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Escherichia Coli Contamination Levels of Water from Unprotected Wells in Chaona Community, Mwachisompola Area of Chibombo District of Zambia

Zgambo Doris*, Munyeme Musso, Kabwali S. Emmanuel, Lysa M. Thendji, Hang'ombe M. Bernard

School of Veterinary Medicine, Disease Control Department, P.O. Box 32379, Main Campus along Great East Road-UNZA, Lusaka, Zambia.
Corresponding author: Doris Zgambo, 0978935250, email: doris.zgambo@gmail.com

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ABSTRACT

A quantitative cross-sectional study was conducted to detect the presence of *E. coli* in unprotected water wells of Chaona community in Mwachisompola area, Chibombo District of Zambia. 48 wells drawn from four villages were sampled from the study area. Laboratory processes of culturing, isolation and identification of *E. coli* confirmed the occurrence of the bacterium in well water. The identified *E. coli* was subjected to antibiotic resistance testing, and PCR was used to detect the resistant genes further. Of the 48 unprotected wells sampled, 38 (79%, 95% CI: 77.3 – 80.7%) were found contaminated with *E. coli*. Meanwhile, 16/48 (33.3%; CI: 31.4 – 35.2%) samples were found with an average CFU of between 1000 and 10,000, the highest range. *E. coli* isolates were also tested for Multi-Drug Resistance (MDR), of which one isolate indicated being resistant to eight antibiotics and another to five antibiotics presenting (5.88%; CI: 3.2 – 8.6%) for each. Meanwhile, seven isolates were resistant to four antibiotics (41.2%; CI: 35.5 – 46.9%), and eight isolates were resistant to three antibiotics (41.1%, CI: 35.4 – 46.9%). In addition, 30.9% (17/55) of the isolated *E. coli* organisms were found to be resistant to three or more classes of antibiotics primarily; Ampicillin, Streptomycin, Tetracycline, Cefotaxime, Nalidixic Acid, Norfloxacin and Ciprofloxacin. The study revealed that *E. coli* contamination was highly possible, and it is recommended that water should be boiled and or treated with chlorine before use at the household level.

KEYWORDS: *Escherichia coli, contamination, unprotected wells and water*

INTRODUCTION

Escherichia coli is a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* and is mostly present in the lower part of the intestine of warm-blooded organisms [1]. Many *E. coli* strains live peacefully in the gut to help keep the growth of more harmful microorganisms in check and aid in digesting food [1]. *E. coli* is also found in the environment, food, and untreated water associated with living animals [2]. *E. coli* is also found in the environment through the faecal-oral route, constituting about 0.1 per cent under aerobic conditions and can grow massively for a few days before the numbers decline slowly [3]. Certain strains of *E. coli*, however, can cause illness and infections such as urinary tract infections, neonatal meningitis sepsis, and abscesses in several organ systems, apart from common diarrhoeal infections [4].

The quality of drinking water is one of the most important variables that influence human health. Poor quality drinking water, particularly in poorer nations, has increased waterborne infections [5]. The most significant factor that led to affecting the quality of the drinking water is the presence of microorganisms that cause diarrhoeal and other illnesses in the water. Water is a vital resource for human life and well-being. It is a fundamental human right to have access to clean and safe drinking water, but a large portion of the world's population still struggles with poor quality drinking water, which has serious health repercussions. Poor quality drinking water directly endangers vulnerable groups, especially the elderly, pregnant women, children, and the chronically ill; therefore, its consequences on human health and public health cannot be understated [6].

Sustainable Development Goals (SDGs) seek to build on the Millennium Development Goals (MDGs) as the universal agenda with 169 targets, some of which aim to achieve universal and equitable access to safe and affordable drinking water for all by 2030 [7]. Despite global efforts, reports from developing countries indicate that about 1 billion people have limited access to safe drinking water and for domestic purposes. This situation contributes to diarrhoeal conditions, particularly in children under five years of age, and is a major cause of morbidity and mortality, especially in sub-Saharan Africa [8].

In Zambia, a developing country, over one-third of the population has limited access to safe drinking water, contrary to progress reports on MDGs indicating that 95 per cent of countries reported meeting the MDG target of achieving clean water for all by 2015 [8]. Unfortunately, people in the study area continue to source water from hand-dug wells for domestic and drinking purposes. Inadequate sanitation and poor quality water are the main causes of diarrhoeal diseases in Zambia due to the consumption of contaminated water, which may contain different pathotypes of *E. coli*, putting many people at risk either directly or indirectly [9].

