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Detection of *Escherichia coli* and Harmful Enteric Bacterial Pathogens in Domestic Hand-Dug Wells in the Cuvelai Etosha Basin of Namibia

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Abstract

The Cuvelai Etosha Basin of Namibia is characterised by complex aquifer systems with multi-layered aquifers and various water qualities. Some parts of the basin have been covered with a pipeline system that supplies purified surface water from the Kunene River. Locations that lack a pipeline system utilise hand-dug wells as a source of drinking water. These wells draw water from shallow perched aquifers and are not protected from surface contamination nor is the water quality monitored. Sanitised water supply is relevant for the growth and development of societies and is a priority of the United Nations Millennium Development Goals. A bacteriological water quality study aimed at investigating the presence and seasonal variation of; *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species was conducted on 44 hand-dug wells in the Ohangwena and Omusati regions of the Cuvelai Etosha Basin. Samples were collected from both the wet and dry seasons. Results disclosed the presence of *Salmonella*, *Shigella*, *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, and *Pseudomonas* species. Chi-square confirmed a significant seasonal variation in *Salmonella* ($P < 0.05$) and *Shigella* ($P < 0.05$) species, and no significant seasonal variation in *Citrobacter* ($P > 0.05$), *Escherichia* ($P > 0.05$), *Klebsiella* ($P > 0.05$), *Enterobacter* ($P > 0.05$), *Proteus* ($P > 0.05$) and *Pseudomonas* ($P > 0.05$) species. Water from these hand-dug wells is not safe for drinking unless it is subjected to appropriate treatment. It is recommended that hand-dug wells should be properly constructed at safe distances from contaminating structures such as

pit latrines and routinely assessed for pathogens, and the water should be sanitized prior to consumption.

Keywords

Citrobacter, Cuvelai Etosha Basin, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, Water

1. Introduction

The Cuvelai Etosha Basin (CEB) of Namibia hosts complex aquifer systems with multi-layered aquifers containing various water qualities [1]. This basin is shared between Angola (36%) and Namibia (64%), and is mainly comprised off our Namibian administrative regions; Oshikoto, Omusati, Ohangwena and Oshana. Namibia has no piped water supply in some rural areas of the CEB and thus communities rely on rain water harvesting or groundwater sources such as boreholes, open deep wells and shallow wells [2]. However, water shortages are exacerbated by Namibia's climatic character of high temperatures, short rain seasons and long dry seasons that lead to increased rain water evaporation rates [3]. Namibia's wet season is variable but mostly occurs between December and May, and the dry season occurs between June and October.

Some parts of the basin with high population densities have been covered with a pipeline system that supplies purified surface water from the Kunene River [4]. Communal boreholes in place are not generally utilised due to long distances from homesteads, and water quality problems such as high total dissolved solids (TDS) or fluoride concentrations [4]. Other parts of the basin use hand-dug wells as a source of drinking water. These hand-dug wells draw water from shallow perched aquifers and are not protected (**Figure 1**) from surface contamination nor is the water quality monitored. This is undesirable since water borne

Figure 1. Hand-dug well used for domestic purposes in the CEB of Namibia.

diseases are one of the major health concerns in developing countries [5] and water ecosystems support the growth of various bacterial, fungal, protozoal and viral pathogens. Namibia displays a high prevalence of diarrhoea and cholera mostly emanating from drinking contaminated water [6]. Agatemor and Agatemor argued that clean water supply is relevant for the growth and development of societies and is a priority of the United Nations Millennium Development Goals [7]. Therefore, a bacteriological water quality study aimed at investigating the presence and seasonal variation of; *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species was conducted on hand-dug wells in the CEBin Namibia.

2. Materials and Methods

2.1. Sampling

Purposive sampling was employed in this study. Samples were collected from hand-dug wells which have been monitored for hydrochemical water quality by a PhD candidate Josephine Hamutoko. Forty-four (44) hand-dug wells from Ohangwena and Omusati regions of Namibia were studied in order to generate information on the microbiological water quality of the hand-dug wells during the dry and wet season. The water samples were collected in sterile 200 ml bottles from the hand-dug wells by lowering them into the hand-dug wells using a rope which was tied to the bottles. The bottles were then placed on ice during transportation to the University of Namibia for analysis.

