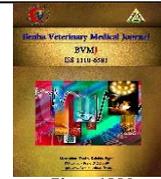




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Prevalence of *Yersinia Enterocolitica* and other species in some retailed chicken meat products

Safia Zahran^{1,*}, Abobakr M. Edris¹, Nermeen F. Elshophary², Walid S. Arab¹

¹ Department of Food control, Faculty of Veterinary Medicine, Benha University, Egypt

² Animal Health Research Institute, Tanta, Egypt

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ABSTRACT

The purpose of the current work is to learn more about *Yersinia* spp. Isolated from chicken meat products. A total of 100 samples were obtained from various retail markets at Tanta, Gharbia governorate, Egypt. Represented by chicken burger, Pane, luncheon, and nuggets (25 of each). *Yersinia* spp was isolated from chicken burger, Pane, luncheon, and nuggets at 8%, 32%, 8%, and 16% respectively. Antimicrobial tolerance has been studied by using the disk dispersion methods, *Yersinia* isolates' levels of resistance were assessed to the seven various antimicrobial drugs. Sixteen isolates indicated resistance to Vancomycine (43.75%), gentamycin, and Cefotaxime, respectively, as well as Doxycycline (93.75), Amoxicillin-Clavulanic acid (62.5), ciprofloxacin (50%) and erythromycin (50%) in that order. Detection of *Yersinia* strains was successfully performed with traditional and recent methods (PCR technique) to investigate these genes 16S rRNA, ystA, ystB. The discovery of *Yersinia* spp. in products containing chicken meat showed that the need for more knowledge about the danger connected with the transportation and preparation of chicken.

1. INTRODUCTION

Poultry meat is regarded as a particularly nutritious diet with little fat and cholesterol amount at a reasonable cost as well as used all over the globe. Its limited shelf-life, even at cool temperatures, is due to its extreme expiration (Mantilla et al., 2011) In Egypt, chicken meat provides fast, cheap meat dinners and resolves the issue of fresh meat shortage. The whole healthy birds and animals tissues that have been slaughtered are essentially aseptic, however the meat become contaminated throughout preprocessing by workers, hands, their clothes, the surroundings or external factors making it inappropriate for human consumption. A risk to society might be presented by infected chicken and its derivatives (Ahmed and Ismail, 2010). More pathogenic and spoilage-causing bacteria are present in chicken carcasses compared to many other kinds of food, and the body can become contaminated during processing procedures such as de-padding, burning, and removing, in addition through cross-contamination with other birds and equipment used for handling (González-Fandos and Dominguez, 2006). There is currently lacking information on the frequency of food-borne illnesses in Egypt, including Yersiniosis, a gastroenteritis infection, which is believed to be the third-most common gastroenteritis infection worldwide after salmonellosis and campylobacteriosis. A psychoactive enteropathogen that may survive at cold temperatures as *Y. enterocolitica* that found in water and food (Nesbakken, 2015) . People who ingest *Y. enterocolitica*-contaminated animal products, milk and cheese run a severe danger to their health, particularly newborns and young children (Bonardi et al., 2010).

According to Bottone et al. (2005), *Yersinia enterocolitica* is a gram negative, short, facultative anaerobic, psychrotrophic, linear coccobacilli or rod bacterium (0.5-1-2 m) that does not produce spores. It may develop in a broad variety of temperatures, 22-25°C the ideal limit for its development. Certain *Yersinia* strains have the capacity to endure temperature which are as cold as 5 °C, however its developing is very poor lower than 0 °C (Bergann et al., 1995) . According to Ryan et al. (2004), enterocolitis, enteritis, fever, chronic diarrhea, abdominal discomfort, mesenteric lymphadenitis and pseudo appendicitis are the major typical clinical signs of *Y. enterocolitica* infection. The recognition of such organisms using conventional bacteriological tests is hard as well as time consuming and is not appropriate with regular handling of a substantial number of samples, which is required due to growing public concern connected to the food borne illness and the economy. By concentrating on certain genes that code for the development of the organism's pathogenicity elements, genotype-based diagnostic tests may quickly and accurately diagnose food-borne illnesses. Microorganisms may be rapidly and accurately detected using the PCR approach (Krajinović et al., 2007). The aim of this study is to find *Y. enterocolitica* using conventional and molecular approaches, including the finding of 16S rRNA gene, and to estimate the prevalence of *Yersinia* spp. in chicken meat products. *Y. enterocolitica*'s biotypes, morphological pathogenicity characteristics, the presence of plasmids (ystA and tstB), and antimicrobial-resistant patterns of all *Yersinia* spp. from these chicken meat products were also subjected on this study.

