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Genotype and Biofilm Patterns of Yersinia enterocolitica Isolated from

Milk and Some Milk Products in Mansoura City, Dakahlia, Egypt



Heba Allah Mohamed Dowidar¹ and Amira Hussein El-Baz²

¹Department of Medical Laboratory, Higher Institute of Technology for Applied Health Science, Badr Institute

for Science and Technology, Cairo, Egypt

²Department of Food Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Mansoura University,

Mansoura 35516, Egypt

Abstract

THE OBJECTIVE: to study the incidence, characteristics, virulence factors, and drug resistance assessment of Yersinia (Y.) Enterocolitica present in cow's milk, sheep milk, milk powder, and infant formula. Samples were collected between May and October 2024 from street vendors and well-known markets spread throughout Mansoura, Dakahlia, Egypt. 15 out of 150 samples (10%) tested positive for *Yersinia enterocolitica* in cow milk, sheep milk (50 samples each), milk powder, and newborn formula milk (25 samples each); the frequency was greater in milk powder (20%). According to estimates, significant phenotypic resistance to amoxicillin-clavulanic acid (100%), cefazolin (93.3%), and ampicillin (66.6%) were observed in Y. enterocolitica isolates. Interestingly, isolates with 11 distinct resistance profiles and 93.3% multidrug resistance were found. All Y. enterocolitica strains demonstrated the potential to produce biofilms, with ten strains exhibiting a moderate capacity and five additional strains exhibiting a weak capacity.The identification of Y. enterocolitica strains from milk as well as milk products that are presumably pathogenic suggests a risk to consumer safety, particularly because of the possibility of pathogenicity and antibiotic resistance, which need the use of control measures.

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

Introduction

Many foodborne bacteria are very dangerous and lethal. Foodborne illnesses delay the development of society through insertion a problem on healthcare schemes, upsetting national economies, besides creating harms for travel internationally and trade [1]. Key zoonotic infections that lead to a variety of illnesses in together persons as well as animals are Yersinia species, particularly Yersinia enterocolitica. One of the Enterobacteriaceae, Y. enterocolitica remains a facultative anaerobic, Gram-negative ,psychrotrophic zoonotic bacterium [2]. It results in yersiniosis, an intestine zoonotic infectious illness that infects an extensive variety of animals, as well as persons. This raises the rate of infection. Humans can contract yersiniosis, a foodborne infection, by consuming undercooked meat, fresh or pasteurized

milk, besides its products [3]. Additionally, inadequate hygiene during manufacturing, distribution, marketing, and storage can allow Y. enterocolitica to infect milk. Eating such tainted milk could cause food poisoning or at the very least reduce the meal's shelf life [4]. Furthermore, opportunistic and competitive pathogens like *Y. enterocolitica* would profit more from pasteurised milk than from raw milk when psychrotrophic bacteria are suppressed by pasteurization [5].

Ileitis, diarrheal infections besides mesenteric lymphadenitis, are the main signs of yersiniosis illness. Once infected food is eaten, the Yersinia colonizes in the digestive tract then causes illness. Furthermore, *Y. enterocolitica* is a bacterium that can be fatal and endanger human health. It is difficult to locate in samples because it develops at a low

*Corresponding authors: Heba Allah Mohamed Dowidar, E-mail: hebadowidar2@gmail.com Tel.: +201016320280 (Received 20 March 2025, accepted 21 April 2025)

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resembles other temperature, **b**acteria morphologically, and is difficult to detect by quick and precise methods [6]. In the 1960s, Y. enterocolitica remained initially a significant human enteropathogen [7]. Reported to be the greatest prevalent microbe in milk, this pathogen mostly comes from animal-based foods, including milk products [8]. Furthermore, the high-water activity and rich nutritional profile of milk besides milk products promote the proliferation of harmful bacteria, making them an ideal environment for bacteria that break down food then lead to foodborne illnesses [9,10].

The progress of a polymerase chain reaction technique that can classify Y. enterocolitica- samples that are positive to a specific biogroup has important effects for clinical analysis, bacteriological research, besides epidemiological investigates [11]. The techniques and resources of molecular biology and functional genomics enable researchers to examine the molecular machinery and processes that underpin all biological systems. The molecular identification of targets in nucleic acids to identify Y. enterocolitica strains has been developed as a solution to the problems with traditional culture-based methods. [12]. Conferring to an EFSA view since 2007 [13], the discovery of Ye in diet, environmental, besides clinical specimens must apply molecular analysis ways. Methods like real-time PCR are very complex in addition to purpose through amplifying particular genetic factor fragments [14].

Biofilms, which are extracellular polymeric proteins, materials composed of lipids, polysaccharides, and nucleic acids that microbial cells develop on their own cells and are essential to their survival, are collections of one or more organisms adhered to a surface and embedded in an independent matrix [15]. This matrix can be used for biological elements (milk, meat, fruits, and vegetables) or durable surfaces (equipment, dispensing, transportation, storage surfaces, and soil). The pathogens in a food industry setting are significantly impacted by biofilm formation in ways such as chemical resistance (against chemicals, disinfectants, and antimicrobials used in the industry), resistance to motion (against strong water impulses during washing), and biological resistance (against dryness) [16]. Additionally, biofilms are important in chronic infections and exhibit multidrug-resistant characteristics, which makes them more resistant to phagocytes, bacteriophages, antibiotics, disinfectants, and antibacterial agents [13]. The phenomena were noted in the food industry's dairy and vegetable sectors, encompassing ice cream, soft cheeses, melons, sprouts, and celery [16].

