

Isolation of Bacterial Pathogens Associated with Commercially Available Spices in Mangaluru City, India

Chaithra S. Hebbar¹ , Apoorva R. Kenjar¹ , Juliet Roshini Mohan Raj^{1,3} ,
Iddya Karunasagar¹  and Rajeshwari Vittal^{1,2*} 

¹Nitte University Centre for Science Education and Research, Nitte (DU), Deralakatte, Mangaluru, Karnataka, India.

²Division of Environmental Health and Toxicology, Nitte University Centre for Science Education and Research, Deralakatte, Mangaluru, Karnataka, India.

³Division of Infectious Diseases, Nitte University Centre for Science Education and Research, Deralakatte, Mangaluru, Karnataka, India.

Abstract

Spices are important sources of natural flavouring, colouring and antimicrobial agents in food and medicine. In India, spices are widely produced, consumed and exported across the world. Like many other agricultural commodities, spices are exposed to a wide range of bacterial contamination during their harvesting, processing and transportation causing foodborne illnesses. Spices in their desiccated form offer an environment conducive to the survival of many pathogenic bacteria which becomes challenging for spice manufacturers to control or mitigate any bacterial contamination. The present study aimed at the isolation, phenotypic and genotypic identification of bacterial pathogens namely *Salmonella spp.*, *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* associated with spices collected in and around Mangaluru, Karnataka. Isolation of bacterial pathogens was performed using a modified standard FDA BAM methodology. A total of 140 spice samples inclusive of pepper, clove, cumin, red chillies, turmeric, coriander, clove and fennel in whole and powdered form were screened for pathogens. No targeted bacterial pathogens were present in the samples collected. It can be inferred that good agricultural, manufacturing and hygienic practices were maintained in the commercial supply of spices. The absence of bacteria could also be attributed to the inherent antimicrobial properties of spices.

Keywords: Bacterial Pathogens, Contamination, Genotype, Isolation, Phenotype, Spices

*Correspondence: rajeshwari.vittal@nitte.edu.in

Citation: Hebbar CS, Kenjar AR, Raj JRM, Karunasagar I, Vittal R. Isolation of Bacterial Pathogens Associated with Commercially Available Spices in Mangaluru City, India. J Pure Appl Microbiol. 2023;17(2):993-999. doi: 10.22207/JPAM.17.2.28

© The Author(s) 2023. **Open Access.** This article is distributed under the terms of the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, sharing, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

INTRODUCTION

India, the world's largest spice producer, consumer and exporter, is rightly known as "The Land of Spices." The Spice Board of India estimates 10.12 million tonnes of spices were produced in the year 2019-20. Pepper, clove, cinnamon, cardamom, fenugreek, fennel, cumin, coriander, turmeric, clove and nutmeg are the most common spices grown in India. Spices are frequently used for culinary purposes because of their distinct flavour and aroma. They are harvested and marketed in a variety of forms, including fresh, dried, whole or powdered, fried or roasted. Spices are also used as medications, in perfume-making industries, cosmetics, incense, soaps and as preservatives in a variety of foods and beverages.^{1,2} Thus, spice's multi-beneficial use has resulted in growing demand in India and other nations.

Spices are classified as Low Moisture Foods (LMFs). LMFs have low water activity (a_w), which hinders microbe development, yet some food-borne pathogens can persist in this environment.³ Microorganisms found in the spices can, not only survive but also proliferate and reproduce to infectious levels when exposed to the nutrient-rich food matrix with higher a_w posing a significant health risk to consumers. Careless handling of spices during processing leads to microbial contamination and even environmental conditions such as dust, warm and humid climate promote microbial development.⁴ When LMFs or the food environment gets contaminated, it becomes difficult to reduce contamination for manufacturers and consumers. Some of the reasons include, dry heat increasing the resistance of various microorganisms compared to wet heat, microbes having a longer survival period in LMFs and the process of rehydration favours the growth of pathogens and spore germination.^{3,5} Dried spices were formerly assumed to be not inhabitable by foodborne microorganisms because of their low moisture and water activity conditions. Nonetheless, in recent years they are linked with foodborne outbreaks and recalls due to microbial pathogens and their testing has found an unacceptably high occurrence rate. Spore-forming pathogens such as *Clostridium* spp. and *B. cereus*, as well as non-spore formers like *Salmonella* spp., *E. coli*, *S.*

