

Characterization and antibiotic resistance profile of pathogenic bacteria from drinking and surface water in Odisha



Asima Das^a  | Sunita Kabi^a  | Lipika Jena^a  | Kundan Kumar Sahu^a 

^aDepartment of Microbiology, IMS & SUM Hospital, Siksha O Anusandhan (Deemed to be University), Kalinga nagar, Bhubaneswar, Odisha, India.

Abstract The study was aimed at identifying pathogenic bacteria from various water sources, such as drinking water distribution channels, groundwaters, and various surface water bodies. The water samples were analyzed for their physicochemical parameters and analyzed for the presence of both fecal coliforms and nonfermentors, including *Aeromonas* and *Pseudomonas aeruginosa*. Most of the isolates were multiple antibiotic resistant (MAR), and the organisms tested were resistant to cefoperazone/sulbactam (CFS), cefuroxime (CXM), norfloxacin (NX) and cefepime/tazobactam (CPT). All isolates were susceptible to ceftriaxone (CTR), and fecal coliforms and *Pseudomonas spp.* were susceptible to imipenem/cilastatin (IC) and ceftazidime (CAZ). Moreover, the findings indicate multiple antibiotic resistance (MAR) and high-level resistance to antibiotics in nonfermentors, such as *Aeromonas* and *Pseudomonas spp.* These organisms have a high prevalence in surface water bodies and a high potential to form biofilms in pipelines. Therefore, it has become important to include these bacteria in the surveillance of the water quality index. We found 13.33% nonfermentors in our study. Since contamination of drinking water can occur at the source or during supply, the exact cause needs to be explored. In our study, we tried to determine the extent of bacterial contamination in various sources, with alarming findings for surface water, supply water, bore wells, and tube wells, which yielded 100%, 80%, 40%, and 20% positive results, respectively.

Keywords: water quality, water analysis, microbial, total coliform, fecal coliform, physicochemical parameters, bacterial analysis, WHO

1. Introduction

Water is the most essential requirement for all living organisms. Water is used for drinking and other household activities. It is at most required to be clean and safe from microbes, but most of the time, water is usually contaminated with opportunistic pathogenic bacteria and other microbes.

Safe water is a human right, and if contaminated, it can lead to various health implications. Unprotected water sources are mainly contaminated with sewage effluents, rendering them unacceptable for human use. Antibiotic resistance is a major public health problem due to the presence of antibiotics in surface and drinking water. Since antibiotics are extensively used, the risk of human exposure to antibiotics has increased. There are reports mentioning hotspot sources such as fishery and animal farming where high doses of antibiotics are used.

There is an increased incidence of infection with antibiotic-resistant bacteria (ARB). The most common source of ARB and resistance genes is sewage (Adomako et al 2021). The genes and bacteria from treated and untreated sewage water reach water bodies such as ponds, dams, lakes, streams, and underground waters. Then, antibiotic-resistant bacteria acquire resistance genes via horizontal gene transfer (HGT).

Traditionally used water treatment via chlorination is not usually effective in removing the whole bacterial population (Cizmas et al 2015; Sun et al 2013; Khan et al 2013; Khan et al 2019). Additionally, resistant bacteria are reported to be more resistant to chlorination. There is research showing the combination of UV and chlorination to be more effective in killing ARB than either process alone (Duarte et al 2022; Sharma et al 2019). In the country, studies indicate that chlorination promotes the amount of free DNA and ARG in the water.

In 2015, the World Health Organization launched a strategy to combat antibiotic resistance. This global plan has been established since 2015 with a primary objective of obtaining in-depth knowledge of the spread of antibiotic resistance, mainly via active surveillance research.

In our study, we tried to determine the water quality with various physical parameters and the prevalence of various resistant bacteria in different drinking and consumable water in Bhubaneswar town. It is a medium-sized city with various



accesses to consumable water. The town receives its major water supply from the Naraj dam after treatment. There are many ponds and lakes that are used for bathing and other religious purposes. Throughout the town, there are scattered groundwater assessment points that include tube wells and bore wells. Since sewage treatment systems are not fully functional, there are possibilities of sewage seepage to ground water. This raises the question of both surface water and ground water contamination.

In this study, the main objective was to determine the physical parameters of various sources of drinking water and consumable water, the bacteriological profile of various water sources and the antibiotic resistance profile of the isolates. This type of study has not been undertaken in this area, so the study findings will definitely provide information about the water quality of the area and possible sources of contamination and may provide knowledge about their prevention.