The misconduct of drugs in farms, antibioticresistant bacterial genes have proliferated across many species as strains of the current studied *Y*. enterocolitica pathogen in the environment and in diet. Also when MDR plasmids are transferred from one Y. enterocolitica to a different one, they encourage the spread of antibiotic resistance. Consequently, in settings where bacteria are constantly in contact with antibiotics, these strains acquire resistance to many drugs at the same time. Additionally, MDR hinders efforts to reduce resistance because so many distinct medications are selected for the same resistant bacteria or plasmids, and restricting the use of a single antibiotic type does not adequately reduce that drug's resistance [17]. Research on the effects of antibiotic confrontation and the discovery of factors that are virulence has effected on the clinical evaluation. Penicillin, amoxicillin-clavulanic ampicillin, acid, and cephalosporins (first-generation) are the only antibiotics to which Y. enterocolitica has previously been designed to be hypersensitive [18].

According to the best available data, not much is known about the number of isolated cases of Y. enterocolitica in Mansoura, Dakahlia, Egypt, from cow milk, sheep milk, milk powder, and infant formula. To ascertain the frequency of genes and antibiotic susceptibility in Mansoura city, Dakahlia, Egypt, such research on the identified Y. enterocolitica strains was carried out.

Material and Methods

Ethics guidelines

The respective protocols were reviewed and approved by the ethics committee of Mansoura University, Egypt, under permit numbers MU-ACUC (VM.R.25.02.210)

Samples collection

From May 2024 to October 2024, 150 randomly selected representative samples of cow milk(50), sheep milk (50), milk powder (25), as well as infant formula (25) collected from street vendors besides well-known markets in Mansoura city, Dakahlia, Egypt (31.0500°N 31.3833°E). From the beginning of sample collection to the end findings, hygiene precautions are considered during sample weighing, labelling, transportation, and storage.

Isolation and identification

Twenty-five grammes of each sample were incubated in 225 milliliters of phosphate-buffered saline (PBS, Oxoid, Hampshire, UK) with addition of 0.15% bile salts (Oxoid, Hampshire, UK) plus 1% sorbitol (Sigma, Germany). The mixture was then homogenized for two minutes in a bag mixer (BagMixer®, Interscience, France). After that, the bags were incubated for two to three days at 25 °C. Then the samples were combined with 0.5% potassium hydroxide (KOH, Sigma-Aldrich, Germany) and cultivated for 48 hours at 25°C to 28°C on Yersinia Cefsulodin–Irgasan–Novobiocin selective agar (CIN, Oxoid, Hampshire, UK). The colonies resemble bull eyes with sharp edges and bright crimson or purple centers, surrounded by a translucent border that is thought to be suspicious. *Y. enterocolitica* [19,20].

Gramme staining, motility testing, and other biochemical assays, including catalase, oxidase, triple sugar iron, urease, indole, Voges-Proskauer, besides methyl-red tests, were performed on fresh CIN agar after the suspicious colonies were selected and purified (Oxoid) [21].

Molecular identification of Y. enterocolitica

Y. enterocolitica isolates were examined for a specific 16s rRNA gene. To extract DNA, a loop of Y. enterocolitica that had been cultured on CIN agar (Oxoid) for 18 hours, vortexed, and cooked in a heat block (BIOBASE, USA) for 20 minutes at 95°C was mixed with 250 μ L of sterile distilled water (MEPACOMEDIFOOD, Egypt). After that, the examined tubes were centrifuged for five minutes at 10,000 rpm. Following centrifugation, about 200 μ L of the supernatant was aspirated and sent to a separate 1.5 mL tube (sterilized), where it was kept until analysis at -20°C [22].

In a heat cycler (Applied Biosystem 2720, USA), the extracted DNA was amplified with a final volume of 25 μ L made up of 12.5 μ L of 2X PCR master mixture (Emerald Amp GT PCR Mastermix (Takara), India), 1 μ L of every primer (Metabion, Germany), 5.5 μ L of PCR-grade water, and 5 μ L of DNA template. The collected DNA was amplified using an Applied Biosystem 2720 heat cycler (USA). They used positive control such as *Escherichia coli* ATCC 9610. After the PCR products were amplified, they were put on a 1.5% agarose gel, discolored by 0.5 g/mL of 1% ethidium bromide, then examined under a Ultra violet lamp (UV gel documentation system, Cleaver scientific Ltd, USA) [23].

Biofilms production

The formation of a biofilm on polystyrene was assessed on the abiotic surface. To put it briefly, five millilitres of trypticase soy broth (TSB; Becton Dickinson, Sparks, USA) were used to inoculate a loopful of each isolate independently. Following a 24-hour incubation period at 28°C, 1 millilitre of the incubated broth was transferred into a second, sterilised 4-milliliter TSB and incubated for an additional 24-hour period at 28°C. The control was a TSB-inoculated tube. The previously inoculated broth was carefully disposed of after incubation, and the tubes were stained for 15 minutes with 1% crystal violet; any surplus stain was disposed of and cleaned with deionised water. To assess biofilm formation, the stained tubes were inverted and allowed to dry before being photographed. [24].

