Damanhour Journal of Veterinary Sciences

Journal homepage: https://djvs.journals.ekb.eg/

E-ISSN 2636-3003 | ISSN 2636-3011

The impacts of ginger and olive oils on competing multidrug resistant *Bacillus cereus* in beef mince, luncheon, sausage, and pasterma

Abdelazim Elsayed Elhelaly[1], Wafaa M. Gad[2], Nashwa M. Zaki[3], Lamiaa M. Reda[4], Amany M. Shosha[5], Wageh Sobhy Darwish[6]*

[1]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt
[2]Department of Bacteriology, Animal Health Research Institute (AHRI), Mansoura Branch, Agriculture Research Center (ARC), Egypt.
[3]Food Hygiene Department, Animal Health Research Institute (AHRI), Mansoura Branch, Agriculture Research Center (ARC), Egypt.
[4]Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt
[5]Bacteriology, Mycology, Immunology Department, Faculty of Veterinary, Medicine, Mansoura University, Egypt.
[6]Department of Food Hygiene, Safety, & Technology, Faculty of Veterinary Medicine, Zagazig University, Egypt

Abstract

Meat products, like beef mince, luncheon, sausage, and pasterma, are vital sources of necessary amino acids, vitamins, minerals, and other nutrients. Nevertheless, these products could serve as potential reservoirs for foodborne pathogens, including *Bacillus cereus (B. cereus)*. The primary goal of the present study is to investigate the prevalence of *B. cereus* in meat products sold in Egyptian local markets. Furthermore, the antibiotic resistance of the recovered *B. cereus* isolates was investigated. In addition, the antibacterial properties of olive and ginger oils against *B. cereus* were evaluated. The data collected indicated that *B. cereus* was present in 4%, 6%, 10%, and 16% of the analyzed samples of luncheon, sausage, pasterma, and minced meat, respectively. The obtained isolates presumably exhibited a multidrug resistance profile. It is notable that ginger and olive oils had significant antibacterial activity against *B. cereus*, particularly at a concentration of 2%. Hence, it is imperative to implement appropriate hygiene protocols while manufacturing such meat products. It is highly recommended to incorporate 2% ginger and olive oils in the culinary sector.

Keywords: *Bacillus cereus*, antibiotic sensitivity testing, ginger oil, olive oil.

*Correspondence: Wageh Sobhy Darwish
Email: wagehdarwish@gmail.com
P ISSN: 2636-3003
EISSN: 2636-2996
DOI: 10.21608/DJVS.2024.314200.1136
Received: August 20, 2024; Received in revised form: September 21, 2024; accepted: October 03, 2024
Editor-in-Chief:
Ass. Prof Dr/Abdelwahab A. Alsenosy (dr_alsenosy_2010@vetmed.dmu.edu.eg)

1. Introduction

Beef mince, sausage, luncheon, and pasterma are examples of red meat products that are high in essential amino acids, fats, vitamins, and minerals like iron and zinc. Children also favor these meat items because of their distinct flavor and aroma. However, microbial contamination may occur during the production of such meat products, affecting their safety and quality. Furthermore, microbiological contamination of these products might happen during storage, transport, or even post-processing **(Morshdy et al., 2024)**.

Meat products are frequently the source of *Bacillus cereus (B. cereus)*, one of the most challenging foodborne pathogens **(Rahnama et al., 2023)**. *B. cereus* is a facultative anaerobic, aerobic, rod-shaped, gram-positive bacterium that forms spores, according to **Liu et al. (2015)**. *B. cereus* has been linked to food poisoning cases worldwide because of its ability to produce enterotoxins **(JeBberger et al., 2014)**.

According to **Darwish et al. (2013)**, livestock farms commonly use antimicrobials drugs or agents to prevent and control bacterial infections, as feed additives to raise the feed conversion ratio, and as preservatives in the meat products industry. However, the overuse of these antimicrobials has led to the development of drug resistance in foodborne bacteria **(Alsayqh et al., 2021)**.

