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Prevalence of *Bacillus cereus* in food and hand swabs of night market food vendors and its relationship with their food safety knowledge, attitude, and practices

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Abstract

This paper aimed to determine *Bacillus cereus* prevalence in food and hand swab samples and its relationship with the level of knowledge, attitude, and practices (KAP) of the food vendors in the night market in northern Perak, Malaysia. A total of 140 cooked food and hand swab samples were collected in this study. About 28% (N = 83) of food and 42% (N = 57) of the food handlers' hands were detected with *B. cereus.* The KAP results of the food handlers showed a (mean ± SD) moderate knowledge score (73.96±15.79%), good attitude score (91.61±7.48%), and high practices score (81.70±11.94%). Further analysis revealed that the attitude of vendors independently influenced their practices. In addition, knowledge and practice scores were found to be significantly related to *B. cereus* hand carriage. Hence, the findings from this study act as an eye-opener for the authorities to improve awareness of food safety and stress the importance of good attitude and hygiene practices, especially among small food vendors.

1. Introduction

Food safety remains a major concern around the globe. At least 10% of the world's population falls ill yearly after consuming spoiled food, with about 30% of the deaths from foodborne diseases being children under the age of five (Centers for Disease Control and Prevention Disease [CDC], 2016; World Health Organization [WHO], 2019). Foodborne diseases can cause short-term symptoms (nausea, vomiting, and diarrhoea) and long-term illnesses (kidney or liver failure, brain, and neural disorders). These maladies may become more serious in children, pregnant women, elders, or those with a compromised immune system (WHO, 2013). Additionally, about 97% of food and waterborne diseases in Malaysia were attributed to food poisoning (Department of Standard Malaysia [DOSM], 2020).

Bacillus cereus, a pathogenic bacteria capable of growing in a wide range of temperatures, is often associated with foodborne disease (Carlin, 2016; Tirloni *et al*., 2019). Even though *B. cereus* is prevalent in cooked rice, it is also commonly found in a variety of foods (Schneider *et al.*, 2017; Osimani *et al.*, 2018). This is because besides naturally existing in the environment, its heat-resistant endospores render it a likely contaminant of cooked and chilled foods (Abee *et al*., 2011; Guérin *et al.,* 2017). Extended holding time for food prepared earlier and serving time with uncontrolled temperatures such as at night markets may increase the risk of food poisoning (Kalyoussef and Feja, 2014). An episode of food poisoning reported in a night market in Malaysia involved three stalls which caused 101 patients hospitalized, while a five-year-old boy died. Ready-to-eat food involved was fried rice, fried *kueytiow* and fried noodle (The Star, 2014).

Cross-contamination in the food establishment is often associated with poor food handling. Therefore, food handlers' training is deemed necessary and has been identified as an effective method to improve food safety by providing food handlers with adequate awareness and knowledge related to food hygiene requirements (Ko, 2013). However, previous studies found that poor

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handling practices and unhygienic hands of food handlers have caused contamination of local ready-to-eat food in Taiwan, Nigeria and Malaysia (Fang *et al.*, 2003; Okareh and Erhahon, 2015; Lee *et al.,* 2017). Buying and eating night market foods have become a norm due to the open-air location, accessibility, low prices, and the higher cost of living among consumers (Mazlan *et al.,* 2017). Therefore, it is relevant to measure the extent of food safety knowledge, attitude, and practices (KAP) towards night market food handlers due to the increased demand for cheap food varieties and RTE meals (Alimi, 2016). Hence, this study aimed to assess the presence of *B. cereus* on food and hand swab samples at the night market in Northern Perak, Malaysia, the vendors' level of KAP towards food safety and its association with the hand carriage of *B. cereus.*

2. Materials and methods

2.1 Ethical consideration

Ethical approval (JKEUPM-2019-043) for this project was obtained from the Ethics Committee of Universiti Putra Malaysia on June 11, 2020. The assurance of confidentiality and anonymity was maintained throughout the study.

