Open Veterinary Journal, **(2024), Vol. 14(1): 470-480**
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Original Research
DOI: *10.5455/OVJ.2024.v14.i1.43*

Submitted: 01/10/2023 Accepted: 15/12/2023 Published: 31/01/2024

Inhibitory effect of some probiotic strains and essential oils on the growth of some foodborne pathogens

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Abstract
Background: *Bacillus cereus* and *Yersinia enterocolitica* are implicated in foodborne diseases that have major effects on human health; therefore, it is considered universal public health disorders. Essential oils and essential oils nano emulsions have a sufficient antibacterial performance against a variety of bacteria, especially multi-drug resistant bacteria. Probiotics showed several health benefits via moderating the GIT microbiota and their metabolites.
Aim: The study was designed to evaluate the biocontrol ability of cinnamon essential oil (CEO) nano emulsion and probiotics as natural antibacterial additives and reveal their bactericidal mechanism.
Methods: 250 random samples (50 raw milk, 50 rice pudding, 50 kariesh cheese, 50 yogurt, and 50 ice cream) were purchased separately from different areas in Mansoura city, Egypt, and exposed to bacteriological analysis.
Results: *Bacillus cereus* was found with the highest mean value of $66 \times 10^7 \pm 1.3 \times 10^8$ CFU/g in raw milk and the lowest mean value of $28 \times 10^7 \pm 2.6 \times 10^7$ CFU/g in kariesh cheese while *Y. enterocolitica* was found in 64% of the total inspected samples with the highest incidence (84%) in yogurt. The toxinogenic potential of the tested pathogens has been evaluated by multiplex PCR pointing *nhe A* and *ces* genes for *B. cereus* isolates while targeting in *Y. enterocolitica 16s rRNA*, and *YST* gene. Different concentrations (0.17%, 0.25%, 0.5%, 0.8%, 1%, 1.5%, and 2%) of cinnamon oil nano emulsion were employed in this study. CEO nano emulsion had the highest reduction rate at a concentration of 1.5% in the case of *B. cereus* and 2% in the case of *Y. enterocolitica*. Among different types of probiotics, the best one which showed inhibitory potential against *B. cereus* and *Y. enterocolitica* was *L. plantarum.*
Conclusion: *Lactobacillus plantarum* and CEO nano emulsion at a concentration of 2% have the highest reduction rate against *Y. enterocolitica*, while *L. plantarum* and CEO nano emulsion at a concentration of 1.5% has the best antibacterial effect against *B. cereus*. In conclusion, more attention is required for both safety and quality in dairy products through the application of natural additives such as essential oils and probiotics.
Keywords: Essential oils, Cinnamon oil, *Bacillus cereus*, *Y. enterocolitica*, *L. plantarum*.

Introduction

Bacillus cereus is considered one of the utmost common food-borne pathogens in foodstuffs (Rahnama *et al.*, 2023). They were mentioned as “aerobic endospore-forming micro-organisms” which broadly blow out over the world (Zhou *et al.*, 2023). The pathogenicity of this organism rests on frequent exogenic enzyme creation, the capability of forming biofilms, and the existence of toxin-encoding genes. It is related to a food poisoning syndrome characterized by diarrhea and gastrointestinal disturbances (Li *et al.*, 2023). *Bacillus cereus* ranked as the fourth greatest reason for foodborne diseases by EFSA and ECDC in the European Union (Elafify *et al.*, 2023).

Yersinia enterocolitica is involved in different human disorders such as gastro-enteritis, sepsis, appendicitis, and lymphadenitis (Ahmed *et al.*, 2023). Yersiniosis as a gastrointestinal contagion comes afterward campylobacteriosis and salmonellosis (Zadernowska *et al.*, 2014) demonstrated by lymphadenitis, diarrhoea, appendicitis, and arthritis (Tavassoli *et al.*, 2019). *Yersinia enterocolitica* resists numerous antibacterial agents and is considered one of the pathogenic drug-resistant bacteria with a public health importance (Sadek *et al.*, 2014; Bonardi *et al.*, 2018).

