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Full Length Research Paper

Antimicrobial resistance pattern in *Salmonella enterica* from clinical and poultry sources in Calabar, Nigeria

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Limited treated options to Salmonellosis exist due to the emergence of extended spectrum beta lactamases (ESBLs). This study determined the prevalence of common ESBL genes (bla_{SHV}, bla_{CTX-M}, bla_{OXA}) and profiled their antimicrobial resistance drug pattern. A descriptive cross-sectional study that involved 16 *Salmonella enterica* isolates from diarrhoeic children at the University of Calabar Teaching Hospital and 39 isolates from healthy birds in poultry farms within the Calabar metropolis. Standard disc diffusion method determined antimicrobial susceptibility while Polymerase Chain Reaction (PCR) using specific primers identified the beta-lactamase genes. All 55 isolates phenotypically demonstrated ESBL production. All 55 salmonella isolates were resistant to Cefotaxime and Nalixidic acid. All the clinical isolates were susceptible Ciprofloxacin and Norflaxacin. The poultry isolates however exhibited varied degrees of resistance to Ciprofloxacin and Norflaxacin. All 55 *Salmonella* isolates carried the bla_{SHV} genes, bla_{CTX-M} gene (94.5%) bla_{OXA} gene (18.2%). Molecular characterization identified the bla_{SHV} gene as the most prominent. This study provides relevant data that would help in the implementation of policies regarding the use of antimicrobial agents in clinical and veterinary settings as well provide antimicrobial treatment guidelines that would strengthen infection control processes.

Key words: Salmonella enterica, Antimicrobial-resistance, ESBL-genes, Poultry-sources.

INTRODUCTION

Salmonella enterica serovars remains one of the major causes of food borne gastroenteritis. Non-typhoid salmonellosis usually causes diarrhoea that may not necessarily require antimicrobial therapy (Andino and Hanning, 2015; Cohen Stuart et al., 2012; Nadimpalli et al., 2019). Nonetheless, in cases where the non-typhoid

infection becomes invasive, severe complications may result hence requiring antimicrobial treatment options (Cohen Stuart et al., 2012). The emergence and spread of antibiotics resistance especially with the detection of extended spectrum beta lactamases (ESBLs) in *Salmonella enterica* serovars has however limited

treatment options because of their acquired resistance to most beta-lactam antibiotics and fluoroquinolones which would have been sufficient first line treatment for both mild and severe salmonellosis (Al Naiemi et al., 2008; Ifeanyi et al., 2015).

Studies have shown ESBL production by *Salmonella enterica* to be plasmid mediated and readily transmitted amongst members of the Enterobacteriaceae family as a result (Rotimi et al., 2008). This potential exacerbates the spread of antimicrobial resistance amongst the other members of the Enterobacteriaceae family against beta-lactam antibiotics and other commonly used antibiotics (Oghenevo et al., 2016). Studies have also shown that ESBLs occur because of mutations in the bla_{TEM-1}, bla_{TEM-2} or bla_{SHV-1} genes, which are commonly found in the Enterobacteriaceae family. Others enzymes, notably from the cefotaxime resistance family (CTX-M), represent plasmid acquisition of broad-spectrum beta-lactamases originally determined by chromosomal genes (Bajpai et al., 2017). These mutations gave rise to the alterations in their amino acid configuration and conferred on these enzymes the ability to hydrolyze a broader spectrum of antibiotics (Bajpai et al., 2017).

More than 350 different naturally occurring ESBL variants have been discovered and classified into nine distinct structural and evolutionary families based on their amino acid sequence comparisons (Sharma et al., 2010). They include, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER}, bla_{VEB}, bla_{GES}, bla_{BES}, bla_{TLA}, and bla_{OXA} (Sharma et al., 2010).

Not much data have been documented regarding the prevalence of the ESBL-producing genes responsible for the antimicrobial drug resistance, especially in Nigeria. This study determined the prevalence of the common ESBL genes (bla_{SHV}, bla_{CTX-M}, bla_{OXA}) in Calabar state, Nigeria and profiled their antimicrobial resistance drug pattern.

