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Microbiological Analysis of Lemon Juice Samples Collected from Local Markets in Dhaka City, Bangladesh

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Lemon juices, widely consumed for their nutritional benefits and sensory appeal, may pose significant public health risks when prepared and sold under unhygienic conditions. This study investigated the microbiological quality of lemon juices sold across various locations in Dhaka, Bangladesh, with a focus on identifying potential contamination sources and assessing the prevalence of bacterial pathogens. While formal establishments such as restaurants and cafes generally maintain acceptable hygiene standards, juices sold by roadside vendors, in parks, and at busy marketplaces often lack proper quality control measures. Microbiological analysis revealed the presence of contaminants, including coliforms, faecal coliforms, and pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, largely attributable to factors such as the use of untreated water, inadequate refrigeration, and unhygienic handling practices. The antibiotic susceptibility profiles of five bacterial isolates revealed that Chloramphenicol was the most effective, demonstrating nearly 100% sensitivity with minimal resistance. In contrast, Piperacillin showed the lowest efficacy, with only 60% susceptibility and higher rates of resistance and intermediate responses. These findings highlight the urgent need for stricter enforcement of food safety regulations and public health interventions to reduce the risk of foodborne illnesses associated with the consumption of lemon juice in urban areas. The study underscores the importance of comprehensive monitoring and improved hygiene practices in safeguarding consumer health.

Keywords: Lemon juice, Contamination, Local market, Antibiotic resistance, Bangladesh

INTRODUCTION

Fruit juices are widely recognized for their nutritional value, appealing flavor, and health-promoting properties (1). Nevertheless, multiple studies worldwide have documented 48 million outbreaks of foodborne illnesses linked to the consumption of contaminated fruit juices (2). Contamination may arise from various factors, including the use of untreated water during preparation, the addition of ice from questionable sources, inappropriate storage without refrigeration, and preparation in unhygienic environments often exposed to houseflies, fruit flies, and airborne particulates. Under such conditions, fruit juices can serve as reservoirs for pathogenic bacteria, notably *Escherichia coli, Salmonella* spp*., Shigella* spp*., and Staphylococcus aureus* (3).

In particular, water employed in the preparation of lemon juice has been identified as a significant source of microbial contamination, harboring organisms such as coliforms, faecal coliforms, and faecal streptococci (4). Additionally, variations in pH levels can further promote the proliferation of these pathogens as they are diluted with varying amounts of water (5). While stringent regulatory frameworks in developed countries help ensure the microbiological safety of fruit juices, the situation remains concerning in many developing nations, including Bangladesh, where enforcement of food safety regulations is often insufficient. Consequently, beverages like lemon juice may act as vectors for the transmission of human diseases (6).

Further, A study that examined samples of vended fresh fruit juice, including lemon juice, that were gathered from different parts of Dhaka city discovered alarming patterns of antibiotic resistance among pathogenic isolates as well as substantial microbial contamination. Total viable bacterial loads were between 10^4 and 10^7 CFU/mL, and *Escherichia coli, Klebsiella* spp*., Salmonella* spp*.,* and *Vibrio* spp. were detected in half of the samples, which had coliform levels above allowable limits. Interestingly, these isolates showed a variety of resistance patterns to several antibiotics, such as ampicillin, amoxicillin, azithromycin, erythromycin, imipenem, and vancomycin. This highlighted the significant spread of drug-resistant pathogens in Dhaka's widely consumed lemon juice (as well as other juices) (7).

In Dhaka city, the demand for lemon juice is particularly high during the summer season. Although restaurants and cafes typically adhere to acceptable hygiene practices, products sold by roadside vendors, in recreational parks, and at bustling marketplaces frequently present considerable microbiological risks. In light of these public health concerns, the present study was conducted to systematically evaluate the microbial safety of lemon juices available in Dhaka, and determining the antibiotic resistance pattern of the responsible organism is also an alarming issue here, thereby contributing to better-informed strategies for safeguarding public health.

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MATERIALS AND METHODS

Collection of samples: A total of 10 samples of lemon juice were collected according to the guidelines set by APHA (8) from 5 locations around Dhaka City from March 2025 to April 2025. These locations were chosen based on consumer demand. Samples were tested within an hour after procurement.