Food additives that are used in the food industry to prolong shelf life may cause intoxication, and severe diseases as cancer. To avoid this problem, the application of natural extracts from herbs to limit the growth of micro-organisms might be a practical solution (El-Sayed and El-Sayed, 2021)

Lactic acid bacteria are commonly reflected as probiotics which have great health importance via moderating the metabolites and microbiota (Fang et *al.*,

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2023). Therefore, they were used as preservatives in the food chain (Hamad *et al.*, 2022). *Lactobacillus plantarum* has an aggressive influence against food micro-organisms (Tian *et al.*, 2022). Finally, efficient foods or probiotic-supplemented food received more attention because of the awareness of healthy and beneficial products targeting good wellbeing (Ali *et al.*, 2023; Hussain *et al*., 2023). The number of cells per milliliter and its therapeutic doses determine the probiotic potential (Begum *et al.*, 2017). A probiotic food should not exceed 1.0×10^6 cfu/g of alive microbes. In accumulation to being a biopreservative, EOs mentioned as generally recognized as safe due to their effective antibacterial role in the food industry (Es *et al.*, 2017). They can inhibit spoilage and pathogenic bacteria through their phenolic constituents and polypeptides (Bakhtiary *et al.*, 2018) and improve nutritive value and organoleptic properties (Kholif and Olafadehan, 2021). Among them, cinnamon essential oil (CEO) has been used as an antifungal and antimicrobial agent (Paudel *et al.*, 2019) due to the presence of cinnamic acid, eugenol, and cinnamaldehyde (Siripatrawan, 2016). It has broad-spectrum antimicrobial activities; in addition, it is characterized by its special aroma and medicinal properties (Nazzaro *et al.*, 2013). Nano emulsions are biphasic dispersions of water and oil either water in oil microemulsions or oil in water microemulsions or (O/W), alleviated by an emulsifier. The exceptional features of these nanostructures diverge from their parent structures chemically, biologically, and physically. This potential request of nanotechnology is to prolong shelf-life, nutritional additives, coloring, flavoring, and food safety via using antimicrobial ingredients for food (Oladipo *et al.*, 2020). Therefore, this study examined the preservative action of CEO nano emulsion and probiotics and how those could affect pathogenic bacteria.

Materials and Methods

Collection of samples

Two hundred and fifty samples of raw milk, yogurt, kariesh cheese, ice cream, and rice budding (50 each) were purchased randomly from different markets at localities in Dakahlia Governorate, Egypt, from September 2022 to March 2023. In sterile containers, samples were collected and transferred immediately to an ice box for isolation and examination.

Microbiological analysis

Preparation of samples

Samples collected were 25 ml of milk and 25 g of yogurt, kareish cheese, rice pudding, and ice cream then homogenized in a sterile jar containing 225 ml of tryptone soya broth. Kareish cheese was mixed with 2% sodium citrate to accelerate homogenization (Salfinger, 2015).

Isolation and quantification of *B. cereus*

It was done through duplicates of BcSAB plates with polymyxin B supplement first then add the egg yolk emulsion. Incubated at 35°C for 24–48 hours. Suspected colonies of *B. cereus* appeared crenate, about 5 mm in diameter, and had a unique turquoise to peacock blue color. Then the recovered colonies were counted and calculated as the total *B. cereus* count. Three to five typical colonies were purified on TSA slants and incubated at 35°C for 24 hours (Oxoid, 2002).

Identification of *Y. enterocolitica*

Pre-enrichment of the sample (1 ml) by 10 ml of PBS supplemented with cefsulodin and novobiocin is necessary. Tubes were incubated at room temperature for 5–7 days. Streaking on selective CIN agar plates. Then incubated at 37°C for 48 hours. Characteristic colonies appeared as bull's eyes (Drake *et al.*, 2018).

Molecular confirmation of isolate

By following manufacturer instructions, the DNA of *Bacillus* and *Yersinia* isolates have been extracted by QIAamp DNA Mini Kit. Amplification of the specific bacterial targets was done by PCR using DreamTaq™ Green PCR Master Mix (2×), Emerald Amp GT PCR master mix (TaKaRa, Japan), respectively (Koua *et al.*, 2014; Owusu-Kwarteng *et al.*, 2017) (Table 1).