aureus have been associated with spices resulting in food-borne diseases.⁶⁻¹⁰ Safety concerns regarding spice pathogens have now become a priority. Maintenance of good manufacturing and hygienic practices during processing is important to maintain the quality and safety of spices. The present study aimed to examine pathogenic microorganisms in common spices used in and around Mangaluru city, Karnataka, India.

MATERIALS AND METHODS

Sample collection

A total of 140 samples of commercially available spices including pepper, clove, cumin, red chillies, turmeric, coriander and fennel in whole and powdered form (n=10 each of whole and powdered) were collected from different supermarkets, sale outlets and local vendors in and around Mangaluru city, Karnataka.

Sample preparation as per FDA BAM protocols

Twenty-five grams of whole and powdered spices were weighed and added to 225ml of sterile Butterfield's phosphate buffer (BPB), blended to prepare a homogenate. The homogenate was serially diluted in 9 ml portions of BPB to obtain decimal dilutions.¹¹

Genotypic and Phenotypic identification of foodborne pathogens

Isolation of *Bacillus cereus*

The homogenate (0.1ml) was spread onto Nutrient agar (NA) and incubated at 37°C for 24 hours. Culture grown were subjected to phenotypic identification by preliminary and biochemical tests. Genotypic identification was performed using Polymerase Chain Reaction (PCR) to identify the toxin gene primers hblC and CER (Table 1) specific to *Bacillus cereus*.

Isolation of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* as per modified FDA BAM protocols

Isolation of *Escherichia coli*

A pour plate technique was used, which involved the addition of 1 ml of the sample dilution over molten Violet Red Bile Agar (VRBA), solidifying it and incubating it at 37°C for 24-48 hours. After incubation, typical purple-red colonies on VRBA

were inoculated into Brilliant Green Lactose Broth (BGLB) and incubated at 37°C for 24-48 hours. Positive cultures were streaked onto Eosin Methylene Blue (EMB) agar for identification. Phenotypic identification was performed using preliminary tests, biochemical analysis and genotypic confirmation of the genus-specific *uidA* (Table 1) gene was conducted using PCR.

Isolation of *Staphylococcus aureus*

Homogenate (1ml) was divided into three portions (0.3ml, 0.3ml and 0.4 ml) and spread on Baird parker Agar (BPA). After 24-48 hours of incubation at 37°C, plates were examined for characteristic colonies.

Isolation of *Salmonella*

Twenty-five grams of sample were added to 225ml of pre-enrichment media-Tryptic Soy Broth (TSB) at 35°C for 24 hours, followed by selective enrichment at 42°C using 0.1ml pre-enriched mixture to 10 ml Rappaport-Vassiliadis Soya (RVS) broth and Tetrathionate Bile Brilliant Green (TTBBGB) broth. After 24 hours, incubated broths were streaked onto Xylose Lysine Deoxycholate (XLD), Bismuth Sulphite (BS) and Hektoen Enteric (HE) agar incubated at 37°C for 24 hours and observed for typical *Salmonella* like colonies.

Crude DNA extraction

Overnight bacterial culture was centrifuged at 10,000 rpm for 10 minutes. Reseeded the pellet with 150 µl 1X TE buffer, heated to 95°C for 10 minutes, flash frozen on ice for 5 minutes and centrifuged at 10,000 rpm for 3 minutes. The crude DNA containing supernatant was transferred to a new vial and used as a template for PCR amplification.

