ANS-LHS

OPEN ACCESS

Advanced Natural Sciences: Life and Health Sciences,
ISSN: 3107-636X, Vol-2, Issue-2,
July-Dec, 2025, PP-64-73
https://www.indjournals.com/index.php/ans

Research Article

Seasonal Prevalence and Molecular Characterization of Enterotoxigenic *Bacillus cereus* in Cooked Rice and Culinary Spices from Indian Public Restaurants

Cherekar Makarand N[1]. and Rameshwar Kuptekar[2]

[1]Department of Biotechnology, [2]Department of Bioinformatics,
MGM's college of computer science and IT, Nanded, Maharashtra, India.

DOI: 10.5281/zenodo.18161529

Article Info:

***Correspondence:**
Dr. Cherekar Makarand N
Department of Biotechnology, MGM's college of computer science and IT, Nanded, Maharashtra, India

---

[Received 22-11-2025,
Accepted 27-12-2025,
Published 31-12-2025]

Publisher's Note: Natural Science Association stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Bacillus cereus is a significant agent of foodborne intoxication, often linked to starchy matrices. This study evaluated the role of culinary spices as primary reservoirs for introducing enterotoxigenic endospores into prepared restaurant meals. Twenty samples,10 cooked rice variants and 10 spices were procured from seven dining establishments in Bengaluru, India[3]. Isolates were recovered on Mannitol Egg Yolk Polymyxin (MYP) agar and confirmed via phenotypic profiling[4]. Molecular characterization targeted the 16S rRNA gene (548 bp) for taxonomic verification and the non-hemolytic enterotoxin (*nhe*) gene (500 bp) to assess pathogenic potential. Critical microbial titers were identified in raw spices, specifically Saunf (Fennel) at 41.2 x 10^5 CFU/g and Meat Masala at 37.5 x 10^5 CFU/g. Cooked rice exhibited significant seasonal escalation ($p < 0.05$), with loads rising from log10 2.08 CFU/g in February to log10 4.25 CFU/g in April. Genomic screening revealed a 100% prevalence of the *nhe* gene across isolates, establishing an epidemiological link between raw ingredients and finished products. Spices serve as persistent vectors for enterotoxigenic *B. cereus*, facilitating survival through standard thermal processing. Effective risk mitigation requires validated spice decontamination and stringent "danger zone" (5°C–60°C) thermal management to disrupt the germination-to-intoxication cycle.

Keywords: *Bacillus cereus*, Spices, *nhe* gene, Food Safety, Seasonal Variation, 16S rRNA.

LIST OF ABBREVIATIONS:

Abbreviation	Full Form
PCR	Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic Acid
MYP	Mannitol Egg Yolk Polymyxin (Agar)
CFU	Colony Forming Units
DNA	Deoxyribonucleic Acid
VP	Voges-Proskauer (Test)
bp	Base Pairs

INTRODUCTION

Foodborne infections arise primarily from the presence of pathogenic microorganisms in food. Food products are highly susceptible to contamination by spoilage and toxin-producing bacteria, which can compromise their safety for human consumption [1]. Raw fruits and vegetables may become contaminated at various stages, including cultivation in the field and post-harvest handling. In contrast, cooked foods are often exposed to contamination as a result of insufficient heat treatment, improper refrigeration, or inadequate hygienic practices during processing, leading to toxin formation [2,3]. The ability of foodborne bacteria to produce toxins has become a significant public health concern due to its direct and indirect role in food poisoning outbreaks. *Bacillus cereus* has been found in various natural habitat and plays a different important ecological role [4]. Frequently this microorganism led to food contamination, it has been isolated from many foodstuffs such as vegetables, meat, milk and dairy product, water, pastry, noodle, and rice [5]. As a highly adaptable organism, *B. cereus* is widely dispersed in nature, colonizing everything from agricultural yields to freshwater ecosystems. Its presence is further noted in sediments, dust, and various forms of vegetation, with occasional occurrences reported in fish species [6].

Bacillus cereus is responsible for two distinct clinical manifestations of foodborne illness: the emetic (vomiting) syndrome and the diarrheal syndrome. The emetic type is triggered by a singular, highly heat-stable toxin, whereas the diarrheal type results from the activity of three or four heat-labile enterotoxins. Clinically, emetic cases are defined by rapid-onset vomiting occurring just a few hours post-ingestion. In contrast, diarrheal symptoms, including abdominal cramping and watery stools, typically emerge between 8 and 16 hours after consuming contaminated food [7]. While both conditions are generally self-limiting and resolve within a 24-hour window, their food associations differ significantly. Emetic illness is predominantly linked to starchy, plant-based products such as pasta, potatoes, and pastries; notably, contaminated fried or cooked rice accounts for approximately 95% of all reported emetic cases [8].

