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Antibiotic Susceptibility Pattern of Bacteria Isolated from Stored Water in Some Residential Homes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The right to water is a basic human right, but if the water is contaminated with microbes, it defeats the purpose of providing safe water and good health. Containers used to store water at home can be sources of microorganism. This study assesses the bacterial pollution of stored water in different homes and the antimicrobial pattern of the bacterial isolates. About 30 samples comprising of 15 storage water cans and 15 swabs from respective storage cans in 5 different homes were used in this study. Spread plate technique was used to enumerate the microbial distribution. Biochemical tests were also conducted to confirm the presence of microorganisms. The total heterotrophic bacterial count ranged between $1.4x10^6$ to $1.55x10^7$CFU/ml, total coliform count ranged between 0.00 $\times10^3$ to 2.0×10^5 CFU/ml, total fungal count ranged between 1.0×10^3 to 8.0×10^4 CFU/ml. The nine bacterial isolates from water samples belonged to *Staphylococcus sp, Micrococcus sp, Proteus sp, Bacillus sp, Klebsiella sp, Escherichia coli*, *Salmonella sp, Enterobacter sp, and*

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Shigella sp. The frequency occurrences of the isolates were 11.5%, 7.6%, 11.5%, 19.2%, 7.6%, 7.6%, 3.8%, 19.2% and 11.5% respectively. The 8 bacterial isolates from swab samples belonged to *Bacillus sp, Staphylococcus sp, Shigella sp, Vibro sp, Escherichia coli, Proteus sp, Enterobacter sp and Aeromonas sp.* The frequency occurrences of the isolates were 19.2%, 19.2%, 11.5%, 7.6%, 19.2%, 11.5%, 7.6% and 3.8% respectively. The presence of *Klebsiella sp., Proteus sp, Enterobacter sp.* and *S. aureus* in the water source have been known to cause diverse disease on human beings, such as skin infections, wound infections, urinary tract infections, gastroenteritis and respiratory tract infections, therefore there is need for regular washing of water containers used for water storage. The antibiogram showed that ciprofloxacin, ofloxacin and gentamicin are the most effective antibiotics, thus, they are recommended for treatment of infections.

Keywords: Water; bacterial pollution antibiogram.

1. INTRODUCTION

The environment in which we live is undergoing tremendous change. An essential resource, clean and safe water is becoming more and more difficult to get by as the world's population is rising, consumption is expanding, pollution is increasing, urbanization is spreading, and megacities have become the new ecological norm [1]. In Europe and North America, a clean and treated water supply is the standard, but in underdeveloped nations, access to both clean water and sanitation is not the norm, and waterborne diseases are widespread [2]. Most regions of the developing nations are experiencing shortage of potable water supply as improved water sources are only limited to urban areas [3]. This shortage of water supply has led to the storage of water in cans and other storage tanks to make the water readily available. In Nigeria, many people in the rural areas have large plastic cans where they store water for domestic uses including drinking. According to Lambin et al. [4], water storage is a broad phrase that refers to the storage of both potable water for human use and non-potable water for agricultural use. During the dry season, both poor countries and some developed countries with tropical climates requires the storage of potable drinking water.

The quality of drinking water is a major concern for human beings, however there is a long history of faecal coliforms and other bacteria contaminating water systems [5]. According to the WHO, the annual death toll from water-related diseases surpasses 5 million people. More than half of them are microbial intestinal infections [2]. High incidence of waterborne disease have also been reported to emanate from consuming water of poor quality [6]. Mulamattathil et al. [7] opined that water is one of the readily available means of spreading water borne diseases and that potable water is a fundamental human right. Thus, if contaminated with opportunistic pathogenic environmental bacteria, it could cause serious health problems to consumers [7].