MATERIALS AND METHODS

Study area

This study was carried out within the Calabar Metropolis in Cross River state, Nigeria. Calabar is the capital city of Cross River State in the South-South geopolitical zone of Nigeria. Cross River State shares boundaries with Benue State to the North, Ebonyi and Abia States to the west and to the east by the Republic of Cameroun. The city is administratively divided into Calabar Municipal and Calabar South Local Government Areas (LGAs). Calabar covers a surface area of about 406 km^2 (157 sq mile) and a population of 371,022 at the 2006 census.

Study design

This was a descriptive cross-sectional study designed to investigate the prevalence of common ESBL (bla_{OXA}, bla_{SHV}, bla_{CTX-M}) genes among *Salmonella* strains obtained from diarrheic patients in the University of Calabar teaching hospital and local poultry farms within the Calabar metropolis. Study period was between November 2017 and February 2018.

Ethical consideration

The Ethical Committees of the selected hospital approved the protocol for this study. Approval was also obtained from the Cross River State Ministry of Health, conveyed via CRS/MH/ CGSE-H/018/Vol/123 and the Health Research Ethical Committee of University of Calabar. Consent was sorted from all study participants and guardians of the poultry farms prior to the collection of samples.

Isolation and identification of *Salmonella* species

Sixteen (16) *Salmonella enterica* isolates were randomly recovered from the faeces of diarrhoeic children attending the University of Calabar teaching hospital and 39 *Salmonella enterica* isolates obtained from healthy birds randomly selected in some local poultry farms within the Calabar Mertopolis. This gave a total of 55 *Salmonella* isolates. Each of the isolates was sub-cultured on MacConkey agar prior to confirmation. Each typical *Salmonella enterica* colony was confirmed by means of the 16s rDNA amplification.

The typical *Salmonella* colonies were further sub-cultured overnight in 5 ml of Luria Bertani (LB) broth and centrifuged at 14000 rpm for 3 min. The cells were re-suspended in 500 µl of physiological saline and heated by means of a heating block at 95°C for 20 min. The heated bacterial suspensions were then brought to cool on ice and re-centrifuged at 14000 rpm for 3 min. The supernatant (containing the DNA) was transferred to a 1.5 ml micro-centrifuge tube and stored at -20°C for further analysis. DNA quantification of the extracted genomic DNA was carried out using the NanoDrop 1000 Spectrophotometer.

The DNA of each typical *Salmonella* isolate was extracted and used for the amplification of the 16S rRNA region of the isolates' DNA using a specific primer set (27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3'), by means of the ABI 9700 Applied Biosystem thermal cycler at a final volume of 50 microliters for 35 cycles. The Polymerase Chain Reaction (PCR) mix included: X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, $MgCl_2$), the primer sets at a concentration of 0.4 M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation at 95ºC for 5 min, denaturation at 95ºC for 30 s, annealing temperature at 52ºC for 30 s, extension at 72ºC for 30 s for 35 cycles and final extension at 72ºC for 5 min. The product was resolved on a 1% agarose gel at 120 V for 20 min and viewed by means of a UV trans-illuminator.

DNA sequence analysis was performed using direct sequencing of both strands by means of the BigDye Terminator kit on a 3510 ABI sequencer (Inqaba Biotechnological, Pretoria - South Africa). The obtained DNA sequences were edited using TraceEdit. Highly similar sequences were downloaded from GenBank in the National Center for Biotechnology Information using BLASTn (http://www.ncbi.nlm.nih.gov/blast). The *Salmonella enterica* isolates were confirmed by a 100% match with the 16S rDNA of the downloaded sequences of *Salmonella enterica*.

Antimicrobial susceptibility testing and detection of ESBL production

This study considered the use of thirteen commercially available antibiotic discs types (LiofilChemDiagnostici, LD) which included; Norfloxacin (NOR-10 µg), Nalidixic acid (NA-30 µg), Clotrimoxazole (CLO-50 µg), Amikacin (AK-30 µg), Ciprofloxacin (CIP-5 µg), Imipenem (IMI-10 µg), Chloramphenicol (C-30 µg), Ceftazidime (CAZ-30 µg), Cefotaxime (CTX-30 µg), Cefepime (FEP-30 µg), Aztreonam (ATM-30 µg), ESBL screen disc kit (Cefotaxime,

