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Prevalence, Antimicrobial Susceptibility Profile of *Citrobacter* and Risk Factors Associated with Diarrheal Diseases in Water Wells in Urban West Region, Zanzibar

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Abstract

Citrobacter are Gram-negative bacteria that give public health challenges due to their zoonotic infections. Diarrheal diseases and antimicrobial resistance are global concerns that increase the burden of implementing infectious disease monitoring, especially in poor economic countries. This study was conducted to determine the prevalence and antimicrobial susceptibility and assess the risk factors of diarrheal diseases caused by bacteria from drinking water wells in the Urban West Region of Zanzibar. A cross-sectional study design was conducted where samples and data were collected from different locations in the Urban West Region of Zanzibar. A total of 250 water samples and respondents were randomly selected. *Citrobacter* spp. was identified by culture media, gram staining, biochemical tests, MALDI-TOF, PCR and sequencing. Antimicrobial susceptibility test to common antimicrobials was done using disc diffusion methods on Mueller Hinton agar. Antimicrobial resistance genes were detected by using PCR. Descriptive statistics were used for data analysis and evolutionary analysis was conducted in MEGA XI whereby a phylogenetic tree was constructed. A total of 13 (5.2%) *Citrobacter* spp were isolated from wells water samples, namely, *C. amalonaticus*, *C. freundii*, *C. braakii*, *C. werkmanii* and *C. farmeri*. Antimicrobial susceptibility tests revealed the potential of isolates to resist antimicrobials. Whereas genomic detection of resistance genes revealed the existence of *tetA*, *blaSHV*, *bla-CTXM* and *sul*1 and *sul*2. 87.6% of respondents were knowledgeable about diarrheal diseases. 54.8% of respondents expressed a positive attitude and 50.4% demonstrated effective practice for preventing diarrheal diseases. This study recommends that the best public health measures be taken in order to reduce the contamination of enteric pathogens

that are potentially resistant to antimicrobials in well water. Water authorities and health officials should minimize the risk of diarrheal diseases, by providing knowledge, positive attitude and monitoring preventive practices to drinking water consumers.

Subject Areas

Biotechnology, Microbiology, Molecular Biology

Keywords

Prevalence, Antimicrobial Susceptibility, Risk Factors, *Citrobacter*, Drinking Water Source

1. Introduction

In Zanzibar Urban West Region, water that is being used for domestic and other purposes comes from wells that are either publicly owned by people or the government. All wells present in the region are monitored by the government water authority (ZAWA) to ensure that they supply safe and quality water to the community. Consumption of water coming from wells might put the public at risk of being infected by a range of bacteria species, mainly from family *Enterobacteriacea* [1]. As reported by [1] *Citrobacter* spp. has been identified to be the dominant *Enterobacteriacea* genera detected in wells water thus making these bacteria of public health concern. *Citrobacter* is a genus of Gram-negative bacteria, it is found in soil, water, and the gastrointestinal tracts of animals and humans. Several species of *Citrobacter* exist but *C. freundii*, *C. werkmanii*, *C. braakii*, *C. koseri*, *C. youngae C. amalonaticus* and *C. farmeri* have been highlighted to cause diseases in humans [2]. *Citrobacter* spp. is less common than other Gram-negative bacterial infections but they can still cause a range of clinical complications such as diarrhoea, urinary tract infections, and sepsis in severe cases [3]. According to the Clinical and Laboratory Standards Institute (CLSI), commonly and currently used antimicrobial agents for the treatment of *Enterobacterales* such as *Citrobacter* infections, are tetracycline (tetracycline) penicillin (ampicillin), quinolones and fluoroquinolones (ciprofloxacin), cephalosporin (cefotaxime/ceftriaxone), aminoglycosides (gentamicin), and folate pathway antagonists (sulfamethoxazole/trimethoprim) [4]. The effectiveness of these agents might be limited by the existence of antimicrobial-resistant strains in this genus [5]. In general, *Citrobacter* spp. is capable of developing resistance to antimicrobial agents through several mechanisms, as shown in **Figure 1**, but the acquisition of genetic mutations or the exchange of resistance genes with other bacteria has been the prominent ones as it was reported by [5].

The transmission of antimicrobial-resistant bacteria among the community is a public health threat leading to hospitalizations, increased treatment costs and

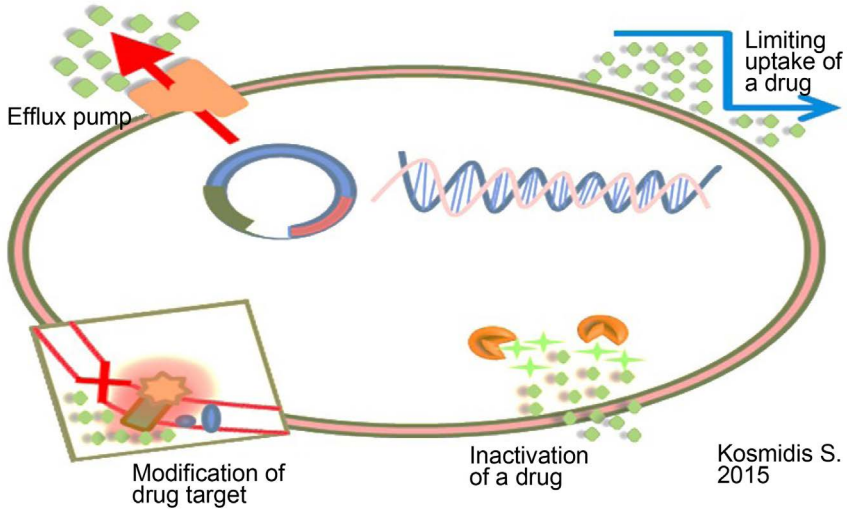

Figure 1. Mechanisms employed by bacteria to resist antimicrobials. Source: Kosmids S, (2015) [6].

or mortality. Hence surveillance of environments, animals and humans through one health approach has been adopted to provide information on these environment-animals-humans transmissions of pathogenic microorganisms.

