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Detection and Antibiogram of Bacteriological Contaminants in Commonly Consumed Sachet Water in Dutse, Jigawa State, Nigeria

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Potable water serves as an important source for primary prevention of diseases and the foundation for the prevention and control of water borne diseases. This study was conducted to determine the bacteriological quality of ten (10) brands of sachet water commonly consumed in Dutse urban, Jigawa state, Nigeria using Membrane Filtration Technique method for enumeration of both total coliform and fecal coliform counts. Eosin methylene blue, *Salmonella Shigella* and plate count media as well as membrane filters with 0.45µm pore size were used. Total bacterial counts for all the water samples were not within the recommended limits by regulatory bodies; Environmental Protection Agency, World Health Organization, and National Agency for Food and Drugs Administration and Control. Results revealed that the sampled sachet water was contaminated with *Klebsiella aerogenes, Pseudomonas aeruginosa, Salmonella paratyphi* A, *Shigella flexneri, Proteus vulgaris, Proteus mirabilis* and *Streptococcus pyogenes*. However, Ofloxacin and Ciprofloxacin revealed 100% potency against all the seven bacterial isolates while Augmentin have no effect (0%) on any of the bacterial isolates. Appropriate treatment processes should be utilized for the production of packaged water meant for human consumption. Again, drinking water regulatory bodies should be proactive in monitoring the adherence of drinking water manufacturers to regulated processing standards.

Keywords: *Sachet water, bacterial isolates, total coliform, faecal coliform, membrane filtration technique*

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1. Introduction

Human health and development can be better enhanced with access to potable water (Pruss *et al*., 2002). The deployment of water with a view to undertaking many activities on a daily basis is unquestionably the uppermost need that cannot be replaced by human beings (Adeleye *et al*., 2020). The drinking of packaged water has since become full-fledged in recent times, most especially in high-income countries and recently in middle and even low income countries of the world. Packaged water is typically a drinking water that is packed in a wide range of receptacles; plastic, glass bottles, sachets or bags. It usually comes in many sizes and sold in shops, vended on the street or delivered to homes. The gauge of packaged water consumption was extensive in 2011 as documented global bottled water sales did exceed 225 billion liters (Fisher *et al*., 2015). While statistics on the sales of packaged sachet water are more problematic to assess, its consumption is swiftly increasing most particularly in low and middle income countries (Stoler *et al*., 2012).

The rapid growth in the consumption of packaged sachet water these days is becoming a public health burden as many countries witnessing the surge are struggling to efficiently regulate its safety within their borders (Dada *et al*., 2011; Vapnek *et al*., 2014). Some packaged sachet water producers willingly adhere to the recommended quality, safety and hygiene standards, but others lack the aptitude or will intentionally produce sub-standard sachet water knowing fully well that they will get away with the practice. However, there is serious concern amongst governments and international organizations in that unregulated packaged sachet water may constitute public health concern (Williams *et al*., 2014). In recent times, serious apprehensions have been raised regarding possible association of packaged sachet water with outbreaks of cholera (Oluwafemi and Oluwole, 2012).

In Nigeria, consumption of packaged sachet water is a norm on daily basis. Dutse urban is equally witnessing a surging increment in the number of packaged water manufacturers. National Agency for Food and Drugs Administration and Control (NAFDAC) as well as Non-Governmental Organizations (NGOs) working in the country are concerned about potential health risks that can be traceable from packaged sachet water products of unknown quality (Williams *et al*., 2014). It is based on this background that this study was conducted with view to examining the bacteriological quality of commonly consumed sachet water in Dutse so as to confirm its conformity to regulated standards.

2. Materials and Methods

2.1 Study Area

This research was carried out in Dutse urban of Jigawa state, Nigeria. According to Peel *et al.* (2007), Dutse is the capital city of Jigawa state. The authors reported that Dutse lies on latitude of $11^0$42'8.46" N and longitude of $9^0$20'2.46" E.