According to established microbiological surveys, a 3.0% prevalence rate of high-titer *B. cereus* contamination has been documented across various herbs and spices [9]. Current molecular research has categorized the toxins responsible for *B. cereus* infections based on their specific syndromes. The diarrheal form is associated with the production of three primary enterotoxins: non-haemolytic enterotoxin (Nhe), haemolytic enterotoxin (Hbl), and cytotoxin K (CytK). Conversely, the emetic syndrome is mediated exclusively by the cyclic peptide toxin known as cereulide (Ces) [10]. A review of causative agents of the “fried rice syndrome” emphasizes the prevalence of B. cereus in starchy food contamination and the possibility of contamination in protein-rich food [11]. Rice and pasta meals are the most important sources of B. cereus spores causing intoxication [12]. Rice is consumed by almost half of the human population and is classified as a staple food. Rice can be classified into several types – raw, brown and white according to the degree of processing. Raw rice is unhusked and contains husks, hulls and bran. Brown rice (husked) is produced when the husk is removed. White rice (milled) is dehulled and dehusked [13]. Members of the genus *Bacillus cereus* are ubiquitous throughout the world. They are found in soil, water, plants and in the digestive tracts of mammals and insects [17]. Rice can be contaminated with micro-organisms during the

growing, harvesting, milling, storage or other processing operations. The heat-resistant spores of Bacillus spp. then germinate, reproduce and contaminate the rice, which can lead to foodborne illness [14]. As a prevention of diseases caused by *Bacillus cereus*, it is recommended to cool cooked food as quickly as possible and to avoid so-called danger zone temperatures, i.e. 5-60 °C [15]. According to the requirements of current legislation, the finished food must be kept at a temperature of at least 60 °C after cooking; foods that are not consumed shortly must be cooled quickly to a temperature not exceeding 4 °C [16]. Molecular biology–based investigations provide definitive confirmation of bacterial isolates and play a crucial role in the accurate documentation of genetic data in public gene banks. Such approaches greatly facilitate reliable differentiation among toxigenic strains of foodborne pathogens implicated in food poisoning outbreaks [18]. Relatively few microbial toxins have been definitely implicated in disease, and the modes of action have been identified for even fewer of these toxins [19]. Therefore, effective monitoring and reliable detection methods are essential for identifying bacterial contamination in processed foods, raw ingredients, and food processing environments. The present study was designed to detect toxigenic *Bacillus cereus* in cooked rice samples in combination with commonly used spices, representing traditional Indian cuisine, and to evaluate their potential risk to consumers.

Statistical Analysis

All microbiological analyses were performed in triplicate to ensure the reproducibility of the results. The bacterial population densities, initially recorded as Colony Forming Units (CFU) per gram, were converted into $\log_{10}$ CFU/g to achieve a normal distribution for statistical comparisons. Quantitative data were expressed as the Mean ± Standard Deviation (SD). The variation in microbial loads across different seasons (February, March, and April) and food matrices (spices vs. rice) was evaluated using One-way Analysis of Variance (ANOVA). A p-value of $p < 0.05$ was considered the threshold for statistical significance. All data processing and graphical representations were generated using SPSS (Version 25.0), ensuring a rigorous mathematical foundation for the observed trends in *B. cereus* proliferation.

2. MATERIALS AND METHODS

2.1 Sample Collection

A systematic sampling approach was conducted across 8 local restaurants and food stalls within Bengaluru, India, following formal briefing of establishment owners regarding the research objectives. A total of 20 samples were procured for analysis, comprising 10 cooked rice variants (including Biryani, Steamed, Fried, and Pulao) and 10 diverse spice samples. The spice cohort included Turmeric, Red Chili, Garam Masala, Coriander, and Black Pepper.

2.2 Isolation and Cultivation

For spice analysis, samples were sourced from local markets and rehydrated using sterile distilled water. To isolate *Bacillus cereus*, aliquots (500 µL) of the resulting suspensions were treated with an equal volume of 100% ethanol (C_2H_6O) and incubated for 30 minutes to facilitate spore selection. Cooked rice samples were collected directly from the dining establishments and preserved under controlled conditions for subsequent processing.

All samples were homogenized in Peptone Water at a 1:10 dilution ratio. Serial dilutions were performed and subsequently spread onto Mannitol Egg Yolk Polymyxin (MYP) Agar, a selective medium for *B. cereus*. The plates were incubated at 37°C for 24 hours. Morphologically typical colonies distinguished by a pink-red hue and a surrounding lecithinase-positive zone of precipitation were isolated for further sub-culturing and molecular validation [25].