The microbiological quality of drinking water is among the prerequisites for the provision of safe and clean water for human consumption [7]. This is because drinking water contaminated with pathogenic microorganisms exposes an individual to various water-borne diseases, including bacterial diseases such as cholera, typhoid fever, dysentery, and gastroenteritis, which are characterized by diarrhoea [8] [9]. Diarrhoea is a gastrointestinal tract (GIT) condition in which individual suffering passes or loose watery stools, sometimes accompanied by fever [10]. According to (WHO/UNICEF 2017), about 1.7 billion people experience diarrhoea cases with 37.7% morbidity and 1.5% mortality worldwide [11] [12]. A number of deaths caused by diarrhoea occur in infants and children due to their inability to withstand losing large amounts of water through stools. Moreover, several diarrhoea cases come from developing countries due to low sanitation, unhealthy altitudes, and practices such as defecating outdoors, poverty, and weak health facilities [13] [14]. Different factors have been associated with incidences of diarrhoea worldwide, but leaving and/or consuming foods, water, and other materials contaminated with pathogenic microorganisms such as bacteria, parasites and viruses have been found to correlate with experiencing diarrhoea [15] [16]. The previous study [17] revealed that bacteria have been frequently associated with diarrhoea cases globally, whereas bacteria, in general, such as *E. coli*, *Klebsiella* spp, *Shigella* spp, *Salmonella* spp, *Vibrio cholera*, and *Citrobacter* spp have been prominently detected and/or isolated in diarrhoea cases. Following the isolation of *Citrobacter* bacteria from the blood of patients attending Mnazi Mmoja Hospital [18] and more recently by [11] who emphasized that drinking well water was the risk for diarrhoea among under five years children prompted the speculation that drinking water contaminated by *Citro-*

bacter spp. might be the risk for observed diarrhoea cases to under-fives and recovery of *Citrobacter* spp. from patients at Mnazi Mmoja hospital. Hence this study was conducted to isolate and characterize *Citrobacter* spp from water wells in Zanzibar and determine their susceptibility to antimicrobials both phenotypically and at the molecular level since this information has not been previously established in Zanzibar.

In Zanzibar, a few reports have been established so far on the prevalence of diarrhoea infections [19] and that bacteria are the most commonly detected agents in diarrhoea cases [20] [21]. In light of this, the owners of wells used to supply water for human consumption were recruited for the study to assess their knowledge, attitudes, and practices that might be accelerating the prevalence of diarrhoea in the region. This population was chosen because individuals owning wells in Zanzibar are the ones responsible for cleaning, maintaining, and monitoring all the activities related to their wells.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted in the Urban West Region (Mjini Magharibi) of Zanzibar (**Figure 2**). The Urban West Region of Zanzibar is located in Zanzibar city on Unguja Island in the Indian Ocean, lying 22 miles (35 km) off the coast of

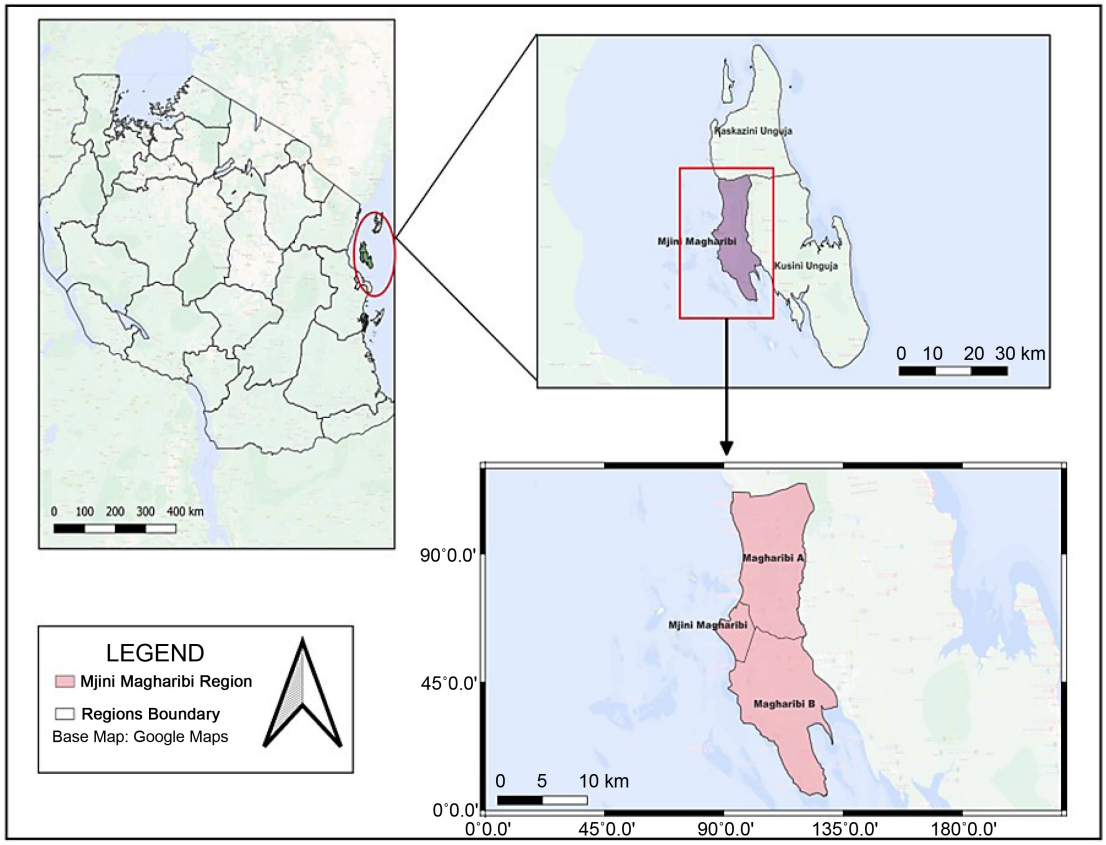

Figure 2. Urban west region (Mjini Magharibi) and the neighbour districts where this study was conducted.

east-central Africa [22]. This is the largest region among those three (3) regions of Unguja Island and consists of three districts: Magharibi A, Magharibi B, and Urban District. According to the 2022 human census, this region has 893,169 inhabitants. Zanzibar City has a tropical climate with an average temperature of 26.9˚C (80.4˚F) and an average annual rainfall of 1512 mm (59.5 in) [23]. The selection of the West Urban Region was based on the fact that the more recent cases of diarrheal-associated bacteria were reported in hospitals of the Urban West Region of Zanzibar [19].

