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SHORT COMMUNICATION

Biofilm Forming Antibiotic Resistant Enteric Bacteria from Drinking Jar Water in Kathmandu Valley, Nepal

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ABSTRACT

Objectives: To determine bacteriological quality of drinking jar water from Kathmandu valley, Nepal and detect biofilm forming antibiotic resistant enteric bacteria.

Methods: A cross-sectional study was conducted during February-March 2023. Drinking jar water samples (n=36) were collected from Kathmandu valley. Isolation and enumeration of coliform were done by membrane filtration method. Bacterial isolates were characterized by biotyping, antibiotic susceptibility pattern was assessed and biofilm formation was detected by tube adhesion method.

Results: Bacteriological analysis revealed the contamination of 97.2% of water samples with total coliform and 11.1% of water samples with thermotolerant coliform. Among 78 bacterial isolates, *Klebsiella* spp (32.1%) was the most common bacteria followed by *Escherichia coli* (15.4%). Most of *E. coli* and *Klebsiella* spp isolates were resistant to antibiotics. Multiple antibiotic resistant (MAR) *Klebsiella* spp and thermotolerant *Klebsiella* spp were detected. Biofilm forming *E. coli* (38.5%) and *Klebsiella* spp (37.9%) were more resistant to antibiotics tested.

Conclusion: The higher number of drinking jar water in Kathmandu valley were contaminated with coliform and presence of biofilm forming antibiotic resistant enteric bacteria in water showed a high risk of waterborne infections to consumers. Quality control of drinking jar water is essential to improve water quality and prevent waterborne diseases.

Keywords: Antibiotic resistant, biofilm, coliform, drinking jar water, MAR

INTRODUCTION

Water pollution is a worldwide problem and poses a serious threat to human life. Nepal has been facing challenges on both the accessibility and quality of drinking water (Adhikari et al. 2021). Kathmandu valley is the most densely populated area in Nepal including the capital city and affected by rapid and unplanned population growth, lack of sustainable water sources and a poor water management system that resulted in low availability of safe water (Udmale et al. 2016; CBS 2022). Kathmandu Upatyaka Khanepani Limited (KUKL) which manages the water supply in Kathmandu valley reported water demand of 506 million liters per day (MLD) in 2025. However, there is an average supply of 240 MLD of water including existing water sources and Melamchi water to fulfill the water requirement of consumers (KUKL 2025). Many countries with water crises and poor quality drinking water depend on packaged/bottled drinking water instead of public water supply and bottled water is considered as safe (Dindarloo et al. 2016). In Kathmandu valley, because of the scarcity of adequate public water supplies, many people use jar water as an alternative drinking water source. Jar water in Kathmandu valley was highly contaminated with coliform and *Escherichia coli* which is a public health issue (Bhandari et al. 2009; Burlakoti et al. 2020; Gautam 2021). Contamination of drinking water with faeces and poor sanitation are linked to diarrhoeal disease transmission and is estimated to cause approximately 505,000 diarrhoeal deaths each year (WHO 2023a).

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Antimicrobial resistance (AMR) is a global health concern and bacterial pathogens are becoming highly resistant to most of the first and some second-line antibiotics in Nepal (Acharya et al. 2019). Antibiotic resistant bacteria have the potential to contaminate drinking water sources that intensify health risks associated with antimicrobial resistance (Dhengesu et al. 2022). Coliform from drinking water sources of Kathmandu valley showed high resistance to antibiotics and are more often multiple drug resistance (MDR) (Gautam 2021; Chaudhary et al. 2011). Biofilm formation in drinking water is a potential source of bacterial contamination including pathogens (Liu et al. 2016) and plays a crucial role in increasing antibiotic resistance. In packaged/bottled water, biofilm forming bacteria were reported previously (Effiok et al. 2019; Shrestha et al. 2024). Therefore, this study was conducted to assess the bacteriological quality of different brands of drinking jar water marketed in Kathmandu valley, Nepal and detect biofilm forming antibiotic resistant enteric bacteria from water.