Preparation of essential oils nano emulsion

Colloidal nano suspension from cinnamon seeds was prepared by ethanolic extract using solvent evaporation/ micelles formation with some modification (Singh

Table 1. Oligonucleotide sequences of the primers used in the present study.

Bacteria	Gene	Sequence	Amplified product	Reference
B. cereus	*B. nhe*	F: TACGCTAAGGAGGGGCA R: GTTTTTATTGCTTCATCGGCT	500 bp	Owusu-Kwarteng *et al.* (2017)
	B. ces	F: GGTGACACATTATCATATAAGGTG R: GTAAGCGAACCTGTCTGTAACAACA	1,271 bp	Ehling-Schulz *et al.* (2006)
Y. enterolitica	*Y. enterocolitica 16S rRNA*	F: AAT ACC GCA TAA CGT CTT CG R: CTT CTT CTG CGA GTA ACG TC	330 bp	Wannet *et al.* (2001)
	Y. enterocolitica yst	F: AATGCTGTCTTCATTTGGAGC R: ATCCCAATCACTACTGACTTC	145 bp	Koua *et al.* (2014)

et al., 2023). CEO nano emulsions with concentrations of 0.17%, 0.25%, 0.5%, 0.8%, 1%, 1.5%, and 2% (v/v) for each were purchased from Nakaa Nanotechnology Network. Experiments and interpretation were done according to previous studies (NCCLS 2012; Kaskatep *et al.*, 2016).

Antimicrobial activity analysis

Disc diffusion was done by using Mueller-Hinton agar following the guidelines of CLSI (2015, 2017). Then bacteria were spread on the plate with 1.5×10^7 CFU/ml density according to Lahtinen *et al.* (2007. Ampicillin (AM 10 µg), ciprofloxacin (Cip), amoxicillin-clavulanic acid (AMC 20/10 µg, and erythromycin (E15) discs were tested.

Antibacterial activity of different probiotic strain

Preparation of probiotic

Lactobacillus plantarum, *L. rhamnosus*, and *L. acidophillus* were evaluated via agar well diffusion method following previous studies (Hamad *et al.*, 2023). Wells (6 mm) were bored on the plates and 100 µl free supernatant containing antibacterial activity was added, ampicillin was used as a positive control, while the nutrient broth was utilized as a negative control. Incubation was done for 24 hours at 37°C and the inhibition zone was measured and a clear zone was noted in mm referring to the antibacterial influence of different probiotic strains.

Antibacterial activity of CEO nano emulsion

Different concentrations of CEO nano emulsion were presented in wells that were swabbed before with overnight cultures of bacteria. The test was done on triplicates as probiotic inoculation (Hulankova, 2022).

Statistical analysis

Data were represented as Mean ± SD. ANOVA was done with SPSS and statistical significance was estimated at $p < 0.05$ (Feldman e*t al.*, 2003).

Ethical approval

Not required for this study.

Results

Occurrence of B. cereus in the examined samples

Bacillus cereus was found in milk, yogurt, ice cream, kareish cheese, and rice pudding samples by 72%, 70%, 32%, 30%, and 44%, respectively (Table 2). Although *nhe*A and *ces* genes were detected at 100% of rice pudding, *nhe*A gene were found only at 50% of yogurt and raw milk as mentioned in Tables 3–5 and Figure 1.

Occurrence of Y. enterocolitica and virulence genes

Yersinia enterocolitica was found in 160 samples out of 250 (64%) with the highest incidence of 84% followed by 76%, 66%, 62%, and 32% in yogurt, kariesh chees, raw milk, rice pudding, and ice cream, respectively, as shown in Table 4. Both *16s* rRNA and *yst* gene were detected in 100% of tested isolates as illustrated in Table 5 and Figures 2 and 3.

Antibacterial susceptibility against B. cereus and Y. enterocoltica isolates

Bacillus cereus isolates were highly sensitive to ampicillin, middle sensitive to Amoxicillin-clavulanic and ciprofloxacin with no effect of erythromycin, while, *Y. enterocolitica* was highly sensitive to ampicillin, intermediate to Amoxicillin-clavulanic, and resistant to ciprofloxacin and erythromycin as depicted in Table 6.

