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Research Article

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A Cross-Sectional Study on the Detection of Extended Spectrum Beta-Lactamase (ESBL) *E. coli* Producers in Groundwater in Lusaka District

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Abstract

Background: *Most of the peri-urban communities in the Lusaka district have a significant number of shallow well water points which part of the local community access drinking water from. The objectives of the research were to detect the presence of extended-spectrum beta-lactamase producing Escherichia coli in shallow well water in the Lusaka district. A total of 145 shallow well water samples from the community were collected between September 2014 and March 2015 and later submitted for laboratory analysis to the University of Zambia, School of Veterinary Medicine, Paraclinical Department Microbiology Laboratory.*

Results: *Overall 9/77 (11.6%, 95% CI; 6.8 – 34.5%) of the total samples analyzed indicated that extended-spectrum beta-lactamase producers were present in water samples. 13.6% (95% CI; 8 – 24.3%) of the total samples analyzed for antibiotic disk sensitivity test indicated that extended-spectrum beta-lactamase producing Escherichia coli isolates had conferred resistance to beta-lactam antibiotics.*

Conclusions: *The study has revealed the presence of ESBL-producing E. coli isolates in shallow well water and could be one of the potential reservoirs for the antimicrobial resistant genes. The results obtained require an urgent need for concerned stakeholders and government wings to immediately bury all the shallow wells in the peri-urban communities and provide them with safe drinking water.*

Keywords: Antimicrobial, *E. coli*, ESBL-producers, Enterobacteriaceae, Shallow well

Background

Extended-spectrum beta-lactamase (ESBL) producers are Gram-negative bacteria that produce enzymes that bestow resistance to most beta-lactam antibiotics which include penicillins, cephalosporins, and the monobactam aztreonam [1]. These ESBL producers have been noticed mainly in the *Enterobacteriaceae* family of bacteria and the commonly encountered ones are *E. coli* and *K. pneumoniae* [2]. These ESBL-producing bacteria inhabit the bowel and cannot be exterminated by commonly used antibiotics [3].

An increased number of shallow well water points located in various peri-urban communities within the Lusaka district are one of the major sources of water. Waste effluents are regularly discharged into these water points resulting in serious environmental and public health hazards [4]. This contributes to frequent outbreaks of water-borne diseases particularly cholera and typhoid fever which results in severe morbidity and mortality. The presence of faecal matter in the water bodies necessitates the dissemination of resistant antibiotic ESBL producers [5].

ESBL has been established to be a serious threat, particularly as a cause of nosocomial infections [6]. These organisms are frequently resistant to many antimicrobial agents usually recommended for the treatment of infections caused by E. coli, such as gentamicin, fluoroquinolones, and trimethoprim-sulfamethoxazole [7]. Heavy usage of antibiotics has been reported to be a risk factor for acquisition of ESBL producing organisms [8]. The existence of ESBL resistant strains in the environment constitutes a serious threat to disease treatment [9]. The occurrence of ESBL pathogenic bacteria in drinking water harbouring resistant genes creates a greater risk of community-acquired infections which could lead to disease outbreaks and later transfer of drug-resistant bacteria to humans causing a public health hazard [10]. In Zambia, the data on ESBL is limited particularly in the environment.

Aim of the study

Therefore, this study was conducted to determine the presence of ESBL-producing E. coli bacteria in shallow well water in the selected community of the Lusaka district.

Methods

The study was conducted in the Lusaka district which is the capital city of Zambia. It covers an estimated area of 360 km^2 and is located at 15º25' Latitude South and 28º17' Longitude East, the city sits on a plateau at 1280m in altitude [11]. The selected peri-urban communities picked in the study are densely populated and also record a high number of diarrhoea cases on annual basis. A cross-sectional study design was used and the duration was from September 2014 to March 2015. A total of 145 shallow well water was sampled.

In each peri-urban community, 10% of shallow well water was sampled. The sampling was done using a string which was first disinfected with 70% alcohol and later tied around the neck of a sterile bottle. The sterile bottle was carefully lowered into the well ensuring that it does not have contact with the walls of the well. When full, the bottle was pulled out and immediately capped. Samples were labelled appropriately and placed in a box with ice packs and transported to the School of Veterinary Paraclinical laboratory, University of Zambia. Samples were processed within 24 hours of collection.

