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RESEARCH ARTICLE OPEN ACCESS

Seasonal Health Risks Due to Zoonotic Pathogens from Hand-dug Well Water in Ohangwena and Omusati Regions of Namibia

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Abstract

Water is a global need for the health and survival of all life forms and a habitat to some. However, approximately one billion people in developing countries do not have access to safe drinking water in which Namibia is not an exception. Rural communities of Ohangwena and Omusati regions of Namibia construct hand-dug wells as a source of water. However, these hand-dug wells are not monitored for water quality and are generally poorly constructed. In addition, these hand-dug wells are easily accessed by both humans and animals making them a potential reservoir for zoonotic bacteria partly known to aid antimicrobial resistance development, a global health concern. A metagenomics analysis targeting the 16S rRNA gene was used to detect the bacterial communities in a total of 40 hand-dug well water samples, with 20 being from the wet season and the rest from the dry season. A total of 57 bacterial pathogens were detected with a diverse of zoonotic assemblage. Analysis revealed significant differences in diversity and evenness, and no significant difference in richness of zoonotic bacterial pathogens in hand-dug wells between the wet and dry seasons ($P < 0.05$) with the dry season having a higher diversity and evenness compared to the wet season. The analysis showed that the hand-dug well water was not definitely safe for human and livestock consumption and their exposure to zoonotic pathogens was pronounced in the dry season.

Keywords: Bacteria, Diversity, Hand-dug wells, Metagenomics, Water, Zoonotic.

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Abbreviations: AMR: Antimicrobial resistance; OTU: Operational taxonomic units; H': Shannon-Wiener diversity; D: Simpson diversity; R: Richness; E: Evenness; UTI: urinary tract infections; PCR: Polymerase Chain Reaction.

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INTRODUCTION

Water is a universal need for survival across all life forms and serves as a habitat to some creatures. Although important, water can be a source and driver of diseases to humans and livestock if contaminated and this highlights the need to ensure the safety of drinking water supplies. However, approximately one billion people in developing countries do not have access to safe drinking water in which Namibia is not an exception.[1] The safety and quality of drinking water sources are a global concern especially in rural areas where water scarcity and contamination can be alarming. Odonkor and Addo[2] argued that rural areas of developing countries experience high rates of waterborne diseases compared to other ailments due to bacteriological contamination. The water shortages in developing countries have led to communities depending on the use of groundwater to supplement the surface water supplies. Since groundwater microbial water quality assessment is not prioritised in most developing countries, water related diseases account for 10% of the disease burden in developing countries.[3]

Rural communities of Ohangwena and Omusati regions within the Cuvelai Etosha Basin (CEB) of Namibia construct hand-dug wells as a primary source of water. However, these hand-dug wells are not monitored for water quality and are generally poorly constructed. This increases the likelihood of incidences of severe endemic water borne diseases and possess a public health threat. Moreover, shallow perched aquifers are not appropriate water resources for human consumption due to high vulnerability to contamination.[4] General observed description of hand-dug well aspects that potentiate contamination include plant materials that grow inside the walls of hand-dug wells, birds and small mammals that die in hand-dug wells, and when faecal matter gains entry. This potentially provides carbon deposits to bacterial communities in hand-dug wells thereby supporting growth since carbon is known to be among the principle determinants of microbial growth.[5]

Since hand-dug wells form part of freshwater environments which are known to be a habitat for microbial life, it is likely that the general status of the hand-dug wells in Ohangwena and Omusati regions possess variable resources and conditions that promote microbial growth and pose a health risk to consumers. Hand-dug well water can be a habitat for pathogenic viruses, bacteria, fungi and protozoa to both humans and livestock leading to death in severe cases.[6,7] This water resource can also be a reservoir for zoonotic bacteria which is currently an emerging global health concern. Zoonotic diseases are infectious diseases that can be transmitted between vertebrate animals and humans with or without an arthropod intermediate.[8]

Zoonotic bacterial pathogens are problematic because they can aid the development and transfer of antimicrobial resistance.[9] This resistance is known partly to be the result of inappropriate use of antibiotics in animal husbandry and agriculture. Hence the detection of zoonotic pathogens in water used for both humans and livestock consumption serves as a sentinel surveillance for possible antimicrobial resistance gene reservoirs. This is especially of concern when abundant zoonotic bacteria are detected with *E. coli* species which are known to possibly be a source, assimilator and disseminator of AMR plasmids/genes from the environment.[10] This study investigated the seasonal health risks to humans and livestock of consuming none sanitised hand-dug well water in Ohangwena and Omusati regions of Namibia using metagenomics detection of zoonotic bacterial pathogens. Metagenomics provides detailed information regarding the bacterial communities in a habitat compared to the culture based approaches by bypassing limitations of culturing based methods that lead to the inability to quantify the total natural diversity within a given habitat.

