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

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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. enterocolitica* isolates. These findings highlighted the emergence of virulent and multidrug-resistant pathogenic *Y. enterocolitica* 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 línguica 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. Entretanto, o *ystB* gene foi detectado com uma alta porcentagem (78,1%), seguido pelo gene *inv* (70,7%), *ail* genes (14,6%), *ystA* gene (12,2%) e *yadA* gene (2,4%). O alto teor de resistência foi estimado ao amoxicilina-clavulánico (100%), seguido por 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. enterocolitica* 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
Yersinia enterocolitica in meat and meat products

(YEFSA, 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 (Falcao 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 (Paixao 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; Peruzi 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 H2S-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); ceftazolin (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...
Table 1. Target genes, primer sequences and amplified segments.

| Target genes | Primer Sequences | Amplified segments (bp) | Primary denaturation | Amplification (35 cycles) | Final extension | References |
|--------------|------------------|-------------------------|----------------------|--------------------------|-----------------|------------|
| Gen 16s rRNA | AGAGTTTGATCMTGGCTCAG | 1485 | 94 °C | 94 °C | 56 °C | 72 °C | 72 °C | Lagace et al. (2004) |
| Inv          | TACGGYTACCTTGTACGACTT | 5 min | | 30 sec | 30 sec | 30 sec | 10 min |
| Inv          | CGTTGGGAGAGTGGGAGTTTGG | 570 | 94.6 °C | 55.6 °C | 72.6 °C | | Rasmussen et al. (1994) |
| Ail          | CTGTGGGGGAGGCTGGGAAGTTTGG | | 94.6 °C | 55.6 °C | 72.12 °C | | Nakajima et al. (1992) |
| Ail          | GACTGACTAAGACTGAGGAG | 170 | 94.6 °C | 55.6 °C | 72.12 °C | | |
| Ail          | CCCCAGTAATCCATAAAGG | | 30 sec | 30 sec | 30 sec | | |
| ystA         | AATGCTGTCTTCATTGAGGAC | 145 | 94.6 °C | 55.6 °C | 72.6 °C | | Ibrahim et al. (1997) |
| ystB         | ATCCCAATCACTGACTTC | 180 | 94.6 °C | 46.6 °C | 72.12 °C | | Platt-Samoraj et al. (2006) |
| yadA         | TGTGAGATATCTGCTGCAATGCTG | 849 | 94.6 °C | 60.3 °C | 72.9 °C | | Thoerner et al. (2003) |
| yadA         | ATGCCCTGACTAGACGATATCC | | 30 sec | 30 sec | 30 sec | | |
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 \textit{Y. enterocolitica} isolates.