2. Materials and Methods

For sampling, we collected water from various sites well scattered over the whole city. It included many municipality supply taps, tube wells, bore wells, and dug wells for groundwater. For surface water, the ponds and lake water were examined. Water samples were collected during a one-year period from September 2021 to August 2022. Water was collected using sterile 500 ml borosil glass bottles (HIMEDIA). The samples were properly labeled and sent to the laboratory in a cold chain using the standard procedure described in Mackie and McCartney (J G et al 14th ed). The physical parameters of pH, turbidity (NTU), electrical conductivity-EC (s/cm), and total solids were analyzed. A digital pH meter (TOA HM-10P) was used to measure the pH. A nephelometer (ELICO India) and a conductivity meter (WTW LF91) were used to measure the turbidity and EC, respectively, and analyze the total solids using the gravimetric method. The chloride (Volhard's method) and dissolved oxygen (Winkler's method) contents of the water were also analyzed by chemical analysis (Prajapati et al 2018; Bhandari et al 2021).

The water was processed by both the membrane filter method and the MPN (most probable number) method. The enteric isolates were isolated by the MPN (most likely number) method using MacConkey broth (HIMEDIA). For the membrane filter method for all water samples, 100 ml of each was filtered through a 0.45 µm pore-sized filter (Gridded Cellulose Nitrate Membrane, Sterile (HIMEDIA)), and the filter was kept on the plate for incubation at 37^o °C. The next day, the filter was removed, and the colonies were inoculated on M-FC agar and incubated overnight at 45^o °C for 24 hours. The isolates from the MPN (most likely number) method were characterized and tested for fecal coliforms. Isolates were considered fecal coliform when they were able to grow in M-FC agar at 45^o c.

Antimicrobial susceptibility testing (AST) was performed using the Kirby Bauer disk diffusion method using 25 antibiotics belonging to different classes of commonly used antibiotics. The following antibiotic discs at the final concentrations that are indicated were used: amikacin (AK)-30 mcg, ampicillin (AMP)- 30 mcg, chloramphenicol (C)- 30 mcg, colistin (CL)- 10 mcg, ceftazidime (CAZ) - 30 mcg, cefoperazone/sulbactam (CFS) – 50 mcg, ciprofloxacin (CIP) – 5 mcg, cefepime/tazobactam (CPT) – 30 mcg, cotrimoxazole (COT) – 25 mcg, ceftriaxone (CTR) – 30 mcg, cefotaxime (CTX) – 30 mcg, cefuroxime (CXM) – 30 mcg, gentamicin (HLG) – 120 mcg, imipenem/cilastatin (IC) – 10 mcg, minocycline (MI) – 30 mcg, meropenem (MRP) – 10 mcg, nitrofurantoin (NIT) – 300 mcg, norfloxacin (NX) – 10 mcg, ofloxacin (OF) – 5 mcg, polymyxin B (PB) – 300 units and piperacillin. These antibiotics were selected either because they are frequently used in both human and animal veterinary treatments or because microbiological resistance to them has been reported in previous research (CLSI 2019).

From each isolate, 2-3 colonies were picked, and antibiotic susceptibility testing was performed according to the Kirby Bauer disk diffusion method. After 18 hours of incubation, the zone size was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI 2019).

For isolates that showed resistance to three or more antibiotics, multiple antibiotic resistance (MAR) phenotypes were generated.

3. Results

The aim of the study was to determine the physicochemical and microbiological profiles of different water sources in an important town located in the eastern part of Odisha. We collected 120 water samples from various sites, which included supply water, bore wells, tube wells, and surface water bodies such as ponds and lakes. The main water supply of Bhubaneswar town is from the Kuakhai River and Naraj dam through pipelines. The water was then subjected to various tests to determine different physical and bacteriological profiles.