2.3 Molecular Identification (16S rRNA PCR)

To confirm the taxonomic identity of the isolates, genomic DNA was extracted using the HiPurA™ Bacterial Genomic DNA Purification Kit (HiMedia Laboratories, Mumbai, India) in

accordance with the manufacturer's specified protocol. Polymerase chain reaction (PCR) was subsequently performed to amplify the 16S rRNA gene region using a group-specific primer set targeted at *Bacillus cereus*. This molecular approach provided a definitive diagnostic tool, ensuring high-resolution identification of the strains recovered from both spice and rice matrices.

Forward Primer:

5'-AGGAGAAGCTTGCTTTTCCA-3'

Reverse Primer:

5'-TAGCTAACACATGCAAGTCG-3'

The thermal cycling conditions were: initial denaturation at 95°C for 5 minutes; 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute; followed by a final extension at 72°C for 7 minutes.

RESULTS AND DISCUSSION

Evaluation of Microbial Loads in Regional Spices

The investigation into various spices sourced from Indian public restaurants and food stalls uncovered a broad spectrum of *B. cereus* contamination, indicating significant variability depending on the spice type and its typical handling process. As detailed in Table 1, the quantitative analysis shows that bacterial titers fluctuate across a wide range of common culinary ingredients and traditional masalas.

Table 1: Quantitative Analysis of *B. cereus* in Spices from Indian Public Dining Establishments

Indian Common Name	English Name	Bacterial Count (CFU/g)	Risk Level
Saunf	Fennel	$41.2x10^5$	Critical
Meat Masala	Kabab Spices	$37.5 x10^5$	High
Curry Podi	Curry Powder	$24.8 x10^5$	High
Jeera	Cumin	$15.4 x10^5$	Moderate
Kali Mirch	Black Pepper	$13.2 x10^5$	Moderate
Lal Mirch	Chili Pepper	$10.9 x10^5$	Moderate
Haldi	Turmeric	$3.1 x10^5$	Low-Moderate
Kabab Chini	Cubeb	$1.8 x10^5$	Low
Jaiphal	Nutmeg	$1.5 x10^5$	Low
Dalchini	Cinnamon	$1.1 x10^5$	Low (Inhibitory)
Dolma Masala	Dolma Spices	$0.8 x10^5$	Low
Bastirma Masala	Bastirma Spices	$0.45 x10^5$	Minimal
Laung	Cloves	$0.39 x10^5$	Minimal (Inhibitory)
Biryani Masala	Biryani Spices	$0.25 x10^5$	Minimal
Shawerma Masala	Shawerma Spices	$0.19 x10^5$	Minimal

Note: Risk Level Assessment of Contaminated Spices

The "Risk Level" assigned to each spice variety (Table 1) is determined by a combination of the raw bacterial titer and the traditional culinary application of the ingredient within Indian gastronomy. Samples designated as Critical or High Risk, such as Saunf (Fennel) and Meat Masala, exhibit bacterial counts exceeding 10^6 $CFU.mg^{-1}$, a threshold that significantly surpasses standard food safety permissible limits. The classification particularly highlights the danger of spices like Fennel, which are frequently consumed without prior thermal processing, thereby serving as a direct vector for *B. cereus* endospores. Conversely, Minimal Risk classifications are reserved for spices that not only show lower microbial density but are also typically subjected to prolonged boiling or frying during the preparation of dishes like Biryani or Shawerma, which serves to reduce the vegetative pathogen load.

The quantitative assessment of bacterial loads across fifteen varieties of spices collected from Indian public restaurants reveals a concerning spectrum of contamination, as detailed in Table 1. The highest microbial concentrations were recorded in Saunf (Fennel) at $41.2x10^5$ $CFU.mg^{-1}$, and Meat Masala at $37.5 x10^5$ $CFU.mg^{-1}$, suggesting that these agricultural products and complex blends are highly susceptible to soil-borne spore survival and environmental cross-contamination. Of particular concern is the high load found in Saunf, which is traditionally served raw as a digestive aid in Indian dining, thereby bypassing the thermal processing steps required to eliminate *B. cereus* vegetative cells or

heat-resistant endospores. Conversely, spices such as Laung (Cloves) and Dalchini (Cinnamon) exhibited significantly lower counts 0.39 $x10^5$ and 1.1 $x10^5$ respectively), likely due to the natural inhibitory effects of their constituent essential oils like eugenol and cinnamaldehyde. Overall, the presence of such high bacterial titers across the majority of samples underscores the resilience of *B. cereus* and highlights the urgent need for stricter hygiene protocols and improved sterilization techniques within the Indian spice supply chain and restaurant industry.

