Bacteriological Analysis of Household Water from Hand-Dug Wells in The Cuvelai-Etosha Basin of Namibia

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Abstract

Communities in Oshikoto, Omusati, Ohangwena and Oshana regions of Namibia widely utilize the Cuvelai-Etosha Basin by constructing hand-dug wells to provide water to sustain agriculture and households. Since these regions lack a developed water pipeline system and deeper groundwater may be saline in large parts of the Basin, people rely on domestic water supply from private hand-dug wells which are near their houses for convenience and preference. However, the microbial water quality and safety of hand-dug wells being utilized for house hold consumption in the Cuvelai-Etosha Basin is unknown and this is undesirable since water is a habitat for some pathogenic microorganisms thereby posing a health risk. Thus, a bacteriological water quality study that focuses on the identification of microbial contaminants was conducted on 25 wells in the Cuvelai-Etosha Basin of Northern Namibia during two sampling campaigns. Molecular methods indicated the presence of *Bacillus aerophilus, Bacillus amyloliquefaciens, Bacillus aquimaris, Bacillus aryabhattai, Bacillus cereus, Bacillus licheniformis, Bacillus pumilus, Bacillus safensis, Bacillus samanii, Bacillus sp. M37, Bacillus sp. M26, Bacillus stratophericus, Bacillus subtilis, Pseudomonas mendocina, Staphylococcus haemolyticus* and *Streptomyces celuloflavus*.

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1 Introduction

Namibia is a desert country with high temperature that leads to an increased evaporation of rain water. The country experiences short rain seasons and long dry seasons which cause water scarcity especially in rural areas that lack developed water pipelines and rely on rain water harvesting or groundwater sources such as boreholes, open deep wells and shallow wells (Msangi, ed., 2013). The Cuvelai Etosha Basin is shared between Angola and Namibia. In Angola, the basin covers 36% with Cunene province having a larger portion of the northern Cuvelai while Cuando Cubango and Huila provinces share a minor piece (DRFN and HIWAC, 2013). As for Namibia, Oshikoto, Omusati, Ohangwena and Oshana regions contribute 64%, while Kunene and Otjozondjupa regions have an intersection with minor areas in the southern part of the Basin (DRFN and HIWAC, 2013).

Rural communities in Namibia utilize hand-dug wells as a source of water for household purposes. Hence, the Cuvelai system serves as a water resource for the communities in Oshikoto, Omusati, Ohangwena and Oshana regions (Christelis and Struckmeier, 2011). In some areas, community boreholes have been set up, but are often not used as they are far from homesteads or have water quality problems such as high total dissolved solids (TDS) or fluoride concentration (Wanke et al., 2014). While water-related diseases continue to be one of the major health concerns globally, statistics in Namibia show high prevalence of diarrhoea as a result of consuming contaminated water (UNICEF Namibia, 2014, January 14) especially in infants, the old aged and people with compromised immunity since they are more vulnerable to infection. Although wells often have visible debris floating in them, they are nevertheless utilized as drinking water without treatment. Contamination is enhanced by a lack of sanitation or waste water treatment systems in the rural areas. This lack of a developed water supply system in some parts of the region increases the risk of water borne infection in these areas because people utilize water from hand-dug wells for house hold use regardless of its quality and safety (Wanke et al., 2014).

Hand-dug well water may harbor microorganisms such as viruses, bacteria, fungi and protozoa which may be pathogenic and induce diseases leading to death in severe cases (Samuel, 2013). Most hand-dug wells in the Cuvelai Etosha Basin of Namibia are not covered and lack a protection zone which allows animals to access the water troughs which are often placed besides the well (Christelis and Struckmeier, 2011). These hand-dug wells

tap water from shallow perched aquifers and are not protected from surface contamination nor is the water quality monitored. This is not desirable given the outbreaks of cholera (UNICEF Namibia, 2014 January 14; Smith, Keddy & De Wee 2008), polio (Schoub, 2006), and diarrhea (Sibeen, 2007 April 18) experienced in Namibia. Safe water supply is crucial to societal development and growth, and therefore forms part of the United Nations Millennium Development Goals (Agatemor & Agatemor, 2010). The objective of this study was to investigate the bacteriological (Culturable) water quality of hand-dug wells.

