ORIGINAL ARTICLE

FOOD ENVIRONMENT AT MARANHÃO MULTIMODAL TERMINAL: MICROBIOLOGICAL QUALITY OF MULTIPURPOSE WATER

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Highlights: (1) All samples analyzed showed *E. coli* levels < 1.0 MPN/100 mL. (2) A high proportion of samples contained bacteria belonging to total coliform group. (3) Seven enterobacterial species, including some clinical relevance, were identified.

PRE-PROOF

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FOOD ENVIRONMENT AT MARANHÃO MULTIMODAL TERMINAL: MICROBIOLOGICAL QUALITY OF MULTIPURPOSE WATER

ABSTRACT

Objective: This study aimed to evaluate microbiological quality of multipurpose water at Maranhão Multimodal Terminal and to determine occurrence of enterobacteria as support for hygienic-sanitary control in production processes. Method: Individual water samples were collected from 23 food stalls and three drinking fountain taps available for users, totaling 27 samples. An enzymatic chromogenic method was applied to quantify most probable number (MPN) of total coliforms and *Escherichia coli*. Enterobacteria were identified through bacterial isolation followed by morphotintorial and biochemical characterization of isolates. Results: All samples analyzed showed absence of E. coli, whereas 59.25% was positive for total coliforms, with counts ranging from 1.0 to >2,419.6 MPN/100 mL. Thirty bacterial strains were isolated from coliform-positive samples. These isolates comprised genus *Enterobacter* sp. and species *Shimwellia blattae*, *Citrobacter koseri*, *Klebsiella pneumoniae* subsp. *pneumoniae*, *Serratia fonticola*, *Enterobacter cloacae*, *Proteus myxofaciens*, and *Proteus mirabilis*. Conclusion: Although multipurpose water samples did not contain *E. coli*, they may function as reservoirs of ubiquitous enterobacteria with clinical relevance (*E. cloacae*, *K. pneumoniae*, and *P. mirabilis*), indicating potential risk to users and need for adequate water treatment. The findings presented here may support effective surveillance and control actions aimed at ensuring microbiological safety of water intended for human consumption at Maranhão Multimodal Terminal.

Keywords: Water microbiology; public health; waterway terminal.

INTRODUCTION

Eating outside the home has become increasingly common in Brazil. Results from the 2017 Household Budget Survey (POF) indicate that approximately one-third (32.8%) of total household food expenditure is allocated to meals consumed away from home[1].

Influence of food environment on dietary practices extends beyond availability of food outlets near residences and workplaces and encompasses routine commuting routes, a factor that has only recently received greater research attention[2-5].

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A study conducted in city of São Paulo demonstrated that high-circulation areas, such as bus terminals and train and subway stations, present higher density of restaurants, bars, and snack outlets[6]. Nevertheless, investigations addressing relationship between eating out and food environment in such transport facilities[7-8], including inland waterway terminals, remain limited.

Evidence from high-income countries indicates that a substantial proportion of meals consumed outside home occurs during daily travel between residence and workplace or educational institutions[4]. Furthermore, foods and beverages marketed in public transport settings often show low nutritional quality [7]. Similar patterns have been observed in low- and middle-income countries; however, research in these contexts remains scarce and has focused primarily on food availability[9-10], with limited assessment of sensory, physicochemical, and microbiological quality.

Although scientific evidence on influence of food environments on individual behaviour is well established[11-12], important gaps persist in characterization of food environments within public transport infrastructure in Latin American countries, including Brazil[12]. In this context, water plays a critical role in food production, being used throughout all processing stages[13], as well as for direct consumption and for hygiene practices among food handlers. According to Ministry of Health guidelines cited by Microambiental[14], water used in food handling and preparation must meet potability standards.

Potable water is defined as water that complies with physicochemical and microbiological criteria established in Annex I of Ordinance GM/MS No. 888, dated 4 May 2021[15]. Consequently, commercial food establishments and food service providers are required to perform periodic monitoring of water quality[14]. Therefore, this study aimed to evaluate microbiological quality (total coliforms and *Escherichia coli*) of multipurpose water within food environment of Maranhão Multimodal Terminal and to determine the occurrence of enterobacteria as a basis for hygienic and sanitary control in food production processes.

Methods

Study Site:

This cross-sectional study was conducted during the first half of 2024 at Cujupe Terminal, located in the state of Maranhão, Brazil. This multimodal facility integrates the Alcântara inland waterway transport (ferry service) with road transport infrastructure[16] and serves as a strategic mobility hub for passengers and residents of the Baixada Maranhense region, contributing to regional socioeconomic development.