The 145 shallow well water samples were inoculated on MacConkey agar containing 2 mg/L of cefotaxime for preliminary screening of ESBL-producing bacteria. The plates were incubated at 37°C for 24 hours. The colonies that grew on MacConkey agar were identified as lactose fermenters or non-lactose fermenters. The lactose fermenting was identified and selected for further analysis. Identification of E. coli lactose fermenting positive colonies was done using phenotypic characteristics and confirmed by the Triple sugar iron (TSI) and IMViC tests as described by [12]. For genetic detection and characterization, *E. coli* isolates were cultured on brain heart broth at 37°C for 24 hours. After incubation, DNA was prepared by boiling methods. 1.5 ml of bacterial suspension was later centrifuged at 5, 800 x g for 5 minutes. After the centrifugation, the supernatant was discarded. The cell pellet was washed with 500 µl normal saline and centrifuged at 13, 000 x g for 15 minutes and the supernatant was later discarded. Immediately after washing, the cell pellet was suspended in 500 µl of TE buffer (pH 8.0) boiled for 10 minutes and then immediately transferred to the ice for 10 minutes. Cell debris was removed by centrifugation at 13,000 x g for 15 minutes. The supernatant was transferred into a new microfuge tube and kept at - 20°C until use.

The *E. coli* isolates were subjected to PCR for confirmation of resistance genes TEM (Temoniera), SHV (Sulfhydryl variable) and CTX-M (Cefotaxime –Munich). Thus 10 µl of PCR mixture containing 5 µl Phusion, 2µl sterile distilled water, and 2 µl primers (Forward and Reverse) was prepared and 1 µl of bacterial DNA template was later added. The PCR (Finnzymes Piko) was performed using 10 µl total reaction volumes. PCR reaction parameters involving the thermal cycling protocol were performed using the rapid cycle DNA amplification method and this comprised of an initial denaturation step at 98ºC for 30 seconds, followed by 35 cycles of template denaturation at 98ºC, primer annealing at 60ºC for 5 seconds, 72ºC for 1 second and further extension at 72ºC for 10 seconds. The PCR products were viewed with ethidium bromide after electrophoresis through 1.5% agarose gels into 100ml TAE buffer.

The antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion method based on the Clinical Laboratory Standard Institute (CLSI) guideline (CLSI, 2011). The antibiotic discs tested include ampicillin (10 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg), streptomycin (300µg), ciprofloxacin (5µg), tetracycline (30 µg), gentamicin (10µg), nalidixic acid ((30 µg), chloramphenical (30 µg), ceftazidime (30 µg), norfloxacin (10µg) and cefotaxime (30 µg). The phenotypic confirmation of ESBL isolates was done by the combination of disc approximation method using either ceftazidime (30 µg) or cefotaxime (30 µg) alone followed by over- night incubation at 37°C for 18 – 24 hrs. ESBL production whereas interpretation of susceptibility patterns on other anti-microbial disks was done using guidelines laid down in the CLSI, which provides breakpoints corresponding to the zone of inhibition diameter. An increase in antibiotic zone diameter (5 – 12 mm) for either ceftazidime or cefotaxime indicated ESBL production [13]. Quality control Standard laboratory procedures were strictly adhered to avoid contamination. E. coli ATCC 25922 was used as a quality control organism.

Mapping the distribution of ESBLs in shallow wells was done using a GPS reader and Garmin e30 equipment. The parameters recorded include the location of shallow well water, the number of shallow wells and the associated coordinates and altitude.

The laboratory data were entered into Microsoft excel and then exported to the STATA version 13.0 software for analysis according to the objectives of the study. For descriptive statistical analysis of quantitative bacterial counts, measures of location were used to describe the outcome. Results were presented in percentages/pro-

portions and the difference in the distribution of predictor variables was considered significant if the p-value was less than 0.05. The authority to conduct the study was approved by the University of Zambia and permission to collect water swabs was obtained from the Ministry of Health.

