Microbial Assessment of the Ice Cream: A focus on *Bacillus cereus* Contamination in Pokhara, Nepal

Mamta Thapa[1,2,*], Mala Sapkota[1], Pooja Thapa Chhetri[1], Subina Nepali[1], Sunita Lamichhane[1], Mahesh Baral[3], Krishna Gurung[1], Mamita Khaling Rai[1]

[1]Prithvi Narayan Campus, Pokhara, Nepal

[2]Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

[3]Lambda Food Lab, Pokhara, Nepal

***Corresponding author:** Mamata Thapa, Central Department of Microbiology, Kathmandu, Nepal; E-mail: mamtathapabhandari@gmail.com

ABSTRACT

Objectives: The study aims to assess microbial contamination in ice cream sold in Pokhara, with a specific focus on *Bacillus cereus* and to determine its antibiotic susceptibility pattern.

Methods: The cross- sectional study was conducted on 108 ice cream samples of different brands collected from different shops in Pokhara. The samples were cultured on plate count agar for heterotrophic count and were also cultured on Mannitol Egg Yolk Polymyxin Agar for *B. cereus* identification. *B. cereus* was identified through cultural characteristics, gram staining and various biochemical tests. Antibiotic susceptibility test was performed by Kirby Bauer disc diffusion technique.

Results: Heterotrophic plate count revealed 91.67% (n=99) ice-cream samples were contaminated with average count of 5.25±0.78 log CFU/gm. Open cup ice-cream samples of all nine brands showed the maximum bacterial count with average of 5.49±0.73 log CFU/gm whereas that of minimum was found in sealed cone samples (4.99±0.51 log CFU/gm). B. *cereus* contamination was observed in 16.67% (n=18) ice-cream samples with average count of 3.52±0.25 log CFU/gm. Out of nine brands, 5 brands were contaminated with *B. cereus.* Sealed cone ice cream did not show any growth of *B. cereus* and heterotrophic plate count was also minimum. All the isolates were susceptible toward used antibiotic except Piperacillin/Tazobactum.

Conclusion: The present study reveals the occurrence of microorganism in the ice cream which is the frozen dairy product. Sealed cone ice cream is generally considered safer compared to other types.

Keywords: Ice-cream, Bacterial contamination, *Bacillus cereus*, Antibiotic susceptibility

INTRODUCTION

Ice cream is one of the widely consumed frozen dairy products enjoyed by people of all ages across the globe. It is made by combining and processing various ingredients, including cream, milk, and flavoring agents, while incorporating air during the freezing process. If proper care is not taken in treating the ingredients or handling equipment during production and distribution, there is a risk of microbial contamination (Manandhar and Sharma 2017). Intrinsic factor such as proportion of ingredients used and extrinsic factors such as manufacturing procedure and storing conditions is responsible for the contamination of the ice cream (Hossain et al 2012).

Date of Submission: September 7, 2024 **Date of Acceptance:** December 15, 2024

Published Online: December 31, 2024 **DOI:** https://doi.org/10.3126/tujm.v11i1.81954

Food poisoning microorganisms may gain entrance to dairy products during processing, handling and distribution, which may lead to public health hazard and cause economic losses by rendering the product unmarketable (Nazem et al 2010). The psychrophiles and psychrotolerant bacteria in ice cream includes *Listeria monocytogens, Staphylococcus aureus, Bacillus, Salmonella, Shigella, Streptococcus, Pseudomonas, Campylobacter, Brucella* (Hossain et al 2012). Among various pathogens, food poisoning strains of *Bacillus cereus* are more significant because of its ubiquitous nature and ability to occur in a wide range of foods (Agwa et al 2012).

B. cereus is a gram positive, rod-shaped facultative anaerobic, beta-hemolytic, non-lactose fermenting and spore forming bacteria. On egg-yolk agar *B. cereus* gives a strong lecithinase reaction. And rapidly liquefies gelatin stabs (Cheesbrough 2006). Due to their spore-forming capacity, they can escape pasteurization, survive high temperatures, and germinate under favorable conditions, potentially leading to food poisoning. (Tewari and Abdullah 2014). *B. cereus* members are ubiquitous in nature. Thus, can result in the spoilage of food products. *B. cereus* is also found in rice, rice products, pasteurized milk, pasta, cereals, fresh acids and cheese (Pluta et al 2019).