Isolation and estimation of microorganisms from juice samples: Serial dilutions of the collected lemon juice samples were prepared up to 10^{-7} using sterile normal saline. From each dilution, an aliquot of 0.1 ml was aseptically spread onto nutrient agar plates, which were then incubated at 37°C for 24 hours. Following incubation, plates were examined for discrete colonies, and bacterial counts were expressed as colony-forming units per milliliter (cfu/ml). To detect and quantify specific groups of microorganisms, selective media were employed: total coliform counts (TCC) were determined on MacConkey agar, while total staphylococcal counts (TSC) were assessed using Mannitol Salt Agar (MSA).
Additionally, samples were cultured on a range of other media, including Salmonella Shigella (SS) agar and Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar, to isolate and identify different bacterial species. The identification of isolates was carried out based on the criteria described in Bergey's Manual of Determinative Bacteriology (9) and the Manual for the Identification of Medical Bacteria (10). Bacterial load estimation followed standard microbiological procedures.
To assess the microbiological safety and hygienic quality of the lemon juice samples, the results were evaluated against the recommended microbiological standards for fruit juices specified in the Gulf Standard.

Enrichment for enumeration of *Salmonella* spp., *Shigella* spp., and *Vibrio* spp.: To enumerate relatively stressed cells and viable but non-culturable (VBNC) microorganisms, 1 ml of each sample was inoculated into 9 ml of enrichment broths: selenite cysteine broth (SCB) for *Salmonella* and *Shigella* spp., and alkaline peptone water (APW) for *Vibrio* spp. The enrichment cultures were incubated at 37°C for 6 hours. Following enrichment, serial dilutions were prepared up to 10^{-5}, and 0.1 ml aliquots from the 10^{-3} and 10^{-5} dilutions were aseptically spread onto *Salmonella Shigella* (SS) agar and Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar to isolate *Salmonella, Shigella,* and *Vibrio* species, respectively. The inoculated plates were then incubated at 37°C for 48 hours to allow the development of characteristic colonies. Finally, presumptive isolates were subjected to biochemical tests and identified according to standard microbiological protocols (11).

Microscopic analysis: Microscopic analysis of the isolates was done through bacterial size, shape, and staining properties. Initial identification of selected isolates was performed by the Gram staining procedure followed by biochemical tests. The cultural and morphological characteristics of selected isolates were identified according to standard microbiological protocols (12).

Biochemical test for confirmatory identification: All isolated bacteria were identified by standard laboratory biochemical tests according to the methods described elsewhere. The biochemical tests for bacterial pathogens were the TSI test, MR-VP test, and citrate utilization test as an indicator test (13).

Determination of antimicrobial susceptibility: The antibiotic susceptibility of the bacterial isolates was evaluated using the disc diffusion method on Mueller-Hinton Agar (Difco, Detroit, MI), following established protocols. Briefly, a single medium-sized colony from each isolate was inoculated into 2 ml of Mueller-Hinton broth and incubated for 4 hours to achieve logarithmic growth. The culture turbidity was then standardized to match the 0.5 McFarland standard. Using sterile cotton swabs, the standardized suspensions were evenly spread across the entire surface of Mueller-Hinton agar plates (14).
Commercial antibiotic discs (Neo Sensitabs, Rosco, Denmark) containing Levofloxacin (10 µg), Pefloxacin (10 µg), Ceftriaxone (30 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), and Piperacillin (10 µg) were aseptically placed on the inoculated agar. Following incubation, the diameters of the inhibition zones were measured, and the results were interpreted to classify the isolates as susceptible, intermediate, or resistant in accordance with standard guidelines, M100, 32nd edition, 2022.

RESULTS AND DISCUSSION

Fruit juices are mostly eaten raw. So, they may lead to the outbreaks of human diseases causing the overall public health at a serious threat. Many health-related problems are associated with the propagation of etiological agents as well as their drug-resistance abilities. Based on these facts, the current study aims to find out the total bacterial count in lemon juice samples from various places, biochemical and microscopic observation of microorganisms of selected samples, and finally, the antimicrobial susceptibility of some commonly consumed lemon juice varieties of Bangladesh.