Antibiotic susceptibility testing

Eleven commercially available antibiotic discs (Oxoid, Ltd.) were examined using the conventional disc diffusion method against strains of Y. enterocolitica on Mueller-Hinton agar (Difco, USA). considering their usefulness in veterinary and human medicine. Eleven antibiotic agents were chosen, including ciprofloxacin (CIP) (5 µg), gentamicin (GN) (10 µg), ampicillin (AM) (10 µg), , trimethrim/sulfamethoxazole (SXT) (25 μg), cephalotin (KF) (30 µg), amoxicillin-clavulanic acid (AMC) (30 µg), cefazolin (CFZ) (30 µg), , , doxycycline (DOX) (30 µg), kanamycin (K) (30 µg, chloramphenicol (C) (30 µg), and fosfomycin (FOS) (50 µg). The dish was incubated at 30°C for 24 hours. The diameter of the inhibitory zone was then measured and categorised as resistant, moderate, or sensitive in agreement with the rules provided via the Clinical plus Laboratory Standards Institute [25]. Escherichia coli ATCC 9610 was utilized as a reference strain.

Results

The incidence of Y. enterocolitica in cow milk, sheep milk, milk powder, and infant formula

Ten percent (n=15) of the 150 samples contained *Y. enterocolitica* strains, which were identified and confirmed using a PCR assay from samples of cow milk, sheep milk, milk powder, and infant formula. Regarding sample types, the *Y. enterocolitica* strains showed a noteworthy presence of 20% (5/25) in milk powder, 10% (5/50) in cow milk, 8% (2/25) in infant formula, and 6% (3/50) in sheep milk.

Biofilms production

The capacity of the harmful *Y. enterocolitica* strains to form biofilm was investigated. Table (4) demonstrates that each strain produces biofilm to a different extent. Ten (66.6%) strains exhibited a moderate biofilm producer, derived from milk powder (4), cow milk (3), and sheep (3). Five (33.3%) bacteria exhibited weak biofilm, derived from infant formula and cow milk (2 each) and only one from milk powder (1). according to Table (4) and Fig. (2).

Antibiotic susceptibility testing

Y. enterocolitica isolates exhibited great phenotypic resistance to Amoxicillin-clavulanicacid (100%), after that Cefazolin (93.3%) and Ampicillin (66.6%), according to the disc diffusion experiment. Conversely, Ciprofloxacin and Gentamicin achieved the highest level of sensitivity (93.3% each), followed by Chloramphenicol (86.6% each), SXT (80%), Cephalotin and Kanamycin (73.3% each), and Fosfomycin (66.6%) (Table 5). Additionally, eleven resistance profiles varied across the isolates under study (Tables 6,7). The most common pattern among these resistance patterns was AMC/CFZ/AM, which was represented by three strains (20%), followed by

AMC/CFZ/AM/FOS and AMC/CFZ/AM/DOX, which were represented by two strains (13.3%).

Remarkably, all examined strain showed a clear resistance pattern, and 14 of the 15 bacteria (93.3%) showed multidrug resistance (MDR) to at least two classes of antibiotics. Interestingly, different strains of *Y. enterocolitica* found in samples of infant formula, cow milk, sheep milk, and milk powder showed varying patterns of antibiotic resistance.

Discussion

Food poisoning is caused by broad variety of microorganisms that are dangerous to people. *Y. enterocolitica* is one of the most well-known types of Gram-negative microbes. It is extensively present in the environment and in broad range of products, as meat and its derivatives, milk products, besides different fruits and vegetables. Compared to adults, children are more likely to get *Y. enterocolitica*, and illness is more common in the winter[26]. The disease can occasionally be acquired by eating contaminated food, eating undercooked pork. Other times, humans get the illness by consuming tainted milk or unclean water, or by meeting diseased animals or their excrement [27].

Yersinia enterocolitica (*Ye*) is often underreported because of the little concentration of *Yersinia* in the specimens and the numerous besides varied accompanying microorganisms in the enrichment stage that outgrow the *Yersinia*, making finding more difficult besides producing falsenegative results [28].

As demonstrated through the data, the prevalence of *Y. enterocolitica* in fresh cow milk samples was 10%, and other researchers assessed the same percentage to be 10.9% and 10.3%, respectively [29,30]. However, our results are larger than those of certain other studies [31], at 7.6% and 4%, respectively. However, other research found greater incidence rates of *Y. enterocolitica* in Egypt (36%), India (64%), and Egypt [32], where they assessed a higher incidence of *Y. enterocolitica* (34%) in cow milk samples. In contrast, in Egypt had a 5.71% frequency of *Y. enterocolitica* in sheep milk seen [33]. The primary cause of fecal pollution of fresh milk by *Y. enterocolitica* is unsanitary conditions on dairy farms, particularly through milking [34].