The great ability of the spores of *B. cereus* to adhere to surfaces, particularly hydrophobic surfaces cause problems in the food and dairy industry (Tewari and Abdullah 2014). Toxin produced by *B. cereus* causes food-poisoning. The toxin is produced when the bacilli sporulate if not stored at proper temperature. *B. cereus* may also cause opportunistic infections in immunocompromised individuals, e.g. pneumonia, bacteraemia, wound infections (Cheesbrough 2006).

B. cereus causes mainly two type of food poisoning, diarrheal and emetic type. Diarrheal disease is caused by the production of enterotoxins such as hemolysin BL (HBL), non-hemolytic enterotoxin (NHE), cytotoxin K, while the virulence of emetic strains is due to the production of a heat stable cereulide (Kwarteng et al 2017). Usually, the emetic syndrome shows rapid effects within 1-5 hours of ingestion and cause symptoms such as nausea, vomiting and abdominal cramp. In contrast, the diarrheal syndrome shows delayed effects within 8-16 hours which is characterized by watery diarrhea, cramp and abdominal pain. The illness is mild and self limiting for both syndromes (Perera 2012). *B. cereus* was established as an organism of foodborne disease in the 1950s, with the first described outbreaks of the diarrheal type of disease in hospitals in Norway in 1947–1949 (Arnesen et al 2008).

Antibiotic resistance is a worldwide problem which is mainly due to the overuse, misuse and abuse of drugs (Tewari et al 2012; Khasnabis et al 2017; Yibar et al 2017). The antimicrobial pattern of resistance of *B. cereus* from foods is useful in epidemiological studies but its effectiveness decreases due to the ignorance in utilization (Agwa et al 2012). The main aim of the study is to identify the *B. cereus* in ice cream available in Pokhara valley. People of different age groups are fond of ice cream, but most of the people are unaware about the fact that even the frozen item might contain microorganism which may cause mild to severe health hazards. Also *B. cereus* is considered as the most common food poisoning bacteria, as they can thrive wide range of temperature. In context of Nepal, research on *B. cereus* has not been done much, so this research is mainly focusing on *B. cereus* and especially on frozen items.

METHODS

Study design, study site and sample size

The cross-sectional study was done in 108 ice cream samples from January 2021 to March 2021. The samples were collected from the retail shops, departmental shops and factories that were commonly sold in the Pokhara. The samples were processed in the Lambda Food Lab, Nayabazar, Pokhara and Microbiology Laboratory of Prithvi Narayan Campus, Pokhara.

Sample collection and transportation

Samples were collected randomly from different shops, factories and from different localities in Pokhara city. The collected samples were transferred to the laboratory in the ice bag. Processing was done as soon as possible to minimize the temperature fluctuation. In case of delay, samples were refrigerated at 4-6˚C until the further processing.

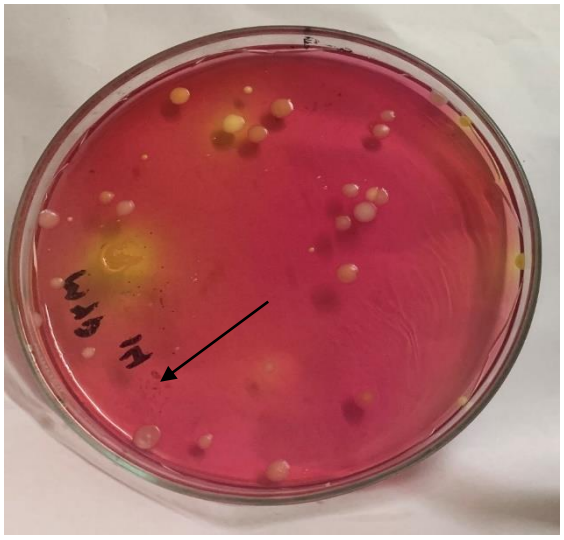
Isolation of *B. cereus*

Samples were serially diluted up to five folds, and was spread plated on MYPA and PCA and were incubate at 35˚C for 48 hours and at 37˚C for 24 hours respectively. The colonies were enumerated and CFU/gm was calculated. Characteristic pink colonies from MYPA plate were sub cultured on NA. The morphological characteristic like colony color, size, shape, texture, elevation, margin, consis-

tency and opacity were noted. The presumptive colonies were confirmed by Gram staining, endospore staining, hemolytic activity and biochemical test like motility test, indole test, oxidase test (Cheesbrough, 2006; Ludwig et al., 2009).

