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Virulence Genotyping Profiles of Cefazolin Resistance Yersinia enterocolitica Isolated From Milk and Milk Products in Egypt

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THE aim of this study was to investigate the incidence rate, characteristics, genes related with virulence, and determination of antibiotic resistance of pathogenic *Yersinia (Y.) enterocolitica* isolated from raw milk, Karish cheese, and ice cream. From July 2021 to May 2022, samples were obtained from popular markets, and street vendors in various places in Mansoura city, Dakahlia, Egypt. Milk, Karish cheese, and ice cream (50 samples each) were positive in 26 of 150 samples (17.3%) for *Yersinia enterocolitica*, with a higher prevalence in ice cream (26%). Among the 26 *Y. enterocolitica* isolates, three virulence genes (*ail*, *inv*, and *yadA*) had different frequency profiles. The inv gene was found in a significant majority of the strains tested (73.1%), followed by the ail gene (50%) and the yadA gene (26.9%). Gentamicin and ciprofloxacin were estimated to have a high sensitivity rate ((96.2% each), followed by sulphamethoxazol-trimethoprim and chloramphenicol (92.3%), then cephalotin and kanamycin (84.6%), finally fosfomycin (73.1%). Interestingly, 53.8% of strains were identified as the multidrug resistance, with 11 different resistance patterns. Nineteen strains of *Y. enterocolitica* showed biofilm-forming ability, of which seven strains had the high biofilm-forming capacity and eight other strains of *Y. enterocolitica* had moderate biofilm-forming capacity. The discovery of presumably pathogenic *Y. enterocolitica* isolates from milk and milk products implies a hazard to consumer safety especially due to the occurrence of pathogenicity potential and antibiotic resistance which necessity the implementation of control measures.

Keywords: *Yersinia enterocolitica*, Antibiotic resistance, Milk, Amoxicillin-clavulanic acid and Cefazolin.

Introduction

Three well-known diseases are included in the Yersinia genus: *Y. enterocolitica*, *Y. pestis*, and *Y. pseudotuberculosis*. *Y. enterocolitica* is a species of great importance in public health that causes disease in mammals (including humans) [1]. It is often isolated from the environment, animals and a variety of food, pretend a potential source of infection to humans [2]. As a zoonotic pathogen, *Y. enterocolitica* causes self-limiting gastroenteritis (Yersiniosis) in humans [2]. The majority of food-poising bacteria cannot live at low temperatures, however, *Y. enterocolitica* can grow and replicate in food at 4°C and can survive at -18°C, which is significant for food hygiene [1].

Y. enterocolitica is linked to foods with an animal origin (milk, milk products, meat, poultry and seafood). The main way that the *Y. enterocolitica* bacteria spread in the food supply is by cross-contamination during food handling.

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These bacteria are prevalent in soil and water [3]. Milk is considered a good medium for growth and multiplication of bacteria, Whole milk could support *Y. enterocolitica* growth at 3 °C [4]. Furthermore, *Y. enterocolitica* can reach milk due to poor hygiene during production, distribution, marketing and storage. Consumption of such contaminated milk may lead to food poisoning condition or at least shorting the shelf-life of the food. In addition, pasteurization of milk when suppressing psychrotrophic bacteria would enable opportunistic and competitor pathogen like *Y. enterocolitica* would benefit more from pasteurised milk than from raw milk [5].

For clinical diagnosis, microbiological research, and epidemiological investigations, the creation of a polymerase chain reaction (PCR) assay that may be used to assign *Y. enterocolitica* positive specimens to a specific biogroup has major consequences [6]. Researchers can study the molecular machinery and pathways that power all biological systems thanks to the tools and resources of molecular biology and functional genomics. To address the issues with conventional culture-based approaches, the molecular identification of specific targets in nucleic acids to identify *Y. enterocolitica* strains has been established [7]. To determine the virulence of isolates of *Y. enterocolitica*, the presence of the virulence genes *inv*, *ail*, and *yadA* was used [8]. The *inv* gene is the major determinant that plays a vital role by improving the entry of *Y. enterocolitica* into epithelial cells. The *ail* gene and pathogenicity have a strong correlation [9], since it has only been detected among pathogenic *Yersinia* strains in comparison with the *inv* gene which is found in all *Y. enterocolitica* isolates [10]. The *ail* gene functions were an adhesin and as an invasion factor for *Y. enterocolitica*. In addition, *ail* gene has been shown to protect the organism against the bactericidal activity of complement [11]. The *yadA* gene has to be qualified as the highest adhesion for attachment, which is essential for induction of disease. This adherence function in addition to internalization, serum resistance, autoagglutinability, resistance to phagocytosis, and cytotoxicity enable protection of the organism to be killed by neutrophils [8].