2.2. Sample Collection, Design and Strategy

A cross-sectional study design was conducted where water well samples were collected from different locations in the Urban West Region of Zanzibar. The study area has a total of 710 wells; thus, by using the sample size formula, $N = Z^2 PQ/E^2/1 + Z^2 PQ/E^2n$, where Z = 1.96, E = 0.05, P = 50%, n = 750, a total of 250 wells were selected and used as the sampling points in this study. A total of 250 water samples (one from each well) were collected and transferred into sterilized Falcon tubes. The samples in falcon tubes were kept in a cool box with ice packs and then transported to Sokoine University of Agriculture (SUA) for laboratory analysis. At the same time, participants to whom samples were collected had to be interviewed on the possible risk factors for water contamination and diarrhoea diseases using a questionnaire. All questions to be asked were first translated into Swahili for easy communication.

2.3. Isolation of Bacteria from a Water Sample

The drinking water sample was inoculated in nonselective enrichment media (buffered peptone water) and then in selective enrichment media (tetrathionate broth media). Then each sample was inoculated on Xylose Lysine Deoxycholate (XLD) and blood agar (BA). The plates were incubated at 37˚C for 24 hrs. The suspected pure colonies of *Citrobacter* spp. were cultured further on nutrient media to have pure colonies. Identification was done by using colonial morphology, gram staining and biochemical tests which included SIM, TSI and IMVIC tests.

2.4. MALDI-TOF Test for Identification and Confirmation of Isolates

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) was further used for bacterial identification and confirmation. Pure 13 suspected *Citrobacter* colonies were recovered from blood agar, smeared on MALDI-TOF slides and entered into the VITEKMS (bioMérieux) instrument at Central Veterinary Laboratory (CVL), Temeke-Dar-es-Salaam. The confidence value of 95% - 100% was used as the cut-off point [24].

2.5. PCR and Sequencing of Bacterial Species

Molecular confirmation of 13 *Citrobacter* colonies was performed by conven-

tional PCR. Bacterial genomes were extracted by using the boiling method as described by [25] and stored at −20˚C waiting for PCR. Universal primers, UN20 (5'-AGA GTT TGA TC (CA) TGG CTC AG-3') and R1438 (5'-GCCC-TAGTTACCAGTTTTAC-3') are designed to give a product of approximately 1500 base pairs and complementary to conserved regions of *Citrobacter* 16S rRNA genes were used for PCR amplification [26]. Each PCR tube had 25 µl of total PCR volume containing 5 µl of bioneer premix, 0.5 µl of forward primer, 0.5 µl of reverse primer, 16 µl of nuclease-free water and 3 µl microliter of DNA. PCR conditions involved an initial denaturation temperature of 95˚C in 5 min and a final denaturation of 94˚C in 30 sec, an annealing temperature of 58˚C in 30 sec and an initial extension temperature of 72˚C in 2 min and a final extension temperature of 72˚C in 10 min with 35X number of cycles. The amplified products were resolved in 1.5% agarose gel run at 80 V for 40 min and visualized by using the gel documentation. Amplicons generated by PCR were then sent for sequencing for more resolved identification of isolates. Sequencing was performed by Microgen Company Ltd in the Netherlands.

2.6. Phenotypic Antimicrobial Susceptibility Testing of the *Citrobacter* spp.

Antimicrobial susceptibility testing was performed by using the disc diffusion method on Mueller Hinton agar, adhering to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2022). Six antimicrobial agents belonging to six different antimicrobial classes, namely gentamicin (CN 10 µg), tetracycline (TE 30 µg), ciprofloxacin (CIP 5 µg), sulfamethoxazole/trimethoprim (SXT1.25/23.75 µg), azithromycin (AT) and ceftriaxone (CRO) were used in this test. Test organisms were suspended in normal saline and suspension adjusted to that of 0.5 McFarland standards and then inoculated on Mueller Hinton agar plates in which antibiotic discs were firmly placed, followed by overnight incubation at 37˚C for 24 hrs [27]. Interpretation of the results was done by measuring zones of inhibitions with reference to CLSI, M100 2022 (32nd Edition).

2.7. Genomic Detection of Antimicrobial Resistance Genes in *Citrobacter* spp.

Confirmed *Citrobacter* spp isolates were further tested for the presence of antimicrobial-resistant genes using conventional PCR. The following genes associated with antimicrobial resistance for tetracycline (*tetA*, *tetB*), sulfonamides (*sul*1, *sul*2), and *β*-lactams (*bla*TEM, *SHV and CTX-M*) were tested. Bioneer premixes of 5 µl, 0.5 µl forward primer, 0.5 µl reverse primer, 16 µl nuclease-free water and 3 µl DNA were used, totalling up to 25 µl volume in each PCR tube. PCR conditions involved initial temperature denaturation at 95˚C for 5 min and final at 94˚C for 30 sec, annealing temperature at 58˚C for 30 sec, initial extension temperature at 72˚C for 2 min and final at 72˚C for 10 min used for *tetA*, *tetB*, sul2, *blaCTX-M*, *blaTEM* and *blaSHV*. However, for *sul*1 initial denaturation temperature was set at 95˚C for 5 min and the final at 94˚C for 1 min, the

annealing temperature at 55˚C for 1 min and the initial extension temperature at 72˚C for 2 min and the final at 72˚C for 10 min. The amplifications were 30X cycled. The amplified products of each gene were resolved in 1.5% agarose gel run at 80 V for 80 min and the gel images were viewed by using the gel documentation system. Primers used in the amplification reaction were obtained from [28]. All primers and their expected product sizes (base pairs) are shown in Table 1.

