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Research Article

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Isolation, Characterisation of Four Gram Negative Environmental Bacteria and Bacteriophage Plaques Formation on Their Lawns

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Abstract: In the recent past a diversity of microorganisms has been isolated from various environmental waters of different regions in Kenya. Owing to the frequent spread of waterborne diseases in different regions where the communities do not have access to clean potable water the objective of this study was to isolate disease causing bacteria especially diarrhoea. In this study we isolated and characterised four species of Gram-negative bacteria from environmental waters used for domestic purposes by some communities in Kenya. Attempts were also made to isolate potential bacteriophages from the environmental waters for application as biocontrol agents in water decontamination. Clear, circular plaques of different sizes were formed on lawns of each of the bacterial species which could form the basis for bacteriophage isolation. The sources of water included Lake Victoria, rivers, wells, ponds, beaches, boreholes, springs and Indian Ocean. The four bacterial species characterized by 16S rRNA partial gene sequences and phylogenetic tree were: *Vibrio cholerae*, *Escherichia coli*: O83, *Providencia sneebia* and *Proteus mirabilis*. The 16S ribosomal RNA gene, partial sequences were deposited in GenBank under accession numbers: MN467398.1 and MN907473.1 *(Escherichia coli*), MN467401.1 *(Providencia sneebia)*, MN467400.1 *(Proteus mirabilis)*, MN467399.1, MN907465.1 and MN907464.1 (*Vibrio cholerae*) bacterial isolates. On the phylogenetic tree, *P. sneebia* grouped most closely to *Morganella* species, a closely related genus. Overall, the phylogenetic analysis also indicates the bacterial species isolated were not identical to bacteria in the database, but rather new isolates of the given species. The presence of these bacteria in the environmental waters of Kenya was an indication that the water was not safe for human consumption. Development of clear phage plaques was an indication of the presence of lytic phages that can be used for subsequent bacteriophage isolation from each bacterial strain after concentration.

Keywords: Environmental, plaques, *Providencia sneebia, Vibrio cholerae, Escherichia coli, Proteus mirabilis*

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1. INTRODUCTION

Availability of clean, potable and quality water for domestic purposes is a fundamental part of life for all human beings. Proper sanitation should accompany the continuous availability of clean potable water. However, human activities have interfered with the natural water cycle affecting the relationship between society and water[1]. Clean water scarcity is a major issue in today's world populace of 7.8 billion people. The strain on the water system will be aggregated by unequal population growth in developing countries first in Africa and then in Asia where scarcity of clean water is already a major issue [2]. Contamination of water bodies can be caused by different types of microorganisms such as bacteria, viruses or protozoa causing diarrhoeal diseases and gastrointestinal illness. Water-borne pathogen contamination in ambient water bodies and related diseases are a major water quality concern throughout the world [3]. *Providencia sneebia*, a Gram-negative bacterium belongs to the genus *Providencia,* family of *Enterobacteriaceae*. The bacterium has been associated with humans as an opportunistic pathogen, commonly found to cause travellers' diarrhoea and urinary tract infections [4]. The different strains of the genus *Providencia* have been isolated from various environments. *Proteus* species are Gram negative, short straight rods, 1.5 to 2µm belonging to the *Enterobacteriaceae* family [5]. *Proteus* species are widely spread in the environment mainly in water, soil and the gastrointestinal tracts of humans and animals. *Proteus mirabilis* is the main cause of all the *Proteus* spp infections accounting for 80-90% of them [6]. It is most noted for infections of the catheterised urinary tract known as catheter associated urinary tract infections [5]. *Escherichia coli* is classified as a rod-shaped, Gram-negative bacterium in the family *Enterobacteriaceae*. The bacterium mainly inhabits the lower intestinal tract of warm-blooded animals, including humans, and is often discharged into the environment through faeces or wastewater effluent. The presence of *E. coli* in environmental waters has long been considered as an indicator of recent faecal pollution [7]. *E. coli* from a small number of O serogroups –O4, O6, O14, O22, O75 and O83 cause 75% of urinary tract infections [8]**.** *Vibrio* spp. are a group of common, Gram-negative, rod-shaped bacteria that are natural constituents of freshwater, estuarine and marine environments [9]. Cholera is a well-known disease since the 19th century and it is topping the list of microbial waterborne diseases. The causative agent of cholera, an acute intestinal water borne or food borne disease common cause of infant and adult deaths, is *Vibrio cholerae* can be isolated from both clinical and environmental sources during epidemics. Isolation of the bacterium from various surface waters in different countries has been summarised [10]. A phage plaque is a clearing in a bacterial lawn. Plaques form via an outward diffusion of phage virions that is fed by bacterial infection. Anything that slows phage diffusion can impede plaque development and thereby plaque size [11]. Plaque morphologies observed during the double agar overlay are used for differentiating the lytic and lysogenic phages because it is believed that lytic phages have a tendency to produce clear plaques while lysogenic phages produce turbid plaques [12,13]. Kenya is among the water scarce countries of Africa and over 80% of the population live in rural areas where water treatment using chlorine or alum is rarely practised. Most of the poor communities rely on unprotected or untreated water for domestic purposes from wells, rivers, lakes, beaches, ponds and boreholes. The spread of waterborne diseases is due to convergence of people in mass numbers at the inadequate water sources as they scramble for the commodity especially during drought. It is against this background that the research sought to assess the microbiological quality of environmental water sources used for various domestic purposes by poor communities in Kenya.

