Diversity of pathogenic bacteria and incidence of antibiotic resistant genes in water used in eateries

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Abstract

Water used in eateries is a potential reservoir for acquisition and dissemination of antibiotic resistance, and human exposure to antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in drinking water may pose an additional health risk. Antimicrobial resistome monitoring is currently not a routine standard check of drinking water by health sector in developing countries. In the present study, culture dependent and illumina sequencing techniques profiled the occurrence of rich bacterial genetic diversity and resistome conundrum in water used by eateries in selected open markets. Analyses were done using R software and QIIME2 software. Heatmap outputs exhibited the correlation contours of morphological, biochemical, antibiotic susceptibility and antibiotic resistance genes (ARG) of culturable bacteria. Results show the following prevalent genera per sampled site; Wakulima Eateries (*Acinetobacter* 44.180%), Wakulima Open Market (*Duganella* 28.201%), Gashororo Eateries (*Acinetobacter* 15.189%), Gashororo Open Market (*Acinetobacter* 30.675%) Mwerevu Eateries (*Acinetobacter* 40.823%) and Mwerevu Open Markets (*Curvibacter* 48.785%). The *qnrD* and *sul2* ARGs were detected in all six samples, *int1* and *FloR* were present in five samples while *strB*, *catA* and *blaTEM* were detected in single sample using qualitative PCR. These findings form critical reference data for development of bacterial pathogen surveillance toolkit in the area.

INTRODUCTION

Drinking water is an indispensable resource for which it is challenging to maintain hygiene (it's an efficient medium for transmission of diseases) in urban areas under persistent anthropogenic influence (Numberger et al*.,* 2019). The availability of biologically safe drinking water is a major public health concern and relies on adequate protection of water sources, effective water treatment and proper distribution system maintenance (Han et al*.,* 2020). Most developing countries require the persistence of residual disinfectant, that is often ineffective, across all water distribution networks to control microbial waterborne pathogens (Han et al*.,* 2020). The presence and growth of microbes in treated drinking water and at the customers' taps is undesirable both for biosafety reasons and because of process-related microbial problems during distribution such as nitrification, bio-corrosion and persistence of pathogens (Liu et al*.,* 2018). Majority of eateries in peri-urban settlements in developing countries depend on unsafe groundwater (wells and boreholes) that are potentially contaminated with antibiotics, ARBs and ARGs for their daily requirements (Sanganyado & Gwenzi, 2019). The quality and Organoleptic features such as flavor, smell and color of groundwater are badly affected by both human activities and natural factors (Singh et al., 2020). The implications of climate change, uncleanness or insalubrious use of water reservoirs, population explosion, the mixing of sewage and agricultural runoff with water supply and the high per capita cost for new hydric infrastructures are few features which auxiliary bound the provision of water (Anand et al., 2016; Gwenzi et al., 2018; Singh *et al.,* 2020). As per WHO, the foremost parameters affecting water quality in terms of fit for utility are microbial (the presence of coliform Enterobacteriaceae like*Escherichia coli* or thermo-tolerant coliforms) and heavy metal contaminants (Waqar & Ali, 2020). Considering the shallow groundwater table, permeable aquifers,

the indiscriminate and abusive prophylactic and therapeutic use of antibiotics, the available untreated under groundwater poses a huge public health risk (Obayiuwana & Ibekwe, 2020). The contaminated under groundwater therefore provides a perfect selective and natural media for interaction between ARB and environmental bacteria for the horizontal shift of ARGs (Anand et al., 2016). ARGs have been detected in various water bodies, including reservoirs (Huerta et al., 2013; Su et al., 2014), source of drinking water (Jiang *et al.,* 2013), river (Ling et al., 2013; Xu et al., 2016), lake (Devarajan et al., 2015; Huerta et al., 2013) and ocean (Chen et al., 2019; Jiang et al., 2018; Zhang et al., 2019).

Although the quality and safety of drinking water are monitored by water authorities based on indicator microorganisms in bulk water, so a simple pass or fail, surveillance of biofilms or microorganisms during distribution is not part of current regulations (Douterelo et al., 2020). For instance, *Escherichia coli* is the World Health Organization (WHO) recommended faecal indicator bacteria for drinking water, while *Enterococci* is the indicator bacteria for bathing water (Acharya, et al., 2019). The routine disinfection of all pathogens is impractical, as each requires a unique period, and there is a demand for more rapid and comprehensive screening methods to detect Faecal Indicator Organisms (FIO) and/or their markers and putative pathogens in water samples (Acharya, et al., 2019). These traditional indicators are therefore limited with respect to their sensitivity, robustness and specificity to detect failures within Drinking Water Distribution Systems (Acharya, et al., 2019). These indicators are also not necessarily correlated with occurrences of new emergent pathogens and are only able to indicate high-intensity disturbances (Douterelo et al., 2020). As a consequence, there is a need to develop alternative bioindicators of water quality that can predict, measure and monitor changes within DWDS (Douterelo et al., 2020). Next-generation sequencing platforms offer high throughput, scalability, efficiency and generate a large dataset with legitimately high taxonomic precision and yields statistically robust assessments of community composition and population structural analysis. This culture independent approach is thus a powerful tool for elucidating the complex microbial diversities and the mechanisms of anti-microbial resistance in drinking water samples (Fang et al., 2019). Quantitative microbial risk assessment (QMRA) is the suitable method for evaluating and quantifying this health risk. However, information about the exposure to ARB and ARGs in drinking water in peri-urban settlement schemes of developing countries is lacking for many scenarios and dose-response models regarding the ARB infections are not developed yet.

