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Influence of essential oil galangal and yellow mustard on multidrug resistant *Bacillus cereus* in chicken meat products

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ABSTRACT

Chicken meat products are a great way to get vitamins, minerals, and nutrients that come from animals. Nonetheless, products containing chicken meat could expose people to foodborne pathogens including *Bacillus cereus (B. cereus)*. The current study's initial goals were to find out how common *B. cereus* was in chicken meat products (burger, pane, nuggets, and luncheon, 50 each) sold in Egypt's local marketplaces. Second, PCR was applied to detect *B. cereus* enterotoxin-coding genes, such as non-haemolytic enterotoxin (*nhe*), haemolysin B (*hbl*), and cereulide (*ces*), which are the causes of diarrheal and emetic illness. In addition, the disk diffusion method was used to test antibacterial sensitivity of the isolates. Lastly, an assessment was conducted on *B. cereus* on the antibacterial properties of essential oils of galangal and yellow mustard. The data collected showed that 26% of the chicken meat products under examination had *B. cereus* isolated from them. In the case of the chicken burger, pane, nuggets, and luncheon under examination, *B. cereus* was isolated at 40%, 32%, 20%, and 12%, respectively. The enterotoxin-coding genes (*nhe, hbl, and ces*) were detected in the recovered *B. cereus* isolates. It's likely that multidrug resistance profiling was present in the retrieved isolates. It's interesting to note that yellow mustard and galangal oils had strong anti-*B. cereus* activity, especially at 2% concentration. Therefore, when producing such chicken meat products, stringent hygiene procedures should be followed. In the food industry, it is strongly encouraged to utilize 2% yellow mustard and galangal oils.

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INTRODUCTION

Chicken meat products contribute significantly since they are a competitively priced alternative to red meat, which is woefully undersupplied in Egypt. High-quality animal protein, necessary amino acids, and trace elements are abundant in this type of meat. The rapid improvements in food processing and technology have led to the production and distribution of a number of chicken meat products into the chicken meat markets, such as chicken burgers, chicken luncheon, chicken nuggets, and chicken pane. These significant products stand out for their unique flavor and scent, which attract consumers, particularly children **(El Bayomi et al. 2018 and Morshdy et al. 2024)**.

One of the most significant foodborne pathogens, *Bacillus cereus (B. cereus)*, is often recovered from meat and poultry meat products **(Rahnama et al. 2023)**. *B. cereus* can contaminate a variety of meals and foodstuffs, such as meat, meat products, chicken meat, and chicken meat products **(Aman et al. 2016)**. According to **Liu et al. (2015)**, *Bacillus cereus* is a rod-shaped, aerobic, spore-forming Gram-positive bacterium. Worldwide, *B. cereus* is linked to numerous incidences of food poisoning. These negative consequences result from the presence of important *B. cereus* toxins, such as cereulide (*ces*), which causes the emetic syndrome, and non-haemolytic enterotoxin (*nhe*), which causes diarrheal syndrome. According to **JeBberger et al. (2014)**, the most virulent enterotoxin of *B. cereus* is the *nhe*.

According to **Darwish et al. (2013)**, antimicrobials are widely used in chicken farms to prevent and manage bacterial infections, as feed additives to increase the feed conversion ratio, and as preservatives in the chicken meat products business. However, foodborne bacteria have developed drug resistance as a result of the unchecked use of such antimicrobials **(Alsayqh et al. 2021)**.

Galangal (*Alpinia officinarum*) is very famous spices especially for Thai foods. This spices gives taste to foods, however its medical characteristics is more important. This plant is used commonly as carminative, stomachic, antispasmodic medicine, and antibacterial drugs **(Özkinali et al. 2017)**.

Yellow mustard (*Sinapis alba*) oil was known for its antimicrobial and antioxidant activity due to its high content of phenolic compounds such as sinapine (the highest content) and sinapic acid (negligible concentration) **(Fahmi, 2016)**.