Table 2: Seasonal Variation of *B. cereus* Load in Cooked Rice Samples

Sampling Date	Sampling Location (Public/Street)	Sample Type	Bacterial Load (CFU/g)	Bacterial Load (CFU/·g−1)	Risk Category
05/02/2024	Railway Station Canteen	Steamed Rice	2.08	$1.2x10^2$	Low
15/02/2024	Local Market Stall	Jeera Rice	2.45	$2.8 \pm 1.2x10^2$	Low
28/02/2024	Bus Terminal Eatery	Pulao	2.65	$4.5 \pm 1.2x10^2$	Moderate
10/03/2024	Highway Roadside Dhaba	Biryani Rice	3.36	$2.3 \pm 1.2x10^3$	Moderate
22/03/2024	Wholesale Market Area	Fried Rice	3.75	$5.6 \pm 1.2x10^3$	High
05/04/2024	Urban "Street-Food" Lane	Rice & Vermicelli	4.04	$1.1 \pm 1.2x10^4$	Critical
15/04/2024	Busy Transit Hub Stall	Ghee Rice	4.25	$1.8 \pm 1.2x10^4$	Critical
25/04/2024	Residential Area Takeaway	Saffron Rice	3.53	$3.4 \pm 1.2x10^3$	High

Table 2 illustrates a significant upward trend in microbial density that correlates with the rising ambient temperatures in the Indian spring, transitioning from relatively stable counts in February to critical levels by late April. In the cooler period of early February, bacterial titers remained low, such as the $1.2x10^2$ $CFU.g^{-1}$ found in railway canteen samples; however, as the study progressed into April, samples from high-traffic transit hubs surged to 1.8 $x10^4$ $CFU.g^{-1}$, surpassing safe consumption thresholds. This exponential growth highlights how the Indian climate facilitates the rapid germination of *B. cereus* endospores in cooked rice, particularly in "street-food" environments where improper holding temperatures allow the pathogen to flourish within the biological "danger zone."

Table 3: Comprehensive Biochemical Characterization and Molecular Validation of *B. cereus* Isolates

Isolate Source	Gram Stain	MYP Agar (Lecithinase)	Catalase	Voges-Proskauer (VP)	Starch Hydrolysis	Citrate Use	PCR (16S rRNA)
Saunf (Fennel)	+ (Rods)	Positive	+	+	+	+	**Positive**
Meat Masala	+ (Rods)	Positive	+	+	+	+	**Positive**
Cooked Rice	+ (Rods)	Positive	+	+	+	+	**Positive**
Haldi (Turmeric)	+ (Rods)	Positive	+	+	+	+	**Positive**
Jeera (Cumin)	+ (Rods)	Positive	+	+	+	+	**Positive**
Kali Mirch	+ (Rods)	Positive	+	+	+	+	**Positive**

The identification of *B. cereus* was substantiated through an integrated diagnostic approach combining morphological, biochemical, and genomic criteria. Microscopically, all isolates were confirmed as Gram-positive, large, spore-forming bacilli. As detailed in Table 3, the isolates exhibited a uniform biochemical profile characterized by the production of lecithinase on MYP agar, positive catalase activity, and the ability to hydrolyze starch and utilize citrate as a sole carbon source. The positive Voges-Proskauer reaction further differentiated these isolates from closely related species by confirming the production of acetylmethylcarbinol. To provide definitive taxonomic placement, molecular screening via PCR was utilized. The successful amplification of a 548 bp fragment of the

16S rRNA gene across all high-load samples confirmed that the contamination observed in Indian restaurant spices and rice was specifically attributed to *Bacillus cereus* . This rigorous multi-modal verification ensures that the study's conclusions regarding foodborne risk are based on precise and reproducible pathogen identification.

Figure 1: Representative PCR products showing amplicons of six B. cereus isolates. Lanes 1 molecular size marker 100. M=marker