2. MATERIALS AND METHODS

2.1 Sampling sites and period

A total of 140 environmental water samples were collected from different regions of Kenya for a period of three years from March 2015 to August 2018. A total of 63 water samples from Kisumu, Kisii and Migori were collected from various sources (rivers, lake victoria, springs and wells) in March 2015, 28 water samples from the coastal region were collected from rivers, wells, ocean and boreholes in October 2016, 32 water samples from Siaya county were collected from rivers, beaches, ponds and wells in June 2016 while 18 water samples from Nairobi and central regions were collected from rivers, well and borehole between May to August 2018. The distribution of the sources of environmental water samples was as follows: 75 water samples were collected from rivers, 21 from beaches, 9 from lakes, 8 from ponds, 8 from springs, 6 from wells, 12 from boreholes and 1 sample from the Indian ocean. These were some sources of surface waters used for various domestic purposes by the surrounding communities especially in the Lake region. The water samples were collected in 500-millimetre sterile plastic bottles with narrow necks and transported in a cool box to the laboratory for isolation of bacteria and for development of phage plaques. At the time of sampling both temperature and pH were measured using a portable thermometer and pHmeter (EZDO, PH5011A), respectively.

2.2 Culturing and identification of bacteria.

Tryptose Soy Agar (TSA) was used for culturing *Providencia sneebia*, *Proteus mirabilis* and *Escherichia coli*: O83. Tryptose soy broth (TSB) (Oxoid, Basingstoke, Hampshire, England) was also used for culturing these bacteria where a colony of each bacteria grown on TSA was emulsified in TSB and incubation done at 37°C for 12-24 hours [36 37 42]. Alkaline peptone water (APW) broth was used for enrichment of *Vibrio cholerae* (HiMedia, Mumbai, India). Tryptose soy broth was also used for routine culturing of *Vibrio cholerae* (CMO 129 Oxoid LTD, Basingstoke, Hampshire, England). As a selective medium for isolation and culturing of *Vibrio cholerae*, Thiosulfate Citrate Bile Salt Sucrose (TCBS) was used (HiMedia, Mumbai, India)[24]. The presences of yellow colonies were suspected to be *V. cholerae*.