Several towns throughout every continent are seeing a decline in water quality as a result of the detrimental effects of human activity [8]. The immediate surroundings of some homes, particularly improvised and other temporary homes, as well as the activities of the occupants or residents, which may be devoid of sanitary and hygienic measures, need to be investigated because these factors may affect the quality of the stored water used for drinking [9]. This could result in health issues or challenges for the people who consume such water. In many shanties and shanty towns/settlements, there is a problem with the lack of water for household and other uses, including drinking. Even with a few sporadic and seasonal options, such as drinking water from commercial water-borne tankers and rainwater, which occasionally only temporarily ease or minimise the issue [10]. According to Akani et al. [11], the difficulties that some residences, particularly shanties or temporary housing in Port Harcourt and its surroundings, encounter are significant. The difficulties include: a lack of facilities for using toilets, poor sanitation, crowded housing with inadequate ventilation, a shortage of water supply, and a polluted atmosphere. Thus, these poor hygienic conditions could aid in contaminating stored water which later becomes a good vehicle for disease transmission and with the increasing rate of antibiotics resistant pathogens, treatment of affected populations becomes a public health challenge. The present study therefore is aimed at investigating the antibiotics susceptibility pattern of bacteria isolated from stored water in residential homes within Port Harcourt, Rivers State.

2. MATERIALS AND METHODS

2.1 Description of Study Area

The study was carried out in different residential homes in Rumuagholu community, Port Harcourt, Rivers state, Nigeria. It is one of the fastest developing communities in Obio/Akpor local Government Area of Rivers State. Their major and easily accessible source of drinking water in the community is borehole.

2.2 Experimental Design

The study was a complete randomized sampling method. Stored water samples were collected randomly from residents within the study area. The water samples as well as swabs of the interior surfaces of the storage tanks where sampled.

2.3 Collection of Water and Swab Samples

Water samples were collected from buckets and Jerry cans used for drinking and other domestic purposes. A total of 30 samples comprising of 15 storage water cans and 15 swabs from respective storage cans were collected. Samples from buckets were collected using a sterile metal cup (sterilized in the hot air oven at 160℃ for 1hour, 30minutes) then transferred into a sterile biological specimen bottle, while samples from Jerry cans were carefully transferred directly into the sample bottles by lowering the Jerry cans. Swab sticks were used to collect swab samples of each storage container by rubbing each swab stick round each storage container. All the collected samples were labelled and put in an ice-pack container before they were transported to the microbiology laboratory of the department of Microbiology, Rivers State University for analysis.

2.4 Microbiological Analysis

The microbiological analysis of the samples involved enumeration and isolation of the bacteria in the different samples. The microbial parameter investigated included; Total Heterotrophic Bacteria (THB), Total Coliform and Total Faecal coliform

2.5 Enumeration and Isolation of Bacteria

Ten-fold serial dilution was carried out on the samples [12,13]. In this method, 1ml of the water sample was transferred into test tubes containing 9mL sterile normal saline, after which 1mL was withdrawn in a step wise fashion unto ssanother sterile 9mL normal saline until a dilution of 10^{-6} was obtained. After the serial dilutions, aliquots of 10^{-4}, 10^{-2} and 10^{-3} dilutions were seeded into prepared Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), and MacConkey agar plates for the enumeration and isolation of total heterotrophic bacteria, faecal coliform and total coliform, respectively. Plates were spread evenly using sterile glass bent rod and incubated at 37 ºC for THB and TCC while faecal coliform plates were incubated at 45 ºC, 24 to 48 hours. After incubation, plates were observed for microbial growth and plates with growth were counted, colonies were characterized morphologically and were subcultured on freshly prepared nutrient agar plates. The counts from the different plates were used in enumerating the microbial load present in the water samples. The swab samples were inoculated directly on the respective media [14] and incubated as described above.

2.6 Characterization and Identification of Bacterial Isolates

The colonies were subcultured to obtain pure isolates. The pure isolates were then characterized by Gram's staining and Biochemical tests such as catalase test, indole test, methyl red test, citrate test, coagulase test, Voges Proskauer test and sugar fermentation tests. Identity of the isolates was matched with the Bergy's Manual of Determinative Bacteriology for confirmation.

2.7 Biofilm Screening

Bacterial isolates obtained from the samples were tested for their biofilm producing capacity using the Congo red test method. Biofilm screening by Congo red agar method is a simple qualitative way to detect biofilm production among bacterial isolates (Rodney & Donlan, 2001). This method was used to determine the bacterial isolates that produced biofilms. The test organisms were inoculated on Congo Red Agar and incubated at 37°C for 24 hours. The formation of black crystalline colonies marks a positive test for biofilm production.