PCR amplification

PCR amplification was performed using a programmable thermocycler (Eppendorf NexusGX2, USA). It was carried out in a 30 µl reaction mixture containing 22.2 µl of nuclease-free water, 3 µl of 10X buffer (HiMedia, India), 0.6 µl of dNTPs (10mM/µl) (HiMedia, India), 0.5 µl forward and reverse primers (10 PM/µl)^{12,13}, 0.2 µl of Taq polymerase (5U/µl) (HiMedia, India) and 2 µl of DNA template. The PCR products after amplification were resolved using gel electrophoresis (2% agarose gel), stained with ethidium bromide (0.5 µg/ml) were visualized under ultraviolet light using a gel documentation system (Bio-Rad, CA, USA).

RESULTS

Isolation of *Bacillus cereus*

Greyish-white, irregular, granular colonies were detected and verified by gram staining and endospore staining. Gram-positive bacilli were found in 99 (70%) of the 140 spice samples tested. Endospore staining showed the presence of spore, followed by the catalase test being positive for all 99 samples. A total of 52 whole samples and 47 powdered samples showed the presence of gram-positive bacilli (Table 2). However, PCR amplification using toxin genes (*hblC* & *CER*) specific primers for *B. cereus* showed negative amplification for all samples tested.

Isolation of *Escherichia coli*

In a total of 140 samples, 43 (30.71%) samples had typical coliform colonies on VRBA. 24 (17.14%) samples were confirmed as coliforms by gas production in BGLB broth after 48h incubation. A total of 10 whole samples and 14 powdered samples were confirmed to harbour

Table 1. Primers used in the study

Gene target	Sequences (5' - 3')	Annealing temp.	Product size (bp)	Ref.
<i>UidA</i>	AAAACGGCAAGAAAAGCAG ACGCGTGGTTACAGTCTTGCG	63°C	146	12
<i>hblC</i>	CGCAACGACAAATCAATGAA ATTGCTTCACGAGCTGCTTT	54°C	421	13
<i>CER</i>	GCGTACCAAATCACCCGTTT TGCAGGTGGCACACTTGTTA	54°C	546	13

coliforms (Table 3). However, none of the samples showed the presence of *E. coli* as characterised by biochemical and molecular techniques.

Isolation of *Salmonella* and *Staphylococcus aureus*

No typical colonies of *Salmonella* (XLD, BS and HE Agar) or *Staphylococcus aureus* (BPA) were observed on the respective selective agar plates.

DISCUSSION

Food that is safe and nutritious is essential for living a better life and promoting good health. More than 200 illnesses, ranging from diarrhea to cancer are caused by contaminated food. According to WHO, food contamination causes 600 million instances of foodborne illnesses and 4,20,000 deaths globally each year.¹⁴ Spices are becoming more common in cuisines, with the substantial intake of spices in the worldwide human diet both for flavour and their therapeutic benefits. It is thus, necessary to inspect food safety aspects regarding spice consumption. The current study aimed to identify pathogenic bacteria from common spices used in Mangaluru, India.

E. coli, a commensal organism is the most common coliform bacteria that are associated with spices.¹⁵⁻¹⁹ Contamination of coliforms in spices indicates poor sanitary or careless handling practices by spice handlers, improper facility design, the use of non-potable water during processing, mix-ups and cross contaminations, all of which results in opportunistic illnesses. In the present study, though *E. coli* was absent in

Table 2. Qualitative analysis for isolation of gram-positive bacilli

No.	Sample type	No. of Whole samples (n=10) showed positive for gram positive bacilli	No. of Powdered samples (n=10) showed positive for gram positive bacilli
1.	Pepper	6	9
2.	Coriander	10	10
3.	Turmeric	7	8
4.	Chilli	6	6
5.	Cumin	10	10
6.	Fennel	8	9
7.	Clove	0	0

all the spice samples, 24 (17.14 %) of the 140 samples tested positive for total coliforms. The total coliform presence in powdered samples was higher than in whole samples regardless of whether the spices were industrially packed or loosely sold in temporary packets. Unlike other studies conducted in India, Ethiopia and Lebanon, where non-packaged spices had greater microbial burdens than packaged goods.^{7,19,20} This might be due to various factors; unlike powdered forms, bacteria if present in whole samples might not be homogeneously distributed or due to a lack of GMP and HACCP practices during collection, processing or manufacturing. Employment of unapproved chemical additives and adulterants in spices cause contamination which might interfere with the purity of spices. A study by Karam et al. observed that locally packaged spices in companies with Food Safety Management Systems (FSMS) had lesser microbial load than those locally packaged in companies without FSMS.¹⁹