The incidence of *Y. enterocolitica* in powdered infant formula samples was 8% besides milk powder samples that was 20%. **Sotohy et al.** [30], found a substantially identical result (8.2%, 20%). This study demonstrated the significant contribution of powdered milk and infant formula to human *Y. enterocolitica* infection. Contamination can happen when milk is being processed into powder. This covers air, personnel, and equipment pollution. Failure to maintain hygienic standards can result in

post-processing contamination even after heat treatment. As an organism that is psychrophilic, *Y. enterocolitica* may endure and even thrive at refrigerator temperatures. Because of this, it is especially problematic for food items that aren't always kept at high temperatures [35]. Enterobacteriaceae contamination is made possible by inadequate hygienic practices during the production of these products [36]. Due to their insufficient immunity, children who ingest large quantities of powdered milk and newborn formula are more vulnerable to digestive system disorders [37].

Despite being a crucial part of modern treatment, antibiotics are being less effective because of bacteria's capability to progress resistance [38]. Our results showed that all isolated strains were resistant to AMC, followed by cefazolin (93.3%) and ampicillin (66.6%), with results that were almost identical to those of Frazão et al. [39]. However, the isolated strains were sensitive to gentamicin and ciprofloxacin (93.3%), followed by trimethoprim/ sulfamethoxazole (80%), chloramphenicol (86.6%), and kanamycin and cephalothin (73.3%). These results are matched with the results of numerous researchers [39,40]. The way antibiotics are used around the world needs to alter. A deal to cut back on unnecessary antibiotic use would give the fight against antibiotic resistance the global governance it desperately needs and give all countries a clear way forward. Interestingly, MDR strains of *Y*. enterocolitica bacteria have been linked to higher infection rates than sensitive bacteria, and they cause sclerosis when used to treat human and animal illnesses [39].

The results of the tests on the sensitivity of the antimicrobials were often in agreement with those of research from China [17, 41], but different results were reported in previous studies in Egypt; 70.7% [42]. Younis et al., [43] found a low incidence of MDR *Y. enterocolitica* strains (23.33%). Unfortunately, multidrug resistance for more than two classes of antimicrobials was found in 14 of 15 strains (93.3%) for ten resistances. Unfortunately, 14 of 15 strains (93.3%) for ten resistances had multidrug resistance for more than two classes of antimicrobials.

The formation of biofilms is a vital bacterial existence method in together natural besides artificial locations. To recognize and stop systemic illness caused by microorganisms that procedure biofilms, it is crucial to identify the attachment of pathogenic microorganisms to medical roofs. These organised microbial groups buried in an amorphous medium exhibit great resilience to ecological stresses, antibiotics, disinfectants, and the host immune system [44]. All strains in this investigation had variable levels of biofilm development, which were separated into five strains that produced biofilms with week and ten strains that produced biofilms with moderate levels. The finding that isolation stains can develop biofilm, as described by Ahmed et al. [45], is somewhat comparable to this one. However, there are differences in the incidence of biofilm development; in certain studies, it might approach 70% of isolated samples [43].

MDR development happens when antibiotics are administered for longer than is required or when they are not required. At first, only a small percentage of bacteria may be resistant to antibiotic therapy. The likelihood of acquiring antibiotic resistance increases with the frequency of antibiotic use. The first is when bacteria acquire genetic traits like virulence and antibiotic resistance thanks to genes from plasmids. Since pumping the antibiotic out of the cell is one of the resistance strategies used by bacteria, the second theory holds that a single resistance mechanism confers resistance to several antibiotics. Occasionally, a wide range of chemicals, including various antibiotic kinds, can be detected by these pumps. To put it another way, the bacteria produced a variety of antibiotics using a single pump. Crossresistance is another term for this [42].

In this investigation, were found in several *Y. enterocolitica* isolates with different patterns of antibiotic resistance. The dissemination of virulence characteristics and patterns of antibiotic resistance among the isolates under investigation is supported by this study. Plasmid-mediated horizontal gene transmission is a key contributor to the ongoing rise of bacterial antibiotic durability [17,46,42].

Conclusion

Information on the incidence of *Y. enterocolitica* pathogenic strains in cow milk, sheep milk, milk powder, and infant formula is now being researched. These activities could all be sources of drug-resistant and pathogenic strains of *Y. enterocolitica* that pose a threat to our public health in Egypt. Reducing *Y. enterocolitica* contamination of milk as well as dairy products requires the proper application of stringent sanitary protocols. Important decision-makers in the health sector will be informed by the study's conclusions and suggestions to implement the best preventive measures.

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Conflict of interest

The authors declare that there is no conflict of interest.

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TABLE 1. Target genes, primer sequences besides amplified segments.

Gene	Primer	Primer Sequence 5'-3'	Amplified product	Reference
16S Rrna	F27	AGAGTTTGATCMTGGCTCAG	1485bp	[47]
	R1492	TACGGYTACCTTGTTACGACTT		

TABLE 2. The Yersinia enterocolitica 16s rRNA gene amplification cycle.

Gene	Primer	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
16S	F27	94°C	94°C	56°C	72°C	35	72°C
rRNA	R1492	15 min.	30 sec.	1 min.	1 min.		10 min.