METHODS

Study sites and sample collections

The study investigated hand-dug wells in rural Omusati and Ohangwena regions of Namibia. These hand-dug wells ranged in depth from shallow which could allow animal entry to deep with a depth of at least 18 m. Fig. 1 and 2 shows the general structure of the hand-dug wells found in the Omusati and Ohangwena regions of Namibia. The sampling strategy employed was convenience sampling which targeted the areas in which hand-dug wells were monitored for

hydrochemical water quality by The Southern African Science Service Centre for Climate Change and Adaptive Land Management. A total of 40 water samples were collected in sterile 200 ml bottles from the hand-dug wells, half the total number were collected from the wet and 20 from the dry season respectively. The same hand-dug wells were assessed in both the wet and dry seasons. The water samples were collected by lowering a rope tied to the sterile bottles into the hand-dug wells, and subsequently transported on ice to the University of Namibia for analysis. Preceding transportation, the temperature of the water was measured.

DNA extraction and 16S rRNA gene amplification

Water samples containing each a volume of 200 ml were centrifuged at a speed of 7 000 *xg* for 1 hour in order to concentrate the bacteria. Each of the sample volumes were then reduced to 10 ml after centrifugation by disposing the supernatant. DNA was extracted using SEEPREP 12 ™ kit (Seegene, Rockville, USA), and the respective concentrations and quality were determined by the NanoDrop-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The 16S rRNA gene was amplified using PCR with universal primer sets 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3'). The thermo-cycler (Bio-Rad, Hercules, CA) was used with reaction conditions of; 1 cycle of pre-denaturation at 94°C for 4 minutes, 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 30 seconds, an extension at 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. Next generation sequencing and diversity assay using Illumina 16S sequencing of the amplicons was performed at Mr. DNA Next Generation Sequencing provider in Texas, United States of America.

PCR product preparation and sequencing

The amplified amplicons were then prepared for sequencing as described by the Illumina TruSeq DNA library preparation protocol. Sequencing (20k 2x300bp Illumina 16s) was performed at MR. DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq using the manufacturer's guidelines, and further processing of sequence data was done using a proprietary analysis pipeline (MR. DNA, Shallowater, TX, USA).

16S rRNA Metagenomics data collection and analysis

The Metagenomics sequence data attained from Mr. DNA Next Generation Sequencing provider (Texas, United States of America) was processed and edited using a proprietary analysis pipeline (www.mrdnalab.com, MR. DNA, Shallowater, TX). The Q25 sequence data derived from the sequencing process were depleted of barcodes and primers, and short sequences less than 150 bp were removed. In addition, sequences with ambiguous base calls, and homopolymer runs exceeding 6 bp were removed. The sequences were then denoised and chimeras also removed. Operational taxonomic units (OTUs) were defined after removal of singleton sequences, and clustering at 3% divergence (97% similarity) was performed as described by Capone *et al.*[11] OTUs were then taxonomically classified by performing a BLASTn against a curated GreenGenes, RDPII (http://rdp.cme.msu.edu) and NCBI (www.ncbi.nlm.nih.gov) databases and compiled into each taxonomic level according to DeSantis *et al.*[12] The files were compiled based on counts and percentages with counts revealing the actual number of sequences while the percentages displayed the relative proportion (in percentage) of sequences within each sample that map to the designated taxonomic classification. Hence, the bacterial communities and the percentage of each species in the community were explored.