In addition to being consumed in its purest form on toast and in fresh salads, olive oil is also added to a number of homemade recipes and commercially produced items, including tuna, tomatoes, and mayonnaise. According to **Capapasso et al. (1995) and Markin et al. (2003)**, polyphenols present in olive fruits, waste fluids from olive oil, and olive leaves possess antibacterial capabilities that can combat a diverse array of infections. However, only a few studies have looked into olive oil's antibacterial capabilities against *B. cereus* **(Keceli and Robinson, 2002)**.

Because of its distinct flavor and aroma, as well as its proven antibacterial properties, ginger rhizome is widely used as a spice in herbal medicine and the food industry. Researchers found that *Ginger rhizome* essential oil effectively prohibit bacterial growth such as *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Streptococcus faecalis* **(Abdalla and Abdallah, 2018)**.

This study, taking into account the previously mentioned information, aimed to ascertain the prevalence of *B. cereus* in meat products sold in Egypt, including sausage, pasterma, luncheon, and beef mince. Besides, the antimicrobial susceptibility testing of the recovered isolates was screened. Finally, we studied the effect of the ginger and olive oils on *B. cereus*.

2. Material and Methods:

Sample collection:

We randomly collected two hundred samples of meat products, including pasterma, luncheon, sausage, and beef mince (n = 50 per each product), from local markets in Sharkia governorate, Egypt. We collected the samples, cooled them in ice box, and immediately brought them to the laboratory of Food Hygiene, Faculty of Veterinary Medicine, Zagazig University for the isolation and identification of *B. cereus*.

Isolation and identification of B. cereus:

Bacillus cereus was isolated using the methods described by **APHA (2001) and Tallent et al. (2012**). Peptone water was used for enrichment, while polymyxin-pyrovate-egg yolk-mannitol-bromothymol blue agar base (PEMBA) supplemented with polymyxin B and egg yolk (Oxoid) was used as selective media. The isolates were subjected to biochemical testing, as described by **Markey et al. (2013)**. Confirmation of the recovered isolates and their virulence associated genes was done using PCR as described before (**Ehling-Schulz et al., 2006)**.

Antibiotic Resistance of B. cereus:

We employed the single diffusion method to assess antibiotic susceptibility, following the guidelines set by the Clinical and Laboratory Standards Institute **(Wayne, 2013).** The susceptibility of the isolated bacterial strains was evaluated by employing sensitivity discs at various concentrations (Oxoid Limited, Basingstoke, Hampshire,UK). The agar plate method employed nutritional agar as a growing medium to assess the antibiotic susceptibility of the tested bacterium. The bacterial culture was uniformly dispersed throughout the surface of the nutritional agar. Subsequently, we applied the antibiotic discs over the surface of the inoculation plate. In addition, the plate was subjected to incubation for a period of two to seven days at a temperature of 25°C, considered suitable for the experiment. The progression of bacterial growth around the antibiotic discs was seen and recorded. We categorized the strains that were evaluated as resistant, intermediate, and susceptible. The formula presented by **Singh et al. (2010)** was utilized to calculate the Multiple Antibiotic Resistance (MAR) index for each strain. The MAR index is determined by dividing the total number of tested antibiotics by the number of resistance isolates. It is important to note that isolates categorized as intermediate are considered sensitive for the MAR index calculation.

The anti-B. cereus activity of olive and ginger oils

The anti-growth properties of olive and ginger oils on *B. cereus* were evaluated. The oils were purchased at 100% concentration from Orgnic company, Egypt and added to corn oil (act as a negative control) at two distinct concentrations: 1% and 2%. Meatballs were made with minced meat that was devoid of *B. cereus*. Twenty-five meat balls, weighing 50 g each, were divided into five groups, with five balls in each group. *B. cereus* (recovered from the present study) was inoculated into the balls at a concentration of 6 log cfu/g. Group 1 served as the control group and was immersed in corn oil for thirty minutes. Group 2 spent 30 minutes immersed in a 1% olive oil solution, while Group 3 spent the same amount of time submerged in a 2% olive oil solution. For thirty minutes, Group 4 was immersed in 1% ginger oil, while Group 5 was immersed in 2% ginger oil as well. Following this, *B. cereus* was counted on Tryptic Soya Agar (TSA, Oxoid). The methodology described by **Bourdoux et al. (2018)** was used to evaluate the sensory qualities and rates of deterioration.

