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Antibiotics Susceptibility Pattern of Bacterial Isolates from Selected Boreholes and Hand-dug Wells Water in Senior Staff Quarters, Obafemi Awolowo University, Ile-Ife, Nigeria

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Abstract:- This study assessed the antibiotic susceptibility pattern of bacterial isolates from selected boreholes and hand-dug wells water in Senior Staff Quarters, Obafemi Awolowo University, Ile-Ife, Nigeria. It isolated and identified the bacterial contaminants in the water samples. It also evaluated the total coliforms and the total heterotrophic bacteria in the water samples and determined the susceptibility of the isolates to conventional antibiotics. These were with a view to providing information on the quality of water from different water sources in the study area. Total bacterial (TBC) and total coliform counts (TCC) were assessed by pour plate technique on nutrient and MacConkey agar plates, respectively, at 37°C for 24 h. Preliminary identification of bacterial isolates was based on cultural and morphological characteristics. Identity of isolates was confirmed using conventional biochemical tests. Antibiotic susceptibility testing of isolates was done on Mueller-Hinton agar plates using Kirby-Bauer's disk diffusion techniques after incubation at 37°C for 24 h. Diameters of zones of inhibition were recorded and compared with the Clinical and Laboratory Standards Institute (CLSI) interpretative guidelines. The data obtained were analyzed using descriptive, One-sample T Test and Analysis of Variance (ANOVA). The TBC and TCC were above permissible standards. Bacterial isolates belonging to 12 genera including *Escherichia coli* were recovered from the water samples. All the Gram-negative bacterial isolates were susceptible to gentamycin and chloramphenicol except *Aeromonas hydrophila, Pseudomonas aeruginosa* and *Enterobacter intermedius* while Gram-positive isolates were susceptible to streptomycin and gentamycin. Most of the isolates from both boreholes and wells displayed multiple antibiotic resistance to more than 4 classes of conventional antibiotics.

Keywords:- *Antibiotics, Boreholes, Hand-dug Wells.*

I. INTRODUCTION

Availability and accessibility of clean freshwater is vital to sustainable development, poverty reduction and healthy living in developing countries (Akrong and Banu, 2017)[3]. Poor resource management and inadequate source protection is said to be a major cause for inaccessibility and deterioration of water sources in developing nations (Nkrumah, 2011)[15].

Groundwater contamination may be due to improper dreggy of well and improper waste disposal (Nkrumah, 2011)[15]. Microbial contaminations have been detected in groundwater due to anthropogenic activities (Pritchard, *et al*, 2008)[17]. The source of water contamination is numerous and includes, land disposal of sewage effluents, sludge and solid waste, septic tank effluents, urban runoff, industrial practices and agricultural mining (Allamin *et al.*, 2015)[4].

The use of untreated and inadequately treated groundwater may be attributed to water borne diseases including gastroenteritis, cholera, hepatitis, typhoid fever, and giardiasis; whose causative agents are bacterial and viral pathogens as well as protozoan parasites (Mile *et al.*, 2012)[13].

This study aimed at evaluating the total coliforms and the total heterotrophic bacteria in the selected boreholes and hand-dug wells water in Senior Staff Quarters, Obafemi Awolowo University, Ile-Ife, Nigeria, and characterize phenotypically the bacterial contaminants in the water samples.

II. MATERIALS AND METHOD

- *Description of the study area and sampling location*

Obafemi Awolowo University, Ile-Ife, is a Federal University, located in the ancient city of Ile-Ife, Osun State, Nigeria. Obafemi Awolowo University, Ile-Ife, Nigeria lies between latitudes 7° 31' 14.7612" N and 7° 31' 14.7612" N and longitudes 4° 32' 3.161" E and 4° 32' 2.591" E of the Greenwich Meridian (Akinsanya and Adewusi, 2017)[2]**.** The Staff Quarters is located within the University and it occupies an expanse of land with efficient road networks in a serene environment. It covers eighteen (18) out of twenty-four (24) roads that are within the built up area of the University. At present, the university has about 35,000 students, 13 Faculties and two colleges (i.e. the Postgraduate College and the College of Health Sciences) (Akinsanya and Adewusi, 2017)[2].

