Brazilian Journal of Biology
ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

https://doi.org/10.1590/1519-6984.229998
Original Article

Virulence genotyping and antimicrobial resistance profiles of *Yersinia enterocolitica* isolated from meat and meat products in Egypt

G. A. Younis[a], *R. M. Elkenany*[a]* and *H. A. Dowidar*[b]

[a]Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Mansoura University, 35516, Mansoura, Egypt

[b]Al-Azhar University, Mansoura, Egypt

*e-mail: dr_rashavet22@yahoo.com

Received: October 14, 2019 – Accepted: March 18, 2020 – Distributed: May 31, 2021
(With 7 figures)

Abstract

Pathogenic *Yersinia enterocolitica (Y. enterocolitica)* is one of the food-borne entero-pathogen responsible for yersiniosis in humans. The purpose of this research was to survey the prevalence, virulence-associated genes, and antimicrobial resistance of *Y. enterocolitica* isolated from meat and meat product samples in Egypt. Forty-one (5.9%) out of 700- samples of chicken meat, beef, ground beef, and sausage were positive *Y. enterocolitica* with a high prevalence in chicken meat (12%). Five virulence genes (*ail*, inv, *ystA*, *ystB,* and *yadA*) were characterized among 41 *Y. enterocolitica* isolates with variable frequencies. Among the strains tested, the *ystB* gene was detected with a high percentage (78.1%), followed by *inv* gene (70.7%), *ail* gene (14.6%), *ystA* gene (12.2%), and *yadA* gene (2.4%). A high resistance rate was estimated to amoxicillin-clavulanic acid (100%), followed by cefazolin (95%), ampicillin (65.9%), and doxycycline (51.2%), whilst a high sensitivity rate was observed to gentamicin and ciprofloxacin (97.6% each). Interestingly, the multidrug resistance was specified in the 70.7% of strains and showing 13 resistance patterns. Based on nucleotide sequence analysis of the *16s rRNA* gene, the phylogenetic tree showed the genetic relatedness amongst *Y. enterocolitic*a isolates. These findings highlighted the emergence of virulent and multidrug-resistant pathogenic *Y. entrocolitica* in retailed meat and meat products in Egypt.

Keywords: Yersinia enterocolitica, meat and meat products, virulence genes, antimicrobial resistance, *16s rRNA* gene.

Genotipagem de virulência e perfis de resistência antimicrobiana de *Yersinia enterocolitica* isolados de carne e derivados no Egito

Resumo

A *Yersinia enterocolitica* patogênica (*Y. enterocolitica*) é um dos enteropatógenos de origem alimentar responsáveis pela yersiniose no ser humano. O objetivo desta pesquisa foi avaliar a prevalência, genes associados à virulência e resistência antimicrobiana de *Y. enterocolitica* isolada de amostras de carne e produtos à base de carne no Egito. Quarenta e um (5,9%) de 700 amostras de carne de frango, carne bovina, moída e linguiça foram *Y. enterocolitica* positivas, com alta prevalência em carne de frango (12%). Cinco genes de virulência (*ail, inv*, *ystA*, *ystB* e *yadA*) foram caracterizados entre 41 isolados de *Y. enterocolitica* com frequências variáveis. Entre as cepas testadas, o gene *ystB* foi detectado com uma alta porcentagem (78,1%), seguido pelo gene *inv* (70,7%), *ail* genes (14,6%), gene *ystA* (12,2%) e gene *yadA* (2,4%). Foi estimada uma alta taxa de resistência ao ácido amoxicilina-clavulânico (100%), seguida de cefazolina (95%), ampicilina (65,9%) e doxiciclina (51,2%), enquanto uma alta taxa de sensibilidade foi observada para gentamicina e ciprofloxacina (97,6% cada). Curiosamente, a resistência a múltiplas drogas foi especificada em 70,7% das cepas e mostrando 13 padrões de resistência. Com base na análise da sequência nucleotídica do gene *rRNA 16s*, a árvore filogenética mostrou a relação genética entre isolados de *Y. enterocolitica*. Esses achados destacaram o surgimento de *Y. entrocolitica* patogênica virulenta e multirresistente em carnes e produtos à base de carne no Egito.

Palavras-chave: Yersinia enterocolitica, carne e produtos à base de carne, genes de virulência, resistência antimicrobiana, gene *rRNA 16s*.

1. Introduction

Yersinia genus belongs to the Enterobacteriaceae family and encompasses three well-recognized human pathogens which are *Y. enterocolitica*, *Yersinia pseudotuberculosis,* and *Yersinia pestis* (Carniel, 2006). *Y. enterocolitica* is one of the most important pathogens responsible for foodborne gastroenteritis (Yersiniosis) in Western and Northern Europe

(EFSA, 2018). Other clinical syndromes associated with *Y. enterocolitica* are enterocolitis, mimicking appendicitis, acute mesenteric lymphadenitis, post- infectious arthritis, and systemic infections, so occasion fatal sepsis (Neubauer et al., 2001). *Y. enterocolitica* is often isolated from humans, a variety of animals, food and the environment (Falcão et al., 2006). Pigs are carriers of *Y. enterocolitica* without clinical signs in their oral cavity, on tongues, and then excrete these bacteria in their feces (Paixão et al., 2013).

The presence of virulence genes and virulence plasmids were applied for the estimation of pathogenic *Y. enterocolitica* strains (Platt-Samoraj et al., 2006; Peruzy et al., 2017). Virulence genes, *ail, ystA,* and *ystB* are located on the bacterial chromosome (Thong et al., 2018). The Ail protein is encoded by the *ail* gene and only occurs in pathogenic *Y. enterocolitica*, it contributes to bacterial adhesion to the host cell as well as strengthens resistance to the bactericidal effects of complement (Thoerner et al., 2003). Moreover, the *yst* gene, which encodes the thermostable enterotoxin Yst protein, advanced the invasion of the *Y. enterocolitica* into host cells (Atkinson and Williams, 2016). The *ystA* and *ystB* are produced by pathogenic and non-pathogenic *Y. enterocolitica*, respectively (Howard et al., 2006). The *yadA* gene is one of the most important virulence plasmids of *Y. enterocolitica*, its product is implicated in auto-agglutination, serum resistance, in addition to adhesion (Atkinson and Williams, 2016). Also, *virF* (*lcrF)* codes transcriptional activators of the *yop* regulon (Cornelis et al., 1989).

The estimation of Yersinia species is commonly occurred by an examination of the biochemical profile. In contrast, biochemical identification is laborious and restricted, as biochemically atypical strains might be hard to assign to a species (Nanni et al., 1991). Thus, trials were applied to improve the capability to study this bacterium in samples through the development of sensitive and specific PCR assays for the recognition of *Yersinia* species. The *Y. enterocolitica* is popular reason in cases of food poisoning and is involved in a wide range of gastrointestinal diseases, the evolution of a PCR assay that could be employed to designate *Y. enterocolitica* positive sample to a particular bio-group has considerable implications for microbiological research, and epidemiological research (Havens et al., 2003). The sequence analysis of small subunit ribosomal RNA (*16S rRNA*) or the correlated genes (*16S rDNA*), then comparing the sequence with the other bacterium is more specialized analysis to appoint genus of bacterium (Woese, 1987; Schmidt and Relman, 1994). The sequence determinants have been simplified by the practice of PCR assay to produce the targets, then direct sequence analysis of the products, consequently terminate the requirement for cloning (Bottger, 1989).

Over several years, many antibiotics have been synthesized, resulting in satisfaction with the risk of bacterial resistance. Resistant of microorganisms to antimicrobial agents as a consequence of chromosomal changes or the interchange of genetic material via plasmids and transposons (Neu, 1992). Determination of drug resistance and the detection of virulence genes have influenced a clinical investigation (Li and Fanning, 2017). *Y. enterocolitica* was previously detailed to be extremely susceptible to many antimicrobial agents exclude penicillin, ampicillin, amoxicillin-clavulanic acid, and the first-generation cephalosporins (Bolton et al., 2013). Though, the high prevalence of drug-resistant *Y. enterocolitica* strains in food and the environment have been stated in recent years, because of excessive use of antibiotics in animal farms and antibiotic-resistance bacteria/gene transmission amongst dissimilar species (Ye et al., 2016).

To the best of our data, a limited investigation is available on the estimate of *Y. enterocolitica* isolated from meat and meat products in Egypt. Therefore, in such investigation, *Y. enterocolitica* isolated from meat and meat products were tested for their virulence genes, antibiotic susceptibility as well as *16s rRNA* gene sequence analysis in Egypt.

2. Material and Methods

2.1. Samples collection

A total of 700 random representative samples of chicken meat, beef, ground beef, and sausage samples (175 samples, for each) were purchased from 100 different supermarkets and retail outlets in different localities at Dakahlia Governorate, Egypt, from October 2017 to April 2019. Each sample was weighed, marked clearly, put in a separate sterile plastic bag and kept in icebox during transportation to the laboratory. Each sample was estimated to bacteriological examination.