Statistical analysis:

To do the statistical analysis, the SPSS-21 software, a statistical package for social sciences headquartered in Chicago, IL, USA, was used. The differences between each group were examined using the Duncan multiple range test. P<0.05 was considered statistically significant, and a 95% confidence level was used to apply significance.

3. Results and Discussion

Red meat products might act as potential sources of human exposure to *B. cereus*. The latter can find the way to the animal products during any stage of meat processing starting from the slaughter process to dressing, evisceration, manufacture, and packaging or during distribution and storage.

The obtained results of the present study revealed isolation of *B. cereus* from the examined samples at 4% (2 out of 50 samples), 6% (3 out of 50 samples), 10% (5 out of 50 samples), and 16% (8 out of 50 samples) from the examined luncheon, sausage, pasterma, and minced meat, respectively **(Fig. 1)**. Minced meat had the highest contamination rate which can be attributed to the contamination from the mincing machine; while other products were either exposed to heat treatment as luncheon and sausage, or had some additives as in pasterma. In agreement with our findings, **Guven et al. (2006)** isolated *B. cereus* from beef, ground meat, Soudjouck, and Pastrami retailed in Turkey at 56%, 40%, 12%, and 4%, respectively. Besides, **Rather et al. (2012)** isolated *B. cereus* from 97 (37.45%) of the 259 meat product samples collected from markets in India. The prevalence of *B. cereus* in chicken, chevon, mutton, meat products, and swab samples was 33.33%, 37.20%, 39.47%, 51.85%, and 26.08%, respectively. The prevalence of B. cereus infection in meat product samples in China was 26.37% (159 out of 603) **(Kong et al., 2021)**. Toxin coding genes were detected in the recovered isolates in the present study. Hospitalizations due to *B. cereus* are commonplace worldwide. Fever, diarrhea, stomach pain, vomiting and dehydration are signs of *B. cereus* infection **(Guven et al., 2006; Rather et al., 2012; Kong et al., 2021).**

Antibiotic abusage is causing *B. cereus* to become more resistant to drugs over time, and different regions are seeing distinct pandemic trends. Previous reports **(Alsayeqh et al., 2021)** have indicated that antibiotic-resistant *B. cereus* has been linked to outbreaks of foodborne disease, particularly multidrug-resistant (MDR) *B. cereus*, which present a threat to public health security **(Darwish et al., 2013)**.

In this study, antimicrobial resistance was screened for the recovered *B. cereus* as seen in **Tables 2, and Fig. 2**. Multidrug resistance was very apparent in the current study. The screened isolates of *B. cereus* showed the highest resistance (100%) to nalidixic acid, cephalothin, and Sulphamethoxazol, while the lowest resistance was shown towards Linezolid and Daptomycin at 11.1%, and 5.6%, respectively, with an average MAR index of 0.579. These results agree with previous reports that studied the antimicrobial resistance of *B. cereus* **(Alsayeqh et al., 2021)**. Multidrug resistant forms of bacteria are becoming more frequently as a result of the widespread use of antibiotics in recent decades, posing serious risks to public health. *B. cereus* is able to quickly develop resistance to almost all antibiotics due to its ability to adapt to its surroundings. In agreement with the obtained results of the present study, **Guven et al. (2006)** observed a high rate of resistance to oxacillin and amoxicillin in meat products retailed in Turkey. In addition, **Rather et al. (2012)** reported that *B. cereus* isolates recovered from meat product samples retailed in India showed multidrug resistance towards Penicillin G (91.75%), Ampicillin (42.26%), tetracycline (32.98%) and Linezolid (31.95%), respectively.