Table 1 shows the physicochemical parameters of the collected samples. In each sample, the pH ranged from 6.0 -7.7 on average. The pH of the sample collected from surface water (pond and lake) was found to be the highest (7.7) among all collected samples. The turbidity in water showed a significant difference and ranged from 0.78 to 8.07 NTU. Out of 120 samples, the turbidity of the sample collected from surface water was found to be the highest (8.07 NTU) among all collected samples and the lowest (0.78 NTU) turbidity of the sample collected from the supply water and bore well. The EC ranged from 43.8 to 518.6 us/cm. Dissolved oxygen levels were found to be low in boring and supply water and high in surface water, followed by tube well water. The total dissolved solids (TDS) values of the water samples ranged from 222 to 800 mg/l. Among all the

collected samples, the highest TDS value was found in surface water. Chlorides were measured at every sampling site and ranged from 201.395 to 554.977 mg/l. The sampled surface water had a high chlorine content.

Table 1 Water quality status based on physicochemical parameters in different sources of water.

Sample sites	PH	Turbidity (NTU)	EC (nm hos/m)	DO	TDS	Chloride
Supply water	6.1	0.78	43.8	3.41	222	201.395
Tube well water	6.8	6.57	518.6	6.61	571	330.868
Boring water	6.0	0.78	48.0	5.50	500	536.110
Surface water	7.7	8.07	331.1	7.76	800	554.977

Coming to microbiological investigations, all samples were subjected to various microbiological analyses. In the study, out of 120 samples, 40 samples had excellent or satisfactory results according to the MPN (most likely number) count. The remaining 80 samples had unsatisfactory MPN (most likely number) counts, which were ≥ 10 . These samples were also processed via the membrane filter method to isolate the bacterial species (Table 2).

Table 2 Bacterial isolates were identified from various sources of water.

Site	No. of culture	No. of positive culture	Isolates	Growth	NG
Supply water	60	80%	<i>Klebsiella pneumonia</i>	24	12
			<i>Escherichia coli</i>	16	
			<i>Pseudomonas aeruginosa</i>	8	
Bore well	20	40%	<i>Citrobacter</i>	8	12
Tube well	20	20%	<i>Aeromonas</i>	4	16
Surface water	20	100%	<i>Pseudomonas aeruginosa</i>	4	0
			<i>Klebsiella oxytoca</i>	12	
			<i>Klebsiella pneumonia</i>	4	

Regarding the distribution of positive out of 120 water samples, 60 were collected from supply water, and 20 tube wells and 20 bore wells were collected from local water bodies. Of these, 100% positivity for microbiological evaluation was found in surface water, followed by supply water, bore wells, and tube wells. This yielded 80%, 40%, and 20% positive results, respectively (Table 2).

Out of 80 positive samples, there were 80 bacterial isolates, which included 28 (23.33%) *Klebsiella pneumonia* isolates, 16 (13.33%) *Escherichia coli*, 12 (10%) *Klebsiella oxytoca*, 8 (6.66%) *Citrobacter* and 16 (13.33%) nonfermentors. The nonfermentors included *Pseudomonas aeruginosa* (12, 10%) and *Aeromonas* (4, 3.33%). In our study, out of 52 coliforms, 36 (69.23%) were fecal coliforms, and 16 (30.76%) were total coliforms (Figure 1)

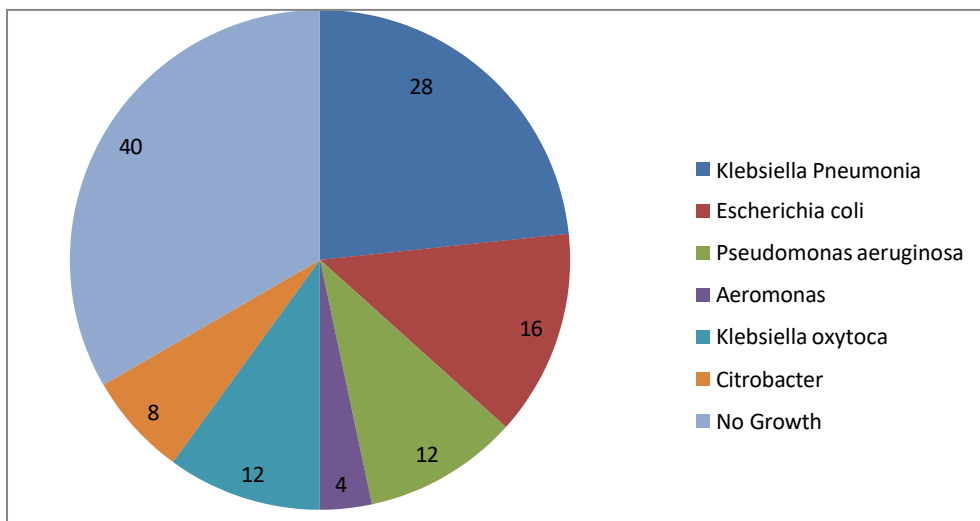


Figure 1 Graphical representation of bacterial isolates identified from various sources of water.