Prevalence of pathogenic microorganisms: Samples of lemon juice taken from different street sellers were subjected to microbiological investigation, which showed varied levels of bacterial contamination in various culture media (Table 1). Most samples had incredibly high bacterial loads on nutrient agar (NA), which is used to assess total viable bacterial counts. The microbiological analysis of the lemon juice samples revealed total viable bacterial (TVB) counts ranging from 1.8×10^5 to 7.8×10^5 cfu/ml, indicating heavy microbial contamination far above levels considered acceptable for beverages.
Staphylococcus spp. enumerated on Mannitol Salt Agar (MSA), were detected in almost all samples, with the highest load recorded in Shantinagar-2 (2.4×10^5 cfu/ml), suggesting significant post-preparation contamination likely due to poor handling and inadequate temperature control. The fact that Staphylococcus aureus can create heat-stable enterotoxins that result in food poisoning makes this finding troubling.
Escherichia coli, isolated on Mac Con key agar, was present in Mouchak-1 (6.4×10^4 cfu/ml), Mouchak-2 (1×10^3 cfu/ml), Kakrail-1 (2.6×10^4 cfu/ml), and Kakrail-2 (2.8×104cfu/mL), which is a strong indicator of fecal contamination and highlights the use of unsafe water or unhygienic preparation conditions.
Klebsiella spp. was detected in the same MacConkey agar —Mouchak-1 (1.4×10^4 cfu/ml) and Mouchak-2 (1×10^3 cfu/ml)—further suggesting environmental or waterborne contamination.
No growth was observed on Salmonella-Shigella Agar (SS) and Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) across all samples, suggesting the absence or undetectable levels of *Salmonella, Shigella,* and *Vibrio* species, which are important food- and waterborne pathogens.
The microbiological analysis of street-vended lemon juice samples revealed alarmingly high levels of bacterial contamination, which is consistent with findings from similar studies conducted in Bangladesh and other countries. This aligns with research conducted in Dhaka, where street juice samples showed total plate counts ranging from 8.58×10^2 to 4.38×10^4 cfu/ml, and coliform counts of 6–34 cfu/ml, indicating similarly poor microbial quality and sanitation practices (15).

Table 1: Isolation and enumeration of bacteria in the local market's lemon juice.

Sample	Total Viable Bacteria (TVB) (cfu/ml)	*Staphylococcus* spp. (cfu/ml)	*Escherichia coli* (cfu/ml)	*Klebsiella* spp. (cfu/ml)	*Salmonella* spp. (cfu/ml))	*Shigella* spp. (cfu/ml)	*Vibrio* spp. (cfu/ml)
Shantinagar 1	$1.8x10^5$	$4.8x10^4$	0	0	0	0	0
Shantinagar 2	$2.8 x10^5$	$2.4 x10^5$	0	0	0	0	0
Mouchak 1	$3.6 x10^5$	$2.2 x10^4$	$6.4 x10^4$	$1.4 x10^4$	0	0	0
Mouchak 2	$3.8 x10^5$	$3 x10^3$	$1 x10^3$	$1 x10^3$	0	0	0
Farmgate 1	$3.4 x10^5$	$8 x10^3$	0	0	0	0	0
Farmgate 2	$6.6 x10^5$	$1.6 x10^4$	0	0	0	0	0
Mogbazar 1	$7.8 x10^5$	$1 x10^4$	0	0	0	0	0
Mogbazar 2	$3.9 x10^5$	$3.1 x10^4$	0	0	0	0	0
Kakrail 1	$1.8 x10^5$	$1.6 x10^4$	$2.6 x10^4$	$1.3 x10^4$	0	0	0
Kakrail 2	$2 x10^5$	$2x10^4$	$2.8 x10^4$	$1.7 x10^4$	0	0	0

Table 2: Biochemical tests of representative bacterial pathogens.

Isolate ID	Gram staining	IMVIC Test				Triple Iron Sugar				Catalase test	Presumptive Organisms
		Indole	Methy l red	VP Test	Citrate Test	Slunt	Butt	Gas	H_2S		
MAC-S1	Gram (-)	+	+	-	-	Acid	Acid	+	-	+	*Escherichia coli*
MAC-S2	Gram (-)	-	-	-	+	-	-	-	-	+	*Pseudomonas aeruginosa*
MSA-S1	Gram (+)	-	+	-	-	Acid	Acid	+	-	+	*Staphylococcus* spp.
MSA-S2	Gram (+)	-	-	-	-	-	-	-	-	+	*Staphylococcus* spp.

Morphological & biochemical characterization: The phenotypic characteristics of some representative organisms and their tentative identification based on biochemical tests (Gram staining, methyl red, Voges-Proskauer test, citrate utilization test, and triple sugar iron test) are presented in Table 2. Based on these characteristics, 4 genera of bacteria were identified. The probable genus of the bacterial isolates is summarized in Table 2.