Baixada Maranhense comprises 21 municipalities and Alcântara is recognized as one of the regions in the state of Maranhão characterized by low Human Development Index (HDI) values[17].

Research Design and Water Sample Collection:

All food and beverage sales booths operating during technical visits to Maranhão Multimodal Terminal were included in water sampling procedures. A total of 23 booths (B1–B23) were evaluated out of 48 existing units. Therefore, sampling followed a non-probabilistic, purposive design.

Individual samples were also collected from drinking-fountain outlets (T1, T2, T3, and T4) available for terminal users. In total, 27 water samples were obtained using sterile 250 mL borosilicate glass bottles, in accordance with recommendations of the Water Analysis Manual of National Health Foundation (FUNASA)[18].

Prior to sampling, external surfaces of taps were disinfected with 70% ethanol solution. Subsequently, taps were fully opened, allowing water to run for approximately 2–3 minutes to flush distribution lines. Flow was then reduced to collect approximately 250 mL of water, avoiding external contamination or splashing during sampling. All procedures were conducted under aseptic conditions, with research team members wearing appropriate personal protective equipment (PPE), including laboratory coats and closed footwear.

After collection, samples were properly labelled, placed in insulated containers containing reusable ice packs, and transported to the Food and Water Microbiology Laboratory

(LMAA) at Universidade Estadual do Maranhão – UEMA (Maranhão State University) within a maximum period of four hours. Microbiological analyses were performed on the same day as collection to ensure analytical reliability.

Microbiological Analyses:

The most probable number (MPN) of total coliforms and *Escherichia coli* were quantified using an enzymatic chromogenic system (Colisure®, IDEXX, USA). This method employs nutrient indicators chlorophenol red-*β*-D-galactopyranoside (CPRG), metabolized by *β*-galactosidase produced by coliform bacteria, and 4-methylumbelliferyl-*β*-D-glucuronide (MUG), hydrolyzed by *β*-glucuronidase produced by *E. coli*, enabling simultaneous detection of both microbial groups.

From each collected sample, 100 mL of water was aseptically transferred into sterile vessels containing enzymatic chromogenic substrate. Samples were then dispensed into Quanti-Tray® plates, sealed using Quanti-Tray Sealer Plus® (IDEXX), and incubated in bacteriological incubator at 35 ± 0.5 °C for 24 h.

The presence of total coliforms was indicated by color change from yellow to magenta, while *E. coli* was identified based on emission of blue fluorescence under ultraviolet light (365 nm wavelength) (IDEXX Laboratories Inc.).

Isolation of Total Coliforms:

Isolation and confirmation of total coliform bacteria followed conventional microbiological procedures. A 10 µL aliquot from chromogenic substrate-positive samples was inoculated onto MacConkey agar (Merck®) and incubated at 37 °C for 24 h. From representative plates, colonies displaying distinct morphotypes and up to five colonies with similar morphology were subcultured onto Tryptic Soy Agar (Merck®) and incubated at 37 ± 0.5 °C for 24 h to obtain pure cultures for subsequent identification of enterobacterial species.

Enterobacteria Identification:

The identification of bacterial isolates was performed by Gram staining (morphotintorial test) and by application of a commercial biochemical identification panel comprising 23 analytical parameters[19-20].

Data Analysis:

Results were expressed as MPN/100 mL following interpretation using standard conversion tables. Values were compared with drinking-water quality criteria established in Ordinance GM/MS No. 888 (2021)[15]. Data were analyzed using descriptive statistical methods, with emphasis on distribution of absolute and relative frequencies.

RESULTS

All analyzed samples (100%; n = 27) showed *Escherichia coli* counts < 1.0 MPN/100 mL, complying with requirements of Ordinance GM/MS No. 888/2021, which establishes absence of this bacterium in 100 mL of water intended for human consumption (Table 1).

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Table 1 - Microbiological results for 27 multipurpose water samples collected at Maranhão Multimodal Terminal