TABLE 3. Prevalence of Y. enterocolitica in different kinds of collected animals milk and dried mill
samples

Examined samples	Total No.	Positive No.	Positive %
Cow milk	50	5	10%
Sheep milk	50	3	6%
Milk powder	25	5	20%
Infant formula	25	2	8%
Total	150	15	10%

TABLE 4. Biofilms production degree from *Y. enterocolitica* strains (N= 15)

Biofilms production degree	No. of strains	Percentage	
Moderate	10	66.6%	
Weak	5	33.3%	
Total	15	100%	

	No. of Y. enterocolitica isolates $(n = 15)$		
A	Sensitive	Intermediate	Resistant
Antibiotic	N (%)	N (%)	N (%)
Amoxicillin-clavulanicacid (AMC) (30 µg)	0	0	15 (100)
Cefazolin (CFZ) (30 µg)	0	1 (6.6)	14 (93.3)
Ampicillin (AM) (10 µg)	0	5 (33.3)	10 (66.6)
Doxycyclin (DOX) $(30 \ \mu g)$	4 (26.6)	4 (26.6)	7 (46.6)
Fosfomycin (FOS) (50 µg)	10 (66.6)	2 (13.3)	3 (20)
Cephalotin (KF) (30 µg)	11 (73.3)	2 (13.3)	2 (13.3)
Kanamycin (K) (30 µg)	11 (73.3)	3 (20)	1 (6.6)
Trimethoprim/sulfamethoxazole (SXT) (25µ)	12 (80)	1 (6.6)	2 (13.3)
Chloramphenicol (C) (30 µg)	13 (86.6)	1 (6.6)	1 (6.6)
Ciprofloxacin (CIP) (5 µg)	14 (93.3)	1 (6.6)	0
Gentamicin (GN) (10 µg)	14 (93.3)	1 (6.6)	0

TABLE 5. Antimicrobial resistance test.

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

Strain No.	Serotype	Source of isolated Strain	Resistance pattern	Biofilms production degree
1	Y.enterocolitica	Cow milk	AMC/ CFZ/ AM/ DOX	Moderate
2	Y.enterocolitica	Cow milk	AMC/ CFZ/ AM/ DOX/ KF/ C	Week
3	Y.enterocolitica	Cow milk	AMC/ CFZ/ AM/ FOS	Moderate
4	Y.enterocolitica	Cow milk	AMC/ CFZ/ DOX/ FOS	Moderate
5	Y.enterocolitica	Cow milk	AMC/ CFZ/ AM	Week
6	Y.enterocolitica	Sheep milk	AMC/ CFZ/ AM/ DOX	Moderate
7	Y.enterocolitica	Sheep milk	AMC/ CFZ/ AM/ FOS	Moderate
8	Y.enterocolitica	Sheep milk	AMC/ CFZ/ DOX	Moderate
9	Y.enterocolitica	Milk powder	AMC/ CFZ/ AM	Week
10	Y.enterocolitica	Milk powder	AMC/ CFZ/ AM/ SXT	Moderate
11	Y.enterocolitica	Milk powder	AMC/ CFZ/ AM	Moderate
12	Y.enterocolitica	Milk powder	AMC/ CFZ/ DOX/ SXT	Moderate
13	Y.enterocolitica	Milk powder	AMC/ CFZ	Moderate
14	Y.enterocolitica	Infant formula	AMC/ CFZ/ AM/ KF	Week
15	Y.enterocolitica	Infant formula	AMC/ DOX/ K	Week
AMC		· · · · · · · · · · · · · · · · · · ·		

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

Table 7. Resistance pattern in isolated Y. enterocolitica

Resistant pattern	Number of antibiotics	Number of isolates
AMC/ CFZ	2	1(6.6%)
AMC/ CFZ/ AM	3	3 (20%)
AMC/ DOX/ K	3	1 (6.6%)
AMC/ CFZ/ DOX	3	1(6.6%)
AMC/ CFZ/ AM/ FOS	4	2 (13.3%)
AMC/ CFZ/ DOX/ SXT	4	1(6.6%)
AMC/ CFZ/ DOX/ FOS	4	1(6.6%)
AMC/ CFZ/ AM/ SXT	4	1(6.6%)
AMC/ CFZ/ AM/ DOX	4	2 (13.3%)
AMC/ CFZ/ AM/ KF	4	1(6.6%)
AMC/ CFZ/ AM/ DOX/ KF/ C	6	1(6.6%)

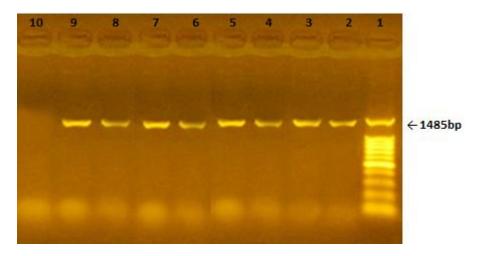


Fig. 1. Descriptive agarose gel electrophoresis viewing amplified PCR products in *Y. enterocolitica* isolated of 16s rRNA gene (1485bp). Lane M: 100 bp ladder; lane 1: positive control; lane 9: negative control Yersinia enterocolitica; lanes -Positive samples.



Fig. 2 J Discovery of the grade of biofilm making by tube test. (A) Moderate biofilm maker, (B) Weak biofilm maker, (C) Non-biofilm maker.