Antibiotic susceptibility testing

Antibiotic susceptibility test was performed using Kirby-Bauer disc diffusion method. Bacterial suspension was prepared on nutrient broth by adding 2-3 similar colonies and incubating at 37°C for 4-5 hours. The turbidity was adjusted to equivalent to 0.5 McFarland standard solution. Carpet culture was performed on sterile Muller Hinton Agar. With the help of sterile forceps, antibiotic discs were applied on the agar surface. The plates were incubated at 37°C for 18 hours, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI 2020; Agwa et al., 2011; Kumari and Sarkar, 2014; Luna et al., 2007).



Photograph 1: Growth of pink colored colonies on MYP Agar (Sample C8)

RESULTS

Total bacterial count of different brands

Total 108 ice cream samples of 9 different brands (labeled as I, II, III, IV, V, VI, VII, VIII and IX) and packed in 4 different manners (3 consecutives samples each B: Bar, C: Cup, OC: Open cone and SC: Sealed cone) were processed (Fgure 1). Heterotrophic plate count revealed 91.67% ice-cream samples were contaminated with average count of 5.25±0.78 log CFU/gm which was above the acceptable limit (5 log CFU/gm). The highest bacterial count was observed in open cone ice-cream samples of all nine brands with average of 5.49±0.73 log CFU/gm., while the minimum was found in sealed cone samples (4.99±0.51 log CFU/gm).

Total *B. cereus* count on MYPA

B. cereus contamination was observed in 16.67% ice-cream samples with average count of 3.52±0.25 log CFU/gm. Out of nine brands, 5 brands showed the growth of *B. cereus. B. cereus* was not obtained from sealed cone in any of the brands (Figure 2).

Incidence of Bacillus cereus in the ice- cream sample

Cup and open cone of brand V showed highest occurrence of *B. cereus* with 16.7 % of the total prevalence (Table 1).

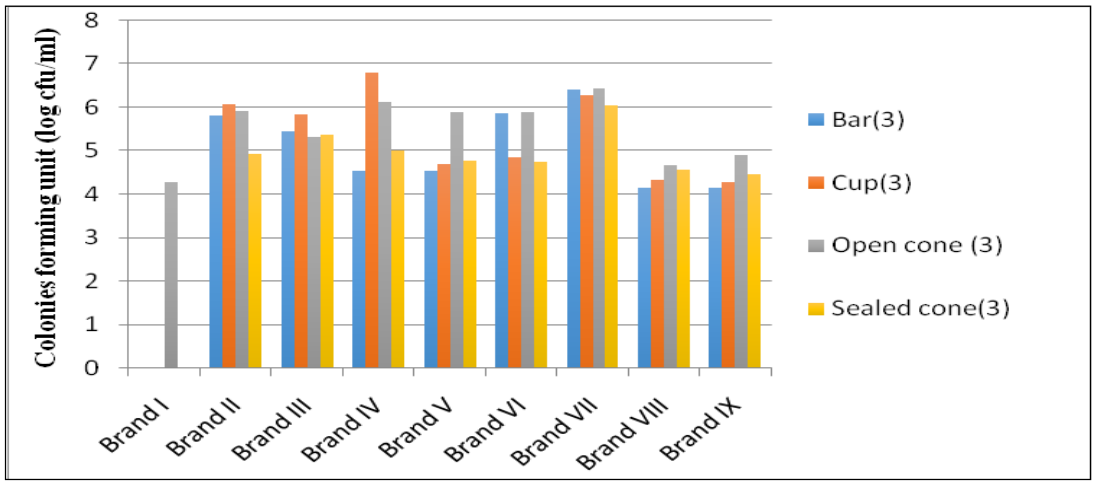


Figure 1: Average colony forming unit by heterotrophic plate (log CFU/mg)