2.8. Data Analysis

Descriptive data analysis, such as proportions, was used to compute prevalence of *Citrobacter* spp and or antimicrobial resistance genes. On the other hand, the raw sequences generated after sequencing were edited using Bio Edit version 7.2 and Sequence Scanner version 2.0 to generate consensus sequences which were aligned to other sequences in the NCBI database to evaluate their similarity. Evolutionary analyses were conducted in MEGA XI whereby a phylogenetic tree was constructed to show an association between *Citrobacter* spp isolates and other *Citrobacter* spp. from the genebank [29]. Other side, the questionnaire survey data were organized by using Kobo software, entered into Microsoft Excel version 2019 for cleaning and coding and then imported into Statistical Package for Social Sciences (SPSS) version 20.0. In SPSS, the chi-square test was used to determine if there was statistical significance between the respondent's knowledge, attitudes, and practices and their demographic characteristics. All the results were considered significant at p-value < 0.05.

2.9. Ethical Consideration

The permission to conduct this study was obtained from Sokoine University of

Table 1. Set of primers used to detect antimicrobial-resistant genes in *Citrobacter* spp. and their amplicon size.

Genes	Primer sequences	Product size (bp)
tetA	F-5'-GGTTCACTCGMCGACGTCA-3' R-5'-CTGTCCCACMGTTGCATGA-3'	372 bp
tetB	F-5'-GAGACGCAATCGAATTCGG-3' R-5'-TTTAGTGGCTATTCTTCCTGCC-3'	228 bp
blaCTX-M	F-5'-SCSATGTGCAGYACCAGTAA3' R-5'-CCCGCRATATGRTTGGTGGTGGTG-3'	554 bp
blaTEM	F-5'-ATGAGTATTCMCATTTCCG-3' R-5'-CCMTGCTTMTCAGTGAGG-3'	858 bp
blaSHV	F-5'-ATGCGTTATATTCGCCTGTG-3' R-5'-AGCGTTGGCCAGTGCTCGATC-3'	862 bp
*Sul*1	F-5'-CGGCGTGGGCTACCTGMCG-3' R-5'-GCCGATCGTGMGTTCCG-3'	450 bp
*Sul*2	F-5'-GCGCTCAAGGCAGATGGCATT-3' R-5-GCGTTTGATACCGGCACCCGT-3'	625 bp

Agriculture (Ref. No. SUA/ADM/R.1/8/933) and the Office of Second Vice President Office (Ref. No. OMPR/M.95/C.6/3/VOL.XI/106).

3. Results

3.1. Cultural Identification of *Citrobacter* spp.

Bacterial cultures were observed after overnight incubation. *Citrobacter* spp. were identified on Xylose Lysine Deoxycholate (XLD) as red colonies with a black centre; this is due to the degradation of xylose leading to a change of the pH from acidic to alkaline, whereas hydrogen sulfide production under alkaline conditions caused colonies to develop black centres. After inoculation on nutrient agar (NA), colonies appeared grey-white, moist, circular discs with a smooth convex surface, while when observed on blood agar bacteria, showed entire red-coloured colonies, and stained negatively when observed microscopically after gram staining. Figure 3 shows *Citrobacter* colonies on XLD media, while Figure 4 shows *Citrobacter* colonies on nutrient agar.

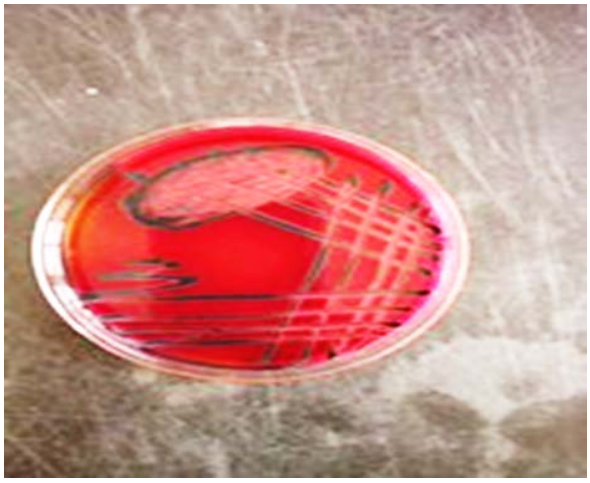

Figure 3. *Citrobacter* colonies on XLD media.

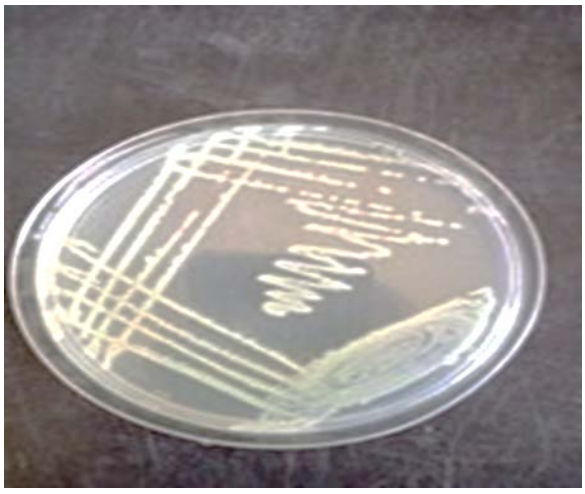

Figure 4. *Citrobacter* colonies on nutrient agar.

3.2. Biochemical Tests for Bacterial Identification

Three Biochemical tests, namely sulfide, indole and motility (SIM), triple sugar iron (TSI), and indole, methyl red, Voges-Proskauer, and citrate (IMVIC), were conducted against the 13 Citrobacter spp. suspected isolates. The results of these tests against the suspected colonies of *Citrobacter* spp. are summarized in Table 2.

3.3. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) for Identification and Confirmation of *Citrobacter* into Species Level

Citrobacter was identified using MALDI-TOF testing to specie level. This validated the suspected colonies identified using previous tests as *Citrobacter* spp. Table 3 contains a summary of the MALDI-TOF test results.