Biofilms are clusters of one or more microorganisms attached to a surface and embedded in independent matrix, biofilms are extracellular polymeric substances made of nucleic acids, lipids, proteins, and polysaccharides that are produced by the microorganisms on their own and are necessary for their survival. [12].This matrix can constant to hard surfaces (equipment, dispensing, transport, storage surfaces, and soil) or biological components (milk, meat, fruits, and vegetables). Biofilm formation has significant effects on the pathogens in a food industry environment like physical resistance (against dryness), mechanical resistance (against powerful water impulse during washing), and chemical protection (against disinfectants, chemicals and antimicrobials used in the industry) [9]. Moreover, biofilms have significant in chronic infection and show multidrug-resistant features, consequently, that becoming more resistant to antibacterial agents, phagocytes, bacteriophages, antibiotics and disinfectants [12]. The phenomenon was observed in the dairy and produce sectors of the food industry, including soft cheeses, cantaloupe, sprouts, celery, and ice cream [13].

Antibiotic-resistant bacterial genes have spread throughout numerous species as strains of *Y. enterocolitica* in the environment and in food as a result of overuse of antibiotics in dairy and beef farms. Multidrug-resistance (MDR), on the other hand, promotes the spread of antibiotic resistance in *Y. enterocolitica* when the MDR plasmids are passed from one to another. As a result, these strains develop resistance to numerous antibiotics simultaneously in environments where bacteria are continuously exposed to antibiotics. Further, MDR impedes progress to lower resistance, because so many different medicines are chosen for the same resistant bacteria or plasmids, limiting the usage of one type of antibiotic does not sufficiently lessen that drug's resistance [14].

A clinical examination has been affected by research into the impact of medication resistance and the identification of virulence genes. First-generation cephalosporins, amoxicillin-clavulanic acid, ampicillin, and penicillin are the only antibiotics that *Y. enterocolitica* has previously been engineered to be hypersensitive to these antibiotics [9,15].

The best data indicate that little is known about the amount of solitary instance of *Y. enterocolitica* from cow milk, Karish cheese, and ice cream in Mansoura city, Dakahlia, Egypt. Hence, such research on the detected *Y. enterocolitica* strains was conducted to determine their prevalence, virulence genes and antibiotic susceptibility in Mansoura city, Dakahlia, Egypt.

Material and Methods

Samples collection

One hundred and fifty random representative samples of cow milk, Karish cheese and ice cream (50 samples each) were purchased from popular markets and street vendors in various markets in Mansoura city, Dakahlia, Egypt (31.0500°N 31.3833°E) from July 2021 to May 2022. Hygiene measures are considered at all stages of sample analysis, from initiation of sample collection to final results, through sample weighing, labeling, transportation and storage.

Isolation and identification

Ninety mL of phosphate-buffered saline (PBS, Oxoid, Hampshire, UK) were used to incubate ten grammes of each sample, with the addition of 0.15% bile salts (Oxoid, Hampshire, UK), and 1% sorbitol (Sigma, Germany) and homogenised for 2 minutes in a bag mixer (BagMixer®, Interscience, France).The bags were then incubated for 2–3 days at 25 °C. Subsequently, samples was mixed with 0.5% potassium hydroxide (KOH, Sigma-Aldrich, Germany) and then, cultured onto *Yersinia*-selective Cefsulodin–Irgasan–Novobiocin agar (CIN, Oxoid, Hampshire, UK) at 25°C- 28°C for 48 h. [16]. The colonies appear as bull eyes with deep red or purple centers and sharp edges enclosed by a translucent border considered as suspected *Y. enterocolitica* [17]. On fresh CIN agar, the suspicious colonies were chosen and purified (Oxoid) and subjected to Gram staining, motility test, and some biochemical test as: catalase, oxidase, triple sugar iron, Indole, Methyl-red, Voges- Proskauer (IMViC) and Urease tests [18].

Molecular identification of the virulence-related genes in Y. enterocolitica

A specific *16s rRNA* gene, and virulence genes (*ail*, *inv*, and *yadA*) were investigated in *Y. enterocolitica* isolates. For DNA extraction, 250 µL of sterile distilled water (MEPACO-MEDIFOOD, Egypt) was combined with a loop of *Y. enterocolitica* that had been grown on CIN agar (Oxoid) for 18 hours, vortexed, and heated in a heat block (BIOBASE, USA) for 20 minutes at 95°C. The tubes were then centrifuged for 5 minutes at 10,000 rpm. Around 200 µL of the supernatant was aspirated after centrifugation and transferred to a different sterile 1.5 mL tube, where it was stored at -20°C until analysis [19]. The DNA extracted was amplified in the thermal cycler (Applied Biosystem 2720, USA) with a final volume of 25 µL consisting of 12.5 µL of 2X PCR master mix (Promega, Madison, USA), 1 µL of individual primer (Metabion, Germany), 5.5 µL PCR-grade water, and 5 µL DNA template, The DNA extracted was amplified in the thermal cycler (Applied Biosystem 2720, USA). As a positive control, *Escherichia coli* ATCC 9610 was utilised. The PCR products were amplified, then placed on a 1.5% agarose gel, stained with 1% ethidium bromide (0.5 g/mL), and seen under UV light (UV gel documentation system, Cleaver scientific Ltd, USA) [20].