2.2. Culture Based Isolation and Identification of Bacteria

Water samples were centrifuged at a speed of 7000 *xg* for an hour in order to concentrate the bacteria which was then cultivated on selective and differential MacConkey agar. MacConkey agar (CM0007) was used to isolate and identify the selected gram negative bacteria and to determine coliform counts. Inoculation was performed and the plates were inverted and incubated at 35˚C for 48 hours for total coliform counts. It also distinguished between lactose-fermenting and lactose non-fermenting gram-negative enteric bacilli. The two groups namely; 1) lactose fermenting and 2) non-lactose fermenting were then subjected to a different flow of biochemical tests as outlined in **Figure 2**.

1) Lactose positive isolates which appeared pink in colour on the MacConkey agar were tested for indole production using the differential SIM media according to manufacturer's guidelines (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The SIM media was then dispensed into tubes and sterilized by autoclaving at 121˚C for 15 minutes, andthe bacteria were then inoculated into the tubes by stubbing down the centre of the medium using an inoculating loop to within the bottom ⅓ of the tube. The tubes were incubated with loosened caps at 35˚C for 18 - 24 hours and observed for H_2S production and motility. To detect indole production, 3 - 4 drops of Kovac's reagent were added to the tubes and observed for a red colour development. The indole positive strains were

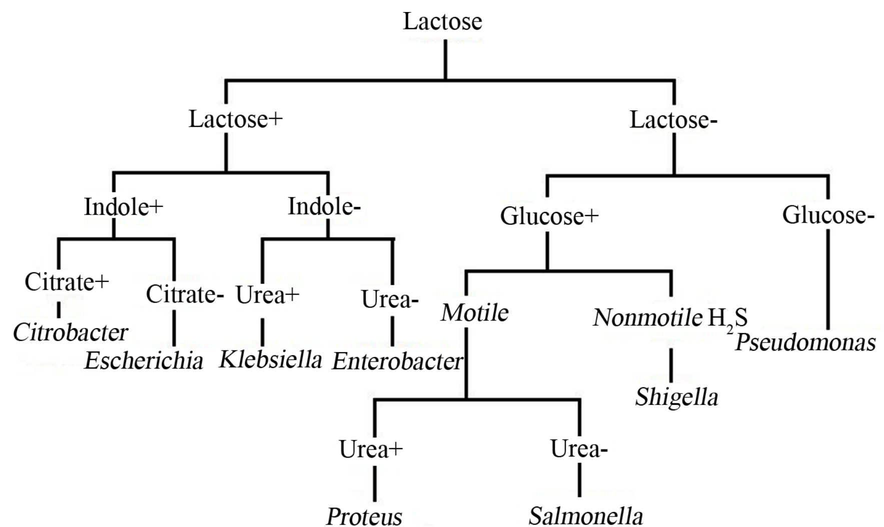

Figure 2. An illustration of the steps used to isolate and identify selected gram negative bacterial species (modified afterHolt *et al.,* 1994).

then tested for citrate utilization using Simmons citrate agar slants according to the manufacturer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to separate those capable of using citrate as a sole carbon source from those that could not.

Pure colonies of respective bacteria were then streaked on the medium with a light inoculum and tubes incubated at 35˚C for 48 hours with loosened caps. The tubes were then observed for a positive reaction indicated by growth on the slant with a colour change of green to blue (alkaline reaction) while a negative reaction was indicated by lack of growth or poor growth without change in colour (medium remained green). The indole negative strains were then tested using Urea agar base medium slants following protocol (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to isolate strains that could hydrolyse urea using the enzyme urease. The respective bacteria were inoculated by streaking back and forth over the entire slant surface, and the tubes were incubated at 35˚C with loosened caps. The tubes were then left for observation for about 6 days with daily inspections. This provided the identity of the lactose fermenting, indole negative bacterial species (**Figures 4-7**).

2) None lactose fermenting gram-negative strains were tested for the ability to metabolize glucose to form acid using Dextrose casein-peptone agarplates following manufacturer's guidelines (Merck, Kenilworth, USA). A positive test showed bacterial colonies that metabolize dextrose to form acid by causing the indicator Bromocresol purple in the medium to change its colour from purple to yellow. The glucose positives were subsequently tested for motility using SIM media as described in the section above while the glucose negative species were identified at this point. The glucose positive motile strains were then tested for the ability to hydrolyse urea using the enzyme urease on Urea agar base medium slants as described in the section above. At this point, the identity of the none

lactose fermenting, glucose negative, gram-negative bacterial species was revealed (**Figures 4-7**).

The outline and flow chart below was modified from Bergey's Manual of Determinative Bacteriology [8] for the identification of unknown bacteria. The scheme below shows the steps that were employed to identify the bacterial isolates.