Among all the isolates, the most resistant antibiotics were cefoperazone/sulbactam (CFS), norfloxacin (NX), imipenem/cilastatin (IC), ceftriaxone (CTR), cefepime/tazobactam (CPT), cotrimoxazole (COT), ciprofloxacin (CIP), and minocycline (MI), which were 100, 95, 94, 90, and 85% resistant, respectively. The least resistance was found in colistin (CL), cefotaxime (CTX), polymyxin B (PB), gentamicin (HLG), and piperacillin/tazobactam (PIT) antibiotics, which were 18,44,45,50,55% resistant. Out of 120 isolates, all the isolates belonged to the MDR category, as they were resistant to more than 3 classes of antibiotics. Most of the MDR isolates belonged to supply and surface water. Groundwater had the fewest MDR bacterial isolates (Table 3, Figure 2).



Table 3 Resistance pattern of antibiotic isolates from different sources of water.

SI NO	Antibiotic	Resistant	Susceptibility
1	Amikacin (AK)	44 (50%)	44 (50%)
2	Ampicillin (AMP)	73 (82%)	15 (18%)
3	Chloramphenicol (C)	66 (75%)	22 (25%)
4	Colistin (CL)	15 (18%)	73 (82%)
5	Ceftazidime (CAZ)	75 (85%)	13 (15%)
6	Cefoperazone/Sulbactam (CFS)	88 (100%)	0
7	Ciprofloxacin (CIP)	80 (90%)	8 (10%)
8	Cefepime/Tazobactam (CPT)	80 (90%)	8 (10%)
9	Cotrimoxazole (COT)	80 (90%)	8 (10%)
10	Ceftriaxone (CTR)	82 (94%)	6 (6%)
11	Cefotaxime (CTX)	38 (44%)	50 (56%)
12	Cefuroxime (CXM)	88 (100%)	0
13	Gentamicin (HLG)	44 (50%)	44 (50%)
14	Imipenem/cilastatin (IC)	83 (95%)	5 (5%)
15	Minocycline (MI)	74 (85%)	14 (15%)
16	Meropenem (MRP)	80 (90%)	8 (10%)
17	Nitrofurantoin (NIT)	80 (90%)	8 (10%)
18	Norfloxacin (NX)	88 (100%)	0
19	Ofloxacin (OF)	80 (90%)	8 (10%)
20	Polymyxin-B (PB)	39 (45%)	49 (55%)
21	Piperacillin/tazobactam (PIT)	49 (55%)	39 (45%)

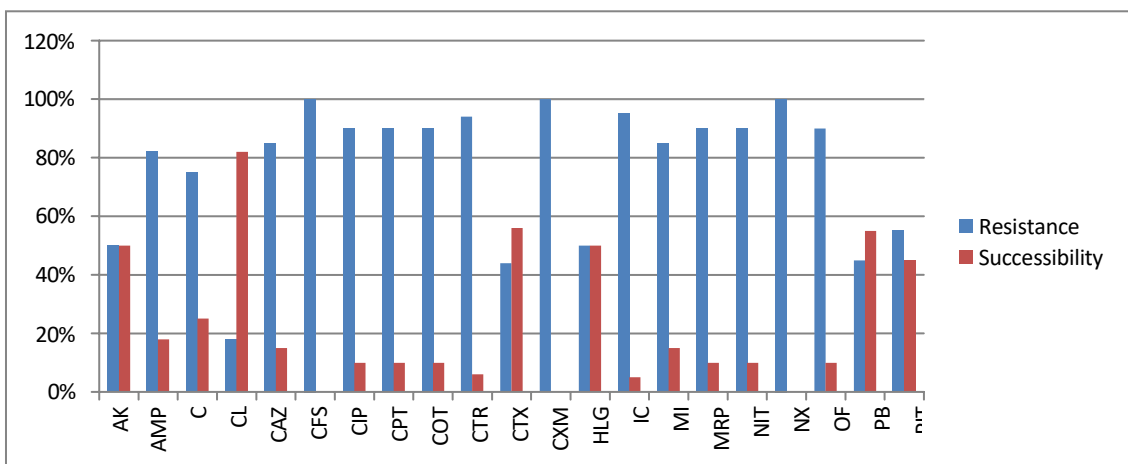


Figure 2 Graphical representation of an antibiotic pattern of various sources of water.