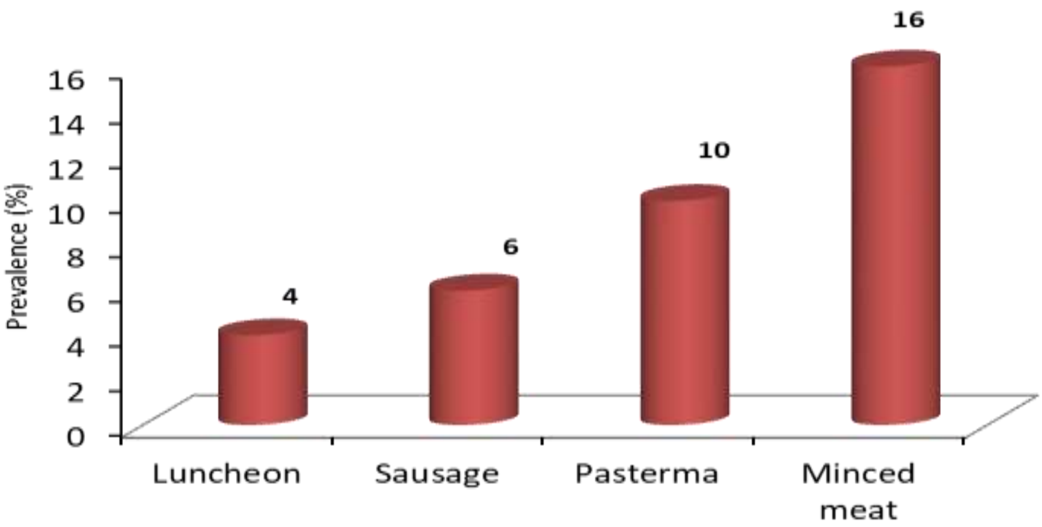

Fig. 1: Prevalence rate (%) of B. cereus in the examined chicken meat products.

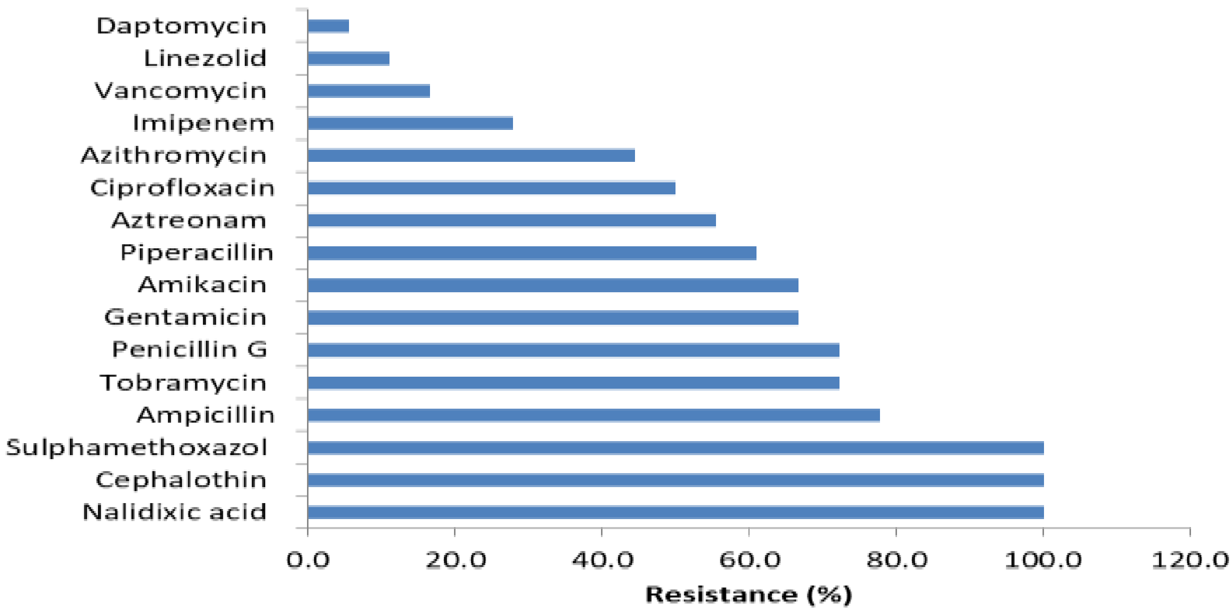

Fig. 2: Antimicrobial resistance rates in the recovered *B. cereus* isolates

Table 2: Antimicrobial resistance profile of *B. cereus* (n=18).