2.2. Isolation and Identification of Y. enterocolitica

A 25 g aliquot of each sample was put into sterile bags containing 225 mL of phosphate-buffered saline pH 7.6 added with 1% sorbitol and 0.15% bile salts and homogenized by bag mixer for 2 min. These diluted samples were incubated at 25 °C for 2-3 d in a shaker incubator. Subsequently, 0.5 mL of the enriched samples was mixed with 4.5 ml of potassium hydroxide (KOH) 0.25% and inoculated onto *Yersinia*-selective agar (Cefsulodin–Irgasan–Novobiocin (CIN) [Oxoid, UK] agar) plates which then were incubated at 30 °C for 1-2 d. The suspected small colonies with a deep red center and sharp border surrounded by a clear colorless zone with the entire edge in the CIN agar plates were selected. Such characteristic colonies for *Yersinia* were biochemically confirmed by catalase, oxidase, *triple sugar iron*, and urease tests (Wang et al., 2009; Liang et al., 2012) *Y. enterocolitica* were oxidase and H_2S-negative, catalase- and urease positive, glucose fermenter, and non-lactose-fermenter. Moreover, the determination of virulence plasmid of *Y. enterocolitica* was performed by auto-agglutination test and Congo red absorption by Congo red magnesium oxalate agar (Oxoid) (Mastrodonato et al., 2018) and were examined for its capability of biofilms production using the tube method (Hassan et al., 2011).

2.3. Molecular determination of Y. enterocolitica and virulence encoded genes

PCR assays were completely performed to detect specific *16s rRNA* gene for *Y. enterocolitica*, chromosomal-encoded virulence genes (*ail, inv, ystA,* and *ystB*) and plasmid-coded virulence genes (*yadA*). The extraction of bacterial genome DNA from purified suspected colonies was occurred by the conventional boiling method (Zeinali et al., 2015). The amplification of extracted of DNA was done in Applied Biosystem, 2720 Thermal Cycler (USA) in a total volume of 25 µL consisted of 12.5 µL of 2× PCR master mix (Promega, Madison, USA), 1 µL of individual primer (Metabion, Germany), 4.5 µL PCR-grade water, and 6 µL DNA template. The specific primers used and the PCR conditions were summarized in Table 1. The amplified PCR products were arranged on a 1.5% agarose gel which was stained by 1% ethidium bromide and photo-documented under UV illumination. *Y. enterocolitica* (ATCC 9610) was used as a model of positive control.

2.4. Sequencing of the 16s rRNA in Y. enterocolitica

The purification of amplified product was formed from one representative strain by a QIAquick PCR product extraction kit (Qiagen, Valencia, CA) and was sequenced with Bigdye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA) using an Applied Biosystems 3130 Genetic Analyzer (Hitachi, Tokyo, Japan) according to the instructions of the manufacturer.

2.5. Sequence analysis

The comparison of sequences of this strain was achieved with other strains on GenBank using BLAST 2.0 and PSI-BLAST research programs, (NCBI). A comparative analysis of sequences was made by the CLUSTAL W multiple sequence alignment program, version 1.83 of the MegAlign module of Lasergene DNAStar software Pairwise, which was intended by Thompson et al. (1994). The phylogenetic analyses were performed using maximum likelihood, neighbor-joining process, and maximum parsimony in MEGA6 (Tamura et al., 2013).

2.6. Nucleotide accession number

In this research, the nucleotide sequence of the *Yersinia enterocolitica* strain YEDH88, comprising the *16s rRNA* gene was deposited in GenBank under accession no. **MK910030**.

2.7. Antibiotic susceptibility testing

Convinced *Y. enterocolitica* strains were examined for their sensitivity to eleven commercially available antibiotic disks (Oxoid, Ltd.) on Mueller–Hinton agar (Difco) by the standard disk diffusion method as stated by the referenced of the Clinical and Laboratory Standards Institute (CLSI, 2016). Taking into consideration their clinical usage in humans and veterinary medicine, the subsequent 11 antibiotic agents were selected: amoxicillin-clavulanic acid (AMC) (30 µg); cefazolin (30 µg); ampicillin (10 µg); trimethoprim/sulfamethoxazole (SXT) (25 µg); doxycycline (30 µg); cephalotin (30 µg); kanamycin (30 µg); chloramphenicol (30 µg); ciprofloxacin (5 µg); fosfomycin (50 µg) and gentamicin (10 µg). The diameter of the inhibition zone was measured and interpreted as resistant, intermediate, or susceptible according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2016). Usage of *Escherichia coli* ATCC 9610 as a reference strain was done.

3. Results

3.1. Prevalence of Y. enterocolitica strains in meat and meat products

Of the 700 samples, 5.9% (41) *Y. enterocolitica* strains were isolated from meat and meat product samples and confirmed by PCR assay (Figure 1). Regarding types of samples, *Y. enterocolitica* strains showed a high prevalence of 12% (21/175) in chicken meat, followed by 5.1% (9/175) ground beef, sausage, and 1.1% (2/175) beef.

3.2. Determination of virulence genes in Y. enterocolitica strains

Y. enterocolitica isolates were screened for the existence of chromosomal virulence genes (*ail*, inv, *ystA*, and *ystB*) and plasmid virulence gene (*yadA)* (Figures 2-6). From all samples, the *ystB* gene was detected with a high percentage of 78.1% (32/41), followed by *inv* gene with

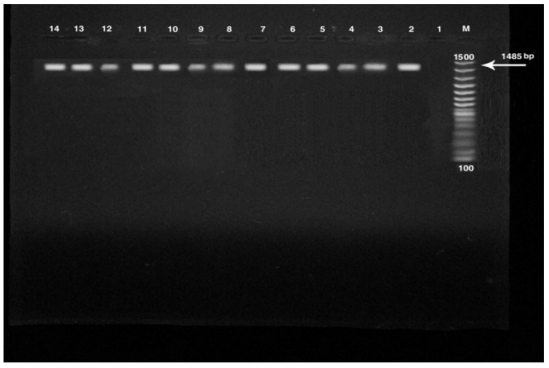

Figure 1. Prevalence of *Yersinia enterocolitica* strains *16s rRNA* gene (1485bp). Lane M: 100 bp ladder; lane 1: negative control; lane 2: positive control *Yersinia enterocolitica* subsp. *enterocolitica* ATCC 9610; lanes 3-14: positive samples.

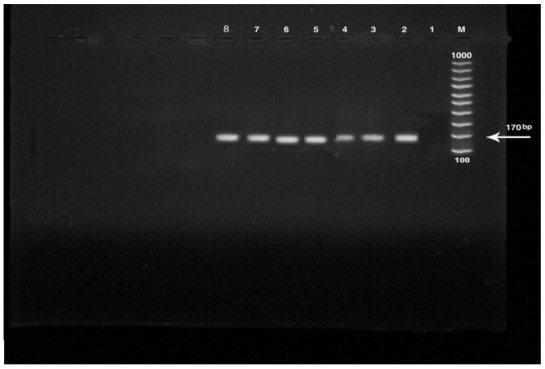

Figure 2. Prevalence of *Yersinia enterocolitica virulence ail* gene (170 bp). Lane M: 100 bp ladder; lane 1: negative control; lane 2: positive control *Yersinia enterocolitica* subsp. *enterocolitica* ATCC 9610; lanes 3-7: positive samples.

Table 1. Target genes, primer sequences and amplified segments.

Target genes	Primer Sequences	Amplified segments (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	References
				Secondary denaturation	Annealing	Extension		
Gen *16s*	AGAGTTTGATCMTGGCTCAG	1485	94 °C	94 °C	56 °C	72 °C	72 °C	Lagace et al. (2004)
rRNA	TACGGYTACCTTGTTACGACTT		5 min	30 sec	30 sec	30 sec	10 min	
Inv	CTGTGGGGAGAGTGGGGAAGTTTGG	570		94.6 °C	55.6 °C	72.6 °C		Rasmussen et al. (1994)
	GAACTGCTTGAATCCCTGAAAACCG			30 sec	30 sec	30 sec		
Ail	ACTCGATGATAACTGGGGAG	170		94.6 °C	55.6 °C	72.12 °C		Nakajima et al. (1992)
	CCCCCAGTAATCCATAAAGG			30 sec	30 sec	30 sec		
ystA	AATGCTGTCTTCATTTGGAGCA	145		94.6 °C	55.6 °C	72.6 °C		Ibrahim et al. (1997)
	ATCCCAATCACTACTGACTTC			30 sec	30 sec	30 sec		
ystB	TGTCAGCATTTATTCTCAACT	180		94.6 °C	46.6 °C	72.12 °C		Platt-Samoraj et al. (2006)
	GCCGATAATGTATCATCAAG			30 sec	30 sec	30 sec		
yadA	CTTCAGATACTGGTGTCGCTGT	849		94.6 °C	60.3 °C	72.9 °C		Thoerner et al. (2003)
	ATGCCTGACTAGAGCGATATCC			30 sec	30 sec	30 sec		

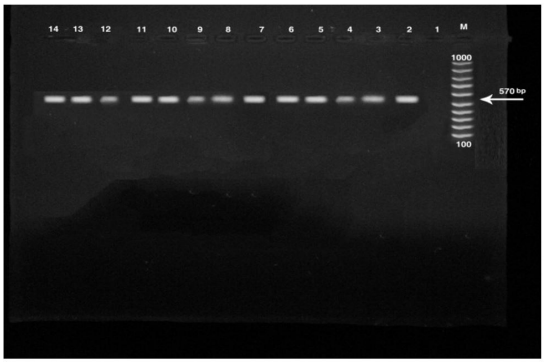

Figure 3. Prevalence of *Yersinia enterocolitica virulence inv* gene (570bp). Lane M: 100 bp ladder; lane 1: negative control; lane 2: positive control *Yersinia enterocolitica* subsp. *enterocolitica* ATCC 9610; lanes 3-14: positive samples.