2.8 Antimicrobial Susceptibility Test

Antimicrobial susceptibility pattern of each isolate was done using conventional disc diffusion

method according to Clinical laboratory standard institute [15] recommendation. In this method, a turbid suspension of 0.5 McFarland Standard of the isolates was made in sterile normal saline. A sterile swab was dipped into the bacteria suspension, pressed on the side of the bottles to allow excess drip-off, and then used to evenly streak the entire surface of the Mueller Hinton agar. Sterile forceps were then used to place the multiple antibiotic discs in on the media. The process was carried out for all the identified isolates, and the plates incubated at 37°C for 24 hours. After incubation, the zone of inhibition for each antibiotic was measured and interpreted as susceptible, intermediate or resistant (CLSI, 2020). The test was carried out using commercial multiple antibiotic discs. The discs used included Gentamicin (10µg), Ampicillin (30 µg), Ofloxacin (5 µg), Chloramphenicol (25 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Norfloxacin (30 µg), Cefuroxime (30 µg), and Amoxicillin (30 µg) for Gram-negative and Gentamicin (10 µg), Cephalexin (30 µg), Cloxacillin (5 µg), Ceftriaxone (30 µg), Amoxicillin-clavulanic acid (30 µg), Cotrimoxazole (25 µg), Erythromycin (10 µg), Clindamycin (10 µg), and Ciprofloxacin (5 µg) for Gram-positive bacteria.

3. RESULTS

Results of the bacterial counts is presented in Table 1. Results showed that the THB of the samples were 1.55 x 10^7, 4.8 x 10^6, 2.0x10^2, 1.4x10^6 and 9.9x10^6 CFU/mL. The coliform counts were 2.0x10^5, 4.9x10^4, 1.2x10^3, 2.5x10^3 and 0.00 x10^3 CFU/mL, and the faecal coliform count was 1.0x10^3, 4.2x10^4, 6.2x10^4, 0 and 8.0x10^4 CFU/mL. There was significant difference ($P<0.05$) in the total heterotrophic bacterial coliform and faecal coliform counts of the different samples.

A total of 26 bacterial isolates distributed among three Gram-positive genera *Staphylococcus, Bacillus* and *Micrococcus,* and seven Gram negative genera *Escherichia, Shigella, Aeromonas, Salmonella, Enterobacter, Proteus* and *Klebsiella,* were isolated from the water and swab samples in the study locations. Results of the bacterial isolates of the water and swab samples showed that *Micrococcus* sp was isolated from water samples but not in the swab.

Results of the percentage occurrence of bacterial isolates from the water samples is presented in Fig. 1, while the bacterial isolates from the swabbed surfaces is presented in Fig. 2. In Fig. 1, *Bacillus* sp and *Enterobacter* sp were the most dominant with percentage of 19.2% followed by *Staphylococcus* sp, *Shigella* and *Proteus* sp (11.5%) while *Klebsiella, E. coli,* and *Micrococcus* sp were the third dominant isolates (7.6%). The percentage occurrence of *Salmonella* sp which was the least isolate in water sample was 3.8%. In Fig. 2, the percentage occurrence of swabbed samples showed that *Bacillus, Staphylococcus* and *Proteus* sp were the most dominant isolates (19.5%), followed by *E. coli* and *Shigella* sp (11.5%), *Vibrio* and *Enterobacter* sp were the third most dominant (7.6%) bacterial isolates on the surfaces of the storage tanks while *Aeromonas* was the least with a percentage of 3.8%.

Results of the biofilm is presented in Table 2. Results showed that all the isolates with the exception of *Micrococcus* sp and *Enterobacter* sp produced biofilm.

Results of the antibiogram of gram positive and negative bacterial isolates is presented in Tables 3 and 4, respectively. In Table 3, the antibiogram showed that susceptibility of the *Staphylococcus* isolates to Augmentin, Ceftriaxone, ampicillin, cotrimoxazole, Ceproflaxin, gentamicin, Clindamycin and erythromycin was 4 (50%), 8 (100%), 2 (25%), 8 (0), 3 (37.5%), 8 (100%), 5 (62.5%) and 7 (87.5%), respectively. Susceptibility of *Bacillus* isolates was 5 (50%), 7 (70), 7 (70), 4 (40%), 10 (100), 2 (20%), 6 (60%) and 4 (40%) to Augmentin, Ceftriaxone, ampicillin, cotrimoxazole, Ciprofloxacin, gentamicin, Clindamycin and erythromycin. While susceptibility of *Micrococcus* sp to Augmentin, Ceftriaxone, ampicillin, cotrimoxazole, Ciprofloxacin, gentamicin, Clindamycin and erythromycin was 1 (50), 2 (0), 2 (100), 1 (50%), 1 (50), 2 (100%), 2 (0%) and 2 (100), respectively.