METHODS

This cross-sectional study was conducted for the bacteriological quality analysis of drinking jar (processed) water during February-March 2023. The different brand of sealed drinking jar water samples (n=36) were collected randomly from Kathmandu valley including Kathmandu (Thamel, Samakhusi, Swoyambhu, Raniban), Lalitpur (Lagankhel) and Bhaktapur (Madhyapur Thimi). Water samples were collected and transported to the laboratory of Department of Microbiology, Amrit Campus, Thamel, Kathmandu according to standard methods of American Public Health Association (APHA), 1998.

Isolation, enumeration and identification of coliform

Isolation and enumeration of total coliform and fecal coliform from drinking jar water samples were done by membrane filtration (MF) method. The measured volume of water sample was vacuum filtered through 0.45 µm membrane filter, the filter paper was transferred on eosin methylene blue (EMB) agar plates and plates were incubated one at 35±0.5°C for total coliform and another at 44±0.2°C for thermotolerant coliform, for 24-48 hours (APHA 1998; Aneja 2023). Then, the number of colony was counted and colony forming units (CFU)/100 mL was calculated. Enteric bacterial isolates from jar water on EMB agar plate were subculture on MacConkey agar and nutrient agar plates. From pure culture of bacteria, colony morphology was studied and then Gram staining and various biochemical tests were done for identification of bacteria (Cheesbrough 2019; Forbes et al. 2007).

Antibiotic susceptibility testing

Antibiotic susceptibility pattern of enteric bacteria from drinking jar water was assessed by modified Kirby-Bauer disc diffusion method. The isolated colony was inoculated into nutrient broth and incubated at 35±0.5°C for 4 hours. The turbidity of inoculum was adjusted to 0.5 McFarland standard. Then, bacterial suspension was inoculated on Mueller Hinton agar (MHA) plate using a sterile cotton swab and the surface of agar was allowed to dry for 5 minutes. Different antibiotic discs, amikacin (30mcg), ampicillin (10mcg), azithromycin (15mcg), ceftriaxone (30mcg), chloramphenicol (30mcg), ciprofloxacin (5mcg), cotrimoxazole (25mcg), gentamycin (10 mcg), nalidixic acid (30 mcg) and tetracycline (30 mcg), were placed on MHA plate inoculated with bacteria and the plate was incubated at 35±2°C for 16-18 hours. The diameter of zone of inhibition was measured after incubation and results were interpreted according to zone size interpretation chart (CLSI 2022, Cheesbrough 2019).

Detection of biofilm forming Bacteria

Biofilm formation by *E. coli* and *Klebsiella* spp from drinking jar water was detected by tube adhesion method. The bacterial isolate from overnight culture was inoculated into 5 mL of tryptone soya broth (TSB) contained glass test tube and incubated at 37°C for 24 hours. The test tube was decanted, washed with phosphate buffer saline (PBS) (pH 7.2) and air dried. Then, test tube was stained with 0.1% crystal violet solution for 10 minutes and washed with distilled water. In inverted position, test tube was left for air dry and the visible biofilm formation on the wall and at bottom of test tube was observed. The assay was performed in triplicates at three different times. Bacterial isolates were classified based on biofilm formation as strong positive, moderate positive and weak positive/negative. *Staphylococcus aureus* ATCC 25923 was used as reference bacterial culture for biofilm assay (Christensen et al. 1985; Harika et al. 2020).