| Strain No. | Serotype       | Source     | Resistance pattern | Virulence gene |
|------------|----------------|------------|-------------------|----------------|
|            | \textit{Y.enterocolitica} | Ground beef | AMC/CFZ/AM/DOX/SXT/KF/C | \textit{All} inv ystA YstB yadA |
| 1          | \textit{Y.enterocolitica} | Ground beef | AMC/CFZ/AM/FOS/KF/K | + - + - - |
| 2          | \textit{Y.enterocolitica} | Ground beef | AMC/CFZ/AM/FOS/DOX/KF/C | + - + - - |
| 3          | \textit{Y.enterocolitica} | Beef       | AMC/CFZ/AM/FOS/DOX  | - + - + - |
| 4          | \textit{Y.enterocolitica} | sausage    | AMC/CFZ/AM/FOS/DOX/  | + + + + - |
| 5          | \textit{Y.enterocolitica} | sausage    | AMC/CFZ/AM/DOX      | - + - + - |
| 6          | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM/DOX/ SXT | - + - + - |
| 7          | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM/DOX/ SXT | - + - + - |
| 8          | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM/DOX      | - + - + - |
| 9          | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM/DOX/FOS  | - + - + - |
| 10         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM/DOX/FOS  | - + - + - |
| 11         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM/DOX      | - + - + - |
| 12         | \textit{Y.enterocolitica} | Ground beef | AMC CFZ/AM/FOS      | - + - + - |
| 13         | \textit{Y.enterocolitica} | Ground beef | AMC/CFZ/AM/DOX      | - + - + - |
| 14         | \textit{Y.enterocolitica} | Beef       | AMC/CFZ/AM/DOX      | - + - + - |
| 15         | \textit{Y.enterocolitica} | Sausage    | AMC/AM/DOX/K        | - + - + - |
| 16         | \textit{Y.enterocolitica} | Sausage    | AMC/CFZ/AM          | - + - + - |
| 17         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM          | - - - + - |
| 18         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM/DOX/FOS  | + + + + - |
| 19         | \textit{Y.enterocolitica} | Sausage    | AMC/AM/DOX          | - + - + - |
| 20         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM/DOX      | - + - + - |
| 21         | \textit{Y.enterocolitica} | Ground beef | AMC/CFZ/AM/DOX      | - - - + - |
| 22         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM/DOX      | - - - + - |
| 23         | \textit{Y.enterocolitica} | Sausage    | AMC/CFZ/AM/DOX      | - + - + - |
| 24         | \textit{Y.enterocolitica} | Sausage    | AMC/CFZ/AM/DOX      | - + - + - |
| 25         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM          | - + - + - |
| 26         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ/AM          | - + - + - |
| 27         | \textit{Y.enterocolitica} | Ground beef | AMC/CFZ/AM          | - - - + - |
| 28         | \textit{Y.enterocolitica} | Sausage    | AMC/CFZ             | - - - + - |
| 29         | \textit{Y.enterocolitica} | Chicken meat | AMC CFZ/FOS/DOX    | + + + + - |
| 30         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ             | - - - + - |
| 31         | \textit{Y.enterocolitica} | Ground beef | AMC/CFZ             | - - - + - |
| 32         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ             | - - - + - |
| 33         | \textit{Y.enterocolitica} | Sausage    | AMC/CFZ/DOX         | - + - + - |
| 34         | \textit{Y.enterocolitica} | Ground beef | AMC/CFZ             | - + - + - |
| 35         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ             | - - - + - |
| 36         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ             | - - - + - |
| 37         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ             | - - - + - |
| 38         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ             | - - - + - |
| 39         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ             | - - - + - |
| 40         | \textit{Y.enterocolitica} | Chicken meat | AMC/CFZ             | - - - + - |
| 41         | \textit{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-clavulanic acid; CFZ: cefazolin; AM: Ampicillin; SXT: Sulphamethoxazole-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 \textit{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 enterocolitica 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 | Human faeces    | 99.7%                           |
| FE80919        | HE803741.1       | Finland | 2012 | Human faeces    | 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-packaged broiler | 99.5%                           |
| KM1            | EU523225.1       | China   | 2008 | refrigerator of a meat factory | 99.4%                           |
| FE81198        | HE803743.1       | Finland | 2012 | human faeces    | 99.4%                           |
| FE801535       | 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     | AB662267.1       | 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 enterocolitica strains based on nucleotide sequence analysis of the 16s rRNA gene.
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 lower 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 (Sirken, 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), whereas it is lower than those detected by other authors (32.5% in Italy (Bonardi et al., 2010), and 55% in Spain (Capita et al., 2002).

| Antibiotics                        | Sensitive No. (%) | Intermediate No. (%) | Resistant 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                |

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* 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

Table 4. Results of antimicrobial resistance susceptibility test
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 TS1A14.1 (KM888064.1) (Murro et al., 2016), Y. enterocolitica FE81536 (HE803738.1) (Silvonen et al., 2012), Y. enterocolitica DSM 13030 (NR_116786.1) (Murro-Kontiainen et al., 2011) Y. enterocolitica FE81198 (HE803743.1) (Wortberg et al., 2012), Y. enterocolitica 2516-87 (CP009983.1) (Johnson et al., 2015).

Antibiotic resistance in pathogenic strains has been increasingly developed around the world in particular Y. enterocolitica (Ozdemin 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., 2015). 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 to the susceptible bacterium (Droz dov 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 (Dekhordi 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 (Tsukahashita 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.

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