Cefotaxime+Clavilanic acid, Cefotaxime+Clxacillin and Cefotaxime+Clavilanic acid+ Cloxacillin). The susceptibility of the isolates to antimicrobial agents was determined using the Kirby-Bauer disk diffusion method (Cheesbrough, n.d.; Fallah et al., 2013). A bacterial lawn was prepared by transferring 4 to 5 colonies of the same morphological type in a tube containing 2.5 ml sterile normal saline by means of a sterile inoculating loop. The suspension was vortexed and its turbidity compared with barium chloride (0.5 McFarland Turbidity Standard; 1.0 x 108 CFU/µL). The optical density of the standard was regularly monitored with a spectrophotometer at λ=625 nm and 1 cm light path (ODλ=0.08 - 0.1) (Cheesbrough, n.d.). One hundred micro liters of the inoculum was spread on Iso-sensitivity test agar plates. The excess inoculum was siphoned with sterile Pasteur pipettes. Plates were allowed to dry at room temperature in a laminar flow. Disks containing predetermined amounts of antibiotics were then dispensed unto the bacterial lawn using a pair of sterile forceps and gentle pressure applied to ensure complete contact with the agar. The disks were placed 15 mm away from the edge of the plate and 25 mm away from each other. The plates were inverted within 15 minutes after the disks were dispensed, and incubated at 37°C for 16 to 18 h. After incubation, they were examined by reading the diameters of the inhibition zones and interpreted in accordance with the description of the United States Clinical and Laboratory Standard Institute (Cheesbrough, n.d.). The isolates showing resistance towards more than three different groups of antimicrobials such as quinolones, aminoglycosides, and cephalosporins, etc., were considered multidrug resistant strains.

The detection of ESBLs producers using the ESBL screen disc kit was performed on the isolates that showed resistance to the third generation cephalosporin antibiotics by disc diffusion method. Briefly, a disc of amoxicillin-clavulanic acid-30 µg was placed on the center of the Muller-Hinton agar plate inoculated with the resistant strain, followed by placing three different cephalosporin discs (cefotaxime-30 µg, ceftrixone-30 µg and ceftazidime-30 µg) around the amoxicillin-clavulanic acid-30 µg, 20 mm each and incubated for 24 h at 37°C.

Examination/interpretation of plates

Enhancement of the inhibition zone, indicating synergy between clavulanic acid and any one of test antibiotics, will be regarded as presumptive for the production of ESBLs. Zone diameters of Ceftriaxone ≤ 26 mm, Cefotaxime ≤ 24 mm and Ceftazidime ≤ 28 mm will be considered presumptive for ESBLs production by screening.

Quality control

Klebsiella pneumoniae 700603 and *Escherichia coli* 25922 were used as positive and negative control strains respectively for ESBLs production.

Detection of *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{OXA}$ genes by PCR

The CTX-M gene was amplified using the primer set CTX-M/F: 5'-CGCTTTGCGATGTGCAG-3' and CTX-M/R: 5'-ACCGCGATATCGTTGGT-3' on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 µl for 35 cycles. The PCR mix included: X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 52ºC for 30 s; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5

min. The PCR product was resolved on 1% agarose gel at 120V for 20 min and visualized on a UV trans-illuminator.

The SHV gene was amplified using the primer set SHV/F: 5'-CGCCTGTGTATTATCTCCCT-3' and SHV: 5'-CGAGTAGTCCACCAGATCCT-3' on an ABI 9700 Applied Biosystem thermal cycler at a final volume of 25 microliters for 35 cycles. The PCR mix included: X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 56ºC for 30 s extension, 72ºC for 30 s for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on 1% agarose gel at 120V for 15 min and visualized on a UV trans-illuminator.

The OXA gene was amplified using the primer pair OXA/F: 5'-AGCCGTTAAAATTAAGCCC-3' and OXA/R: 5'-CTTGATTGAAGGGTTGGGCG-3' on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 microliters for 35 cycles. The PCR mix included: X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 s; annealing, 55ºC for 30 s; extension, 72ºC for 30 s for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel at 120V for 15 min and visualized on a UV trans-illuminator.

Statistical analysis

The data generated in the cause of this study from the antimicrobial susceptibility tests were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA, version 22.0.). P-values of less than 0.05 (< 0.05), was considered of statistical significance.