MATERIALS AND METHODS

A quantitative cross-sectional analytical study was conducted to investigate the occurrence of *E. coli* in unprotected water wells in Chaona community of the Mwachisompola area in Chibombo District.

Study Area

This study was conducted in Chaona community of Mwachisompola area located in Chibombo rural district of Central Province (14° 55' 0" South, 27° 57' 0" East), Zambia. Chibombo District which is among the seven districts of Central Province of Zambia situated along Great North Road.

Study Population

The study population were unprotected wells in Chaona community of Mwachisompola area in Chibombo District of Central Province, Zambia.

SAMPLE SIZE

The sample size was calculated from the single proportion of fifty-five (55) unprotected water wells in the study area, as described by Charan [10], and 48 unprotected wells were sampled.

Sample Collection

Water samples were collected into sterile falcon tubes (50 ml) from the wells using the same containers which the community members use to draw water from each Well. The collected samples were labelled with identification numbers for traceability. Samples were transported in cooler boxes with ice packs to maintain the temperature until they reached the Microbiology Laboratory of the School of Veterinary Medicine at the University of Zambia for analysis.

Enumeration of Bacteria

Each collected sample was serially diluted to a 10th, 100th and 1000th fold and later plated on MacConkey agar and incubated at 37º C for 24 hours. This was done to enable quantification of the colonies. The process started by obtaining 1 ml of the original sample and suspending it in 9 ml of normal saline, giving a 10^{-1} dilution. Secondly, 1ml from the first test tube containing 10^{-1} dilution was pipetted into the second test tube containing 9 ml of normal saline giving 10^{-2} dilution, and thirdly, 1ml from the second test tube was pipetted into the third tube containing 9 ml of normal saline giving 10^{-3} dilution. Thereafter, 0.1ml from each of the three (3) test tubes was pipetted onto MacConkey plates and spread using a microbe spreader. The plates were incubated for 24 hours at 37ºC according to ISO 6887. After 24 hours, the developed colonies were visually counted on the MacConkey plate and multiplied by the reciprocal of the dilution factor. To find the total Colony Forming Units (CFUs) occurring for each sample, the average was calculated [11].

Isolation and Confirmation of *E. coli*

After obtaining 1 ml from each raw sample for serial dilution, 49ml of each sample remained in falcon tubes and centrifuged at 3000xg for 5 minutes. After centrifugation, the supernatant was decanted to remain with 5mls of the sediment. Then the sediment was vortexed (Mixed), and 1ml was obtained and pipetted into a test tube containing 9ml Brain Heart Infusion (BHI) broth. The BHI tubes with the samples were incubated at 37ºC for 24 hours. After 24hrs, a loopful of the sample was inoculated on MacConkey Agar for 24hrs from each of the 48 plates. Suspected *E. coli* colonies were gram-stained to observe a Gram reaction of the bacteria. *E. coli*, which is Gram-negative, appeared pink/red under the microscope. Further, confirmation of the suspected *E. coli* colonies was done using four biochemical tests: 1. Triple Sugar Iron (TSI), 2. Sulfide-Indole-Motility (SIM), 3. Urease and 4. Citrate. Suspected *E. coli* bacteria were further subcultured onto MacConkey Agar and incubated for 24 hours at 37ºC. Pure *E. coli* colonies (typically, they are small, round, smooth, and opaque on agar plates with a slightly raised appearance and pink to red due to lactose fermentation) were grown

on MacConkey-containing cefotaxime (CTX) and incubated for 24hrs.