Insight of the previous facts, this study targeted an investigation of the prevalence of B. cereus in chicken meat products retailed in some Egyptian markets including luncheon, nuggets, burger and pane. Besides, examining the recovered isolates for the diarrheal toxin: haemolysin BL (*hbl*), and non-hemolytic enterotoxin (*nhe*), and the emetic toxin: cereulide toxin (*ces*). Furthermore, the antibiogram of the recovered isolates was screened. Lastly, the influence of the galangal and yellow mustard oils on *B. cereus* was investigated.

MATERIALS and METHODS

Sample preparation:

Collection of samples

Two hundreds of chicken meat product samples including luncheon, nuggets, burger, and pane (n = 50 per each product) were randomly collected from Egyptian local markets. The collected samples were transferred cooled directly to the Food Hygiene Department of the Animal Health Research Institute, Mansoura Branch, where they were tested for isolation and identification of *B. cereus*.

Isolation and identification of *Bacillus cereus*:

Using peptone water as enrichment and Polymyxin - pyruvate - Egg yolk - Mannitol - Bromothymol blue Agar base (PEMBA) with Polymyxin B and Egg yolk supplement (Oxoid) as a selective media, *Bacillus cereus* was isolated in accordance with **APHA (2001)** and **Tallent et al. (2012)**. Biochemical testing was done on the isolates according to **Markey et al. (2013)**.

Genotypic detection of some virulence genes in Bacillus cereus using polymerase chain reaction (PCR):

The virulence genes of 10 selected recovered *B. cereus* isolates were screened using PCR. For the purpose of genotyping the virulence genes of the ten recovered *B. cereus* strains, three sets of primers were employed **(Table 1)**. Haemolysin BL (*hbl*), non-hemolytic enterotoxin (*nhe*), and cereulide toxin (*ces*) were the genes associated with diarrheal and emesis toxins, respectively. Using the QIAamp® DNA Mini Kit (Catalogue no. 51304), the Emerald Amp GT PCR master mix (Takara, Japan) with Code No. RR310A, and 1.5% agarose gel electrophoreses **(Sambrook et al. 1989)**, DNA was extracted from the chosen isolates.

Table 1. Oligonucleotide primers sequences:

Primer	Sequence	Amplified product	Reference
hbl	F- GTA AAT TAI GAT GAI CAA TTTC R- AGA ATA GGC ATT CAT AGA TT	1091 bp	Ehling-Schulz *et al.*, 2006
nhe	F- AAG CIG CTC TTC GIA TTC R-ITI GTT GAA ATA AGC TGT GG	766 bp	
ces	F- GGTGACACATTATCATATAAGGTG R-GTAAGCGAACCTGTCTGTAACAACA	1271 bp	

Antibiotic Resistance of B. cereus (Antibiogram):

Antimicrobial susceptibility was tested by the single diffusion method according to CLSI guidelines **(Wayne, 2013)**. Sensitivity discs with variable concentrations were used to determine the susceptibility of the isolated bacterial strains (*Oxoid Limited, Basingstoke, Hampshire, UK*).

Agar plate method was applied by using nutrient agar as a substrate for growth of the tested bacterium for its antibiotic sensitivity. The bacterial culture was uniformly spread on the surface of nutrient agar. Then the antibiotic discs were placed over the surface of inoculated plate. Moreover, the plate was then incubated at suitable temperature (25°C) for 2-7 days and checked for the growth of the bacterium around the antibiotic discs.

The tested strains were evaluated as susceptible, intermediate and resistant. Multiple Antibiotic Resistance (MAR) index for each strain was determined according to the formula stipulated by **Singh et al. (2010)** as follow: MAR index= No. of resistance (Isolates classified as intermediate were considered sensitive for MAR index) / Total No. of tested antibiotics

Anti-B. cereus activity of galangal and yellow mustard oils:

Galangal and yellow mustard oils were tested for their ability to inhibit the growth of *B. cereus*. The oils were utilized at two different concentrations, specifically 1% and 2% in corn oil. Such concentrations were reported to have no alteration on the sensory attributed of different food subjects (Nguyen, 2020).