Salmonella, a non-coliform pathogen is a leading cause of acute gastroenteritis and was absent in all samples screened in the present study. Similar results were observed by Karam L et al.¹⁹ and Bedada et al. studies.²⁰ Globally, many recent studies reported the occurrence of *Salmonella*, a bacteria of particular public health concern.^{16,21,22} A study investigated that imported

Table 3. Qualitative analysis for isolation of total coliforms

No.	Sample type	No. of samples showing typical colonies on VRBA	No. of samples confirmed for coliforms by BGLB
1.	Pepper whole	3	3
2.	Pepper powder	5	4
3.	Coriander whole	1	1
4.	Coriander powder	8	4
5.	Turmeric whole	5	2
6.	Turmeric powder	4	3
7.	Chilli whole	2	0
8.	Chilli powder	0	0
9.	Cumin whole	4	1
10.	Cumin powder	4	3
11.	Fennel whole	5	3
12.	Fennel powder	2	0
13.	Clove whole	0	0
14.	Clove powder	0	0

herbs and spices accounted for 1,677 cases i.e., 16% of all foodborne illnesses in the United States between 1996 and 2014.²³ Furthermore, the FDA determined that imported dried spices had a contamination rate of 6.6% between 2007 and 2009 and also according to the researchers, the contamination rate has been relatively constant over the last three decades. Also, they reported that spices had a 190% higher risk of *Salmonella* contamination when compared to other imported food commodities that are subjected to FDA regulation.²⁴ Between 1973 and 2010, Van Doren et al. recorded 14 foodborne disease outbreaks connected with spices among 10 countries, resulting in 2 fatalities, 128 hospitalizations and 1,946 illnesses. Infants and toddlers were disproportionately affected in these epidemics, accounting for 36% of all infections. *Salmonella* was the major agent responsible for 71% of outbreaks and 87% of illnesses, whereas *Bacillus* spp. was responsible for 29% of outbreaks and 13% of illnesses.²⁵

Spices therefore must endure the decontamination process to lower the risk of illnesses and product recalls. The primary industrial treatments used to lower microbial burdens in spices are mainly physical methods such as steam sterilization, irradiation, microwave and chemical fumigation methods, and each of these approaches has advantages and disadvantages. Thermal sterilisation is a widely accepted decontamination method by consumers. Yet, essential oils and polyphenols in spices are highly volatile, heat treatments can have a severe impact on the colour and flavour of certain spices.²⁶ According to Nyhan et al. heat treatment is less successful with low-moisture foods like spices and powders and irradiation has an impact on sensory characteristics and fumigation with ethylene oxide causes cancer.²⁷

The study also targeted two most common gram-positive microorganisms often encountered as food contaminants, namely *S. aureus* and *B. cereus*. *S. aureus* causes staphylococcal food poisoning when the bacteria contaminated with spices gets ingested. The current study found the absence of *S. aureus* growth in all the samples tested, similar to the Demir et.al study which is encouraging.¹⁸ Yet, many studies have reported the incidence of *S. aureus* in their studies.^{7,16,20,28}

Bacillus species constitute the most frequently encountered bacteria in a variety of spice samples from various geographical origins.^{7,29} *B. cereus*, a group of mesophilic bacteria was found to be absent in the present study, even though gram-positive sporulating bacteria was detected in 99 (70%) of the spice samples in which the powdered form showed a higher number when compared with the whole samples. A recent study conducted in India reported 89% *B. cereus* contamination of the 100 samples tested.²⁸ Berthold-Pluta et al. identified 63.3% prevalence rate of *B. cereus* among nine spices in Poland.³⁰ In Turkey, *B. cereus* was isolated in 31.5% of the 203 packaged and loosely sold spice samples.³¹ Bacterial spores are major contamination factors involved with all heat-treated foods; however, they can sustain food processing treatments. Studies have proven that *B. cereus* can withstand high temperatures of 100°C for about 3.4 to 100 minutes.^{32,33} Endospore germination may occur under favourable conditions such as accessible water content, pH, ambient temperature and their intake of food results in food intoxication.⁹ Traditional spice processing techniques that may lead to contamination, still appear to be commonly practised in small farms of major spice-producing countries. It is consequently essential to comprehend bacterial spore contamination associated with spices and also to decrease potential contamination caused during the harvesting and post-harvesting process.