2.3. Bacterial Culturing Data Collection and Analysis

The results from culturing were recorded as binary data. The bacterial cultures from each water sample were screened for the presence of; *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species. The data was then manually scored into a binary matrix for subsequent analysis. Each water sample was obtained from a single well and was scored for the presence (1) of any of the aforementioned species or absence (0) of the specie (s). The binary data was then entered into IBM SPSS statistics for windows version 24.0 software for Frequency and Crosstab calculations (Corp, I.B.M., 2016). The Frequency variables entered were season, *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas*. The Crosstab tables were generated by entering season and relating (crossing) it to; *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species. The Chi-Square statistical method was used in the Crosstab analysis.

2.4. Bacterial Isolate DNA Extraction and Amplification of 16S rDNA

Genomic DNA was extracted from a total of 80 bacterial isolates using Zymo Research's ZR Fungal/Bacterial DNA mini prep kit according to the manufacturer's protocol. The primers 27F and 1492R were then used to amplify the bacterial 16S rDNA region in a polymerase chain reaction (PCR) reaction. PCR reaction mixturecontained 4 µl of template DNA, 2 µl of a 1 µM concentration of 27F primer, 2 µl of a 1 µM concentration of 1492R primer, 17 µl of nuclease free water and 25 µl of 2× Dream *Taq* master mix.The Dream *Taq* master mix was composed of Dream *Taq* DNA polymerase, 2× Dream *Taq* buffer, dATP, dCTP, dGTP and dTTP of 0.4 mM each, and 0.4 mM $MgCl_2$. The PCR reaction profile setup was; initial denaturation temperature of 94˚C for 4 min, followed by 35 cycles of denaturation temperature at 95˚C for 1 min, annealing temperature of 53˚C for 1 min, and an extension temperature at 72˚C for 2 min. The final extension was then performed at 72˚C for 10 min. PCR products were held at 4˚C and viewed on 2% agarose gel to confirm amplification.

2.5. Sequencing Data Analysis

The PCR amplicons were subsequently sent for sequencing at Inqaba Biotechnical Industries (Pty) Ltd. The resulting sequences from Inqaba Biotechnical

Industries were cleaned using BioEdit (Biological Sequence Alignment editor for Windows 99/98/NT/2K/XP/7) sequence alignment editor software [9]. All the 1492R sequences from each sample were reversed and pairwise aligned with their respective complementary 27F sequences using the option that allows ends to slide. A single sequence was obtained from each aligned pair by merging the overlapping sections of the two respective ends, forminga single good quality sequence for analysis. The edited sequences were then aligned and used to generate a phylogenetic tree with MEGA (Molecular Evolutionary Genetics Analysis) software version 6.0 [10]. The phylogenetic tree was determined using the Maximum Likelihood method based on the Tamura-Neimodel [11] and the bootstrap consensus tree was determined from 1000 replicates which represented the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates were collapsed and initial trees for the heuristic search were obtained by applying the Neighbour-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. Of the 80 isolates, the analysis involved 21 nucleotide sequences because similar sequences were discarded. The use all sites function was used in the missing data treatment and the 1st + 2nd + 3rd + Non-Coding codons were included in the analysis.

3. Results

This study recorded total coliform counts ranging from 160 CFU/ml to 297 CFU/ml in the wet season, and 140 CFU/ml to 273 CFU/ml in the dry season (**Figure 3**). *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species were detected in hand-dug wells from the CEB (**Figures 4-7**). *Citrobacter* species were present in six (6) hand-dug wells from the wet season and five (5) hand-dug wells from the dry season, and*Escherichia* species were present in five (5) hand-dug wells from the wet season and four (4) hand-dug wells from the dry season. *Klebsiella* species were present in twenty-one (21) hand-dug wells from the wet season and eighteen (18) hand-dug wells from the dry season, and *Enterobacter* species were present in twenty-two (22) hand-dug wells from the wet season and fifteen (15) hand-dug wells from the dry season. *Proteus* species were present in only two (2) hand-dug wells from the wet season while *Salmonella* species were present in only six (6) hand-dug wells from the wet season. *Shigella* species were present in four (4) hand-dug wells from the wet season and fifteen (15) hand-dug wells from the dry season, and *Pseudomonas* species were present in three (3) hand-dug wells from the wet season and seven (7) hand-dug wells from the dry season. Chi-square showed that there was no significant variation in the presence of*Citrobacter* ($P > 0.05$), *Escherichia* ($P > 0.05$), *Klebsiella* ($P > 0.05$), *Enterobacter* ($P > 0.05$), *Proteus* ($P > 0.05$) and *Pseudomonas* ($P > 0.05$) species between the wet and dry season, and a significant difference in the presence of *Salmonella* ($P < 0.05$) and *Shigella* ($P < 0.05$) species between the wet and dry seasons (**Table 1**).