RESULTS

Table 1 revealed the antimicrobial resistance profile of the *Salmonella enterica* isolates from clinical and poultry sources. All the *Salmonella enterica* isolates (n=55) from both clinical and poultry sources produced ESBL. All the *Salmonella* isolates from the clinical source (n=16) were resistant to Clotrimoxazol. All 55 the *Salmonella* isolates were resistant to Cefotaxime and Nalidixic acid. All the clinical *Salmonella enterica* isolates showed no resistance to Ciprofloxacin and Norfloxacin.

Table 2 showed the distribution of *bla*$_{SHV}$, *bla*$_{OXA}$ and *bla*$_{CTX-M}$ genes. All 55 *Salmonella enterica* isolates carried the *bla*$_{SHV}$ gene while *bla*$_{OXA}$ and *bla*$_{CTX-M}$ were observed in 18.2%(10) and 94.5%(52) of the isolates, respectively. The proportions of occurrence of the *bla* genes in both the clinical and the poultry *Salmonella* isolates were not of statistical significance (P>0.05). The *bla*$_{SHV}$ and *bla*$_{CTX-M}$ occurred well in predominance over the *bla*$_{OXA}$ gene in both the clinical and poultry *Salmonella enterica* isolates.

Figure 1 characterized the combination of *bla* genes possessed by the *Salmonella enterica* isolates from clinical and poultry sources. From the clinical sources, 7 and 9 *S. enterica* isolates carried only *bla*$_{CTX-M}$ and *bla*$_{SHV}$ while 4 *S. enterica* isolates carried both the *bla*$_{CTX-M}$ and *bla*$_{SHV}$ genes in combination. Also, all three *bla* genes

Table 1. Resistance patterns of clinical and poultry Salmonella isolates to tested antimicrobial agents.

Antimicrobial agent	No.(%) resistance	
	Clinical isolates (n=16)	Poultry isolates (n=39)
Amikacin	1(6.3)	12(30.8)
Chloramphenicol	15(93.8)	23(59.0)
Clotrimoxazol	16(100.0)	25(64.1)
Cefepime	7(43.8)	27(69.2)
Cefotaxime	16(100.0)	39(100.0)
Ceftazidime	8(50.0)	33(84.6)
Imipenem	13(81.3)	36(92.3)
Aztreonam	7(43.8)	33(84.6)
Nalidixic acid	16(100.0)	39(100.0)
Ciprofloxacin	0(0.0)	18(46.2)
Norfloxacin	0(0.0)	12(30.8)

Table 2. Distribution of *bla*-genes among clinical and poultry Salmonella isolates.

Source of *S. enterica*	No. (%) *bla*-genes		
	OXA	SHV	CTX-M
Human (n=16)	3(18.8)	16(100)	14(87.5)
Poultry (n=39)	7(17.9)	39(100)	38(97.4)
Total (n-55)	10(18.2)	55(100)	52(94.5)
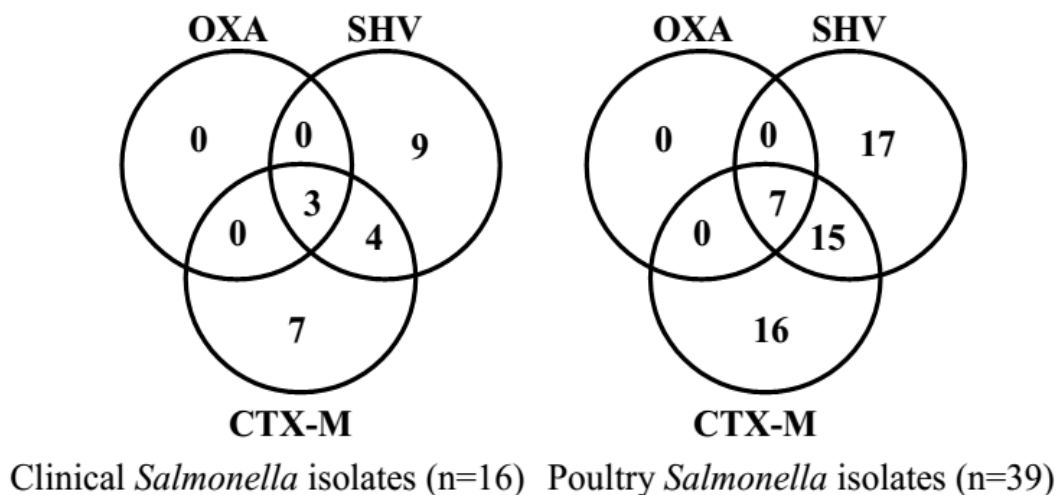
Figure 1. Combination of *bla* genes among the clinical and poultry *Salmonella* isolates.

under study were carried by 3 clinical Salmonella isolates.