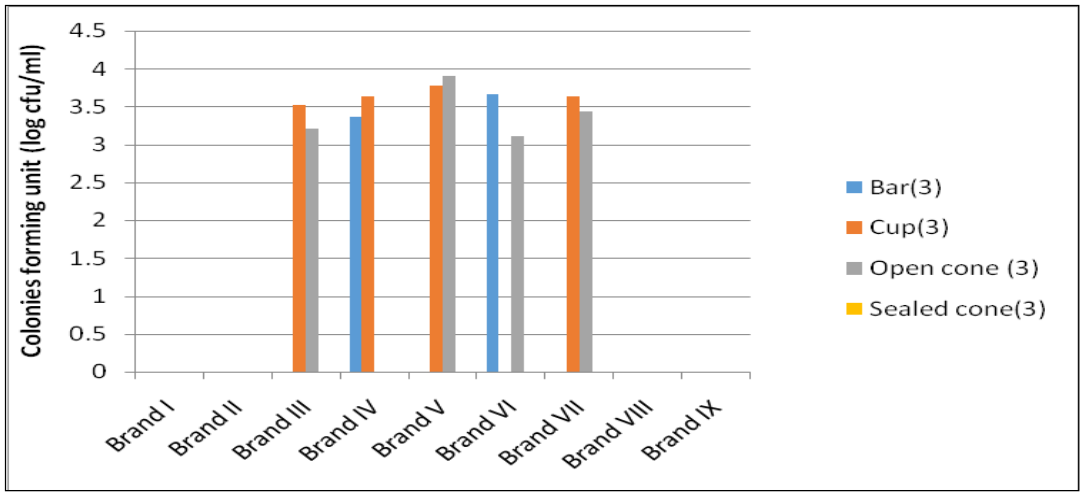


Figure 2: Total *B. cereus* count (log CFU/mg)

Table 1: Incidence of Bacillus cereus in the ice- cream sample

Brands	Incidence of *B. cereus* (%)							
	Bar		Cup		Sealed Cone		Open cone	
	frequency	%	frequency	%	frequency	%	frequency	%
I	0	0	0	0	0	0	0	0
II	0	0	0	0	0	0	0	0
III	0	0	2	11.1	0	0	1	5.5
IV	1	5.5	2	11.1	0	0	0	0
V	0	0	3	16.7	0	0	3	16.7
VI	2	11.1	0	0	0	0	0	0
VII	0	0	2	11.1	0	0	2	11.1
VIII	0	0	0	0	0	0	0	0
IX	0	0	0	0	0	0	0	0

Distribution of *Bacillus cereus* with respect to the ice cream brands in positive samples

B. cereus was found only in five brands, namely Brand III, IV, V, VI and VII. Out of 18 positive isolates, 17% were from Brand III, 17% were from Brand IV, 33% from Brand V, 11% from Brand VI and 22% from Brand VII with highest occurrence in brand V Figure 3).

Distribution of *Bacillus cereus* with respect to the packaging manners in positive samples

B. cereus was found only in bar, cup and open cone. There was no growth of *B. cereus* in sealed cone. 50% of the isolates were from cup, 33% from Open cone and 17% from bar (Figure 4).

Distribution of *Bacillus cereus* in open type and closed type

From total 27 samples of open type, *B. cereus* was obtained from only six samples which is 22.22 % of the total open type sample. And from total 81 samples of closed type, *B. cereus* was obtained from only 12 (14.81 %) samples from total closed type samples (Table 2).