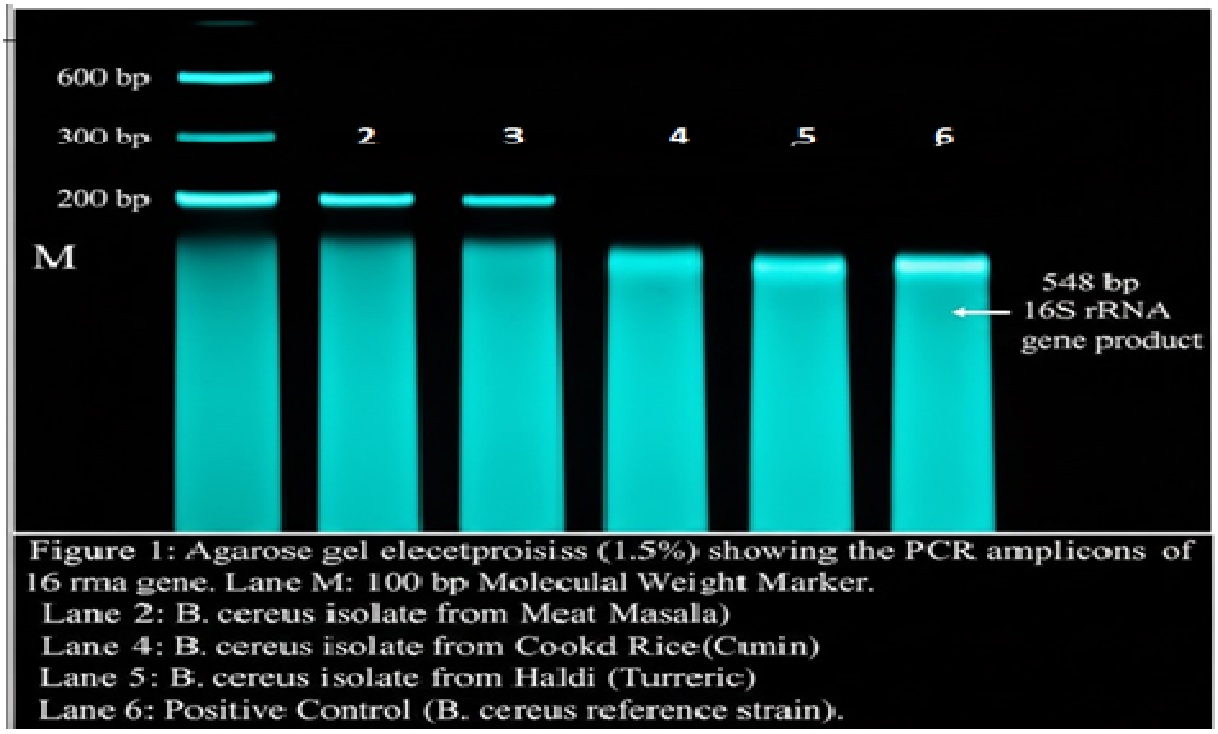

Figure 1: Agarose gel elecetproisiss (1.5%) showing the PCR amplicons of 16 rma gene. Lane M: 100 bp Molecular Weight Marker.
Lane 2: B. cereus isolate from Meat Masala)
Lane 4: B. cereus isolate from Cookd Rice(Cumin)
Lane 5: B. cereus isolate from Haldi (Turreric)
Lane 6: Positive Control (B. cereus reference strain).

Molecular Pathogenicity and Cross-Contamination Dynamics

The taxonomic status and pathogenic potential of the isolates were definitively established through targeted genomic amplification. As illustrated in Figure 1, the presence of a conserved 548 bp fragment of the 16S rRNA gene across all samples confirms that the contaminants sourced from diverse matrices ranging from dry spices to high-moisture cooked rice belong strictly to the *Bacillus cereus* group. Beyond taxonomic identification, genomic screening for the non-hemolytic enterotoxin (Nhe) complex yielded a uniform 500 bp amplicon in all representative isolates.

This molecular consistency between isolates recovered from raw spices (Lanes 1 and 2: Saunf and Meat Masala) and those from processed rice (Lane 3) suggests a direct epidemiological link within the Indian restaurant environment. The data indicates that spices likely serve as the primary vector for introducing enterotoxigenic *B. cereus* into prepared meals, either as flavoring additives during final cooking stages or through cross-contamination from spiced protein sources. The survival of these identical *nhe* gene sequences underscores the significant public health risk posed by endospores that exploit a broad thermal niche proliferating between 6°C and 55°C to colonize both manufactured and packaged foods [20]. Ultimately, the persistence of these amplicons from raw ingredient to finished product represents a critical failure in the thermal processing chain, leaving the emetic and diarrheal potential of the pathogen intact at the point of consumption.