Antimicrobial Susceptibility Test

The colonies that grew on CTX MacConkey were subjected to antibiotic testing using the disc diffusion method [12]. Drugs used were Cefotaxime, Nalidixic Acid, Streptomycin, Cotrimoxazole, Ciprofloxacin, Tetracycline, Gentamicin, Chloramphenicol, Ampicillin and Norfloxacin. The colonies resistant to Cefotaxime were subjected to DNA extraction for Polymerase Chain Reaction (PCR). Based on recommendations from the Clinical Laboratory Standard Institute (CLSI), the Kirby-Bauer disc diffusion method was used for the antimicrobial susceptibility testing (CLSI, 2009). Ampicillin (10 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg), streptomycin (300 µg), ciprofloxacin (5 µg), tetracycline (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), ceftazidime (30 µg), norfloxacin (10 µg), and cefotaxime (30 µg) were among them. Using recommendations established by CLSI, breakpoints matching the zone of inhibition diameter were used, and the interpretation of susceptibility patterns on different antimicrobial disks was performed. To prevent contamination, normal laboratory practices for quality control were carefully followed. The organisms utilised for quality control were *E. coli* (ATCC®) 25922.

Molecular detection of Antibiotic Resistance Genes

After culture, 2-3 pure colonies from the nutrient agar plate were placed in 1ml of distilled sterile tube. The Eppendorf tube was vortexed for 10 seconds. Bacteria DNA was extracted using a heat-lysis protocol. Molecular confirmation was done by PCR to confirm the resistance of genes Temoniera (TEM), Sulphydryl Variable (SHV), and Cefotaxime-Munich in *E. coli* isolates (CTX). A total reaction volume of 20µl was used for the PCR (Finnzymes piko), which included 5µl Phusion, 2µl sterile distilled water, 2µl primers (forward and reverse), and 1µl bacterial DNA template. The rapid cycle DNA amplification method was used for the PCR reaction, which included an initial denaturation step at 94º C for 1 minute, followed by 35 cycles of template denaturation at 94º C for 30 seconds, primer annealing at 60º C for 30 seconds, primer annealing at 72º C for 30 seconds, and a final extension at 72º C for 5 minutes. After electrophoresis through 1.5% agarose gel, the products were visualised with ethidium bromide.

RESULTS

E. coli load determined in sampled water

Forty-eight unprotected wells were sampled across four villages, independent of each other by distance. Out of 48 unprotected wells, 38 were indicative of *E. coli*, considering strata and within individual variation results presented 79% (95% CI: 77.3 -80.7%). The 38 wells were distributed among all four villages in the study area, and the following results were obtained: Chilumbwa 13% (95% CI: 2.3 – 23.7%), Chabwa 26% (95 CI: 12.1 -39.9%), Kafwilo 29% (95% CI: 14.6 – 43.4%) and Katobole 32% (95% CI: 17.2 – 46.9%).

Microbial Bacterial Count

After dilution, the average number of colony-forming units was relatively high in the sampled wells. The dilutions were in 10 folds, 100 folds and 1000 folds. 16/48 (33.3%; CI: 31.4-35.2%) samples had an average CFU of between 1000 and 10,000, which was the highest range. Meanwhile, 14/48 (29.2%; CI: 27.3 -31.1%) of the samples indicated the average number of CFUs ranging from 1-1000 and 10,000 plus. Only 4/48 (8.3%; CI: 7.2 -9.4%) samples were found with Zero CFU.

Antimicrobial Susceptibility

All 55 *E. coli* isolates were resistant to one or more antibiotics. All isolates were resistant to Ampicillin (100%), Cefotaxime (22/55; 40% CI: 38.3 - 41.8%), Nalidixic Acid (34.6% CI: 32.9 - 36.2%), Cotrimoxazole (12/55; 21.6% CI: 20.1 - 23%), and Tetracycline (8/55; 14.6% CI: 13.3 - 15.8%). Norfloxacin 3/55 (5.5%; CI: 4.6 - 6.26%), Ciprofloxacin (5) 2/55 (3.6%; CI: 2.97 - 4.3%), and Streptomycin (101) 1/55 providing (1.8%; CI: 1.3 - 2.3%). Generally, the antimicrobial susceptibility results indicate varying degrees of resistance among *E. coli* isolates to different antibiotics. The resistance to Ampicillin and Cefotaxime underscores the importance of reasonable antibiotic use and the need for alternative treatment strategies for the exposed population. Additionally, the relatively low resistance rates observed for norfloxacin, Ciprofloxacin, Streptomycin, Gentamicin and Chloramphenicol suggest their potential utility in treating *E. coli* infections. Thus, monitoring antimicrobial resistance patterns is essential to combat the emergence of multi-drug resistance.