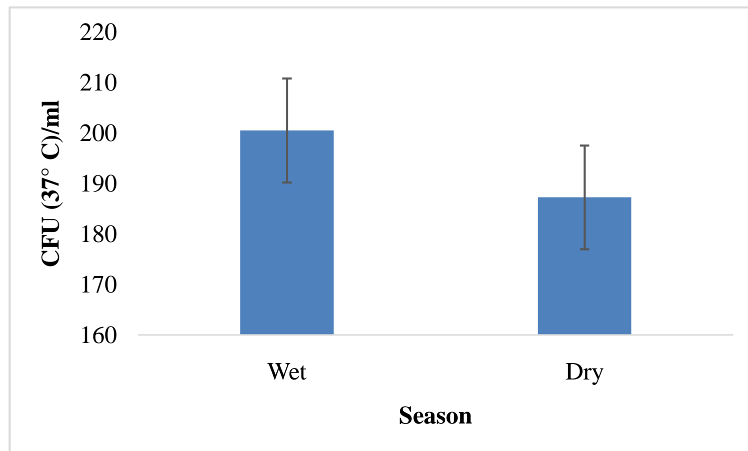

Figure 3. Total coliform counts (CFU's) grown at 37˚C from hand-dug well water samples of the CEB in the wet and dry seasons.

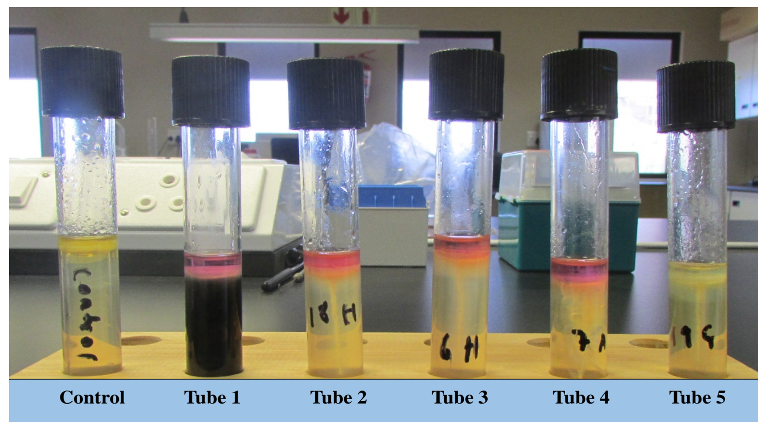

Figure 4. A depiction of gram-negative bacteria tested for hydrogen sulphide production, indole formation and motility on SIM agar. Test tube 1 indicated the presence of *Proteus* species, tubes 2, 3 and 4 had *Enterobacter* species while tube 5 indicated the presence of *Salmonella* species.

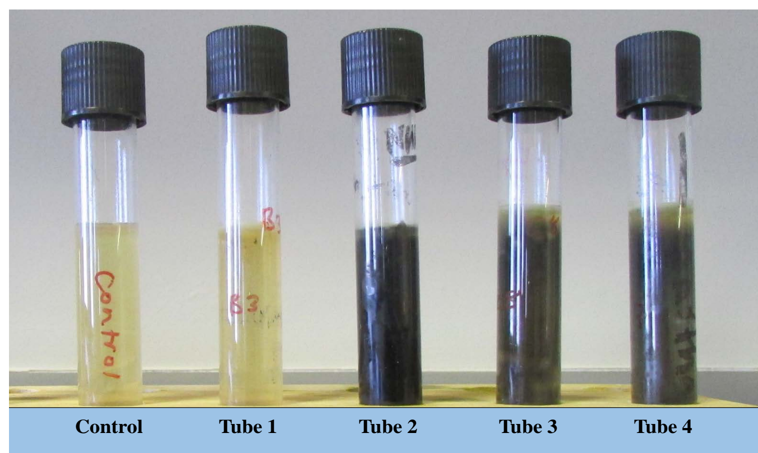

Figure 5. A depiction of gram-negative bacteria tested for hydrogen sulphide production and motility on SIM agar. Test tube 1 showed no growth while tubes 2, 3 and 4 showed the presence of *Shigella* species (H_2S +).