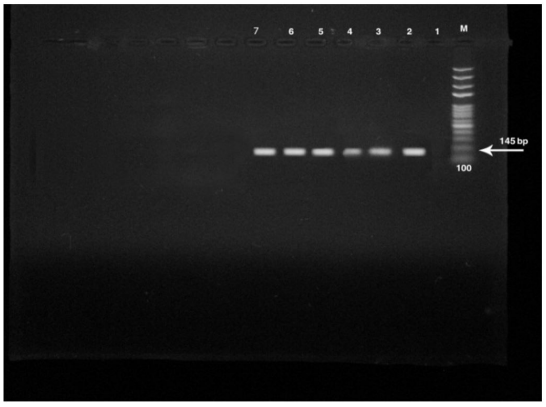

Figure 4. Prevalence of *Yersinia enterocolitica virulence ystA* gene (145 bp). Lane M: 100 bp ladder; lane 1: negative control; lane 2: positive control *Yersinia enterocolitica* subsp. *enterocolitica* ATCC 9610; lanes 3-6: positive samples.

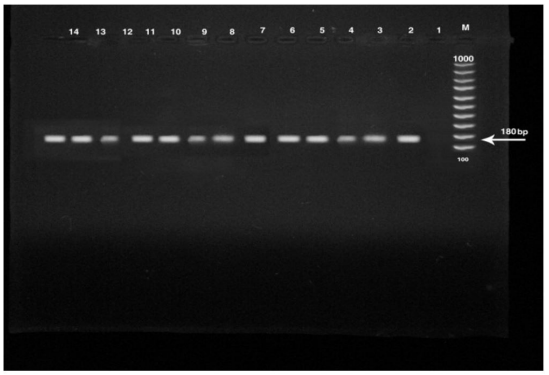

Figure 5. Prevalence of *Yersinia enterocolitica virulence ystB* gene (180bp). Lane M: 100 bp ladder; lane 1: negative control; lane 2: positive control *Yersinia enterocolitica* subsp. *enterocolitica* ATCC 9610; lanes 3-14: positive samples.

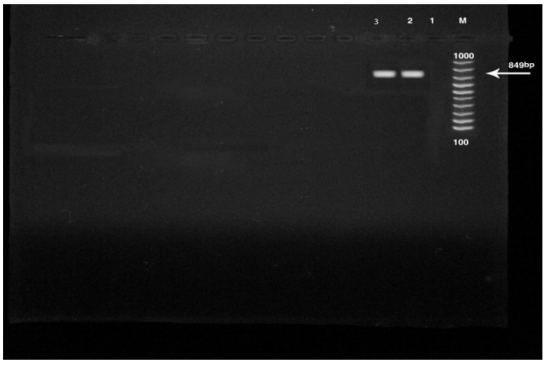

Figure 6. Prevalence of *Yersinia enterocolitica virulence yadA* gene (849bp). Lane M: 100 bp ladder; lane 1: negative control; lane 2: positive control *Yersinia enterocolitica* subsp. *enterocolitica* ATCC 9610; lanes 3: positive sample.

70.7% (29/41), *ail* gene 14.6% (6/41), *ystA* gene 12.2% (5/41) and *yadA* gene 2.4% (1/41).

Virulence genes of *Y. enterocolitica* strains were distributed as shown in Table 2. The chromosomal (*ail*, *inv*, *ystA*, and *ystB)* encoded virulent genes were demonstrated in the several categories of *Y. enterocolitica* strains in meat and meat products. Of note, plasmid *(yadA)* encoded virulence gene was determined only in one isolate (No. 1), obtained from ground beef. Twelve isolates from chicken meat, two isolates from beef, nine isolates from ground beef and sausage carried the gene *ystB*. Ten isolates from chicken meat, two isolates from beef, nine isolates from ground beef, and eight from sausage had the *inv* gene. Two isolates from chicken meat and ground beef, one isolate from beef and sausage contain the *ail* gene.

3.3. Identification of the sequence of the 16s rRNA gene in Y. enterocolitica strain

Sequencing of the *16s rRNA* gene from one purified PCR product of one *Y. enterocolitica* strain YEDH88 isolated from ground beef was achieved. This *Y. enterocolitica* strain YEDH88 was identical to several strains of *Y. enterocolitica subsp. enterocolitica* with 98.7-99.8% similarity and then, the sequence was recorded in GenBank under the accession number **MK910030**. Furthermore, the phylogenetic tree based on nucleotide sequence analysis of the *16s rRNA* genes is indicating the genetic relationship among 26 *Y. enterocolitica* strains found in different countries (Table 3) (Figure 7).

3.4. Results of antibiotic susceptibility testing

The phenotypic resistance of *Y. enterocolitica* isolates using the disk diffusion assay displayed a high resistance to AMC (100%), followed by cefazolin (95%), ampicillin (65.9%), and doxycycline (51.2%). On the other hand, the highest susceptibility rate to gentamicin and ciprofloxacin (97.6% each) was observed, followed by SXT and chloramphenicol (92.7% each), kanamycin and cephalotin (85.4% each), and fosfomycin (70.7%) (Table 4). Furthermore, thirteen resistance patterns were identified in the examined isolates (Table 2). Among

Table 2. Source, resistance pattern and virulence genes of *Y. enterocolitica* isolates.

Strain No.	Serotype	Source	Resistance pattern	Virulence gene				
				Ail	*inv*	*ystA*	*Ystb*	*yadA*
1	*Y.enterocolitica*	Ground beef	AMC/CFZ/AM/ DOX/ SXT/KF/C	+	+	+	+	+
2	*Y.enterocolitica*	Ground beef	AMC/CFZ/AM/ FOS /KF/K	+	+	-	+	-
3	*Y.enterocolitica*	Ground beef	AMC/CFZ/AM /FOS/DOX/KF/C	+	+	+	-	-
4	*Y.enterocolitica*	Beef	AMC/CFZ/AM/ FOS/DOX	-	+	-	+	-
5	*Y.enterocolitica*	sausage	AMC/CFZ/AM/ FOS/ DOX/	+	+	+	+	-
6	*Y.enterocolitica*	Sausage	AMC /CFZ/AM / DOX	-	+	-	+	-
7	*Y.enterocolitica*	Chicken meat	AMC/CFZ/AM/ DOX/ SXT	-	+	-	+	-
8	*Y.enterocolitica*	Chicken meat	AMC/CFZ/AM/ DOX/ SXT	-	+	-	+	-
9	*Y.enterocolitica*	Chicken meat	AMC/CFZ/AM/ DOX	-	+	-	+	-
10	*Y.enterocolitica*	Chicken meat	AMC/CFZ/AM/DOX/FOS	-	+	-	+	-
11	*Y.enterocolitica*	Chicken meat	AMC /CFZ/AM/DOX	-	+	-	+	-
12	*Y.enterocolitica*	Ground beef	AMC CFZ/AM/FOS	-	+	-	+	-
13	*Y.enterocolitica*	Ground beef	AMC /CFZ/AM/DOX	-	+	-	+	-
14	*Y.enterocolitica*	Beef	AMC /CFZ/AM/DOX	-	+	-	+	-
15	*Y.enterocolitica*	Sausage	AMC /AM/DOX/K	-	+	-	-	-
16	*Y.enterocolitica*	Sausage	AMC / CFZ /AM	-	+	-	-	-
17	*Y.enterocolitica*	Chicken meat	AMC / CFZ /AM	-	-	-	+	-
18	*Y.enterocolitica*	Chicken meat	AMC / CFZ/AM/FOS	+	+	+	+	-
19	*Y.enterocolitica*	Sausage	AMC /AM/DOX	-	+	-	-	-
20	*Y.enterocolitica*	Chicken meat	AMC / CFZ/AM/DOX	-	+	-	-	-
21	*Y.enterocolitica*	Ground beef	AMC / CFZ/AM/DOX	-	-	-	+	-
22	*Y.enterocolitica*	Chicken meat	AMC / CFZ/AM/DOX	-	-	-	+	-
23	*Y.enterocolitica*	Sausage	AMC / CFZ/AM/DOX	-	+	-	-	-
24	*Y.enterocolitica*	Sausage	AMC / CFZ/AM/DOX	-	+	-	-	-
25	*Y.enterocolitica*	Chicken meat	AMC / CFZ /AM	-	+	-	+	-
26	*Y.enterocolitica*	Chicken meat	AMC / CFZ /AM	-	+	-	-	-
27	*Y.enterocolitica*	Ground beef	AMC / CFZ /AM	-	+	-	+	-
28	*Y.enterocolitica*	Sausage	AMC / CFZ	-	-	-	+	-
29	*Y.enterocolitica*	Chicken meat	AMC CFZ/FOS/DOX	+	+	+	-	-
30	*Y.enterocolitica*	Chicken meat	AMC / CFZ	-	-	-	+	-
31	*Y.enterocolitica*	Ground beef	AMC / CFZ	-	-	-	+	-
32	*Y.enterocolitica*	Chicken meat	AMC / CFZ	-	-	-	+	-
33	*Y.enterocolitica*	Sausage	AMC / CFZ/DOX	-	+	-	+	-
34	*Y.enterocolitica*	Ground beef	AMC / CFZ	-	+	-	+	-
35	*Y.enterocolitica*	Chicken meat	AMC / CFZ	-	-	-	+	-
36	*Y.enterocolitica*	Chicken meat	AMC / CFZ	-	-	-	+	-
37	*Y.enterocolitica*	Chicken meat	AMC / CFZ	-	-	-	+	-
38	*Y.enterocolitica*	Chicken meat	AMC / CFZ	-	+	-	+	-
39	*Y.enterocolitica*	Chicken meat	AMC / CFZ	-	+	-	+	-
40	*Y.enterocolitica*	Chicken meat	AMC / CFZ	-	-	-	+	-
41	*Y.enterocolitica*	Chicken meat	AMC / CFZ	-	-	-	+	-
Total %	41	41	41(100)	6(14.6)	29(70.7)	5(12.2)	32(78.1)	1(2.4)