Correspondence to: walids444@gmail.com

2. MATERIAL AND METHODS

2.1. Ethical Approval

This research was approved by Institutional Animals Care and Use Committee of faculty of veterinary medicine, Benha university (approved number BUFVTM) 08-07-23)

2.2. Collection of samples

A total of 100 samples of chicken meat products from various marketplaces (50g) were obtained, rapidly transferred to the microbiological lab (Animal Research Center, Tanta) under aseptic factors, and then submitted to bacterial tests.

2.3. Isolation and purification of *Yersinia* spp (ISO 10273:2003)

The chicken meat product samples (10g) were stomached with a 90 mL of aseptic Peptone Sorbitol Bile (PSB) broth (Fluka, Steinheim, Germany) by using a stomacher device (BagMixer400; Inter science, Paris, France) in accordance with ISO 10273:2003 procedure. This mixture was divided into 10 mL and added to 90 mL of Irgasan Ticarcillin Chlorate (ITC) broth base (Fluka) with ticarcillin and potassium chlorate supplements. The specimens were cultured in ITC and PSB broth for a period of 48 hours each at 25 °C. Then, a loop of each of the sample obtained from each enriched culture was transferred onto MacConkey agar (MAC; Merck, Darmstadt, Germany) and Cefsulodin-Irgasan-Novobiocin (CIN) agar (*Yersinia* Selective Agar Base supplemented with *Yersinia* Selective Supplement, Oxoid, Basingstoke, UK). 0.5 ml of the improved PSB broth was added to 4.5 ml of a 0.5% potassium hydroxide (KOH) solution for the alkali treatment, and the mixture proceeded to stir for twenty seconds, (Aulisio et al., 1980). The cultures were subsequently set on CIN and MacConkey agar plates.

2.5. Characterization and identification of bacterial culture

For morphological characteristics (Mahesh et al., 2017), the gram-stain was used to differentiate between Gram negative and Gram-positive bacteria in all cultures of bacteria. Microscopic data were used to characterize morphological features. For biochemical examination; the following biochemical experiments were conducted for further characterization. Tests for movement, Oxidase, Urease, Triple Sugar Iron, Indole, and Simmon's Citrate Agar were performed according to (Schriefer and Petersen 2011).

2.6. Antibiotic sensitivity assay (ISO 10273:2003)

The antibiotic tolerance test was carried out using the Kirby-Bauer disc diffusion method in accordance with Clinical and Laboratory Standard Institute (CLSI) standards (2019). Muller Hinton Agar (MHA) media was made and placed onto the plates after being sanitized. On MHA plates, the bacterial solution was dispersed. The agar was covered with 16 antimicrobial discs from Oxoid (England): ciprofloxacin (30 µg), cefotaxime (30 µg), erythromycin (5 µg), amoxicillin clavulanic acid (20/10 µg), gentamicin (15 µg), voncomycin (30 µg), and doxycycline (5 µg). The plates were incubated overnight at 37°C. Each antibiotic inhibitory zone surrounding each disc was measured. Non-susceptibility to one or more agents in at least 3 antimicrobial classes is known as multidrug resistance (MDR)

2.7. Molecular identification of *Yersinia* spp. gene sequences (Neubauer and others 2000)

In line with the guidelines provided by the manufacturer, genomic DNA was obtained from *Yersinia* spp. Strains that had been grown in BHI broth (Merck) at 28 °C for a period of 18 hours. The DNA was extracted and kept at 20 °C. The 16S rRNA gene was used to identify the bacteria. PCR was carried out in 50 µL reaction mixtures that included 1 µL of DNA template (50 ng/µL), 5 µL of 10× PCR buffer (100 mM Tris-HCl pH 8.8, 500 mM KCl, 0.8% (v/v) nonidet P40; Fermentas, Life Sciences Corporation, Canada), 1 mM of MgCl₂ (Fermentas), 0.2 mM of dNTP mix (Fermentas), 0.15µM each of the primers (Biomers, Ulm, (Fermentas). By including molecular level water (AppliChem, Darmstadt, Germany), the overall volume was changed to 50 L. A thermal cycler (XP Thermal Cycler, Bioer Technology Co.,Ltd, Tokyo, Japan) was used to process the mixture. The subsequent procedures were used throughout the amplification process: 30 cycles of denaturation at 94 °C for one minute, warming at 55 °C for a period of 30 s, extensions at 72 °C for 30 s, and finally extension at 72 °C for 5 min were performed after the initial denaturation at 94 °C for 5 min. 100 bp DNA molecular marker to analyze the amplicons, 1.5% (w/v) agarose gel electrophoresis in 1% TBE buffer was used. Also provided as usual was DNA Analyzer (Fermentas). UV trans illumination was used to see them (DNR Minilumi Bio-imaging Systems, Jerusalem, Israel). In the current work, the 16S rRNA gene was determined using products of the PCR of 5 microbes as representations of dietary samples (2 from chicken flesh and 1 each from minced meat, raw milk, and open white cheese). The two reverse and forward primer pairs were employed by Ref Gen Biotechnology (METU Technopolis, Ankara, Turkey) to purify and analyze the selected PCR products. The results of the gene sequencing were compared to the sequences of nucleotide in the National Center for Biotechnology Information (NCBI) collections utilizing BioEdit 7.2.5.0 (Germany), 1.25 U of Taq DNA polymerase and the results of the sequencing of genes