The present study used NGS, PCR-based molecular markers for screening antibiotic resistance genes and biochemical approaches to catalogue molecular origin of known and emerging antibiotic resistance in water used by eateries in selected open markets.

MATERIALS AND METHODS

Study area and Sampling

The study was done in Juja town (1.1018° S, 37.0144° E) Kiambu county (1.0314° S, 36.8681° E) in Kenya. Purposive sampling technique was used to collect water samples used in eateries and open markets of Wakulima, Gachororo center and Mwerevu in Juja. The samples were collected during the outbreak of Covid-19 pandemic when washing of hands was encouraged and the study was seeking to know if Juja residents were using clean water. The sampling also considered nearness to dumping sites of wastes from these markets which are likely source of contamination of the water. A total of 82 water samples; Wakulima Eateries (WE)-17, Wakulima Open Market (WOM)- 12, Gashororo Eateries (GE)- 21, Gashororo Open Market (GOM)-12, Mwerevu Eateries (ME)-9 and Mwerevu Open Markets (MOM)- 11 were collected. Sterile falcon tubes and small water containers were used to collect water either from taps or water containers from each of the sampling points. The samples were labelled properly and kept on ice then transported to the Molecular Biology Laboratory at the Institute of Biotechnology Research in Jomo Kenyatta University of Agriculture and Technology (JKUAT) for immediate use and stored at -20 for subsequent experiments.

Characterization of culturable antibiotic resistant bacteria

Isolation of bacteria from water

Isolation was done using; MacConkey media (MaC 51.53g/l), Triple Sugar Iron medium (TSIA, 64.5 g/l, Thioglycollate medium (brewer) (TM) (26 g/l), Lauryl Tryptose Broth (LTB, 35.60 g/l and Nutrient Agar (NA, 28 g/l). From each water sample, 50 µl of water was pipetted and placed on each of the five different types of media in petri dishes and then spread using a sterile glass spreader till dry (Mbithi, 2013). The petri dishes were kept in the incubator for 24 hours at 37°C. Discrete colonies were isolated and sub-cultured on nutrient agar using streak plating method to obtain pure cultures (Temitope et al., 2013). A total of 42 pure cultures were obtained and preserved using as sterile loop to pick a colony from the plate and placing it in 20%(v/v) glycerol solution in cyro vials then vortexed and stored at -80 °C (Akinbankole et al., 2015) which were morphologically and biochemically characterized (Marzan et al., 2017).

Morphological characterization

Gram staining was done on all the isolates while the cell shape and Colony morphology (form, elevation, elevation, margin, size, color, surface, opacity and cell shape) were viewed and recorded as pictures using computerized microscope fitted with Scope Image 9.0 application. (Supplementary Table 2).

Biochemical characterization

Citrate utilization test

This was done using Simmons's citrate test protocol (Akinbankole et al., 2015). *Klebsiella pneumoniae* culture was used as a positive control while *Escherichia coli* culture was used as a negative control. The positive reaction showed a green color that changed to intense blue color while the negative reaction showed no growth and no color change along the slants.

Methyl red test and Voges Proskauer test

The tubes containing sterile Methyl Red-Voges Proskauer Broth were inoculated with freshly prepared cell colonies of the isolates (24 hours old) on nutrient agar in petri dishes. The tubes were incubated at 37°C for 24 hours after which aliquots of 1 ml of the broth were transferred to clean test tubes. A volume of 0.6mL of 5% α-naphthol was added, followed by 0.2 mL of 40% dipotassium phosphate to the broth. The tubes were shaken gently to expose the medium to atmospheric oxygen were allowed to remain undisturbed for 30 minutes. *Klebsiella aerogenes* culture was used as a positive control while*Escherichia coli* culture was used a negative control. The positive test showed the development of a red color after the 15 minutes while the negative result indicated a yellow-brown color.

Motility Indole Urease test

Motility Indole and Urea test was done using Motility Indole agar according to (Arya et al., 2020). The tubes were observed for urease test through change of color from yellow-orange to pink- red for a positive reaction and no color change for a negative reaction upon addition of the Kovac's reagent. *Escherichia coli* culture was used as a positive control while *Klebsiella pneumonia* was used as a negative control for indole test.