2.3 Enrichment of environmental water samples, Plaque formation and purification

Enrichment of the environmental water samples for bacteriophage isolation was done according to the procedure described by Van Twest et al. [14]. Each of the four bacterial strains was grown in single strength TSB (Tryptose Soy Broth) for 12hr, 10ml of the host bacterium mixed with 10ml of the environmental water samples in a 250ml Erlenmeyer flask, 20ml double strength TSB that had been supplemented with 2mM$CaCl_2$ added, mixed well and incubated for 48h at

37°Cin a shaker at 100 revolutions per minute (rpm). After 48hr, 30ml was transferred onto a falcon tube, centrifuged at 3400rpm for 15minutes and supernatant passed through a sterile syringe mounted on 0.45µm pore size filter. Serial dilutions were performed using SM buffer (100 mM NaCl, 8.1 mM MgSO4, 0.05 mM Tris-Cl [pH 7.5], 0.01% gelatin), 100µl of selected dilution mixed with 500µl of each of the 12h culture bacterial strain, 4ml of top agar (Tryptose soy broth containing 0.6% Bactoagar, Difco)) was added, mixed well by inversion and the mixture overlaid on the TSA plates. After settling, plates were incubated for 12h at 37°Cand examined for clear zones (plaques) which were indicative of bacterial lysis. A sample was scored positive for plaques when a clear zone was observed on the bacterial lawn in the plates. Double layer method was used for plaque assays as described by Kropinski *et al.* [13] and the subsequent purification. Purification of the phage plaques was done by picking a single plaque from each of the bacterial lawns, suspended in 1ml SM buffer, mixed thoroughly by inversion then passed through 0.45µm pore size filter. The filtrate was serially diluted, 500µl of each of the 12h culture respective bacteria added, mixed with 4ml top agar by inversion the mixture overlaid on the TSA plates. Incubation was done at 37°C for 12h to obtain separate plaques. Three rounds of purification were done to obtain clonal phage plaques with potential for phage isolation of each of the four bacterial strains. For future analysis a clear zone was picked with a sterile pipette, suspended in 1ml SM buffer, mixed by inversion, passed through 0.45µm pore size and stored at 4°C.

2.4 Molecular analysis of the bacterial isolates

For further identification of the bacterial strains isolated from the environmental waters of Kenya, sequencing of 16S ribosomal RNA gene was performed according to the method described by Rainey *et al.* [15]. Bacterial DNA was extracted for polymerase chain reaction (PCR) in order to amplify the 16S rRNA gene using the universal primers 27F (5'-AGA GTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTAC CTTGTTACGACTT-3') Specific primers, Y1 (5'-TTACCGG ACGCCGAGCTGTGGCGT-3') and Y2 (5'-CAGGAAGA TGCGTTATCGCGAGT-3'). For extraction of bacterial DNA, a boiling method was used where one big colony or several small colonies were mixed with 50µl of ddH_2O and subjected to 4 cycles of 98°C for 2 min and 4°C for 1min. PCR in both cases was prepared using 50µl mixture containing 10X Taq buffer, dNTP Mix (2mM each), Primers (10µM), 25mM $MgCl_2$, Template DNA, 1.25U Taq DNA polymerase and ddH_2O. PCR conditions for the universal primers were: 95°C for 3 min, followed by 34 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min with the final extension being set at 72°C for 5 min. Similar conditions were used for Y1/Y2 primers except for the annealing temperature which was adjusted to 60°C. DNA was detected by gel electrophoresis in 1% Tris-acetate-EDTA (TAE) buffer and stained with Gel Red. The PCR products were sequenced by Sangon Biotech co. Ltd (Shanghai, China), China, followed by analysis of the sequences on NCBI Standard Nucleotide BLAST.

2.5 Bioinformatics and construction of phylogenetic trees of the bacteria species

The 16S partial gene regions were used to query the NCBI RefSeq Representative genomes database using BLASTn (accessed October 21 2020, organism: Proteobacteria). The top 10 hits for each sequence were taken to be used for the phylogenetic tree. Redundant hits were removed. *Lactococcus lactis* strain IL1403 (CP033607.1) 16S rRNA gene sequence was added as an outgroup for tree rooting. MAFFT (v7.388) [16] was used to align 16S sequences and raxmlHPC-PTHREADS (v8.2.4, -N 100 -f a -m PROTCATLG) was used to construct phylogenetic trees [17]. The tree was visualised using FigTree (v1.4.3) [18].