NO	Antimicrobial resistance profile	MAR index
1	NA, CN, SXT, AM, TO, PG, G, AK, P, AT, CP, AZ, IPM, V, LZ, DA	1
2	NA, CN, SXT, AM, TO, PG, G, AK, P, AT, CP, AZ, IPM, V, LZ	0.937
3	NA, CN, SXT, AM, TO, PG, G, AK, P, AT, CP, AZ, IPM, V	0.875
4	NA, CN, SXT, AM, TO, PG, G, AK, P, AT, CP, AZ, IPM	0.812
5	NA, CN, SXT, AM, TO, PG, G, AK, P, AT, CP, AZ, IPM	0.812
6	NA, CN, SXT, AM, TO, PG, G, AK, P, AT, CP, AZ	0.75
7	NA, CN, SXT, AM, TO, PG, G, AK, P, AT, CP, AZ	0.75
8	NA, CN, SXT, AM, TO, PG, G, AK, P, AT, CP, AZ	0.75
9	NA, CN, SXT, AM, TO, PG, G, AK, P, AT, CP	0.687
10	NA, CN, SXT, AM, TO, PG, G, AK, P, AT	0.625
11	NA, CN, SXT, AM, TO, PG, G, AK, P	0.562
12	NA, CN, SXT, AM, TO, PG, G, AK	0.5
13	NA, CN, SXT, AM, TO, PG	0.375
14	NA, CN, SXT, AM	0.25
15	NA, CN, SXT	0.187
16	NA, CN, SXT	0.187
17	NA, CN, SXT	0.187
18	NA, CN, SXT	0.187
	Average = 0.579	

AM: Ampicillin V: Vancomycin PG: Penicillin G
CN: Cephalothin DA: Daptomycin SXT: Sulphamethoxazol
AZ: Azithromycin NA: Nalidixic acid AK: Amikacin CP:Ciprofloxacin G: Gentamicin
LZ: Linezolid TO: Tobramycin P: Piperacillin AT: Aztreonam IMP: Imipenem

Both ginger and olive oils showed a pronounced and potent anti-*B. cereus* effects in the protection testing, according to the current investigation. 1% and 2% ginger oil, respectively, decreased *B. cereus* at 11.16% and 31.11%, respectively. Similarly, *B. cereus* was lowered by 1% and 2% of olive oil, respectively, at 8.48% and 29.84% **(Fig. 3)**. In agreement with the obtained results of the present study, ginger had clear inhibitory effects against *B. cereus* using either the disk diffusion or agar well diffusion methods **(Chand et al., 2013)**. The antimicrobial activities of olive oil also agree with **Aliabadi et al. (2012)** who illustrated the antimicrobial activities of olive oil against *B. cereus, Salmonella Typhimurium, S. aureus* and *E. coli*. In addition, **Fei et al. (2019)** confirmed the antimicrobial activities of olive oil against the vegetative *B. cereus*. Such activities were attributed to the richness of the olive oil with polyphenols such as flavonoids, carotenoids, and alkaloids. The samples under examination retained their sensory qualities after using the oils **(Nadarajah et al., 2005; Tomar and Shrivastava, 2014)**. The antibacterial effects of the oils employed against *B. cereus* may be attributed to the decrease in intracellular ATP levels, depolarization of the cell membrane, reduction in bacterial protein content, and leakage from the cytoplasm **(Fei et al., 2019)**. The findings demonstrated that olive and ginger oils have the potential to inhibit the proliferation of *B. cereus* cells in the meat products industry.