From the poultry isolates, 16 and 17 isolates respectively carried only the *bla*$_{CTX-M}$ and *bla*$_{SHV}$ gene while 15 isolates carried both the *bla*$_{CTX-M}$ and the *bla*$_{SHV}$ gene combination. Also, 7 isolates carried all three *bla* genes under study in combination.

DISCUSSION

It became imperative to profile antibiotic resistance patterns because incorrect identification of antibiotic resistance usually leads to inappropriate antibiotic prescription, which may in turn select and give rise to new resistant genes. This study profiled antibiotic

resistance and determined the prevalence of the common ESBL-producing genes (bla_{SHV}, bla_{CTX-M}, bla_{OXA}) in Calabar state, Nigeria.

S. enterica isolates were extracted from 55 isolates of poultry and clinical sources. Based on the result of the ESBL phenotypic screening test kit carried out in this study, all the *S. enterica* isolates were ESBL producers. All 55 isolates demonstrated 100% resistance to nalixidic acid and 100% susceptible to ciprofloxacin and norflaxacin antibiotics. This agrees with several studies carried out in India where salmonella isolates demonstrated 100% resistance to nalixidic acid with susceptibility to ciprofloxacin (Crump et al., 2003; Kownhar et al., 2007; Ye et al., 2018). These antibiotics belong to the quinolone/fluoroquiniolone drug class. Studies have shown Quninolone resistance to be as a result of mutations in the DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV encoding (*parC* and *parE*) genes (Heisig et al., 1995; Ye et al., 2018). Other studies have also reported the presence of plasmid mediated quinolones resistant (PMQR) genes carried by the ESBL-producing plasmid, which facilitates the selection of higher-level resistance to quinolone drugs (Ahamed Riyaaz et al., 2018; Carfora et al., 2018; Jacoby et al., 2014; Kongsoi et al., 2015). A combination of both factors with the possible presence of PMQR genes to a greater extent, we believe could be responsible for the observed resistance to Nalixidic acid and the increased resistance to Ciprofloxacin and Norfloxacin in the poultry isolates.

Other studies have also suggested that the in vitro resistance to nalixidic acid could be used as a pointer to the actual level of in vitro resistance to ciprofloxacin (Campioni et al., 2017; Klemm et al., 2018). According to the Clinical and Laboratory Standard Institute (CLSI) guidelines, the resistance to any antimicrobial agent in the fluoroquinolone drug class has an impact on the resistance of other antimicrobial agents within this drug class (Sahu et al., 2018). This implies that the resistance observed by the *Salmonella enterica* to nalidixic acid in this study is a pointer to the development of resistance to other members of the fluoroquinolone class of antimicrobial agents such as ciprofloxacin and norfloxacin in humans in the Calabar region. This is important information given that fluoroquinolones are regarded as the antimicrobial class of first choice for the treatment of severe infections caused by *S. enteica* as well as other pathogenic Enterobacteriaceae in humans.

Results further indicated that fluoroquinolone resistance was higher in poultry isolates in comparison to the clinical isolates. This agrees with several studies carried out in Europe, Asia and North America where resistance to fluoroquinolones by *S. enterica* was far higher in animals than in humans (Britto et al., 2018; Gouvêa et al., 2015; Nelson et al., 2007). Given that poultry is one of the most preferred foods of animal origin, this study suggests that the resistant *S. enterica* isolates in humans could have come from animals via the food chain.

This study showed high rates (93.8 and 59% from clinical and poultry sources respectively) of resistance by the *Salmonella* isolates to chloramphenicol. This finding was in consonance with a similar study carried out in Brazil were the sensitivity of chloramphenicol was found to be as high as 95% among children with salmonellosis (Brown et al., 2018; Ferreira et al., 2011). The high resistance observed suggests the frequent use of chloramphenicol in both the clinical and veterinary setting.

