Detection of Indicator Organisms from Potable Jar Water Samples in Dhaka City, Bangladesh

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DOI: https://doi.org/10.51584/IJRIAS.2025.10030026

Received: 26 February 2025; Accepted: 04 March 2025; Published: 05 April 2025

ABSTRACT

Aim: This present study was conducted to determine the presence of indicator microorganisms from potable jar water samples.

Methods: A total of 30 jar water samples were collected from different areas in Dhaka city, Bangladesh. Samples were analyzed using conventional standard microbiological method such as membrane filtration technique and biochemical tests and VITEK 2 method for presumptive identification of the bacterial isolates. The outcomes from both methods were compared.

Results: The high number of coliform and fecal coliform were found in Manda and Siddheswari area which were 225 CFU/100 ml and 166 CFU/100 ml respectively. The antibiotic susceptibility patterns of these isolates were further confirmed by Kirby Bauer disk diffusion method and automated system such as VITEK-2 compact. Antibiotic susceptibility testing showed that indicator organisms such as Escherichia coli and Klebsiella pneumoniae were 100% resistant to Cefuroxime, Vancomycin and Cefepime.

Conclusions: This study indicated that some water samples were polluted by both coliform and fecal coliform which meant those potable jar water samples were of poor quality that may cause water borne diseases in consumers. The constant surveillance for drug resistance and microbiological analysis is crucial for efficient water borne disease prevention and treatment.

Keywords: Indicator Organisms, Potable jar water, Conventional Microbiological Methods, VITEK-2 compact

INTRODUCTION

Water is a vital element in our lives, and access to safe drinking water is essential for maintaining good health. Although water covers approximately two-thirds of the Earth's surface, the majority of it is undrinkable. Freshwater constitutes only 2.7% of the total water on Earth, and just 1% of that freshwater—found in lakes, rivers, and groundwater—is usable for human consumption [1].

The World Health Organization (WHO) defines improved water sources as those free from fecal contamination and safe for human use [2]. According to WHO reports, approximately 9.1% of global diseases are linked to the consumption of unsafe drinking water [3]. Potable water refers to water that is clean, safe, and free from microbial contamination. Ensuring access to safe drinking water is crucial for public health. The quality of potable water is a significant public health concern, as contaminated water can cause severe health issues, particularly in children, infants, pregnant women, and individuals with weakened immune systems [4].

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue III March 2025

In urban areas, bottled or jarred water has become a common source of drinking water. However, microbial contamination remains a major issue. Contamination can occur due to sewage leaks infiltrating water supply pipelines, which then mix with the water distribution system, leading to unsafe drinking water [5].

Pathogenic contamination of drinking water includes a variety of harmful microorganisms such as Rotavirus, Entamoeba histolytica, Campylobacter, Escherichia coli (E. coli), and other pathogens, posing significant health risks to humans [6],[7],[8].Water contains a lot of pathogenic microorganisms such as Vibrio cholerae, Salmonella typhi, Salmonella paratyphi, Salmonella typhimurium, Escherichia coli [9],[10],[11],[12]. These microorganisms are becoming resistant to different antimicrobials day by day [12],[13]. Microbiological water quality indicators are specific species or groups of microorganisms that serve as proxies for fecal contamination in water. These indicators are easier to detect and quantify than the full spectrum of pathogenic microorganisms that pose risks to human health. Monitoring these indicators is crucial for assessing water safety and preventing waterborne diseases.

Antimicrobial resistance (AMR) has been recognized by the World Health Organization (WHO) as one of the top ten global health threats, necessitating immediate and coordinated action across human health, animal health, and environmental sectors—a multidisciplinary strategy known as the *One Health* approach. The WHO warns that the world may be on the brink of a post-antibiotic era, where the efficacy of antibiotics is significantly diminished, endangering human lives. In such a scenario, routine medical procedures that rely on prophylactic antibiotic administration may become impractical, posing severe challenges to modern healthcare. Addressing AMR requires comprehensive surveillance, responsible antibiotic stewardship, and global policy interventions to mitigate its impact on public health [12].