Antibacterial activity of probiotic

Bacillus cereus showed high sensitivity to *L. plantarum*, intermediate *L. acidophillus* then *L. rhamnosus* with

Table 2. Prevalence of *B. cereus* in the examined samples.

Type of examined Samples	No of examined samples	Positive samples		Min	Max	Mean	± SE
		No	%				
Raw milk	50	36	72	12×10^7	50×10^8	66×10^7	1.3×10^8
Yogurt	50	35	70	16×10^8	52×10^8	30×10^8	1.6×10^8
Ice cream	50	16	32	29×10^7	42×10^8	73×10^7	2.3×10^8
Kareish cheese	50	15	30	1×10^8	45×10^7	28×10^7	2.6×10^7
Rice pudding	50	22	44	21×10^8	77×10^8	52×10^8	3.1×10^8
Total	250	124	49.6				

Table 3. Molecular identification of certain *B. cereus* among the examined samples.

	Raw milk	Yogurt	Ice cream	Kareish cheese	Rice pudding
Total nO. of isolates	2	2	2	2	2
*nhe*A gene	1 (50%)	1 (50%)	0	0	2 (100%)
Ces gene	0	0	0	0	2 (100%)

Table 4. Prevalence of *Yersinia* spp. in the examined samples.

Type of examined samples	No of examined samples	Positive samples	
		No	%
Raw milk	50	33	66
Yogurt	50	42	84
Ice cream	50	16	32
Kareish cheese	50	38	76
Rice pudding	50	31	62
Total number	250	160	64

Table 5. Molecular identification of *Y. enterocolitica* among the examined samples.

	Raw milk	Yogurt	Kareish cheese	Rice pudding
Total no. of isolates	5	2	2	1
16s rRNA	5 (100%)	2 (100%)	2 (100%)	1 (100%)
Yst	5 (100%)	2 (100%)	2 (100%)	1 (100%)

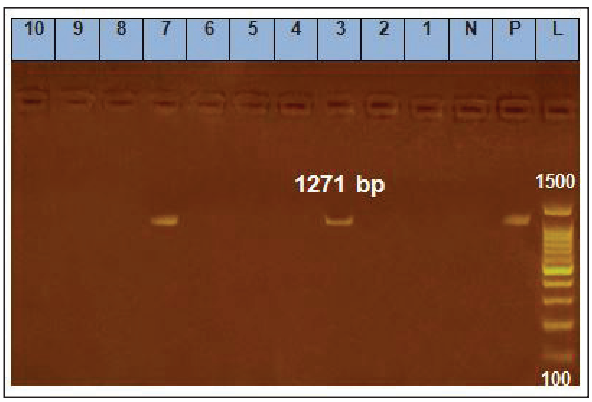

Fig. 1. Amplification of *B. cereus* isolates on agarose gel using *ces* (1,271 bp). Lane (1–10) lane ladder (100–1,500) *B. cereus* lanes 3 and 7 show positive results while other lanes show no expression.

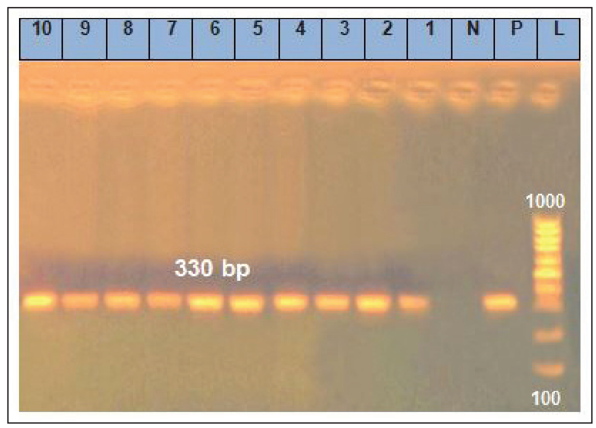

Fig. 2. Amplification of *Y. enterocoltica* isolates on agarose gel using *16srRNA* (330 bp). Lane (1–10) *Y. enterocoltica* isolates shows positive results.