Biofilms production

On the abiotic surface, the production of a biofilm on polystyrene was determined. Bacteria were cultivated in blood agar medium for 24 hours at 30°C and 37°C after being grown overnight on nutrient broth (Oxoid, UK) at 30°C and 37°C. Bacteria adhered to the inner surface of the tube were rinsed three-times with water, fixed in 2.5% glutaraldehyde, then rinsed with water and dyed for 15 minutes with 0.25% crystal violet, after the bacteria cultures had been incubated. The tubes were finally photographed to assess *Y. enterocolitica*'s capacity to build biofilms on their walls [21].

Antibiotic susceptibility testing

On Mueller-Hinton agar (Difco, USA), eleven commercially available antibiotic discs (Oxoid, Ltd.) were tested against *Y. enterocolitica* strains using the traditional disc diffusion method. Considering their practical applications in both human and veterinary medicine. Eleven antibiotic agents were selected: ciprofloxacin (CIP) (5 µg); ampicillin (AM) (10 µg); gentamicin (GN) (10 µg); trimethoprim/sulfamethoxazole (SXT) (25 µg); amoxicillin-clavulanic acid (AMC) (30 µg); cefazolin (CFZ) (30 µg); cephalotin (KF) (30 µg); chloramphenicol (C) (30 µg); doxycycline (DOX) (30 µg); kanamycin (K) (30 µg); fosfomycin (FOS) (50 µg).

Incubated the plate at 30°C for 24 hours. According to the Clinical and Laboratory Standards Institute›s recommendations, the inhibitory zone›s diameter was then measured and classified as resistance, intermediate, or sensitive. [22]. As a reference strain, *Escherichia coli* ATCC 9610 was utilised.

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 rRNA*	AATACCGCATAACGTCTTCG CTTCTTCTGC-GAGTAACGTC TACGGYTACCTTGTTACGACTT	330	94°C 5 min	94°C 30 sec	62°C 40 sec	72°C 40 sec	72°C 10 min	[50]
inv	CTGTGGGGAGAGTGGGGAAGTTTGG GAACTGCTTGAATCCCTGAAAACCG	570		94.6 °C 30 sec	55.6°C 30 sec	72.6°C 30 sec		[51]
ail	ACTCGATGATAACTGGGGAG CCCCCAGTAATCCATAAAGG	170		94.6°C 30 sec	55.6°C 30 sec	72.12°C 30 sec		[52]
yadA	CTTCAGATACTGGTGTCGCTGT ATGCCTGACTAGAGCGATATCC	849		94.6°C 30 sec	60.3°C 30 sec	72.9°C30 sec		[53]

Results

Occurrence of Y. enterocolitica strains in raw cow milk, Karish cheese and ice cream

From the 150 samples, 17.3 % (n=26) *Y. enterocolitica* strains were identified and verified by PCR assay from cow milk, Karish cheese, and ice cream samples. About sample types, *Y. enterocolitica* strains indicated a significant occurrence of 26.0% (13/50) in ice cream**,** followed by 16.0% (8/50) Karish cheese, finally 10.0 % (5/50) raw cow milk.

Detection of virulence genes in Y. enterocolitica strains

The existence of (*ail inv*, and *yadA*) virulence genes in *Y. enterocolitica* isolates was examined. A significant percentage of all samples [73.1% (19/26)] showed the presence of *inv* gene, followed by 50.0% (13/26) and 26.9% (7/26) of the ail gene and *yadA* gene. The dissemination of the virulence genes in the *Y. enterocolitica* strains is presented in Table (3). In numerous categories of *Y. enterocolitica* strains found in cow milk, Karish cheese, and ice cream, the chromosomal (*ail* and *inv*) encoded virulent genes were demonstrated.

Overall, six strains of examined samples containing all genes (*ail, inv* and *yadA),* which classified as 4 strains from ice cream and one strain from each of cow milk and Karish cheese. It is noticeable that the *yadA* gene (virulence gene encoded by the plasmid) was identified in seven isolates divided into five isolates obtained from ice cream, one isolate from raw cow milk and the other from Karish cheese. Nine isolates from ice cream, six isolates from Karish cheese, and four isolates from raw cow milk carried the *inv* gene. Eight isolates from ice cream, three isolates from Karish cheese and two isolates from raw cow milk had the *ail* gene.

Biofilms production

The pathogenic strains of *Y enterocolitic*a were examined for its ability to produce biofilm. As shown in Table (4), 73.1% (19/26) strains produce biofilm to varying degrees. Seven (26.9%) strains showed strong biofilm producer obtained from ice cream (5) and Karish cheese (2), eight (30.8%) strains show moderate biofilm producer obtained from ice cream (5) and Karish cheese (3) and four (15.4%) strains showed weak biofilm obtained from cow milk only. On the other hand, seven (26.9%) strains were non-biofilm producer as shown in Fig. (5) and Table (4).

TABLE 2. Detection of *Y. enterocolitica* strains (n=26) in Cow milk, Karish cheese and Ice cream

Types of samples	No. of samples	No. (%) of positive isolates
Cow milk	50	5 (10%)
Karish cheese	50	8 (16%)
Ice cream	50	13 (26%)
Total	150	26 (17.3%)

TABLE 3. Prevalence of virulence genes in *Y. enterocolitica* strains .