2.2 Collection of food and hand swab samples

This study was conducted in northern Perak, Malaysia, i.e., Larut, Matang and Selama district, and Kerian district, from July to August 2020. The selection of sampling location was based on night markets registered under Taiping City Council and Kerian District Council while the types of food to be sampled were rice, meat, poultry and vegetables. Sample size calculations were based on a study by Sandra *et al*. (2012) for food samples and Shilla *et al*. (2011) for hand swab samples with a prevalence of 0.73 and 0.18, respectively. Two to three food samples were randomly bought from each stall while hand swab samples of the food vendors were collected using a 3M Quick Swab. Both food and swab samples were collected during the mid-time of the stall operation hour. The characteristics respondents selected for hand swab sample collection were 18 years old and above and did not wear gloves during food handling. After food samples were collected, each sterile swab was rubbed over the palm and fingertips of the food handler's hands (Lee *et al.*, 2017). One swab stick was used aseptically on both hands of each food handler during food preparation. Each swab was labelled as S1, S2, S3,.. Sn to maintain anonymity. Collected samples were kept in an ice-filled, sealed box and transported to the laboratory for microbial analysis.

2.3 Questionnaires distribution

The KAP questionnaire was distributed after the collection of food and swab samples. The questionnaire was written according to Abdul Mutalib *et al.* (2012), Asmawi *et al.* (2018) and the Malaysian Food Act 1983 and regulations (Ministry of Health Malaysia, 1983). A pilot test was conducted among 21 street-food handlers in Batu Kurau, a city in the district of Larut, Matang and Selama, Perak, before the actual study and resulted in acceptable Cronbach's alpha results (knowledge: 0.84, attitude: 0.83, practice: 0.71) (Tavakol and Dennick, 2011). The questionnaire is comprised of four sections which are section I (socio-demographic background), section II (knowledge of food safety), section III (attitude towards food safety), and section IV (food handling practices). A Likert scale was used for sections III and IV of the questionnaire. The survey was self-administered and conducted with a face-to-face approach, and the project's objectives were explained to the respondents before answering questions. Unique codes (e.g. R1, R2, R3,..Rn) were used to represent respondents to maintain anonymity. A total of 57 respondents from 25 night market food facilities were involved.

2.4 Isolation and identification of Bacillus cereus from food and hand swab sample

Approximately 25 g of each food sample was homogenized with 225 mL of sterilized buffered peptone water (BPW) for 30 s in a stomacher. For the swab sample, each swab tip was vigorously vortexed for 10 s to transfer the sample from the swab tip into the diluent (Lee *et al.*, 2017). An amount of 0.1 mL of each diluent was inoculated on duplicated plate count agar (PCA) to enumerate the growth of aerobic bacteria and COMPASS *B. cereus* (BC) selective agar. The COMPASS plates were incubated for 24 to 27 hrs at 30° C, while the samples on the total plate count agar were incubated for 24 hrs at 35°C (Biokar Diagnostic, 2019; Tallent *et al*., 2019). Haemolytic activity was assessed by inoculating trypticase soy-sheep blood agar with a loopful 24 hrs of *B. cereus* culture suspension and incubated at 35°C for 24 hrs (Tallent *et al.,* 2019).

2.5 Data analysis

All data were analyzed using MINITAB for Windows version 19.0. Prevalence of *B. cereus* in food, hand carriage and demographic data of all respondents were presented in percentages. The mean scores for food safety KAP were categorized as low (<50%), moderate (51-79%) and high (>80%) (Abdullah Sani and Siow, 2014). The comparison of the differences in the demographic background on KAP scores was analyzed

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using t-test and ANOVA. The cut-off for a statistically significant effect has been set at $p < 0.05$ overall. Association between KAP and hand carriage were analysed using correlation (Pearson test and chi-square) to identify the level of relationship with a correlation scale of 0 to 1.

3. Results and discussion

3.1 Food contamination and hand carriage of Bacillus cereus

About 28% of the food samples were found to be contaminated with *B. cereus* ranging from 1.0×10^2 CFU/g to 4.2×10^4 CFU/g, while 89% of samples were positive with aerobic bacteria ranging from 5.0×10^2 CFU/g to 1.5×10^7 CFU/g from a total of 83 food samples tested. *B. cereus* colonies produced a green colour with a precipitate zone on the COMPASS agar (Figure 1). The types of foods tested in this study were rice-based, poultry, meat and gravy dishes. The analysis of the hands' swab showed that 42% of the samples were detected with *B. cereus*, while 82% showed an unsatisfactory level of aerobic bacteria (Table 1).