Construction of a phylogenetic tree

Selection of cleaned zoonotic bacterial sequences from the total identified OTU's, was performed. A phylogenetic tree was then constructed using the Maximum Likelihood Method.[13] Only the trees with the highest log likelihood were chosen and percentages of trees in which the associated taxa clustered together were shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Bootstrap was performed and the consensus trees inferred from 1000 replicates were taken to represent the evolutionary history of the taxa analysed.[14] Branches corresponding to

Table 1. Wilcoxon rank test performed to determine the influence of season on the abundance of the detected zoonotic bacterial pathogens

Bacterial species	Z value	P – Value
Actinomyces spp.	-0.938	0.348
Actinomyces viscosus	-1.34	0.180
Aerococcus viridans	-2.65	0.008
Afipia sp.	-2.92	0.004
Alcaligenes faecalis	-3.92	0.000
Alcaligenes sp.	-1.84	0.066
Anabaena spp.	-1.00	0.317
Anaerorhabdus spp.	-1.00	0.317
Anaplasma phagocytophilum	-1.34	0.180
Arcobacter butzlerii	-2.03	0.042
Arcobacter cryaerophilus	-3.93	0.000
Arcobacter spp.	-1.76	0.079
Bacillus pumilus	-3.93	0.000
Bacillus spp.	-3.81	0.000
Bacillus subtilis	-3.20	0.001
Bordetella sp.	-3.92	0.000
Brucella spp.	-2.81	0.005
Chlamydia spp.	-2.21	0.027
Clostridium perfringens	-2.06	0.039
Clostridium spp.	-2.30	0.022
Corynebacterium spp.	-3.93	0.000
Corynebacterium urealyticum	-3.44	0.001
Dietzia maris	-1.69	0.092
Dietzia spp.	-3.47	0.001
Enterobacter cloacae	-2.12	0.034
Enterococcus sp.	-3.84	0.000
Erysipelothrix spp.	-0.35	0.726
Escherichia coli	-1.34	0.180
Fusobacterium nucleatum	-1.60	0.109
Fusobacterium spp.	-3.22	0.001
Hafnia sp.	-3.92	0.000
Helicobacter spp.	-0.923	0.356
Klebsiella sp.	-1.34	0.180
Legionella spp.	-2.69	0.007
Leptospira interrogans	0.000	1.000
Leptospira spp.	-3.21	0.001
Mycobacterium spp.	-3.37	0.001
Mycoplasma sp.	-3.23	0.001
Nocardia nova	-1.34	0.180
Paenibacillus polymyxa	-1.73	0.083
Paenibacillus spp.	-3.31	0.001
Porphyromonas spp.	-2.24	0.025
Propionibacterium acnes	-2.56	0.011
Pseudomonas aeruginosa	-1.00	0.317
Pseudomonas spp.	-3.21	0.001
Rhodococcus spp.	-1.63	0.102
Rickettsia spp.	-1.72	0.086
Salmonella enterica	-3.43	0.001
Sphingobium paucimobilis	-1.60	0.109
Sphingobium spp.	-3.08	0.002
Sphingomonas spp.	-0.728	0.467
Staphylococcus epidermidis	-3.58	0.000
Staphylococcus spp.	-3.83	0.000
Stenotrophomonas spp.	-3.20	0.001
Treponema spp.	-0.445	0.656
Vibrio spp.	-1.99	0.046
Waddlia sp.	-1.34	0.180

partitions reproduced in less than 70% bootstrap replicates were collapsed and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were also indicated next to the branches. The evolutionary analyses were conducted in MEGA 7[14] The *B. anthracis* sequence with accession number *AJ516943.1* was retrieved from the NCBI website and used as the outgroup to root the zoonotic pathogen's phylogenetic tree.

Influence of season on zoonotic bacterial pathogens abundance, diversity, evenness and richness

The distribution of the zoonotic bacterial pathogens in the wet and dry seasons was evaluated by entering the data into SPSS version 24 and subsequently generating the Shapiro-Wilk test and Kolmogorov-Smirnov test values, visually inspecting histograms, Normal Q-Q Plots, and calculation of Z scores from Skewness and Kurtosis. A Wilcoxon test was then performed to investigate the influence of season on the abundance of the identified zoonotic bacterial pathogens. The ecological concepts of species diversity and species evenness were used in this study to investigate the seasonal health risk possed to humans and livestock in consumption of unsanitized hand-dug well water. Zoonotic bacterial species richness, evenness, and both the Shannon-Wiener diversity indices and Simpson's diversity indices were calculated as described by Pielou.[15] Following a normality test, a Paired sample t-test was used to investigate statistically significant differences on Shannon-Wiener diversity indices and species richness data between the wet and dry seasons while a Wilcoxon test was used for the same purpose on Simpson's diversity indices and species evenness data between the wet and dry seasons.