Source of strains	*inv* gene	*ail* gene	*yadA* gene
Cow milk (5)	4	2	1
Karish cheese (8)	6	3	1
Ice cream (13)	9	8	5
Total (26)	19 (73.1%)	13 (50%)	7 (26.9%)

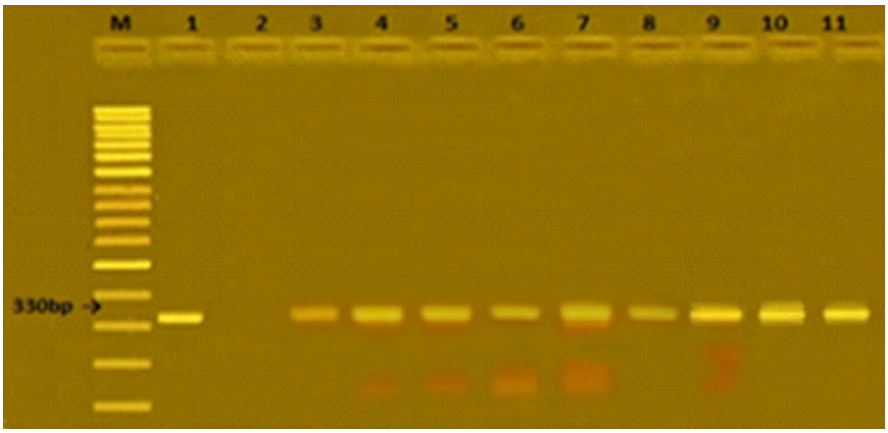

Photo 1. Representative agarose gel electrophoresis showing amplified PCR products in Y. enterocolitica isolated of 16s rRNA gene (330bp). Lane M: 100 bp ladder; lane 1: positive control; lane 2: negative control Yersinia enterocolitica; lanes -Positive samples.

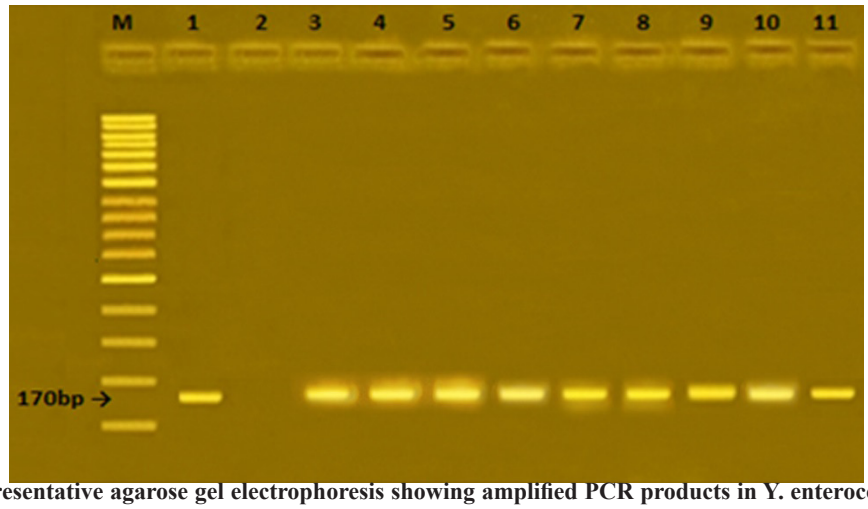

Photo 2. Representative agarose gel electrophoresis showing amplified PCR products in Y. enterocolitica isolated of ail gene (170 bp). Lane M: 100 bp ladder; lane 1: positive control; lane 2: negative control Yersinia enterocolitica; lanes 3-11: positive samples.

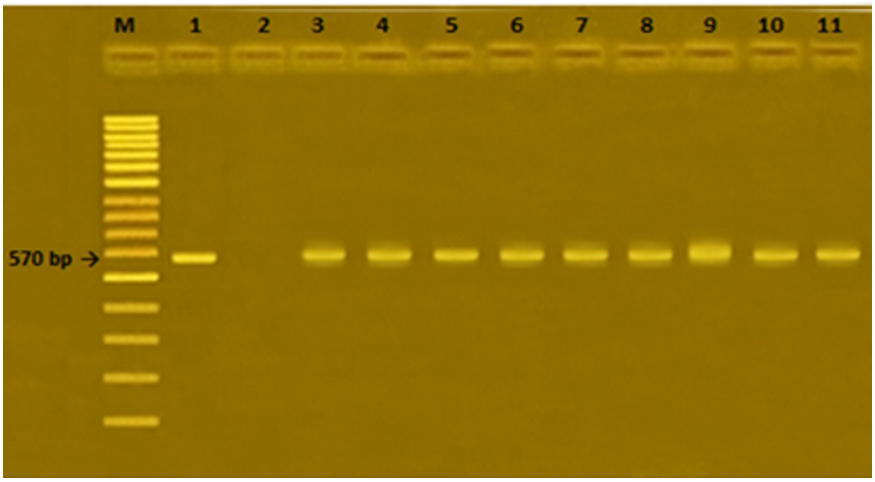

Photo 3. Representative agarose gel electrophoresis showing amplified PCR products in *Y. enterocolitica* isolated of *inv* gene (570bp). Lane M: 100 bp ladder; lane 1: positive control; lane 2: negative control *Yersinia enterocolitica*; lanes 3-11: positive samples.