3. RESULTS

Table 1 shows the four strains of gram negative bacteria isolated from different environmental water sources in this study ie *V. cholerae, E. coli, P. mirabilis* and *P. sneebia.* Among the sampling sites was River Kuja where all the four strains of bacteria were isolated, *V. cholerae* was also isolated from Koleche pond in Siaya and River Nsongoni in Mombasa County while *E.coli* was also isolated from Nairobi river. These were some of the sources of environmental waters used by residential communities for various purposes.

Table 1: Characteristics of the bacterial strains isolated in this study

No.	Strain	Code	Sampling site	Source	Year of isolation	Accession No.
1	*Vibrio cholerae*	VC_ke	River Kuja	Environmental	2015	MN467399.1
2	*Vibrio cholerae*	Vc_Koleche	Koleche pond	Environmental	2016	MN907464.1
3	*Vibrio cholerae*	Vc_Nsongoni	River Nsongoni	Environmental	2016	MN907465.1
4	*E. coli*	Ec_Kuja	River Kuja	Environmental	2015	MN907473.1
5	*E. coli*	Ec_ke	Nairobi river	Environmental	2018	MN467398.1
6	*Proteus mirabilis*	PREM_ke	River Kuja	Environmental	2015	MN467400.1
7	*Providencia sneebia*	PROS_ke	River Kuja	Environmental	2015	MN467401.1

Fig 1: Plaques formed on a lawn of E. coli isolate Ec_Kuja

Figure 1 shows plaques on a lawn of *E .coli* were clear, round approximately 0.5mm in size. The clear plaques were an indication of bacterial killing by the bacteriophage.

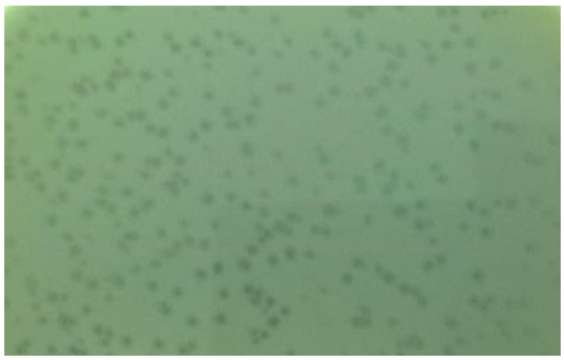

Fig 2: Plaques on a lawn of P. mirabilis isolate PREM_ke

The clear zones on the lawn of *P. mirabilis* bacterium shown above in figure 2 were an indication of bacterial lysis by the bacteriophage

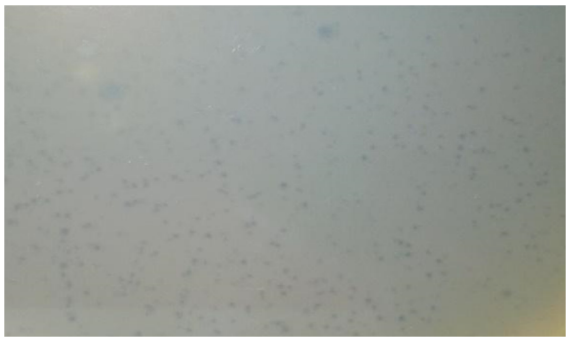

Fig 3: Plaques on a lawn of Vibrio cholerae isolate (Vc_kuja) displaying pinpoint plaques

The small clear zones shown in figure 3 were zones of cell lysis by the bacteriophages on a lawn on *V. cholera*