RESULTS

Zoonotic pathogens phylogenetic tree

A total of 57 zoonotic pathogens were detected. The relationship between the detected zoonotic pathogens was investigated by generating a phylogenetic tree (Fig. 3). It was disclosed that *E. cloacae, Klebsiella sp., Hafnia sp., E. coli, S. enterica* and *Vibrio spp.* formed a cluster at 79% bootstrap. *A. faecalis*, *Alcaligenes sp.* and *Bordetella sp.* formed a cluster at 100% bootstrap while *P. aeruginosa* and *Pseudomonas spp.* formed a cluster at 96% bootstrap. *Chlamydia spp.* and *Waddlia sp.* formed a cluster at 82% bootstrap while *A. butzleri, A. cryaerophilus* and *Arcobacter spp.* formed a cluster at 95% bootstrap, and *Anaerorhabdus spp.* and *Erysipelothrix spp.* formed a cluster at 97% bootstrap. *P. polymyxa* and *Paenibacillus spp.* formed a cluster at 80% bootstrap while *B. pumilus* and *B. subtilis* formed a cluster at 84% bootstrap. *Anabaena spp.*, *Rhodococcus spp.*, *Actinomyces spp.*, *P. acnes*, *A. viscosus*, *Mycobacterium spp.*, *N. nova*, *Corynebacterium spp.*, *C. urealyticum*, *D. maris* and *Dietzia spp.* formed a cluster at 73% bootstrap while the rest did not form any clusters. The *B. anthracis* sequence with accession number *AJ516943.1* was retrieved from the NCBI website and used as the outgroup to root the zoonotic pathogen's phylogenetic tree.

Influence of season on zoonotic bacterial pathogens abundance, diversity, evenness and richness

The Kolmogorov-Smirnov and Shapiro-Wilk tests revealed that zoonotic pathogens data was not normally distributed ($P < 0.05$). Wilcoxon rank test was performed to determine the influence of season on the abundance of the detected zoonotic bacterial pathogens (Table 1). Notable pathogens; *Brucella spp.*, *Bacillus spp.*, *Chlamydia spp.*, *Enterococcus sp.*, *Legionella spp.*, *Leptospira spp.*, *Mycobacterium spp.*, *Salmonella enterica* and *Staphylococcus spp.* showed a significant difference in abundance between the wet and dry season ($P < 0.05$, Table 1). *Brucella spp.*, *Bacillus spp.*, *Chlamydia spp.*, *Enterococcus sp.*, *Legionella spp.* and *Salmonella enterica* had higher abundances in the dry season compared to the wet season. *Leptospira spp.*, *Mycobacterium spp.* and *Staphylococcus spp.* had higher abundances in the wet season compared to the dry season. However, there was no significant difference in the abundance of *Escherichia coli*, *Helicobacter spp.*, *Treponema spp.* and *Klebsiella sp.* between the wet and dry seasons.

Shannon-Wiener diversity indices and species richness data were normally distributed ($P > 0.05$) as per Kolmogrov-Smirnov test, and a subsequent Paired sample t-test disclosed a significant difference between Shannon-Wiener diversity (H') indices, and no significant difference in species richness of the detected zoonotic bacterial pathogens between the wet and dry seasons (Table 2). Simpson diversity indices and species evenness data were not normally distributed ($P < 0.05$) as per Kolmogrov-Smirnov test and a subsequent Wilcoxon rank test disclosed a significant difference in Simpson diversity (D) and evenness data between the wet and dry seasons.

Fig. 1. An illustrative diagram of the shallow hand-dug wells found in the Ohangwena and Omusati regions of Namibia in which animals have access to the water.

Fig. 2. An illustrative diagram of the deep hand-dug wells found in the Ohangwena and Omusati regions showing potential seepage of surrounding water into the hand-dug wells.