The presence of *Escherichia coli* and *Staphylococcus aureus* in the current study reflects common contamination routes, namely fecal matter and human handling. This is supported by findings from Gazipur, Bangladesh, where *S. aureus* was found in 58% and *E. coli* in 48% of fruit juice samples, both of which are strong indicators of unsafe handling and the use of contaminated water (16). Although no growth of *Salmonella* spp, *Shigella* spp, or *Vibrio* species was observed in this study, previous studies in Dhaka have reported their presence in some street-vended juices, albeit at low frequencies (17).

Antibiotic susceptibility profiles: Five representative isolates were analyzed for their antibiotic resistance properties. This bar chart represents the antibiotic susceptibility profile of different bacterial isolates (MSA-S1, MSA-S2, MSA-S3, MAC-S1, MAC-S2) against five antibiotics—Levofloxacin, Pefloxacin, Chloramphenicol, Ceftriaxone, and Piperacillin—measured as inhibition zone diameters (mm) from a disk diffusion test presented in Figure 1. Chloramphenicol showed the highest level of bacterial sensitivity, with nearly 100% susceptibility and negligible resistance or intermediate responses, indicating it is the most effective antibiotic in this study.

Levofloxacin, Pefloxacin, and Ceftriaxone each exhibited approximately 80% sensitivity, with around 20% of isolates showing resistance, suggesting they are generally effective but with some resistance emerging.

In contrast, Piperacillin demonstrated the lowest sensitivity, with only 60% of isolates being susceptible, while 20% were resistant and another 20% showed intermediate susceptibility. This indicates that Piperacillin may be less reliable for treatment unless guided by susceptibility testing.

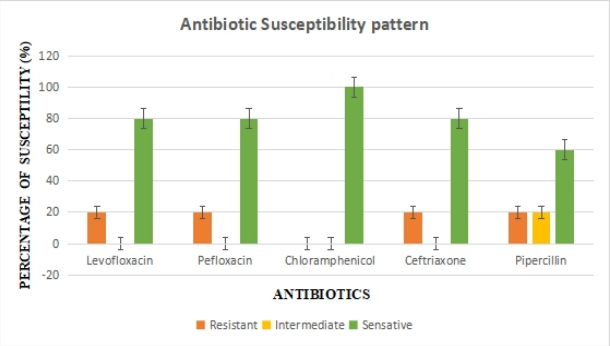

Figure 1: Antibiotic Susceptibility pattern for bacterial isolates collected from different samples

The antibiotic susceptibility profiles in this study revealed that chloramphenicol was the most effective antibiotic, with nearly 100% susceptibility, while Piperacillin showed the lowest effectiveness, with only 60% of isolates being sensitive. Similar resistance patterns have been reported in other studies, such as in Delhi, where *E. coli* and *S. aureus* were highly resistant to beta-lactam antibiotics like ampicillin and cefotaxime, but remained largely sensitive to chloramphenicol and ciprofloxacin (18). Additionally, studies focusing on hand borne bacteria from Dhaka vendors also confirmed a high resistance to amoxicillin (~64%) but greater sensitivity to ciprofloxacin and azithromycin (19, 20).

CONCLUSIONS

The present study clearly demonstrates that street-vended lemon juice in Dhaka, Bangladesh, harbors an alarmingly high microbial load, with total viable bacterial counts (TVB) far exceeding acceptable safety limits for beverages. A major public health danger is presented by the frequent discovery of *Staphylococcus* species and *Escherichia coli*, which highlights inadequate hygiene practices, unsafe water use, and post-preparation contamination. While *Staphylococcus aureus* infection indicates direct human interaction and the possibility of toxin-mediated foodborne disease, *E. coli* is a powerful sign of fecal contamination. Even though no *Shigella, Vibrio*, or *Salmonella* species were found, the danger still exists because these diseases have been found in comparable items in other research.

Antibiotic susceptibility testing revealed that chloramphenicol remains the most effective drug against the tested isolates, exhibiting near-complete sensitivity. In contrast, piperacillin demonstrated the lowest activity, with only 60% susceptibility, indicating significant resistance. Levofloxacin, pefloxacin, and ceftriaxone retained moderate-to-high effectiveness but showed signs of emerging resistance in some isolates. These findings are consistent with regional and international reports that highlight growing antimicrobial resistance among foodborne bacteria, particularly to beta-lactam antibiotics. It also emphasizes the urgent need for improved food safety practices, vendor hygiene training, and regular monitoring to mitigate public health risks associated with street-vended beverages.