Results

Of the 77 suspected *E. coli* isolates analyzed for the presence of ESBL-producing isolates, a total of 9/77 (11.6%, 95% CI; 6.8 – 34.5%) ESBL-producing *E. coli* isolates were found in water (shallow wells) (Figure 1).

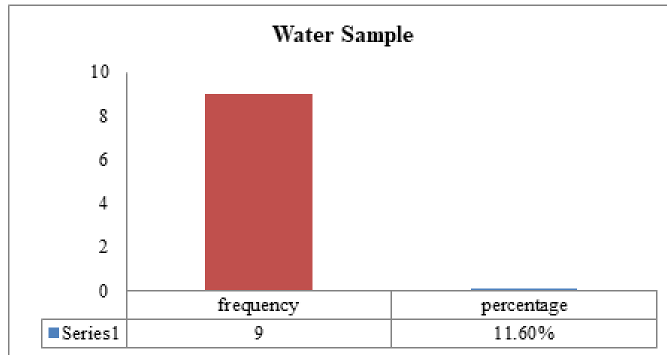

Figure 1: ESBL producers in shallow well water

The results showed that 9/77 (11.6%) ESBL-producing *E. coli* isolates analyzed on PCR confirmed the presence of ESBL-producing *E.coli* isolates and the most frequently encountered gene was a combination of bla_{CTX-M} + bla_{TEM}-cluster 5.2% (4 isolates), followed by bla_{CTX-M}-cluster, bla_{SHV}-cluster with 2.6% (2 isolates) respectively and bla_{TEM}-cluster (Table 1).

Table 1: Confirmed ESBL producers (using PCR and ESBL genes) from Water

Detected gene(s)	No. of *E. coli* isolates	% *E. coli* isolates (n = 9) [a]
SHV	2	2.6
CTX-M	2	2.6
TEM	1	1.3
CTX-M and TEM	4	5.2
Noneb	0	0
Proven ESBL producers	9	9

[a]Seventy seven *E. coli* isolates suspected of being ESBL producers were examined.

[b]Negative in all PCRs

Of the 145 unprotected shallow well water sampled from the peri-urban communities in Lusaka district, 77 were identified to have suspected ESBL *E. coli* isolates and that 9 confirmed the presence of ESBL *E. coli* producers as indicated in figure 2, Map showing the locations of shallow well water samples collected from peri-urban communities in seven study sites (Chawama, Chipata, Garden, Mazyopa, Kanyama, George and Chunga) in Lusaka district.