Thus, the current study agrees with most of the other studies that reported *B. cereus*, *Salmonella*, *E. coli*, and *S. aureus* in spices are seemingly rare and in irregular intervals. Monitoring the microbiological safety and quality of spices before selling would limit public health risks, expenses and losses linked with food spoilage during storage, which eventually extends the shelf life.²⁶

Spice antimicrobial compounds are associated with the inhibition of virulence factor encoding gene expression, biofilm synthesis, bacterial migration and adhesion. Among all the spices analysed, clove was the only spice sample that showed a complete absence of any pathogens tested. This can be attributed to its strong antimicrobial compounds (eugenol) which hinder the growth of microorganisms and

exhibits antiseptic, antioxidant and antibacterial activities.³⁴ The lack of or the low microbial load of different spices could be attributed to the bioactive components with antimicrobial properties.^{35,36}

According to the Codex Code of Hygienic Practice (CAC, 1995), any toxin-producing bacteria in spices should not exceed in quantities that cause public health risks. *Salmonella* should be completely devoid in 25 g of the sample (Codex Alimentarius Commission, 2014), and the bacterial count in the same sample should be within limits of 102 CFU/g for *E. coli* and 104 CFU/g for *B. cereus* (European Commission (EC), 2004).³⁷⁻³⁹ In correlation to this, the present study detected no pathogenic bacterial contamination of major concern that may pose a threat to public health.

CONCLUSION

Among the seven spices, food-borne pathogens like *E. coli*, *Salmonella*, *B. cereus* and *S. aureus*, the main bacterial pathogens causing significant public health burden were found to be absent in the current study. All the samples collected which impart post-harvesting methods, processing and storage conditions were ideal for the products. These results also convey that the spice samples were safe for consumption and can be used for culinary, pharmaceutical and cosmetic purposes. The presence of coliforms can be reduced by maintaining good hygienic, manufacturing and handling practices by following HACCP principles, aseptic packaging of spices by using Modified Atmosphere Packaging (MAPs) techniques or use of irradiation during pre- and post-packaging, heat treatments, environmental monitoring by controlling moisture to maintain the dry storage conditions can lead to improved purity and quality of spice products in India.

ACKNOWLEDGMENTS

The authors would like to thank Nitte (Deemed to be University) for providing laboratory facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

REFERENCES

- Hosamani SV, Thyagaraja CM. Challenges of marketing Indian spices in India. *Int J Commer Bus Manag.* 2017;10(2):99-103. doi: 10.15740/HAS/IJCBM/10.2/99-103
- Rathore MS, Shekhawat NS. Incredible Spices of India: from Traditions to Cuisine. *J Bot.* 2008;1(3):85-89.
- Beuchat LR, Komitopoulou E, Beckers H, et al. Low-water activity foods: Increased concern as vehicles of foodborne pathogens. *J Food Prot.* 2013;76(1):150-172. doi: 10.4315/0362-028X.JFP-12-211
- Bakobie N, Addae AS, Duwiejua AB, Cobbina SJ, Miniyila S. Microbial profile of common spices and spice blends used in Tamale, Ghana. *Int J Food Contam.* 2017;4(1):10. doi: 10.1186/s40550-017-0055-9
- Komitopoulou E, Penaloza W. Fate of *Salmonella* in dry confectionery raw materials. *J Appl Microbiol.* 2009;106(6):1892-1900. doi: 10.1111/j.1365-2672.2009.04144.x
- Geeta H, kulkarni PR. Survey of the Microbiological Quality of Whole, Black Pepper and Turmeric Powder Sold in Retail Shops in Bombay. *J Food Prot.* 1987;50(5):401-403. doi: 10.4315/0362-028X-50.5.401
- Banerjee M, Sarkar PK. Microbiological quality of some retail spices in India. *Food Res Int.* 2003;36(5):469-474. doi: 10.1016/S0963-9969(02)00194-1
- Klimesova M, Horacek J, Ondrej M, et al. Microbial contamination of spices used in the production of meat products. *Potravin Sci J Food Ind.* 2015;9(1):154-159. doi: 10.5219/440
- Moore RE, Cherie Millar B, Panickar JR, Moore JE. Microbiological safety of spices and their interaction with antibiotics: Implications for antimicrobial resistance and their role as potential antibiotic adjuncts. *Food Qual Saf.* 2019;3(2):93-97. doi: 10.1093/fqsafe/fyz008
- Zhang G, Hu L, Pouillot R, et al. Prevalence of *Salmonella* in 11 spices offered for sale from retail establishments and in imported shipments offered for entry to the United States. *J Food Prot.* 2017;80(11):1791-1805. doi: 10.4315/0362-028X.JFP-17-072