Catalase Test

A sterile wooden stick was used to transfer a small amount of freshly grown colony on the surface of a clean, dry glass slide. A drop of 3% of hydrogen peroxide was place in the colony on the glass slide. The slides were observed for evolution of bubbles. A positive reaction showed active bubbling while a negative reaction showed no or few bubbles. A positive control used was *Salmonella* while the negative control was *Enterococcus faecalis.*

Triple Iron Sugar (TSI) Agar Test

This was used to test for lactose, sucrose and glucose sugar fermentation and production of gases of hydrogen, carbon IV oxide and Hydrogen Sulphide. The TSI agar was prepared by dissolving 65.524g of this agar in 1000ml of distilled water. It was distributed into test tubes and sterilized in an autoclave at 121°C for 15 minutes and left to solidifying in a slanted position to give a 2.5 cm butt and a 3.8 cm slant in the safety

cabinet. A sterile wire loop was used to touch the top of a colony (24 hours old) followed by stabbing the butt through the middle of the agar to the bottom of the tube and then streaking of the slant. The test tubes were loosely closed with sterile cotton wool and incubated at 37 °C for 24 hours. A yellow color of both the butt and the slant indicated lactose and sucrose fermentation. A red slant and a yellow butt indicated glucose fermentation. Both the slant and butt remained red in some tests which indicated lactose, sucrose and glucose non-fermenters. A black precipitate in the butt showed production of Hydrogen Sulphide gas. Production of hydrogen and carbon IV gases were indicated by presence of bubbles, cracks or lifting of the media.

Antimicrobial susceptibility/resistant testing

All the pure culture isolates obtained during isolation were subjected to antimicrobial susceptibility test to determine the sensitivity or resistance of the bacteria using commercially available standard impregnated antibiotic disks (Biyela et al., 2018) containing; Ampicillin (AMP) 25mcg, Tetracycline (TE) 25mcg, Co-Trimoxazole (COT) 25mcg, Streptomycin (S) 10mcg, Kanamycin (K) 30mcg, Gentamycin (GEN) 10mcg, Sulphamethoxazole (SX) 200mcg, Chloramphenicol (C) 30mcg antibiotics. The isolates were spread on nutrient agar in petri dishes using spread plate method and then using a sterile forceps, the disks were laid on the spread bacterial isolates and incubated at 37^0C for 24 hours. Antibiotic susceptibility was determined by measuring diameters of zones of inhibition using a ruler. The isolates were classified as resistant or susceptible to a particular antibiotic depending on the inhibition zone results that were obtained (Bhargav et al., 2016).

Molecular characterization.

DNA extraction

DNA extraction was done from the 82 raw water samples collected from eateries and open markets of Wakulima, Gachororo center and Mwerevu-Juja. The extraction was done using Isolate II Genomic DNA Kit from Meridian Bioscience Company. The extracted DNA was stored in the freezer at -20 ^{0}C in PCR tubes for further analysis.

Polymerase Chain Reaction for amplification of 16S rRNA

Amplification of 16S rDNA of genomic DNA obtained from all the 82 raw water samples was performed using the 16S universal bacterial primer 515F-5' GTGCCAGCCGCCGCGGTAA and 806R-5' GGACTACT-CGGGTTTCTAAT to amplify the approximately 300bp of the 16S rRNA gene. PCR was prepared in a 40 µl reaction mixture per tube containing 0.5µl of 0.25 µmol/L of each primer, 18 µl of sterile ddH2O, and 20 µl of 5X MyTaq Reaction Buffer to which 1 µl of template DNA was used. The amplification of the template DNA was done in a programmed thermocycler.

DNA sequencing and phylogenetic analysis

A total of six samples containing pooled PCR products were sent for sequencing in Scot DNA Laboratory Company in the United States of America. The amplified DNA were pooled into six tubes according to the sites of collection; Wakulima Eateries (WE), Wakulima Open Market (WOM), Gashororo Eateries (GE), Gashororo Open Market (GOM), Mwerevu Eateries (ME) and Mwerevu Open Markets (MOM). The samples were then sent to Scot DNA Laboratory Company in the United States of America for sequencing. Sequencing wad done on the Illumina MiSeq

platform.

Phylogenetic analysis was done using Quantitative Insights into Microbial Ecology (QIIME) software to determine the genetic diversity of bacteria present in the untreated water. The Next Generation Sequencing (NGS) raw data (already demultiplexed and CASSAVA 1.8 format) were received from Scot DNA Laboratory Company and confirmed the quality of the reads (Phred score > 25) in the QIIME pipeline by obtaining the interactive quality plots. DADA2 was used for denoising and merging of the sequences to achieve the Operational Taxonomic Unit (OTUs) for downstream processing of alpha and beta analyses. The samples had different number of sequences and so had to be standardized by sub-sampling without replacement

through rarefaction. A sampling depth of 105230 that considered the lowest number of sequences among the samples was chosen to avoid losing any of the samples.

Alpha analysis was done utilizing Faith Phylogenetic Diversity (a measure of community richness) and evenness metrics (a measure of distribution of number of features per taxa in a sample). The was followed by Beta diversity analysis utilizing Emperor plots that allowed plot Principal Coordinates Analysis (PCoA) of the data. It generated Emperor plots for Weighted and unweighted Unifrac with PCoA showed distances of the Operational Taxonomic Units (OTUs) in terms of diversity between the three markets. The last step was taxonomic classification which utilized Naive Bayes classifier to assign a class label to the samples. The classifier generated interactive bar plots which were visualized at phylum level to determine the dominant phyla in each sample.