AMC: amoxicillin-clavulanicacid; CFZ: cefazolin; AM: Ampicillin; SXT: Sulphamethoxazol-Trimethoprim; DOX: doxycyclin; KF: Cephalotin; K: Kanamycin; C: Chloramphenicol; FOS: fosfomycin.

these resistance patterns, the most common pattern was AMC/CFZ represented by 12 (29.3%) strains followed by AMC/CFZ/AM/DOX displayed by 10 (24.3%) strains. Remarkably, multidrug resistance (MDR) to more than two antimicrobial classes was demonstrated in 29 out of 41 (70.7%) strains to show 13 resistance patterns. Of note, the presence of virulence determinants (*ail*, inv, *ystA*, *ystB,* and *yadA)* in different *Y. enterocolitica* strains recovered from meat and meat product samples showed different antimicrobial resistance patterns as illustrated in Table 2. The detailed analysis exhibited relations of resistance phenotypes with potential virulence genes.

Table 3. Detailed information of *16s rRNA* gene sequence of *Yersinia enterocolitic*a used in this study with identity % of other strains in other countries.

Isolate number	Accession number	Country	Year	Isolation source	Identity with strain in this study
2516-87	CP009838	USA	2015	unknown	**99.5%**
8081	CP009846.1	Russia	1993	Fatal septicemia	**99.0%**
WA	CP009367.1	USA	2015	Homo sapiens	**98.7%**
FC1820	MH174080.1	China	2018	Waste	**99.3%**
FE80313	HE803739.1	Finland	2012	human faeces	**99.6%**
FE81536	HE803738.1	Finland	2012	Humanfaeces	**99.7%**
FE80919	HE803741.1	Finland	2012	Humanfaeces	**99.6%**
CVUAS	HQ222845.1	Germany	2012	Salmo trutta	**99.6%**
DSM 13030	NR_116786.1	Finland	2019	unknown	**99.5%**
B-4-3	JF922124.1	China	2011	bamboo shoot spoilage	**99.7%**
Arma5a-a	KM888075.1	Finland	2014	modified atmosphere-packaked broiler	**99.5%**
KM1	EU523225.1	China	2008	refrigerator of a meat factory	**99.4%**
FE81198	HE803743.1	Finland	2012	human faeces	**99.4%**
FE81535	HE803744.1	Finland	2012	human faeces	**99.4%**
WSTY 161ON	KM888073.1	Finland	2014	wild boar tonsils	**99.5%**
FYE155	KM888020.1	Finland	2014	vole, intestine	**99.5%**
FE81651	HE803746.1	Finland	2012	human faeces	**99.4%**
WSTY 3D2	KM888074.1	Finland	2014	wild boar tonsils	**99.7%**
T51A14.1	KM888064.1	Finland	2014	vole, tongue	**99.8%**
PUFSTb04	KT266804.1	India	2015	beef meat	**99.5%**
HYE9180	KM888038.1	Finland	2014	human feces	**99.7%**
FC1790	MH177866.1	China	2018	Waste	**99.5%**
FC1783	MH174076.1	China	2018	Waste	**99.5%**
NBRC 10569	AB682267.1	Japan	2012	unknown	**98.7%**
ATCC 9610	NR_116785.1	USA	2014	unknown	**98.7%**
FE80890	HE803740.1	Finland	2012	human faeces	**99.6%**

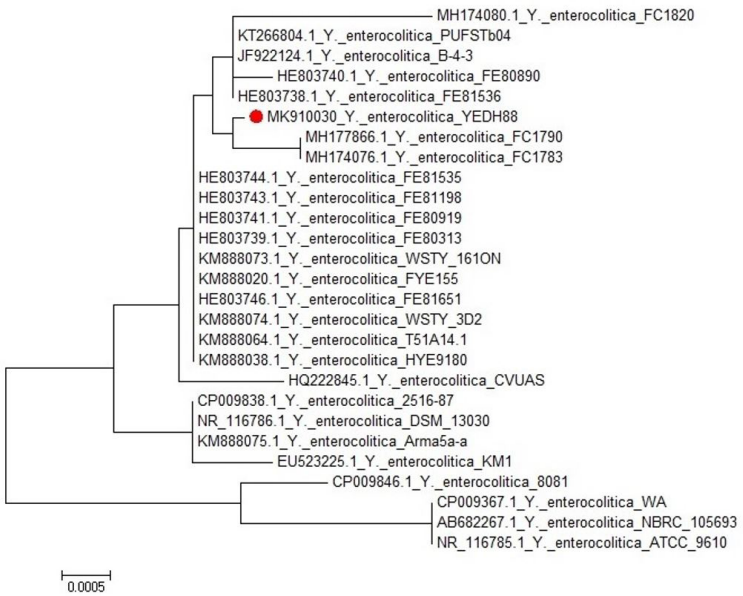

Figure 7. Phylogenetic tree showing the genetic relatedness among *Yersinia enterocolitic*a strains based on nucleotide sequence analysis of the *16s rRNA* gene.

Table 4. Results of antimicrobial resistance susceptibility test

Antibiotics	Sensitive	Intermediate	Resistant
	No. (%)	No. (%)	No. (%)
Amoxicillin-clavulanicacid (AMC)	0	-	41(100)
Cefazolin (CFZ)	0	2(4.8)	39(95)
Ampicillin (AM)	0	14(34)	27(65.9)
Trimethoprim/sulfamethoxazole (SXT)	38(92.7)	0	3(7.3)
Doxycyclin (DOX)	10(24.4)	10(24.4)	21(51.2)
Cephalotin (KF)	35(85.4)	3(7.3)	3(7.3)
Kanamycin (K)	35(85.4)	4(9.8)	2(4.8)
Chloramphenicol (C)	38(92.7)	2(4.8)	2(4.8)
Ciprofloxacin (CIP)	40(97.6)	1(2.4)	0
Fosfomycin(FOS)	29(70.7)	4(9.8)	8(19.5)
Gentamicin (G)	40(97.6)	1(2.4)	0

4. Discussion

Y. enterocolitica is one of the most common Gram-negative bacteria causing food poisoning, widespread in The environment, water, meats, and dairy products. Meat and meat products had been suggested as the main source of *Y. enterocolitica* for humans. In the United States and Canada, the high incidence of *Y. enterocolitica* was reported, even though this might be a result of the improvement of investigation and detection methods (Wesley et al., 2008). In this work, *Y. enterocolitica* overall prevalence was 5.9% in the meat and meat products. *Y. enterocolitica* prevalence in chicken meat (12%) was slightly lesser than previous investigations: 16.7% in Turkey (Ozdemir and Arslan, 2015), 21.6% in Iran (Dallal et al., 2010), 32.5% in Italy (Bonardi et al., 2010), and 55% in Spain (Capita et al., 2002).

Moreover, the prevalence in ground beef (5.1%) was consistent with Ozdemir and Arslan (2015), while this is lower than those detected by other authors (Sırıken, 2004). Also, the occurrence of *Y. enterocolitica* in beef (1.1%) was compatible with previous studies (Dzomir, 2006), whereas it is lower than that reported in previous investigations as 27.9% in Turkey (Sırıken, 2004) and 5.5**%** in Poland (Zadernowska and Chajęcka-Wierzchowska, 2016). Furthermore, the low frequency (5.1%) of sausage contamination found is expected and following other reports (Ramirez et al., 2000) since it is a cooked and vacuum-packed product. It is well known that the risk of contamination is difficult to be eliminated in this type of product (Logue et al.. 1996). The contamination of meat and other meat products with bacteria from a slaughterhouse as well as from many equipments during processing, workers, air, and water could have occurred (Ray, 2004). In this study, chicken meat had the highest prevalence of *Y. enterocolitica* due to slaughter the poultry outside the slaughterhouse under unhygienic condition, also high levels of bacterial contamination occur especially during defeathering and water chilling. further, elevate the contamination levels during evisceration of the carcasses, washing, and processing due to contamination by workers (Pieniz et al., 2019). On the other hand, the beef has the lowest percentage of isolates where the slaughter is done in a slaughterhouse of Mansoura city at Dakahlia Governorate, Egypt, showed lower contamination due to absence of section to slaughter pigs which are the carrier of *Y. enterocolitica* and excrete this bacterium in their feces (Paixão et al., 2013).