References

- 1. WHO. World health organization, food safety (2022)
- Dowidar, H. A. and Khalifa, M.I. Virulence genotyping profles of Cefazolin resistance Yersinia enterocolitica isolated from milk and milk products in Egypt. *Egypt. J. Vet. Sci.*, **54** (4),555–69 (2023)
- Chlebicz, A. and Slizewska, K. Campylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as Zoonotic Foodborne Diseases: A Review.

International Journal of Environmental Research and Public Health, **15**, 863 (2018)

- 4. Khalifa, M. and Dowidar, H. Load of coliforms and fecal coliforms in dairy desserts in Aswan City. *International Journal of Life Science Research Archive*, **02**, 041–046 (2022)
- Gruber, J., Morris, S., Warren, K., Kline, K., Schroeder, B., Dettinger, L., Husband, B., Pollard, K., Davis, C. and Miller, J. Yersinia enterocolitica Outbreak Associated with Pasteurized

Egypt. J. Vet. Sci. Vol. 56, (Special issue) (2025)

Milk. Foodborne Pathogens and Disease, 18, 448–454 (2021)

- Shoaib, M., Shehzad, A., Raza, H., Niazi, S., Khan, I.M., Akhtar, W., Safdar, W. and Wang, Z. A comprehensive review on the prevalence, pathogenesis and detection of *Yersinia enterocolitica*. *RSC Adv.*, 9, 41010-41021(2019)
- Schiemann, D.A. Yersinia enterocolitica in milk and dairy products. Journal of Dairy Science, 70, 383-391(1987)
- Bursová, Š., Necidová, L., Haruštiaková, D. and Janštová B. Growth potential of *Yersinia enterocolitica* in pasteurised cow's and goat's milk stored at 8 °C and 24 °C. *Food Control*, **73**, 1415-1419 (2017)
- Salman, K.H., Mehany, T., Zaki, K.G. and Al-Doury, M.K. Development of functional probiotic yogurt from buffalo milk supplemented with red beetroot (*Beta vulgaris* L.) as an antioxidant, natural colorant, and starter growth stimulant. *Food Chemistry Advances*, 5, Article 100776 (2024)
- Sharif, M.K., Javed, K. and Nasir, A. Chapter 15 foodborne illness: Threats and control A.M. Holban, A.M. Grumezescu (Eds.), Foodborne diseases, Academic Press, 501-523 (2018)
- Havens, J. M., Montgomery, E., Greenson, J. K., Scott, M. A. and Lamps, L. W. Pathogenic Y. enterocolitica DNA is detected in gastrointestinal malakoplakia. *Modern Pathology*, 16, (Abstr.)1200A (2003).
- Cocolin, L. and Comi, G. Use of a culture independent molecular method to study the ecology of *Yersinia* spp. in food. *International Journal of Food Microbiology*, **105**, 71–82 (2005).
- 13. EFSA. Monitoring and identification of human enteropahogenic *Yersinia* spp. *EFSA Journal*, **595**, 1-30 (2007).
- Petsios, S., Fredriksson-Ahomaa, M., Sakkas, H., Papadopoulou, C. Conventional and molecular methods used in the detection and subtyping of *Yersinia enterocolitica* in food. *International Journal* of Food Microbiology, 237, 55-72 (2016).
- 15. Del Pozo, J.L. Biofilm-related disease. *Expert. Rev.* Anti Infect. Ther., **16** (1), 51-65 (2018).
- Abbasi, K., Tajbakhsh, E. and Momtaz, H. Antimicrobial resistance and biofilm encoding genes amongst the Staphylococcus aureus bacteria isolated from meat and meat products. *Egypt. J. Vet. Sci.*, **52**, 55-62 (2021).
- Ye, Q., Wu, Q., Hu, H., Zhang, J. and Huang, H. Prevalence and characterization of *Yersinia enterocolitica* isolated from retail foods in China. *Food Control*, **61**, 20-27 (2016).
- Kot, B., Piechota, M. and Jakubiak, K. Virulence genes and antibiotic resistance of *Yersinia enterocolitica* strains isolated from children. *European Journal of Biological Research*, 7, 366-373 (2017).