2.8. Identification of *Yersinia* species' genotypic pathogenicity traits

According to Thoerner et al., (2003a) overview, seven *Yersinia* spp. samples were subjected to PCR analysis to check for the absence or existence of ystB and ystA using the primer pairs (Biomers). Each pathogenicity gene has been amplified using the PCR reaction combination described above that used to determine the genes ystA and ystB.

3. RESULTS

Table (1) revealed to that prevalence of *Yersinia* species in chicken meat samples were 32% 8%, 16%, 8% and 16% isolated from Panea , Burger, Nuggets and Luncheon. Table (2) revealed that serotyping of *Yersinia* species in chicken meat samples were (7) of *Y.enterocolitica* O:5.7.8.3,27 , *Pestis* O:27 (3) , *Pseudotuberculosis* O:3.O:5 (3) and one from *Intermedia* O:7, *Kristensenit* O:33 and *Ossiliensis* O:27 respectively.

Table (3) showed that *Yersinia* isolates were resistant to Doxycycline resistance (93.75), Amoxicillin-clavulanic acid resistance (62.5), Ciprofloxacin resistance (50%) and Erythromycin resistance (50%) as well as resistance to Vancomycin (43.75%), Gentamycin, and Cefotaxime (34.5%). Table (4) demonstrated multiple resistance of *Yersinia spp* isolated from chicken meat products, where most types 8 (50%) were resistance to GN, VA, CIP while 5 (31.25%) of *Yersinia* isolates were resistance to seven antimicrobial used in this study including, AMC, CTX, CIP, E, GN, VA, DO.

Table (1): prevalence of *Yersinia* species in chicken meat product samples

Types of samples	Number of examined samples	Number of positive samples	Percentage of positive samples%
Panea	25	8	32%
Burger	25	2	8%
Nuggets	25	4	16%
Luncheon	25	2	8%
Total	100	16	16%

Table (2): Serotyping and biotyping of *Yersinia* spp. Isolates from chicken meat products

Bio typing of the isolates	Serotyping of the isolate	Number
A1	<i>Y. enterocolitica</i> O:5,7,8,3,27	7
A1	<i>Pestis</i> O:27	3
A1	<i>Pseudotuberculosis</i> O:3, O:5	3
A2	<i>Intermedia</i> O:7	1
A1	<i>Kristensenit</i> O:33	1
A4	<i>ossiliensis</i> O:27	1
Total		16

Table (3): Antibiotic resistance assay of the isolated *Yersinia* spp. from chicken meat products

Antimicrobial agent	App. And Conc. (µg/disc)	Resistance N0 (%)	Susceptible N0 (%)
Amoxicillin - clavulanic acid	AMC (20/10)	10 (62.5%)	6 (34.5%)
Cefotaxime	CTX (30)	6 (34.5%)	10 (62.5%)
Ciprofloxacin	CIP (30)	8 (50%)	8 (50%)
Erythromycin	E (5)	8 (50%)	8 (50%)
Gentamicin	GN (15)	6 (34.5%)	10 (62.5%)
Voncomycin	VA (30)	7 (43.75%)	9 (56.25%)
Doxycycline	DO 54	15 (93.75%)	1 (6.25%)

Table (4): Multiple resistance of *Yersinia* spp. Isolated from chicken meat products

Antimicrobial resistance profile	Number of antimicrobials	Number of strains% <i>Yersinia</i> spp. (n=16)
AMC, CIP, E	3	3 (18.8)
GN, VA, CIP	3	8 (50)
AMC, CTX, CIP	3	3 (18.8)
AMC, CTX, CIP, E	4	4 (25)
AMC, CTX, CIP, E, GN	5	6 (37.5)
AMC, CTX, CIP, E, GN, VA	6	7 (43.8)
AMC, CTX, CIP, E, GN, VA, DO	7	5 (31.25)