3.4. Molecular Confirmation of the Isolates by Conventional PCR and Genomic Sequencing

A total of 13 isolates were confirmed by PCR giving the expected amplicon size of 1500 bp (Figure 5). PCR products were sent for sequencing. The generated sequences were assembled and later to obtain the consensus sequences. The

Table 2. Biochemical tests for confirmation of *Citrobacter* isolates.

SIM			TSI				IMVIC			
Sulphur	Indole	Motility	Slant	Butt	CO_2	H_2S	Indole	Methyl red	Voges-Proskauer	Citrate
+	−	+	Alkali	Acid	+	+	−	+	+	−

NOTE: + stands for positive and − stands for negative.

Table 3. *Citrobacter* species confirmed by MALDI-TOF test.

No. of isolate	Genus	Species	Confidence value (%)
1	*Citrobacter*	*amalonaticus*	95
2	*Citrobacter*	*freundii*	99
3	*Citrobacter*	*freundii*	98
4	*Citrobacter*	*freundii*	98
5	*Citrobacter*	*werkmanii*	97
6	*Citrobacter*	*werkmanii*	99
7	*Citrobacter*	*werkmanii*	99
8	*Citrobacter*	*werkmanii*	95
9	*Citrobacter*	*braakii*	95
10	*Citrobacter*	*braakii*	96
11	*Citrobacter*	*farmeri*	98
12	*Citrobacter*	*farmeri*	99
13	*Citrobacter*	*farmeri*	99

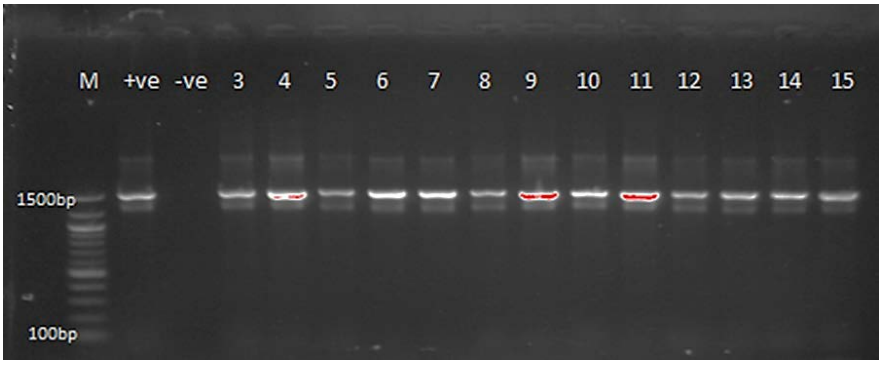

Figure 5. PCR amplification of 16 SrRNA from bacteria. Lane 1 contained DNA markers; lanes 2 and 3 were positive and negative controls, whereas samples were placed from lanes 3 - 15.

sequences were blasted to find the most similar reference sequences in GenBank. Following BLAST analysis, all the sequences were found to belong to the genus *Citrobacter* with percentage similarity ranging from 96% - 98%. The *Citrobacter* species and the location where they were isolated are presented in **Table 4**.

3.5. A Phylogenetic Analysis

A phylogenetic tree was constructed by aligning 16S rRNA sequences of different *Citrobacter* spp. and the sequences of the isolates from this study, are shown in **Figure 6**. Three sequences of the isolated *Citrobacter* among 13 isolates in this study were represented in the construction of a phylogenetic tree in which these sequences of isolated *Citrobacter* spp. were given names of their isolated areas as shown in the brackets in the phylogenetic tree including Shangani, Sogea and Magomeni.

3.6. Antimicrobial Susceptibility Profile of the Isolated *Citrobacter* spp.

All 13 *Citrobacter* isolates were assayed to assess their potential to resist six antimicrobials. Tetracycline, gentamicin, ciprofloxacin, sulfamethoxazole/ztrimethoprim, ceftriaxone and Azithromycin were used in the test. **Table 5** summarizes the results of the antimicrobial susceptibility of *Citrobacter* spp. against six antimicrobial agents.

3.7. Confirmation of Antimicrobial Resistance Genes in *Citrobacter* by PCR

Antimicrobial resistance genes corresponding to three antimicrobial agents, namely, tetracycline, ceftriaxone and sulfamethoxazole/trimethoprim were confirmed in 13 *Citrobacter* isolates using PCR. *C. braakii* amplified for *blaSHV* and sul2 genes, *C. freundii* for *tetA*, *blaSHV*, *sul*1 and *sul*2 genes, and *C. amalonaticus* for *blaCTX-M* and *sul*2 genes. Nevertheless, it was only for *C. werkmanii* and *C. farmeri* where there was no amplification of either gene. The results of the amplification of antimicrobial resistance genes in 13 tested isolates are

Table 4. *Citrobacter* spp. isolated from collected samples and the streets they were isolated.

Citrobacter species	No of isolates	Street name	Proportion (%)
C. amalonaticus	1	Sogea	7.69
C. freundii	1	Magomeni	23.07
	2	Kihinani	
	3	Sogea	
C. werkmanii	1	Shangani	30.79
	2	Mwera	
	3	Mikunguni	
	4	Tomondo	
C. braakii	1	Kijichi	15.38
	2	Kilimahewa	
C. farmeri	1	Kijichi	23.07
	2	Kijichi	
	3	Magogoni	

Table 5. Phenotypic antimicrobial susceptibility profile of the Citrobacter species.

Phenotypic	R		I		S	
	N	%	N	%	N	%
Gentamicin	0	0	0	0	13	100.0
Tetracycline	1	7.7	0	0	12	92.3
Ciprofloxacin	0	0	1	7.7	12	92.3
sulfamethoxazole/trimethoprim	5	38.5	0	0	8	61.5
Ceftriaxone	4	30.8	1	7.7	8	61.5
Azithromycin	4	30.8	0	0	9	69.2

NOTE: (R) = Resistance (I) = Intermediate (S) = Susceptible (N) = Number of *Citrobacter* isolates (%) = Percentage of *Citrobacter* isolates.

shown in **Figure 7**, whereas **Table 6** summarizes the results of the amplification of antimicrobial resistance genes in the tested isolates.