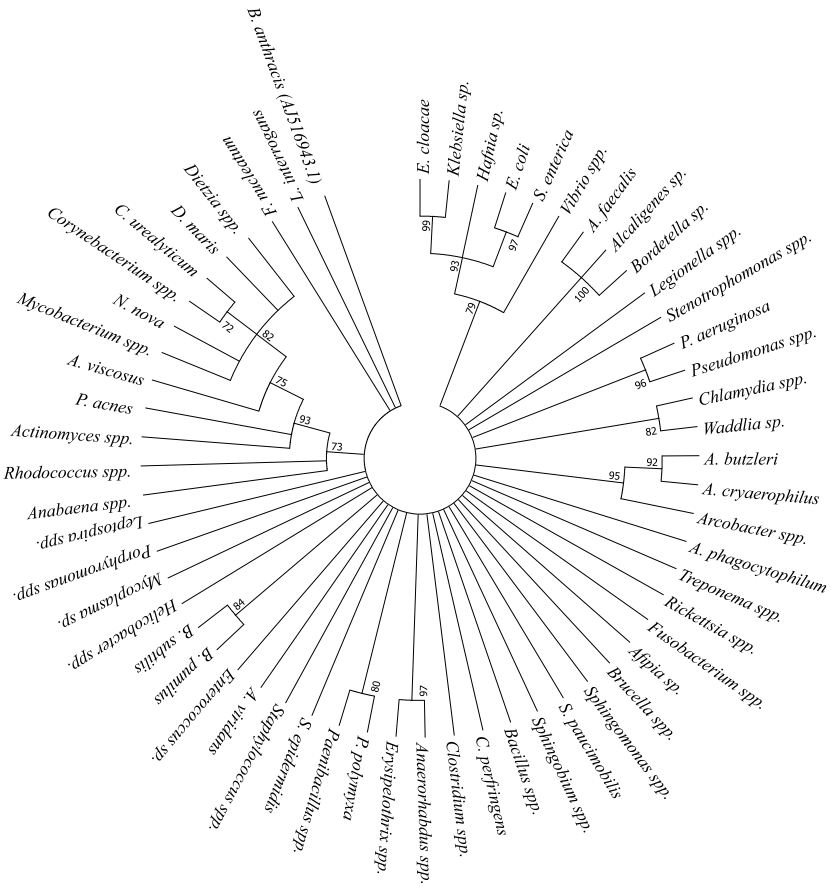

Fig. 3. Phylogenetic tree depicting the evolutionary history of the detected zoonotic bacterial pathogens. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates were collapsed.

Table 2. Tests performed to determine the influence of season on human bacterial species richness (R), evenness (E) and diversity [Shannon (H') and Simpson (D)]

	Wet season				Dry season			
Parameters	H'	D	R	E	H'	D	R	E
Mean	1.15	0.532	22.9	0.101	2.08	0.713	24.75	0.252
St.Dev	0.564	0.253	2.67	0.0373	0.717	0.136	4.54	0.0990
Test statistics value	-3.823	-2.128	-1.386	-3.808	-3.823	-2.128	-1.386	-3.808
Df	19		19		19		19	
P-value	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P>0.05	P<0.05

DISCUSSION

Health risks of detected zoonotic bacterial pathogens

Most clusters of the zoonotic pathogens phylogenetic tree were formed by species belonging to the same family indicating their close relation. The detection of multiple species in each family and their close relation confirmed the complexity and versatility of families. Among others, the notable zoonotic pathogens of public health concern were; *Brucella spp.* known to cause Brucellosis.[16] *B. anthracis* causes anthrax[17] while *Chlamydia abortus* and *Chlamydia psittaci* from the genus *Chlamydia* may cause abortion and psittacosis respectively in animals, birds and humans.[18] *E. faecalis* and *E. faecium* belonging to

the genus *Enterococcus* can cause mastitis and bacteraemia in humans and livestock.[19]

Escherichia coli is known to cause diarrhoea, haemorrhagic colitis, haemolytic uremic syndrome, thrombotic thrombocytopenic Purpura, urinary tract infections, bacteraemia, wound infections, meningitis, enteric infection, uraemic syndrome in humans and livestock.[20] *H. pylori* belonging to the genus *Helicobacter* is known to cause chronic gastritis and ulcerations[21] while *K. pneumoniae* from the genus *Klebsiella* causes intra-mammary infections, and Donovanosis (*K. granulomatis*) as revealed by Umeh and Berkowitz[22] and Bannerman *et al.*[23] *L. jordanis*, *L. lytica*, *L. pneumophila* and *L. sainthelensi* from the genus *Legionella* are known to cause pneumonia, Legionnaire's disease and Pontiac fever[24] while *Leptospira interrogans* belonging to the genus *Leptospira* is known to cause leptospirosis.[25]