2.2 Sampling Procedure and Collection of Samples

Local markets and retail shops in Dutse urban of Jigawa state selling sachet and bottled water were visited to identify most purchased and consumed sachet water. The retail shops and street vendors within the city were selected for point-of-sale sample collection. Ten (10) retail shops and point-of-sale were purposively selected for sampling of sachet water owing to their location and population of residents purchasing the specific brand. At each selected retail shop and point-of-sale, one brand of sachet water after purchasing was sampled at random (by drawing numbers out of a master bag). All the sampled sachet water were retained in their original sealed containers and transported to the laboratory for onward analyses as done by Fisher *et al.* (2015).

2.3 Laboratory Analyses

Samples collected from the sachet water were subjected to bacteriological assay with a view to detecting possible faecal coliforms, total coliforms, total aerobic mesophilic count (TAMC) and *Salmonella* spp and *Shigella* spp following the procedures described by AOAC (2007); APHA (2012).

2.3.1 Bacteriological Assessment

Nutrient agar, eosin methylene blue agar, plate count agar, *Salmonella Shigella* agar, cetrimide agar were used for possible isolation of bacteriological contaminants in the sampled sachet water (APHA, 2012). Eosin methylene blue agar was used for the enumeration and identification of *Escherichia coli* and other coliforms. Plate count agar was used for detecting total bacterial and its enumeration. *Salmonella Shigella* agar was used for the enumeration and identification of *Salmonella* and *Shigella* spp. Cetrimide agar was used for the enumeration and identification of *Pseudomonas* spp while nutrient agar was used for the storage of pure cultures obtained for future use.

2.3.2 Media Preparation

Considering the quantity needed, all the media used for isolating possible bacteriological contaminants in the samples sachet water were prepared following the instructions of the respective manufacturers.

2.3.3 Procedure for Total Bacterial Detection and Enumeration

Pour-plate inoculation method was adopted using sterile molten plate count agar medium. A set of appropriately labeled sterile disposable petri-dishes were set out, into each labeled plate one milliliter (1 mL) of each sachet water sample was dispensed. Approximately 15 mL of freshly prepared plate count agar medium was poured into each inoculated plate. Inoculated plates were rocked gently and allowed to solidify in the upright position. After which it were then incubated in an incubator in reverse position at 37 °C for 24 hours. After incubation, the bacterial colonies were counted using an illuminated colony counter and results were recorded as described in APHA (2012).

2.3.4 Procedure for Detection and Enumeration of Salmonella spp. and Shigella spp.

Membrane filtration technique as described in APHA (2012) was employed for the detection and enumeration of *Salmonella* spp. and *Shigella* spp. in the sampled sachet water. Erlenmeyer (side-arm) flask was connected to the vacuum source (turned off) and the porous support was placed in position. The filtration unit was assembled by placing a sterile membrane filter on the porous support using forceps sterilized by flaming, after which the upper container was placed in position and secured it. About 100 mL of the sachet water sample was then poured into the upper container and the off vacuum source was immediately turned on. It sucked the 100 mL water through the placed sterile filter paper placed on the porous support into the Erlenmeyer flask after which the filtration unit was taken apart. Using sterile forceps, the membrane filter was then removed from the porous support and placed in the petri dish containing *Salmonella Shigella* agar. During this

process no air bubbles was allowed to be trapped between the *Salmonella Shigella* agar and the membrane filter. The dish was then placed in an incubator at 37°C for 24 hours. After this incubation, the bacterial colonies were counted using an illuminated colony counter and number of *Salmonella* spp and *Shigella* spp were recorded accordingly.

2.3.5 Procedure for Detecting Escherichia coli and Coliform Bacteria

Membrane filtration technique as described by APHA (2012) was employed for the detection of *Escherichia coli* and other coliforms in the sampled sachet water. Erlenmeyer (side-arm) flask was connected to the vacuum source (turned off) and the porous support was placed in position. The filtration unit was assembled by placing a sterile membrane filter on the porous support using forceps sterilized by flaming after which the upper container was placed in position and secured the membrane filter sterile paper. About 100 mL of the sachet water sample was then poured into the upper container and the off vacuum source was immediately turned on. It sucked the water through the sterile filter paper placed on the porous support into the Erlenmeyer flask, after which the filtration unit was taken apart. Using sterile forceps, the membrane filter was then removed from the porous support and placed in the petri dish containing Eosin Methylene Blue agar. During this process no air bubbles was allowed to be trapped between the Eosin Methylene Blue agar and the membrane filter. The dish was then placed in an incubator at 37 °C for 24 hours. After this incubation, the bacterial colonies were counted using an illuminated magnifying colony counter and number of coliforms in 100mL of the water was then recorded accordingly.