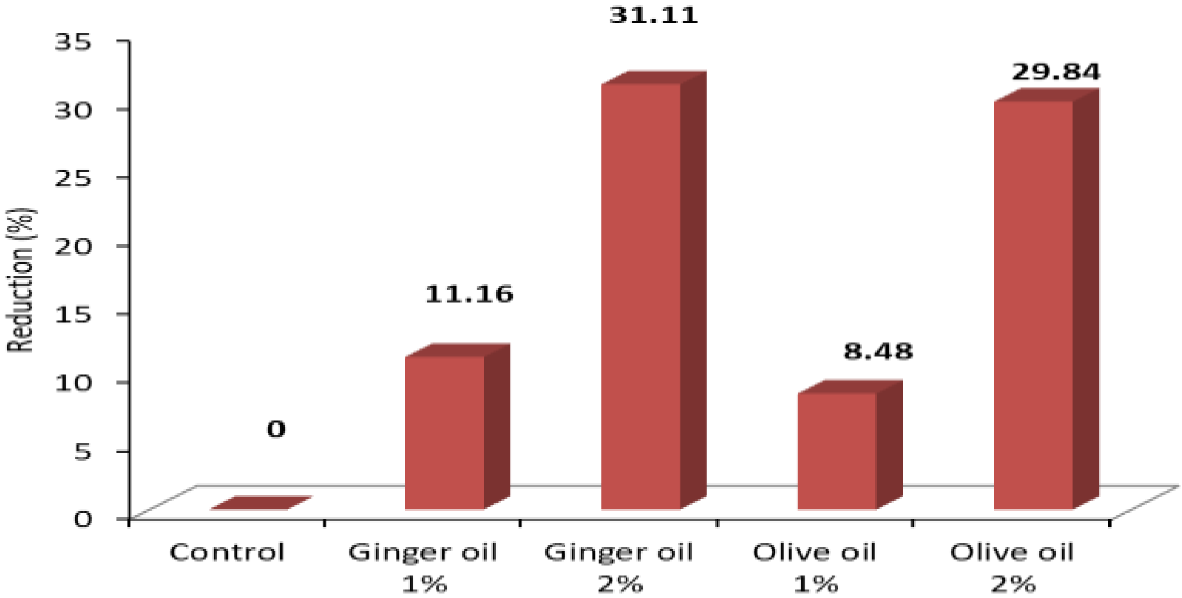

Fig. 3: Anti-B. cereus activities of ginger and olive oils

4. Conclusion:

The findings of this study demonstrated that improper sanitation practices during the handling of chicken meat products resulted in the contamination of those items with *B. cereus*. As a result, stringent sanitation regulations must be adhered to when processing chicken meat products. Using 2% olive and ginger oils is highly recommended in order to lower the *B. cereus* load that contaminates chicken meat products.

Conflict of interest: The authors have no conflicts of interest.

5. References

Abdalla, W.E. and Abdallah, E.M., 2018. Antibacterial activity of ginger (*Zingiber Officinale Rosc.*) Rhizome: a mini review. Inter. J. Pharmacogn. Chin. Med., 2(4), p.000142.

Alsayeqh, A.F., Baz, A.H.A. and Darwish, W.S., 2021. Antimicrobial-resistant foodborne pathogens in the Middle East: A systematic review. Environ. Sci. Pollut. Res., 28(48), 68111-68133.

Aliabadi, M.A., Darsanaki, R.K., Rokhi, M.L., Nourbakhsh, M. and Raeisi, G., 2012. Antimicrobial activity of olive leaf aqueous extract. Ann. Biol. Res., 3(8), 4189-4191.

APHA "American Public Health Association"., 2001. Compendium of Methods for the Microbiological examination of Foods. 4th edition. APHA. Washington D.C., USA.

Bourdoux, S., Rajkovic, A., De Sutter, S., Vermeulen, A., Spilimbergo, S., Zambon, A., Hofland, G., Uyttendaele, M. and Devlieghere, F., 2018. Inactivation of Salmonella, *Listeria monocytogenes* and *Escherichia coli* O157: H7 inoculated on coriander by freeze-drying and supercritical CO2 drying. Innov. Food Sci. Emerg. Technol., 47, 180-186.

Budiati, T., Suryaningsih, W., Umaroh, S., Poerwanto, B., Bakri, A., Kurniawati, E., 2018, December. Antimicrobial activity of essential oil from Indonesian medicinal plants against food-borne pathogens. In IOP Conference Series: Earth and Environmental Science (Vol. 207, p. 012036). IOP Publishing.

Capasso, R., A. Evidente, L. Schivo, G. Orru, M. A. Marcialis, and G. Cristinzo. 1995. Antibacterial polyphenols from olive mill waste waters. J. Appl. Bacteriol., 79, 393–398.

Chand, B., 2013. Antibacterial effect of garlic (Allium sativum) and ginger (Zingiber officinale) against *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Bacillus cereus*. J. Microbiol. Biotechnol. Food Sci., 2(4), 2481-2491.