The key role in the *Y. enterocolitica* pathogenicity is the virulence genes (chromosome and plasmid) (Zheng et al., 2008). In the current research, the presence of chromosomal virulence genes (*ail, inv, ystA,* and *ystB*) and plasmid virulence genes (*yadA*) in *Y. enterocolitica* was occurred by PCR assay. The high occurrence of the *ystB* (78.1%) and *inv* (70.7%) gene in the examined strains was detected. In accordance, Bhagat and Virdi (2007) found a high prevalence of *inv* (100%) and *ystB* (79%) genes in pathogenic isolates of *Y. enterocolitica*. In contrast, Kot et al. (2007) determined the low occurrence of *inv* (13.75%) and *ystB* (4.35%) genes. Also, Ozdemir and Arslan (2015) recorded *ystB* in 20% of the isolates. Furthermore, the low percentage of other examined genes (*ail,* 14.6% and *ystA,* 12.2%) detected by this investigation. There was a lot of study that had a low or negative incidence of such virulence genes (*ail* and *ystA*) in pathogenic strains of *Y. enterocolitica* isolated from meat and other meat products (Falcão et al., 2006; Kot et al., 2007; Bhagat and Virdi, 2007; Ozdemir and Arslan, 2015). On the other hand, previous reports have a high prevalence of such virulence genes isolated from humans (Zheng et al., 2008; Frazão and Falcão, 2015). Besides, The *yadA* gene was identified in 2.4% of *Y. enterocolitica* strains in this research, while Tadesse et al. (2013) detected *yadA* gene in 12.8% of *Y. enterocolitica* strains isolated from porcine. There was no detection of the *yadA* gene in any samples isolates from chicken meat (Shabana et al., 2015).

Generally, pathogenic strains should have whole virulence genes (*ail, inv, ystA,* and *yadA*) such virulence genes were perhaps cooperating to cause a public health hazard (Zheng et al., 2008). However, in this work, only one isolate containing all tested virulence genes. A lot of

the examined isolates in this research were positive for *ystB* and *inv*. On the other hand, other isolates were positive for only some of them, which could still be representative of public health hazards.

In contrast to methods of identifying bacteria using the phenotype, the genetic-based approach stands out for its consistency. The small subunit ribosomal RNA (16S rRNA), highly preserved and seldom variable within species, is one desirable candidate and is becoming a principal method for phylogeny study and species classification (Woese, 1987). Therefore, in this study, DNA sequence analysis of the *16s rRNA* gene of *Y. enterocolitica* isolate YEDH88 showed the genetic relatedness amongst 26 *Y. enterocolitica* strains isolated from many countries as shown in the phylogenetic tree (Figure 7). Similar *16s rRNA* genes were previously specified in *Y. enterocolitica* as *Y. enterocolitica* T51A14.1 **(KM888064.1)** (Murros et al., 2016), *Y. enterocolitica* FE81536 **(HE803738.1)** (Sihvonen et al., 2012), *Y. enterocolitica* DSM 13030 **(NR_116786.1)** (Murros-Kontiainen et al., 2011) Y. *enterocolitica* FE81198 **(HE803743.1)** (Wortberg et al., 2012), *Y. enterocolitica* 2516-87 **(CP009838.1)** (Johnson et al., 2015).

Antibiotic resistance in pathogenic strains has been increasingly developed around the world in particular *Y. enterocolitica* (Ozdemir and Arslan, 2015). In this study, assay results from the antimicrobial sensitivity of *Y. enterocolitica* isolates provided high resistance to AMC followed by cefazolin, ampicillin, and doxycycline that were frequently reported (Bucher et al., 2008; Fredriksson-Ahomaa et al., 2010; Frazão et al., 2017). Such high resistance to AMC and ampicillin is due to the wide distribution of β-lactamases amongst *Y. enterocolitica* isolates (Fredriksson-Ahomaa et al., 2011). In contrast, the sensitivity of *Y. enterocolitica* to gentamicin, ciprofloxacin, SXT, chloramphenicol, kanamycin, and cephalotin was noticed in previous studies (Hadef et al., 2015; Frazão et al., 2017). Gentamicin and ciprofloxacin, the most clinically important antimicrobial, has been used very successfully in *Y. enterocolitica* osteomyelitis and septic arthritis (Carniel, 2006).

Interestingly, MDR pathogenic bacteria cause stiffness in the treatment of diseases affecting humans and animals and strains MDR of *Y. enterocolitica* were associated with the rise of the the morbidity, compared to the susceptible bacterium (Drozdov et al., 1992; Jean and Hsueh, 2011). Unfortunately, the results obtained in this research detected MDR against more than two antibiotics in 70.7% of isolates with 13 resistance patterns. However, there is little studies on the multidrug-resistant strains of *Y. enterocolitica* in meat and meat products in Egypt compared to other countries. Similar results in MDR *Y. enterocolitica* isolates were observed by many investigators (Bonardi et al., 2010; Thong et al., 2018). Younis et al. (2019) identified a low prevalence of MDR *Y. enterocolitica* isolates (23.33%) from retail and processed meat in Egypt, while Ye et al. (2015) and Peng et al. (2018) demonstrated the high occurrence of MDR *Y. enterocolitica* strains (94.3%, 92.3%) in China, respectively. There are many reasons for the elevation of percentages of multidrug-resistant pathogenic bacterium including the illegal and inaccurate prescription of antibiotics, the long term use and even abuse of feed antibiotics (Dehkordi et al., 2014). While the global use of antibiotics in modern animal husbandry plays an important role in improving the prevention and control of animal diseases, the elevation of animal growth and high feed utilization rate, frequent use of veterinary antibiotics much more than the essential treatment of animal disease, and most of these antibiotics are used to improve the feed conversion and feed additives (Tsubakishita et al., 2010; Silva et al., 2011). Elevation of the microbial resistance problem and expectations for future use of antimicrobial drugs remains uncertain. Consequently, measures must be taken to reduce this problem, for example, to control the antibiotics used, to develop the research to better understand the genetic mechanisms of resistance, and to continue studies to improve new drugs. The ultimate goal is to provide the patient with appropriate and effective antimicrobial drugs (Höfling et al., 2010). Whatever, diversity in the use of many antibiotics in the treatment of humans and animals causes elevation of the microbial resistant bacterium to the human beings. The transmission of antibiotic-resistant bacteria to humans may be caused by the means of food, so the antimicrobial resistance of isolates in human and animal foods should be monitored continuously to avoid public health hazards (McDermott et al., 2002).

Generally, the acquirement of the antimicrobial resistance in the bacteria influences their virulence according to two alternative ways; elevated resistance is followed by elevated virulence (a positive effect) or raising antimicrobial resistance decreases a bacterium virulence (seemingly negative effect) (Beceiro et al., 2013). The presence of virulence determinants (*ail*, *inv*, *ystA*, *ystB,* and *yadA)* in different *Y. enterocolitica* isolates displayed various antimicrobial resistance patterns in this investigation. This research verified the dissemination of antimicrobial resistance patterns and virulence factors in the examined isolates. These results are important concerning public health and had been formerly reported (Sacchini et al., 2018). The antimicrobial resistance of bacterium is regularly developing and horizontal gene transmission by plasmids plays the main role (Rozwandowicz et al., 2018).

5. Conclusion

Meat and meat products might be a source of virulent and multi-drug resistant strains of *Y. enterocolitica* that might have a potential public-health hazard in Egypt. Accordingly, strict hygienic measures should be applied to minimize *Y. enterocolitica* contamination in meat and meat products.

References

ATKINSON, S. and WILLIAMS, P., 2016. *Yersinia* virulence factors: a sophisticated arsenal for combating host defences. *F1000Res*, vol. 5, pp. F1000. PMid:27347390.

BECEIRO, A., TOMÁS, M. and BOU, G., 2013. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? *Clinical Microbiology Reviews*, vol. 26, no. 2, pp. 185-230. http://dx.doi.org/10.1128/CMR.00059-12. PMid:23554414.

BHAGAT, N. and VIRDI, J.S., 2007. Distribution of virulence-associated genes in *Yersinia enterocolitica*biovar 1A correlates with clonal groups and not the source of isolation. *FEMS Microbiology Letters*, vol. 266, no. 2, pp. 177-183. http://dx.doi.org/10.1111/j.1574-6968.2006.00524.x. PMid:17233728.

BOLTON, D.J., IVORY, C. and MCDOWELL, D., 2013. A small study of *Yersinia enterocolitica* in pigs from birth to carcass and characterisation of porcine and human strains. *Food Control*, vol. 33, no. 2, pp. 521-524. http://dx.doi.org/10.1016/j.foodcont.2013.03.039.