11. United States. Food and Drug Administration. Division of Microbiology, Association of Official Analytical Chemists. Bacteriological analytical manual. 1998.
12. Bej AK, DiCesare JL, Haff L, Atlas RM. Detection of *Escherichia coli* and *Shigella* spp. in water by using the polymerase chain reaction and gene probes for uid. *Appl Environ Microbiol.* 1991;57(4):1013-1017. doi: 10.1128/aem.57.4.1013-1017.1991
13. Ngamwongsatit P, Buasri W, Pianariyanon P, et al. Broad distribution of enterotoxin genes (*hblCDA*, *nheABC*, *cytK*, and *entFM*) among *Bacillus thuringiensis* and *Bacillus cereus* as shown by novel primers. *Int J Food Microbiol.* 2008;121(3):352-356. doi: 10.1016/j.ijfoodmicro.2007.11.013
14. Food safety. WHO. 2022. <https://www.who.int/newsroom/factsheets/detail/food-safety>. Accessed on October 12th 2022.
15. Flynn DM, Weinstein RA, Nathan C, Gaston MA, Kabins SA. Patients' endogenous flora as the source of "nosocomial" Enterobacter in cardiac surgery. *J Infect Dis.* 1987;156(2):363-368. doi: 10.1093/infdis/156.2.363
16. Ogur S. Microbiological Quality and Safety of Some Dried Spices Obtained from Markets, Spice Shops and Homes. *Braz Arch Biol Techn.* 2022;65(1). doi: 10.1590/1678-4324-2022220315
17. Bitira LM. Microbial safety of locally produced spices in Uganda (Doctoral dissertation, Makerere University). 2022. <https://dissertations.mak.ac.ug/handle/20.500.12281/4200>. Accessed on October 29th 2022
18. Demir P, Erkan S, Oksuztepe G, Incili GK. Microbiological Quality of Open Spices in Elazığ. *Erciyes Medical J.* 2019;16(3):204-212. doi: 10.32707/ercivet.648568
19. Karam L, Salloum T, El Hage R, Hassan H, Hassan HF. How can packaging, source and food safety management system affect the microbiological quality of spices and dried herbs. The case of a developing country. *Int J Food Microbiol.* 2021;353:109-295. doi: 10.1016/j.ijfoodmicro.2021.109295
20. Bedada TL, Derra FA, Gebre SG, et al. Microbial evaluation of spices in Ethiopia. *Open Microbiol J.* 2018;12(1). doi: 10.2174/1874285801812010422
21. Islamoglu AH, Korkmaz BI. Assessment of *Salmonella* spp. and mold-yeast contamination in packaged and unpackaged spices. *Acta Pharm Sci.* 2021;59(2):207-214. doi: 10.23893/1307-2080.APS.05912
22. World Health Organization. Microbiological hazards in spices and dried aromatic herbs: Meeting report. Food & Agriculture Org.; 2022.
23. Gould LH, Kline J, Monahan C, Vierk K. Outbreaks of disease associated with food imported into the United States, 1996-2014. *Emerg Infect Dis.* 2017;23(3):525-528. doi: 10.3201/eid2303.161462
24. Van Doren JM, Kleinmeier D, Hammack T, Westerman A. Prevalence, serotype diversity, and antimicrobial resistance of *Salmonella* in imported shipments of spice offered for entry to the United States, FY2007-FY2009. *J Food Microbiol.* 2013;34(2):239-251. doi: 10.1016/j.fm.2012.10.002