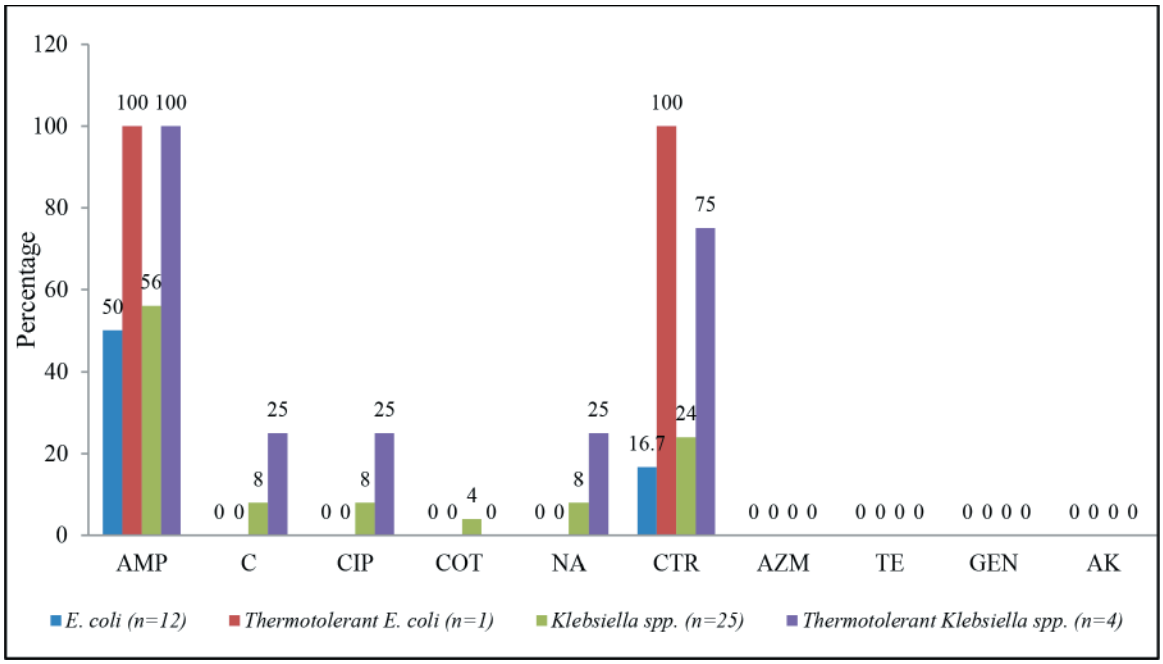
RESULTS

Out of 36 drinking jar water samples from Kathmandu valley, 97.2% (n=35) of samples were contaminated with total coliform and 11.1% (n=4) of samples were

contaminated with thermotolerant coliform which exceeded the World Health Organization (WHO) guidelines as well as National Drinking Water Quality Standards (NDWQS) of zero CFU/100 mL. The maximum total coliform count and thermotolerant coliform count was found to be 632 CFU/100 mL and 12 CFU/100 mL respectively. All drinking jar water samples from Samakhusi, Swoyanbhu, Raniban, Lagankhel and Madhyapur Thimi were found to be contaminated with total coliform whereas 83.3% and 16.7% of water samples from Thamel showed the contamination with total coliform and thermotolerant coliform respectively. Among 78 bacterial isolates from drinking jar water, *Klebsiella* spp (32.1%) was the most common bacteria followed by *E. coli* (15.4%), *Pseudomonas* spp (9.0%), *Enterobacter* spp (6.4%), *Citrobacter* spp (3.8%) and *Proteus* spp (3.8%). Thermotolerant *E. coli* (n=1, 20%) and *Klebsiella* spp (n=4, 80%) were also detected from jar water.

Antibiotic resistance pattern of *E. coli* and *Klebsiella* spp

The most of *E. coli* isolates showed resistance to ampicillin (50%) and ceftriaxone (16.7%). Similarly, the higher number of *Klebsiella* spp were resistant to ampicillin (56%) and ceftriaxone (24%). Thermotolerant *Klebsiella* spp showed resistance to most of antibiotics tested. Multiple antibiotic resistant (MAR) *Klebsiella* spp (12%) and thermotolerant *Klebsiella* spp (25%) were also detected (Figure 1).



AMP- Ampicillin, C- Chloramphenicol, CIP- Ciprofloxacin, COT- Cotrimoxazole, NA- Nalidixic acid, CTR- Ceftriaxone, AZM- Azithromycin, TE- Tetracycline, GEN- Gentamycin and AK-Amikacin

Figure 1: Antibiotic resistance pattern of *E. coli* and *Klebsiella* spp from drinking jar water in Kathmandu valley

Biofilm formation and antibiotic resistance pattern of biofilm forming bacteria

Of *E. coli* isolates (n=13), 15.4% were strong positive, 23.1% were moderate positive and 61.5% were weak positive/negative for biofilm formation. Similarly, among *Klebsiella* spp isolates (n=29), 13.8% were strong positive, 24.1% were moderate positive and 62% were weak positive/negative for biofilm formation. Biofilm forming *E. coli* isolates were resistant to ampicillin and ceftriaxone while majority of biofilm forming *Klebsiella* spp isolates were more resistant to antibiotics (Table 1).