Twenty-five cubes of pane samples free from *B. cereus*, each weighing 50 g, were partitioned into five groups, with each group including 5 cubes. The cubes were inoculated with *B. cereus* (recovered from the current study) at a concentration of 6 log cfu/g. All examined samples were submerged in corn oil (has no antibacterial activity) for 30 minutes and samples were assigned as follow: Group 1 was set as a control group. Group 2 was submerged in 1% galangal for 30 minutes. Group 3 was submerged in 2% galangal. Group 4 was submerged in 1% yellow mustard in corn oil for 30

minutes. Group 5 was submerged in 2% yellow mustard. Subsequently, the enumeration of *B. cereus* was performed on Tryptic Soya Agar (TSA, Oxoid). The sensory attributes and rates of decline were assessed based on the methodology outlined by **Bourdoux et al. (2018)**.

Statistical analysis:

The statistical analysis was done using the SPSS-21 software, a statistical package for social sciences, based in Chicago, IL, USA. The Duncan Multiple Range test was used to analyze the differences among individual groups. A significant level of 95% confidence was applied, with $P<0.05$ deemed as statistically significant as **(Feldman et al. 2003)**.

RESULTS

The obtained results of the current study revealed isolation of *B. cereus* from the examined samples at 40%, 32%, 20%, and 12% from the examined chicken burger, pane, nuggets, and luncheon, respectively **(Fig. 1)**.

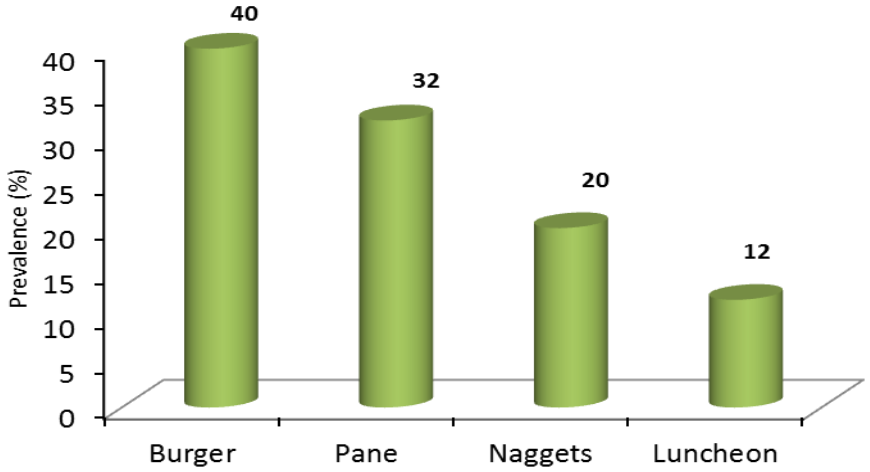

Fig. 1: Prevalence rate (%) of B. cereus in the examined chicken meat products.

The obtained results in **Fig. 2** revealed detection of the *hbl* and *nhe*-coding genes responsible for diarrhea in all screened isolates, while *ces*-coding gene responsible for vomiting was detected in 8 out of 10 examined isolates.

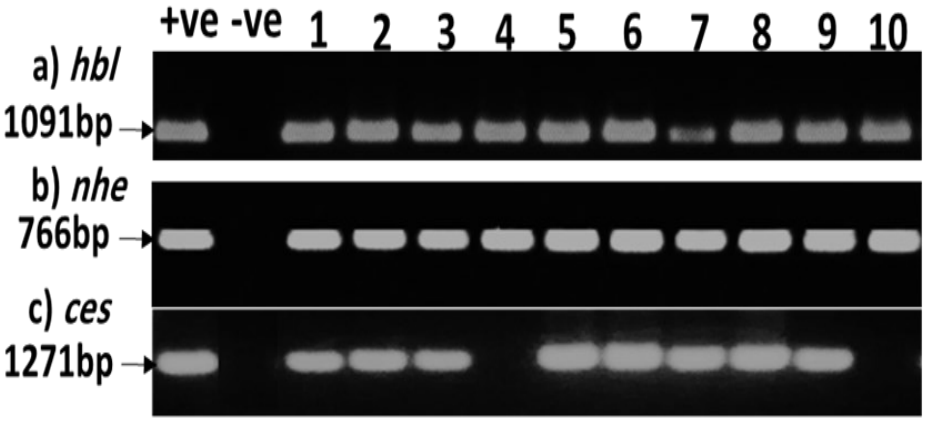

Fig. 2: Detection of hbl, nhe, and ces coding genes in the recovered B. cereus isolates