Detection of antibiotic resistance genes

The DNA from raw water samples were pooled into six tubes according to site of collection same as the DNA amplicons that were sent for sequencing. Polymerase chain reaction (PCR) was performed using sixteen ARG primers on all extracted genomic DNA (Supplementary Table 7). PCR reaction volume of 20 µl mixture containing 0.4µl of 0.2 µmol/L of each primer, 8.2 µl of sterile ddH2O, and 10 µl of One Taq® quick-load 2X master mix and 1 µl of template DNA was used. The thermal cycling protocol involved 3 minutes of initial denaturation at 94 °C followed by 30 cycles of denaturation for 30 seconds for at 94°C, 40 seconds annealing set at gradient PCR of between temperature 47-64 °C to determine the optimum annealing temperature of each primer and 1 minute extension at 68 °C. The final extension was performed for 5 minutes at 68 °C. Upon optimization, PCR was done using all primers on all the extracted DNA. PCR products were then separated by electrophoresis on 1.5 % agarose run at 150A and 160V for 45 minutes. Amplicons were visualized under UV light and recorded.

RESULTS

Isolation of bacteria

A total of 42 bacterial isolates were obtained from the water samples collected from the eateries (E) and open markets (OM) of Wakulima (W) 24 isolates, Gachororo (G) 9 isolates and Mwerevu (M) 9 isolates. The isolates were coded as follows; Wakulima market (WE1, WE2, WE3, WE4, WE5, WE6, WE7, WE8, WE9, WOM13, WOM14, WOM15, WOM16, WOM17, WOM18, WOM19, WOM20, WOM21, WOM22, WOM23, WOM25, WOM26, WOM27, WOM28), Gachororo market (GE10, GE11, GE12, GE24, GE29, GE30, GE37, GE38, GE40) and Mwerevu market (MWV31, MWV32, MOM33, MOM 34, MOM35, MOM36, ME39, ME41, MOM42). Gram staining technique was used to profile the morphology of bacterial isolates (Supplementary Table 2).

Morphological characterization

The correlation between morphological descriptors and their contribution on the phylogeny of the isolates is clearly profiled in Figure 1. Twenty-four out of the 42 bacterial isolates were gram negative bacteria while 18 were gram positive. Thirty-three of the bacterial isolates were rod shaped while nine were cocci shaped (Supplementary Table 2).

Biochemical characterization

The ability of isolates to utilize different substrates was equally useful in studying relationship between isolates (Figure 2). The differential strength in utilizing the substrates significantly clustered isolates as shown in figure 2.

Antimicrobial susceptibility testing

The antibiogram of tested isolates showed susceptibility or resistance to the eight antibiotics used. Out of the 42 isolates, 12 (28.57%) were resistant to ampicillin, 27(64.29%) were resistant to co- trimoxazole and sulphamethoxazole each, 8(19.05%) were resistant to streptomycin and kanamycin each, 3(7.143%) were

resistant to gentamycin and 9(21.43%) were resistant to chloramphenicol (Supplementary Table 5). On the other hand, 30 (71.49%) were susceptible to ampicillin, 15(35.71%) were susceptible to co- trimoxazole and sulphamethoxazole each, 34(80.95%) were susceptible to streptomycin and kanamycin each, 39(92.86%) were susceptible to gentamycin and 33(78.57%) were susceptible to chloramphenicol (Supplementary Table 5). All the 42 isolates were susceptible to tetracycline as shown in Figure 3 below.

The results of antimicrobial resistance pattern of the isolates showed that 16(38.10%) were resistant to 2 antibiotics, 7(16.67%) were resistant to 3 antibiotics, 3(7.143%) were resistant to 4 antibiotics, 2 (4.762%) were resistant to 5 and 6 antibiotics each. From this analysis it was evident that 30(71.43%) isolates were multidrug resistant when considered resistant to 2 or more drugs.

Molecular characterization.

Assessment of bacterial genetic diversity in water samples

Quality filtering of the sequence data from the water samples yielded 1,591,729 high-quality reads with a per sample read mean of 265,288. A total of 2,589 features were generated based on 97% similarity. The Faiths phylogenetic alpha diversity metric boxplots showed (p-value= 0.651) indicating that there was no statistically significant difference in terms of bacterial diversity in samples collected within the same market (Supplementary material, Figure 3). Pielou's evenness alpha diversity boxplots also showed (p-value= 0.180) indicating that there was no statistically significant difference in terms of distribution of bacterial species in samples collected within the same market as compared to another market (Supplementary material, Figure 4). Beta diversity analysis using PCoA plots of Bray-Curtis distances showed no significant distinction between the water samples (Supplementary material, Figure 5).

Water from all the three markets was dominated by bacterial phylum Protobacteria with; WE (96.057%), WOM (94.763%), GE (91.851%), GOM (88.955%), ME (95.285%) and MOM (86.063%) (Figure 4). Other Phyla that were found in the samples and highest in their respective

sites are Bacteroidota (MOM=9.967%), Verrucomicrobiota (GOM=1.646%), Deinococcota

(GOM=4.200%) and Firmicutes (WOM=1.229%) (Figure 4).

The genera belonging to Proteobacteria dominant in the six sites were; WE (*Acinetobacter*44.180%), WOM (*Duganella* 28.201%), GE (*Acinetobacter*15.189%), GOM (*Acinetobacter* 30.675%) ME (*Acinetobacter*40.823%) and MOM (*Curvibacter* 48.785%). The genus of*Acinetobacter* was the most abundant across the samples 4(66.67%) (Figure 5). The species of*Acinetobacter baumanniii*were detected in samples GE (2.975%) and WE (5.495%) while that of *Acinetobacter iwoffi*were detected in samples GOM (0.093%) and ME (5.296%) (Supplementary material Figure 6).