BONARDI, S., PARIS, A., BASSI, L., SALMI, F., BACCI, C., RIBOLDI, E., BONI, E., D'INCAU, M., TAGLIABUE, S. and BRINDANI, F., 2010. Detection, semiquantitative enumeration, antimicrobial susceptibility of *Yersinia enterocolitica* in pork and chicken meats in Italy. *Journal of Food Protection*, vol. 73, no. 10, pp. 1785-1792. http://dx.doi.org/10.4315/0362-028X-73.10.1785. PMid:21067665.

BÖTTGER, E.C., 1989. Rapid determination of bacterial ribosomal sequences by direct sequencing of enzymatically amplified DNA. *FEMS Microbiology Letters*, vol. 65, no. 1-2, pp. 171-176. http://dx.doi.org/10.1111/j.1574-6968.1989.tb03617.x. PMid:2482222.

BUCHER, M.C., MEYER, C., GRÖTZBACH, B., WACHECK, S., STOLLE, A. and FREDRIKSSON-AHOMAA, M., 2008. Epidemiological data on pathogenic *Yersinia enterocolitica* in Southern Germany during 2000-2006. *Foodborne Pathogens and Disease*, vol. 5, no. 3, pp. 273-280. http://dx.doi.org/10.1089/fpd.2007.0076. PMid:18564908.

CAPITA, R., ALONSO-CALLEJA, C., PRIETO, M., GARCÍA-FERNÁNDEZ, M.C. and MORENO, B., 2002. Incidence and pathogeniticity of *Yersinia* spp. isolates from poultry in Spain. *Food Microbiology*, vol. 19, no. 4, pp. 295-301. http://dx.doi.org/10.1006/fmic.2002.0492.

CARNIEL, E., 2006. *Y. enterocolitica* and *Y. pseudotuberculosis*. In: M. DWORKIN, S. FALKOW, E. ROSENBERG, K.H. SCHLEIFER and E. STACKEBRANDT, eds. *The prokaryotes*. New York*:* Springer, vol. 6, pp. 270-398. http://dx.doi.org/10.1007/0-387-30746-X_13.

CLINICAL AND LABORATORY STANDARDS INSTITUTE – CLSI, 2016. *Performance standards for antimicrobial susceptibility testing*. 26th ed. Wayne: CLSI.

CORNELIS, G., SLUITERS, C., DE ROUVROIT, C.L. and MICHIELS, T., 1989. Homology between *VirF*, the transcriptional activator of the *Yersinia* virulence regulon, and AraC, the *Escherichia coli* arabinose operon regulator. *Journal of Bacteriology*, vol. 171, no. 1, pp. 254-262. http://dx.doi.org/10.1128/JB.171.1.254-262.1989. PMid:2644192.

DALLAL, M.M., DOYLE, M.P., REZADEHBASHI, M., DABIRI, H., SANAEI, M., MODARRESI, S., BAKHTIARI, R., SHARIFIY, K., TAREMI, M., ZALI, M.R. and SHARIFI-YAZDI, M.K., 2010. Prevalence and antimicrobial resistance profiles of *Salmonella* serotypes, *Campylobacter* and *Yersinia spp.* isolated from retail chicken and beef, Tehran, Iran. *Food Control*, vol. 121, no. 4, pp. 388-392. http://dx.doi.org/10.1016/j.foodcont.2009.06.001.

DEHKORDI, F.S., YAZDANI, F., MOZAFARI, J. and VALIZADEH, Y., 2014. Virulence factors, serogroups and antimicrobial resistance properties of *Escherichia coli* strains in fermented dairy products. *BMC Research Notes*, vol. 7, no. 217, pp. 217. http://dx.doi.org/10.1186/1756-0500-7-217. PMid:24708594.

DROZDOV, A.V., BELOKUROVA, T.V., IL'INA, T.S., DZIUBAK, V.F. and DOMARADSKIĬ, I.V., 1992. A new R-plasmid from *Yersinia pseudotuberculosis. Molekuliarnaia Genetika, Mikrobiologiia i Virusologiia*, vol. 5, no. 6, pp. 31-32. PMid:1454082.

DZOMIR, A.Z.M., 2006 [viewed 30 July 2020]. *Isolation, identification and characterization of Yersinia spp. from meat and meat products* [online]. Malaysia: School of Graduate Studies, Universiti Putra Malaysia. Thesis in Philosophy. Available from: http://psasir.upm.edu.my/id/eprint/6712/1/FSTM_2005_3(1-24).pdf

EUROPEAN FOOD SAFETY AUTHORITY – EFSA, 2018. The european union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. *EFSA Journal*, vol. 16, no. 12, pp. 5500.

FALCÃO, J.P., FALCÃO, D.P., PITONDO-SILVA, A., MALASPINA, A.C. and BROCCHI, M., 2006. Molecular typing and virulence markers of *Yersinia enterocolitica* strains from human, animal and food origins isolated between 1968 and 2000 in Brazil. *Journal of Medical Microbiology*, vol. 55, no. 11, pp. 1539-1548. http://dx.doi.org/10.1099/jmm.0.46733-0. PMid:17030914.

FRAZÃO, M.R. and FALCÃO, J.P., 2015. Genotypic diversity and pathogenic potential of *Yersinia enterocolitica* biotype 2 strains isolated in Brazil. *Journal of Applied Microbiology*, vol. 118, no. 4, pp. 1058-1067. http://dx.doi.org/10.1111/jam.12751. PMid:25639495.

FRAZÃO, M.R., ANDRADE, L.N., DARINI, A.L.C. and FALCÃO, J.P., 2017. Antimicrobial resistance and plasmid repliconsin *Yersinia enterocolitic* astrains isolated in Brazilin 30 years. *The Brazilian Journal of Infectious Diseases*, vol. 21, no. 4, pp. 477-480. http://dx.doi.org/10.1016/j.bjid.2017.04.006. PMid:28558260.

FREDRIKSSON-AHOMAA, M., CERNELA, N., HÄCHLER, H. and STEPHAN, R., 2010. *Yersinia enterocolitica* strains associated with human infections in Switzerland 2001–2010. *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 31, no. 7, pp. 1543-1550. http://dx.doi.org/10.1007/s10096-011-1476-7. PMid:22071910.

FREDRIKSSON-AHOMAA, M., WACHECK, S., BONKE, R. and STEPHAN, R., 2011. Different enteropathogenic *Yersinia* strains found in wild boars and domestic pigs. *Foodborne Pathogens and Disease*, vol. 8, no. 6, pp. 733-737. http://dx.doi.org/10.1089/fpd.2010.0711. PMid:21288132.

HADEF, K.Z., MEDJAHDI, K., BENAMAR, I. and BOUDJEMAA, B.M., 2015 [viewed 30 July 2020]. Safety characterization of Yersinia enterocolitica strains isolated from raw milk in Western Algeria. *African Journal of Microbiological Research* [online], vol. 10, no. 1, pp. 15-22. Available from: https://www.researchgate.net/profile/Khawla_Hadef/publication/293800570_Safety_characterization_of_Yersinia_enterocolitica_strains_isolated_from_raw_milk_in_Western_Algeria/links/5b1169450f7e9b498101c030/Safety-characterization-of-Yersinia-enterocolitica-strains-isolated-from-raw-milk-in-Western-Algeria.pdf

HASSAN, A., USMAN, J., KALEEM, F., OMAIR, M., KHALID, A. and IQBAL, M., 2011. Evaluation of different detection meth¬ods of biofilm formation in the clinical isolates. *The Brazilian Journal of Infectious Diseases*, vol. 15, no. 4, pp. 305-311. http://dx.doi.org/10.1016/S1413-8670(11)70197-0. PMid:21860999.

HAVENS, J.M., MOTOGOMERY, E., GREENSON, J.K., SCOTT, M.A. and LAMPS, L.W., 2003. Pathogenic *Y. enterocolitica* DNA is detected in gastrointestinal malakoplakia. *Modern Pathology*, vol. 16, pp. 1200A.

HÖFLING, J.F., ANIBAL, P.C., OBANDO-PEREDA, G.A., PEIXOTO, I.A., FURLETTI, V.F., FOGLIO, M.A. and GONÇALVES, R.B., 2010. Antimicrobial potential of some plant extracts against *Candida* species. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 70, no. 4, pp. 1065. http://dx.doi.org/10.1590/S1519-69842010000500022. PMid:21180915.

HOWARD, S.L., GAUNT, M.W., HINDS, J., WITNEY, A.A., STABLER, R. and WREN, B.W., 2006. Application of comparative phylogenomics to study the evolution of *Yersinia enterocolitica* and to identify genetic differences relating to pathogenicity. *Journal of Bacteriology*, vol. 188, no. 10, pp. 3645-3653. http://dx.doi.org/10.1128/JB.188.10.3645-3653.2006. PMid:16672618.

IBRAHIM, A., LIESACK, W., GRIFFITHS, M.W. and ROBINS-BROWNE, R.M., 1997. Development of a high specific assay for rapid identification of pathogenic strains of *Yersinia enterocolitica* based on PCR amplification of the *Yersinia*heat-stable enterotoxin gene (*yst*). *Journal of Clinical Microbiology*, vol. 35, no. 6, pp. 1636-1638. http://dx.doi.org/10.1128/JCM.35.6.1636-1638.1997. PMid:9163505.