➢ *Collection of samples and bacteriological analysis*

Water samples were collected from identified fixed sources, namely: hand-dug wells and boreholes. Fifty percent (to a maximum of 25 samples) consisting of households with five boreholes and twenty hand-dug wells within Senior Staff Quarters, Obafemi Awolowo University, Ile-Ife, were randomly selected for the investigation.

Microbial counts were carried out using pour plate method (Dubey and Maheshwari, 2002)[10]. From each water sample, 1 ml of 10^{-3} – 10^{-4} dilution was poured into sterile plate. Then, 20 ml of prepared sterile nutrient agar and MacConkey agar were poured into each plate aseptically for enumeration of total viable bacteria and coliform counts, respectively. After solidifying, the plates were incubated at 37°C for 24 h. The microbial counts were recorded and expressed as the number of Colony Forming Units (CFU) per milliliters (ml), (cfu/ml). Pure colonies of bacterial isolates were preliminarily identified by cultural and morphological characteristics. Morphological characterization on the agar plates was based on the cell shape, size, colour, elevation, margin, surface appearance and optical observation (Dubey and Maheshwari, 2002)[10].

Isolates were further identified by conventional biochemical tests with reference to Bergey's Manual of Determinative Bacteriology (2010)[8].

The biochemical tests carried out to confirm the identity of test bacteria isolates included Gram's stain, catalase test, Methyl Red- Voges- Proskauer test, oxidation-fermentation, citrate utilization test, indole test for specific carbohydrate (glucose, lactose, mannitol, maltose and sucrose), and motility test.

➢ *Antibiotic susceptibility test*

Antibiotic susceptibility of the isolates was carried out on Mueller-Hinton agar (Lab M ltd, UK) plates using the Kirby-Bauer's disc diffusion method as described by Bauer *et al.* (1966)[6] and interpreted according to the guidelines of Clinical Laboratory Standard (CLSI, 2013)[9]. Sterile molten Mueller-Hinton agar (Lab M ltd, UK) plate was seeded with standardized inoculum (0.5 Mcfarland-10^7cfu/ml). The antibiotic disks consisting of Gram positive disks; gentamycin (10 µg), penicillin (10 µg), erythromycin (5 µg), ampicillin (25 µg), streptomycin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), cloxacillin (5 µg) and Gram negative disks; gentamycin (10 µg), augmentin (30 µg), cotrimoxazole (25 µg), erythromycin (5 µg), amoxicilin (25 µg), chloramphenicol (30 µg), tetracycline (30 µg) and cloxacillin (5 µg) were separately placed on the seeded plates using sterile forcep. The plates were then incubated at 37^0C for 18-24 h. The diameter of the zones of inhibition were measured with a calibrated transparent ruler to the nearest millimeter and recorded. The results were recorded as resistant, intermediate and susceptible according to the guideline of Clinical Laboratory Standard Institute (CLSI, 2013)[9].

➢ *Statistical analysis*

The results are expressed as Mean ±SD. Difference in means were also determined by Analysis of Variance (ANOVA) ($P<0.05$).

III. RESULTS AND DISCUSSION

Sources variation in the concentration of bacteriological properties of boreholes and hand-dug wells water in the Senior Staff Quarters, O. A. U, Ile-Ife is presented in table 1. The mean Total Bacterial Count (TBC) value of boreholes was 7.30±8.51 cfu/ml while that of hand-dug wells was 12.45±16.46 cfu/ml. The difference was not statistically significant ($p< 0.05$). The mean TCC value of boreholes was 0.40±0.97 cfu/ml while that of hand-dug wells was 3.55±11.85 cfu/ml. The difference was also not statistically significant ($p< 0.05$)

Seasonal variation in the concentration of bacteriological properties of boreholes and hand-dug wells Water is presented in table 2. The mean TBC value during the rainy season was 15.52±15.92 cfu/ml and 7.32±13.70 cfu/ml during the dry season. The difference was statistically different ($p >0.05$) in both seasons. The mean TCC value during the rainy season was 5.36±14.78 cfu/ml and 0.48±0.93 cfu/ml during the dry season. The difference was not statistically different ($p< 0.05$) in both seasons.