40.33 ± 5.48, while in *Y. enterocoltica L. plantarum* showed the strongest antibacterial activity with DIZs 38 mm, followed by *L. acidophillus* and *L. rhamnosus* (Table 7).

Antibaterial activity of CEO nano emulsion against B. cereus and Y. enterocoltica isolates

Bacillus cereus shows high sensitivity for 1.5% CEO nano emulsion reaching 38.00 ± 4.35, while *Y. enterocoltica* showed inhibition at 2% with 38.67 ± 2.4. $p < 0.05$ showed a significant decrease in values (Mean ± SE) in Table 7 which assumed that *L. plantarum*, 1.5% CEO, and 2% have a more powerful effect against the examined isolate.

Discussion

Dairy foods contaminated by contagious microorganisms are considered the most critical matter of human-being health and safety worldwide. Spoilage of dairy foodstuffs is highly risky because of the ability of raw milk and its dairy products to accelerate progression. *Bacillus cereus* is recognized as a significant pathogenic microorganism that is characterized by diarrheal and emetic syndrome (Radmehr *et al.*, 2020; Han *et al.*, 2023).

Thus, *B. cereus* is a significant microbe that threatens human beings whether their toxins are present or not. In comparison with our findings, Hefny *et al.* (2020) and Osama *et al.* (2020) isolated *B. cereus* from 40% of tested Kareish cheese while (Heikal and Al-wakeel, 2014; Ibrahim *et al.*, 2015) failed to isolate *B. cereus* in their examined cheese samples. In opposite to our result, Adam *et al.* (2021) and Fetouh *et al.* (2022) indicated that the occurrence of *B. cereus* in yogurt samples was at 8.0%, and 4.0%, respectively, while it could not be detected in yogurt samples by Tirloni *et al.* (2017). The absence of growth or even low incidence observed might be owing to the low pH < 5.

Lower results were reported by Owusu-Kwarteng *et al*. (2017) who found that *B. cereus* is isolated at 47.00% from raw cow milk in Ghana. Also, Mohamed *et al*. (2016) declared that *B. cereus* was found in 60.00% of raw cow milk samples. Abraha *et al*. (2017) reported that *B. cereus* rate exists at 8.80% in raw cow milk samples in Ethiopia.

Although *B. cereus* was detected at 32% in ice cream, a higher rate (62.7%) was defined by Messelhäusser *et al.* (2010). Consequently, variation in *B. cereus* rate is owing to different bacterial loads and poor hygiene standards during the preparation and production process. *Bacillus cereus* is recurrently isolated from raw milk because of pasteurization process resistance (Tirloni *et al.*, 2017).

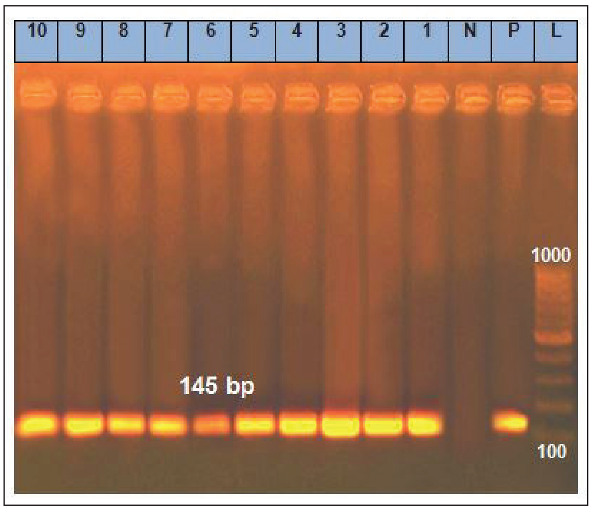

Fig. 3. Amplification of *Y. enterocoltica* isolates on agarose gel using *YST* (145bp). Lane (1–10) *Y. enterocoltica* isolates show positive results.