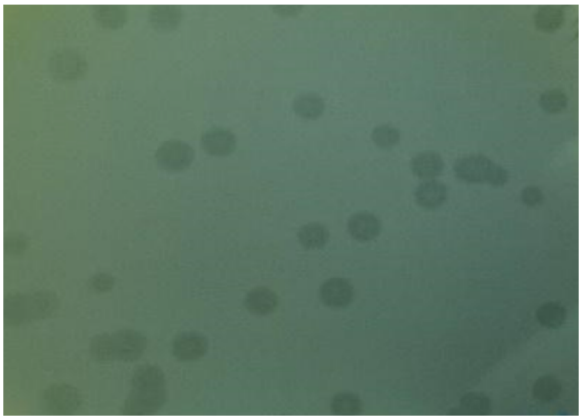

Fig 4: Plaques on a lawn of P. sneebia isolate PROS_ke

Figure 4 shows clear zones indicative of cell lysis by bacteriophage on a lawn of *P. sneebia*. The plaques formed were large more than 0.5mm.

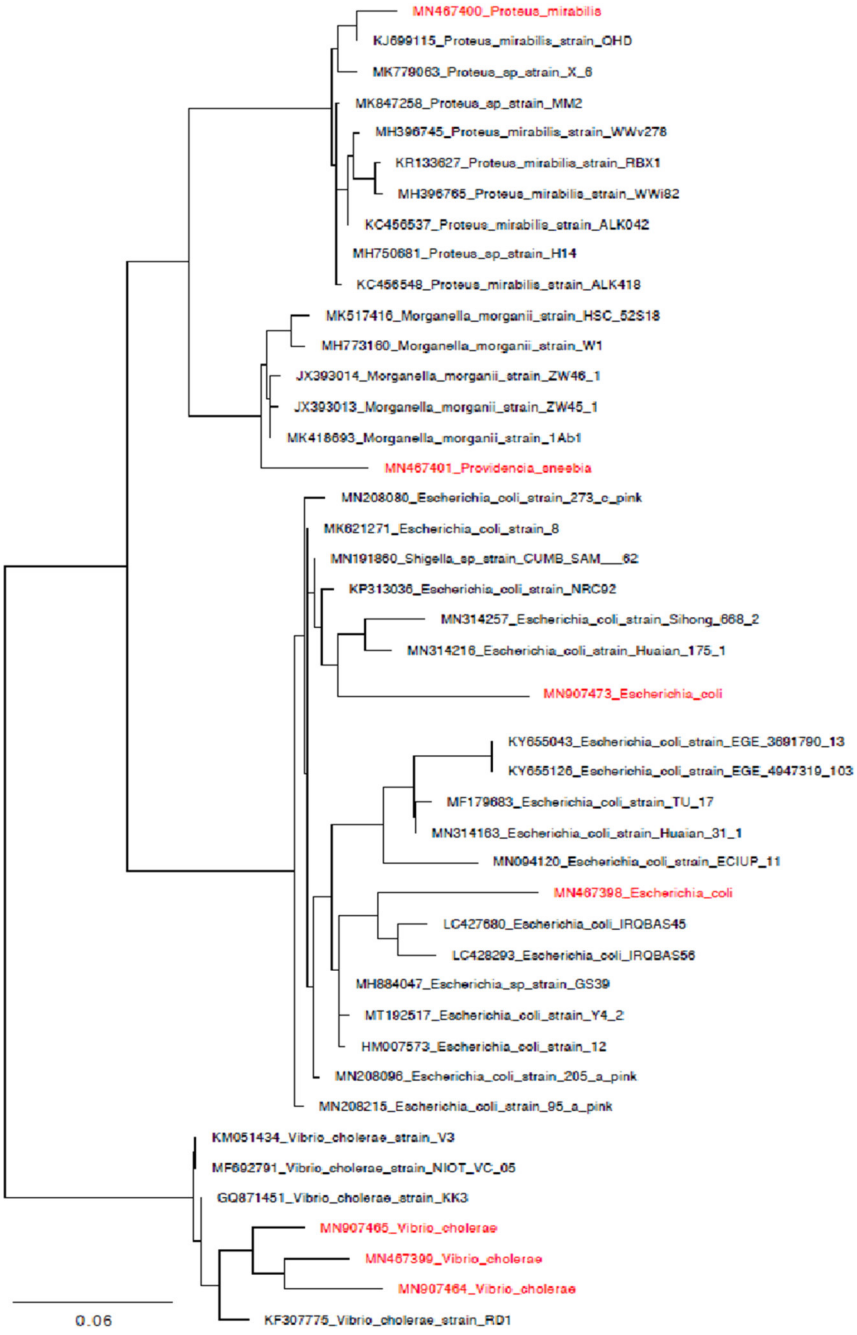

Fig 5:Phylogenetic relationship of the four environmental bacterial strains isolated in the study (red) to reference bacteria (black).