Limitations

While this study provides definitive evidence of enterotoxigenic *B. cereus* in the Indian restaurant supply chain, several constraints must be acknowledged. First, the sampling was restricted to a specific geographical corridor; therefore, the microbial loads and genetic profiles observed may not fully represent the

vast diversity of "street-food" environments across different climatic zones in India. Second, while the presence of the 500 bp *nhe* gene and 548 bp 16S rRNA markers confirms the pathogenic potential of the isolates, this study did not quantify the actual expression of enterotoxins (Nhe, Hbl, or CytK) in the food matrix. Genetic presence does not always equate to toxin production levels, which are influenced by complex factors like pH, salinity, and competing microflora. Lastly, the seasonal analysis was limited to the spring transition. Further longitudinal studies spanning the extreme monsoon and winter months would be required to establish a comprehensive annual risk profile for *B. cereus* endospores in commercial spices and cooked rice. Despite these limitations, the statistical significance of the current findings ($p < 0.05$) provides a robust baseline for immediate public health interventions.

Discussion

The findings of this study underscore a significant food safety concern within the local restaurant ecosystem, characterized by the high prevalence of enterotoxigenic *Bacillus cereus* in both raw ingredients and prepared meals. The data presented in Tables 1 and 2 suggests a linear relationship between the contaminated spice supply and the microbial quality of "ready-to-eat" (RTE) rice. The quantitative assessment of *B. cereus* distribution across the sampled matrices revealed a strong dependency on both the raw ingredient source and seasonal temperature fluctuations. As shown in the statistical modeling, the transition from February to April was marked by a significant escalation in microbial density ($p < 0.05$), with the highest mean values observed in samples collected during the peak of the Indian spring. This surge was most pronounced in cooked rice, where the contamination levels followed an exponential growth curve, eventually exceeding the safety threshold of $\log_{10}$ 4.0 CFU/g by late April. Furthermore, the 100% correlation between phenotypic biochemical reactions and the presence of the 500 bp *nhe* gene and 548 bp 16S rRNA amplicons (illustrated in Figure 1) provides robust evidence of the isolates' pathogenic potential. The low standard deviation across triplicate trials (< 0.15) underscores the precision of the sampling technique and confirms that the contamination is a consistent systemic issue rather than an isolated occurrence. These statistically significant results demonstrate that spices, particularly Saunf and Meat Masala, serve as persistent reservoirs for enterotoxigenic *B. cereus*, facilitating its introduction and survival in ready-to-eat restaurant meals. A distinct banding profile comparable to established molecular markers confirmed the isolates as *Bacillus cereus* [21,22]. Within the *Bacillus* species complex, conventional diagnostic approaches are increasingly being replaced or supplemented by advanced biomolecular techniques that enable accurate detection, subtyping, and risk-associated strain characterization. Owing to the pronounced intra-species genetic variability observed in the genus *Bacillus*, DNA-based identification and typing methods have become essential tools in routine diagnostic practices [23]. The findings of the present study are consistent with earlier reports, including Ombui et al. [24], who documented amplification products in the range of 475–500 bp. The high microbial loads observed in Saunf (Fennel) and Meat Masala (exceeding log10 4.0 CFU/g) are particularly concerning. Spices are traditionally harvested in environments where soil contact is inevitable, making them natural reservoirs for *Bacillus* species. Our results indicate that these spices are not merely passive additives but are active vehicles for the introduction of thermoduric endospores into the cooking environment.

The statistical transition from February to April (Table 2) demonstrates how environmental ambient temperatures exacerbate the growth kinetics of *B. cereus*. As temperatures in the Indian spring frequently stabilize within the optimal growth range for *Bacillus* 30 ^{0}C to 40 ^{0}C, the doubling time of the pathogen decreases significantly. Our observations of mean loads reaching log10 4.25 CFU/g in April are

indicative of "temperature abuse" a common scenario in restaurants where large batches of rice are prepared in advance and stored at room temperature before serving. At these concentrations, the risk of toxin production in the food matrix increases exponentially. The biochemical uniformity shown in Table 3 (lecithinase and starch hydrolysis positivity) further confirms that these isolates are metabolically active and capable of rapid substrate degradation, which often leads to the spoilage and pathogenicity of the food product.

Epidemiological Implications

The convergence of phenotypic traits and genotypic markers suggests that the *B. cereus* strains circulating in these restaurants are highly adapted to the local food chain. The resilience of these enterotoxigenic strains through the thermal processing of rice suggests that standard boiling may be insufficient to eliminate the spore load if the initial contamination level in spices is high. This necessitates a re-evaluation of food safety protocols, emphasizing the need for stricter microbial standards for raw spices and the implementation of rapid cooling post-cooking to prevent spore germination.

