2018).

According to our data, the highest prevalence occurred in the winter (p=0.0001), which is consistent with findings from Mahmoud *et al.* (2023) in Egypt (higher winter prevalence) (Mahmoud *et al*., 2023) and Lü *et al*. (2022) in China (12% in winter). The bacterium's capacity to endure and proliferate in cold environments may be the cause of the elevated winter prevalence (Bari *et al*., 2011). Also, the alternations of the animals' farming regime in winter (such as switching diet and housing) can undermine the animals' immunity and create hazards of bacterial contamination (Chen *et al*., 2024).

Discussion on biotype and serotype

Biotype 1A accounted for 65.5% of the 58 *Y. enterocolitica* isolates, while biotype 1B made up 34.5%. O:5 (5.2%) and O:8 (43.1%) were the most prevalent serotypes. Notably, bio/serotypes 1A/O:8 (28.1%), 1B/O:8 (17.5%), and 1A/O:5 (5.3%) were identified. Previous studies in Iran have reported variable biotype prevalence: Soltan Dallal *et al.* (2022) found 80% 1A and 20% 1B in chicken meat; Sirghani *et al*. (2018) identified only biotype 1A; in chicken liver samples, Hassanzadeh *et al.* (2022) reported primarily non-pathogenic 1A with serotypes O:8 and O:5; and 1B with serotype O:3, while Momtaz *et al*. (2013) isolated biotypes 2, 3, 4, and 5 but not 1A or 1B. Although the geographical region, the animal host studied, and regional hygiene are involved in the prevalence of *Y. enterocolitica* biotypes, the predominance of biotype 1A in chicken meat is also supported by international studies, such as those conducted by Capita *et al.* (2002) in Spain, Lü *et al*. (2022) in China, Bonardi *et al*. (2010) in Italy, and Ye *et al.* (2015) in China. Biotype is important in epidemiology because it is used to differentiate between pathogenic and non-pathogenic strains. The trend of biotype 1A predominating in raw chicken meat is also supported by our findings. Although the prevalence of *Y. enterocolitica* in Iran has been decreasing, comparing our results with a study conducted in Iran (Soltan Dallal *et al*., 2022) shows that the prevalence of biotype 1B has been increasing. Also, previous studies show that biotype 1B was not prevalent in Iran, but unfortunately, recently, the prevalence of biotype 1B has been reported. These data indicate that necessary measures should be taken urgently in terms of hygiene standards.

Discussion on antibiogram data

The isolates showed great susceptibility to the majority of the antibiotics tested, but they were resistant to ampicillin and ceftazidime. This profile of resistance is similar to the ones reported earlier that signify susceptibility to ciprofloxacin, chloramphenicol, nalidixic acid, streptomycin, tetracycline, azithromycin, and gentamicin and universal resistance to ampicillin (Łada *et al*., 2023; Gkouletsos *et al*., 2019; Bonardi *et al*., 2010; Peng *et al*., 2018; Lü *et al*., 2022). Of interest, the resistance rates to ceftazidime and trimethoprim-sulfamethoxazole were increased compared to those recorded before, which could be a result of overuse and misuse of the antibiotics as well as inappropriate antibiotic prescriptions in both human and veterinary practices.

Discussion on virulence factors data

Regarding virulence genes, 1B/O:8 and 1B/O:NI isolates harbored *ystA*+, *virF*+, *myfA*+, *ail*+, and *inv*+ genes but lacked *ystB*. Conversely, the 1A/O:8 and 1A/O:NI strains were negative for *ystA*, *virF*, *myfA*, and *ail*, but positive for *inv*. The 1A/O:5 bio/serotype lacked all virulence genes except *inv*. Momtaz *et al*. (2013) in Iran reported that the prevalence of *yadA* was 82.60%, *inv* 100%, *ail* 95.65%, *ystA* 93.47%, and *virF* 58.69%. In 2022, Lü *et al*. reported in China that in bio/serotype 1A/O:8, virulence factors were *ystA*-, *ystB*+, *virF*-, *ail*-, and *yadA*-. In Italy, Bonardi *et al*. (2010) reported that in bio/serotype 1A/O:8, the most abundant virulence factors were *ystA*-, *ystB*+, *ail*-, and *inv*±. In 2015, Ye *et al*. (2015) isolated *Y. enterocolitica* from frozen meat varieties in China, and in bio/serotype 1A/O:8, most strains lacked the virulence genes *ail*, *virF*, and *ystA* but contained *ystB* and *inv*. Our findings were similar to the results of the aforementioned studies.

Discussion on polymerase chain reaction ribotyping data

In our study, three ribotypes (IRT1, IRT2, IRT3) were identified using PCR ribotyping, so that all strains of biotype 1B were clustered in IRT1 and IRT2, and the whole group of biotype 1A in IRT3. There was no association between a ribotype and serotypes. The Discriminatory Index was 0.48. In a study conducted by Estrada *et al*., PCR ribotyping showed that 35 strains of *Y. enterocolitica* isolated in Argentina had 9 ribotypes, and the fragment sizes ranged between 750 and 1400 bp. Their strains were divided into two clusters: Cluster A (including biotype 1A and bio/serotype 3/O:5) and Cluster B (including bio/serotype 2/O:9) with a DI of 0.885 (Estrada *et al*., 2011). Similar to Estrada's study, our study demonstrated the fact that this clustering helps to determine the genetic differences between pathogenic and non-pathogenic strains, in which pathogenic strains were classified into different groups than non-pathogenic strains. Estrada's study had a higher value of the Discriminatory Index as compared to our study. Comparing our study with that of Estrada *et al*., we found that a wider biotype range of isolates increases the chances of band pattern variation, resulting in a higher DI. Thus, the relatively low DI we observed in our study should not be viewed as a drawback of the PCR ribotyping technique, but instead as an indicator of the shallow underlying genetic diversity among the strains.

Studies have shown that some techniques, such as ribotyping,

PFGE, and PCR ribotyping have good discriminatory power for genetic diversity studies of *Y. enterocolitica* strains (Estrada *et al*., 2011). In general, recent studies have introduced whole genome sequencing (WGS) as the gold standard for genetic diversity studies of *Y. enterocolitica* (Huang *et al*., 2023), which we also believe is a suitable method due to the biotypic diversity of this bacterium.

Conclusions

This paper shows that virulence factors are important in distinguishing between *Y. enterocolitica* biotypes 1A and 1B but fail in distinguishing between serotypes. Some markers that were found to be specific to biotypes 1B and 1A were the genes *ail*, *ystA*, *ystB*, *virF,* and *myfA*. Our results indicate bio/serotypes 1A/O:NI and 1A/O:8 to be the highest ones that were isolated in chicken meat. Not surprisingly, all the isolates were found to be 100% ampicillin-resistant, and resistance to trimethoprim-sulfamethoxazole and nitrofurantoin is increasing in Iran. The prevalence of highly pathogenic strains of *Y. enterocolitica* (1B/O:8) increases the importance of effective measures to control the risk of infection through eating contaminated poultry meat. Also, PCR ribotyping identified a new ribotype among biotype 1B strains, as well as made it clear there was clear genetic differentiation between highly pathogenic and non-pathogenic biotypes.

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Online supplementary material:
Supplementary Table 1. Biochemical reaction of 58 strains of *Yersinia enterocolitica* isolated from raw chicken samples.
Supplementary Table 2. Sequence of primers used.
Supplementary Figure 1. Polymerase chain reaction product electrophoresis of virulence factor genes.

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