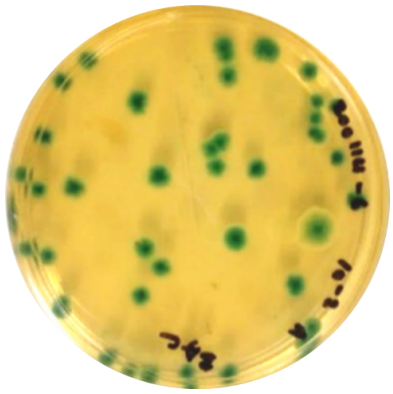

Figure 1. *Bacillus cereus* colony on COMPASS selective *Bacillus cereus* agar after 24-hour incubation

Bacillus cereus was detected in the highest percentage involving meat and rice dishes, followed by poultry and curry dishes. However, no significant difference was shown (Table 1) between the types of food to *B. cereus* count, which suggested the same possibility of *B. cereus* contamination regardless of any type of food. Previous studies by Ali *et al*. (2017), Yu *et al.* (2020) and Sornchuer and Tiengtip (2021) also showed incidences of *B. cereus* related to the same types of food. A study by Tirloni *et al.* (2019) found rapid spore germination and toxin-producing of *B. cereus* in cooked rice stored at room temperature even though its precooked rice was only contaminated at very low levels. The percentage of *B. cereus* detected in this study was almost the same as to study done by Navaneethan and Effarizah (2021) who found that 34% of fried rice tested positive in Penang, Malaysia. A low percentage was also reported by Tirloni *et al.* (2019) who found only 8% of fried rice meals were detected with *B. cereus* in Milan, Italy. Other studies involved various cooked foods that were contaminated with *B. cereus* with a prevalence rate of 21% and 35% respectively (Yu *et al.*, 2020; Sornchuer and Tiengtip, 2021). The samples were taken from various markets in major cities in China and Pathum Thani Province, Thailand. In this study, about 43% of meat dishes were detected with *B. cereus*, which is almost similar to a previous study done in China and Serbia which reported a detection at 34% and 31%, respectively (Savic *et al.,* 2016; Yu *et al.*, 2020). The open-air style of stalls and unwrapped food sold at night markets may cause cooked food exposed to dust-containing spores (Yu *et al.*, 2020), thus leading to a high prevalence of *B. cereus* in cooked meat samples.

The Food Standard Australia New Zealand (FSANZ) guideline on microbiological limits states that less than 10^5 CFU/g for total plate count and less than 10^4 CFU/g for *B. cereus* are considered satisfactory for ready-to-eat food. This guideline is also used in Europe and many other countries (Bolton, 2009; FSANZ, 2018). Meanwhile, Malaysia Food Regulation 1985 has clearly stated that "*no person shall import, prepare or advertise for sale any food ready for consumption that is contaminated with pathogenic microorganism*", which means no food pathogen is allowed in ready-to-eat food.

The microbial detection in aerobic plate count showed a higher bacteria count compared to *B. cereus* isolated from a food handler hand's swab. The same

Table 1. Detection of *Bacillus cereus* in food and hand swab

	No of sample	Aerobic plate count				*Bacillus cereus*			
		Detection	Mean bacterial count	SD	*p-value*	Detection	Mean bacterial count	SD	*p-value*
Food sample					0.383				0.494
Rice	40	37(93%)	3.5×10^6 CFU/g	1.5×10^6		15(38%)	2.9×10^3 CFU/g	1.8×10^3	
Poultry	11	9 (82%)	3.9×10^6 CFU/g	4.3×10^6		2(18%)	3.9×10^3 CFU/g	1.4×10^3	
Meat	7	7(100%)	8.9×10^5 CFU/g	7.3×10^5		3(43%)	9.0×10^2 CFU/g	1.8×10^2	
Curry	25	21(84%)	6.8×10^6 CFU/g	2.1×10^6		3(12%)	6.6×10^2 CFU/g	6.7×10^2	
Hand swab*	78	64(82%)	> 10^3 CFU/mL			33(42%)	>1 CFU/mL		

*Unsatisfactory limit for hand swab is more than 10^3 CFU/mL for APC and any detection of *Bacillus cereus*
p-value is statistically significant different if $p < 0.05$.