Genes Detected on Molecular Analysis

The main genes detected on PCR were *bla*TEM-cluster and *bla*CTX-M cluster, which are beta-lactamase coffering genes, while the *bla*SHV gene was not detected on any isolates. The twenty-two *E. coli* isolates that were further analysed on PCR

revealed that (72.7%; CI: 68.7 – 76.7%) sixteen isolates were Extended Spectrum Beta Lactamase (ESBL)-producing *E. coli* isolates carrying β-lactamase genes of *bla*TEM and (18.2%; CI: 14.7 – 21.6%) four isolates were ESBL-producing *E. coli* isolates carrying the β-lactamase genes of *bla*CTX-M.

DISCUSSION

The study's results indicate that out of 48 unprotected wells sampled across four villages, 38 were found to indicate the presence of *E. coli*. This suggests that approximately 79 per cent of the sampled wells were contaminated with *E. coli*. These findings are important because as they highlight a high prevalence of bacterial contamination in the sampled wells, posing potential health risks to the communities relying on them for water. The results vary in terms of *E. coli* contamination across all four villages. The village-specific contamination rates provide insights into the spatial variability of *E. coli* contamination within the study area. The differences observed among the villages may be attributed to various factors such as geographical location, population density, land use practices, and proximity to potential sources of contamination, as observed by Begum and others [13] on the isolation of *E. coli* from surface water in rural areas of Bangladesh.

Among the samples analysed, most were within the 1000 to 10,000 CFUs after dilution. This range represents the high CFU counts, indicating a significant presence of bacterial colonies in these samples. These high percentages of CFUs found in sampled wells were in line with a study by Phiri [14] on risks of domestic underground water sources in informal settlements in Kabwe, Zambia, revealing 70% of poor water quality in Africa. Addressing the issues on the quality of water is crucial for safeguarding public health and reducing the risk of waterborne diseases associated with bacterial contamination.

Antibiotic-sensitive testing revealed fascinating resistant patterns because all the tested isolates were found to be resistant to three (3) or more classes of antibiotics. These findings were similar to a study conducted in Lusaka, Zambia, by Chishimba and others [115] on ESBL, where high antibiotic-resistant profiles were established and also a study done by Larson and others [16] on antibiotic resistance *E. coli* in drinking water in Peru. These resistant patterns observed in this study could be attributed to the high misuse of antibiotics in treatments as indicated by WHO [20]. These enteric bacteria organisms that may acquire resistance through this system may end up in a pit latrine, increasing the risks of water contamination with antibiotic-resistant bacteria in unprotected water wells. AMR is of great concern as the issue was deliberated upon in the tripartite joint Secretariat for AMR, where a political declaration was made to which the heads of state at the United Nations General Assembly in New York in 2016 committed. These heads of state agreed to focus on a broad, coordinated approach that engages human, animal, and plant environments and health in what is known as the "One Health Approach" [20].

This study further revealed the high prevalence of the isolates analysed on PCR carrying β-lactamase genes of the *bla*TEM cluster. Meanwhile, a relatively low percentage of isolates were carrying β-lactamase genes of *bla*CTX-M. This picture was not very different from the study done by Chishimba and others [15] in Lusaka, Zambia, despite β-lactamase genes for *bla*SHV in this study were not detected on any isolates analysed by PCR. The difference could be due to differences in sample size. It was further interesting to comprehend that the predominant gene detected in this study was *bla*TEM, as opposed to the other study by Chishimba [15]. The occurrence of these ESBL-producing *E. coli* bacteria in water wells is of great concern because bacterial pathogens have been reported to be associated with serious human infections with symptoms of diarrhoea globally and especially in children, as indicated in a study conducted by Thani and others [18] on isolation and characterisation of *E. coli* on phenotypic factors associated with well and borehole water contamination in India. These findings necessitate that water drawn from unprotected wells could play a major role in spreading ESBL-producing *E. coli*, hence the need to improve water sources in the study area.

CONCLUSION

Finally, *E. coli* was isolated from water in 38 (79%) of the 48 unprotected wells in Mwachisompola community in Chibombo District. The *E. coli* counts were found to be higher than the typical limit recommended by WHO water recommendations. The study also found a high (91%) Colony-Forming Unit (CFU) count in the examined wells, indicating faecal contamination. The identified *E. coli* organisms demonstrated antibiotic resistance, and some were even multi-drug resistant.

