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BACTERIAL COMPOSITION OF ICE BIRIYANI: PROFILING THE MICROFLORA OF A REGIONAL FERMENTED RICE DISH

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ABSTRACT

Food safety remains a critical concern for ready-to-eat (RTE) foods, which are prone to microbial contamination from improper handling, inadequate storage, and environmental exposure. Ice Biriyani, a cold-stored rice-based dish popular in South India, lacks scientific study of its microbial safety despite widespread consumption. This study assessed the microbial profile of Ice Biriyani to evaluate potential food safety risks. Eight bacterial strains isolated from Ice Biriyani samples were characterized using Gram staining, Methyl Red, Voges-Proskauer, Citrate utilization, Triple Sugar Iron (TSI), Catalase, and Nitrate Reduction tests. Results revealed both Gram-negative (*Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp.) and Gram-positive (*Bacillus cereus a*nd *Bacillus* spp.) bacteria. The detection of E. coli suggests fecal contamination from poor water quality or unhygienic handling, while Bacillus cereus, a known foodborne pathogen capable of surviving cooking and proliferating under improper storage, raises concerns about toxin production and food poisoning. These findings underscore the need for strict hygiene measures, safe handling practices, and proper temperature control to minimize microbial risk in Ice Biriyani and similar RTE foods. Future research should incorporate molecular techniques such as PCR and next-generation sequencing (NGS) for more comprehensive microbial risk assessment.

Keywords: Ice Biriyani, food safety, microbial contamination, *Bacillus cereus, Escherichia coli, Enterobacteriaceae*, foodborne pathogens.

INTRODUCTION

Ice Biriyani is a popular cold-stored RTE street food in South India. Unlike conventional hot-served biriyani, it undergoes a cooling and storage phase that can favor survival of spore-forming pathogens that persist through cooking, such as *Bacillus cereus* (Tambekar et al., 2008; Mensah et al., 2002). Despite its popularity, scientific data on its microbial safety are lacking. Rice itself is a high-moisture, high-carbohydrate medium well suited to microbial growth when stored improperly (Doyle & Buchanan, 2013), and common contaminants of rice-based dishes include *B. cereus, E. coli, Salmonella spp., and Staphylococcus aureus,* especially when food sits in the 5°C–60°C temperature "danger zone" (Aycicek et al., 2004; FAO, 2019). Water quality, ingredient handling, and

environmental exposure during preparation and sale further influence the microbial load of street-vended dishes (Mensah et al., 2002; Muinde & Kuria, 2005; Barro et al., 2006).

Traditional culture-based microbiological methods remain the standard for detecting foodborne contaminants, though they may miss viable-but-non-culturable organisms; molecular methods such as PCR and next-generation sequencing (NGS) offer more comprehensive detection (Jay et al., 2005; Doyle & Buchanan, 2013). A clear gap remains in research on cold-stored rice dishes specifically, as most existing literature addresses freshly prepared, hot-served rice foods rather than the extended cold-storage conditions unique to Ice Biriyani. Given its high moisture content and frequent exposure to improper handling and storage, Ice Biriyani may harbor pathogens such as *Bacillus cereus, E. coli,* and *Salmonella*. This study aims to assess the microbial contamination of Ice Biriyani and evaluate the associated food safety risks using conventional microbiological methods.

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

Samples were collected from day-old Ice Biriyani. Serial dilution was performed to isolate individual colonies: after 24 hours, morphologically distinct colonies were picked and streaked onto nutrient agar plates, then inoculated into 10 ml sterile nutrient broth and incubated (Elamathy and Kanchana, 2012). After a further 24 hours, 1 ml aliquots from dilutions 10^{-4} to 10^{-7} were transferred aseptically onto fresh sterile nutrient agar plates and incubated at 30°C ± 2°C for 24 hours for isolation of bacterial colonies (Winny Fred Crossia *et al.*, 2025).

Identification of Bacterial Isolates

Isolated colonies were subjected for identification by microscopy and standard biochemical tests. Grams staining and Biochemical tests such as Methyl red, Voges Proskauer, Citrate Utilization, TSI, Catalase and Nitrate Reduction were performed (Winny Fred Crossia *et al.*, 2019).

RESULTS

After Incubation morphologically distinct colonies were picked, streaked, and stored for further use. A total of eight colonies were isolated, samples were labelled as 1–8, and maintained on nutrient agar slants at 4°C for further analysis.