JEAN, S. and HSUEH, P.R., 2011. Current review of antimicrobial treatment of nosocomial pneumonia caused by multidrug-resistant pathogens. *Journal Expert Opinion on Pharmacotherapy*, vol. 12, no. 14, pp. 2145-2148. http://dx.doi.org/10.1517/14656566.2011.599320. PMid:21895553.

JOHNSON, S.L., DALIGAULT, H.E., DAVENPORT, K.W., JAISSLE, J., FREY, K.G., LADNER, J.T., BROOMALL, S.M., BISHOP-LILLY, K.A., BRUCE, D.C., COYNE, S.R., GIBBONS, H.S., LO, C.C., MUNK, A.C., ROSENZWEIG, C.N., KOROLEVA, G.I., PALACIOS, G.F., REDDEN, C.L., XU, Y., MINOGUE, T.D. and CHAIN, P.S., 2015. Thirty-two complete genome assemblies of nine *Yersinia* Species, Including *Y. pestis, Y. pseudotuberculosis,* and *Y. enterocolitica. Genome Announcements*, vol. 3, no. 2, pp. e00148-e15. http://dx.doi.org/10.1128/genomeA.00148-15. PMid:25931590.

KOT, B., TRAFNY, E.A. and JAKUBCZAK, A., 2007. Application of multiplex PCR for monitoring colonization of pig tonsils by *Yersinia enterocolitica*, including biotype 1A, and *Yersinia pseudotuberculosis. Journal of Food Protection*, vol. 70, no. 5, pp. 1110-1115. http://dx.doi.org/10.4315/0362-028X-70.5.1110. PMid:17536668.

LAGACÉ, L., PITRE, M., JACQUES, M. and ROY, D., 2004. Identification of the bacterial community of maple sap by using amplified ribosomal DNA (rDNA) restriction analysis and rDNA sequencing. *Applied and Environmental Microbiology*, vol. 70, no. 4, pp. 2052-2060. http://dx.doi.org/10.1128/AEM.70.4.2052-2060.2004. PMid:15066796.

LI, F. and FANNING, S., 2017. Antimicrobial resistance in food-related bacteria. In: J.J. JEN and J. CHEN, eds. *Food safety in China: science and technology, management and regulation*. Chichester: John Wiley & Sons, pp. 163-184. http://dx.doi.org/10.1002/9781119238102.ch11.

LIANG, J., WANG, X., XIAO, Y., CUI, Z., XIA, S., HAO, Q., YANG, J., LUO, L., WANG, S., LI, K., YANG, H., GU, W., XU, J., KAN, B. and JING, H., 2012. Prevalence of *Yersinia enterocolitica* in pigs slaughtered in Chinese abattoirs. *Applied and Environmental Microbiology*, vol. 78, no. 8, pp. 2949-2956. http://dx.doi.org/10.1128/AEM.07893-11. PMid:22327599.

LOGUE, C.M., SHERIDAN, J.J., WAUTERS, G., MCDOWELL, D.A. and BLAIR, I.S., 1996. *Yersinia spp.* and numbers, with particular reference to *Y. enterocolitica* bio/serotypes, occurring on Irish meat and meat products, and the influence of alkali treatment on their isolation. *International Journal of Food Microbiology*, vol. 33, no. 2-3, pp. 257-274. http://dx.doi.org/10.1016/0168-1605(96)01164-6. PMid:8930710.

MASTRODONATO, A.C., FAVIER, G.I., LUCERO ESTRADA, C.S.M., VIDAL, R. and ESCUDERO, M.E., 2018. Bioserotypes, virulence genes, antimicrobial susceptibility and genomic diversity of *Yersinia enterocolitica* isolates from Argentina and Chile. *Journal of Food Safety*, vol. 38, no. 5, e12491. http://dx.doi.org/10.1111/jfs.12491.

MCDERMOTT, P.F., ZHAO, S., WAGNER, D.D., SIMJEE, S., WALKER, R.D. and WHITE, D.G., 2002. The food safety perspective of antibiotic resistance. *Animal Biotechnology*, vol. 13, no. 1, pp. 71-84. http://dx.doi.org/10.1081/ABIO-120005771. PMid:12212946.

MURROS-KONTIAINEN, A., FREDRIKSSON-AHOMAA, M., KORKEALA, H., JOHANSSON, P., RAHKILA, R. and BJÖRKROTH, J., 2011. *Yersinia nurmii* sp. Nov. *International Journal of Systematic and Evolutionary Microbiology*, vol. 61, no. 10, pp. 2368-2372. http://dx.doi.org/10.1099/ijs.0.024836-0. PMid:21037032.

MURROS, A., SADE, E., JOHANSSON, P., KORKEALA, H., FREDRIKSSON-AHOMAA, M. and BJORKROTH, J., 2016. Characterization of European *Yersinia enterocolitica* 1A strains using restriction fragment length polymorphism and multilocus sequence analysis. *Letters in Applied Microbiology*, vol. 63, no. 4, pp. 282-288. http://dx.doi.org/10.1111/lam.12626. PMid:27473388.

NAKAJIMA, H., INOUE, M., MORI, T., ITOH, K., ARAKAWA, E. and WATANABE, H., 1992. Detection and identification of *Yersinia pseudotuberculosis* and pathogenic *Yersinia enterocolitica* by an improved polymerase chain reaction method. *Journal of Clinical Microbiology*, vol. 30, no. 9, pp. 2484-2486. http://dx.doi.org/10.1128/JCM.30.9.2484-2486.1992. PMid:1401022.

NANNI, F., PACIFICO, L., RENZI, A.M., VOLTERRA, L. and CHIESA, C., 1991. Urease-negative strains of *Yersinia enterocolitica* and related species isolated from human and nonhuman sources. *Contributions to Microbiology and Immunology*, vol. 12, pp. 44-49. PMid:1935112.

NEU, H.C., 1992. The crisis in antibiotic resistance. *Science*, vol. 257, no. 5073, pp. 1064-1073. http://dx.doi.org/10.1126/science.257.5073.1064. PMid:1509257.

NEUBAUER, H., SPRAGUE, L.D., SCHOLZ, H. and HENSEL, A., 2001. The diagnostic of *Yersinia enterocolitica*infections: a review on classical identification techniques and new molecular methods. *Berliner und Munchener Tierarztliche Wochenschrift*, vol. 114, no. 1-2, pp. 1-7. PMid:11225491.

ÖZDEMIR, F. and ARSLAN, S., 2015. Genotypic and phenotypic virulence characteristics and antimicrobial resistance of *Yersinia* spp. isolated from meat and milk products. *Journal of Food Science*, vol. 80, no. 6, pp. M1306-M1313. http://dx.doi.org/10.1111/1750-3841.12911. PMid:25969137.

PAIXÃO, R., MORENO, L.Z., SENA DE GOBBI, D.D., RAIMUNDO, D.C., FERREIRA, T.S., SPINDOLA, M.G., HOFER, E., FALAVINA DOS REIS, C.M., MATTÉ, M.H. and MICKE MORENO, A., 2013. Genotypic characterization of *Yersinia enterocolitica* biotype 4/O:3 isolates from pigs and slaughterhouses using SE-AFLP, ERIC-PCR, and PFGE. *Journal of Pathogens*,

vol. 2013, pp. 1-8. http://dx.doi.org/10.1155/2013/521510. PMid:23819054.

PENG, Z., ZOU, M., LI, M., LIU, D., GUAN, W., HAO, Q., XU, J., ZHANG, S., JING, H., LI, Y., LIU, X., YU, D., YAN, S., WANG, W. and LI, F., 2018. Prevalence, antimicrobial resistance and phylogenetic characterization of *Yersinia enterocolitica* in retail poultry meat and swine feces in parts of China. *Food Control*, vol. 93, pp. 121-128. http://dx.doi.org/10.1016/j.foodcont.2018.05.048.

PERUZY, M., MURRU, N., PERUGINI, A.G., CAPUANO, F., DELIBATO, E., MERCOGLIANO, R., KORKEALA, H. and PROROGA, Y.T.R., 2017. Evaluation of virulence genes in Yersinia enterocolitica strains using SYBR Green real-time PCR. *Food Microbiology*, vol. 65, pp. 231-235. http://dx.doi.org/10.1016/j.fm.2017.03.004. PMid:28400007.

PIENIZ, S., RODRIGUES, D.F., ARNDT, R.M., MELLO, J.F., RODRIGUES, K.L., ANDREAZZA, R., CAMARGO, F.A.O. and BRANDELLI, A., 2019. Molecular identification and microbiological evaluation of isolates from equipments and food contact surfaces in a hospital Food and Nutrition Unit. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 79, no. 2, pp. 191. http://dx.doi.org/10.1590/1519-6984.175350. PMid:30365634.

PLATT-SAMORAJ, A., UGORSKI, M., SZWEDA, W., SZCZERBA-TUREK, A., WOJCIECH, K. and PROCAJŁO, Z., 2006. Analysis of the presence of *ail, ystA* and *ystB* genes in *Yersinia enterocolitica*strains isolated from aborting sows and aborted fetuses. *Journal of Veterinary Medicine, Series B*, vol. 53, no. 7, pp. 341-346. http://dx.doi.org/10.1111/j.1439-0450.2006.00969.x.