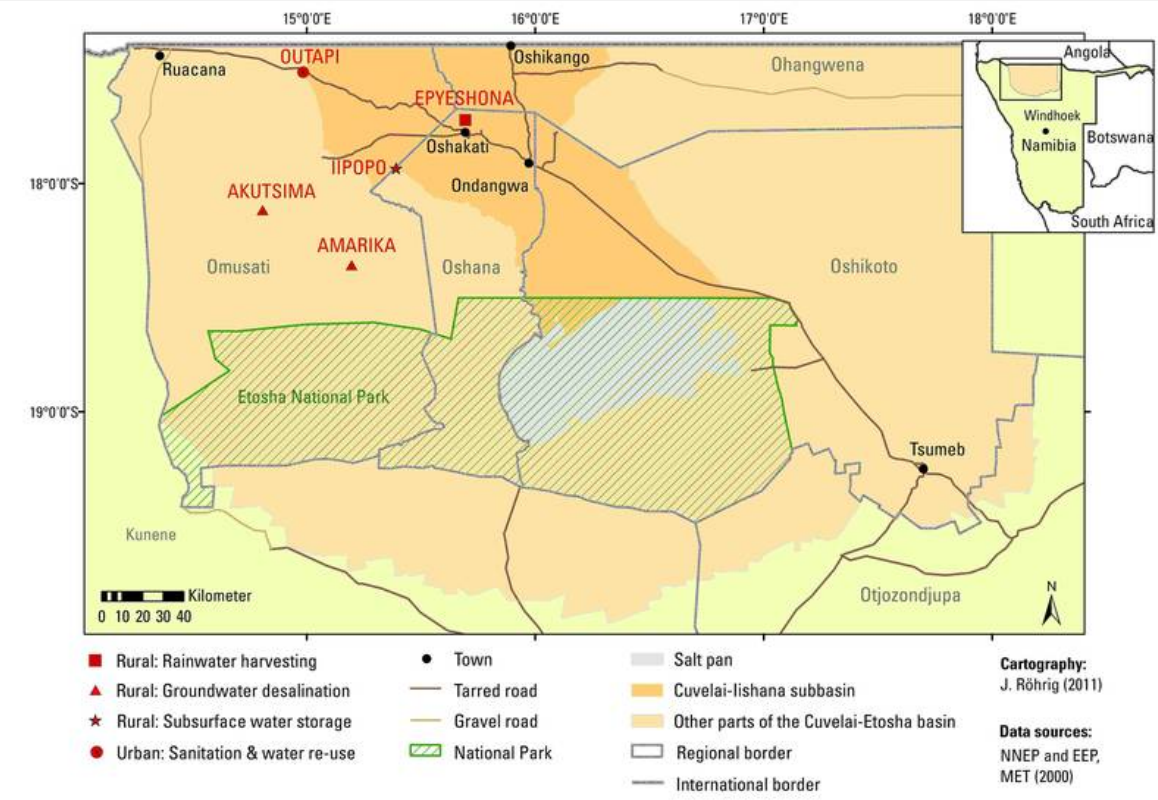

Figure 1: Location of the four regions sharing the Cuvelai-Etosha basin in Namibia (retrieved from `https://www.google.com.na/Cuvelai-Etosha+Basin+Atlas&biw`)

2 Materials and Methods

2.1 Isolation of Bacteria

Water samples were collected from a total of 25 wells in the Cuvelai-Etosha basin of Namibia (Figure 1). Figure 2 shows a typical unprotected well. Sampling was conducted during two sampling campaign trips in March and May in order to account for the period before the rainy season and after the rain season. The water samples were filtered with membranes of pore size of 0.1 - 10 μm in order to concentrate the bacteria. The bacteria were then cultivated on a general purpose medium (Nutrient agar) at an incubation of 37°C for 24 hours. After bacterial growth, single colonies were isolated and grown as pure cultures. Gram stain was performed on the pure cultures to distinguish gram negative and gram positive (results not shown). Bacteria isolation was performed at the University of Namibia (UNAM).

Figure 2: An illustrative diagram of a hand-dug well in the Cuvelai-Etosha basin.