Comparison of investigated boreholes and hand-dug wells water quality in the Senior Staff Quarters O.A.U, Ile-Ife with WHO drinking water standard is presented in table 3.The mean TBC and TCC values of boreholes and hand-dug wells water were higher than WHO recommended standard and they are not statistically significant ($p> 0.05$).

Parameters	Boreholes	Hand-dug Wells	P-Value
No Investigated	5	20	
TBC (cfu/ml)	7.30±8.51	12.45±16.46	NS
TCC (cfu/ml)	0.40±0.97	3.55±11.85	NS
Key: TBC: Total Bacteria Count, TCC: Total Coliform Count, cfu: Coliform Forming Unit			

Table 1:- Sources Variation in the Concentration of Bacteriological Properties of Boreholes and Hand-dug Wells Water in the Senior Staff Quarters, O.A.U, Ile-Ife .

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Parameters	Rainy Season		Dry Season		P-Value
	BH	DW	BH	DW	
No Investigated	5	20	5	20	
TBC (cfu/ml)	15.52±15.92		7.32±13.70		>0.05
TCC (cfu/ml)	5.36±14.78		0.48±0.93		NS
Key: BH: Boreholes, DW: Dug Wells, cfu: Coliform Forming Unit TBC: Total Bacteria Count, TCC: Total Coliform Count					

Table 2:- Seasonal Variation in the Concentration of Bacteriological Properties of Boreholes and Hand-dug Wells Water in the Senior Staff Quarters, O.A.U, Ile-Ife.

Parameters	N	Mean±SD	WHO Standard	P-Value
TBC (cfu/ml)	25	11.42±15.27	10	NS
TCC (cfu/ml)	25	2.92±10.66	0	NS
Key: TBC: Total Bacteria Count, TCC: Total Coliform Count, cfu: Coliform Forming Unit				

Table 3:- Comparison of Investigated Boreholes and Hand-dug Wells Water Quality in the Senior Staff Quarters, O.A.U, Ile-Ife with WHO Drinking Water Standard

The percentage distribution of the bacterial isolates is shown in table 4. A total of sixty-four (64) bacteria species were isolated from both boreholes and hand-dug wells sampled. *Aeromonas hydrophila* has the highest percentage distribution of (18.8%), followed by *Bacillus* spp with 15.6%, *Staphylococcus* spp with 10.9%, *Listeria* spp has 9.4%, *Escherichia coli* has 7.8%, *Acinetobacter* spp has 7.8%, *Enterobacter intermedius* has 6.3%, while *Corynecbacterium* spp 4.7%, *Arthrobacter* spp 4.7%, *Pseudomonas aeruginosa* 4.7%, *Klebsiella* spp 4.7% and *Micrococcus* spp 4.7% had the least percentage distribution.

The susceptibility of the bacterial isolates to various antibiotics is shown in tables 5 – 6. *Klebsiella* spp recorded the highest (17%) susceptibility to gentamycin. Meanwhile *Acinetobacter* spp and *Enterobacter intermedius* were resistant to gentamycin. Susceptibility to cotrimoxazole was highest in *Klebsiella* spp (18.5%) but *Acinetobacter* spp, *Escherichia coli* and *Klebsiella* spp were resistant to cotrimoxazole. *Klebsiella* spp recorded highest susceptibility (22.5%) to chloramphenicol but *Aeromonas hydrophila, Pseudomonas aeruginosa* and *Enterobacter intermedius* were resistant to this antibiotic.. Similarly, *Acinetobacter* spp, *Escherichia coli, Aeromonas hydrophila, Pseudomonas aeruginosa, Klebsiella* spp and *Enterobacter intermedius* were resistant to augmentin, amoxicillin, erythromycin, tetracycline and cloxacillin. The antibiotic susceptibility profile of Gram positive bacterial isolates revealed that *Listeria* spp had the highest susceptibility (18.5%) to gentamycin. Similarly, *Staphylococcus* spp, *Bacillus* spp, *Arthrobacter* spp, *Listeria* spp, *Micrococcus* spp and *Corynecbacterium* spp were resistant to penicillin. Susceptibility to streptomycin was highest in *Bacillus* spp (16%) but *Staphylococcus* spp, *Arthrobacter* spp, *Listeria* spp, *Micrococcus* spp and *Corynecbacterium* spp were resistant to streptomycin. *Staphylococcus* spp, *Bacillus* spp, *Arthrobacter* spp, *Listeria* spp, *Micrococcus* spp and *Corynecbacterium* spp. were equally resistant to tetracycline, ampicillin, chloramphenicol, cloxacillin, and erythromycin.