The 16S partial gene regions were used to query the NCBI RefSeq Representative genomes database using BLASTn (accessed Oct 21 2020, organism: Proteobacteria). The top 10 hits for each sequence were taken to be used for the phylogenetic tree. Redundant hits were removed. *Lactococcus lactis* strain IL1403 (CP033607.1) 16S rRNA gene sequence was added as an outgroup for tree rooting

4. DISCUSSION

Out of the 140 environmental water samples, four different bacterial species were isolated. The isolates, all Gram-negative rods were identified as *V. cholerae, E. coli:* O83, *P. sneebia* and *P. mirabilis*. Table 1 summarises characteristics of the bacterial species isolated in this study**.** According to the results displayed in Table 1, the four different bacterial species were isolated from river Kuja in Migori county, *V. cholerae* was also isolated from river Nsongoni in Mombasa county and Koleche pond in Siaya county. *E. coli* was also isolated from the Nairobi river, Nairobi County. In these three counties, residents obtain water from individual connections, public tap connections, boreholes, springs, wells, beaches, ponds, rivers or water vendors which remain unmonitored. Safety of this water is not entirely guaranteed and may be harmful to human health. According to our study findings, occurance of these bacteria in the environmental waters can result in water-borne illness with subsequent health illness among residencial communities consuming such contaminated water. Surface waters in most countries are polluted with pathogens and this is widely recorded in the