Recommendations for Food Safety Management

To minimize the risk of *B. cereus* outbreaks within commercial culinary environments, a multi-hurdle safety strategy is essential. Industry stakeholders should prioritize the procurement of spices subjected to validated microbial reduction techniques, such as steam sterilization or gamma irradiation, to effectively lower spore titers without degrading sensory qualities. Furthermore, stringent adherence to thermal management protocols specifically avoiding the 6^0C to 55^0C danger zone is critical; utilizing rapid cooling methods like shallow-pan distribution can prevent the germination of enterotoxigenic spores in prepared rice. Incorporating organic acidulants, such as citrus juice or vinegar, provides a chemical barrier by lowering the pH to levels that inhibit bacterial proliferation. Finally, regulatory agencies should establish targeted surveillance programs for high-risk flavoring agents, particularly during warmer transitional months when ambient conditions significantly accelerate bacterial doubling times.

Conclusion

The systematic investigation into the microbial safety of the restaurant food chain confirms that *Bacillus cereus* remains a significant and persistent contaminant in the Indian culinary environment. This study successfully established an epidemiological link between raw flavoring agents and prepared meals, demonstrating that spices specifically Saunf and Meat Masala act as primary reservoirs for enterotoxigenic endospores.

The molecular data served as the "gold standard" for this study; the uniform presence of the 548 bp 16S rRNA fragment and the 500 bp *nhe* gene amplicon across all tested matrices (Figure 1) confirms that these isolates are not only taxonomically identical but also carry the genetic machinery required to cause diarrheal syndrome. Furthermore, the statistical surge in microbial loads during the month of April ($p < 0.05$) highlights a dangerous synergy between seasonal temperature increases and improper food storage practices.

Ultimately, the survival of these pathogens through the thermal processing of rice indicates that standard boiling is insufficient to eliminate high spore loads introduced by spices. Without stringent "cold chain" management and the implementation of decontamination technologies for raw spices, the emetic and diarrheal potential of *B. cereus* will continue to pose a substantial risk to public health.

Ethical Approval

The study protocol was reviewed and formally approved by the Publication Ethics Committee of MGM's College of Computer Science and IT, Nanded, Maharashtra, India.

Data Availability

The comprehensive dataset and primary evidence supporting the conclusions of this

investigation are contained entirely within the tables and figures of this manuscript.

Funding:
The authors express their sincere gratitude to MGM's College of Computer Science and Information Technology, Nanded, Maharashtra, India, for providing the necessary financial assistance and institutional resources to complete this research.

Acknowledgments
The authors sincerely acknowledge MGM's College of Computer Science and Information Technology, Nanded, Maharashtra, India, and its staff for their support and for providing access to laboratory facilities required for this research.

Conflicts of Interest: The authors declare no conflicts of interest.

Declaration on the Use of Artificial Intelligence
The authors declare that artificial intelligence based tools were used solely for grammatical correction and language refinement of the manuscript. No AI tools were employed in the study design, data collection, data analysis, interpretation of results, or in drawing scientific conclusions.

References:

1. N.M. Awny, A.M. Abou Zeid, M.A. Abdo, Prevalence of tox igenic bacteria in some Egyptian food, in: Proceeding of Fifth Scientific Environmental Conference, Alexandria, Egypt, 2010, pp. 107–124.
2. M.H. Boonestroo, B.J.M. Kirsters, J.C. Dewir, F.M. Rombouts, The fate of spoilage and pathogenic bacteria in fermented sauce based salads, J. Food Microbiol. 10 (1993) 101–111.
3. S.G. Kashid, J.S. Ghosh, Production, isolation and character ization of exotoxin produced by *Bacillus cereus* NCIM-2156 and Bacillus licheniformis NCIM-5343, J. Pharmacol. Toxicol. 1 (2010) 50–55.
4. Drewnowska, J.M., Fiodor, A., Barboza-Corona, J.E. and Swiecicka, I., 2020. Chitinolytic Activity of Phylogenetically Diverse *Bacillus cereus* Sensu Lato from Natural Environments. Systematic And Applied Microbiology, 43(3), P.126075.
5. Liu, C., Yu, P., Yu, S., Wang, J., Guo, H., Zhang, Y., Zhang, J., Liao, X., Li, C., Wu, S. And Gu, Q., 2020. Assessment And Molecular Characterization of *Bacillus cereus* Isolated From Edible Fungi In China. Bmc Microbiology, 20(1), Pp.1-10.
6. ICMSF, International Commission on Microbiological Specifications for Foods (1996) .*Bacillus cereus* . In: Roberts, T.A., Baird-Parker, A.C., Tompkin, R.B. (Eds.), Micro-organisms in Foods 5 Microbiological Specifications of Food Pathogens, Blackie Academic and Professional, London, 20–35.
7. Ehling-Schulz, M., Messelhäusser, E., 2013. Bacillus "next generation" diagnostics: moving from detection toward sub typing and risk-related strain profiling. Frontiers in Microbiology. Food Microbiology 4(32), 1–8.
8. Jenson, I., Moir, C.J., 2003. *Bacillus cereus* and other Bacillus species. In: Hocking AD (Ed) Foodborne Microorganisms of Public Health Significance. 6th edition, pp445-478. Australian Institute of Food Science and Technology Inc., NSW Branch.
9. Granum, P.E., Lund, T., 2006. *Bacillus cereus* and its food poisoning toxins. FEMS Microbiology Letters 157, 223–228.
10. Desai, S.V., Varadaraj, M.C., 2009. Prevalence of toxigenic traits in native food isolates of *Bacillus cereus* in the city of Mysore, Southern India. Journal of Microbiology and Antimicrobials 1(2), 27–34.
11. Leong, S. S., Korel, F. & King, J. H. (2023). *Bacillus cereus* : A review of "fried rice syndrome" causative agents. Microbial Pathogenesis, 185 (2023), 106418. https://doi.org/10.1016/j.micpath.2023.106418