2.2 Amplification of 16S rDNA

Genomic DNA from 25 bacterial isolates was extracted using the Zymo Research's ZR Fungal/Bacterial DNA mini prep kit. The 16S rDNA was successfully amplified from the 25

isolates using PCR with primers; 27F and 1492R. The PCR reaction mixture contained 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 which contained: 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 consisted of 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 and lastly the PCR products were held at 4°C. A 2% agarose gel was then prepared in order to visualize PCR products and determine the success of the amplification (photo not shown).

2.3 Sequencing and data analysis

The PCR products were sent for sequencing at Inqaba Biotechnical Industries (Pty) Ltd. The sequences obtained from Inqaba Biotechnical Industries were then edited in BioEdit (Biological Sequence Alignment editor for Windows 99/98/NT/2K/XP/7) sequence alignment editor software (Hall, 1998). A pairwise alignment in BioEdit (Hall, 1998) which allows ends to slide was performed on each 27F sequence from each sample with its complementary 1492R reversed respective sequence. From each pair, a single sequence was obtained by merging the overlap and the two respective ends of the pair of sequences in order to form one edited sequence for analysis. The edited and aligned sequences were then used to construct a phylogenic tree in MEGA (Molecular Evolutionary Genetics Analysis) software version 6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The phylogenetic tree was resolved by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993) and the bootstrap consensus tree was determined from 1000 replicates which represented the evolutionary history of the taxa analyzed. 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 Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. 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. A *Streptomyces bacillaris* strain retrieved from the National Centre for Biotechnology Information (NCBI) Website with accession number KJ571045.1 was also included in the analysis and used as the out-group to root the phylogenetic tree.

3 Results and Discussion

A BLAST search of the bacterial sequences revealed the identity (Figure 3) of the bacteria as *Bacillus aerophilus, Bacillus amyloliquefaciens, Bacillus aquimaris, Bacillus aryabhattai, Bacillus cereus, Bacillus licheniformis, Bacillus pumilus, Bacillus safensis, Bacillus samanii, Bacillus sp. M37*, *Bacillus sp. M26*, *Bacillus stratophericus, Bacillus subtilis, Pseudomonas mendocina, Staphylococcus haemolyticus* and *Streptomyces celuloflavus. Bacillus* species were the most common inhabitants of the hand-dug wells.

Table 1: The bacteria isolates and their corresponding identity retrieved from NCBI

Bacterial isolate‡	Place of isolation	NCBI Identity	% Identity	Accession #
2	UNAM*	*Bacillus aerophilus*	99	JX049585.1
3	UNAM	*Bacillus amyloliquefaciens*	98	KP334099.1
4	UNAM	*Bacillus aquimaris*	99	KJ009414
5	UNAM	*Bacillus aryabhattai*	98	KM051114
6	UNAM	*Bacillus cereus*	99	KP729612.1
7	UNAM	*Bacillus cereus*	98	HQ683909.1
8	UNAM	*Bacillus licheniformis*	98	LC006127.1
9	UNAM	*Bacillus licheniformis*	98	LC006127.1
10	UNAM	*Bacillus pumilus*	97	EU863189
11	UNAM	*Bacillus safensis*	98	KP717556
12	UNAM	*Bacillus samanii*	99	EU240367
13	UNAM	*Bacillus samanii*	98	EU240367
14	UNAM	*Bacillus M 26*	99	EU240373.1
15	UNAM	*Bacillus M37*	98	GQ495062.1
16	UNAM	*Bacillus M 26*	99	GQ495051
17	UNAM	*Bacillus M37*	99	GQ495062
18	UNAM	*Bacillus M 37*	98	GQ495062.1
19	UNAM	*Bacillus M 26*	98	GQ495051.1
20	UNAM	*Bacillus stratophericus*	98	KM277362
21	UNAM	*Bacillus subtilis*	98	KF758384
22	UNAM	*Bacillus subtilis*	99	KF758384
23	UNAM	*Pseudomonas mendocina*	98	DQ345122.1
24	UNAM	*Staphylococcus haemolyticus*	99	KT003269.1
25	UNAM	*Bacillus M37*	98	GQ495062.1
26	UNAM	*Streptomyces celluloflavus*	99	KP235209.1