In surface water, 13.33% were the major species that showed the growth of nonfermenters, which included *Pseudomonas spp.* and *Aeromonas spp.* The majority of which were supply water followed by Bindu Sagar water. For the resistance pattern of nonfermentors, the isolates were 100% resistant to cefoperazone/sulbactam (CFS), cefepime/tazobactam (CPT), meropenem (MRP), and norfloxacin (NX) (Table 4).

Table 4 Antibiotic pattern of total number and percentage of *Pseudomonas aeruginosa* and *Aeromonas*.

SI NO	Antibiotic	Resistance	Susceptibility
1	Amikacin (AK)	8 (50%)	8 (50%)
2	Ceftazidime (CAZ)	12 (75%)	4 (25%)
3	Cefoperazone/Sulbactam (CFS)	16 (100%)	0
4	Ciprofloxacin (CIP)	8 (50%)	8 (50%)
5	Cefepime/Tazobactam (CPT)	16 (100%)	0
6	Cotrimoxazole (COT)	8 (50%)	8 (50%)
7	Gentamicin (HLG)	8 (50%)	8 (50%)
8	Imipenem/cilastatin (IC)	12 (75%)	4 (25%)
9	Meropenem (MRP)	16 (100%)	0
10	Norfloxacin (NX)	16 (100%)	0
11	Ofloxacin (OF)	8 (50%)	8 (50%)
12	Polymyxin-B (PB)	8 (50%)	8 (50%)
13	Piperacillin/tazobactam (PIT)	8 (50%)	8 (50%)



There was no significant difference in the resistance pattern of amikacin (AK) in either hospital drinking water or collected surface water. There was a significant resistance pattern to colistin (CL) observed in hospital drinking water when compared to collected surface water.

4. Discussion

The pH value ranged from 6.0-7.7 in our study, which is similar to that reported by P. Bhandari et al., whose finding was 6.38-6.9. In our study, the highest pH of 7.7 was found in surface water (pond and lake). The groundwater (tube well, boring) was measured in the range of minimum pH 6.0-6.8. According to the WHO, the acceptable range is from pH 6.5 to 8.5, and all pipeline water samples in our study are almost within WHO (World Health Organization) and BIS (Bureau of Indian Standards) standards. Only in a few cases does groundwater have a lower pH than needed, possibly due to the infiltration of rainwater and high iron contents in the soil (Bhandari et al 2021; WHO 2016).

In our study, the turbidity ranged from 0.78 to 8.07 NTU. This is met by BIS (Bureau of Indian Standards), which allows a maximum of 10 NTU for consumption. All water samples thus come under consumable limits. In many areas, turbidity ranged higher, as P. Bhandari et al. found 320.6 NTU in their study. The mixture of suspended particles, colloidal particles, clay particles, asbestos minerals, leaching of organic components, and domestic wastes from many sources can increase the turbidity in water. The turbidity of the water is a sign of water pollution (Bhandari et al 2021). In P. Bhandari et al., the EC ranged from 43.6 to 1,012.3 $\mu\text{S}/\text{cm}$. A high EC is often observed in groundwater. The EC ranged in our study from 43.8 to 518.6 $\mu\text{S}/\text{cm}$ of the tested water samples, which complies with the BIS (Bureau of Indian Standards) guidelines. Higher EC levels can be found in ground waters and surface waters than in tap water (Bhandari et al 2021). Dissolved oxygen was low in supply water and boring water but high in surface water followed by tube well water in our study, which is similar to the results of Prajapati DN et al. Samples 3 and 1 had low dissolved oxygen levels, whereas sample 4 had the highest levels, followed by samples 7, 6, and 2. It was brought on by groundwater contamination, which will not harm consumer health (Prajapati et al 2018).