The detected *M. aeruginosa* from the genus *Microcystis* is known to cause poisoning[26] while *M. bovis*, *M. tuberculosis* and *M. leprae* belonging to the *Mycobacterium* genus are known to cause tuberculosis and leprosy.[27,28] *Salmonella enterica* is known to cause gastroenteritis, enteric fever, osteomyelitis and diarrhoea in humans and livestock[29,30] while *S. aureus*, *S. epidermidis* and *S. saprophyticus* from the genus *Staphylococcus* are known to cause skin disease, bacteraemia, wound infections, endocarditis, catheter-related sepsis, UTI, toxic shock syndrome, food poisoning, eye infection and osteomyelitis.[31,32] *T. pedis* and *T. pallidum* from the genus *Treponema* are known to cause dermatitis and syphilis in humans and livestock.[33,34]

Seasonality of zoonotic bacterial pathogens of public health concern

Brucella spp., *Bacillus spp.*, *Chlamydia spp.*, *Enterococcus sp.*, *Legionella spp.*, *Leptospira spp.*, *Mycobacterium spp.*, *Salmonella enterica* and *Staphylococcus spp.* showed a significant difference in abundance between the wet and dry seasons. *Brucella spp., Bacillus spp.*, *Chlamydia spp.*, *Enterococcus sp., Legionella spp.* and *Salmonella enterica* had higher abundances in the dry season compared to the wet season, indicating that the exposure of the populace and livestock in Ohangwena and Omusati regions to these species is pronounced in the dry season although disease cases are not documented. It can be argued that since pathogens show seasonality in aquatic environments and correlate with higher temperatures prevailing in the dry, it cannot be ruled out that the warmer temperatures in the wet season can also support the growth of *Brucella spp., Bacillus spp.*, *Chlamydia spp.*, *Enterococcus sp., Legionella spp.* and *Salmonella enterica* since bacterial species respond quickly to higher temperatures when appropriate resources are available.[35]

Leptospira spp., Mycobacterium spp. and *Staphylococcus spp.* had higher abundances in the wet season compared to the dry season, demonstrating that exposure to these species is pronounced in the wet season compared to the dry season because of surface runoff. Moreover, these species are mesophilic in nature and so the water temperatures are mostly within their optimal growth or survival range since hand-dug well water temperature values ranged from 13.2°C to 26.3°C in the wet season and 20.5°C to 34.6°C in the dry season which supports the growth of mostly mesophilic bacteria. However, *Escherichia coli*, *Helicobacter spp.*, *Treponema spp.* and *Klebsiella sp.* did not show a significant difference in abundance between the wet and dry seasons demonstrating that the Ohangwena and Omusati rural populace and livestock experience a continuous exposure to these pathogens. This leads to the conclusion that some water related diseases that occur in these communities can be predicted and appropriate prevention measures ascertained based on pathogen's seasonal variations in abundance.

Influence of season on zoonotic bacterial pathogens diversity, evenness and richness

This study revealed a significant difference in the diversity and evenness, and no significant difference in richness of zoonotic bacterial pathogens in hand-dug wells between the wet and dry seasons ($P < 0.05$) with the dry season having a higher diversity and evenness compared to the wet season. The higher diversity in the dry season translates into increased bacterial loads of each detected zoonotic bacterial pathogenic species in these hand-dug wells. The dry season is characteristic of water scarcity with thirsty animals having no access to flowing water as is the case in the rainy season. This compels livestock and other animals or birds to walk into the water in the case

of shallow hand-dug wells thereby potentially depositing the zoonotic species which can provide a basis for the transfer of enteric zoonotic pathogens between humans and livestock since the hand-dug well water is consumed by both life forms.