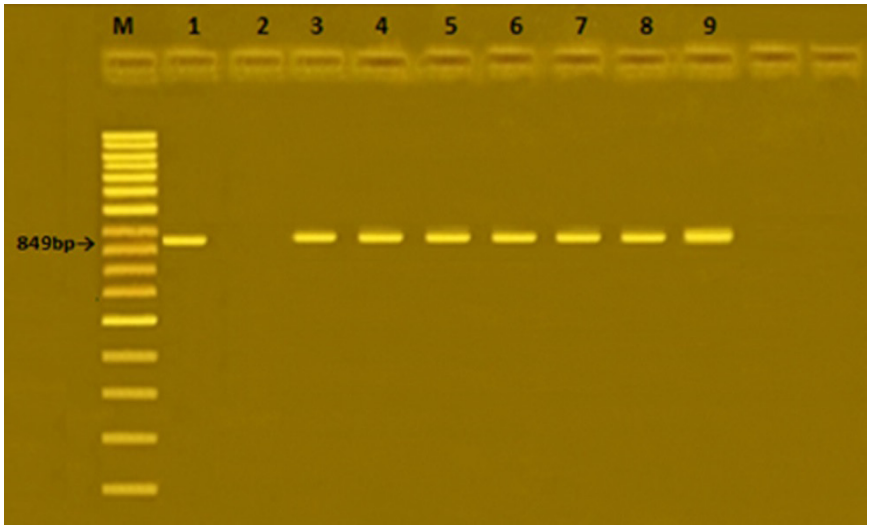

Photo 4. Representative agarose gel electrophoresis showing amplified PCR products in *Y. enterocolitica* isolated of *yadA* gene (849bp). Lane M: 100 bp ladder; lane 1: positive control; lane 2: negative control *Yersinia enterocolitica*; lanes 3: positive sample.

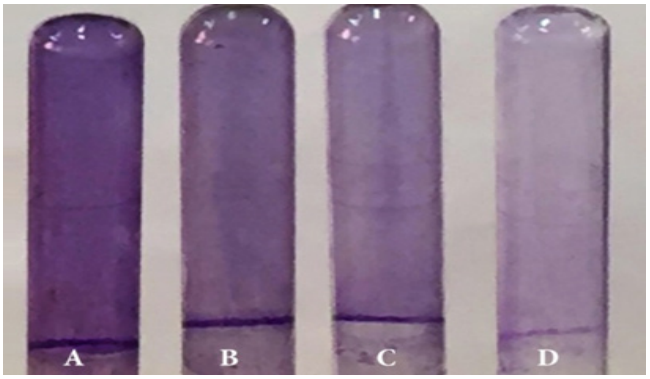

Photo 5. Detection of the degree of biofilm production using tube test. (A) Strong biofilm producer, (B) Moderate biofilm producer, (C) Weak biofilm producer, (D) Non-biofilm producer.

TABLE 4. Biofilms production degree from *Y. enterocolitica* strains (N= 26).

Biofilms production degree	No. of strains	Percentage
Strong A	7	26.9%
Moderate B	8	30.8%
Weak C	4	15.4 %
Total	19	73.1%

TABLE 5. Antimicrobial resistance test.

Antibiotic	No. of *Y. enterocolitica* isolates (n = 26)		
	Sensitive	Intermediate	Resistant
	N (%)	N (%)	N (%)
Amoxicillin-clavulanicacid (AMC) (30 μg)	0	0	26 (100)
Cefazolin (CFZ) (30 μg)	0	(7.7) 2	24 (92.3)
(**Ampicillin (AM)** (10 μg	0	9 (34.6)	17 (65.4)
(**Doxycyclin (DOX)** (30 μg	6 (23.1)	7 (26.9)	13 (50)
Fosfomycin (FOS) (50 μg)	19 (73.1)	2 (7.7)	5 (19.2)
(**Cephalotin (KF)** (30 μg	(84.6) 22	2 (7.7)	2 (7.7)
Kanamycin (K) (30 μg)	22 (84.6)	3 (11.5)	1 (3.8)
Trimethoprim/sulfamethoxazole (SXT) (25μ)	24 (92.3)	0	2 (7.7)
Chloramphenicol (C) (30 μg)	24 (92.3)	1(3.8)	1(3.8)
Ciprofloxacin (CIP) (5 μg)	25 (96.2)	1 (3.8)	0
(**Gentamicin (GN)** (10 μg	25 (96.2)	1(3.8)	0

Antibiotic susceptibility testing

The disc diffusion experiment revealed that Y. enterocolitica isolates had strong phenotypic resistance to Amoxicillin-clavulanicacid (100%), then Cefazolin (92.3%) and Ampicillin (65.4%). On the opposite side, the maximum level of sensitivity obtained by Ciprofloxacin and Gentamicin (96.2% each), then Chloramphenicol and SXT (92.3% each), Cephalotin and Kanamycin (84.6% each), and Fosfomycin (73.1%) **(**Table 5**)**. Also, the studied isolates showed variation in eleven resistance profiles (Tables 6,7). The most frequent pattern among these resistance patterns was AMC/CFZ, AMC/CFZ/AM and AMC/CFZ/AM/DOX represented by 5 (19.2 %) strains then AMC /AM/ DOX, AMC/CFZ/DOX and AMC/CFZ/ FOS displayed by 2 (7.7 %) strains.