In this study, the total dissolved solids (TDS) values of the water samples ranged from 222 to 800 mg/l. According to the WHO (World Health Organization), guidelines set an acceptable level of 500 mg/l. All supply water in our study maintained the WHO (World Health Organization) standards limit. In a study conducted by Prajapati DN et al., the water sample's total dissolved solids (TDS) concentrations ranged from 200.00 to 1500.00 mg/l. In our study, chlorides were detected at all sampling sites ranging from 201.395 to 554.977 mg/l. The chloride level recorded in the supply water was within the drinking water quality of WHO (World Health Organization) (250 mg/l), whereas the rest of the sample showed higher chloride levels but within the maximum limit of BIS (Bureau of Indian Standards), which is 1000 mg/l, similar to Prajapati DN et al., who found that the chloride values ranged from 82.260 to 555.966 mg/l at all sampling sites. (Prajapati et al 2018; WHO 2016).

Among the bacterial isolates, 64/80, i.e., 80%, belonged to the family Enterobacteriaceae, which included 28 *Klebsiella pneumoniae* (23.33%), 16 *Escherichia coli* (13.33%), 12 *Pseudomonas aeruginosa* (10%), 4 *Aeromonas* (3.33%), 12 *Klebsiella oxytoca* (10%) and 8 *Citrobacter* (6.66%). A similar distribution of bacteria was found by P. Bhandari et al. The isolates were 21.51% *E. coli* spp., 20.93% *Citrobacter* spp., 19.77% *Klebsiella* spp., 8.72% *Enterobacter* spp., and 4.07% *P. aeruginosa*, which is similar to our findings (Bhandari et al 2021).

Regarding the resistance pattern, the antibiotics showed >80% resistance to cefoperazone/sulbactam (CFS) (100%), cefuroxime (CXM) (100%), imipenem/cilastatin (IC) (95%), ciprofloxacin (CIP) (90%), ampicillin (AMP) (82%), ceftazidime (CAZ) (85%) and chloramphenicol (C) (75%). The lower resistance of our isolates to colistin (CL) (18%), cefotaxime (CTX) (44%), polymyxin-B (PB) (45%), and amikacin (AK) (50%). Similar findings in a study conducted by B. Hooban et al. revealed high resistance to ampicillin (97%), ciprofloxacin (71%), and nalidixic acid (70%) and lower resistance to cefotaxime (64%) and ceftazidime (56%) (Hooban et al 2021). Our finding is supported by malamattathil, where the researchers found that 100% of isolates were multiple antibiotic resistant (MAR). Additionally, the study finding is supported by studies by A. Łuczkiwicz et al. and J. E. Moore et al. with similar results (Luczkiwicz et al 2010; Moore et al 2010).

This is similar to our finding by Kichana et al. that 96.2% of isolates were resistant to cefuroxime (CXM) (Kichana et al 2022). In a similar study conducted by Hawa Ahmed et al. (Ahmed et al 2022), 88.7% resistance to cefuroxime (CXM) was observed, and 100% resistance to cefoperazone/sulbactam (CFS), cefuroxime (CXM) and norfloxacin (NX) in our study may be due to excessive use of these antibiotics locally, which can promote resistance in bacteria by selective pressure (Duarte et al 2022).

In this study, 13.33% of the total isolates were nonfermentors, which included *Aeromonas* spp. and *Pseudomonas aeruginosa*. *Aeromonas* was isolated in both tube wells and lake water. *Aeromonas* is an organism that can grow in water distribution systems by causing biofilms, although free *Aeromonas* are killed in chlorination (Emekdas et al 2006). We have not found any supply water possibly for that reason. A similar finding was found by Suma George Mulamattathil et al. *Aeromonas* spp. were isolated from raw water samples. The nonfermenter isolates in our cases were resistant to the antibiotic cefoperazone/sulbactam (CFS) and were susceptible to the antibiotic ciprofloxacin. All isolated *Aeromonas* species were erythromycin resistant. These species were all susceptible to the antibiotic ciprofloxacin (Mulamattathil et al 2014). There have been reports of *Aeromonas* wound infection and gastroenteritis. The finding in any source of water is alarming and needs intervention (Emekdas et al 2006).