developing world. Consumption of these waters leads to waterborne disease outbreaks (WBDOs) [19]. People could get exposed to these microorganisms while drinking water, by eating food prepared with contaminated water, bathing, during recreational activities or even sometimes in healthcare facilities during dialysis [20]. Water, sanitation, and hygiene were responsible for 829,000 deaths from diarrheal disease in 2016. It is estimated that every year, 361,000 children under 5 years of age die because of diarrhea. In addition, poor sanitation and contaminated water are also linked to transmission of waterborne diseases such as cholera, dysentery, hepatitis A, and typhoid [21]. Previous studies have reported the existence of *V. cholerae* in surface environmental waters. An investigation carried out in Bepanda Cameron, well, tap and stream water were found to be reservoirs of *V. cholerae* and 33% of the 96 isolates were confirmed as *V. cholerae* O1 [22]. Studies done in Accra, Ghana confirmed presence of *V. cholerae* in environmental waters (streams and wells) [23]. During the cholera outbreak in Haiti in 2010, water collected from irrigation canals where local population used it for drinking purposes, *V. cholerae* O1 serotype ogawa, *ctx*A-positive strains were found and the communities near the canals were heavily affected by the outbreak [24]. In South Africa, surface water samples collected from Lenge dam, the Tyme River, the Sityi River and the Naikina River, *V. cholerae* were isolated in all the surface water samples by culture methods[25]. The study found out that 25% of the isolated organisms were found to be potentially toxigenic *V. cholerae.* In another study, *V. cholerae* was found to be present in Gutshwa, Komati and Crocodille Rivers of Mpumalanga Province in South Africa [26]. In Argentina, 18 water samples collected from Sali River (In canal norte and Banda) and Lules River, the isolated *V. cholerae* corresponded to *V. cholerae* non-O1 and non-O139 (Lules 26%, canal Norte 33% and Banda 41%) [27] . In Mozambique, water collected included 3 fresh water Lakes, 15 river water samples, 5 pond water samples and 4 estuarine water samples [28]. The results showed that presence of *V. cholerae* O1 only in nine (32%) of the total of 28 water samples from the river, estuarine, lake and pond water. These studies are therefore inline with the current study on occurance of *V. cholerae* in surface waters. In 2015, following an outbreak of cholera in Western Kenya, *V. cholerae* was isolated from river Riana in Migori. The same serotype of *V. cholerae* confirmed to be the source of the outbreak was also isolated from a patients' rectal swab[29]. The river was the source of water used for domestic purposes by the Migori people. According to WHO [29]. Kenya experienced an outbreak of cholera that started in Tana river at the Coast and later reported in other counties: Garissa, Vihiga, Nairobi, Mombasa, Turkana, Kericho, Nakuru, Kiambu and Narok. The outbreak was reported both in the general public and in refugee camps. A total of 1216 cases and 14 deaths was reported by July 2017, according to Unicef bulletin on cholera and Acute watery disease (AWD) outbreaks in Eastern and Southern Africa (ESAR) a total of 1198 cases including 4 deaths had been reported since the beginning of 2019 cholera cases being confirmed in Nairobi and Machakos [30]. Kenya was among the seven countries in Sub-Saharan that experienced cholera outbreaks between 2017 to 2018 [31]. This is evidence that the presence of pathogenic *V. cholerae* found its route to a certain population of Kenyan people either through consumption of contaminated food or water with the pathogen. Ramamurthy et al.[32] reported that only *V. cholerae* serogroup O1 Classical or El Tor biotypes and serogroup O139 are known to cause cholera epidemics. Other studies that showed presence of *V. cholerae* in environmental waters of Kenya include reports by [8]. Cholera disease appears not to be eradicable due to the fact that *V. cholerae* is a natural inhabitant of the aquatic environment [30]. In this study *V. cholerae* isolation for three environmental sources: river Kuja, Koleche pond and River Nsongoni was an indication that the pathogen continues to thrive in the environmental waters of Kenya. Apart from *V. cholerae*, *P. mirabilis* was also isolated from the environmental waters of Kenya from rivers Kuja in Migori county. Other studies have reported that *Proteus mirabilis* could be isolated from the environmental waters[32]. Their presence in water and soil may indicate faecal pollution from human and animal faeces into the natural environment. In Nigeria *Proteus* spp bacteria were detected in two of five studied well waters treated as a source of drinking waters [33]. Alarming presence of multidrug resistant *P. mirabilis* and *P. vulgaris* strains among other bacteria in drinking water from the springs and streams in rural areas of Sikkim, India, was reported [34]. Besides causing diarrhoea, a case of food poisoning in a restaurant in Beijing, China was reported to have been caused by *P. mirabilis* [35]. Isolation of *P. mirabilis* from river Kuja in Migori county, is inline with the previous studies and therefore drinking water at such a time of sampling could cause diarrhoeal diseases. A study in Nigeria showed that borehole water was a source of *V. cholerae*, *E. coli* and *Providencia sneebia* during rainy and dry seasons [36]. These three bacteria also present in the surface waters of the current study have been reported to cause different forms of diarrhoea hence a health hazard. Their presence in water was an implication that the water was of poor microbiological quality. Faecal coliforms and *E. coli* have been used for decades for identification of faecal contamination to indicate presence of microbial pathogens in water[37]. Therefore, the presence of *E. coli*:O83 in surface waters of rivers: Kuja in Migori and Nairobi River in Nairobi county as shown in Table 1 was a clear indication that the water had been contaminated with pathogenic bacteria, hence not fit for human consumption. The possible contamination could have been attributed to heavy rains experienced in the country in the year 2018. Faecal contamination of river Kuja, Migori county with *E. coli* :O83 could have been due to various human activities taking place in the river like bathing, washing clothes, swimming, domestic animals like cows taken to the river for drinking water among others. Prior studies on the bacteriological quality of water of the river of the metropolitan capital city of Kenya, Nairobi river are in agreement with the current study results where *E. coli* was also isolated[38]. Other bacteria isolated from the Nairobi river included *V. cholerae*, *P. mirabilis* and *P. aeruginosa*[38]. According to WHO, 80% of all diseases in developing countries result from contaminated water. The spectrum of pathogenic and potentially pathogenic microorganisms spread by water is extensive [1]. This is supported by the current study as it has proved the existence of potential pathogens in Kenyan environmental waters. Figures 1 to 4 display the morphology of the plaques on the bacterial lawns that depicted clear, round plaques of different sizes classified as ranging from very small/pinpoint, medium and large. Plaques formed on a lawn of *P. sneebia* were classified as large, plaques on a lawn of *E. coli* were classified as medium while on lawns of *V. cholerae* and *P. mirabilis* plaques formed were described as pinpoint or very small. Previous studies by Elves et al. [39] on *P. mirabilis* bacteriophages showed plaques that were small, circular and clear with consistent diameter. The taxonomy of host bacteria was determined according to 16S partial gene