Multiple antibiotic resistance (MAR) profile of the bacteria isolates is shown in table 7. *Acinetobacter* spp, which constituted (18.8%) of the isolated bacteria displayed high multiple antibiotic resistance to more than four classes of the antibiotics tested whereas all the *Escherichia coli, Aeromonas hydrophila and Pseudomonas aeruginosa* were resistant to the six classes of antibiotics used. Similarly, *Klebsiella* spp and *Enterobacter intermedius* displayed multiple antibiotic resistance to more than four classes of the antibiotic. Among the Gram positive bacterial isolates, *Staphylococcus* spp, *Arthrobacter* spp, *Listeria* spp and *Corynecbacterium* spp displayed multiple antibiotic resistance to five classes of antibiotics while *Bacillus* spp, and *Micrococcus* spp. exhibited multiple antibiotic resistance to four classes of the antibiotic (Table 7). The isolates displayed various MAR patterns.

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Isolated Bacteria	No in Borehole	No in Hand-dug Wells	(%) of Isolates
Aeromonas hydrophila	4	8	18.8
Bacillus spp.	3	7	15.6
Staphylococcus spp.	NA	7	10.9
Listeria spp.	2	4	9.4
Escherichia coli	NA	5	7.8
Acinetobacter spp.	NA	5	7.8
Enterobacter intermedius	NA	4	6.3
Corynecbacterium spp.	NA	3	4.7
Arthrobacter spp.	NA	3	4.7
Pseudomonas aeruginosa	NA	3	4.7
Klebsiella spp.	NA	3	4.7
Micrococcus spp.	1	2	4.7
Total	**10**	**54**	**100.0**
Key: No- Number,	%- Percentage,	spp- Species, NA- Not Available	

Table 4:- Percentage Distribution of the Isolated Bacteria from Boreholes and Hand-dug Wells in the Senior Staff Quarters, O.A.U, Ile-Ife

Disc		**Gram-Negative Bacterial Isolates**																	
Classes of Antibiotic	**Types of Antibiotic**	**Acinetobacter spp (n = 5)**			**Escherichia coli (n = 5)**			**Aeromonas hydrophila (n = 12)**			**Pseudomonas *aeruginosa* (n=3)**			**Klebsiella sp (n=3)**			**Enterobacter *intermedius* (n=4)**		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Aminoglycosides	GEN (10 µg)	12	0	0	15	0	0	15	0	0	16	0	0	17	0	0	13.5	0	0
Beta-lactam	AMX (25 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CXC (5 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	AUG (30 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0
FPI	COT (25 µg)	0	0	0	0	0	0	15	0	0	17	0	0	18.5	0	0	13.5	0	0
Macrolides	ERY (5 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phenicols	CHL (30 µg)	21.5	0	0	18	0	0	0	16.5	0	0	0	0	22.5	0	0	0	16	0
Tetracyclines	TET (30 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key: S = Sensitive R = Resistant I = Intermediate n = Number of Isolates
GEN = Gentamycin COT = Cotrimoxazole CHL = Chloramphenicol AUG = Augmentin
AMX = Amoxicilin ERY = Erythromycin TET = Tetracycline CXC = Cloxacillin
FPI = Folate Pathway Inhibitor