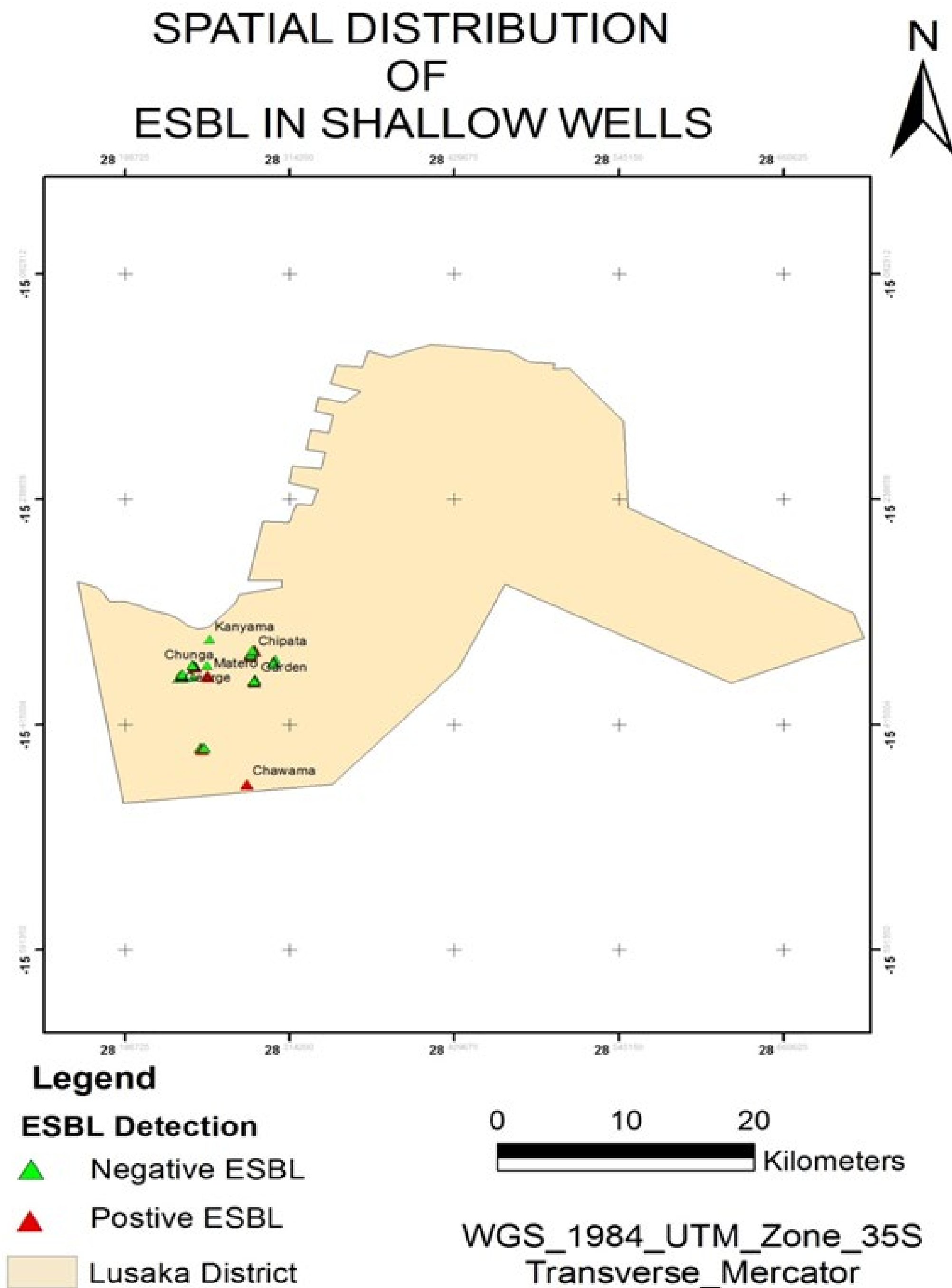

Figure 2: Spatial distribution of ESBL- producing *E. coli* in unprotected shallow wells in the peri-urban communities of the Lusaka district.

77 suspected *E. coli* isolates were subjected for analysis to 11 antimicrobials namely ampicillin (10 µg), chloramphenical (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), norfloxacin (10 µg), streptomycin (300 µg), sulfamethazole/trimethoprim and tetracycline (30 µg). Of 77 suspected *E. coli* isolates analyzed, 11.7% (9/77; CI: 8 – 24.3) were resistant to one or several antimicrobial compounds. The diversity of the antibiotic resistance and susceptible *E. coli* isolates are presented in Table 2. Multidrug-resistance (MDR) *E. coli* isolates to six or more drugs was most frequent (3.9%, 3/77) together with resistance to five drugs (3.9%, 3/77) followed by four drug resistance respectively
(2.6%, 2/77) (Table 2).

Table 2: Antibiotic resistance patterns of *E. coli* isolates from Water

Antibiotic combination	Number of resistant isolates	%	Observation
AMP+CXT+GEN	1	1.3	Resistant to three antibiotics
AMP+CAZ+CHL+SXT	2	2.6	Resistant to four antibiotics
AMP+STR+GEN+TET+CXT	3	3.9	Resistant to five antibiotics
AMP+STR+TET+CXT+NAL+CAZ+NOR+CIP	3	3.9	Resistant to six or more antibiotics