sequence identity and verified using phylogenetic relationships to reference bacteria (Figure 5). On the phylogenetic tree, *P. sneebia* grouped most closely to *Morganella* species, a closely related genus. Overall, the phylogenetic analysis also indicates the bacterial species isolated were not identical to bacteria in the database, but rather new isolates of the given species. Oliveira et al. 2017 isolated and characterized a phage named PR1 (vB_PreS_PR1) from sewage enriched with *Providencia* strains from different human clinical specimens. PR1 produced very small plaques on a layer of all sensitive bacteria (0.1-mm diameter) unlike in our current study where [Plaques were large. More studies can be done on the plaques formed on a lawn of *P. sneebia* in our study forming the basis for bacteriophage isolation [40]. Recently, Necel et al. 2020 isolated bacteriophage, vB_Eco4M-7, which effectively infects many, though not all, *Escherichia coli* O157 hosts with relatively small uniform plaques (1mm diameter). Three phages JSF3, JSF4 and JSF7 were initially isolated from different samples of river water in Dhaka, Bangladesh produced clear plaques with a diameter of ~ 1 mm on a lawn of their respective *Vibrio cholerae* host bacteria [41]. The plaques formed by *E. coli* in our study were relatively medium. Several studies have been attempted for isolation of bacteriophages with the potential of combating *P. mirabilis* and an application of *P. mirabilis* bacteriophages to prevent blockage of catheters and biofilms has been demonstrated [42]. According to WHO, 80% of all diseases in the developing countries results from contaminated water[1] The spectrum of pathogenic and potentially pathogenic microorganisms spread by water is extensive. This is supported by the current study as it has proved existence of potential pathogens in Kenyan environmental waters. Majority of the rural communities in Kenya depend on surface water sources for all their water needs and the presence of these bacteria especially *V.cholerae* indicates that these communities are at risk of cholera infection since proliferation of the pathogen is favoured by the environmental conditions. According to census results of August 2019, the population of Kenya stands at 47 million people and constant supply of clean potable water should be a priority. Follow up on the microbial safety and quality of the surface water through periodic bacteriological analysis, diarhoeal diseases surveillance, proper sanitation and implementattion of hygienic standards should be carried out to prevent occurance of these waterborne bacteria in environmental waters.

5. CONCLUSION

The current study revealed the presence of four different Gram negative bacterial species in environmental waters of Kenya in four different regions posing a threat to human health. The four bacterial species formed clear phage plaques that have potential for further studies in characterisation of the bacteriophages that can be used in biocontrol of the respective bacteria. The phage plaques can be concentrated for further characterisation like TEM, full genome analysis and the phages can be used for biocontrol of the respective bacteria in the environment.

6. AUTHORS CONTRIBUTION STATEMENT

ANM conceptualised this manuscript and designed the experiments with the guidance of MJ and EBM. ANM carried out the laboratory experiments. Analysis and data interpretation was done by ANM and KK with guidance of MJ and FBM. Manuscript was written by ANM and verified by MJ, KK and FBM while KK helped with phylogenetic tree analysis. All the Authors helped in shaping the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none

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