The antibiogram of the gram-negative bacterial isolates showed that *Enterobacter* sp were highly susceptible to cefuroxime, gentamicin and norfloxacin while also being susceptible to ofloxacin, ciprofloxacin and tetracycline. They were completely resistant to ampicillin antibiotic. The *E. coli* on the other hand were highly susceptible to ciprofloxacin, norfloxacin, ofloxacin, chloramphenicol and gentamicin. The *Klebsiella* isolates were highly susceptible to ciprofloxacin, ofloxacin, cefuroxime and gentamicin while *Proteus* isolates were completely susceptible to ciprofloxacin and ofloxacin with only 3 (37.5%) being susceptible to gentamicin.

Table 1. Microbial counts (CFU/ml) of the water samples

Samples	Total heterotrophic bacteria	Faecal coliform	Coliform count
Wa	1.55×10^7	1.0×10^3	2.0×10^5
Wb	4.8×10^6	4.2×10^4	4.9×10^4
Wc	2.0×10^2	6.2×10^4	1.2×10^3
Wd	1.4×10^6	0	0.00×10^3
We	9.9×10^6	8.0×10^4	2.5×10^3

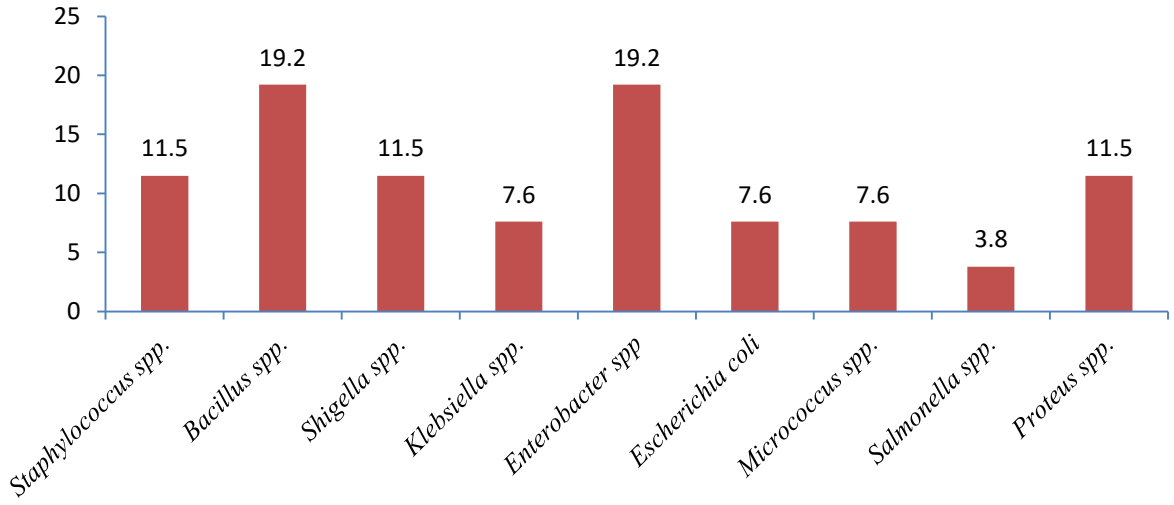

Fig. 1. Frequency occurrence of bacterial isolates from water sample

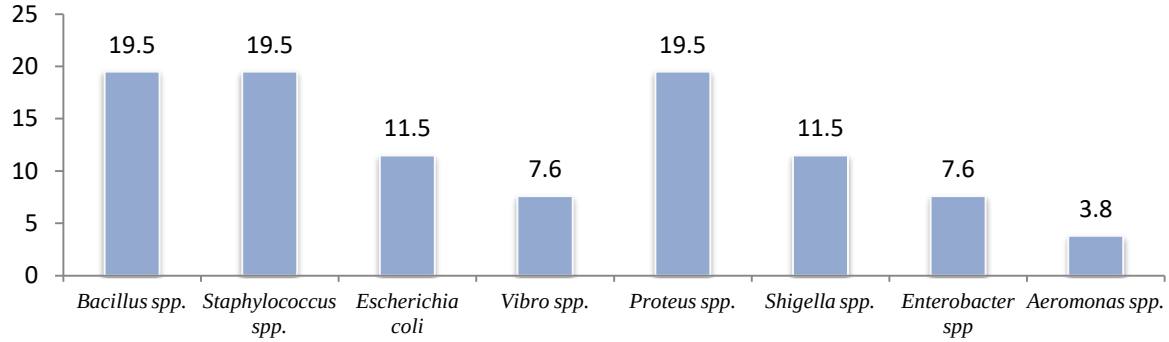

Fig. 2. Frequency Occurrence of Bacterial isolates from swab sample

Table 2. Biofilm producing bacterial isolates

Isolates	Response
Staphylococcus sp	++
E. coli	++
Bacillus sp	++
Shigella sp	++
Salmonella sp	++
Micrococcus sp	-
Vibrio sp	++
Aeromonas sp	++
Enterobacter sp	-
Proteus sp	++

Keys: ++ = strong biofilm former; - = no biofilm formation

4. DISCUSSION

The bacteriological quality of the different stored water showed high bacterial counts. The total heterotrophic bacterial load in all the stored water were very high and exceeds the limit of 1.0×10^2 guideline of the WHO for drinking water [16]. The world health organization standard for drinking water states that the water should be void of any faecal coliform while the limit for total coliform ranged between 0-10CFU/ml [16]. Thus, the coliform and faecal coliforms in the present study exceeds this limit which could imply that the water is not safe for consumption. The

contamination of the water could either be during storage or directly from the source where they were fetched (obtained). More so, the high bacterial load could also be a reflection of poor hygiene. For instance, always filling storage tanks without washing them properly could encourage microbial growth even if the source where the water is obtained from is void