Environmental isolations, including those from water sources, play a critical role in the emergence and dissemination of antibiotic resistance. Resistance mechanisms such as enzymatic degradation of antibiotics (e.g., β-lactamases), efflux pumps, target modification, and horizontal gene transfer contribute to the persistence and spread of resistant bacteria in aquatic environments [13]. The detection and surveillance of antibiotic-resistant microorganisms in water systems are essential for early warning and risk assessment, as these environments can act as reservoirs for resistance genes that may transfer to clinically relevant pathogens [14]. Effective monitoring of environmental AMR is crucial for developing mitigation strategies and informing policies aimed at controlling its global impact on public health [15].

This study focuses on the indicator microorganisms found in water, such as coliform and fecal coliform by using conventional methods to detect indicator microbes, such as microscopic observation, culture-based methods, and microbial indicator-based pathogen estimation, because these techniques are simple, easy to perform, cost-effective, and easy to identify specific organisms. Besides the conventional methods, VITEK-2 was also used in this experiment to ensure more reliable and accurate results on bacterial phenotypic character and their antimicrobial profiling. The aim of this study is to detect indicator organisms from potable jar water samples in Dhaka city, Bangladesh through Conventional Microbiological and VITEK-2 method. This assessment is to provide safe and drinkable water to humans.

MATERIALS AND METHODS

Study area and sample preparation

Samples were collected aseptically from February to May, 2024 from different vendor water supplies in Dhaka city, Bangladesh. A total of 30 such samples were collected between 10 a.m. and 2 p.m. in sterile plastic 500 ml container. The bottles were labeled with sample number, date and place name and transported immediately into the laboratory. Microbiological examination was started promptly to avoid any changes in sample (preferably within 2 hours of arrival) [16].

Conventional methods

Isolation of coliform and fecal coliform

Each water samples (100ml) were filtered through 0.45-μm pore size cellulose ester membrane filters.100 ml

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue III March 2025

volumes of water were filtered for triplicate experiments: one filter paper was placed on Nutrient agar (NA), one on MacConkey agar and one on m-FC agar (Hi Media Laboratories Pvt. Ltd., India). All culture media were prepared according to manufacturers' instructions. Then Nutrient agar and MacConkey agar media plates were incubated at 37°C and m-FC agar media incubated at 44.2°C for 24 hours. After incubation, the total colony of coliform and fecal coliform was counted [17][18]. Selected one or two colonies of coliform and fecal coliform were then cultured into MacConkey and EMB agar media (Hi Media Laboratories Pvt. Ltd., India) and incubated at 37°C for 24 hours [18].

Microscopic analysis and biochemical tests

Microscopic analysis was done to characterize the size, shape and morphology. A bright field microscope with a 1000x magnification was used to study the isolates morphology and Gram reactions [17]. Standard biochemical tests were performed to cross check the identified isolates. The methyl red (MR), citrate utilization, catalase, triple sugar iron (TSI) tests were performed to confirm the presence of *Escherichia coli* and *Klebsiella pneumoniae* [18].

Antibiotic susceptibility test

Antibiogram profiling for the bacterial isolates was determined as recommended by Clinical and Laboratory Standards Institute (CLSI) to determine which isolates are resistant against commercially available antibiotics [16],[17]. Pure culture of isolates were selected to determine the antibiotic susceptibility patterns against 12 different group of antibiotics, such as Cefuroxime (30 µg), Ceftazidime (30 µg), Vancomycin (30 µg), Cefepime (CPM) (30 µg), Rifampicin (30 µg), Netilmicin (30 µg), Cefixime (5 µg) and Nalidixic acid (30 µg) by Kirby Bauer disc diffusion method. Then the plates were incubated at 37°C for 24 hours. After incubation, then plates were observed and noted the zone of inhibition in millimeters [19].