Table 5:- Antibiotic Susceptibility Profile of Gram-Negative Bacterial Isolates from Boreholes and Hand-dug Wells in the Senior Staff Quarters, O.A.U, Ile-Ife

Disc		**Gram-Negative Bacterial Isolates**																	
Classes of Antibiotic	**Types of Antibiotic**	**Staphylococcus sp (n = 7)**			**Bacillus sp (n = 10)**			**Arthrobacter sp (n = 3)**			**Listeria sp (n=6)**			**Micrococcus sp (n=6)**			**Corynecbacterium sp (n=3)**		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Aminoglycosides	GEN (10 µg)	15	0	0	0	0	12	13.5	0	0	18.5	0	0	15	0	0	16	0	0
	STR (10 µg)	10.5	0	0	16	0	0	9.5	0	0	0	14.5	0	0	14	0	0	13.5	0
Beta-lactam	PEN (10 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	AMP (25 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0
	CXC (5 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrolides	ERY (5 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phenicols	CHL (30 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tetracyclines	TET (30 µg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.5	0	0	0

Key: S = Sensitive R = Resistant I = Intermediate n = Number of Isolates
GEN = Gentamycin PEN = Penicillin STR = Streptomycin TET = Tetracycline
AMP = Ampicillin CHL = Chloramphenicol CXC = Cloxacillin ERY = Erythromycin

Table 6:- Antibiotic Susceptibility Profile of Gram-Positive Bacterial Isolates from Boreholes and Hand-dug Wells in the Senior Staff Quarters, O.A.U, Ile-Ife

Isolates	**Multiple Antibiotics Resistant Pattern**	**Number of Antibiotic Class**	**Frequency (n%)**
Gram-Negative Bacterial Isolates			
Acinetobacter spp	AMX, AUG, CHL, COT, CXC, ERY, GEN, TET	6	2
	AMX, AUG, COT, CXC, ERY, TET	5	2
	AMX, COT, CXC, GEN	3	1
	Total		5 (7.8%)
Escherichia coli	AMX, AUG, CHL, COT, CXC, ERY, GEN, TET	6	3
	AUG, COT, CXC, GEN, TET	3	2
	Total		5 (7.8%)
Aeromonas hydrophila	AMX, AUG, CHL, COT, CXC, ERY, GEN, TET	6	5
	AMX, AUG, CHL, CXC, ERY, GEN, TET	5	3
	CHL, COT, CXC, ERY	4	2
	COT, CXC, GEN, TET	3	1
	CXC, GEN, TET	3	1
	Total		12 (18.8%)
Pseudomonas aeruginosa	AMX, AUG, CHL, COT, CXC, ERY, GEN, TET	6	2
	AUG, CHL, ERY, TET	4	1
	Total		3 (4.7%)
Klebsiella spp	AMX, AUG, CHL, COT, CXC, ERY	4	1
	CHL, COT, ERY	3	1
	AMX	1	1
	Total		3 (4.7%)
Enterobacter Intermedius	AMX, AUG, CHL, COT, CXC, ERY, TET	5	2
	CHL, COT	2	2
	Total		4 (6.3%)
Gram-Positive Bacterial Isolates			
Staphylococcus spp	AMP, CHL, CXC, ERY, GEN, PEN, STR, TET	5	4

	AMP, CHL, ERY, TET	4	2
	CXC, ERY	2	1
	Total		7 (10.9%)
Bacillus spp	AMP, CHL, CXC, ERY, PEN, TET	4	3
	CHL, ERY, TET	3	3
	CXC, ERY	3	2
	TET	1	2
	Total		10 (15.6%)
Arthrobacter spp	AMP, CHL, CXC, ERY, GEN, PEN, STR, TET	5	2
	ERY, STR, TET	3	1
	Total		3 (4.7%)
Listeria spp	AMP, CHL, CXC, ERY, GEN, PEN, STR, TET	5	3
	CHL, ERY, TET	3	2
	ERY	1	1
	Total		6 (9.4%)
Micrococcus spp	AMP, CHL, CXC, ERY, GEN, PEN, STR	4	2
	CHL, ERY, STR	3	1
	Total		3 (4.7%)
Corynecbacterium	AMP, CHL, CXC, ERY, GEN, PEN, STR, TET	5	2
	ERY, TET	2	1
	Total		3(4.7%)