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REFERENCES

1. Bhardwaj RL, Nandal U, Pal A, Jain S. 2014. Bioactive compounds and medicinal properties of fruit juices. Fruits, 69(5):391–412.
2. Callejón RM, Rodríguez-Naranjo MI, Ubeda C, Hornedo-Ortega R, Garcia-Parrilla MC, Troncoso AM. 2015. Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. Foodborne Pathogens and Disease, 12(1):32–38.
3. Tambekar DH, Murhekar SM, Dhanorkar DV, Gulhane PB, Dudhane MN. 2009. Quality and safety of street vended fruit juices: a case study of Amravati city, India. Journal of Applied Biosciences, 14(1997-5902):782-7.
4. Tasnim F, Hossain MA, Nusrath S, Hossain MK, Lopa D, Haque KMF. 2010. Quality assessment of industrially processed fruit juices available in Dhaka city, Bangladesh. Malaysian Journal of Nutrition, 16(3):431–438.
5. Food and Drug Administration (FDA). 2001. Chapter 12. In: Bacteriological Analytical Manual Online. p.1–6. USA.
6. Tasnim F, Hossain MA, Nusrath S, Hossain MK, Lopa D, Haque KMF. 2010. Quality assessment of industrially processed fruit juices available in Dhaka city, Bangladesh. Malaysian Journal of Nutrition, 16(3):431–438.
7. Jabin T, Hossain MM, Nasrin S, Tabassum R, Rahman MA, Uddin MA. 2022. Microbiological assessment and detection of drug resistant bacterial isolates in some vended fresh fruit juice samples in Dhaka city, Bangladesh. Food Research, 6:413–419.
8. Al Mamun S, Das KK, Uddin MA. 2016. Microbiological analysis and determination of antibacterial activity of apple samples collected from local markets in Dhaka city, Bangladesh. Stamford Journal of Microbiology, 6(1):11–15.
9. Buchanan RE, Gibbon NE. 1984. Bergey's Manual of Determinative Bacteriology. Baltimore: Williams and Wilkins Co.
10. Cowan ST. 1975. Manual for the Identification of Medical Bacteria. 2nd ed. London: Cambridge University Press.
11. Highmore CJ, Warner JC, Rothwell SD, Wilks SA, Keevil CW. 2018. Viable-but-nonculturable Listeria monocytogenes and Salmonella enterica serovar Thompson induced by chlorine stress remain infectious. mBio, 9(2):10-128.
12. Eipa BR, Islam MR, Sultana R, Alam ST, Mehjabin T, Bushra NN, et al. 2023. Determination of the antibiotic susceptibility pattern of Gram-positive bacteria causing UTI in Dhaka, Bangladesh. arXiv preprint, arXiv:2306.10553.
13. Hafezi A, Khamar Z. 2024. The method and analysis of some biochemical tests commonly used for microbial identification: a review. Comprehensive Health and Biomedical Studies, 3(2):e160199.
14. Chitra SR. 2017. Theoretical investigation on antimicrobial susceptibility testing methods. Bioinformatics, 5(2):12–26.
15. Khan A, Khan S, Khan MA, Qamar Z, Waqas M. 2015. The uptake and bioaccumulation of heavy metals by food plants, their effects on plant nutrients, and associated health risk: a review. Environmental Science and Pollution Research, 22(18):13772–13799.
16. El-Shenawy PM. 2013. Evaluation of the microbiological quality of street-vended juices sold in Cairo. Journal of Food Industry and Nutrition Sciences, 3:69–80.
17. Uddin ME, Akter T, Parvez MAK, Nahar S, Pervin S, Debnath B, et al. 2017. Microbial safety of street vended fruit juices in Dhaka city of Bangladesh. Journal of Advances in Microbiology, 3:1–7.
18. Sharma N, Singh K, Toor D, Pai SS, Chakraborty R, Khan KM. 2020. Antibiotic resistance in microbes from street fruit drinks and hygiene behavior of the vendors in Delhi, India. International Journal of Environmental Research and Public Health, 17:4829.
19. Hassan MM, Chakrabarty RP, Siddique MA, Rahaman MM. 2017. Prevalence of antibiotic resistant enteric bacteria in the hands of street food vendors in Dhaka city. Bangladesh Journal of Microbiology, 34:33–38.
20. Monir SB, Hasan MZ, Uddin MA. 2024. Microbiological and antibiogram profiling of frozen foods and ice cream products from super-shops of Dhaka, Bangladesh. Stamford Journal of Microbiology, 14(1):22–27.