In this study, antimicrobial resistance was screened for the recovered *B. cereus* as seen in **Tables 2, and 3**. Multidrug resistance was very apparent in the current study. The screened isolates of *B. cereus* showed the highest resistance to nalidixic acid (100%) and cephalothin, and Sulphamethoxazol at 80% for both, while the lowest resistance was shown towards Daptomycin at 10%, with an average MAR index of 0.431.

Table 2. Antimicrobial susceptibility of *B. cereus* (n=10).

Antimicrobial agent	S		I		R	
	NO	%	NO	%	NO	%
Nalidixic acid (NA)	-	-	-	-	10	100
Cephalothin (CN)	-		2	20	8	80
Sulphamethoxazol (SXT)	1	10	1	10	8	80
Ampicillin (AM)	3	30	1	10	6	60
Tobramycin (TO)	3	30	2	20	5	50
Penicillin G (PG)	4	40	1	10	5	50
Gentamicin (G)	4	40	2	20	4	40
Amikacin (AK)	6	60	-	-	4	40
Piperacillin(P)	4	40	3	30	3	30
Aztreonam (AT)	5	50	2	20	3	30
Ciprofloxacin (CP)	5	50	2	20	3	30
Azithromycin (AZ)	6	60	1	10	3	30
Imipenem (IPM)	6	60	2	20	2	20
Vancomycin (V)	7	70	1	10	2	20
Linezolid (LZ)	8	80	-	-	2	20
Daptomycin (DA)	9	90	-	-	1	10

Table 3. Antimicrobial resistance profile of *B. cereus* (n=10).

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	0.750
4	NA, CN, SXT, AM, TO, PG, G, AK	0.500
5	NA, CN, SXT, AM, TO, PG	0.375
6	NA, CN, SXT, AM	0.250
7	NA, CN, SXT	0.188
8	NA, CN, SXT	0.188
9	NA	0.062
10	NA	0.062
	Average = 0.431	

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 galangal and yellow mustard oils showed a pronounced and potent anti-*B. cereus* effects in the protection testing, according to the current investigation. 1% and 2% galangal oil, respectively, decreased *B. cereus* at 11.65% and 33.33%, respectively. Similarly, *B. cereus* was lowered by 1% and 2% of yellow mustard oil, respectively, at 8.71% and 18.31% **(Table 4, Fig. 3)**. The samples under examination retained their sensory qualities after using the oils **(Nadarajah et al. 2005; Tomar and Shrivastava, 2014)**. Similarly, galangal oil was effective in the inhibition of *B. subtilis* and *E. coli* in an *in vitro* approach **(Rini et al. 2018)**.

Table 4. *B. cereus* counts (log 10 cfu/g) and reduction percentages after treatment with galangal and yellow mustard oils

	Min	Max	Mean	Reduction %
Control	5.9	6.17	6	0
Galangal 1%	5.17	5.6	5.3	11.65
Galangal 2%	3.69	4.3	4	33.33
Yellow Mustard1%	5	5.69	5.47	8.71
Yellow Mustard 2%	4	4.47	4.3	28.31