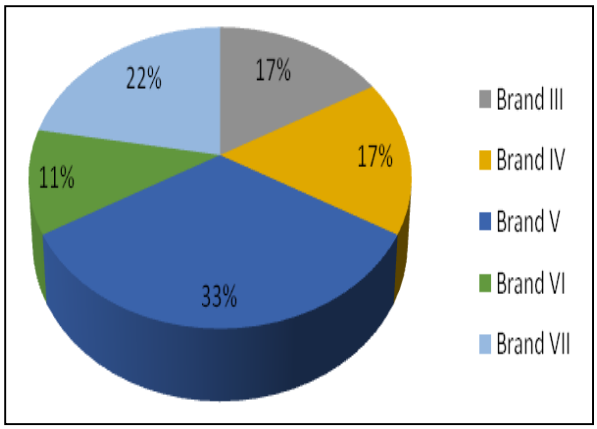


Figure 3: Distribution of *Bacillus cereus* with respect to the ice cream brands in positive samples

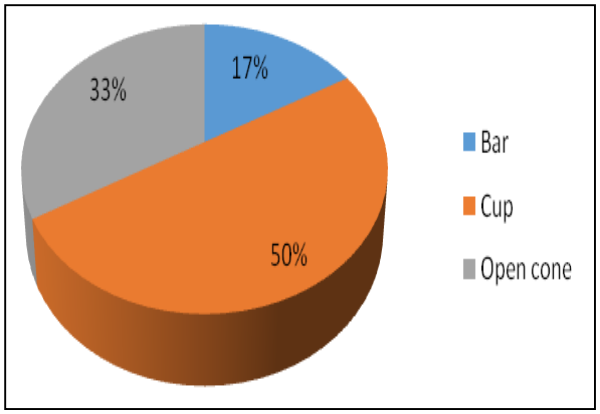


Figure 4: Distribution of *Bacillus cereus* with respect to the packaging manners in positive samples

Table 2: Distribution of *Bacillus cereus* in open type and closed type

Type of ice cream	Total no. of sample	Total no. of positive isolates	Percentage (%)
Open type (open cone)	27	6	22.22
Closed type (bar, cup, sealed cone)	81	12	14.81