Sampling location	Sampling site	Number of water samples collected	*Escherichia coli* (MPN /100 mL)*	Total coliforms (MPN/100 mL)*	Reference standard
B1	Water tap	1	< 1.0	< 1.0	
B2	Water tap	1	< 1.0	< 1.0	
B3	Water tap	1	< 1.0	< 1.0	
B4	Water tap	1	< 1.0	< 1.0	
B5	Water tap	1	< 1.0	**1.0**	
B6	Water tap	1	< 1.0	< 1.0	
B7	Water tap	1	< 1.0	< 1.0	
B8	Water tap	1	< 1.0	< 1.0	
B9	Water tap	1	< 1.0	< 1.0	
B10	Water tap	1	< 1.0	< 1.0	
B11	Water tap	1	< 1.0	< 1.0	*Escherichia coli* and total coliforms <1.0 MPN/100 mL (absence)
B12	Water tap	1	< 1.0	**2.0**	
B13	Water tap	1	< 1.0	**387.7**	
B14	Water tap	1	< 1.0	**490.7**	
B15	Water tap	1	< 1.0	**1299.7**	
B16	Water tap	1	< 1.0	**829.7**	
B17	Water tap	1	< 1.0	**191.0**	
B18	Water tap	1	< 1.0	**>2419.6**	
B19	Water tap	1	< 1.0	**689.3**	
B20	Water tap	1	< 1.0	**980.4**	
B21	Water tap	1	< 1.0	**1553.1**	
B22	Water tap	1	< 1.0	**2419.6**	
B23	Water tap	1	< 1.0	**1046.2**	
T1	Drinking fountain	1	< 1.0	< 1.0	

T2	Drinking fountain	1	< 1.0	**1046.2**
T3	Drinking fountain	1	< 1.0	**1.0**
T4	Drinking fountain	1	< 1.0	**2.0**
Total			**27**	

Reference standard: Annex 1 of Ordinance GM/MS No. 888, of May 4, 2021[15].
Water tap: water for multipurpose use.
*Where: MPN: Most Probable Number.
Source: The authors.

At the evaluated terminal, total coliforms were detected in 59.25% (n = 16) of the samples, with bacterial counts ranging from 1.0 to >2,419.6 MPN/100 mL. A total of 30 bacterial strains were isolated and identified at genus or species level. Accurate identification of enterobacterial species required an extensive set of biochemical assays (Table 2).

Table 2 – Biochemical profiles of 30 enterobacterial isolates obtained from multipurpose water samples collected at Maranhão Multimodal Terminal

Morphotintorial and biochemical tests	**Genera and species of Enterobacteriaceae**							
	Shimwellia blattae	*Citrobacter koseri*	*Klebsiella pneumoniae subsp. pneumoniae*	*Serratia fonticola*	*Enterobacter spp.*	*Enterobacter cloacae*	*Proteus myxofaciens*	*Proteus mirabilis*
Gram staining	-	-	-	-	-	-	-	-
Indole production	-	+	-	-	-	-	-	-
Voges-Proskauer test	-	-	-	-	+	+	-	+
Citrate use (Simmons)	+	+	+	+	+	+	+	+
Hydrogen sulfide (H_2S) production	-	-	-	-	-	-	-	+
Urease activity**	+	+	+	+	+	+	+	+

Tryptophan deaminase activity	-	+	+	+	-	-	-	-
Lysine decarboxylase activity	+	+	+	+	-	-	-	-
Arginine decarboxylase activity	+	+	+	+	-	-	+	+
Ornithine decarboxylase activity	-	+	+	+	+	+	+	+
Malonate utilization	+	+	+	+	+	+	+	+
Glucose oxidation	-	+	+	+	-	-	+	+
Lactose fermentation	-	-	+	+	-	+	+	-

Sucrose fermentation	-	-	+	+	-	-	+	-
Mannitol fermentation	-	+	+	+	-	-	-	-
Adonitol fermentation	-	+	+	-	-	-	-	-
Myo-inositol fermentation	-	+	+	+	-	-	-	-
Sorbitol fermentation	-	+	+	+	-	-	-	-
Raffinose fermentation	-	-	+	+	-	-	-	-
Rhamnose fermentation	-	+	+	+	-	-	+	-
Maltose fermentation	-	+	+	+	-	-	+	-

Melibiose fermentation	-	-	+	+	-	-	+	-
ß-D-galactosidase activity	-	+	+	+	+	+	+	+
Esculin hydrolysis	-	+	+	+	+	+	+	-

Source: The authors.

The study identified isolates belonging to genus *Enterobacter* spp. and seven enterobacterial species (*Shimwellia blattae*, *Citrobacter koseri*, *Klebsiella pneumoniae* subsp. *pneumoniae*, *Serratia fonticola*, *Enterobacter cloacae*, *Proteus myxofaciens*, and *Proteus mirabilis*), all members of family Enterobacteriaceae (Table 3).

Table 3 - Bacterial genera and species isolated from 16 multipurpose water samples collected at Maranhão Multimodal Terminal

Number of isolates	Bacterial genera and species
3	*Shimwellia blattae*
3	*Citrobacter koseri*
3	*Klebsiella pneumoniae subsp. pneumoniae*
3	*Serratia fonticola*
3	*Proteus myxofaciens*
3	*Proteus mirabilis*
6	*Enterobacter* sp.
6	*Enterobacter cloacae*

Source: The authors.