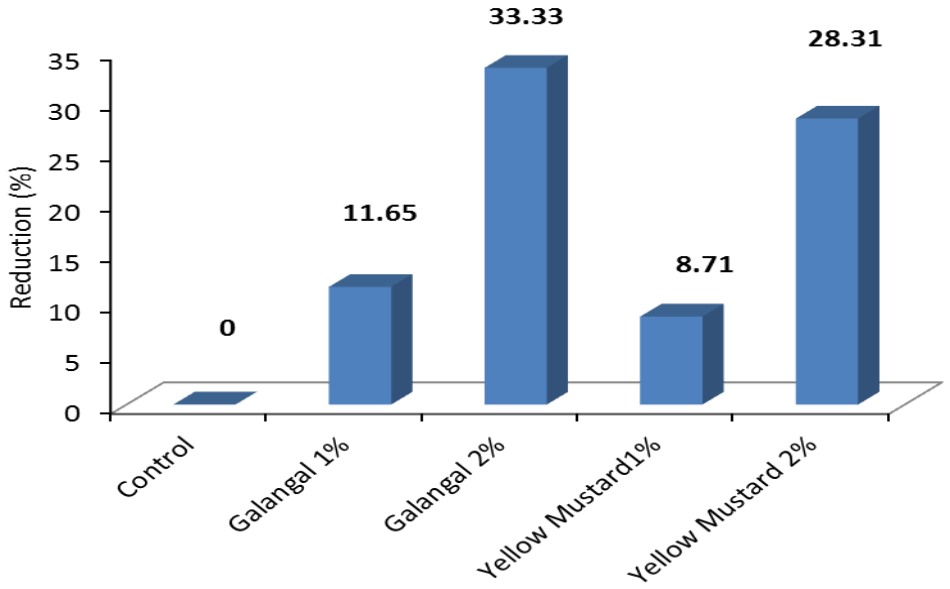

Fig 3. Anti-B. cereus activities of galangal and yellow mustard oils

DISCUSSION

Products made from chicken meat may become contaminated at any stage of the processing, packing, and shipping process with various pathogens. The chicken products become hazardous to customers and inappropriate for human consumption due to these pathogens.

The obtained results of the current study revealed isolation of *B. cereus* from the examined samples at 40%, 32%, 20%, and 12% from the examined chicken burger, pane, nuggets, and luncheon, respectively **(Fig. 1)**. In agreement with our findings, **Smith et al. (2004)** isolated *B. cereus* from chicken meat products including nuggets, fillets, and chicken parts retailed in USA at 45%.

Hospitalizations due to *B. cereus* are commonplace worldwide. Fever, diarrhea, stomach pain, vomiting and dehydration are signs of *B. cereus* infection **(Suthar et al. 2022)**. The obtained results in **Fig. 2** revealed detection of the *hbl* and *nhe*-coding genes responsible for diarrhea in all screened isolates, while *ces*-coding gene responsible for vomiting was detected in 8 out of 10 examined isolates. Likely, **Smith et al. (2004)** detected *hbl, ces, and nhe* in the recovered *B. cereus* isolates from chicken meat products.

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 3**. Multidrug resistance was very apparent in the current study. The screened isolates of *B. cereus* showed the highest resistance to nalidixic acid (100%) and cephalothin, and Sulphamethoxazol at 80% for both, while the lowest resistance was shown towards Daptomycin at 10%, with an average MAR index of 0.431. 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, **Suthar et al. (2022)** reported that *B. cereus* isolates recovered from raw chicken meat samples showed multidrug resistance towards Penicillin G, Ampicillin, Trimethoprim, Cefotaxime and Ceftazidime followed by Clindamycin.

Both galangal and yellow mustard oils showed a pronounced and potent anti-*B. cereus* effects in the protection testing, according to the current investigation. 1% and 2% galangal oil, respectively, decreased *B. cereus* at 11.65% and 33.33%, respectively. Similarly, *B. cereus* was lowered by 1% and 2% of yellow mustard oil, respectively, at 8.71% and 18.31% **(Table 4, Fig. 3)**. In agreement with the obtained results of the present study, yellow mustard oil at 1.5% and 2% could reduce other bacterial species such as *E. coli* and *S. aureus* contamination of meat and reduced the natural flora to undetectable levels **(Abu Zaid et al. 2018)**

The antimicrobial activities of mustard oil also agree with previous reports and were attributed to its richness of phenolic compounds 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)**. Similarly, galangal oil was effective in the inhibition of *B. subtilis* and *E. coli* in an *in vitro* approach **(Rini et al. 2018)**. Likely, galangal oil showed antimicrobial activity in vitro against *B. cereus*, *Staphylococcus aureus*, and *Salmonella Typhimurium* **(Budiati et al. 2018)**.

CONCULSION

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% galangal and/or yellow mustard 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.

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