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result was also reported by Marzano and Balzaretti (2013) and Lee *et al.* (2017) who conducted their studies among food handlers in Italian school catering and local universities cafes, respectively. Other than *B. cereus*, other types of pathogenic bacteria isolated from hand swabs in previous studies were *Staphylococcus aureus, Salmonella* and *Vibrio cholerae* (Okareh and Erhahon, 2015; Lee *et al.,* 2017; Abayneh *et al.,* 2019). Poor hand hygiene practices render the survival of various bacteria from raw food and cause cross-contamination to the RTE food through the food handler's hand (Okareh and Erhahon, 2015).

Even though less than half of the food handlers were found to be *B. cereus* hand carriage, the reported percentage is considered high compared to a previous study by Gholam-Mostafaei *et al.* (2017) who isolated this bacterium from food handlers in Tehran, Iran. They concluded that a proper handwashing practice can bring down the possibility of getting contaminated or spreading infection. To produce safe food and ensure safe food handling, Malaysia's regulation has not allowed food handlers to use bare hands to handle unwrapped food according to the Food Hygiene Regulations (Ministry of Health Malaysia, 2009).

3.2 Demographic profiles

Fifty-seven night market food vendors were involved in this study. Table 2 shows the percentage of respondents according to their demographic characteristics. About 61% of the respondents are female, while the rest are male. The highest percentage of the respondents aged between 20 to 59 years old, and only a small percentage were at and more than 60 years old. A high percentage of respondents (86%) have at least a secondary and upper level educational background. About half of the respondents have working experience between 1 to 10 years (51%), with 61% of them working as a part-timer. About 61% of the respondents had the anti-typhoid vaccination, while the rest were not vaccinated. Only 40% of respondents had attended the food safety training program by the Ministry of Health, Malaysia.

The percentage of trained respondents was less than half even though food handler training was made compulsory by the government of Malaysia. Similar findings were also reported by Abdullah Sani and Siow (2014) and Woh *et al.* (2016) who found that only 28% and 47% of their respondents from different cities of Malaysia received food handlers training, respectively. The respondents with anti-typhoid vaccination in this study were recorded to be 60% of the total food handlers, which is congruent with the data reported by Aziz and Dahan (2013) and Woh *et al*. (2016). The data collected were among food handlers working at the school canteen and restaurants in Malaysia. All food handlers are compulsory to undergo training from a certified institution and must be medically examined and vaccinated by a registered medical practitioner (Food Hygiene Regulations 2009).

Table 2. Demographic profiles of respondents (n = 57).

Variable	Category	Percentage (%)
Gender	Male	39
	Female	61
Age	Youth	23
	Adult	72
	Elderly	5
Marital status	Single	47
	Married	53
Education background	No formal	0
	Primary	4
	Secondary	49
	Higher	47
Working experience	< 1 year	28
	1-10 years	51
	>10 years	21
Employment status	Part time	61
	Full time	39
Food handler training	Yes	40
	No	60
Anti-typhoid vaccination	Yes	61
	No	39

3.3 Knowledge, attitude and practices on food safety and hygiene

The knowledge section of the questionnaire comprised 'Yes', 'No', or 'Not sure' options for 12 statements. One score was given for the correct answer, while a 0 was given to the wrong and/or 'not sure' answers. Meanwhile, for the attitude section, a Likert scale of 1 to 5, with 1 as 'strongly disagree' and 5 as 'strongly agree' options was given comprised of 11 statements. A Likert scale of 1 to 5 with 1 meaning 'never' to 5 as 'very often' was used for the practice section covering 13 statements. The score for the Likert scale type of statements was calculated based on the number of scales they chose for positive statements and vice versa.