Table 7:- Multiple Antibiotic Resistance Profile of the Bacteria Isolated from Boreholes and Hand-dug Wells in the Senior Staff Quarters, O.A.U, Ile-Ife

IV. DISCUSSION

In the study, the TBC and TCC values recorded in hand dug well were higher than those recorded in bore holes. This variation can be attributed to the depth of the sources and their locations being far distance away (above 20 meters) from septic tank. However, the mean total bacteria and total coliform count values obtained in boreholes and hand-dug wells in this research was lower than what was obtained in a report by Jacinta *et al.* [12] on their assessment of microbiological quality of boreholes and wells water sources in Amai Kingdom, Ukwuani Local Government Area of Delta State, Nigeria. As expected, total coliform and total bacterial counts were generally higher during the rainy season than the dry season. Increase in the bacterial count during the rainy season may be attributed to increase in the amount of rainfall which increases the porosity of the soil and makes it easier for the bacteria to penetrate faster. The nature of the hand-dug wells and other wastes from anthropogenic activities could be responsible for the increase in bacterial count during the rainy season. In this study, *E coli* was recovered in hand dug wells which is an indication of feacal contamination that may be attributed to unhygienic practices and anthropogenic activities. Bakare *et al.* [5] reported the presence of coliforms especially *Escherichia coli* as an indication of faecal contamination. The values obtained for the total bacterial count and total coliform count was higher than WHO recommended standard for total bacteria and total coliform count in potable water.

Most of the bacterial isolates from the twelve (12) bacterial genera isolated from the investigated boreholes and hand-dug wells in Senior Staff Quarters, Obafemi Awolowo University had been previously reported as common microbial contaminants in water bodies. Jacinta *et al.* [12] in their assessment of microbiological quality of boreholes and wells water sources in Amai Kingdom, Ukwuani Local Government Area of Delta State, Nigeria reported isolation of eleven (11) bacterial genera. Similarly, Bello *et al.* [7] in their published research work on bacteriological and physico-chemical analyses of borehole and well water sources in Ijebu-Ode, South-western Nigeria reported isolation of eight bacterial genera. However, the persistence presence of these bacterial isolates in any of these sources can result into detrimental effect on the health of its consumers (Nicholas *et al.*)[14].

One of the on-going problems scientists and medical workers face in the fight against infectious diseases is the development of resistance to the agents used for their control. The phenomenon of resistance has been known since almost the beginning of antibiotic use (US National Institute of Health, 2007)[18]. In this study, the resistance of both Gram-positive and Gram-negative bacterial isolates to almost all the classes of antibiotics was similar to what was reported by Akinpelu *et al.* [1] publication on antibiotic resistance pattern of isolated bacteria from Obere River in Orile-Igbon, Oyo State, Nigeria where resistance to minimum of seven antibiotics, indicating the multiple resistance pattern characteristic of the isolated bacterial was observed. Similarly, the present finding agrees with the report of Odeyemi *et al.* [16] who reported multi-drug resistance pattern in Gram-positive bacteria to three classes of antibiotics and two classes in Gram-negative bacteria in well water samples from Osekita Hostels, Iworoko-Ekiti, Ekiti State, Nigeria, The multi-drug resistance pattern displayed among the isolated bacteria in this study may be due to improper or repeated use of antibiotics, poor hygiene and sanitation, over-prescription of antibiotics, failure to finish the entire antibiotics course and drug abuse among others.

V. CONCLUSIONS

The bacteriological quality of some of the water sources fell short of permissible standards suggesting a need for additional treatment before use. Resistance to antibiotics varied among the isolated bacteria and the prevalence of multiple antibiotic resistance (MAR) to conventional antibiotic classes was high. Frequent and proper cleaning of water storage tank, regular treatment of water sources and strict adoption of good hygienic practices are highly canvassed to reduce the risk of water borne diseases.

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