Darwish, W.S., Eldaly, E.A., El-Abbasy, M.T., Ikenaka, Y., Nakayama, S. and Ishizuka, M., 2013. Antibiotic residues in food: the African scenario. Jpn. J. Vet. Res., 61, S13-S22.

Ehling-Schulz, M., Guinebretiere, M.H., Monthán, A., Berge, O., Fricker, M., Svensson, B., 2006. Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*. FEMS Microbiol. Lett., 260, 232-240.

Fei, P., Xu, Y., Zhao, S., Gong, S. and Guo, L., 2019. Olive oil polyphenol extract inhibits vegetative cells of *Bacillus cereus* isolated from raw milk. J. Dairy Sci., 102(5), 3894-3902.

Gueven, K., Mutlu, M.B. and Avci, O., 2006. Incidence and characterization of *Bacillus cereus* in meat and meat products consumed in Turkey. J. Food Saf., 26(1), 30-40.

JeBberger, N., Dietrich, R., Bock, S., Didier, A., Märtlbauer, E., 2014. Bacillus cereus enterotoxins act as major virulence factors and exhibit distinct cytotoxicity to different human cell lines. Toxicon, 77, 49-57.

Keceli, T., and R. K. Robinson. 2002. Antimicrobial activity of phenolic extracts from olive oil. Milchwissenschaft, 57, 436–440.

Kong, L., Yu, S., Yuan, X., Li, C., Yu, P., Wang, J., Guo, H., Wu, S., Ye, Q., Lei, T. and Yang, X., 2021. An investigation on the occurrence and molecular characterization of *Bacillus cereus* in meat and meat products in China. Foodborne Pathog. Dis., 18(5), 306-314.

Liu, Y., Lai, Q., Göker, M., Meier-Kolthoff, J.P., Wang, M., Sun, Y., Wang, L., Shao, Z., 2015. Genomic insights into the taxonomic status of the *Bacillus cereus* group. Sci. Rep., 5, 14082.Markey, B.K., Leonard, F.C., Archambault, M., Cullinane, A., Maguire, D., 2013. Clinical Veterinary Microbiology, Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.

Markin, D., L. Duek, and I. Berdicevsky. 2003. In vitro antimicrobial activity of olive leaves. Mycoses 46:132–136.

Morshdy, A.E.M., Morsy, D.M., Mahmoud, A.F. and Darwish, W., 2024. Antimicrobial activities of coriander in chicken meat products: A review. J. Adv. Vet. Res., 14(2), 326-329.

Nadarajah, D., Han, J.H., Holley, R.A., 2005. Use of mustard flour to inactivate *Escherichia coli O157:H7* in ground beef under nitrogen flushed packing. Inter. J. Food Microbiol., 99, 257-267.

Rahnama, H., Azari, R., Yousefi, M.H., Berizi, E., Mazloomi, S.M., Hosseinzadeh, S., Derakhshan, Z., Ferrante, M., Conti, G.O., 2023. A systematic review and meta-analysis of the prevalence of *Bacillus cereus* in foods. Food Control, 143, 109250.

Rather, M.A., Aulakh, R.S., Gill, J.P.S. and Ghatak, S., 2012. Enterotoxin gene profile and antibiogram of *Bacillus cereus* strains isolated from raw meats and meat products. J. Food Saf., 32(1), 22-28.

Singh, A., Yadav, S., Singh S., Bharti, P., 2010. Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Res. Inter., 43, 2027-2030

Tallent, S.M., Kotewicz, K.M., Strain, E.A., Bennett, R.W., 2012. Efficient isolation and identification of *Bacillus cereus* group. J. AOAC Inter., 95, 446-451.

Tomar, R.S., Shrivastava, V., 2014. Efficacy evaluation of ethanolic extract of Brassica nig potential antimicrobial agent against selected microorganisms. Inter. J. Pharmacol. Sci. Health Care, 4, 3.

Wayne, P. 2013. Performance standards for antimicrobial susceptibility testing. CLSI approved standard M100–S23. Clin. Lab. Stand. Instit., 33, 118-156.