Surprisingly, 14 of 26 bacteria (53.8%) demonstrated multidrug resistance (MDR) a minimum of two classes of antibiotics, and 11 of them displayed a distinct resistance pattern. Notably, distinct *Y. enterocolitica* strains detected from cow milk, Karish cheese, and ice cream samples displayed differential antimicrobial resistance patterns due to the occurrence of virulence gene (*ail*, *inv*, and *yadA*) (7). After careful study, it was shown that there is a connection between the resistance phenotype and possible virulence genes.

TABLE 6. Source of isolated strains of *Y. enterocolitica* and their resistance pattern and virulence genes.

Strain No.	Serotype	Source of Isolated Strain	Resistance pattern	Virulence gene		
				inv	*ail*	*yadA*
1	*Y.enterocolitica*	Cow milk	AMC/CFZ/AM/ DOX/ SXT/KF/C	+	+	+
2	*Y.enterocolitica*	Cow milk	AMC/CFZ/AM/ FOS / KF/K	+	+	-
3	*Y.enterocolitica*	Cow milk	AMC/CFZ/AM /FOS/DOX	+	-	-
4	*Y.enterocolitica*	Cow milk	AMC/CFZ/AM/ DOX	+	-	-
5	*Y.enterocolitica*	Cow milk	AMC/CFZ /DOX	-	-	-
6	*Y.enterocolitica*	Karish cheese	AMC /CFZ/AM / DOX	+	+	+
7	*Y.enterocolitica*	Karish cheese	AMC/CFZ/AM/ DOX/ SXT	+	+	-
8	*Y.enterocolitica*	Karish cheese	AMC/CFZ/AM/ DOX	+	-	-
9	*Y.enterocolitica*	Karish cheese	AMC/CFZ / DOX	-	+	-
10	*Y.enterocolitica*	Karish cheese	AMC/CFZ /DOX/FOS	+	-	-
11	*Y.enterocolitica*	Karish cheese	AMC /CFZ/AM	+	-	-
12	*Y.enterocolitica*	Karish cheese	AMC/CFZ /FOS	+	-	-
13	*Y.enterocolitica*	Karish cheese	AMC /CFZ/AM	-	-	-
14	*Y.enterocolitica*	Ice cream	AMC /CFZ/AM	+	+	+
15	*Y.enterocolitica*	Ice cream	AMC /AM/DOX	+	+	-
16	*Y.enterocolitica*	Ice cream	AMC / CFZ	+	+	-
17	*Y.enterocolitica*	Ice cream	AMC / CFZ	-	-	-
18	*Y.enterocolitica*	Ice cream	AMC / CFZ/AM/FOS	+	+	+
19	*Y.enterocolitica*	Ice cream	AMC /AM/DOX	+	+	-
20	*Y.enterocolitica*	Ice cream	AMC / CFZ/AM	-	+	+
21	*Y.enterocolitica*	Ice cream	AMC / CFZ	+	-	-
22	*Y.enterocolitica*	Ice cream	AMC / CFZ/AM	+	-	-
23	*Y.enterocolitica*	Ice cream	AMC / CFZ/AM/DOX	+	+	+
24	*Y.enterocolitica*	Ice cream	AMC / CFZ/AM/DOX	+	+	+
25	*Y.enterocolitica*	Ice cream	AMC / CFZ	-	-	-
26	*Y.enterocolitica*	Ice cream	AMC / CFZ	-	-	-
Total%	**26%**	--------	----------	19 (73.1%)	13 (50%)	7 (26.9%)

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

TABLE 7. Resistance pattern in isolated *Y. enterocolitica*

Resistant pattern	Number of antibiotics	N &% of isolates
AMC/CFZ	2	5 (19.2 %)
AMC/CFZ/AM	3	5 (19.2 %)
AMC/CFZ/AM/DOX	4	5 (19.2 %)
AMC/CFZ/DOX	3	2 (7.7 %)
AMC /AM/ DOX	3	2 (7.7 %)
AMC/CFZ/ FOS	3	2 (7.7 %)
AMC/CFZ/AM/FOS	4	1(3.8 %)
AMC/CFZ/ AM/DOX/SXT	5	1(3.8 %)
AMC/CFZ/AM/DOX/KF	5	1(3.8 %)
AMC/CFZ/AM/FOS/KF/K	6	1(3.8 %)
AMC/CFZ/AM/DOX/SXT/KF/C	7	1(3.8 %)
Total	-	26 (100%)

AMC, Amoxicillin-clavulanicacid ; CFZ, Cefazolin; AM, Ampicillin; DOX, Doxycyclin; FOS, Fosfomycin; KF, Cephalotin; K, Kanamycin; SXT, Sulphamethoxazol-Trimethoprim; C, Chloramphenicol.