2.4 Identification of Bacterial Isolates

Pure cultures of each bacterial isolate were obtained by aseptically picking distinct colonies which were subsequently cultured on newly prepared nutrient agar. The plates were then incubated at 37 °C for 24 hours. The pure cultures of the bacterial isolates obtained were then subjected to morphological identification using the Gram-staining procedure described in Olutiola *et al.* (2000). Microscopy of the isolates was done under X100 objective of light microscope. Biochemical tests related to the colonial and morphological characteristics were conducted following the description of Barrow and Feltham (1993).

2.5 Antibiotic Susceptibility Testing

A sizeable number of antibiotics; ceftazidime, cefuroxime, cefixime, ofloxacin, nitrofurantoin, streptomycin, chloramphenicol, gentamicin, ciprofloxacin, tetracycline, augmentin, erythromycin and clarithromycin were employed with a view to testing their antibiogram on the bacterial isolates detected in the sampled sachet water.

2.5.1 Inoculation of Agar Plate

As described by Jorgensen *et al*. (2007); Afiukwa *et al*. (2010), Mueller-Hinton agar was used as the culture media. About 12g of the powdered medium was suspended in 315 mL (for 21 plates) of distilled water. This mixture was heated with frequent agitation and boiled for about one minute to completely dissolve the medium. It was then subjected to autoclaving at 121 ^{0}C for 15 minutes. After the autoclaving processes, the agar was then allowed to cool but not solidify before 15 mL of it was dispensed into each sterile petri dish to solidify prior to inoculation. When the media was ready, the test bacteria were picked with the help of a wire loop from an overnight growth on nutrient agar. The inoculum was evenly spread all over the entire surface of the plate by swabbing in different directions and the plate was allowed to get dried before applying discs.

2.5.2 Placement of Discs

The discs were firmly positioned to the dry surface of the inoculated susceptibility plate with the help of sterile forceps. The disc from its cartridge was aseptically picked and positioned on the Mueller-Hinton agar and its contact with the agar was preserved with a view to avoiding unsatisfactory diffusion of the antibiotics on the disc and was instantaneously incubated at 37 ^{0}C for 24 hours.

2.5.3 Measuring Zones and Interpretation of Susceptibility

After incubation, the diameters of zones of inhibition to the nearest millimetre (zone edge was taken at the point of inhibition as judged by the naked eye) with the help of a ruler and the result recorded in each case. All the results observed were recorded.

3. Results and Discussion

3.1 Bacteriological Characteristics of Sachet Water Samples

Results of the colonial, morphological attributes and gram reactions of the bacterial isolates detected in the sampled sachet water are depicted in Table 1. Whereas the results of the various biochemical characterization tests and frequency of the occurrence of the bacterial isolates in the sampled sachet water are presented in Tables 2 and 3.

Table 1. Colonial, Morphological Characteristics and Gram Reactions of the Bacterial Isolates

I	EMB	SSA	PCA	CLED	CA	MC
A	Circular, convex, mucoid, about 2mm in diameter and appeared pink in colour			Mucoid and grayish green colour		Gram negative (pale reddish) rod in single and in pairs
B	Circular, convex about 3mm in diameter with smooth surface and appeared colourless			It appeared pale blue-green in colour	Appearance of yellow-green colony	Gram negative (pink) rod in single and in pairs
C	Circular, 2mm in diameter, some are spread with irregular edge and are colourless			The culture growth appeared transparent greenish blue in colour		Gram negative (pink) rod in single and in pairs
D	Circular, 2mm in diameter, some are spread with irregular edge and are colorless			The culture growth appeared transparent greenish blue in colour		Gram negative (pink) rod in single and in pairs
E		Circular, convex, about 3mm in diameter, smooth and red with black center		Appearance of blue-green growth of colony.		Gram negative pink rod in single and in pairs
F		Circular, convex, about 2mm in diameter, smooth and grayish in colour		Appearance of blue-green growth of colony.		Gram negative pink large rod in single and in pairs
G			Circular with smooth surface, tiny in size and white-grayish colour	Grayish-green and tiny sized colony		Gram positive blue tiny colonies in single and in chains