VITEK-2 Compact

VITEK-2 compact method is an automated system for microbial identification and antibiotics susceptibility test [20]. For this test procedure, three isolates of Escherichia coli and three isolates of Klebsiella pneumoniae were selected as identified by conventional methods. The fully automated VITEK 2 Compact system (Bio Merieux, France) was used both to identify the isolates and for antibiotic susceptibility. The appropriate card was selected based on the microscopic morphology of the isolates and the Gram stain results. The inoculation suspension tube was placed inside the straw to produce a uniform bacterial solution in the cassette. After entering the data into the VITEK system, the tape was put into the holder, and the V2C device compared the virtual cassette with scanned barcodes. The tubes and straws were disposed of in a biohazard container once the tape was taken off [21].

RESULTS

Total count of coliform, fecal coliform by Membrane filtration method

After incubation the culture plates were observed and the total viable colonies of coliform and fecal coliform were counted.

In this table, the total coliform and fecal coliform were counted where the maximum colony count of coliform was 225 CFU/100 ml and, the maximum colony count of fecal coliform was 166 CFU/100 ml found in Manda. The presence of organisms was also observed on Nutrient agar plate which was TNTC (Too numerous to count).

Cultural characteristics of microorganism

The samples tested were analyzed for microbiological tests and isolates were cultured into MacConkey Agar and Eosin Methylene Blue for the presumptive identification of E. coli and K. pneumoniae [22].

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue III March 2025

Biochemical Identification

Several biochemical tests were performed to further confirm E. coli and K. pneumonia [22]. The 6 isolates of *E. coli* showed MR, TSI positive and citrate negative results. On the other hand, 29 isolates of *Klebsiella pneumoniae* showed MR, TSI and citrate positive results.

After incubation, different zones of inhibition were seen around the antibiotic disc. For some antibiotics, there is no inhibitory zone, indicating resistance property of the bacterial isolates [23]. On the other hand, some antibiotics are susceptible to bacterial isolates which are shown in Figure 1 and 2.

Table 1: Total count of coliform, fecal coliform in various samples of potable jar water samples.

Sample ID	Location	Total Coliform CFU/100 ml	Fecal Coliform CFU/ 100 ml
S1	Farmgate, Green Road (Fuska store)	160	80
S2	Farmgate, Green Road (Burger store)	2	0
S3	Kawran Bazar	11	7
S4	Eskatan Road	0	0
S5	Siddheswari (Tea stall)	24	14
S6	Siddheswari (Hotel)	8	4
S7	Baily road (tea stall)	82	60
S8	Siddheswari (Food court)	176	82
S9	Moghbazar tea stall	0	0
S10	Moghbazar (Tea stall)	20	14
S11	Moghbazar	87	34
S12	Moghbazar	120	95
S13	Mayakanan	16	8
S14	Mugdha	92	74
S15	Tikatuli	0	0
S16	Manda	225	166
S17	Maniknagar	84	56
S18	Ittefaq hotel	TNTC	10
S19	Rajdhani tea stall	0	0
S20	Mautali	36	14
S21	Malibagh street food	30	20
S22	Malibagh road side tea stall	0	0
S23	Malibagh tea stall	0	0
S24	Sadarghat	2	0
S25	Dholaikhal	0	0
S26	Wari	168	160
S27	Khilgoan (street food)	0	0
S28	Khilgoan tea stall	22	14
S29	Khilgoan tea stall	100	70
S30	Khilgoan	2	0

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue III March 2025

Table 2. Identification of isolates by VITEK-2 compact

Isolates	Tested	Correctly Identified by VITEK-2	Unidentified by VITEK-2
1. Escherichia coli	3	2 (66.67%)	1 (33.33%)
2. Klebsiella pneumoniae	3	3 (100%)	0

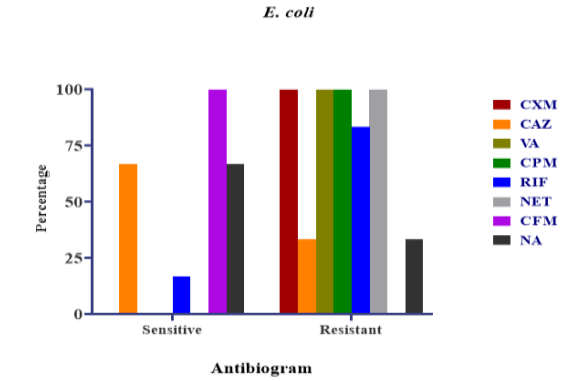

Figure 1. Percentages of antimicrobial resistance patterns on tested isolates (*E. coli)* against different types of antibiotics.