According to our result, *B. cereus* was isolated at 22 (44%) of the examined rice pudding and this disagreed with Morsy *et al.* (2022) who isolated *B. cereus* at 84% in rice pudding samples. The same findings were defined before (Mohamed *et al.*, 2016; Amin, 2017; El-Sherif *et al.*, 2021). While, higher results were also reported (Hussein *et al.*, 2015; El-Karamani, 2017; El-Zmakan and Mubarak, 2017; El-Sherif *et al.*, 2021).

Our study discussed the mechanism of food contamination with *B. cereus*. Certainly, the main source of contamination is poor sanitation and cross contamination. Overall, the raw ingredients and the post-pasteurization contamination might increase the prevalence of *B. cereus*. This concluded that firm cleaning administration must be approved to control *B. cereus* with the aim of assuring high quality and safety products.

According to our result, *nhe*A gene was found in 50% of *B. cereus* isolated from each raw milk and yogurt. Higher occurrence rates were recorded by Eltokhy *et al.* (2021) who detected *nhe*A in all isolates. Fetouh *et al.* (2022) stated that non-hemolytic (*nheA*) and cytotoxic K (*cytK*) genes were involved in food poisoning. Hefny *et al.* (2020) who failed to detect ces gene, detected cytk in 47% of all isolated *B. cereus* while, nhe and hbl were detected at 52% and 33%, respectively. Bianco *et al.* (2020) detected sich cytotoxic genes in 35%–100% of their isolated strains.

Bacillus cereus revealed high sensitivity to ampicillin followed by amoxicillin, clavulanic, and ciprofloxacin

Table 6. Antimicrobial sensitivity test for the isolated *B. cereus* and *Y. enterocolitica.*

Antibacterial agents	Susceptibility			Result	
	Sensitive	Intermediate	Resistant	*Y. enterocolitica*	*B. cereus*
Ciprofloxacin	≥26	22–25	≤21	Resistant	Intermediate
Ampicillin	≥17	14–16	≤13	Sensitive	Sensitive
Amoxicillin-clavulanic acid	≥18	14–17	≤13	Intermediate	Intermediate
Erythromycin	≥15	13	≤12	Resistant	ND

Table 7. Antibacterial activities of different probiotic cell free supernatant (CFS) and CEO nano emulsion against *B. cereus* and *Y. enterocolitica* according to control positive inhibition zone detected by mm.

Isolate	Ampicillin	CEO Nano emulsion	*L. plantarum*	*L. acidophillus*	*L. rhamnosus*
B. cereus	33.00 ± 4.04^{ab}	38.00 ± 4.35^{a}	40.33 ± 5.48^{a}	19.00 ± 2.08^{c}	23.33 ± 1.76^{bc}
Y. enterocoltica	29.33 ± 5.21^{ab}	38.67 ± 2.4^{a}	32.67 ± 4.81^{ab}	21.33 ± 2.40^{bc}	15.67 ± 2.6^{c}

Means in the same row with a different superscript letter are significantly different ($p < 0.05$).

with high resistance toward erythromycin and this might be due to the misuse of antibiotics in dairy farms. This finding differs from Ahmed *et al.* (2023) who said that all examined isolates were 100% sensitive to erythromycin, 100% resistant to tetracycline, and intermediate sensitive to ciprofloxacin. EFAS stated that probiotics to be used in food stuff; it should not have antimicrobial resistance genes (EFSA, 2007).

Yersinia spp., is capable of growing in both anaerobic and aerobic situations. Nguyen *et al.* (2019) concluded that *Y. pseudotuberculosis*, *Y. enterocolitica*, and *Y. pestis* are pathogenic and transferred through contaminated milk. Yersiniosis is stated as a zoonotic syndrome with a public health importance. EFSA (2009) revealed that *Y. enterocolitica* in the whole European Union is considered one of the most informed zoonoses. *Yersinia enterocolitica* proliferated in the lower small intestine and upper large intestine, pusjing the organism establishment (Chlebicz and Śliżewska, 2018). Consequently, Yersiniosis causes gastric infection because of the improper handling and cooking of animal-origin foods during preparation (Tavassoli *et al.*, 2018).