Key: I = Isolates, EMB = Eosin Methylene Blue, SSA = *Salmonella Shigella* Agar, PCA = Plate Count Agar, CA = Cetrimide Agar, MC = Morphological Characterisation

Table 2. Biochemical Characterization Tests of the Bacterial Isolates

I	L	GL	C	M	IN	U	Butt	SLT	GA	H_2S	SC	MR	VP	A	ALK	O	Identity
A	+	+	+	-	+	+	Y	Y	+	-	+	-	-	+	-	+	*Klebseila aerogenes*
B	-	-	+	+	-	-	R	R	-	-	+	-	-	-	+	+	*Pseudomonas aeruginosa*
C	-	+	+	+	-	+	Y	R	+	+	+	+	-	+	+	-	*Proteus mirabilis*
D	-	+	+	+	+	+	Y	R	+	-	+	+	-		+	-	*Proteus vulgaris*
E	-	+	+	-	-	-	Y	R	+	-	+	-	-	+	+	-	*Shigella flexneri*
F	-	-	+	+	-	-	R	R	+	-	-	-	-	-	+	-	*Salmonella paratyphi* A
G	+	+	-	-	-	+	NA	NA	NA	NA	-	-	-	+	+	+	*Streptococcus pyogenes*

Key: += positive, - = negative, I **=** Isolates, L = Lactose Utilization, GL = Glucose Utilization, C = Catalase Test, M =Motility Test, IN = Indole Test, U = Urease Test, SLT = Slant, Y = Yellow Colour Appearance, R = Red Colour Appearance, GA = Gas Production, H_2S = Hydrogen Sulphite Production, MR = Methyl-Red Test, VP = Voges-Proskauer Test, A = Acid, ALK = Alkaline, O = Oxidase Test, NA = Not Applicable

Table 3. Frequency of Occurrence for Bacterial Isolates (%)

Sample	*Klebsiella aerogenes*	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Proteus vulgaris*	*Shigella flexneri*	*Salmonella paratyphy A*	*Streptococcus pyogenes*
01	21.4	10.0	-	-	-	-	-
02	-	18.9	-	-	-	-	-
03	-	34.3	-	-	8.6	-	-
04	45.7	11.4	-	-	-	-	-
05	18.6	12.9	-	-	-	-	-
06	22.9	20.0	-	-	-	-	-
07	18.6	-	15.7	32.9	17.1	7.1	5.7
08	-	25.7	-	-	-	-	-
09	-	20.0	-	14.3	-	-	-
10	-	30.0	17.1	-	-	-	-

It can be observed that bacteria like *Klebsiela aerogenes, Pseudomonas aeruginosa, Proteus mirabilis*, *Proteus vulgaris, Shigella flexneri, Salmonella paratyphi* A and *Streptococcus pyogenes* were present in the water samples (Table 2). However, it was detected that sachet water sampled from sampling point number seven (7) and brand recorded six (6) bacteria out of the total number of seven (7) bacteria detected across the sampling points and brands while the sachet water sampled from sampling points number two (2) and eight (8) respectively recorded only one (1) bacterium out of the total number of seven (7) bacteria detected across the sampling points and brands (Table 3).

Results obtained in this study showed that the sampled water sold at different sampling sites in Dutse urban were contaminated with bacteria such as *Klebsiella aerogenes, Pseudomonas aeruginosa*, *Salmonella paratyphi* A*, Shigella flexneri, Proteus vulgaris, Proteus mirabilis* and *Streptococcus pyogenes*. The detection of majority of these water borne bacteria in the sampled sachet water is in conformity to the reports of Mgbakor *et al*. (2011); Kalpana *et al.* (2011); Adewoye and Adewoye (2013) who reported the presence of bacterial contaminants in samples of sachet water sold and consumed in Nigeria most specifically in the cities of Owerri, Birnin Kebbi and Ogbomoso respectively. Ajayi *et al*. (2008) in their work on sachet water, also reported the presence of bacteria such as *Klebsiella* spp.*, Streptococcus* spp. and *Pseudomonas* spp. It was reported by WHO (2002) that a high total plate count (TPC) concentration does not itself present a risk to human health. Nevertheless, heterotrophic plate counts (HPCs) are used as good indicators of the overall quality of production (Ferreira *et al*., 1994; Obiri-Danso *et al*., 2003).