*UNAM- University of Namibia

‡Isolate 1 is a root sequence downloaded: *Streptomyces bacillaris*

The *Bacillus* genus is a diverse group of Gram positive bacteria that are rod-shaped and have the ability to form endospores that are resilient to harsh environmental conditions (Clause & Berkeley, 1986). Most *Bacillus* species are harmless with the exception of a few being pathogenic to humans and animals. *Bacillus cereus* is among the pathogenic species, it is known for causing food poisoning that is comparable to *Staphylococcal* species food poisoning. *Bacillus cereus* is capable of forming heat-stable toxin in food that is associated

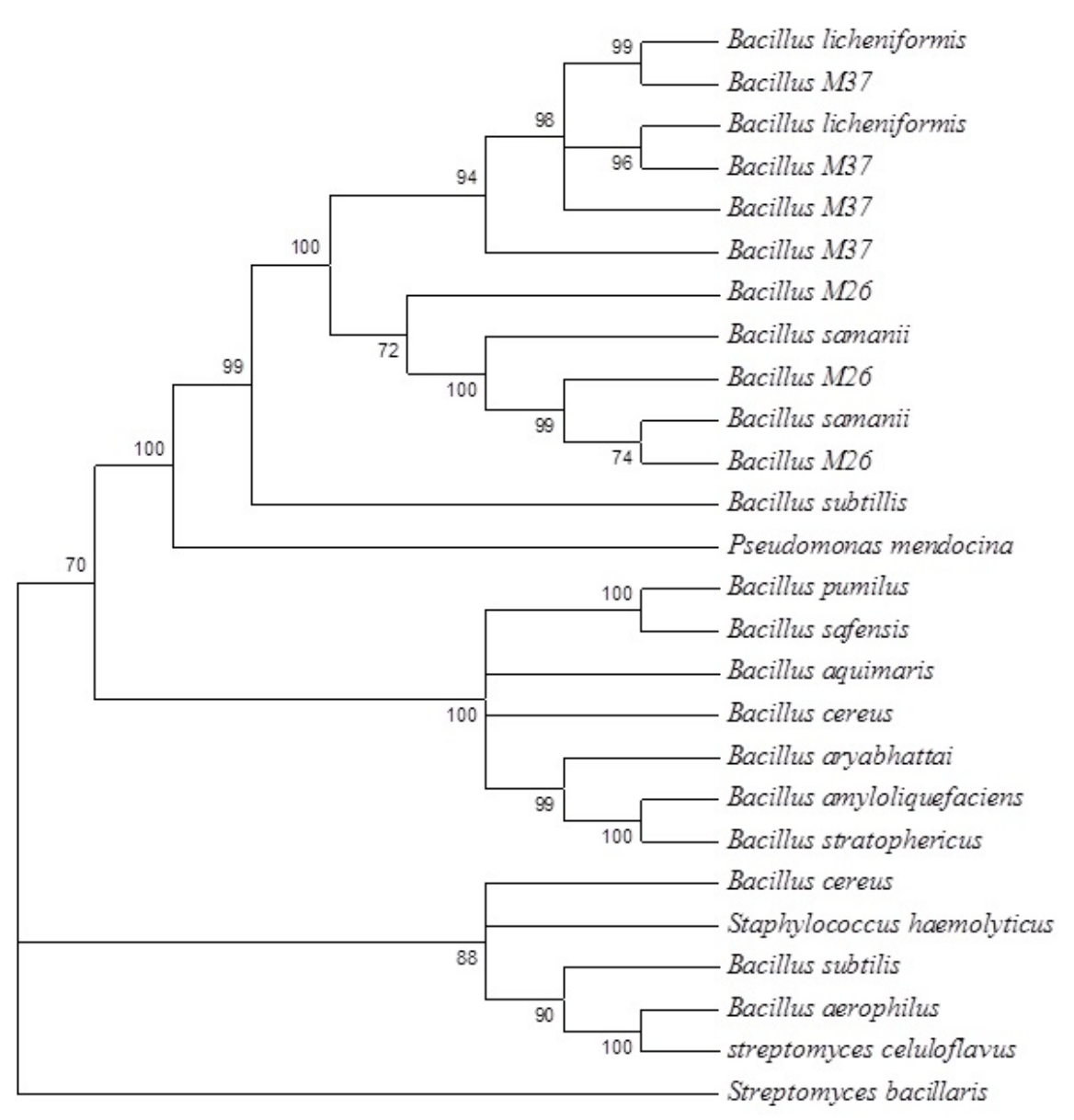

Figure 3: The phylogenetic tree for 25 bacterial isolates sequences inferred using the Maximum Likelihood method. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates are collapsed. The bootstrap values show the confidence in the groupings as a percentage. *Streptomyces bacillaris* was used as the out-group to root the tree generated with MEGA 6.0 software.

with spore germination which induces vomiting after ingestion while other strains produce a heat-labile enterotoxin after ingestion that causes diarrhea (Ashbolt, Grabow & Snozzi, 2001). The presence of *Bacillus* species in the present study also corroborates with the World Health Organization (2004) suggesting that *Bacillus* spp. can be found in diverse natural environments such as soil and water. The existence of *Bacillus cereus* in drinking water supplies has not been reported. Furthermore, World Health Organization (2004) reported that drinking-water has not been identified as a source of infection of pathogenic *Bacillus* spp. However, *Bacillus cereus* was detected in the present study. This is in agreement with the ability of bacillus species to form spores. Hand-dug wells can be found with a diverse form of bacteria due to their vulnerability to contamination from human and animal activities occurring around the top of the well. Contamination of hand-dug wells can also be influenced by structures in the vicinity of the well such as pit latrines, farm animal wastes and septic systems (FMDW, 1997). Furthermore, households in the vicinity of contamination are vulnerable because they share the same aquifer (Centre for Disease Control and prevention (CDCP, 2010). The bacterial contamination in the present study can also be attributed to structure of the wells. Not all hand-dug wells surveyed had a cover to prevent contamination.

The presence of *Staphylococcus haemolyticus* in the hand-dug wells also suggested the possible contamination of the water with selenium (Riadi & Barford, 1994). This study revealed the presence of bacterial contamination in the hand-dug wells of the Cuvelai-Etosha basin and supports the conclusions that; the water from the hand-dug wells is not safe for drinking unless it is subjected to appropriate disinfection methods. These wells are also vulnerable to the spread of bacterial contamination because they use the same aquifers.