Yersinia was detected in 64% (160 out of 250) of the examined products. This result was classified as following: 33(66%) raw milk, 42(84%) yogurt samples, 16(32%) ice cream samples, 38 (76%) kariesh cheese samples, and 31(62%) from rice pudding. However, Gűven *et al*. (2010) isolated *Y. enterocolitica* from raw milk at 1.33%, while Darwish *et al*. (2015) isolated it at 38.5%. Hassan and Afify (2007) secluded the lowest incidence at 2% in Kareish cheese, while Basyoni and Elsheikh (2005) isolated it by 16.67%, and Basha *et al.* (2008) isolated it at 14%. A higher incidence of *Y. enterocolitica* was reported in kariesh cheese and yogurt at 60% and 84%, respectively. This might be due to poor hygiene during manufacture. Basha *et al.* (2008) confirmed that gathering Kareish cheese samples from shops and street retailers could clarify the poor quality.

In small-scale ice cream, Gűven *et al.* (2010) recorded the lowest incidence of *Y. enterocolitica* at 2.67% and Khalifa *et al.* (2007) could isolate it at 12.5%. Nearly similar results have been recorded by AlShammary and Madi (2016) who isolated it with a percentage of 30%. Being psychrotrophic bacteria elevates their prevalence in ice cream which affect badly on cold food chain.

Similar results were recorded by Harakeh *et al.* (2012), and Ali and Al-Samarai (2020) who isolated *Y. enterocolitica* at 12%, and 9.75%; respectively. *Yersinia enterocolitica* was isolated at 5.78%, 5.3%, and 3.3%, respectively (Ali *et al.*, 2015; Ozdemir and Arslan, 2015). While, higher incidence was recorded by Darwish *et al*. (2015) and Ahmed *et al.* (2019) at 46%, and 22%, respectively, whereas Zeinhom and Abdel-Latef (2014) could not isolate it.

PCR was able to identify pathogenic strain genes in the isolates. We focused on virulence-associated genes of 10 isolates of *Y. enterocolitica*. These genes were *16S rRNA* for detection and *yst* for virulence. PCR results declared that genes were found in all isolates. Younis *et al.* (2019) detected *tetA* and *blaTEM* genes in all eight isolates. On the other hand, it is remarkable that the YST gene cannot be detected (Peruzy *et al.*, 2017). *Yersinia enterocolitica* revealed high resistance to ciprofloxacin, and erythromycin. Meanwhile, they were intermediate sensitive to AMC. Moreover, they were highly sensitive to ampicillin. While Abdelwahab *et al*. (2021) reported that *Y. enterocolitica* was highly sensitive to norfloxacin and meropenem (79.0% for each). Then, gentamycin was recovered at 68.4% and middle sensitive to doxycycline by 63.2%. These results demonstrated that *Y. enterocolitica* had multiple antibiotic resistances. Thus, we tried to demonstrate other alternatives as natural probiotics and essential oils.

The misapplication of the antibacterial agents could raise the bacterial resistance. As *B. cereus* and *Y. enterocolitica* were examined against several antibiotics. All tested isolates showed high resistance levels. Probiotics that are applied in some foodstuffs and pharmaceutics are regarded to be safe, and they should not have transportable antibiotic-resistance genes (EFSA, 2007). Montassier *et al.* (2021) stated that antibacterial potential is a critical feature to assess the use of probiotics in the food industry (Aditya *et al.*, 2020). According to the current study, *L. plantarum* is characterized by powerful activity against *B. cereus* and *Y. enterocolitica* strains.

Lactobacillus plantarum (MK850930) showed inhibition zones of 40 mm in *B. cereus* and 38 mm in *Y. enterocolitica*. Ahmed et *al.* (2023) said that *L. plantarum* has an antimicrobial effect toward *B. cereus EMCC1006*. These studies disagreed with Yusra and Likaa (2013) who reported that in the case of *B. cereus*, *L. plantarum* MIC was 0.07 ml. Approaches of probiotics might successfully inhibit pathogens such as *L. monocytogenes* (Wu *et al.*, 2022), other studies stated that *L. casei* IMAU60214 was effective against *Escherichia coli* (Rocha-Ramírez *et al.*, 2023). Similarly, *L. fermentum* LBF433 and *L. casei* LBC 237 have sufficient action against *Salmonella* (Lando *et al.*, 2023).