Discussion

Many different types of bacteria are harmful to humans and cause food poisoning. The Gram-negative bacteria *Y. enterocolitica* is one of the most famous varieties. It's widely spread in the environment and food products of various kinds, such as dairy and its derivatives, meat and its derivatives and various vegetables and fruits. Children have a greater probability to contract the *Y. enterocolitica* than adults are, and the illness is more occurrence in the winter. *Y. enterocolitica* infection induces approximately 117,000 illnesses, 640 hospitalisations, and 35 fatalities per year in the United States [23].

Sometimes people contract the disease by consuming infected food, undercooked pork, or by coming into contact with pig butcher. Other times people contract the disease by coming into contact with infected animals or their faeces or by drinking contaminated milk or untreated water [24].

In this research, the occurrence of *Y. enterocolitica* in cow milk Karish cheese, and ice cream was 17.3% overall. *Y. enterocolitica* prevalence in ice cream (26%) was nearly similar to previous investigations, the recovered prevalence was 26.8% in Nigeria by Amasiani et al. [25], 25% in Egypt by Thabet and Thabet [26]. Other studies isolated *Y. enterocolitica* with lower incidence as (2%) by Rahimi et al. [27], (3.3%) by Özdemir and Arslan [28]**,** and (4.7%) by Mathews [29]. meanwhile, different studies recorded the highest incidence of *Y. enterocolitica* in ice cream disagree with Warke et al.[30] (40.3%) and Darwish et al.[10] (46%).

Moreover, prevalence in Karish cheese (16%) was close to those obtained by Abd El Tawab et al.[31] (14.3%), while this is higher than the results obtained by Rahimi et al.[27] (11.7%) and [32] (6.7%). Also, the prevalence of *Y. enterocolitica* in cow milk (10%) was compatible to previous studies [27,32]. While it is lower than the 20% recorded in earlier investigations for Turkey [33] and higher than that reported 5.8% in Malaysia [24]. On the other hand, Pavlović et al. [34] in Serbia and Zeinhom and Abdel-Latef [35] in Egypt performed poorly to isolate *Y. enterocolitica* from milk and milk products samples. There are likely a number of factors to blame for the discrepancies between this study's conclusions and those of several other writers, such as discrimination in sampling tactics for samples analyzed, methods of analysis, origins of

samples, environment, humidity, health measures, season, and geographic position, which might have effect positive or negative transmission of *Y. enterocolitica* [31].

Plasmids play a key role in pathogenesis of diseases caused by intestinal bacterial pathogens. Virulence plasmids are commonly large transcription elements and encode genes that advance host-pathogen interactions [36]. In the current paper, the used PCR assay to confirm the existence of the virulence genes (*ail*, *inv* and *yadA*) in the isolated strain of *Y. enterocolitica*. The results of the PCR assay show that the chromosomal virulence genes included *inv* (73.1%) and *ail* (50%) and the plasmid-encoded virulence factors included *yadA* (26.9%) were present**.** In Iraq, some authors [11] found a high prevalence of virulence genes previously present in this research included *inv* and *ail* (100%), and *yadA* (82.8%). On the other side, In Egypt, Darwish et al. [10] found a low prevalence of virulence genes previously present in this research included *inv* (34.4%), and the absence of *ail* and *yadA* genes**.** In numerous studies, pathogenic *Y. enterocolitica* examined from milk and other milk products in Lebanon and Turkey had few or no occurrences of virulence genes [37,28].

In general, pathogenic strains should typically contain the virulence genes (*ail, inv*, and *yadA*), which may have been working together to represent a risk to the public's health [38]. Notwithstanding, in this paper, only 23.1% (6/26) isolate containing all examined virulence genes, divided into four strains isolated from ice cream and one strain from each raw cow milk and Karish cheese. Many of the isolates tested in this study were positive for both *ail,* and *inv*. However, several other isolates only tested positive for part of them, which still poses a threat to the public's health.

In both natural and artificial environments, biofilm development is an essential survival strategy for bacteria. It is essential to detect the attachment of pathogenic bacteria to medical surfaces in order to identify and stop systemic infection brought on by bacteria that form biofilms. High tolerance to environmental stressors, antibiotics, disinfectants, and the host immune system is displayed by these structured bacterial populations embedded in an amorphous matrix [39]. In this study, 73.1% of the strains could produce biofilms to varying degrees, which were divided into seven strains that showed strong biofilm producers, eight strains that showed moderate biofilm producers, and four strains that showed poor biofilm production. This result is slightly more than the result reported by Younis et al. [40], 70% of isolate stains can form biofilm. On the other side, the incidence of biofilm formation is low, in some studies reaching 2.1% of isolated samples [3].