The frequency distribution of these bacterial species isolated from the selected water samples showed *Klebsiella aerogenes* and *Pseudomonas aeruginosa* as the the two bacteria isolated in water samples “01”, “04”, ”05”, and ”06”. Although these four different water samples contained the same bacteria, they however differed in frequency level (concentration)**.** Samples “02” and”08” were contaminated with only *Pseudomonas aeruginosa* but with a different frequency level of 18.9% and 25.7% respectively. Sample “03” showed the presence of *Pseudomonas aeruginosa* and *Shigella*

flexneri. In this same, *Pseudomonas aeruginosa* was more prominent with a frequency value of 34.3% than *Shigella flexneri* (8.6%). Water sample "07" was the most contaminated amongst the ten (10) water samples assayed, with the exception of *Pseudomonas aeruginosa,* as it harboured all the bacterial isolates including a gram positive bacterium *(Streptococcus pyogene)***.** Sample "09" harboured *Pseudomonas aeruginosa* and *Proteus vulgaris* with a frequency level of 20% and 14.3% respectively.

The presence of these pathogens in water could account for the incidence of diarrhea, food poisoning and gastroenteritis amongst consumers of the sachet water sampled (Omalu *et al*., 2010). Although *E. coli* was not isolated in any of the assayed water samples, there were still other coliforms like *Klebsiella aerogenes* in some of the sachet water samples which according to WHO (2002) and other relevant regulatory bodies should not be present. Their presence alone was a clear indication that the sachet water sampled was not fit enough for human consumption. Therefore, the presence of coliform suggests that the sampled sachet water with its respective brands might have been contaminated with faeces either of human and/or animal origin (Okonko *et al*., 2011). Also high aerobic colony counts witnessed in this study are indication of poor, unhygienic handling and processing of water (Amoo *et al.,* 2021) and this could also bring about the presence of other pathogenic microorganisms which cannot be detected by simpler methods.

The presence of these bacteria with an inability to ferment lactose in the water samples suggests contamination of the water either through decay or improper sanitization or sterilization of the factory equipment or instrument used in the production processes. It can also result from the use of unsterile polythene which is used for the packaging of the water product. Enteric pathogens such as *Salmonella paratyphi* and *Shigella flexneri* were also detected representing 20% of the total water samples. The detection of *Shigella flexneri* usually suggests serious pathogenic water borne threat liable of causing serious disease to the consumers of such water (Nisa *et al.,* 2020). It could also be as a result of serious bacterial pollution of the factory equipment or from an infected worker, working under unhygienic practices.

3.2 Antimicrobial Sensitivity Profile of the Bacterial Isolates

The results of the antimicrobial susceptibility tests carried out to determine the sensitivity of each of the bacterial isolates to various antimicrobial compounds in order to assist in selecting treatment options for possible victims are shown in Tables 4 and 5. It can be observed that OFL recorded the most efficacy (35 mm) on *Salmonella paratyphi* A while GEN recorded the least efficacy (10 mm) on *Proteus vulgaris* (Table 4).

Table 4. Mean Values (mm) of Zones of Inhibition for Sensitivity for Bacterial Isolates

AB	*Klebsiella aerogenes*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Proteus mirabilis*	*Shigella flexneri*	*Salmonella paratyphi* A
CAZ	-	-	-	-	-	16
CRX	-	-	-	-	-	19
GEN	-	15	10	15	18	24
CXM	-	-	-	-	-	17
OFL	24	22	20	27	30	35
AUG	-	-	-	-	-	-
NIT	-	-	30	20	23	30
CPR	27	25	30	23	28	16

Key: AB = Antibiotic, CAZ = Ceftazidime, CRX = Cefuroxime, GEN = Gentamicin, CXM = Cefixime, OFL = Ofloxacin, AUG = Augmentin, NIT = Nitrofurantion, CPR = Ciprofloxacin

Table 5. Sensitivity Test of *Streptococcus pyogenes* as Compared with Standard Values