3.8. Social Demographic Variables

A total of 250 respondents were interviewed in this study, all were water well owners from the Urban West Region of Zanzibar. The social demographic characteristics of the respondents are summarized and presented in **Table 7**.

3.9. The Study Variables Related to Knowledge, Attitude and Practice

The results showed that variables related to knowledge having good knowledge

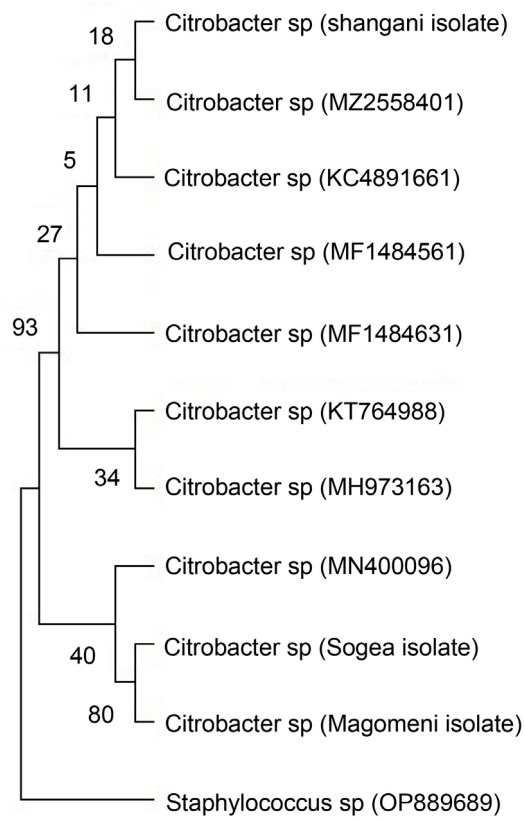

Figure 6. The phylogenetic relationship, on partial 16S rRNA sequence, of the *Citrobacter* spp. isolates, isolated in this study (Shangani, Sogea and Magomeni) to other *Citrobacter* spp. The tree was rooted with *Staphylococcus* spp. The scale bar represents the number of inferred nucleotide substitutions per site. Bootstrap values (1000 replicates) are shown at the nodes.

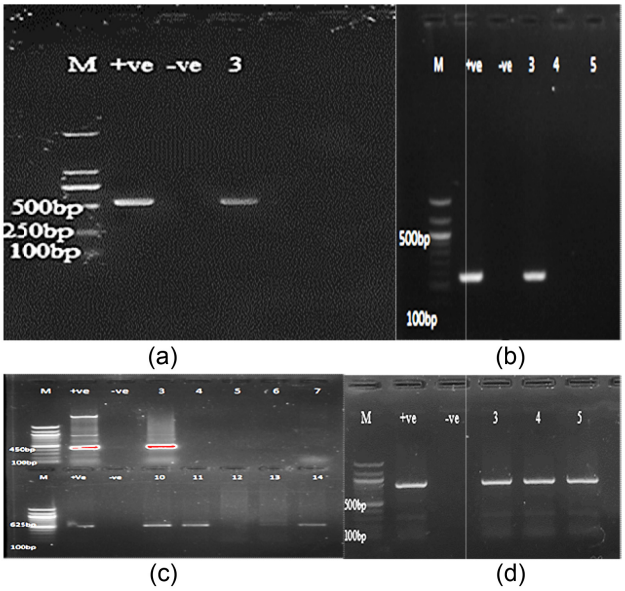

Figure 7. Gel electrophoresis picture showing resistant gene products: (a)-blaCTXM (554 bp), (b)-tetA (372 bp), (c)-sul1 (450 bp, loaded in the upper part) and sul2 (625 bp, loaded in the lower part of the gel), while (d)-blaSHV (862 bp)., M is a DNA ladder (100 bp).

Table 6. Detection of antimicrobial resistance genes in *Citrobacter* species.

Citrobacter species	No.of isolate	Street	Antimicrobial-resistant Genes						
			tetA	*tetB*	*blaCTX-M*	*blaTEM*	*blaSHV*	*Sul*1	*Sul*2
C. amalonaticus	1	Sogea	–	–	+	–	–	–	+
C. freundii	1	Magomeni	–	–	–	–	+	–	+
	2	Kihinani	–	–	–	–	+	–	+
	3	Michungwani	+	–	–	–	–	+	–
C. werkmanii	1	Kilimahewa	–	–	–	–	–	–	–
	2	Mwera	–	–	–	–	–	–	–
	3	Mikunguni	–	–	–	–	–	–	–
	4	Tomondo	–	–	–	–	–	–	–
C. braakii	1	Kijichi	–	–	–	–	+	–	–
	2	Shangani	–	–	–	–	–	–	+
C. farmeri	1	Kijichi	–	–	–	–	–	–	–
	2	Kijichi	–	–	–	–	–	–	–
	3	Magogoni	–	–	–	–	–	–	–

NOTE: (+) = present, (−) = absent.

Table 7. Social demographic characteristics of the respondents and their knowledge, attitude and practices on diarrhoea diseases.