4 Conclusion

This study revealed the presence of bacterial contamination in the hand-dug wells of the Cuvelai-Etosha basin and supports the conclusions that; the water from the hand-dug wells is not safe for drinking unless it is subjected to appropriate disinfection methods. These wells are also vulnerable to the spread of bacterial contamination because they use the same aquifers. In addition, these wells indicated possible contamination with Selenium which when consumed in high amounts can induce a disease called Selenosis. The presence of the health risk bacteria indicated that the water was not fit for drinking in light of WHO and Namibian guideline values for drinking water. However, this water can be consumed with prior treatment.

The findings of this study lead to the recommendations that; the entry/access of animals in the vicinity of hand-dug wells should be restricted in order to prevent the defecating of animals near the wells. This can be implemented by constructing fences around the

wells. Furthermore, wells should be lined with concrete from the top to the bottom. The construction of the wells at higher ground levels can also prevent the entry of contaminants through surface run off especially in the rain season. The wells should be covered and the concrete protection of the well should also be properly constructed. In addition, the wells should be assessed for the water quality periodically by the Ministry of Health and the well owners should be trained on the maintenance of the well. It is further recommended that; the water must be boiled before drinking it in order to kill bacteria. The boiled water must be covered and protected against recontamination. It is also encouraged that bleach should be added to the water to kill bacteria. Iodine can also be added due to its ability to kill bacteria. However, Iodine may cause allergies in some instances. In addition, the addition of water purification tablets also eliminates the bacteria. In cases of financial challenges, solar disinfection can be performed which involves Filling plastic bottles with water and placing them in the hot sun for two hours. Combining these methods is more effective especially in the elimination of resistant bacteria such as cholera bacterium. Since the most common water source for the community in the Cuvelai-Etosha basin is hand-dug well, the construction of more boreholes by the government with an inclusive borehole sinking and water quality education program would reduce chances of bacterial infections.

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