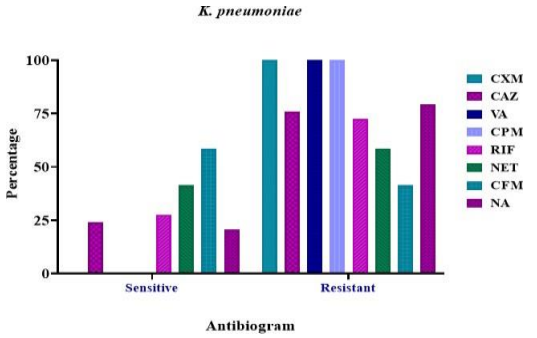

Figure 2. Percentages of antimicrobial resistance patterns on tested isolates (*K. pneumoniae*) against different types of antibiotics.

VITEK-2 method

VITEK-2 method was performed for microbial identification and detection of antibiotics susceptibility (Table 2). The results were compared with the outcomes from conventional methods.

DISCUSSION

Water contamination by notable bacterial pathogens is one of the biggest public health problems worldwide, mostly in developing countries. In this study, conventional and advanced methods (VITEK-2 Compact) were used to identify the indicator microorganisms from potable jar water sample in Dhaka city, Bangladesh which can help to assess the microbiological quality of water.

Total 30 samples of potable jar water were collected from different area in Dhaka city. Table 1 represents the number of Total coliform and fecal coliform into different water samples.

According to the WHO recommendation, the microbial limits for potable jar water should not be exceed one coliform per 100 ml and should contain no colony-forming unit of *E. coli* per 100 ml water [23]. The present study showed that 75% of samples contain coliform and among them, 16.67% of samples contain *E. coli.* A recent study was conducted in Chittagong city, Bangladesh by using PCR and cultural test where jar water sample contain 60.53% and 50% of the bacteria in the sample, respectively [24]. Another study in Gopalganj, Bangladesh showed that 80% of sample commercially jar water contain *E. coli* which exceed the standard limits [25]. Improper personal hygiene of handlers and some environmental factors have been reported to the contamination in commercially supplied drinking water in developing countries [26][27]. The findings of all these previous studies demonstrated that the presence of indicator organisms in the water poses a major risk to the population, and they recommended that probable sources of contamination were by processors and operators. The occurrence of *E. coli* and *K. pneumoniae* in potable jar water is an important indicator because these bacteria are a major part of coliform bacteria. The result showed that the water samples were contaminated with fecal material which is not safe for human consumption. Mainly coliform bacteria are used as an indicator of water quality. According to WHO (World Health Organization, 2011), there should not be any fecal coliforms found in 100 ml of drinking water. In this study, 75% of water samples from various areas showed the presence of fecal coliform (i.e. *E. coli*). Enteropathogenic *E. coli* can cause food borne illness, vomiting and diarrhea [28]. *E. coli* and other coliforms such as *K. pneumoniae* are responsible for urinary tract

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue III March 2025

infection and pneumoniae. Occurrence of coliform bacteria showed the danger of fecal contamination and the consequent hazard of contracting disease through pathogenic organisms [29, 30].

Bangladesh Standards and Testing institute (BSTI) provides guidelines and approval to produce commercially supplied potable jar water for handling, processing as well as distribution from factory to consumer. But many of the processors fail to do so. Consequently, supplied drinking jar water is contaminated with disease-causing pathogens and bacteria. Moreover, many of the defaulting processors may not have been licensed for their operations, which could be the reason for more contamination of supplied drinking water.