This study reported the presence of antibiotic resistant priority pathogens from the WHO list. The most abundant were pathogens belonging to priority 1 (Critical). The dominant group of bacteria, from the WHO priority pathogens list, was*Pseudomonas spp* detected in samples WE (12.163%) and WOM (0.323%) (Supplementary material Figure 6). The second most frequently isolated pathogen was*Acinetobacter baumannii* detected in samples GE (2.975%) and WE (5.495%) (Supplementary material Figure 6). The third most frequently isolated pathogen was*Enterobacteriaceae*detected in samples WE (0.125%), MOM (0.326%), WOM (0.875%), GE (0.452%) and ME (0.206%) (Supplementary material Figure 6). The genus of *Escherichia* , *Klebsiella* ,*Raoultella* and *Enterobacter* dominated in*Enterobacteriaceae* . The pathogen belonging to priority 2 (High) detected was the genus*Staphylococcus* in samples MOM (1.826%) and GE (0.962%) (Supplementary material Figure 6).

Detection of antibiotic resistance genes

The ARGs of *sul2* and *qnrD* were detected in all the six pooled samples. The *FloR* and *Int1* ARGs were present in five samples except in MOM and GOM samples resectively. Genes of*catA* and *strB* were detected in sample MOM while that of*blaTEM* was detected in sample GOM. The genes of *erm* X,*Amp, aac6, qnrC,*

qnrS, blaCTMX, aac3, NleA and TetA were not detected across all the six pooled samples (Figure 6). This is a clear indication that ARGs have been prevalent in water used in the three markets.

DISCUSSION

The present study revealed that a number of culturable antibiotic resistant bacteria were present in drinking water used by eateries in the sampled area. The study area relies heavily on water supplied from wastewater treatment plants and boreholes sources. Earlier studies have shown that drinking water and wastewater treatment processes are incapable of completely removing antibiotic resistance genes (ARGs), (Amarasiri et al., 2020). The observed resistance to antibiotics could possibly arise either from point mutations in the bacterial genome (intrinsic resistance) or through the acquisition of genes encoding resistance determinants (acquired resistance), (Das et al., 2020). The antibiogram results formed basis for screening of the antibiotic resistant genes primer specific PCR assays (Akpan et al., 2020; Zou et al., 2019). PCR assays showed that antibiotic resistance gene *qnrD* was detected in all samples. This ARG is a plasmid-mediated quinolone resistance (PMQR) determinant that encodes a pentapeptide repeat protein, responsible for inducing low susceptibility to quinolone by binding to DNA–DNA gyrase complexes (Zeng et al., 2020). Quinolones (ciprofloxacin, norfloxacin, ofloxacin, etc.) are among the most commonly prescribed antibiotics due to their broad-spectrum antibacterial activity, and the frequent use of quinolones has contributed to the emergence of quinolone resistance worldwide, posing a serious threat to public health (Zhang et al., 2020). The plasmid nature of *qnrD* plays a crucial role in the horizontal transfer of quinolone resistance genes due to their good self-replication and transmission characteristics and hence explains why it was detected in all samples (Zhang et al., 2020).

Sulfonamide resistance gene *sul2* were detected in all of the six samples exhibiting a frequency of 100%, the same as that of *qnrD*genes. This indicates that water used in these markets is highly contaminated with sulfonamides and the bacteria in this water have developed resistance against these antibiotics. *Sul2* is a plasmid borne gene encoding diyhdropteroate synthase that inhibit sulfa-based drugs due to mutations of *folP* gene encoding this enzyme (Wang et al., 2019).

Present in gram negative bacteria such as *Salmonella enterica typhimurium* . Sulfonamides function as broad-spectrum antibacterial agents and their prolonged use is likely to favor the selection of resistant bacteria. This similar occurrence has been shown in the detection of *sul2* genes in water from River Njoro in Nakuru county, Kenya (Itotia et al., 2018).

Previous studies reported domestic water from wastewater treatment plants are 'hot spots' of *sul* genes and MGEs and hence serve as reservoirs for the dissemination of the *sul* genes among bacteria (Lin et al., 2021).

Int genes were detected in all the samples except the Gachororo open market with a frequency of 83.33%. The presence of *Int*genes in the bacteria indicates the possibility to alternate antibiotic resistance among different bacterial strains as integrons are mobile and are capable of integrating and expressing gene cassettes by site-specific recombination (Adesoji et al., 2015). This means that one bacterium can possess multiple cassettes which code for different antibiotic resistance making it a multidrug resistant. This will make it very difficult to treat bacterial infections in Juja residents who use this water due to the presence of these integrons reducing antibiotic drugs potency. These genes have also been detected and reported in wastewater that are likely to mix with underground water used for human consumption (Gwenzi et al., 2020).

The genes of both *strB* and *catA* were detected in only Mwerevu open market while that of *blaTEM* was only detected in Gachororo open market which is a 16.67% frequency each. The genes of*strB* pose aminoglycoside resistance which have been reported as gene cassettes in relation with integron genes (Adesoji et al., 2015). That is why the genes of both *Int* and *strB* were detected in the same sample. The study showed the lowest prevalence of these genes in the water used in this area. An occurrence of these antibiotic residues has been detected in a study conducted on surface water in Nairobi County (Ngigi et al., 2019).