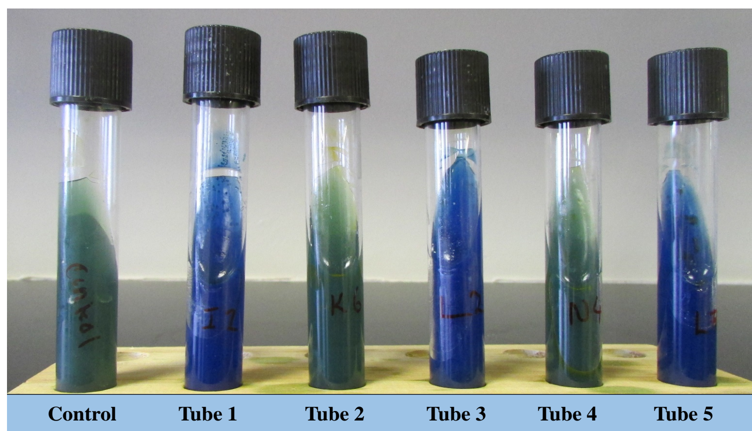

Figure 6. A depiction of gram-negative bacteria tested for citrate utilization by means of Simmons citrate agar slants. Test tubes 1, 3 and 5 showed the presence of *Citrobacter* species (citrate +) while tubes 2 and 4 indicated the presence of *Escherichia* species (Citrate −).

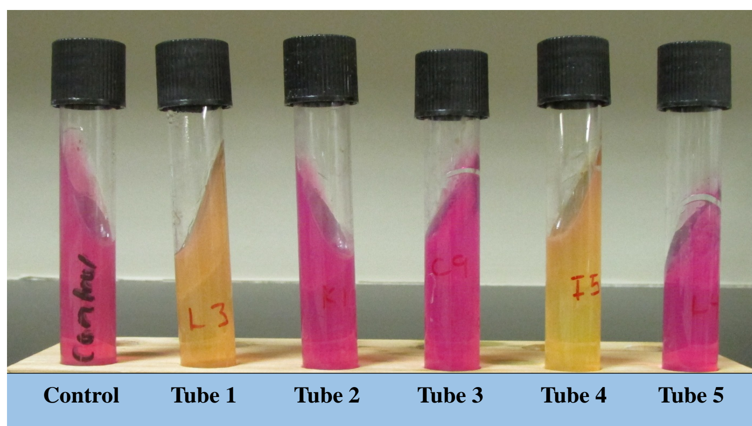

Figure 7. An illustration of gram-negative bacteria tested for urease production using Urea agar base slants. Test tubes 2, 3 and 5 showed the presence of *Klebsiella* species (Urease +) while tube 1 indicated the presence of *Enterobacter* species, (Urease −), and tube 4 the presence of *Salmonella* species.

Table 1. Chi-Square test performed to determine the influence of season on the presence of *Proteus*, *Salmonella*, *Shigella*, *Pseudomonas* and coliform species.

Bacterial species	X^2-value	Deg. of freedom	Asymptotic Sig.
Citrobacter spp.	0.000	1	1.000
Escherichia spp.	0.005	1	0.946
Klebsiella spp.	0.068	1	0.795
Enterobacter spp.	2.296	1	0.132
Proteus spp.	2.504	1	0.186
Salmonella spp.	8.059	1	0.016
Shigella spp.	15.130	1	0.000
Pseudomonas spp.	3.182	1	0.076

A BLAST search of the bacterial sequences with a threshold of 97% similarity revealed that the identity the culture isolates were *Pseudomonas oryzihabitans*, *Pseudomonas pachastrellae, Pseudomonas luteola*, *Pseudomonas aeruginosa*, *Pseudomonas tuomuerense*, *Pseudomonas mendocina*, *Pseudomonas stutzeri*, *Pseudomonas taiwanensis*, *Pseudomonas savastanoi*, *Pseudomonas straminea*, *Pseudomonas umsongensis*, *Citrobacter spp.*, *Proteus myxofaciens*, *Pseudomonas putida*, *Escherichia coli, Salmonella enterica, Shigella sonnei, Enterobacter hormaechei, Klebsiella aerogenes, Enterobacter cloacae* and *Pseudomonas veronni* (Figure 8). At 76% bootstrap, *Pseudomonas luteola*, *Pseudomonas pachastrellae, Pseudomonas oryzihabitans*, *Pseudomonas tuomuerense*, *Pseudomonas aeruginosa*, *Pseudomonas mendocina*, *Pseudomonas stutzeri*, *Pseudomonas taiwanensis*, *Pseudomonas savastanoi, Pseudomonas straminea, Pseudomonas umsongensis, Citrobacter spp., Pseudomonas veronni, Pseudomonas putida, Proteus myxofaciens*, *Klebsiella aerogenes, Enterobacter hormaechei* and *Enterobacter cloacae* formed a cluster. However, *Klebsiella aerogenes, Enterobacter hormaechei* and *Enterobacter cloacae* formed a sub cluster at 98% bootstrap. *Salmonella enterica* and *Shigella sonnei* did not form any cluster while *Escherichia coli* was used as the out group to root the tree (Figure 8).