Nowadays, antibiotic resistance is a major concern in public health worldwide. A recent study showed that human sewage collected from various locations in Chattogram City, Bangladesh, had multi drug-resistant coliforms [31]. Therefore, we also tried to identify in what extent coliform bacteria in drinking water contribute to the prevalence of antibiotic resistance in Bangladesh. In this study, a total of 6 isolates of Escherichia coli and 29 isolates of Klebsiella pneumoniae were subjected to susceptibility testing against 8 commonly used antibiotics from different group. We observed that all isolates showed 100% resistance to cefuroxime (30 µg), Vancomycin (30µg) and Cefepime (30µg). On the other hand, E. coli showed 100% sensitivity to Cefixime (5 µg), 66.67% sensitive to Ceftazidime (30 µg) and Nalidixic acid (30 µg). For Klebsiella isolates sensitivity was observed with Ceftazidime (30 µg), Rifampicin, Netilmicin and Cefixime showed (24.14%), (27.59%), (41.38%) and (58.63%) respectively. The comprehensive results are shown in Figure 1 and 2. A recent study in Peru demonstrated that isolates of E. coli from drinking water showed highest resistance against tetracycline (37.6%), ampicillin (34.2%), sulfamethoxazole–trimethoprim (21.4%), and nalidixic acid (13%) and Multidrug resistance was found in 19.7% (23) for E.coli [32].

In our study the high levels of resistance observed against commonly prescribed antibiotics such as Cefuroxime, Vancomycin and cefepime in *E. coli* and *K. pneumoniae* isolates emphasized the need for careful consideration when selecting antibiotic treatments for diarrhea. In previous study for *E. coli* antimicrobial susceptibility testing Vancomycin has been used even though this is a drug for gram positive organisms and it showed 100% resistance against *E. coli*. So Vancomycin can be suggested to check the susceptibility of gram-negative organisms also [33].

Furthermore, the susceptibility to Cefixime, Ceftazidime, Nalidixic acid, Rifampicin and Netilmicin can be suggested as potential alternative treatment options.

VITEK-2 compact is an efficient tool for identifying the isolates and confirmed the antibiogram profiles of previously screened AST isolates and revealed other resistance phenotypes. *E. coli* and *K. pneumoniae* were positively identified by VITEK-2 compact (Table 2). A total of 20 different groups of antibiotics were used to test antibiotic susceptibility and gave 100% positive results as shown in Figure 3. In this study, VITEK-2 compacts produced accurate results (99.9%) for bacterial identification and found similar to our conventional methods to identify indicator microorganisms. A study showed that, VITEK 2 system represents an accurate and acceptable means for performing identification and antibiotic susceptibility tests with medically relevant Gram-positive cocci [34].

The choice between conventional and VITEK-2 Compact methods will depend on the specific needs of the laboratory and the desired turnaround time for results. For routine monitoring of potable water quality, conventional methods may be sufficient. However, in situations where rapid identification and susceptibility testing are critical, such as during outbreaks or public health emergencies, the VITEK-2 Compact system can be a valuable tool. Future research could explore the potential for integrating molecular techniques, such as polymerase chain reaction (PCR), with the VITEK-2 Compact system to further enhance the speed and accuracy of microbial detection.

CONCLUSION

The overall study indicated that commercially supplied potable jar water of different areas of Dhaka city contained indicator microorganisms and thereby proving the water is contaminated with fecal matter. Thus, people in these areas are having risk of affected with water borne diseases such as diarrhea, dysentery etc.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue III March 2025

Therefore, the processors of commercially supplied potable jar water in Dhaka City should follow the guidelines to avoid fecal contamination, maintaining hygiene. Besides conventional methods, VITEK-2 compact also produces faster accurate results for identification of indicator microorganisms and antibiotic susceptibility testing result.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Microbiology, Stamford University Bangladesh and National Science & Technology Fellowship (2023-24) Group: Biological and Medical Science, Ministry of Science and

Technology for giving support throughout the study.

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