Acinetobacter baumannii is commonly found in environmental samples such as water and soil. There are several species that belong to this genus, however, the species of *Acinetobacter baumannii*accounts for 80% of

infections in humans (Vázquez-López et al., 2020). Other studies have shown that *Acinetobacter baumannii* is highly resistant due to the multiple antibiotic resistance genes that are harbored in this bacterium (Palavecino et al., 2022). For instance,*Acinetobacter baumannii* has been reported to contain resistant genes against Beta-lactam antibiotics and Quinolones and its resistance to Aminoglycosides is increasing (Georgina Solano-Gálvez et al., 2021; Karumathil et al., 2018).

Currently, the microbial indicators for surface water contamination used are: total coliforms, *enterococci* , fecal *streptococci* ,*Escherichia coli* , and *Clostridium perfringens* (Jałowiecki et al., 2022). These bacterial indicators have been widely used in monitoring microbiological water quality. Additionally, these microbiological indicators are both bacteria-specific and multi-antibiotic resistance (Jian et al., 2021). Therefore, the pathogens have acquired a new characteristic of multi-antibiotic resistance which is a threat to public health. The occurrence of these pathogens in the environment have escalated the mortality rate associated with microbial infections particularly those caused by multi-drug resistant species, such as: *Escherichia coli, Acinetobacter spp, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus* , and *Enterococcus* spp (Asokan et al., 2019). The species of *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae* , and *Pseudomonas aeruginosa* harbor antibiotic resistant genes against: penicillins, cephalosporins, carbapenems, monobactams, quinolones, aminoglycosides, tetracyclines, and polymyxins (Jałowiecki et al., 2022).

CONCLUSION.

The bacterial diversity revealed that the phylum Proteobacteria, largely found in groundwater, had the highest relative abundance (more than 85%) with its members having a relative abundance above 40%;*Curvibacter* (48.785%) and *Acinetobacter* (44.180%).

The molecular detection of ARGs in the water samples is a major public health concern and an indicator of poor water hygiene.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this manuscript. The datasets generated and/or analyzed during the current study are not publicly available as they are awaiting publication but are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

Alfrick Makori: Conceptualization (lead); writing – original draft (lead); formal analysis (lead); writing – review and editing (equal); Johnstone Neondo: Conceptualization (supporting); Writing – original draft (supporting); formal analysis (supporting); Writing – review and editing (equal); Ian Mwangi: formal analysis (supporting); Writing – review and editing (equal); Cecilia Mweu: Conceptualization (supporting); Writing –original draft (supporting); formal analysis (supporting); Writing – review and editing (equal).

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CONFLICT OF INTEREST

None declared

ETHICS STATEMENT

No formal consent or approval was necessary.

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MANUSCRIPT RESULTS

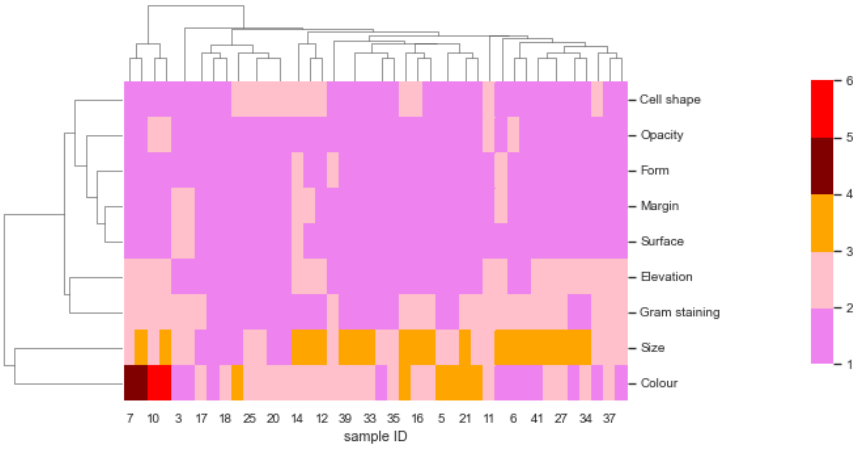

Fig. 1. A heatmap showing the morphological relationship among the bacterial isolates. Heatmap generated using the various phenotypic characteristics obtained from colony morphology of the bacterial isolates.

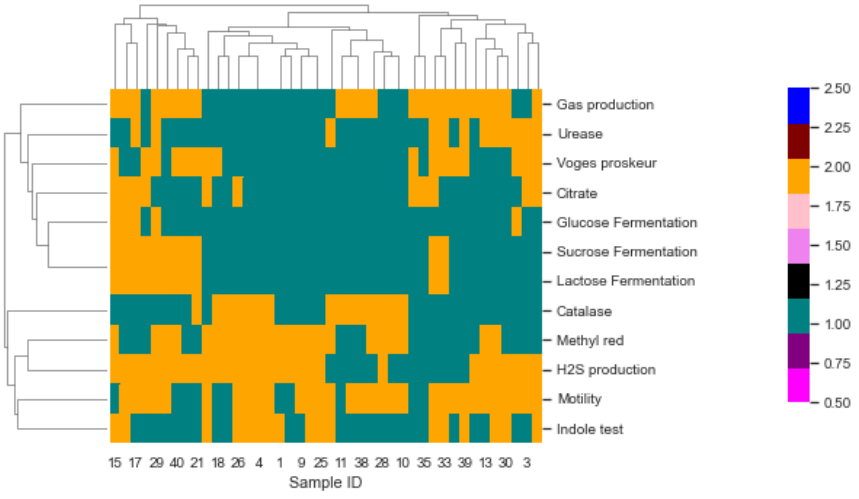

Fig. 2. A heat map showing the biochemical characterization relationship among the bacterial isolates.