Gram staining differentiated the eight isolates into two groups: Samples 1, 2, and 3 were Gram-negative, consistent with enteric bacteria, while Samples 4–8 were Gram-positive, consistent with spore-forming *Bacillus* spp. Biochemical testing (summarized in Table 1 and Figures 1–7) supported genus-level identification of *Escherichia coli* (Sample 1), *Enterobacter* spp. (Sample 2), *Klebsiella* spp. (Sample 3), *Bacillus cereus* (Sample 4), and *Bacillus* spp. (Samples 5–8).

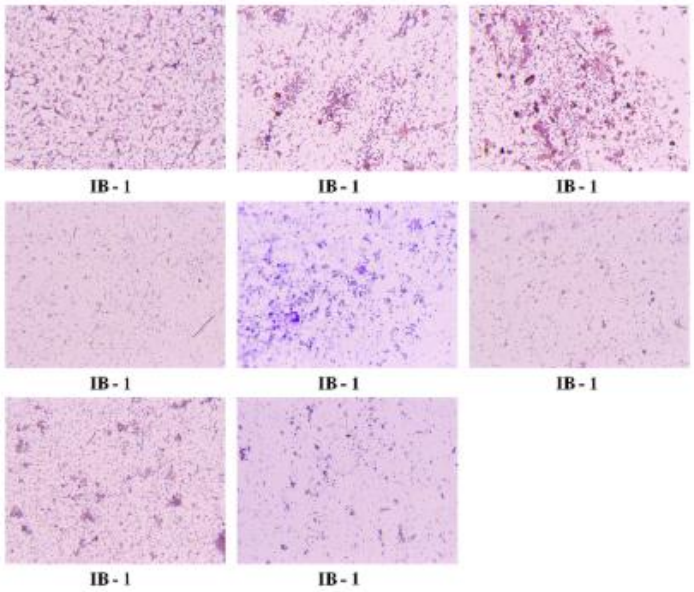

Fig 1: Gram staining test results of isolated microbes

1. Gram Staining Results

Gram staining was performed to differentiate bacterial isolates into Gram-positive and Gram-negative groups. Samples 1, 2, and 3 tested Gram-negative (-), suggesting the presence of enteric bacteria. Samples 4, 5, 6, 7, and 8 tested Gram-positive (+), indicating the presence of spore-forming bacteria such as Bacillus spp.

2. Biochemical Characterization of Isolates

To further identify the bacterial genera, the biochemical tests described above were conducted; results are summarized in Table 1.

Table – 1. Macroscopy, Microscopy and Biochemistry of Gram Positive Bacterial Isolates from Ice Biriyani

CODE	CHARACTERISTICS										PREDICTED GENERA
	MACROSCOPY		MICROSCOPY			BIOCHEMISTRY					
	Colour	Colony morphology	Shape	Gram's nature	Motility	MR	VP	TSI	Citrate	Catalase	
S1	Grey	Smooth, raised, circular colonies	Bacilli	-	-	-	+	A/ A	-	+	***Klebsiella sp***
S2	White	Smooth, large, flat, irregular, filamentous colonies	Bacilli	-	+	-	-	K/ K	+	+	***Pseudomonas sp***
S3	Translucent	Smooth, large, flat, circular colonies	Bacilli	-	+	+	-	A/ A	-	+	***Escherichia coli***
S4	White	Smooth, small and circular colonies	Bacilli	+	-	-	-		-	+	***Bacillus sp***
S5	White	Smooth, small and circular colonies	Bacilli	+	-	-	-		-	+	***Bacillus sp***

S6	White	Smooth, small and circular colonies	Bacilli	+	-	-	-		-	+	***Bacillus sp***
S7	White	Smooth, small and circular colonies	Bacilli	+	-	-	-		-	+	***Bacillus sp***
S8	White	Smooth, small and circular colonies	Bacilli	+	-	-	-		-	+	***Bacillus sp***

NOTE: + = positive; - = negative; A/A = acid slant acid butt; A/K = acid slant alkaline butt; K/K = alkaline slant alkaline butt;

Among the Gram-negative bacteria, *E. coli* (Sample 1) was identified by a positive MR test, negative VP test, and positive citrate test, consistent with mixed-acid fermentation and citrate utilization typical of Enterobacteriaceae. *Enterobacter* spp. (Sample 2) and *Klebsiella* spp. (Sample 3) were differentiated by VP-positive and citrate-positive reactions, consistent with the butanediol fermentation pathway. Samples 4–8 were confirmed as Bacillus spp. based on Gram-positive staining, TSI-positive, and citrate-positive results; Sample 4 was specifically identified as Bacillus cereus based on its nitrate-negative, VP-positive profile. The absence of catalase activity in all samples suggests a predominance of facultative anaerobic or aerotolerant bacteria capable of surviving fluctuating oxygen availability common in improperly stored food.