Although antibiotics are an essential component of contemporary therapy, bacteria's capacity to evolve resistance is making them less and less effective [28]. According to our findings, every isolated strain was resistant to AMC, then cefazolin (92.3%) and ampicillin (65.4%), that result agrees with many authors [41,42]**.** One the other side, the isolated strains were sensitive to gentamicin and ciprofloxacin (96.2%) then, trimethoprim/ sulfamethoxazole and chloramphenicol (92.3%) finally, kanamycin and cephalothin (84.6%), that result agrees with many researchers [42,43]. There must be a change in how antibiotics are used globally. A pact to reduce needless antibiotic usage would provide the global governance that the fight against antibiotic resistance sorely needs, as well as a clear path forward for all nations. Interestingly, multidrug resistance bacteria cause sclerosis in the treatment of human and animal illness and MDR strains of *Y. enterocolitica* bacteria have been associated with increased rates of infection, compared to sensitive bacteria [44]. Unfortunately, multidrug resistance for more than two classes of antimicrobials was found in 14 of 26 strains (53.8%) for eleven resistant. The results of the tests on the antimicrobials' sensitivity were frequently in agreement with those of research from Egypt and other nations. [4,24,45]. [40] found a low incidence of MDR *Y. enterocolitica* isolates (23.33%) in Egypt, while in China [14,46] show a high incidence of MDR *Y. enterocolitica* strains (94.3%, 92.3%), respectively.

When antibiotics are taken for longer than necessary or when they are not necessary, MDR development occurs. Only a few bacteria may at first be resistant to antibiotic treatment. The more repeatedly antibiotics are used, the higher the risk of developing antibiotic resistance. The first is when genes from plasmids give bacteria genetic benefits like virulence and antibiotic resistance. The second theory is that a single resistance mechanism gives resistance to more than one antibiotic, as pumping the antibiotic out of the

cell is one resistance strategy utilized by bacteria. These pumps can occasionally detect a variety of substances, including several antibiotic types. In other words, the bacteria used a single pump to create a range of antibiotics. Another word for this is cross-resistance [47]. In this investigation, *ail*, *inv*, and *yadA*, which are virulence determinants, were discovered in various isolates of the bacteria *Y. enterocolitica* that had various antibiotic resistance patterns. This study supports the spread of virulence traits and antimicrobial resistance patterns across the investigated isolates. Bacterial antibiotic resistance is continuously increasing, and horizontal gene transmission by plasmids is a major factor [9,48,49].

Conclusion

The current research progress information on the incidence of several pathogenic *Y. enterocolitica* strains in cow milk, Karish chess, and ice cream, all of which may be sources of pathogenic and drug-resistant strains of *Y. enterocolitica* that are dangerous to Egypt's public health. The correct implementation of strict sanitary procedures is required to reduce *Y. enterocolitica* contamination of milk and milk products. The findings and recommendations of the study will inform important decision-makers in the field of health to adopt the appropriate preventive measures.

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Conflict of interest

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التركيب الجيني لمكروب اليرسينيا القولونية المقاومة للسيفازولين والمعزولة من الحليب ومنتجات الألبان في مصر

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الهدف من هذه الدراسة هو معرفة معدل انتشار، وخصائص، وجينات الضراوة، ومدى مقاومة المضادات الحيوية المتعلقة بجرثومة **اليرسينيا القولونية** المعزولة من الحليب الخام، وجبن القريش، والآيس كريم. وقد تم تجميع العينات في الفترة من يوليو ٢٠٢١ إلى مايو ٢٠٢٢ من الأسواق والمحال، والباعة الجائلين في أماكن مختلفة في مدينة المنصورة، الدقهلية، مصر. وكانت النتائج تظهر أن الحليب وجبن القريش والآيس كريم (٥٠ عينة لكل منهما) موجبة في ٢٦ من ١٥٠ عينة (١٧,٣٪)، مع انتشار أعلى في الآيس كريم (٢٦٪). من بين العزلات الإيجابية (٢٦) كانت تركيز ثلاث جينات للضراوة (ail, inv, and yadA) فكانت الغالبية من نصيب جين inv بمعدل ٧٣,١٪ يليه جين ailوجين yada.

تم تقدير معدل حساسية الجرثومة للمضادات الحيوية، فكانت الجنتاميسين والسيبروفلوكساسين الأعلى (٩٦,٢٪ لكل منهما)، يليهما سلفاميثوكسازول-تريميثوبريم والكلورامفينيكول (٩٢,٣٪)، ثم السيفالوتين والكاناميسين (٨٤,٦٪)، وأخيراً فوسفوميسين (٧٣,١٪).

من السلالات المعزولة (١٩) وجد منها سلالات متفاوتة القدرة على انتاج الغشاء الحيوي البكتيري، موزعة على ١١ نمطًا مختلفًا، منها سبع سلالات لديها قدرة عالية على تكوين غشاء حيوي وثماني سلالات أخرى من **اليرسينيا القولونية** لديها قدرة لإنتاج غشاء حيوي متوسط. إن اكتشاف **اليرسينيا القولونية** التي يُفترض أنها مسببة للأمراض في الحليب ومنتجات الألبان ينطوي على خطر جسيم على سلامة المستهلك خاصة بسبب تعاظم قدرة الجرثومة على مقاومة المضادات الحيوية. لذا نطالب بضرورة اتخاذ كافة التدابير الطبية والصحية لمواجهة هذا التلوث.

الكلمات الدالة: اليرسينيا القولونية ، مقاومة المضادات الحيوية ، الحليب ، حمض أموكسيسيلين-كلافولانيك وسيفازولين