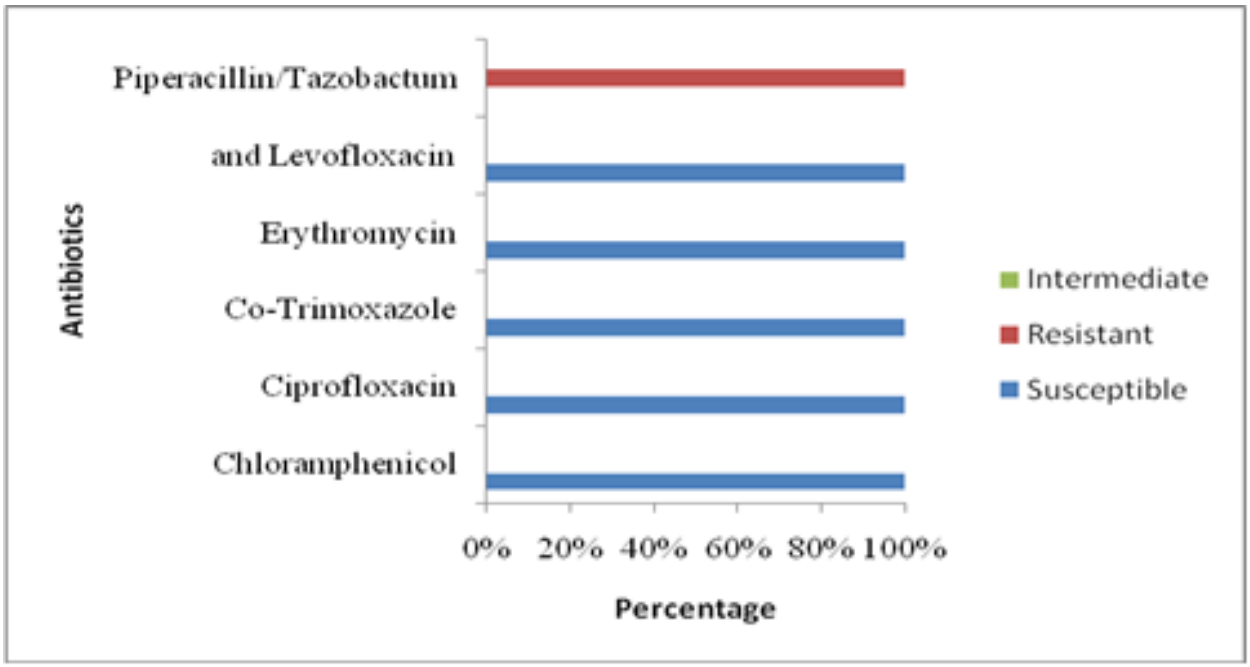


Figure 5: Antibiotic susceptibility pattern of *B. cereus*

Antibiotic susceptibility pattern of *B. cereus*

Out of 18 isolated *B. cereus* all were susceptible toward Chloramphenicol, Ciprofloxacin, Co-Trimoxazole, Erythromycin, and Levofloxacin were resistant toward Piperacillin/Tazobactum. Out of 18 isolates none of the isolate was multidrug resistant (Figure 5).

DISCUSSION

The study was conducted in the area around Pokhara valley to find out the presence of bacteria and to isolate and enumerate *B. cereus* and to perform the antibiotic susceptibility test of isolated colonies. From total of 108 samples of nine different brands and four different packaging manner namely bar, cup, open cone and sealed cone, which were further categorized into closed type (bar, cup and sealed cone) and open type (cone), 27 samples from each type processed, microbial growth was observed in 99 (91.67 %) of the total samples. All brands, except the bar, cup and sealed cone of Brand I showed the growth of microorganisms. The bacterial load in ice cream ranges from 1.4×10^4 CFU/gm (40.15±0.24 log CFU/gm) obtained from bar of Brand VIII and bar of Brand IX to 6.4×10^6 CFU/gm (6.81±0.18 log CFU/gm) obtained from cup of Brand IV. The result was in agreement with Jadhav and Raut (2014) in which the bacterial count ranged from 1.2×10^3 CFU/gm to 8.0×10^7 CFU/gm. Warke et al (2000) found 2.3×10^4 to 8.5×10^6 CFU/ml. Research done by Fadihl et al (2019) also found bacterial count ranging from 3.3×10^5 to 5.2×10^5 CFU/g. The difference in the microbial load may be due to inappropriate handling of ice cream during pre or post manufacturing as the ingredients involved in manufacturing of ice cream have high potential to provide the habitat for the microbial growth. According to the Yusuf et al (2013) ice creams being a dairy product with high nutritional value and the presence of easily useable fats, carbohydrate and protein, provides an ideal environment for spoilage microorganisms. According to Yusuf et al (2013). Bacterial load less than 10^5 CFU/gm in ice cream reflect good hygiene.

In this study, bacterial count indicates that only Brands I, VIII and IX fell within acceptable limits for bacterial load and all other brand exceeded the acceptable limit for ice cream. According to Jannat et al (2016) quality of ice cream depends upon both intrinsic and extrinsic factors hence the presence of microorganism indicates introduction of unhygienic condition at any stage from manufacturing to distribution and storage of the ice cream. Out of nine different brands six brands showed the maximum bacterial growth in open cone. The occurrence of high bacterial growth in open cone might be due to the contamination from air, water or the scoop used during the distribution of ice cream. As for open cone ice cream is frequently exposed to air hence chance of bacterial contamination is high and even the water and scoop used may be contaminated by microorganism which in turn contaminates the ice cream (Warke et al 2000; Yaman et al 2014).

From total of 108, *B. cereus* was isolated from 18 (16.67%) samples ranging ranges from 1.6×10^3 CFU/gm to 8×10^3 CFU/gm. According to Webb et al (2019) the total infective dose for *B. cereus* is 10^5 to 10^8 cells per gram of food. Although the range of the present study is below the infective dose, it should not be overlooked because temperature fluctuation and unhygienic condition can increase the number of bacterial cell capable of causing food borne infection. According to the research conducted by Zhao et al (2020), the ability of *B. cereus* to survive in dairy products and causing contamination is increasing because of its strong tolerance against adverse environmental factors.

The study conducted by Gundogan and Avci (2014) for occurrence and antibiotic resistance of *Escherichia coli, Staphylococcus aureus and B. cereus* in raw milk and dairy products in Turkey found that 20% of the ice cream was contaminated with *B. cereus* which is close to the result obtained in the present study. Another study conducted by Yusuf et al (2018) showed the presence of *B. cereus* in 61/215 samples with an overall prevalence of 28.37%.