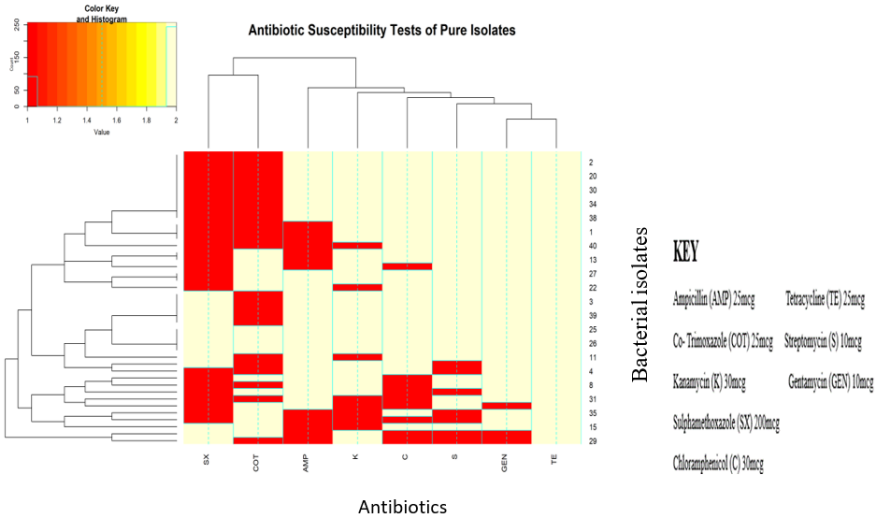

Fig. 3. A heat map showing antibiotic susceptibility test of pure cultures isolated from the water collected from Wakulima, Gashororo and Mwerevu markets.

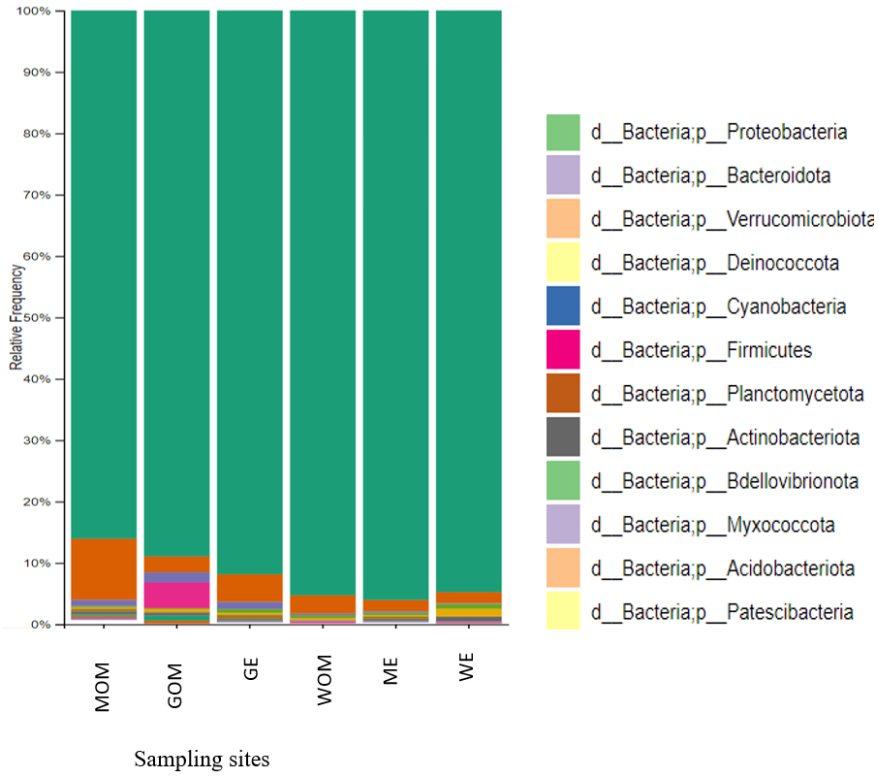

Fig. 4. Bacterial relative abundance of phylum-level taxonomic classification of water microbiota collected from Wakulima, Gashororo and Mwerevu markets.