4. Discussion

There was no significant variation in the presence of *Citrobacter* ($P > 0.05$), *Escherichia* ($P > 0.05$), *Klebsiella* ($P > 0.05$), *Enterobacter* ($P > 0.05$) *Proteus* ($P > 0.05$) and *Pseudomonas* species ($P > 0.05$) between the wet and dry seasons.

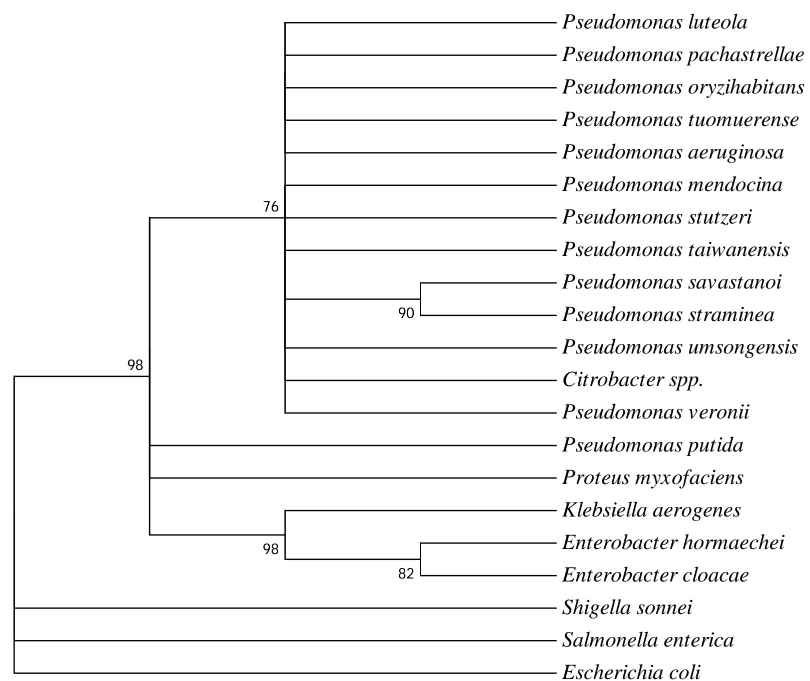

Figure 8. Phylogenetic tree showing the bacterial species detected in 44 hand-dug wells from the Cuvelai-Etosha Basin.

Citrobacter, *Escherichia, Klebsiella*, *Enterobacter*, *Proteus* and *Pseudomonas* species are widely distributed across various ecosystems. *Citrobacter* species are found in soil, food, sewage, water and intestinal tracts of humans and animals [8], and were isolated from both the dry and wet seasons. This agrees with Liu [12] disclosure that *Citrobacter* species are commonly found in water, soil, food, and the intestines of animals and humans. *Escherichia* species abundantly existas normal flora in the intestine of humans and animals, and their detection indicates faecal contamination. The uncontrolled animal access into the hand-dug wells coupled with the surface runoff caused the contamination of these hand-dug wells by *Escherichia* species [13].

The detection of *Klebsiella* species in these hand-dug wells was plausible given their ubiquitous distribution in nature in addition to the gut microbiota in humans and livestock. *Klebsiella* species are part of the coliform indicator organism's list and their detection is described to indicate faecal contamination [14]. It is arguable that these species are not suitable indicators of faecal contamination sincethey inhabit diverse environments ranging from surface waters, gastrointestinal tract of mammals, soil, and plants [8]. The present study's results confirmed earlier reports by Iversen [15] who indicated that *Enterobacter* species are widely distributed in nature, and occur in freshwater, soil, sewage, plants, and animal and human faeces.