- Velázquez, L., Escudero, M. E. and Guzmán, A. M. Prevalence of *Yersinia enterocolitica* in hake (*Merluccius hubbsi*) fillets. *Journal of Food Protection*, **59**, 7, 781-783(1996).
- Markey, B.K., Leonard, F.C., Archambault, M., Cullinane, A. and Maguire, D. Clinical veterinary microbiology (2nd ed.), MOSBY. Elsevier Ltd., Edinburgh, London, New York, Oxford, Philadelphia, St. Louis, Sydney, Toronto (2013)
- FDA/ CFSAN/ BAM. Y. enterocolitica Bacteriological Analytical Manual online chp.8. US Food and Drug Administration Center for Food Safety and Applied Nutrition (2007).
- Garzetti, D., Susen, R., Fruth, A., Tietze, E., Heesemann, J. and Rakin, A. A molecular scheme for Yersinia enterocolitica patho-serotyping derived from genome-wide analysis. *International Journal of Medical Microbiology*, **304** (3-4), 275- 283 (2014).
- Sambrook, J., Fritsch, E.F. and Maniatis, T. Molecular Cloning—A Laboratory Manual, second ed. Cold Spring Harbor Laboratory Press, NY 1, 66– 1.79 (1989).
- Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A. and Iqbal, M. Evaluation of different detection methods of biofilm formation in the clinical isolates. *Brazilian Journal of Infectious Diseases*, 15, 305-311 (2011).
- CLSI (Clinicaland Laboratory Standards Institute). Performance Standards for Antimicrobial Susceptibility Testing. Clinical Lab Standards Institute, 2020 M100s (26th ed.) (2020).
- Rosner, B., Klaus Stark, K., Werber, D., Epidemiology of reported *Yersinia enterocolitica* infections in Germany, 2001-2008, *BMC Public Health*, **10**, (2010)
- 27. Jamali, H., Paydar, M., Radmehr, B. and Ismail, S. Prevalence, characterization, and antimicrobial resistance of *Yersinia* species and *Yersinia enterocolitica* isolated from raw milk in farm bulk tanks. Journal of Dairy Science, **98**, 798-803 (2015).
- Cristiano D., Peruzy M.F., Aponte M., Mancusi A., Proroga Y.T.R., Capuano F. and Murru N. Comparison of droplet digital PCR vs real-time PCR for *Yersinia enterocolitica* detection in vegetables. *International Journal of Food Microbiology*, **354**, 109321 (2021).
- Abdelwahab, A., EL-Tawab, A., Awad, A., Abdallah, F. and Maarouf, A. Phenotypic and genotypic studies on antibiotic resistant *Yersinia enterocolitica* isolated from milk and milk products in Kaliobia, Egypt. *Benha Veterinary Medical Journal*, 40, 149–153 (2021).
- Sotohy, S.A., Diab, M. S., Ewida, R.M., Aballah, A. and Alm Eldin, N. K. An investigative study on *Yersinia enterocolitica* in animals, humans and dried milk in New Valley Governorate, Egypt. *BMC C. Microbiology*, 24,395 (2024)
- 31. Khalid, D. and Abbas, B. Detection of enterotoxin genes and antibiotic susceptibility pattern in *Yersinia*

enterocolitica isolated from cheese in Basrah. *Basrah Journal of Veterinary Research*, **18**, 69–84 (2019)

- 32. Gruber, J.F., Morris, S., Warren, K.A., Kline, K.E., Schroeder, B., Dettinger, L., Husband, B., Pollard, K., Davis, C., Miller, J., Weltman, A., Mattioli, M., Ray, L., Tarr, C. and Longenberger, A.H. Yersinia enterocolitica outbreak associated with pasteurized milk. Foodborne Pathog. Dis., 18(7), 448-454 (2021).
- 33. Mohamed, A.A., Stohy, S.A., Allam Eldin, N.K., Kholief, M.E. and Diab, M.S. Prevalence of *Yersinia enterocolitica* and *Cronobacter sakazakii* in Animals, Human and Dried Milk in New Valley Governorate, Egypt. *New Valley Egypt Journal*, 5, 1 (2025).
- Hanifian, S. and Khani, S. Prevalence of virulent *Yersinia enterocolitica* in bulk raw milk and retail cheese in northern-west of Iran. *International Journal of Food Microbiology*, **155**, 89–92 (2012).
- Wang, Y., Wang, X., Xu, X, and Liu, Y. Detection of *Yersinia enterocolitica* in milk powders by crosspriming amplification combined with immunoblotting analysis. *Journal of dairy science*, 98, 6748–6755 (2015).
- Lucero-Estrada, C., Favier, G. and Escudero, M. An overview of *Yersinia enterocolitica* and related species in samples of different origin from San Luis, Argentina. *Food Microbiology*, 86, 103345 (2020).
- 37. Guner, A., Dogruer, Y., Cebirbay, M., Yalcin, S., Gulsen, S. and Telli, N. An investigation on the prevalence of *Cronobacter sakazakii* in powdered infant formula consumed in Turkey. *Journal Of Food Agriculture And Environment*, 9, 82–84 (2011).
- Özdemir, F. and Arslan, S. Genotypic and phenotypic virulence characteristics and antimicrobial resistance of *Yersinia* spp. isolated from meat and milk products. *Journal of Food Science*, 80, 1306-1313 (2015).
- 39. Frazão, M. R., Andrade, L. N., Darini, A. C. and Falcão J. P. Antimicrobial resistance and plasmid repliconsin *Yersinia enterocolitica* strains isolated in Brazilin 30 years. *The Brazilian Journal of Infectious Diseases*, **21**, 477-480 (2017).
- 40. Hadef, K.H., Medjahdi, K.H., Beldjilali, H., Benamar, I. and Boudjemâa, M.B. Safety

characterization of *Yersinia enterocolitica* strains isolated from raw milk in Western Algeria. *African Journal of Microbiology Research*, **10**, 15-22 (2015).