Table 1: Antibiotic resistance pattern of biofilm forming *E. coli* and *Klebsiella* spp from drinking jar water

Antibiotics	Biofilm forming *E. coli* (n=5)		Biofilm forming *Klebsiella* spp (n=11)	
	Resistance		Resistance	
	Number	Percentage	Number	Percentage
Ampicillin	4	80	11	100
Ceftriaxone	3	60	9	81.8
Chloramphenicol	0	0	3	27.3
Ciprofloxacin	0	0	3	27.3
Nalidixic acid	0	0	3	27.3

DISCUSSION

Contamination of drinking water with coliform and *E. coli* indicates poor water quality and lack of sanitation as well as a serious health risk. In this study, 97.2% of drinking jar water from Kathmandu valley were contaminated with total coliform that exceeded the WHO guideline value. Burlakoti et al. (2020) and Gautam et al. (2021) reported the contamination of 92% and 52% of jar water in Kathmandu valley with total coliform respectively. Majority of water samples contaminated with total coliform and thermotolerant coliform showed a high risk category of coliform contamination according to WHO that has increased the risk of waterborne diseases to consumers in Kathmandu valley and also indicated low quality of jar water as well as lack of appropriate treatment and proper sanitation during production of drinking water. The most common enteric bacteria isolated from drinking jar water was *Klebsiella* spp followed by *E. coli*. The presence of thermotolerant *Klebsiella* spp and *E. coli*. in jar water revealed the faecal contamination of water. Contamination of jar water in Kathmandu valley with *E. coli*, *Klebsiella* spp, *Citrobacter* spp and *Salmonella* spp along with thermotolerant *E. coli* was reported previously (Bhandari et al. 2009; Subedi et al. 2010). The presence of these indicator bacteria showed that human pathogenic bacteria may be present in jar water which cause waterborne diseases. In present study, *E. coli* and *Klebsiella* spp from jar water were mostly resistant to ampicillin and ceftriaxone. In addition, *Klebsiella* spp and thermotolerant *Klebsiella* spp from jar water were MAR bacteria. Gautam (2021) has reported the resistance of fecal *E. coli* to ampicillin and chloramphenicol. This existence of antibiotic resistant and MAR enteric bacteria in drinking water sources are of great concern. The misuse and overuse of antimicrobials in humans, animals and plants as well as prolonged exposure of antimicrobials have lead to the emergence and spread of AMR in microorganisms which is a global public health threat (WHO 2023b).

E. coli (38.5%) and *Klebsiella* spp (37.9%) isolated from drinking jar water were biofilm producers of which 15.4% of *E. coli* and 13.8% of *Klebsiella* spp were strong biofilm producer in our study. Effiok et al. (2019) reported 32% of *E. coli* isolates from packaged water as strong biofilm producing bacteria. Long term use of plastic bottles like jar for marketing of drinking water increases the risk of biofilm formation (Kim & Lee 2022). Antibiotic resistance and its link to biofilm forming bacteria from bottled drinking water is posing a significant health problem. There was a positive correlation between antibiotic resistant profiles and biofilm forming capability in extensively drug-resistant *K. pneumoniae* (Santiago et al. 2020). Shrestha et al. (2024) reported that *E. coli* and *Klebsiella* spp in bottled water were biofilm-producing and drug-resistant bacteria. In present study, biofilm forming *Klebsiella* spp were more resistant to antibiotics, mostly MAR bacteria, than biofilm forming *E. coli*. These findings showed that the bacteria with higher rate of antibiotic resistance were biofilm forming as biofilm provides a protective environment for bacteria.

CONCLUSION

The higher number of drinking jar water was contaminated with coliform which indicated the poor quality of jar water in Kathmandu valley. The common bacteria in drinking jar water was *Klebsiella* spp and *E. coli* which showed biofilm forming ability as well as antibiotic resistance and MAR. Biofilm forming enteric bacteria were more resistant to antibiotics tested, mostly ampicillin and ceftriaxone. The presence of biofilm forming antibiotic resistant bacteria in jar water showed a high risk of waterborne diseases to consumers. Quality control of drinking jar water is essential to improve water quality and prevent waterborne diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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