25. Van Doren JM, Neil KP, Parish M, Gieraltowski L, Gould LH, Gombas KL. Foodborne illness outbreaks from microbial contaminants in spices, 1973-2010. *J Food Microbiol.* 2013;36(2):456-464. doi: 10.1016/j.fm.2013.04.014
26. Mathot AG, Postollec F, Leguerinel I. Bacterial spores in spices and dried herbs: The risks for processed food. *Compr Rev Food Sci Food Saf.* 2021;20(1):840-862. doi: 10.1111/1541-4337.12690
27. Nyhan L, Przyjalowski M, Lewis L, Begley M, Callanan M. Investigating the use of ultraviolet light emitting diodes (Uv-leds) for the inactivation of bacteria in powdered food ingredients. *Foods.* 2021;10(4):797. doi: 10.3390/foods10040797
28. Gulati D, Das N. Microbiological quality of spices marketed in Uttarakhand, India. *JBINO.* 2021;10(3):750-759. doi: 10.46344/JBINO.2021.v10i03.04
29. Chakraborty M, Afrin T, Munshi SK. Microbiological quality and antimicrobial potential of extracts of different spices. *Food Res.* 2020;4(2):375-379. doi: 10.26656/fr.2017.4(2).303
30. Berthold-Pluta A, Pluta A, Garbowska M, Stefanska I. Prevalence and toxicity characterization of *Bacillus cereus* in food products from Poland. *Foods.* 2019;8(7):269. doi: 10.3390/foods8070269
31. Cufaoglu G, Ayaz ND. Potential risk of *Bacillus cereus* in spices in Turkey. *Food Control.* 2022;132:108570. doi: 10.1016/j.foodcont.2021.108570
32. Yehia HM, Al-Masoud AH, Elkhadragy MF, Sonbol H, Al-Dagal MM. Analysis of spore-forming bacterial contaminants in herbs and spices and evaluation of their heat resistance. *J Food Sci Technol.* 2022;42(2). doi: 10.1590/fst.19422
33. Xue R, Wang H, Zhao D, et al. Identification of heat-resistant *Bacillus* strains in peppers in Sichuan Province, China. *LWT.* 2023;114425. doi: 10.1016/j.lwt.2023.114425
34. Hu Q, Zhou M, Wei S. Progress on the antimicrobial activity research of clove oil and eugenol in the food antiseptics field. *J Food Sci.* 2018;83(6):1476-1483. doi: 10.1111/1750-3841.14180
35. Costa MD, Cruz AI, Bispo AS, Ferreira MA, Costa JA, Evangelista-Barreto NS. Occurrence and antimicrobial resistance of bacteria in retail market spices. *Cienc Rural.* 2020;50(4):e20190775. doi: 10.1590/0103-8478cr20190775
36. Thanh MD, Frentzel H, Fetsch A, Krause G, Appel B, Mader A. Tenacity of *Bacillus cereus* and *Staphylococcus aureus* in dried spices and herbs. *Food Control.* 2018;83:75-84. doi: 10.1016/j.foodcont.2016.12.027
37. Codex Alimentarius International food standards: code of hygienic practice for spices and dried aromatic plants. CAC/RCP 42-1995. <https://www.fao.org/fao-who-codexalimentarius/en/>
38. European Spice Association European Spice Association Quality Minima Document. 2004. URL: www.esa-spices.org/download/esaqmdrev1-2nov07.pdf.
39. Muggeridge M, Clay M. Quality specifications for herbs and spices. *Handbook of herbs and spices.* 2001;17(1):13-21. doi: 10.1533/9781855736450.13