B. cereus was obtained only from five brands. The highest occurrence of *B. cereus* was seen in the Brand V (33%) followed by Brand VII (22%), Brand III and IV (17%) and lowest in Brand VI (11%). 50% of total positive isolates was obtained from cup, 33 % from open cone and 17% from bar, while *B. cereus* was not obtained from sealed cone. The higher percentage of occurrence of *B. cereus* in cup might be due to improper practice followed during manufacturing, packaging or storage. As cup comes under closed type packing method, the chance of contamination from outside environment is comparatively less so it must have been contaminated either during manufacturing, during packaging or during storage. But if cups were not closed properly, it might get contaminated from the surrounding environment. Nazem et al (2010) concluded from their research that temperature fluctuation during

storage of the ice cream is also one of the major factors for contamination by psychrotrophs like *B. cereus.* The absence of *B. cereus* in all sealed cone samples indicates that proper and standard practices might have been followed during processing, packaging and storage.

B. cereus was obtained in higher percentage (22.22%) in open type than in closed type (14.81%). The study conducted by Warke et al (2000) for open and closed type of ice cream also showed similar kind of result. The risk of contamination is higher in open-type ice cream because environmental factors play a significant role, as it is frequently exposed to air. Additionally, scoop water is another factor contributing to the contamination of open-type ice cream.

From total isolated *B. cereus* all the isolates were susceptible toward Chloramphenicol, Ciprofloxacin, Co-Trimoxazole, Erythromycin, and Levofloxacin and all were resistant toward Piperacillin/Tazobactum. Similar result was obtained by Kumar and Sarkar (2015). No multidrug resistant *B. cereus* was isolated. Microbial contamination in ice cream is primarily caused by unhygienic practices, poor sanitary conditions, or inadequate sterilization of equipment or packaging systems during its production, processing, storage, and distribution, so proper care during the process can minimize the contamination.

Conclusion

From 108 total samples processed, 91.67% of samples showed bacterial growth and 16.67 % showed the growth of *Bacillus cereus*. Out of nine different brands, six brands showed the bacterial load above the acceptable limit (5 log CFU/gm). Sealed cone ice-cream was comparatively safer as *B. cereus* was not detected in any of the samples. All the isolates were sensitive toward Co- Trimoxazole, Levofloxacin, Chloramphenicol, Erythromycin and Ciprofloxacin but resistant toward Piperacillin. Proper hygienic conditions and proper control measures are most important for ensuring the good quality of ice cream.

ACKNOWLEDGEMENTS

The authors are greatful to Dr. Dev Raj Joshi and Mr. Tikaram Subedi, for their guidance and support.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Agwa, O.K., Uzoigwe, C.I. and Wokoma, E.C., 2012. Incidence and antibiotic sensitivity of Bacillus cereus isolated from ready to eat foods sold in some markets in Portharcourt, Rivers state, Nigeria. *Asian J. Microbiol. Biotechnol. Environ. Sci*, *14*(1), pp.13-18.Arnesen LPS, Fagerlund A, and Granum PE (2008). From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS microbiology reviews **32(4):** 579-606.

Cheesbrough, M., 2006. *District laboratory practice in tropical countries, part 2*. Cambridge university press , pp. 19, 167.

Fadihl, S.J., Mohammad, S.Q. and Al-qrtani, Y.M., 2019, September. Microbiological evaluation of locally produced ice cream in Baquba city. Iraq. In *Journal of Physics: Conference Series* (Vol. 1294, No. 6, p. 062057). IOP Publishing.

Gundogan, N. and Avci, E., 2014. Occurrence and antibiotic resistance of E scherichia coli, S taphylococcus aureus and B acillus cereus in raw milk and dairy products in T urkey. *International journal of dairy technology*, *67*(4), pp.562-569.

Hossain, K.M., Kabir, S.L., Rahman, M.M., Rahman, M.B. and Choudhury, K.A., 2012. Organoleptic and microbial quality of ice cream sold at retail stores in mymensingh, Bangladesh. *Journal of Microbiology Research*, *2*(4), pp.89-94.