of contaminants. This agreed with the present study in which the storage cans of one of the sampled houses showed no coliform or faecal bacterial counts. Other measures of contamination of stored water tanks could be from hands of those accessing the water, the cleanliness of the instrument used in scooping [17]. It has been reported that microorganisms enter indoor environment through winds, food particles and pets [18-20]. Thus, exposure of storage tanks when lid is removed could predispose the water to microbial contamination especially when microorganisms within the room settles in the water. other measures include creeping activities of insects and cockroaches. High bacterial and coliform loads has been reported in a similar study [11] which agreed with the present study.

The bacterial isolates reported in the present study have been reported in previous studies as contaminating microorganisms. Akani et al. [11] in their study isolated *Staphylococcus, Vibrio, Salmonella, E. coli* and *Klebsiella* sp from stored water cans in residential homes. Pant et al. [5] in their study also isolated *Staphylococcus, Proteus, Enterobacter* and *Klebsiella* sp amongst other bacteria from tap water even though these isolates were not found in the bottled water. Thus, this support our report that contamination of water could be from the source. Similarly, Onuorah et al., [21] reported the presence of *Klebsiella, Vibrio, Enterobacter and Staphylococcus* sp from borehole water in Ogbaru Community, Anambra State, Nigeria while Abdullahi et al., [22] reported the presence of *Klebsiella* from Staff school, Science Department and female hostel boreholes in Niger State Polytechnic, Zungeru campus. The residents fetch their water from the bore holes; thus, this could be the primary area of contaminant especially since most of these boreholes are sited without proper geological surveys: proximity to toilet, dumpsites, latrines and septic tanks [23]. The presence of these microbes in the samples could be a public health problem especially in the diseases associated with these microorganisms. *Staphylococcus* sp have been implicated to cause range of diseases both on skins and as food poisons while the coliforms: *Klebsiella, Vibrio, E. coli, Salmonella* and *Shigella* are known to cause many gastroenteritis as well as typhoid in the case of *Salmonella* sp [24].