Social demographic variables	Category	Total frequency (N = 250) and percentage (100%)	Knowledge N (%)		Attitude N (%)		Practice N (%)	
			Poor	Good	Negative	Positive	Poor	Good
Sex	Male	122 (48.8)	15 (6.0)	107 (42.8)	56 (22.4)	66 (27.2)	60 (24)	62 (24.8)
	Female	128 (51.2)	16 (6.4)	112 (44.8)	57 (22.8)	71 (28.4)	64 (25.6)	64 (25.6)
Age group	18 - 25	29 (11.6)	2 (0.8)	27 (10.8)	13 (5.2)	16 (6.4)	13 (5.2)	16 (6.4)
	26 - 35	52 (20.8)	6 (2.4)	46 (18.4)	25 (10.0)	27 (10.8)	23 (9.2)	29 (11.6)
	36 - 45	67 (26.8)	7 (2.8)	60 (24.0)	28 (11.2)	39 (15.6)	28 (11.2)	39 (15.6)
	46 - 55	81 (32.4)	6 (2.4)	75 (30)	31 (12.4)	50 (20.0)	44 (17.6)	37 (14.8)
	55+	21 (8.4)	10 (4.0)	11 (4.4)	16 (6.4)	5 (2.0)	16 (6.4)	5 (2.0)
Educational level	None	9 (3.6)	6 (2.4)	3 (1.2)	7 (2.8)	2 (0.2)	8 (3.2)	1 (0.4)
	Primary	38 (15.2)	16 (6.4)	22 (8.8)	27 (10.8)	11 (4.4)	26 (10.4)	12 (4.8)
	Secondary	156 (62.4)	9 (3.6)	147 (58.8)	79 (31.6)	77 (30.8)	90 (36)	66 (26.4)
	Advanced	12 (4.8)	0 (0.0)	12 (4.8)	0 (0.0)	12 (4.8)	0 (0.4)	12 (4.8)
	College/University	35 (14.0)	0 (0.0)	35 (14.0)	0 (0.0)	35 (14.0)	0 (0.0)	35 (14.0)
Occupation	Employed	50 (20.0)	4 (1.6)	46 (18.4)	15 (6.0)	35 (14.0)	15 (6.0)	35 (14.0)
	Not employed	118 (47.2)	16 (6.4)	102 (40.8)	73 (29.2)	45 (18.0)	78 (31.2)	40 (16.0)
	Self-employed	82 (32.8)	11 (4.4)	71 (28.4)	25 (10.0)	57 (22.8)	31 (12.4)	51 (20.4)

(87.6%) and poor knowledge (44.4%) as shown in Table 8. The variables related to attitude scored positive attitude (54.8%) and negative attitude (45.2%), as shown in Table 9. The variables related to practice scored good practice (50.4%) and poor practice (49.6%), as shown in Table 10.

Table 8. Knowledge of respondents on diarrhoea diseases.

Variables	Category	N (%)
Do you know any disease spread by water?	Yes	223 (89.2)
	No	27 (10.8)
Can you name any diarrhoea diseases and their symptoms	Yes	222 (88.8)
	No	28 (11.2)
Do you know the factors for the occurrence of diarrhoea diseases	Yes	216 (86.5)
	No	34 (13.5)
Can you name factors for the occurrence of diarrhoea	Yes	216 (86.5)
	No	34 (13.5)
Are diarrhoea diseases preventable?	Yes	219 (87.5)
	No	31 (12.4)
Ways to prevent diarrhoea diseases?	Yes	219 (87.5)
	No	31 (13.5)
Knowledge of diarrhoeal diseases	Poor	31 (12.4)
	Good	219 (87.6)

Table 9. Attitude of respondents on water wells sanitation.

Variables	Agree	Neutral	Disagree
Consuming water coming directly from the wells	133 (48.8%)	0 (0.0%)	117 (48)
Boiling drinking water can prevent diarrhoea	176 (70.4%)	14 (5.6%)	60 (24)
Treatment of drinking water with disinfectants can prevent diarrhoea	159 (63.6%)	20 (8%)	71 (28.4)
Treatment of drinking water is only when one gets diarrhoea	74 (29.6%)	23 (9.2%)	153 (61.2)
Water wells should always be cleaned	108 (43.2%)	73 (29.2%)	69 (27.6)
Drinking contaminated water might cause diarrheal diseases	140 (56%)	63 (25.2%)	47 (18.8)
Getting water from water wells by using containers can be a source of contamination	80 (32%)	93 (37.2%)	77 (30.8)
Overall attitude			**N (%)**
Negative attitude			113 (45.2)
Positive attitude			137 (54.8)

Table 10. Practice of respondents on water wells sanitation.

Variables	Category	N (%)
Distance from water well to pit latrine	>15 metre	140 (56)
	<15 metre	110 (44)
Type of water well	Closed drilled well	130 (48)
	Opened borehole	120 (52)
Means of getting water from the wells	Container	130 (52)
	Motor pumping machine	120 (48)
Cleaning water well	Yes	144 (57.6)
	No	106 (42.4)
Frequency of cleaning water well	Once a year	75 (30)
	Twice a year	27 (10)
	Not regularly	42 (17.6)
	Never clean	106 (42.4)
Ways of cleaning water well	Removing wastewater by pumping machine	120 (48)
	Removing wastewater and sand by container	130 (52)
Practice wells water sanitation	Poor	124 (49.6)
	Good	126 (50.4)

4. Discussion

Enterobacteriaceae comprises bacteria that have the potential to cause infections in animals and humans [30]. This study revealed that various species of *Citrobacter* are the prominent bacteria from the *Enterobacteriaceae* contaminating water used for human consumption in the urban west region of Zanzibar. Out of 250 collected water samples *Citrobacter spp*. were recovered in thirteen samples (5.2%). This is the first study to isolate these bacteria from water in Zanzibar, another study by [18] recovered a single isolate of *Citrobacter freundii* out of 470 patients who attended Mnazi Mmoja Hospital. Moreover, the observation during sample collection disclosed that recovery of *Citrobacter* spp. from wells water might be ascertained by the deposition of faecal through pit latrines built near the wells. The same phenomenon was also reported by [31], who explained that pit latrines were the major sources of faecal contaminants in Zanzibar water wells.

On the other side, our study revealed that building bored wells provides a suitable environment for harbouring water-contaminating animals such as snails that might be contributing significantly to the higher prevalence of pathogenic bacteria in wells water, this is proved by the isolation of *C. amalonaticus* from bored well in Sogea street which had significant nucleotides similarity to the bacteria which was once isolated by [32] from gastrointestinal tract of giant African snail (*Achatina fulica*). Giant African snails are also found in Zanzibar. Hence contribution of snails to bacterial contamination of water in the urban

west region should be taken into account. On molecular identification, all isolates belonged to the *Citrobacter* genus, as indicated on the phylogenetic tree. The taxonomic unit (node) in the phylogenetic tree showed that isolates from Sogea and Magomeni were closely related. This is the reason that these two streets are neighbours, thus, it is easy for bacteria sharing between two closed streets. Shangani isolate was not matched with Sogea and Magomeni isolates. This might be due to distance between them.