4. DISCUSSION

Yersinia enterocolitica, is a prevalent worldwide strain and has an extraordinary ability to adapt to a variety of conditions and they are regularly separated from various foods (Logue et al., 1996, Mayrhofer et al., 2004). In the present work, 16% of meat products had *Yersinia* spp. so bacteria can spread to uncooked poultry and meat from the surroundings of the slaughterhouse, in addition to various devices utilized to process, people, water and air (Ray and Bhunia, 2004). The overall incidence of *Yersinia* spp. in chicken meat (16%) had been determined to be decreased in this work than that noticed by other authors as 21.6% (Dallal

et al., 2010) 32.5% (Bonardi et al., 2010) and 55% (Capita et al., 2002). The isolation rates of *Yersinia* spp. in meat samples from chicken meat ranged from 16% to 89% (Logue et al., 1996). However, Guven et al., (2010) revealed that the prevalence rate of *Yersinia* spp. in chicken flesh was just 1.3%. On the other hand, Siriken, (2004) recorded an outcome of (27.9%). The existence of pathogenic bacteria in particular foods presents a risk to users. *YstA* and *YstB* genes, which performed as a characteristic for *Yersinia* spp and a pathogenicity gene specifically which owned *YstB*, were examined using the PCR method utilizing 16rRNA (Thoerner et al., 2003c) when found that just two of the fifty biotype A1 isolates tested positive for *ystA*, whereas 43 (86%) tested positive for *ystB*. In contrast, the prevalence rate of the *ystB* gene was 16% in the *Y. enterocolitica* biotype 1A isolated from meat, which is comparable to our outcomes, and just one isolated (4%) was positive for both the *ail* and the *ystA* gene (Estrada et al., 2011) According to a study recorded by (Bonardi et al., 2010) the majority of *Y. enterocolitica* biotype 1A strains from meat specimens (84.3%) had been *ystB* positive. According to this, all of the *Y. enterocolitica* O:8 strains obtained from foods and animals in China that were classified as biotype 1A owned the *ystB* gene, but none of the virulence genes *ail*, *virF*, *ystA*, and *yadA*. In an additional investigation, all biotype 1A strains from humans and pigs tested negative for the *ystB* gene, but positive for all *yadA*, *ystA* and *virF* genes (Bolton et al., 2013). In conclusion, it was shown that strains collected from various geographical locations differed in their dispersion of the genes related to virulence in biotype 1 strains. Toxin *YstB*'s importance in virulence was demonstrated by the nursing mice test, however Ramamurthy et al., (1997) discovered the *ystB* gene in a large number of biotype 1A strains. As a result, biotype 1A should not be entirely regarded as nonpathogenic. Similar to this, the *ystB* gene was the most common in the *Y. enterocolitica* biotype 1A strains from multiple places. According to medical and other sources of information, biotype 1 strains of *Y. enterocolitica* were the main cause of the diarrhea (Thoerner et al., 2003b). According to the findings of the investigations noted above, virulence-related genes are present in *Y. enterocolitica* biotype 1A strains found in food in a similar amount to those obtained from people or other sources. The outcomes shown in table (3) showed that *Yersinia* isolates were resistant to Ampicillin (52%) and Cephalothine (98%) and that outcomes comparable to ours have been reported by Bonardi et al., (2010) in meat and (Dallal et al., 2010) in water. Similar outcomes concerning 16 isolated strains in the present study included doxycycline resistance (93.75), Amoxicillin-clavulanic acid resistance (62.5), Ciprofloxacin resistance (50%) and Erythromycin resistance (50%) as well as resistance to Vancomycin (43.75%), Gentamycin, and Cefotaxime (34.5% of each). These outcomes were not different from those reported by Kwaga and Iversen (1990). Additionally, *Yersinia* spp septic arthritis and osteomyelitis have been effectively managed with ciprofloxacin, one of the therapeutically major antimicrobials (Carniel et al., 2006) Because *Y. enterocolitica* has -lactamases, which differ based on the bio group, it frequently shows resistance when exposed to first-generation cephalosporins. Based to some researchers, *Yersinia* gastroenteritis can be managed with a fluoroquinolone and trimethoprim-sulfamethoxazole. However, certain *Yersinia* spp infections have considerable resistance to Cephalosporins of the first generation, including Cephazoline and Cephalothine (Schriefer and Petersen, 2011). In the present study, *Yersinia* spp had a Ciprofloxacin susceptibility of 50%, which was lower than

the reported resistance of 95% in Dallal et al.(2010) , in comparison to the soiled antimicrobial discs Antimicrobial resistance may therefore develop in great part due to the use of antimicrobials in human and veterinary treatment. In order to prevent the spread of antibiotic-resistant strains to humans, it is important to frequently assess the level of antimicrobial resistance of food-borne isolates (McDermott et al., 2002).

5. CONCLUSION

From the obtained results it could be concluded that the prevalence, virulence features, and resistance to antibiotics of *Yersinia spp.*, particularly *Y. enterocolitica* in poultry meat products has been identified as a potential food-borne pathogen, are all reported in this study, which makes it remarkable. In this study, it was discovered that every *Y. enterocolitica* strain belonged to the biotype 1A, which is not dangerous to people. However, they might operate as opportunistic pathogens that spread other intestinal diseases and cause diarrhea. Consequently, depending on the virulence-associated genes, *Y. enterocolitica* biotype 1 strains found in meat products may be potentially harmful.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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