RAMÍREZ, E., VÁZQUEZ-SALINAS, C., RODAS-SUÁREZ, O.R. and PEDROCHE, F.F., 2000. Isolation of *Yersinia* from raw meat (pork and chicken) and precooked meat (porcine tongues and sausages) collected from commercial establishments in Mexico City. *Journal of Food Protection*, vol. 63, no. 4, pp. 542-544. http://dx.doi.org/10.4315/0362-028X-63.4.542. PMid:10772223.

RASMUSSEN, H.N., RASMUSSEN, O.F., ANDERSEN, J.K. and OLSEN, J.E., 1994. Specific detection of pathogenic *Yersinia enterocolitica* by two-step PCR using hot-start and DMSO. *Molecular and Cellular Probes*, vol. 8, no. 2, pp. 99-108. http://dx.doi.org/10.1006/mcpr.1994.1014. PMid:7935518.

RAY, B., 2004. *Fundamental food microbiology*. 3rd ed. Florida: CRC Press.

ROZWANDOWICZ, M., BROUWER, M.S.M., FISCHER, J., WAGENAAR, J.A., GONZALEZ-ZORN, B., GUERRA, B., MEVIUS, D.J. and HORDIJK, J., 2018. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *The Journal of Antimicrobial Chemotherapy*, vol. 73, no. 5, pp. 1121-1137. http://dx.doi.org/10.1093/jac/dkx488. PMid:29370371.

SACCHINI, L., GAROFOLO, G., DI SERAFINO, G., MAROTTA, F., RICCI, L., DI DONATO, G., MIRACCO, M.G., PERLETTA, F. and DI GIANNATALE, E., 2018. The prevalence, characterisation, and antimicrobial resistance of *Yersinia enterocolitica* in pigs from Central Italy. *Veterinaria Italiana*, vol. 54, no. 2, pp. 115-123. PMid:30019328.

SCHMIDT, T.M. and RELMAN, D.A., 1994. Phylogenetic identification of uncultured pathogens using ribosomal RNA sequences. *Methods in Enzymology*, vol. 235, pp. 205-222. http://dx.doi.org/10.1016/0076-6879(94)35142-2. PMid:7520119.

SHABANA, S.M., KHALIL, S. and HEGAZY, A.E.H., 2015. Molecular characterization of *Yersinia enterocolitica* isolated from chicken meat samples. *Alexandria Journal of Veterinary Sciences*, vol. 46, no. 1, pp. 124-129. http://dx.doi.org/10.5455/ajvs.184190.

SIHVONEN, L.M., JALKANEN, K., HUOVINEN, E., TOIVONEN, S., CORANDER, J., KUUSI, M., SKURNIK, M., SIITONEN, A. and HAUKKA, K., 2012. Clinical isolates of *Yersinia enterocolitica* Biotype 1A represent two phylogenetic lineages with differing pathogenicity-related properties. *BMC Microbiology*, vol. 12, pp. 208. http://dx.doi.org/10.1186/1471-2180-12-208. PMid:22985268.

SILVA, J., LEITE, D., FERNANDES, M., MENA, C., GIBBS, P.A. and TEIXEIRA, P., 2011. *Campylobacter* spp. as a food borne pathogen: a review. *Frontiers in Microbiology*, vol. 2, pp. 200. http://dx.doi.org/10.3389/fmicb.2011.00200. PMid:21991264.

SIRIKEN, B., 2004 [viewed 30 July 2020]. The presence of *Yersinia enterocolitica*and other *Yersinia* species in ground beef in Aydın, Turkey. *Turkish Journal of Veterinary and Animal Sciences* [online], vol. 28, pp. 489-495. Available from: http://journals.tubitak.gov.tr/veterinary/issues/vet-04-28-3/vet-28-3-5-0209-1.pdf

TADESSE, D.A., BAHNSON, P.B., FUNK, J.A., MORROW, W.E., ABLEY, M.J., PONTE, V.A., THAKUR, S., WITTUM, T., DEGRAVES, F.J., RAJALA-SCHULTZ, P.J. and GEBREYES, W.A., 2013. *Yersinia enterocolitica* of porcine origin: carriage of virulence genes and genotypic diversity. *Foodborne Pathogens and Disease*, vol. 10, no. 1, pp. 80-86. http://dx.doi.org/10.1089/fpd.2011.1120. PMid:23320426.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. and KUMAR, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, vol. 30, no. 12, pp. 2725-2729. http://dx.doi.org/10.1093/molbev/mst197. PMid:24132122.

THOERNER, P., BIN KINGOMBE, C.I., BÖGLI-STUBER, K., BISSIG-CHOISAT, B., WASSENAAR, T.M., FREY, J. and JEMMI, T., 2003. PCR detection of virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and investigation of virulence gene distribution. *Applied and Environmental Microbiology*, vol. 69, no. 3, pp. 1810-1816. http://dx.doi.org/10.1128/AEM.69.3.1810-1816.2003. PMid:12620874.

THOMPSON, J.D., HIGGINS, D.G. and GIBSON, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, vol. 22, no. 22, pp. 4673-4680. http://dx.doi.org/10.1093/nar/22.22.4673. PMid:7984417.

THONG, K.L., TAN, L.K. and OOI, P.T., 2018. Genetic diversity, virulotyping and antimicrobial resistance susceptibility of *Yersinia enterocolitica* isolated from pigs and porcine products in Malaysia. *Science of Food and Agriculture*, vol. 98, no. 1, pp. 87-95. http://dx.doi.org/10.1002/jsfa.8442. PMid:28542807.

TSUBAKISHITA, S., KUWAHARA-ARAI, K., BABA, T. and HIRAMATSU, K., 2010. *Staphylococcal* cassette chromosome mec-like element in Macrococcus caseolyticus. *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 4, pp. 1469-1475. http://dx.doi.org/10.1128/AAC.00575-09. PMid:20086147.

WANG, X., CUI, Z., JIN, D., TANG, L., XIA, S., WANG, H., XIAO, Y., QIU, H., HAO, Q., KAN, B., XU, J. and JING, H., 2009. Distribution of pathogenic *Yersinia enterocolitica* in China. *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 28, no. 10, pp. 1237-1244. http://dx.doi.org/10.1007/s10096-009-0773-x. PMid:19575249.

WESLEY, I.V., BHADURI, S. and BUSH, E., 2008. Prevalence of *Yersinia enterocolitica* in market weight hogs in the United States. *Journal of Food Protection*, vol. 71, no. 6, pp. 1162-1168. http://dx.doi.org/10.4315/0362-028X-71.6.1162. PMid:18592741.

WOESE, C.R., 1987. Bacterial evolution. *Microbiological Reviews*, vol. 51, no. 2, pp. 221-271. http://dx.doi.org/10.1128/MMBR.51.2.221-271.1987. PMid:2439888.

WORTBERG, F., NARDY, E., CONTZEN, M. and RAU, J., 2012. Identification of *Yersinia ruckeri* from diseased salmonid fish by Fourier transform infrared spectroscopy. *Journal of Fish Diseases*, vol. 35, no. 1, pp. 1-10. http://dx.doi.org/10.1111/j.1365-2761.2011.01317.x. PMid:22103737.

YE, Q., WU, Q., HU, H., ZHANG, J. and HUANG, H., 2015. Prevalence, antimicrobial resistance and genetic diversity of *Yersinia enterocolitica* isolated from retail frozen foods in China. *FEMS Microbiology Letters*, vol. 362, no. 24, pp. fnv197. http://dx.doi.org/10.1093/femsle/fnv197. PMid:26472688.

YE, Q., WU, Q., HU, H., ZHANG, J. and HUANG, H., 2016. Prevalence and characterization of *Yersinia enterocolitica* isolated from retail foods in China. *Food Control*, vol. 61, pp. 20-27. http://dx.doi.org/10.1016/j.foodcont.2015.09.016.

YOUNIS, G., MADY, M. and AWAD, A., 2019. *Yersinia enterocolitica*: Prevalence, virulence, and antimicrobial resistance from retail and processed meat in Egypt. *Veterinary World*, vol. 12, no. 7, pp. 1078-1084. http://dx.doi.org/10.14202/vetworld.2019.1078-1084. PMid:31528036.

ZADERNOWSKA, A. and CHAJĘCKA-WIERZCHOWSKA, W., 2016. Prevalence, biofilm formation and virulence markers of *Salmonella sp*. and *Yersinia enterocolitica* in food of animal origin in Poland. *Lebensmittel-Wissenschaft + Technologie*, vol. 75, pp. 552-556. http://dx.doi.org/10.1016/j.lwt.2016.10.007.

ZEINALI, T., JAMSHIDI, A., RAD, M. and BASSAMI, M., 2015. A comparison analysis of Listeria monocytogenes isolates recovered from chicken carcasses and human by using RAPD PCR. *International Journal of Clinical and Experimental Medicine*, vol. 8, no. 6, pp. 10152-10157. PMid:26309714.

ZHENG, H., SUN, Y., MAO, Z. and JIANG, B., 2008. Investigation of virulence genes in clinical isolates of *Yersinia enterocolitica. FEMS Immunology and Medical Microbiology*, vol. 53, no. 3, pp. 368-374. http://dx.doi.org/10.1111/j.1574-695X.2008.00436.x. PMid:18557936.