In this study, 12 *Pseudomonas spp.* 8 were isolated from supply water, and 4 were isolated from pond and lake water. The groundwater in our study is free from *Pseudomonas spp.* This organism is emerging as a water-borne pathogen because of its ability to live within a water system. They often form biofilms in which they are protected from chemicals, the environment, and disinfectants (Singh et al 2017; Ateba et al 2020). This explains the increased finding of *Pseudomonas spp.* in water supply systems even after chlorination of water sources. However, *Pseudomonas spp.* survival in chlorinated water was reported by the same et al. (Odjadjare et al 2012; Mckenzie et al 2003). This might explain our finding of *Pseudomonas spp.* in supply water through pipelines that are chlorinated at the source. The antibiogram profile of *Pseudomonas spp.* isolates in our cases showed 100% resistance to cefepime/tazobactam (CPT), meropenem (MRP), and norfloxacin (NX), followed by 75% resistance to imipenem/cilastatin (IC) and ceftazidime (CAZ), but all were susceptible to amikacin (AK), ciprofloxacin (CIP), cotrimoxazole (COT) and gentamicin (HLG). In a study by Suma George Mulamattathil et al., *Pseudomonas spp.* were isolated from various treated and untreated water sources. All of the isolates in their cases were susceptible to streptomycin, neomycin, and ciprofloxacin but were resistant to up to eight antibiotics (Mulamattathil et al 2014).

The chi-square test was applied to compare the resistance patterns of hospital drinking water and surface water to different antibiotics. The p Value of these resistant pattern of antibiotics Amikacin (AK), Amoxyclav (AMC), Ceftazidime (CAZ), Cotrimoxazole (COT), Cefotaxime (CTX), Minocycline (MI), Meropenem (MRP), and Nitrofurantoin (NIT) were no significant difference in both hospital drinking water and collected surface water. There was a significant resistance pattern observed to p value <0.001 of colistin (CL), 0.004 of gentamicin (HLG), <0.001 of polymyxin-b (PB) and 0.01 of piperacillin/tazobactam (PIT) observed in hospital drinking water when compared to collected surface water in our study setting. Almost all the hospital and surface water samples showed 100% resistance to antibiotics such as cefuroxime (CXM) and norfloxacin (NX) in our study setting. In all hospital drinking water samples collected during the study, bacterial isolates were 100% resistant to cefepime/tazobactam (CPT), chloramphenicol (C), imipenem/cilastatin (IC) and ofloxacin (OF).

In our study, we found 100% contamination of surface water, out of which fecal coliform was found in 36/52 (69.23%) water samples. In a study in Pakistan, 64.29% were fecal coliform, and in a study in South Africa, Mafikeng, there was 100% fecal coliform. In India, although there is not much supportive literature, one study in Mumbai city by Kolawole et al. and Shradha Yelle et al. found that 76.36% of fecal coliform in supply water yielded fecal coliforms (yelle et al 2020). Regarding the resistance pattern of coliforms, we found that the isolates had 100% resistance to cefoperazone/sulbactam, cefuroxime, and norfloxacin. In the study by Suma George Mulamattathil et al., the coliforms exhibited 100% resistance to ampicillin, ciprofloxacin, and cotrimoxazole even in treated water (Mulamattathil et al 2014).

5. Conclusion

According to the WHO (World Health Organization), there can be a 10% reduction in global disease burden by preventing contaminated water supply and improving hygiene and sanitation of water sources. Keeping in mind the WHO global action plan on antimicrobial resistance, the main objective was to have deep knowledge about the spread of antimicrobial resistance via surveillance. Our studies have shed light on the burden of MDR (multidrug-resistant) species in the water system and their resistance patterns.

In our study, although all physical parameters were met by all samples, the alarming finding is about the bacteriological contamination. All the surface water bodies were unfit for human consumption in all cases. Even the supply water was contaminated many times. The groundwater was the least contaminated and in the majority of cases due to environmental bacteria. Traditionally, *Escherichia coli* was taken for the presence of fecal contamination and specific pathogens, such as *Salmonella* and *S. aureus*, for the investigation of particular disease outbreaks. In our study, we considered fecal coliforms as indicators as well as nonfermentors of resistant bacteria, which can have serious health hazards among consumers, particularly immunocompromised persons. In our case, surface and supply water in all cases were contaminated mainly by coliforms, which are of majority fecal origin and nonfermentors. The isolates most importantly belonged to MDR categories with resistance to more than 3 groups of antibiotics. As the majority of isolates belong to fecal origin, indicating sewage contamination of water, sewage contamination should be strictly investigated, the implementation of sewage treatment plants should be rigorously monitored, and supply water disinfection should be more stringently followed.

To conclude, possible sewage contamination should be strictly investigated in all cases of surface water. Supply water disinfection should be more stringently followed.

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Ethical considerations

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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