The highest mean ranking for knowledge statements was regarding cleaning techniques, microbial hazards related to unwashed eggs and the risk of food poisoning. On the other hand, the lowest men ranking for the knowledge section were temperature of the freezer, spore of *Bacillus cereus* and thawing technique (Table 3). Osaili *et al.* (2018) also reported a low percentage of respondents knew the correct operating temperature of the refrigerator and freezer. The failure to determine the

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Table 3. Response to KAP of food handler

Statement		Mean ranking
Knowledge (yes/no/not sure)		
Temperature of cooked food		0.77
Food handling training		0.79
Temperature for freezer		0.53
Anti-typhoid vaccine		0.70
Correct cooking technique		0.75
Correct thawing practice		0.49
Human hand as habitat for various bacteria		0.93
Heat resistance of *Bacillus cereus*'s spore		0.46
Cleaning technique		0.91
Microbial hazard on egg		0.86
Growth temperature for bacteria		0.70
Food poisoning		0.98
	Average mean scale	0.74
Attitude (Likert scale 1-5)		
It is important to follow 7 steps hand washing		4.7
Hands have to be washed before wearing a glove		4.4
Making safe food is my responsibility		4.9
If I have diarrhoea, I will not handle food		4.5
If I have a finger's cut, I will cover it with a band-aid and wear a glove		4.7
Cooked food should be reheated every 4 hours		4.4
I will pat dry my hands after washing my hand		4.5
I am responsible if food poisoning occurs		4.4
I have to routinely check the refrigerator's temperature		4.3
I have to properly clean the knife and cutting board before and after use		4.7
I wear clean and proper clothes during food handling		4.8
	Average mean scale	4.6
Practices (Likert scale)		
I wear a watch/jewellery while working*		3.6
I always wear an apron while working		4.3
I make sure my nails are always short and clean		4.7
If I have food poisoning symptoms, I will be absent until recover		3.9
I eat while working*		3.7
I wash my hands before and after wearing gloves		4.2
I practised 7 steps of hand washing		4.4
I do not change gloves while handling raw and cooked food*		4.4
I touch cooked food using bare hands*		3.4
I wash my hands before and after throwing rubbish		4.7
I use the same cloth for cooking utensils and table*		4.5
I wear a clean apron while working		4.6
I use a handphone while working*		3.0
	Average mean scale	4.1

Negative statements were marked with (*).

exact temperature for a chiller and a freezer may lead to food spoilage due to microbial growth (Ko, 2013). Besides, more than half of the respondents chose the wrong answer about the thawing practice. The wrong thawing technique may increase the number of microorganisms in the food, as during the thawing process, the temperature reaches a suitable condition for bacterial multiplication (Abdul Mutalib *et al.,* 2012).

Most of the respondents did not know about the spore of *B. cereus*. The lack of knowledge about the ability of some microorganisms to form heat-resistance spores can lead to an increase in the food poisoning risk even though the food is properly cooked. Adopting more information about the occurrence of foodborne illnesses and pathogen transmission related to those illnesses in food handler training is important (Bou-Mitri *et al.,* 2018). Besides, the practice of cooking the food earlier and storing it for several hours on the tables without

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proper reheating methods by the food vendors will allow for the growth of microorganisms (Abdul Mutalib *et al.,* 2012).

Overall mean ranking for statements in the attitude section was high with an average of 4.6 (Table 3). This finding is similar to the study by Abdul Mutalib *et al.* (2012), who reported high positive attitudes toward food handlers in restaurants in Kuala Pilah, Malaysia. The highest mean ranking was regarding the responsibility of preparing safe food and proper food handling attire. Meanwhile, the lowest mean ranking for the attitude section was regarding the importance of routine checks of the refrigerator's temperature, reheating food every four hours, the proper way of wearing gloves and responsibility for any occurrence of food poisoning. The finding contrasted with a study by Bou-Mitri *et al.* (2018) in Lebanon who reported that the majority of the respondents recognized the importance of regularly checking the thermometer setting of refrigerators and freezers to reduce the risk of food spoilage.

The highest mean ranking for the practices section was regarding washing hands after handling rubbish, keeping nails short and clean, and wearing a clean apron. On the other hand, the lowest men's ranking was regarding wearing a watch/jewellery, using handphones, touching food with bare hands and eating while preparing food (Table 3). The same results were reported by Abdul Mutalib *et al.* (2012) about wearing jewellery and watches while handling food. Abdullah Sani and Siow (2014) also observed that only a few food handlers assigned to serve at local university cafes wore gloves while preparing food. Continuously using handphones and touching food also could lead to cross-contamination because a previous study by Koscova *et al.* (2018) found the presence of *Bacillus* spp., *Staphylococcus aureus* and *Micrococcus* spp. on handphone surfaces of university workers in Slovakia.