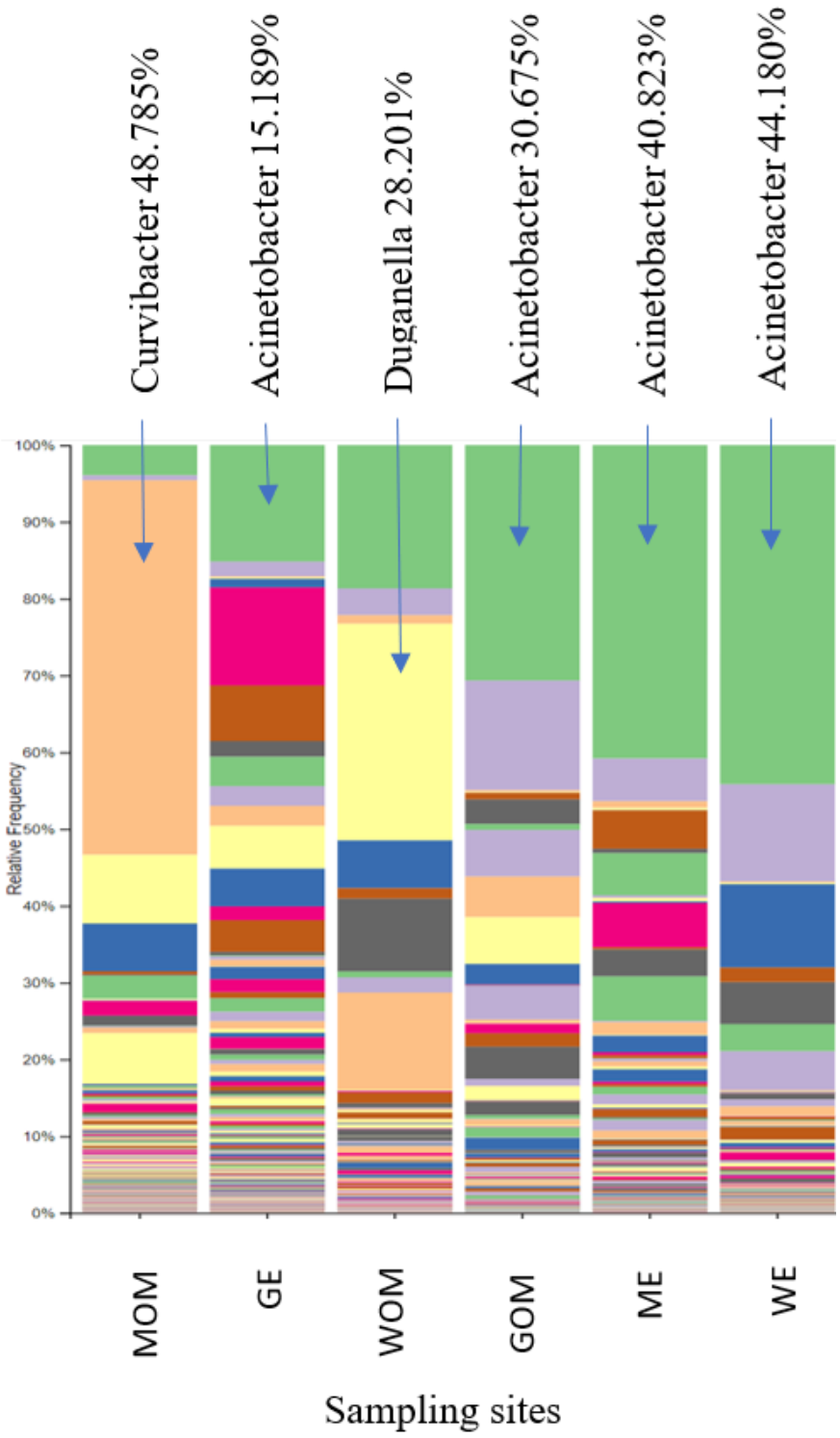

Fig. 5. Relative abundance of *Curvibacter, Actinobacter*and *Duganella* in water collected from Wakulima, Gashororo and Mwerevu eateries and open markets

Detection of antibiotic resistance genes in bacterial DNA.

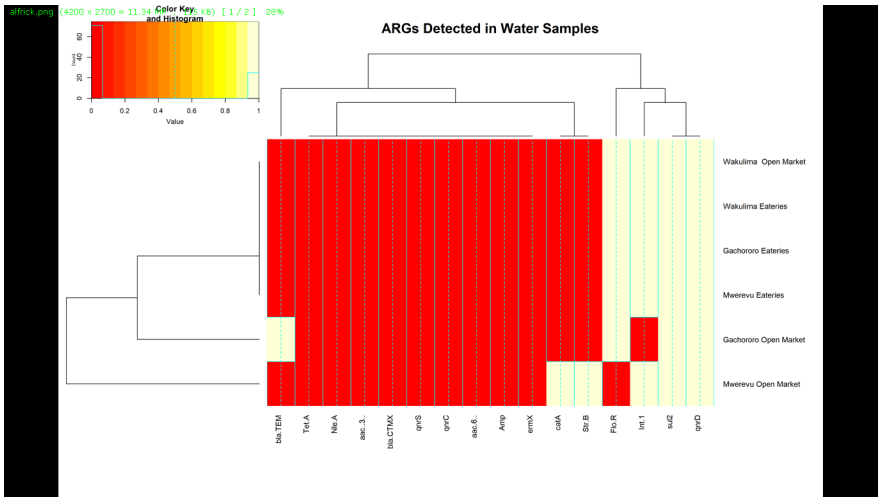

Fig. 6. A heat map showing the detection of antibiotic resistance genes in the water samples collected from Wakulima, Gashororo and Mwerevu markets.