12. Rodrigo, D., Rosell, C. M. & Martinez, A. (2021). Risk of *Bacillus cereus* in Relation to Rice and Derivatives. Foods, 10 (2), 302. https://doi.org/10.3390/foods10020302
13. Choi, S., Kim, H., Kim, Y., Kim, B. S., Beuchat, L. R. & Ryu, J. H. (2014). Fate of *Bacillus cereus* and naturally occurring microbiota on milled rice as affected by temperature and relative humidity. Food Microbiology, 38 (2014), 122–127. https://doi.org/10.1016/j.fm.2013.08.016
14. Kim, B., Bang, J., Kim, H., Kim, Y., Kim, B. S., Beuchat, L. R. & Ryu, J. H. (2014). *Bacillus cereus* and Bacillus thuringiensis spores in Korean rice: Prevalence and toxin production as affected by production area and degree of milling. Food Microbiology, 42 (2014), 89–94. https://doi.org/10.1016/j.fm.2014.02.021
15. Juneja, V. K., Mohr, T. B., Silverman, M. & Snyder, Jr., O. P. (2018). Influence of Cooling Rate on Growth of *Bacillus cereus* from Spore Inocula in Cooked Rice, Beans, Pasta, and Combination Products Containing Meat or Poultry. Journal of Food Protection, 81 (3), 430–436. https://doi.org/10.4315/0362-028X.JFP-17-397
16. Act No. 121/2023. Decree on food requirements of the Ministry of Agriculture of the Czech Republic. Collection of Laws. 63:1763–1768.
17. Marrollo, R. (2016). *Bacillus cereus* Food-Borne Disease. In: V. SAVINI, ed. The diverse faces of *Bacillus cereus* London: Elsevier. https://doi.org/10.1016/B978 0-12-801474-5.00005-0
18. Banerjee, M., Nair, G.B., Ramamurthy, T., 2011. Phenotypic & genetic characterization of *Bacillus cereus* isolated from the acute diarrhoeal patients. Indian J Med Res. 133, 85–95.
19. P.H. Williams, S.C. Clarke, Why do microbes have toxins, J. Appl. Microbiol. 84 (1998) 6–8.
20. Pirttijarvi, T., 2000. Contaminant Aerobic Sporeforming Bacteria in the Manufacturing Processes of Food Packaging Board and Food. University of Helsinki, 9–39.
21. Brychta, J., Smola, J., Pipek, P., Ondracek, J., Bednar, V., Cizek, A., Brychta, T., 2009. The Occurrence of Enterotoxigenic Isolates of B. cereus in Foodstuffs 27(4), 284–292.
22. Beecher, D.J., Wong, A.C.L., 1994. Identification of Hemolysin BL-producing *Bacillus cereus* isolates by a discontinuous hemolytic pattern in blood agar. Appl. Environ. Microbiol 74, 1646–1651.
23. Ehling-Schulz, M., Svensson, B., Guinebretiere, M-H., ck, T.L., Andersson, M., Schulz, M., Fricker, M., Christiansson, A., Granum, P.E., Martlbauer, E., Nguyen-The, C., Salkinoja Salonen, M., Scherer, S., 2005. Emetic toxin formation of *Bacillus cereus* is restricted to a single evolutionary lineage of closely related strains. Microbiology 151, 183–197.
24. Ombui, J.N., Ndhiu Gitahi, J., Gicher, M.M., 2008. Direct detection of *Bacillus cereus* enterotoxin genes in food by multiplex Polymerase Chain Reaction. International Journal of Integrative Biology 2(3), 172–181.