Our study disclosed that *L. plantarum* had a significant inhibitory action on foodborne pathogens such as *Y. enterocolitica* and *B. cereus*. The main effect of the essential oils was attributed to their antimicrobial properties, and the ability to dissolve the cytoplasmic membrane of the bacterial cells (Kaskatep *et al.*, 2016; Ferrari *et al.*, 2012). One of the approaches to deal with these hydrophobic compounds is by dispersing them in nano emulsion delivery system (Singh *et al.*, 2023). Dávila-Rodríguezet *et al.* (2019) reported that one of Eos disadvantages on food is its flavor effect in food products. To solve these issues, CEO can be encapsulated in nano emulsions to increase the stability, solubility, and potential activity of CEO (Akhavan *et al.*, 2018).

Paudel *et al.* (2019) stated that CEO has a proper source of antifungal and antimicrobial compounds. This activity is basically linked to eugenol, cinnamaldehyde, and cinnamic acid (Siripatrawan, 2016). In comparison with Eos nonencapsulation, nanoemulsions were extra active against bacteria, needing less than 50% EOs to decrease 5-log bacterial count. Numerous studies discussed the effectiveness of CEO against food-borne pathogens (Cava-Roda *et al.*, 2010; Aliakbarlu *et al.*, 2013).

CEO nano emulsion has effective power on food-borne diseases. In our study, *B. cereus* was found to be delicate to CEO nano emulsion 0.5% with IZD 34 mm, *Y. enterocolitica* sensitive to CEO nano emulsion 2%. Gupta *et al.* (2008) reported that cinnamon oil is very supportive against *Bacillus* spp. with MIC 1.25%. Azadi *et al.* (2023) concluded that CEO nano emulsion have a notifiable activity toward *Staphylococcus aureus and B. cereus* more than *S. typhimurium and E. coli* (*O157:H7*).

Sharma *et al.* (2022) stated that the CEO was the most operative as an antimicrobial agent. It is recognized by the presence of cinnamaldehyde. Cinnamaldehyde is an effective natural antioxidant that prevents stomach ulcers, preventing both strains of *Helicobacter pylori*, also it can be inhibited through the growth of yeast, molds, and bacteria (Basak *et al.*, 2021). Oregano, basil, rosemary, and thyme were branded as the most favorable active antimicrobial EOs against *Y. enterocolitica* recently which deserve further studies (Durofil *et al.*, 2022). It was found that *Y. enterocolitica* was sensitive to CEO nano emulsion 2% in our study. Hulankova (2022) concluded that *Y. enterocolitica* and *Y. pseudotuberculosis* are multi resistant bacteria against cinnamon oil by MICs (median 414 and 207 µg /ml, respectively). Koua *et al.* (2014) referred to the importance of micro-organisms not to acquire resistance toward essential oils. CEO contains cinnamaldehyde (68.79%) which was proven to have high antibacterial activity against G + ve bacteria (Es *et al.*, 2017). *Yersinia enterocolitica* was the most sensitive bacteria to *Cinnamomum cassia* EOs with a zone of inhibition (16.67 mm). Klūga *et al.* (2021) examined 14 types of Eos against *Y. enterocolitica* and found extreme action against pathogenic microbes.

Conclusion

Entero-pathogenic *Yersinia* and *B.* cereus showed a great resistance level toward some antibiotics. Other approaches were used effectively against *Y. enterocolitica* and *B. cereus* such as *L. plantarum* which have a functional inhibition potential. CEO was effective against both *Yersinia* and *Bacillus* spp. Therefore, EOs and probiotics can be hopeful alternate inhibitors for these multiresistance strains.

Acknowledgments

All appreciation to the Food Control Department, Faculty of Veterinary Medicine, Zagazig University.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

No external funding has been provided.

Authors contributions

All authors contributed to his study. All authors read and approved the final manuscript.

Data availability

All data are provided in the manuscript.

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