The hand-dug wells are poorly constructed in that water is continuously in contact with the soil due to lack of concrete walls and lack top covers. This promotes the survival and unceasing source of soil bacteria, and introduction of enteric microorganisms from animal defecation and this agrees with Ayantobo *et al.*[36] who found that unprotected hand-dug wells had the highest *E. coli* and total coliform counts followed by semi-protected hand-dug wells and protected hand-dug wells. In the case of deep hand-dug wells, the livestock drink water from the troughs that are placed besides the deep hand-dug wells and consequently drop faeces close to the hand-dug wells which can be transported into these wells by wind or water running from the troughs since they lack a top covering. Since Namibia is among countries with the highest rate of open defecation in southern Africa[37], it's logical that hand-dug wells can be polluted with enteric microorganisms especially in the rainy season when the water penetrates the permeable soil layers reaching the aquifers below that are shared by the hand-dug wells within the same vicinity.[38]

The reason for a high zoonotic bacterial diversity in the dry season is most likely due to increased evaporation of hand-dug well water leading to a reduced volume of water with concentrated bacteria. Odonkor and Addo[2] argued that reduced water volumes coupled with increased water-animal contact leads to high bacterial abundance and richness. Hence the soil water exchange interface of bacteria coupled with the effect of evaporation can be viewed as a vicious cycle that potentially maintains the zoonotic bacterial pathogen communities in these hand-dug wells. The soil water interface can potentiate larger genetic and metabolic plasticity by the transfer and exchange of genes owed to microbial interactions thereby enhancing the ability of the microorganisms to cope with various or fluctuating environmental conditions in the hand-dug wells. Evidently, *Escherichia* species were known not to survive lengthy periods outside warm blooded animal intestines making them reliable water quality indicators for faecal contamination and predictors of the potential presence of other contaminant species.[39] However, recent studies have indicated that *E. coli* strains survive in soil and water that's not known to be faecally contaminated.[40]

Overall, the high numbers of detected zoonotic bacterial pathogens are alarming due to possibilities of transferring untreatable bacterial infections between humans and livestock that arise as a result of the inappropriate use of antibiotics in food animals. This leads to untreatable infections when bacteria attain the state of Antimicrobial resistance (AMR). Argudɒn *et al.*[41] revealed that AMR can be transmitted from animals to humans or vice versa either by the direct spread of the resistant bacteria or indirectly through the transmission of resistance genes from animal bacteria to human bacteria. Hence the detection of zoonotic pathogens in hand-dug water used for both humans and livestock consumption also served as a sentinel surveillance deeming these hand-dug wells a possible antimicrobial resistance gene reservoir.

Genetic elements that encode AMR transmission have been documented to exist in commensal bacteria and the intestinal tract is among the core potential sites for the transmission of resistance genes from none pathogenic to pathogenic organisms.[10] Among the pathogens identified in this study, *E. coli* is well documented as a potential source and assimilator of AMR plasmids/genes from the environment, and can disseminate genetic determinants thereby serving as a reservoir for transmissible resistance.[10] Zoonotic pathogens detected in this study known to express multidrug resistance genes include *Escherichia*,[42] *Klebsiella*,[43] *Enterobacter*,[44] *Salmonella*,[45] and *Pseudomonas*.[46] Hence, the high numbers of zoonotic bacterial pathogens in this study signalled the possible upsurge of antibiotic resistant bacterial strains.

Although Metagenomics is a highly informative technique, it has some limitations that are worth noting when used. Metagenomics as opposed to Metatranscriptomics is a DNA based technique, hence the microbial communities detected potentially included DNA from dead bacteria thereby displaying an over representation

of bacterial communities or omitted some bacteria due to DNA extraction difficulties especially in spore forming Firmicutes as described by Filippidou *et al.*[47] This might have led to a low coverage of less abundant taxa known as "depth bias" and under-representation of certain taxa.

CONCLUSIONS

The presence of a diverse assemblage of zoonotic pathogens showed that the water was not definitely safe for human consumption and undoubtedly harmful to livestock as well. The exposure of humans and livestock to zoonotic bacterial pathogens is more pronounced in the dry season compared to the wet season. Furthermore, hand-dug wells were found to be a potential reservoir for antimicrobial resistance genes.

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CONFLICT OF INTERESTS

The authors declares that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

BM collected the water samples and performed the research. WNH and BM analysed microbial diversity, richness, evenness and the statistical analysis. BM, WNH, HW, PMC and BMH wrote the manuscript. BM, PMC and BMH proof read the manuscript.

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DATA AVAILABILITY

The 16S rRNA sequences data that were generated in the current study are available on GenBank, with the following accession numbers: MH155975 - MH156031.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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