Discussion

Detection of ESBL-producing *E. coli* isolates in shallow well water has not been determined in Zambia. This cross-sectional study has revealed the presence of ESBL-producing *E. col*i isolates in shallow well water. The presence of ESBL-producing *E. coli* isolates in water could have been due to excreta discharges gaining access to groundwater thereby contaminating these water sources with faecal pathogens. This is in line with studies conducted by [14, 15]. The frequent use of shallow wells may increase the risk of acquiring ESBL-producing *E. coli* isolates. The uses of shallow well water in almost all the peri-urban communities include laundry and washing of kitchen utensils, and cooking and washing of fruits and vegetables. The presence of Enterobacteriaceae detected in the shallow well water could possibly pose a potential public health hazard as stated by [9]. The incidence of these ESBL-producing bacteria in water supplies is of greater risk without effective treatments and this calls for vital attention because these bacterial pathogens have been reported to be associated with serious human infections globally [10]. This entails that shallow well water could play a significant role in the dissemination of these ESBL-producing *E. coli* organisms.

Antimicrobial susceptibility testing revealed interesting patterns with multiple resistance rates observed in the majority of all the eleven beta-lactam antibiotics tested namely ampicillin (10 µg), chloramphenicol (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), norfloxacin (10 µg), streptomycin (300 µg), sulfamethazole/trimethoprim and tetracycline [16]. For instance, a study on published and unpublished literature for DRC, Mozambique, Tanzania and Zambia revealed an increased trend of resistance to ampicillin, co-trimoxazole, gentamicin, erythromycin, tetracycline and third-generation cephalosporins [17].

These results have detected the presence of ESBL-producing *E. coli* isolates in water. It is therefore evident that contaminated shallow well water could be one of the possible sources for the transmission of ESBL- resistant genes to the general public.

Study Limitations

This study was only done in selected peri-urban communities in Lusaka district and particularly targeted households with shallow well water sources. The results of this study cannot be generalized to the whole Zambian population however, the study provides initial scientific baseline data regarding the detection of ESBL-producing E. coli isolates in groundwater sources.

Conclusions

This is the first study to be conducted in Lusaka district on the detection of ESBL-producing *E. coli* isolates in shallow wells in peri-urban communities. The cross-sectional study has revealed the presence of ESBL-producing *E. coli* isolates in shallow wells. This therefore further confirms that shallow well water can be one of the major and potential reservoirs for the antimicrobial ESBL-resistant genes which could spread into the food chain. The study has also shown a widespread occurrence of multi-drug resistance patterns [18].

List of Abbreviations

AMP: Ampicillin
CAZ: Ceftazidime
CHL: Chloramphenicol
CIP: Ciprofloxacin
CXT: Cefotaxoime
E. coli: Escherichia coli
ECOFF Epidemiological Cut-Off
ESBL: Extended Spectrum Beta-lactamase
ESBL-E: Extended Spectrum Beta Lactamase Enterobacteriaceae
ESC-R: Extended-Spectrum-Cephalosporin-Resistant
EUCAST: European Committee on Antimicrobial Sensitivity Testing
GEN: Gentamicin
GPS: Geographical Positioning System
NAL: Nalidixic acid
NOR: Norfloxacin
PCR: Polymerase Chain Reaction
SHV: Sulphydryl variable
ST: Strain
SXT: Trimethoprim-sulphamethoxazole
STR: Streptomycin
TE Buffer Tris –Ethylenediaminetetraacetic acic buffer
TEM Temoniera
TET: Tetracycline

Ethics Approval And Consent To Participate

The authority to conduct the study was approved by the University of Zambia and permission to collect water swabs was obtained from the Ministry of Health

Consent for publication

Not applicable

Availability Of Data And Materials

Data available by request from Eden University, School of Medicine, Department of Research and Grants, P.O. Box 37727, Lusaka, Zambia. Authors may be contacted at eden@university.com

Competing Interests

All the authors gave approval and no conflict of interest was declared.

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Authors' Contributions

KC and BH conceived and designed the research. KC, MM, LL and EM developed the data collection tool. KC, BH, NM, FNB and KM analysed and interpreted the data. KC, MM, NM and BH wrote the manuscript. All authors read and approved the final manuscript.

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