3.7 Association between knowledge, attitude, and practices of food handlers

The correlation test (Table 4) shows a strong positive relationship (*r*: 0.681, *p*-value: 0.000) between attitude and practice. Thus, the results show that attitude independently influenced practices. Similarly, significant positive correlations between attitudes with practices were also reported by Abdul Mutalib *et al.* (2012), Abdullah Sani and Siow (2014) and Asmawi *et al.* (2018).

Table 4. Correlation between knowledge, attitude and practice score

Variables	N	Correlation (Pearson)	*p*-value
Knowledge α Attitude	57	-0.145	0.283
Knowledge α Practice	57	-0.158	0.240
Attitude α Practice	57	0.681	0.000

Pearson correlation 0 (no correlation) to 1 (perfect correlation),
p-value is statistically significant different if $p < 0.05$.

On the other hand, no association was observed between knowledge-attitude (r: 0.145, *p*-value: 0.283) and knowledge-practice (r: 0.158, *p*-value: 0.240). The result obtained was contrasted with studies among food handlers at local mobile stalls and school canteens who observed positive correlations between knowledge and practices (Tan *et al.,* 2013; Ismail *et al.,* 2016). However, the absence of any significant correlations between knowledge, attitude, or practices did not conclude the less importance of any variable in achieving desirable food safety quality. Many other aspects influenced the food handlers' attitude toward ensuring safe food handlings, such as small working spaces and inconvenient locations for handling equipment (Ko, 2013; Aziz and Dahan, 2013; Woh *et al.,* 2016). Besides, Al-Kandari *et al.* (2019) suggested in their study to adopt a positive attitude with theoretical aspects in food handler training to give more impact on food safety practices.

Table 5. Association between KAP score and *B. cereus* hand carriage (n = 78)

	KAP Level	No. of respondents (%)	*B. cereus* hand carriage		
			No. of respondents (%)	*p*-value	Chi-square (Pearson)
Knowledge				0.007	9.877
	Low	0.87 (5)	1.00 (5)		
	Moderate	0.54 (31)	0.45 (14)		
	High	0.37 (21)	0.24 (5)		
Attitude				NA	NA
	High	1.00 (57)	0.42 (24)		
Practices				0.003	7.406
	Moderate	0.46 (26)	0.62 (16)		
	High	0.54 (31)	0.26 (8)		

p-value is statistically significant different if $p < 0.05$.

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3.8 Association between knowledge, attitude and practice score and Bacillus cereus hand carriage

There was a significant relationship existed between knowledge (*p*-value: 0.007) and practices (*p*-value: 0.006) with *B. cereus* hand carriage (Table 5). The highest percentage of respondents with low knowledge scores and average practice scores were found to be the hand carriers of *B. cereus*. A similar result was obtained by Woh *et al.* (2017) who found the highest hand colony count detected from respondents with only moderate food safety knowledge and average hygiene practice. However, there is no significant association between attitude and *B. cereus* hand carriage. Lee *et al.* (2017) stated that the food safety knowledge level is not necessarily reflected in good hygiene behaviour. For instance, most respondents in this study achieved more than the average KAP score. However, the microbial assessment was not as good as the overall KAP results. Other aspects such as improper hand washing equipment might be the causes of poor hand hygiene rather than food handler training.

4. Conclusion

Overall, a low percentage of RTE food samples were detected with *B. cereus* but no significant difference was found between the types of foods to microbial count. Almost half of the food handlers were identified as *B. cereus* hand carriage. From the KAP survey conducted, the mean knowledge score was moderate, while the mean scores for attitude and practice were good. A strong positive correlation (*r*: 0.681, *p*-value: 0.000) was observed between attitude and practice scores indicating that attitude independently influenced practices. A significant relationship also exists between knowledge and practice scores with *B. cereus* hand carriage, which concluded that knowledge level does portray the hygiene status of food handlers. Further study could include the identification of other pathogenic bacteria in both RTE food and food handler hands' swabs. Therefore, it will help to determine the prevalence of various types of bacteria associated with cooked food and food handler's hands. Besides, future studies could also include other environmental swab samples so the potential sources of contamination can be predicted. The study location could also be expanded to other states across the country to permit better and broader statistical findings.

Conflict of interest

The authors declare no conflict of interest.

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