The antibiogram showed that some of the isolates exhibited multi-drug resistance. Despite this observation, the bacterial isolates were highly susceptible to ciprofloxacin, norfloxacin ofloxacin, gentamicin and chloramphenicol. Susceptibility of the isolates to gentamicin could be attributed to its availability. The drug is not a tablet or capsule as other antibiotics are. Thus, frequent and inappropriate use may not be highly practiced. Furthermore, Gentamicin is an aminoglycoside and carries out its antimicrobial effects by attaching to the 30S ribosomal subunit of the bacteria; thus, altering the proof-reading

Table 3. Antibiogram of gram-positive bacterial isolates

Bacteria	AU	FX	AM	CO	CX	GN	CD	E
Staphylococcus spp (8)	4 (50)	8 (100)	2 (25)	8 (0)	3 (37.5)	8 (100)	5 (62.5)	7 (87.5)
Bacillus spp. (10)	5 (50%)	7 (70)	7 (70)	4 (40%)	10 (100)	2 (20%)	6 (60%)	4 (40%)
Micrococcus sp (2)	1 (50)	2 (0)	2 (100)	1 (50%)	1 (50)	2 (100%)	2 (0%)	2 (100)

CX=Ceproflaxin, GN=Gentamicin, E=Erythromycin, CD=Clindamycin, CO= Cotrimoxazole, AM=Ampicillin, , FX=Ceftriaxone, AU=Augmentin

Table 4. Antibiogram of gram-negative bacterial isolates

Bacteria	CIP	TE	NF	AX	OF	C	CF	AM	GN
Enterobacter sp (7)	5 (71.4%)	5 (71.4%)	7 (100)	4 (57.1)	6 (85.7)	3 (42.9)	7 (100)	7 (0)	7 (100)
E. coli (5)	5 (100)	2 (40)	5 (100)	1 (20)	5 (100)	5 (100)	2 (40)	3 (60)	5 (100)
Klebsiella sp. (2)	2 (100)	1 (50)	2 (0)	1 (50)	2 (100)	1 (50)	2 (100)	2 (0)	2 (100)
Proteus sp. (8)	8 (100)	6 (75)	5 (62.5)	5 (62.5)	8 (100)	2 (25)	6 (75)	1 (12.5)	3 (37.5)

CF=Cefuroxime, AM=Ampicillin, CIP=Ciprofloxacin, OF=Ofloxacin=Chloramphenicol, TE=Tetracycline, NF=Norfloxacin, AX=Amoxicillin

function which leads to the synthesis of toxic proteins caused by wrong interpretation of the mRNA [25]. Ciprofloxacin, norfloxacin and ofloxacin are fluroquinolones which possess broad spectrum activities and is used in treatment of bacterial infections of skin, urinary tract, bronchitis, pneumonia, chlamydia and gonorrhoea [24]. Resistance of bacterial isolates has been reported in previous studies. Mulamattathil et al. [7] in their study reported that all their isolates were resistant to erythromycin while Moore et al. [26] reported that some of their isolates were susceptible to erythromycin. Although the isolates were highly susceptible to the fluroquinolones and aminoglycosides, the level of resistance observed is of great significance as this might be attributed to misuse of antimicrobial drugs. More so, the observed resistance could be via the modification of the isolates having picked up resistant genes from the environment [24,27]. The level of resistance of *Staphylococcus* sp to Gentamicin in this study does not agree with previous work done by Fair and Tor [28] who reported complete susceptibility of *Staphylococcus* isolates to the antibiotic agent.

5. CONCLUSION

In conclusion, the water in storage cans in the studied locations are generally unfit for consumption. The presence of faecal coliforms as well as suspected pathogens clearly showed that disease could be transmitted to consumers especially those with weak or immune compromised systems. Furthermore, the antibiogram showed multi-drug resistance amongst the bacterial isolates and the choice of antibiotics is dependent on the isolate to be treated. Gentamicin is drug of choice for staphylococci, *Enterobacter, E. coli* and *Klebsiella* sp in the present study. Despite the antimicrobial effectivity of other antibiotics, the presence of resistant isolates could be a public health problem. Thus, treatment of water supply is highly recommended. Also, cans should be washed properly before introduction of fresh water. Hygiene of users, the outside and internal environment is of great importance to ensure drinking water void of microbial contaminants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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