		Zone Diameter Interpretation Standards (mm)			
AB	DP (µg)	R	IM	S	TZI (mm)
S	10	-	-	-	-
CH	30	≤ 17	18 – 20	≥ 21	18
GC	10	-	-	-	-
CPX	5	-	-	-	-
TRC	30	≤ 18	19 – 22	≥ 23	30
AUG	10	-	-	-	-
ERT	15	≤ 13	14 – 22	≥ 23	27
CLT	15	≤ 16	17 – 20	≥ 21	25

Key: S = Streptomycin, CH = Chloramphenicol, GC = Gentamicin, CPX = Ciprofloxacin, TRC = Tetracycline, AUG = Augmentin, ERT = Erythromycin, CLT = Clarithromycin, DP = Disc Potency, R = Resistance, IM = Intermediate, S = Susceptibility, TZI = Test Zone of Inhibition

The seven (7) bacterial isolates were screened for antimicrobial susceptibility patterns. The antibiogram tests were conducted using eight (8) antibiotics; Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Cefixime (CXM), Ofloxacin (OFL), Augmentin (AUG), Nitrofurantion (NIT) and Ciprofloxacin (CPR). Among the tested antibiotics on the bacterial isolates, *Klebsiella aerogenes* showed complete resistance to six (6) of the antibiotics but was susceptible to OFL and CPR with CPR being the most effective*. Pseudomonas aeruginosa* was susceptible to GEN, OFL and CPR with CPR recording the most efficacy followed by OFL. The growth of *Salmonella paratyphi* A was inhibited by the effect of seven (7) antibiotics and was only resistant to AUG. CAZ, CRX, CXM and CPR exhibited some level of potency against the bacterial isolates but the diameters of their zone of inhibition were not enough to declare them good for treatment according to the standards of susceptibility table.

On a general note, OFL and CPR were the two antibiotics that showed 100% effectiveness against all the six tested gram negative bacteria. GEN and NIT showed 57.14% potency, CAZ, CRX and CXM showed 14.29% and AUG had no effect (0%) on all the six gram negative isolates. The 100% susceptibility of these bacteria to OFL and CPR conformed to the findings of Oyetayo *et al*. (2007) who had demonstrated the potency of these antibiotics on some gram negative bacteria. In a similar work Losch *et al*. (2008); Lynch *et al*. (2013) also reported antimicrobial resistance of isolates from the Enterobacteriacea family such as some species of *Proteus*, *Klebsiella*, *Citrobacter*, *Salmonella*, *Shigella* and *Escherichia* which are well-known opportunist pathogens causing nosocomial infections.

In a similar finding and as observed in this study, the sensitivity of all the bacterial isolates to OFL and CPR was also reported by Mulamattathil *et al*. (2014)**.** Results from their studies, revealed that all the tested bacterial isolates**;** *Klebsiella* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Shigella* spp. were completely sensitive to Neomycin, Streptomycin and Ciprofloxacin. These authors also documented the resistance of their bacterial isolates to Erythromycin, Trimethoprim, Augmentin and amoxicillin. According to Potron *et al*. (2013), most of this resistance is induced by the over expression of the AmpC *β*-lactamases which are encoded on the chromosomes of these bacteria. AmpC *β*-lactamases is also known to be transferred to organisms lacking or poorly expressing chromosomal AmpC genes such as *Klebsiella pneumonia***,** *Escherichia coli, Proteus mirabilis* and *Proteus vulgaris* through transmissible plasmids (Jacoby, 2009).

4. Conclusion

Results obtained in this study showed that all the ten (10) brands of sachet water (1 to 10) sold

and consumed in Dutse urban recorded bacteriological contaminants. The total bacteriological quality of the water samples did not conform to the standards set by water regulatory agencies. The quest for quick money has resulted in “pure water” business and this, as witnessed in this study has resulted into exposing the final consumers to enormous health risks. Therefore, sachet water business should be critically reviewed by water regulatory agencies to ensure that producers comply with standards at every stage of production and distribution processes. Defaulters should be sanctioned and incessant public sensitization programmes with a view to educating producers as well as consumers on the health hazards associated with contaminated or poorly treated water should be done regularly.

Conflict of interest

The authors declare no conflict of interest.

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