DISCUSSION

The microbial analysis of Ice Biriyani samples revealed a significant presence of both Gram-negative and Gram-positive bacterial genera, with variations in biochemical responses indicating multiple food safety concerns. The identification of *E. coli*, *Enterobacter* spp., *Klebsiella* spp., and *Bacillus* spp. suggests multiple, overlapping contamination routes — poor hygiene, inadequate handling, and improper storage — consistent with the known behavior of rice-based dishes as favorable microbial growth media (Doyle & Buchanan, 2013).

E. coli (Sample 1) is a well-established fecal indicator organism; its MR-positive, VP-negative, citrate-positive profile is consistent with mixed-acid-fermenting Enterobacteriaceae, and its presence points to contamination via poor water quality, unhygienic ingredient handling, or inadequate cooking (Jay et al., 2005). While most E. coli strains are harmless, pathogenic variants such as enterohemorrhagic E. coli can cause severe illness, including hemolytic uremic syndrome in vulnerable individuals (WHO, 2020). *Enterobacter* spp. (Sample 2) and *Klebsiella* spp. (Sample 3), distinguished by VP-positive and citrate-positive reactions, are opportunistic Enterobacteriaceae commonly linked to contaminated water, unwashed produce, and biofilm formation on food-contact surfaces (Mensah et al., 2002). Though less virulent than *E. coli* or *Salmonella*, both are associated with opportunistic infections — UTIs, respiratory and wound infections for Enterobacter, and pneumonia or bloodstream infections, including antibiotic-resistant strains, for *Klebsiella* (Podschun & Ullmann, 1998) — underscoring the importance of clean water and sanitized surfaces in preparation.

Samples 4–8, identified as Bacillus spp. (Sample 4 specifically as B. cereus), are of particular concern because B. cereus forms heat-resistant spores capable of surviving cooking and germinating during improper storage (Adams & Moss, 2008). It produces two toxin types: the heat-stable emetic toxin cereulide, causing rapid-onset vomiting within 1–5 hours, and heat-labile diarrheal enterotoxins producing symptoms 8–16 hours post-ingestion (Stenfors Arnesen et al., 2008). Both are strongly associated with rice left in the 5°C–60°C temperature danger zone. The TSI-positive results across all Gram-positive isolates reflect broad carbohydrate fermentation capacity typical of Bacillus spp., while variable nitrate reduction (negative for Samples 4–5, positive for Samples

6–8) reflects known metabolic diversity within the genus, with nitrate-reducing strains better adapted to oxygen-limited conditions such as densely packed, improperly cooled rice (Jay et al., 2005).

Together, these findings indicate that Ice Biriyani is vulnerable to both fecal-indicator contamination and spore-forming pathogen survival, with the greatest risk arising from unsafe water, cross-contamination during preparation, and temperature abuse during cooling and storage. Mitigation requires consistent hygiene practices among handlers (handwashing, gloves, surface sanitation), use of safe or treated water, rapid cooling and refrigeration of cooked rice below 5°C (or holding above 60°C), thorough reheating to at least 75°C before consumption, and routine microbial monitoring. Traditional culture-based testing remains useful for baseline surveillance, but molecular approaches such as PCR and NGS would allow more rapid, comprehensive detection of both known and emerging pathogens, including non-culturable organisms missed by conventional methods (Bintsis, 2017).

Summary and Conclusion

This study assessed the microbial profile of Ice Biriyani, a rice-based RTE dish, to evaluate its food safety risks. Analysis of eight isolates revealed Gram-negative *E. coli*, *Enterobacter s*pp., and *Klebsiella* spp. (Samples 1–3), linked to fecal contamination from water or handling, and Gram-positive *Bacillus cereus* and *Bacillus* spp. (Samples 4–8), capable of surviving cooking and proliferating within the 5°C–60°C danger zone. Together these findings point to inadequate refrigeration, cross-contamination, and poor hygiene as the primary risk factors. The detection of enteric and spore-forming pathogens in Ice Biriyani highlights the need for improved food safety practices among vendors and consumers, including proper handling, storage, and sanitation. Strengthened hygiene regulations and enforcement in street-food settings, combined with future research using molecular techniques such as PCR and NGS for deeper microbial profiling, are needed to minimize microbial risk and protect public health.