All 13 isolates of *Citrobacter* spp. obtained from different water wells of the Urban West Region of Zanzibar demonstrated susceptibility to ciprofloxacin and gentamicin. Some species of *Citrobacter* spp were resistant to sulfamethoxazole/trimethoprim, ceftriaxone, tetracycline and azithromycin. This variation in susceptibility could be explained by genetic variation of the bacteria [27]. Moreover, the types of antibiotics used had a significant impact on their antimicrobial activity, as all isolates were susceptible to gentamicin due to the fact that gentamicin is relatively infrequently used (reserved drug) compared to other antibiotics. It is worth saying that sulfamethoxazole/trimethoprim was a drug which received robust resistance compared to others, which may be due to its being overprescribed. Interestingly, this study reveals the existence of *Citrobacter freundii* with different antimicrobial susceptibility profiles despite the fact that they were isolated in the same region.

In addition to that, the detection of antimicrobial resistance genes in our isolates might be explained by environmental pollution, such as plastic litter around the wells. This was also observed by [33], who discovered that plastic litter around Unguja streets provided habitats for adherence, biofilm formation and dissemination of antimicrobial-resistant *Citrobacter freundii* in Unguja regions, hence genes and or bacteria could spread into water sources around the Island. Again, the Presence of antimicrobial resistance genes in bacterial genomes or plasmids often results in the expression of molecules, particularly proteins, with physiological effects on altering the intended function of the drug. This is revealed in our study since all phenotypically resistant isolates during sensitivity tests were genotypically harbouring either one or two of the putative-resistant genes for a respective group of drugs. For instance, *C. amalonaticus* showed phenotypic resistance to sulfamethoxazole/trimethoprim, which comes from the folate pathway antagonist family and ceftriaxone from the β-lactams family. During PCR, sul2 and *blaCTXM* genes were amplified from its genome. It is known that *Enterobacteriaceae* members have small outer membranes with a reduced number of porin channels which makes them resistant to hydrophilic molecules such as carbapenems and certain cephalosporin, hence this might explain the phenotypic resistance against ceftriaxone (cephalosporin) [34] [35].

On the other hand, this study evaluated the public's knowledge, attitude, and behaviour regarding many facets of good management and water hygienic practices that may be related to the occurrence of diarrheal diseases in the Urban West Region. Overall results showed that 87.6% of respondents had good knowledge of the questions that were asked, while 54.8% showed a positive attitude

and 50.4% had good practices.

On the knowledge component, 89.2% of the respondents stated that they were aware of some diarrheal diseases, such as cholera, typhoid, and gastroenteritis diseases, which frequently present with watery stools along with stomach pain, fever, vomiting, and headache. Additionally, 86.5% of the respondents were able to name a number of factors that can increase the risk of diarrheal disease occurrence, such as water contamination from improper waste disposal, running sewage from pit latrines, and street sewage and/or animals and their faecal discharges to water wells. 87.6% of respondents are aware of the preventative measures for diarrheal diseases, including the proper disposal of waste. Many respondents also recommended improving the sanitation of water wells by performing routine cleanings and using water disinfectants. This might be explained by the fact that a good number of respondents included in this study had at least secondary school education where these diseases are taught in biological classes.

Comparing our findings with other studies, it was revealed that our respondent's knowledge was less than the one obtained by [36] in which 95% of the respondents had knowledge of diarrheal diseases. This variation might be explained by the fact that [36] over-included mothers and their child caretakers as respondents, and these populations normaly succumb more diarrheal diseases, hence the results were slightly higher. On the other hand, our respondent's knowledge was higher than those included in the studies conducted in other areas such as Ethiopia 41% [37] and Cambodia 85% [38], which is explained by the reason that those studies were done on the indigenous people in rural areas who had less formal education and training on diarrheal diseases.

On the attitude component, the majority of respondents, 67%, believed in boiling and treating their well water before using it for drinking or other health-related purposes, and a majority of respondents, 54.8%, believed that routine water well cleaning can remove diarrheal agent contamination from water. Respondents (65%) think that drinking contaminated water directly from the water well can lead to the development of diarrhoea. The study's findings diverge from those of a previous study by [39] in Mkuranga, Tanzania, which found that 90.48% of respondents did not treat their drinking water because they believed that water coming from their source was safe.

According to the study's findings regarding the practice component, 50.4% of the respondents had good practice, and 56% percent of them dug water wells far than 15 meters away from their pit latrines, as was advised by national regulations on the appropriate distance between latrines and water points in various countries [40]. Also, our study findings showed that 57.6% of respondents clean their water wells. And 48% of the respondents have modern water wells (closed-drilled water wells) where an electric pump supplies the water. This is a less than the previous study done in Central Africa, which found that modern water wells were present in 51% [41]. Due to the high cost of digging modern drilled water wells that use electric pumps to get water, many residents in Zan-

zibar are unable to do so.

5. Conclusion

In conclusion, this study revealed the presence of antimicrobial-resistant *Citrobacter* spp contaminating drinking water in the Urban West Region. On the other hand, the KAP survey conducted in the region regarding the risk factors for contracting diarrhoea, were aware and knowledgeable on causes, transmission, effects and prevention of diarrheal diseases, and this impacted their beliefs and insisted hygiene practices.

6. Recommendations

Sustainable monitoring for antimicrobial resistance pathogens in both hospital and community settings in Zanzibar is hereby emphasized and assessing the role Snails play in ascertaining the prevalence of pathogenic microorganisms in well water, since in our study some Citrobacter species spp had nucleotide sequences similar to the one isolated from snails' GIT in India.

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Conflicts of Interest

There are no conflicts of interest among the authors in this study.

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