DISCUSSION

Results obtained for *Escherichia coli* in this study are relevant from a sanitary perspective, as this species is widely recognized as a primary indicator of fecal contamination in aquatic environments. *Escherichia coli* is commonly present in intestinal tract of warm-blooded animals[21,22] and is therefore considered preferred microbiological indicator of drinking-water safety, including in supply systems operated by rural communities[23]. Considering that Maranhão Multimodal Terminal is supplied by a collective alternative water supply solution (SAC), based on an artesian well, the absence of this indicator suggests adequate maintenance of water source and possibly of distribution infrastructure. Such conditions may contribute to prevention of waterborne and foodborne diseases (WFBDs).

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Ensuring microbiological safety of drinking water requires implementation of multiple protective barriers, including appropriate treatment processes and effective management of distribution systems. Disinfection represents a critical control measure against a wide range of pathogenic microorganisms, particularly bacteria, and commonly involves use of chlorine-based chemical disinfectants[24].

Assessing microbiological water quality is essential in sanitary inspection procedures, as water supplies may contain a high diversity and potential pathogenicity of microorganisms, especially those of fecal origin. Since direct detection of all microbial agents is operationally impracticable, monitoring strategies rely on quantification of indicator organisms, particularly *E. coli*, thermotolerant coliforms, and total coliforms[25].

Public health relevance of pathogenic *E. coli* strains has led to establishment of national and international surveillance programs aimed at monitoring and tracing outbreak events associated with this microorganism. Although epidemiological profiles vary among strains, several lineages have significant disease-causing potential[26] and represent important challenges within One Health framework. In present study, high total-coliform counts observed in 12 samples (191.0 to >2,419.6 MPN/100 mL) suggest possible deficiencies in distribution system integrity[27].

According to Brazilian drinking-water regulations[15], total coliforms serve as indicators of system integrity. Annex I of Ordinance GM/MS No. 888/2021[15] establishes bacteriological standards for water intended for human consumption, requiring absence of *E. coli* in 100 mL at distribution points and points of use. In this respect, all samples analyzed complied with national regulatory criteria. Nevertheless, regulation also stipulates that in supply systems serving fewer than 20,000 inhabitants, only one monthly sample collected by system operator or by collective alternative supply solution may present a positive result for total coliforms.

According to the World Health Organization (WHO)[24], standards for drinking-water quality control may vary across countries and regions; therefore, no single analytical or evaluation approach can be universally applied. Nevertheless, comprehensive understanding of vulnerabilities within water supply systems depends on implementation of appropriate monitoring and control procedures, including inspection of water at distribution points and points of consumption, which constitutes focus of this study.

Carvalho[28] conducted a study in five restaurants in the city of Salgueiro, Pernambuco, evaluating presence or absence of total and thermotolerant coliforms in water samples. In one establishment, water supply source (well) was also assessed. Results showed that only one restaurant had all samples free of total and thermotolerant coliforms, whereas three establishments presented contamination in at least one sample. The restaurant supplied by well water showed presence of these indicators in all analyzed samples. These findings are consistent with those observed in the present study for total coliforms.

Nascimento, Maia, and De Araújo[29] investigated bacterial contamination in water reservoirs in the semiarid region of Rio Grande do Norte and reported that 73.2% of isolates belonged to family Enterobacteriaceae. Among enterobacteria, *Escherichia coli*, *Enterobacter cloacae* complex, and *Klebsiella pneumoniae* were the most frequently identified. Similarly, Martins et al.[30] evaluated antimicrobial resistance of enterobacteria isolated from public water supplies in the central-western region of São Paulo State and reported high frequency of *Enterobacter* sp., *E. coli*, *Klebsiella* sp., *Edwardsiella* sp., *Proteus* sp., *Citrobacter* sp., *Providencia* sp., *Salmonella* sp., and *Serratia* sp. Results of the present study are consistent with these reports, with identification of similar enterobacterial taxa.

According to Cabral[31], family Enterobacteriaceae can be divided into three ecological groups: (i) Group I includes *E. coli*, considered a reliable indicator of recent fecal contamination due to its limited environmental persistence; (ii) Group II comprises ubiquitous organisms found both in intestinal microbiota of humans and animals and in the environment, including species of *Klebsiella*, *Enterobacter*, and *Citrobacter*, whose presence does not necessarily indicate fecal contamination; and (iii) Group III consists of environmental species associated with aquatic systems, plants, and small animals, including *Raoultella planticola*, *R. terrigena*, *Enterobacter amnigenus*, *Kluyvera intermedia* (*Enterobacter intermedius*), *Serratia fonticola*, and genera *Budvicia*, *Buttiauxella*, *Leclercia*, *Rahnella*, *Yersinia*, and most species of *Erwinia* and *Pantoea*.