- Peng, Z., Zou, M., Li, M., Liu, D., Guan, W., Hao, Q., Xu, J., Zhang, S., Jing, H., Li, Y., Liu, X., Yu D., Wang, W. and Li, F. Prevalence, antimicrobial resistance and phylogenetic characterization of *Yersinia enterocolitica* in retail poultry meat and swine feces in parts of China. *Food Control*, 93, 121-128(2018).
- Younis, G.A., Elkenany, R.M. and Dowidar, H.A. 'Virulence genotyping and antimicrobial resistance profiles of *Yersinia enterocolitica* isolated from meat and meat products in Egypt', *Brazilian Journal of Biology*, 81(2), 424–436 (2021).
- 43. Younis, G., Mady, M. and Awad, A. Yersinia enterocolitica: Prevalence, virulence, and antimicrobial resistance from retail and processed meat in Egypt. *Veterinary World*, **12**, 1078-1084 (2019).
- 44. Taha, M., Kalab, M., Yi Q., Landry, C., Stewart, V., Brassinga, A., Sifri, C. and Arcos, S. Biofilmforming skin microflora bacteria are resistant to the bactericidal action of disinfectants used during blood donation. *Transfusion*, 54, 2974-2982 (2014).
- 45. Ahmed, A.S., Diab, H.M., Hendy, B.A. Batiha, G.E., Dandrawy, M.K. and El-Zamkan, M.A. Molecular characterization of *Y. enterocolitica* isolated from dairy environment with special reference to the antimicrobial activity of milk proteins hydrolysates. *Journal of Advanced Veterinary Research*, **12**, 118-127 (2022).
- 46. Rozwandowicz, M., Brouwer, M. S., Fischer, J., Wagenaar, J. A., Zorn, G.B., Guerra, B., Mevius, D.J. and Hordijk, J. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, **73**, 1121–1137 (2018).
- Lagacé, L., Pitre, M., Jacques, M. and Roy, D. Identification of the bacterial community of maple sap by using amplified ribosomal DNA (rDNA) restriction analysis and rDNA sequencing. *Applied* and Environmental Microbiology, 2052–2060 (2004).

الأنماط الجينية والبيوفيلمية لبكتيريا يرسينيا إنتير وكوليتيكا المقاومة للسيفاز ولين المعزولة من الحليب ومنتجاته في مصر

هبة الله دويدار¹، أميرة حسين الباز²

1قسم المختبرات الطبية، المعهد العالي للتكنولوجيا للعلوم الصحية التطبيقية، معهد بدر للعلوم والتكنولوجيا، القاهرة، مصر 2قسم صحة وسلامة وتكنولوجيا الغذاء ، كلية الطب البيطري، جامعة المنصورة، المنصورة 35516، مصر

الملخص

كان الهدف من هذه الدراسة هو دراسة معدل الإصابة والسمات وعامل الضراوة وتقييم مقاومة الأدوية لمرض يرسينيا القولون المعوي الموجود في حليب الأبقار وحليب الأغنام ومسحوق الحليب وحليب الأطفال. تم جمع العينات بين مايو وأكتوبر 2024 من الباعة الجائلين والأسواق المعروفة المنتشرة في جميع أنحاء المنصورة والدقهلية بمصر. 15 من أصل 150 عينة (10٪) أثبتت إيجابية اختبار يرسينيا القولون المعوي في حليب الأبقار وحليب الأغنام (50 عينة لكل منهما) ومسحوق الحليب وحليب الأطفال حديثي الولادة (25 عينة لكل منهما)؛ وكان التردد أكبر في مسحوق الحليب (20%). وفقًا للتقديرات، لوحظت مقاومة ظاهرية كبيرة لحمض أموكسيسيلين-كلافولانيك (100٪) وسيفازولين (3.3%). وفقًا (6.66٪) في عزلات يرسينيا القولون المعوي. ومن المثير للاهتمام أن العزلات التي تم العثور عليها تحتوي على 11 نمطًا مميزًا للمقاومة و3.00% من مقاومة الأدوية المتعددة. وقد أظهرت جميع سلالات التي تم العثور عليها تحتوي على 11 نمطًا الأغشية الحيوية، حيث أظهرت عشر سلالات قدرة معتدلة وخمس سلالات التي تم العثور عليها تحتوي على 11 نمطًا الأغشية الحيوية، حيث أظهرت عشر سلالات قدرة معتدلة وخمس سلالات التي أضار عليها تحتوي على 11 نمطًا الأغشية الحيوية، حيث أظهرت عشر سلالات قدرة معتدلة وخمس سلالات إضافية أظهرت قدرة ضعيفة. يشير تحديد سلالات معافية بسبب احتمالية الإصابة عائر معتدلة وخمس سلالات إضافية أظهرت قدرة ضعيفة. يشير تحديد المنشية الحيوية، حيث أظهرت عشر سلالات قدرة معتدلة وخمس سلالات إضافية أظهرت قدرة ضعيفة. يشير تحديد سلالات 2000، وحود خطر علي سلالات قدرة معتدلة وخمس سلالات إضافية أظهرت قدرة ضعيفة. يشير تحديد المنتهالي المقاومة بسبب احتمالية الإصابة بالأمراض ومقاومة المضادات الحيوية، والتي تتطلب استجدام تدابير الرقابة.

الكلمات المفتاحية: يرسينيا إنتروكوليتيكا، الأغشية الحيوية، مقاومة المضادات الحيوية، الحليب، حمض أموكسيسيلين. كلافولانيك وسيفازولين.