References

1. Adams, M. R., & Moss, M. O. (2008). *Food microbiology* (3rd ed.). Royal Society of Chemistry.
2. Aycicek, H., Oguz, U., & Karci, K. (2004). Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. *International Journal of Hygiene and Environmental Health*, *207*(6), 527–533.
3. Barro, N., Bello, A. R., Savadogo, A., Ouattara, C. A. T., Ilboudo, A. J., & Traoré, A. S. (2006). Hygienic status assessment of dishwater, utensils, hands, and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). *African Journal of Biotechnology*, *5*(11), 1107–1112.
4. Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*, *16*(3), 497–516.
5. Bintsis, T. (2017). Foodborne pathogens. *AIMS Microbiology*, *3*(3), 529–563. https://doi.org/10.3934/microbiol.2017.3.529
6. Bryan, F. L., Teufel, P., Riaz, S., Roohi, S., Qadar, F., & Malik, Z. (1992). Hazards and critical control points of vending operations at a railway station and a bus station in Pakistan. *Journal of Food Protection*, *55*(6), 534–541.
7. Centers for Disease Control and Prevention. (2021). *Foodborne germs and illnesses*. https://www.cdc.gov/foodsafety/
8. Doyle, M. P., & Buchanan, R. L. (2013). *Food microbiology: Fundamentals and frontiers* (4th ed.). ASM Press.
9. Elamathy, S., & Kanchana, D. (2012). Antimicrobial activity of lactic acid bacteria isolated from goat meat against various pathogenic bacteria. *International Journal of Pharmaceutical & Biological Archives*, *3*(4), 930–932.

10. Food and Agriculture Organization. (2019). *Food safety and quality*. http://www.fao.org/food-safety/en/
11. Hammad, A. M., Watanabe, W., Fujii, T., & Shimamoto, T. (2020). Occurrence and characteristics of methicillin-resistant *Staphylococcus aureus* in ready-to-eat food in Japan. *Journal of Food Protection*, *83*(6), 962–969.
12. Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern food microbiology* (7th ed.). Springer Science & Business Media.
13. Kergourlay, G., Taminiau, B., Daube, G., & Champomier-Vergès, M. C. (2015). Metagenomic insights into the dynamics of microbial communities in food. *International Journal of Food Microbiology*, *213*, 31–39.
14. Mensah, P., Yeboah-Manu, D., Owusu-Darko, K., & Ablordey, A. (2002). Street foods in Accra, Ghana: How safe are they? *Bulletin of the World Health Organization*, *80*(7), 546–554.
15. Muinde, O. K., & Kuria, E. (2005). Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, *5*(1), 1–13.
16. Oliver, J. D. (2005). The viable but nonculturable state in bacteria. *Journal of Microbiology*, *43*(1), 93–100.
17. Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (3rd ed.). Springer Science & Business Media.
18. Podschun, R., & Ullmann, U. (1998). *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, and clinical significance. *Clinical Microbiology Reviews*, *11*(4), 589–603.
19. Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15.
20. Stenfors Arnesen, L. P., Fagerlund, A., & Granum, P. E. (2008). From soil to gut: *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiology Reviews*, *32*(4), 579–606.
21. Tambekar, D. H., Kulkarni, R. V., Shirsat, S. D., & Bhadange, D. G. (2008). Bacteriological quality of street vended food panipuri: A case study of Amravati city (MS) India. *Bioscience Biotechnology Research Communications*, *1*(1), 77–79.
22. U.S. Food and Drug Administration. (2020). *Bad bug book: Handbook of foodborne pathogenic microorganisms and natural toxins* (2nd ed.). https://www.fda.gov/food/foodborne-pathogens/bad-bug-book
23. Winny Fred Crossia, A., Priya Dharsini, P., Vijayalakshmi, K., & Mohamed Mahroop Raja, M. (2025). Exploration of *Carica papaya* L. extract infused silver nanoparticles against *Staphylococcus* sp. from acne skin lesions. *Biochemical and Cellular Archives*, *25*, 493–500.
24. Winny Fred Crossia James Rex Prabhu, A., Vijayalakshmi, S., Rajkumar, S., Bhuvaneswari, M., & Chitra Devi Senthilkumar, R. (2019). Screening of *Anogeissus latifolia* for phytochemical and antimicrobial properties against human pathogens. *Journal of Emerging Technologies and Innovative Research*, *6*(5), 120–127.
25. World Health Organization. (2020). *Food safety*. https://www.who.int/health-topics/food-safety