Enterobacter strains are part of the normal flora of the human and animal gastrointestinal tracts, and can also infect animals leading to death in some cases. Although *Enterobacter* species are unlikely to cause serious harm in immunocompetent livestock and humans, Hussain *et al.* [16] argued that consumption of water consisting of *Enterobacter* strains regardless of their source is a major risk to the health of human beings. Boamah *et al.* [17] also isolated *Enterobacter* species from freshwater, and detected *Enterobacter sakazakii* also known as *Cronobacter* species, and *Enterobacter cloacae* in hand-dug wells of which both species are pathogenic to humans [18] and animals [19]. *Enterobacter* species were detected in most hand-dug wells regardless of season due to the versatility of these species allowing them to grow and survive over a wide range of temperatures, pH values and nutrient composition [8] [20]. *Proteus* species are members of the human and livestock gastrointestinal tract [8]. These species are also widely distributed in environments such as water, faeces, and soil, and known to contribute to the decomposition of organic matter of animal origin [21]. Although it is established that *Proteus* species are found in water environments [8] [20], the findings of this study propose that these species have short periods of survival in water unless optimal conditions are available in which case it seldom occurs due to bacterial competition or interaction. Although *Pseudomonas* species are extensively distributed in soil, faeces, water and sewage, they were only detected in 10 hand-dug wells indicating that most species cannot probably grow and survive lengthy periodsin water. However, Vaz-Moreira *et al.* [22] argued that *Pseudomonas* species are residents of various aquatic environments which

agrees with their occurrence in some of the hand-dug wells studied. Vaz-Moreira *et al.* [22] isolated a total of 14 *Pseudomonas* species from 32 water sampled sites in which all the isolates had a distinct genotype based on the type of water from which they were obtained (water treatment plant/distribution system, tap water, cup fillers, biofilm, and mineral water).

It was noted that there was a significant variation in the presence of *Shigella* ($P < 0.05$) and *Salmonella* species ($P < 0.05$) between the wet and dry seasons. *Salmonella* species are widely distributed in the environment and can easily gain entry into the hand-dug wells. *Salmonella* species were not detected in the dry season in the present study and this agrees with Adingra *et al.* [23] who indicated that the presence of *Salmonella* species increased with high levels of rainfall and its prevalence was significantly increased in higher rainfall seasons. Hence, rainfall significantly influences the presence and distribution of *Salmonella* species in the hand-dug wells especially those lacking proper architecture with increased vulnerability to surface runoff or animal entry. *Shigella* species are intestinal pathogens of humans and other primates in which they cause dysentery [8] [14] [20]. Although the present study indicated that these species were readily detected in the dry season compared to the wet season, Phung *et al.* [24] revealed that these species show a high peak in the rainy season. The present study's findings showed that nutrients and climatic conditions favouring *Shigella* species growth and survival were present in these hand-dug wells especially during the dry season. Moreover, *Shigella* species are chemoorganotrophic having both a respiratory and fermentative type of metabolism making them versatile. Phung *et al.* [24] also found that temperature, humidity, and precipitation were positively associated with the incidences of *Shigella* species in the rain season. Although Phung *et al.* [24] argued that *Shigella* species incidence rates are higher in the rain season, it cannot be ruled out that suitable conditions for their growth exist in the dry season as evidenced by the present study.

In this study, water quality assessment by culturing found no resemblance in the presence of *Escherichia* species which is currently recognized as the gold standard for microbial water quality assessment and *Salmonella*, *Shigella*, *Pseudomonas*, *Citrobacter*, *Klebsiella*, *Enterobacter* and *Proteus* species. This is due to the different rates of survival of these species in water and this highlights the inappropriateness of asserting water to be safe based on the presence or absence of *Escherichia coli.* Similar results have been reported although they focused on comparing *Salmonella* to *Escherichia coli* *[25]* [26]. Since water in these hand-dug wells is in contact with the soil, and most of the detected species have a ubiquitous distribution, it is no surprise that these species were detected. Furthermore, this soil-water environment can enhance the ability of the microorganisms to cope with various or fluctuating environmental conditions by the transfer and exchange of genes owed to microbial interactions. This is evident because *Escherichia* species were once described to be unable to survive lengthy periods outside the intestines of warm blooded animals and it's on this basis that *Escherichia coli* is used as a water quality indicator for faecal contamination and

a predictor of the potential presence of other contaminant species [14]. Recent studies have indicated that *E. coli* strains survive in soil and water that's not known to be faecally contaminated [27]. In addition, Van Elsas *et al.* [28] revealed that *Escherichia* species can grow and survive outside their primary hosts (humans and animals) in open environments with appropriate resources and key abiotic conditions.