Among bacterial isolates obtained in this study, those belonging to the genus *Enterobacter* (n = 9/30; 30% *Enterobacter* spp. and *E. cloacae*) were the most frequent. *Enterobacter cloacae* is a Gram-negative bacillus of the Enterobacteriaceae family, and it can be found in soil, water, and plants and is also part of the intestinal microbiota of humans and

animals[32]. This species is considered an opportunistic pathogen and has been increasingly associated with healthcare-associated infections (HAIs), particularly infections of urinary tract, lower respiratory tract, skin and soft tissues, wounds, and central nervous system[33].

Another clinically relevant species identified was *Klebsiella pneumoniae* (Table 3). Gram-negative bacteria are responsible for substantial proportion of antimicrobial-resistant nosocomial infections, and *K. pneumoniae* is notable for its ability to develop enzymatic resistance mechanisms. This species is associated with a wide range of infectious diseases[34], with severity often influenced by host immune status and by strain-specific virulence factors, including multidrug-resistant phenotypes driven by antimicrobial overuse [35].

The genus *Proteus* comprises five species (*P. vulgaris*, *P. mirabilis*, *P. penneri*, *P. myxofaciens*, and *P. hauseri*), two of which were identified in the present study (Table 3). These organisms are commonly found in the intestinal microbiota of humans and animals, as well as in soil and contaminated water. *Proteus mirabilis* is recognized as an opportunistic pathogen[36].

Importantly, the Cujupe Terminal receives vessels from the Ponta da Espera ferry terminal in São Luís, operating route São Luís–Alcântara–São Luís, with an average travel time of approximately two hours. Approximately 1.8 million passengers pass through this terminal annually[37]. Therefore, findings of this study are highly relevant to public health, particularly regarding presence of bacterial genera and species of clinical importance and potential exposure risks for users of this transport hub.

The socioeconomic relevance of the terminal must be highlighted, as dozens of vendors operate standardized stalls at the Cujupe Terminal. This organized structure provides consumers with a revitalized environment offering a wide variety of foods (raw and cooked; of animal and plant origin; liquids, solids, and semi-solid preparations; including snacks and full meals) and beverages. In addition, these activities generate employment and income for local families and support regional labor markets. Consequently, commercialization of food and beverages at this site contributes to continuous improvement in the quality of life of populations working in and residing near the terminal, while also stimulating local production systems.

Given the findings of this study, it is essential to emphasize that, due to its role throughout food production chain, water used in preparation and processing of food must consistently meet potability standards. Although Ordinance GM/MS No. 888/2021[15] does not

directly regulate commercial food establishments and food service operations, it remains primary national reference for drinking-water quality standards.

Furthermore, this study is, to our knowledge, the first to address the microbiological quality of multipurpose water used within food environment of the Maranhão Multimodal Terminal using laboratory-based analyses. These findings underscore importance of ensuring both availability and quality of water as key protective factors against food insecurity. In addition, access to safe drinking water is recognized as a fundamental human right and constitutes Sustainable Development Goal 6 (SDG 6) of the 2030 Agenda, established by the United Nations in 2015, to which Brazil is a signatory, aiming to ensure universal and sustainable management of water and sanitation[38].

Therefore, this study contributes to addressing existing knowledge gaps regarding the microbiological quality of water in this setting and provides evidence to support public health surveillance.

CONCLUSION

Multipurpose water distributed and used at the Maranhão Multimodal Terminal, although free of *Escherichia coli*, may function as a vehicle for transmission of other enterobacteria, including ubiquitous species with clinical relevance (*Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Proteus mirabilis*). These findings indicate potential risk to users and highlight need for effective water treatment and monitoring at distribution and consumption points to prevent exposure to pathogenic microorganisms.

From a regulatory perspective, water quality surveillance systems in Brazil are well established; however, operational challenges persist, particularly regarding communication and dissemination of information to communities. In this context, results presented in this study may support implementation of targeted surveillance and control measures aimed at ensuring safety of water intended for human consumption at the Maranhão Multimodal Terminal.

Studies of this nature are essential for public health, as they contribute to understanding the occurrence of diseases associated with collective-use environments and enable identification of potential contamination sources and microbial hazards.

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