Jadhav, A.S. and Raut, P.D., 2014. Evaluation of microbiological quality of ice creams marketed in Kolhapur city, Maharashtra, India. *Int. J. Curr. Microbiol. App. Sci*, *3*(9), pp.78-84.

Jannat, B., Hasan, M.M., Gomes, M.J., Uddin, M.E., Al Sanjee, S., Fatema, K. and Datta, S., 2016. Comparative analysis of the microbiological quality between industrially produced and street vended ice creams offered for public consumption in Dhaka City, Bangladesh. *Bioresearch Communications-(BRC)*, *2*(2), pp.259-263.

Khasnabis, J., Adhikari, P., Chowdhury, D., Rai, C. and Roy, A., 2017. Incidence of multiple drug resistant Bacillus cereus in some popular snacks and sweets sold in Kolkata city, India. *Indian J Microbiol Res*, *4*(1), pp.14-19.

Owusu-Kwarteng, J., Wuni, A., Akabanda, F., Tano-Debrah, K. and Jespersen, L., 2017. Prevalence, virulence factor genes and antibiotic resistance of Bacillus cereus sensu lato isolated from dairy farms and traditional dairy products. *BMC microbiology*, *17*, pp.1-8.

Ludwig, W., Schleifer, K. H., & Whitman, W. B. (2009). *Bergey's manual of systematic bacteriology. Bergey's Manual of Systematic Bacteriology*, 3.

Manandhar S and Sharma S (2017). *Practical Approach to Microbiology*. Third edition. Bhotahity Kathmandu, pp. 61, 78, 305, 306.

AM, N., Amer, A.A. and Soukayna, A.E.A., 2010. Prevalence of some food poisoning microorganisms in some dairy products.

Berthold-Pluta, A., Pluta, A., Garbowska, M. and Stefańska, I., 2019. Prevalence and toxicity characterization of Bacillus cereus in food products from Poland. *Foods*, *8*(7), p.269.

Tewari, A. and Abdullah, S., 2015. Bacillus cereus food poisoning: international and Indian perspective. *Journal of food science and technology*, *52*, pp.2500-2511.

Tewari, A., Singh, S.P. and Singh, R., 2012. Prevalence of multidrug resistant Bacillus cereus in foods and human stool samples in and around Pantnagar, Uttrakhand. *Journal of Advanced Veterinary Research*, *2*(4), pp.252-255.

Warke, R., Kamat, A., Kamat, M. and Thomas, P., 2000. Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. *Food Control*, *11*(2), pp.77-83.

Webb, M.D., Barker, G.C., Goodburn, K.E. and Peck, M.W., 2019. Risk presented to minimally processed chilled foods by psychrotrophic Bacillus cereus. *Trends in food science & technology*, *93*, pp.94-105.

Yaman, H., Elmali, M., Ulukanli, Z., Tuzcu, M. and Genctav, K., 2006. Microbial quality of ice cream sold openly by retail outlets in Turkey. *Revue de médecine vétérinaire*, *157*(10), p.457

Yaman, G., 2017. Prevalence, enterotoxin production and antibiotic resistance of Bacillus cereus isolated from milk and cheese.

Yusuf, M.A., Abdul, T., Hamid, T.A. and Yusuf, M.M.A., 2013. Assessment of the bacteriological quality of ice cream offered for public consumption in Bauchi, Nigeria. *J. Pharm*, *3*, pp.25-30.

Yusuf, U., Kotwal, S.K., Gupta, S. and Ahmed, T., 2018. Identification and antibiogram pattern of Bacillus cereus from the milk and milk products in and around Jammu region. *Veterinary world*, *11*(2), p.186.

Zhao, S., Chen, J., Fei, P., Feng, H., Wang, Y., Ali, M.A., Li, S., Jing, H. and Yang, W., 2020. Prevalence, molecular characterization, and antibiotic susceptibility of Bacillus cereus isolated from dairy products in China. *Journal of Dairy Science*, *103*(5), pp.3994-4001.