Floating debris and livestock droppings were observed in and near hand-dug wells, and potentially created a propitious ecosystem for microbial growth. This was among the reasons for detecting high levels of contamination. These high levels of contamination can also be viewed as environments with the potential to reveal the interactions within and between bacterial species in ecological studies. The understanding of bacterial interactomics can allow the prediction of which bacteria are most likely to co-exist with the detected bacterial species, and serve as a more accurate molecular marker facilitating the prevention of water related diseases. This is founded on knowledge that some bacterial species inhibit or restrict the growth and survival of others and this has been shown by various studies [29] [30]. Moreover, Thomas [6] disclosed that Namibia recorded the highest rate of open defecation in Southern Africa, and poor architecture of these hand-dug wells promotes the entry of faecal matter especially in the wet season. The current status quo demands the development of a more suitable indicator of faecal contamination and further research to explore emerging patterns of pathogenic bacteria inhabiting water resources.

The detected species in this study signifies a public health threat and emphasizes the need to treat the water prior to consumption. *Citrobacter* species are known to cause infections affecting the urinary tract, liver, biliary tract, peritoneum, intestines, bone, respiratory tract, endocardium, wounds, soft tissue, meninges, and the bloodstream [31] [32]. *Escherichia coli* is documented to cause urinary tract infections, diarrhoea, haemolytic uraemic syndrome (HUS), acute renal failure, hemorrhagic colitis, enteric infection, haemolytic fluoroquinolones, haemolytic anaemia, bacteraemia, wound infection, and meningitis [33] [34].*Salmonella enterica* entirely consists enteric pathogens except *Salmonella typhi,* and clinical presentations include gastroenteritis, bacteraemia, septicaemia, osteomyelitis, diarrhoea, typhoid fever/enteric fever and a carrier state in persons with previous infections [35] [36]. *Shigella sonnei* is known to cause enteric infections [37]. *Enterobacter hormaechei* and *Enterobacter cloacae* are known to cause bacteraemia, respiratory tract infections and UTI [38] [39]. *Enterobacter cloacae* is pathogenic to humans [18] and animals [19].*Pseudomonas* species are also found on the surfaces of plants and animals, and are opportunistic and nosocomial pathogens of the gastrointestinal tract, heart, blood, respiratory system, central nervous system, ear, eye, bone and joint, skin, and soft tissues [40]. *Pseudomonas mendocina, Pseudomonas oryzihabitans, Pseudomonas putida, Pseudomonas stutzeri, Pseudomonas aeruginosa* and other *Pseudomonas species* are known to cause bacteraemia, UTI, wound infection, pneumonia, abscesses, septic arthritis, conjunctivitis, endocarditis, meningitis, otitis, perito-

nitis and pulmonary infections in patients with Cystic fibrosis [41] [42] [43].

5. Conclusions and Recommendations

This study detected the presence of *Salmonella, Proteus, Shigella, Pseudomonas* and coliform bacteria in the hand-dug wells of the Cuvelai-Etosha Basin and supports the conclusions that; water from these hand-dug wells is not safe for drinking unless it is subjected to appropriate disinfection methods. Diseases induced by *Salmonella* and *Shigella* species are likely to be pronounced in the wet season compared to the dry season, and infections caused by *Proteus, Pseudomonas Citrobacter, Escherichia, Klebsiella* and *Enterobacter* species are expected to occur regardless of season. Hand-dug wells close to each other are also vulnerable to the spread of bacterial contamination because they may share the same aquifers. It is recommended that; the entry/access of animals in the vicinity of hand-dug wells should be restricted in order to prevent defecating near the hand-dug wells, hand-dug wells should have a top cover and be properly constructed with inside walls having concrete from the top to the bottom, and located at safe distances from pit latrines. The top part of the hand-dug wells should be elevated from the ground to prevent inflow of surface runoff. Furthermore, water must be subjected to treatment such as boiling before drinking and addition of bleach to kill bacteria, and hand-dug wells should be routinely assessed for water quality and protected against recontamination.

Author Contributions

Billy McBenedict collected the water samples and performed the research. Billy McBenedict performed the statistical analysis. Billy McBenedict, Heike Wanke, Percy Chimwamurombe and Bernard Hang'ombe wrote the manuscript. Billy McBenedict, Percy Chimwamurombe, Heike Wanke and Bernard Hang'ombe proof read the manuscript.

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Availability of Data and Materials

The 16S rRNA sequences data that were generated in the current study are available on GenBank, with the following accession numbers: MH062903 -

MH062920.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests regarding the publication of this paper, and permission was obtained regarding the publishing of photos in this paper.

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