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REFERENCES

1. Percival SL, Williams DW. *Escherichia coli*. Microbiology of Waterborne Diseases: Microbiological Aspects and Risks: Second Edition. 2013:89–117.https://doi.org/10.1016/B978-0-12-415846-7.00006-8.

2. Foster NE. The Microbial Ecology of *Escherichia coli* in the Vertebrate gut. February. 2022:1–22. https://doi.org/10.1093/femsre/fuac008.

3. Ercumen A, Pickering AJ, Kwong LH, Arnold BF, Masud Parvez S, Alam M, *et al*. Animal Feces Contribute to Domestic Fecal Contamination: Evidence from *E. coli* Measured in Water, Hands, Food, Flies, and Soil in Bangladesh. 2017. https://doi.org/10.1021/acs.est.7b01710.

4. Gwimbi P, George M, Ramphalile M. Bacterial contamination of drinking water sources in rural villages of Mohale Basin, Lesotho: exposures through neighbourhood sanitation and hygiene practices. Environmental Health and Preventive Medicine. 2019; 24:1-7.

5. Pal M, Ayele Y, Hadush M, Panigrahi S, Jadhav V.J. Public health hazards due to unsafe drinking water. Air Water Borne Dis. 2018; 7(1000138):2.

6. Babuji P, Thirumalaisamy S, Duraisamy K, Periyasamy G. Human Health Risks due to Exposure to Water Pollution: A Review. Water (Switzerland). 2023; 15(14):1–15. https://doi.org/10.3390/w15142532.

7. Bain R, Johnston R, Slaymaker T. Drinking water quality and the SDGs. npj Clean Water. 2020;3 (1):37. https://www.nature.com/articles/s41545-020-00085-z.pdf.

8. UNICEF. 2015 Update and MDG Assessment. UNICEF. 2015.

9. Palaniappan RUM, Zhang Y, Chiu D, Torres A, DebRoy C, Whittam TS, *et al*. Differentiation of *Escherichia coli* pathotypes by oligonucleotide spotted array. *Journal of Clinical Microbiology*. 2006; 44(4):1495–1501. https://doi.org/10.1128/JCM.44.4.1495-1501.2006.

10. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? *Indian Journal of Psychological Medicine*. 2013; 35(2):121–126.https://doi.org/10.4103/0253-7176.116232.

11. Blount ZD, Barrick JE, Davidson CJ, Lenski RE. Genomic Analysis of a Key Innovation in an Experimental *E. coli* Population HHS Public Access. Nature. 2012; 489(7417):513–518. https://doi.org/10.1038/nature11514.Genomic.

12. Rehm HL, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, *et al*. ACMG clinical laboratory standards for next-generation sequencing. Genetics in Medicine. 2013; 15(9):733–747. https://doi.org/10.1038/gim.2013.92.

13. Begum YA, Talukder KA, Nair GB, Qadri F, Sack RB, Svennerholm AM. Enterotoxigenic *Escherichia coli* isolated from surface water in urban and rural areas of Bangladesh. *Journal of Clinical Microbiology.* 2005; 43(7):3582. DOI: 10.1128/JCM.43.7.3582.

14. Phiri A. Risks of Domestic Underground Water Sources in Informal Settlement in Kabwe – Zambia. 2016; 5(2):1–14. DOI: 10.5539/ep.v5n2p1.

15. Chishimba K. Extended spectrum beta-lactamase (ESBL) Producing *Escherichia coli* in poultry and water in Lusaka District. The University of Zambia; 2015.

16. Larson A, Hartinger SM, Riveros M, Salmon-mulanovich G, Hattendorf J, Verastegui H, Huaylinos ML, Daniel M. Antibiotic-Resistant *Escherichia coli* in Drinking Water Samples from Rural Andean Households in Cajamarca, Peru. 2019; 100(6):1363–1368. DOI: 10.4269/ajtmh.18-0776.

17. WHO. Water safety plan: a field guide to improving. World Health Organisation; 2014.

18. Thani TS, Symekher SML, Boga H, Oundo J. Isolation and characterisation of *Escherichia coli* pathotypes and factors associated with well and boreholes water contamination in Mombasa county. *Pan African Medical Journal*. 2016; 23. DOI: 10.11604/pamj.2016.23.12.7755.