The emergence of ESBL resistance to beta-lactam antibiotics has also introduced a new hurdle in the treatment of salmonellosis as extended-spectrum cephalosporins (beta-lactam antibiotics drug class), which were typically the antibiotics of choice for mild salmonellosis has become insufficient. Results showed an enhanced degree of resistance to the third and fourth generation Cephalosporins (Cefepime, Cefotaxime, Ceftazidime) thus thwarting the reliability of these antibiotics in the treatment of salmonellosis. In judicious use in both humans and poultry, we believe could be the most probable reason for the selective pressure and consequent resistance to these drugs. Therefore, we suggest strict selection and rotation of antimicrobial agents coupled with the continuous monitoring of susceptibility profiles of antimicrobial agents in order to manage the emergence and spread of cephalosporin resistance.

All the salmonella isolates in this study carried the ESBL-producing bla_{SHV} gene in combination with the bla_{CTX-M} and bla_{OXA} genes making bla_{SHV} the most prominent ESBL-producing gene from *S. enterica* isolates in this study.

Conclusion

Other combinations may exist in Calabar, as this study does not account for other known *bla* genes and recommend that more resources be dedicated to fund further research on this area. Nonetheless, the diversity of the *Salmonella* isolates as a result of the dissemination of these genes is a call for concern and emphasizes a need for an extensive investigation for the presence of these genes in Nigeria as well as the implementation of strict antimicrobial policies in a bid to restrict the spread of these resistance genes and prevent the emergence of new resistant strains.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Detection of antibiotic resistant bacteria and sterol concentration in hand dug wells cited near pit latrine in Southwestern Nigeria

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Provision of safe water and improvement in sanitation has led to a reduction in occurrence of diseases, especially water borne diseases but citing of pit latrine near these wells can be of health concern. Assessment of microbiological quality, feacal sterol concentration and antibiotic resistant pattern of isolated bacteria from hand dug well in Oko, Nigeria were carried out during dry and rainy seasons using standard methods. A total of thirty-one and twenty-nine organisms were isolated during rainy and dry seasons, respectively. The total heterotrophic count of the water samples for dry and rainy seasons ranged from 1.14 to 5.53×10^5 Cfu/100 mL and 0.54 to 7.06×10^5 Cfu/100 mL respectively, while total enterobacteriaceae count ranged between 1.18 to 4.62×10^5 Cfu/100 mL and 4.58 to 14.1×10^5 Cfu/100 mL during dry and rainy season, respectively. All the isolates showed multiple antibiotics resistant (MAR) to the eleven antibiotics used in this study. U.V spectrophotometric analysis revealed the concentrations of coprostanol to be within the range of 1.654 to 2.676 abs which is an indication of contamination from human feacal sources. There was a significant relationship between the resistant pattern of both Cephalosporin and Penicillin classes of antibiotics, a justification from heavy pollution and possession of multidrug (commonly used antibiotics) resistant organisms of the studied well water samples, these calls for a major concern of public health workers.

Key words: Water, antibiotic, resistant, Enterobacteriaceae, sterol.

INTRODUCTION

Rural towns and villages in Africa are confronted with huge challenges and multiple issues that adversely affect public health. One of the major challenges is the ability of both rural and urban inhabitants to access clean water supply (WHO, 2006). Not only is there poor access to portable drinking water, even when water is available in

these small towns, there are risks of contamination due to several factors. When wells are dug and water sanitation facilities are developed, they are not properly maintained due to limited financial resources (Jasmin and Malikarjuna, 2014; WHO/UNICEF, 2008). Both pit latrines and hand dug well are necessities in rural areas of low-income countries like Nigeria. Leachate from pit latrines to nearby wells may cause human and ecological health hazards associated with microbiological and chemical contamination of groundwater (Nwachukwu and Otokunefor, 2006). In rural areas, well water are not properly planned before drilling and are often times located near unlined septic tanks or pit latrines which are majorly not properly covered (Tukur and Amadi, 2014).

The quality of water is majorly affected by microbial pollution and human health can be affected by many pathogen-contaminated water resources from agricultural produce, body contact and drinking of this water (Azzam et al., 2017). Water borne diseases include many gastrointestinal disorders as well as urinary tract infections and associated skin diseases, though people are not affected the same way but immunocompromised people are more affected (Pillai and Rambo, 2014).

The use of antibiotics is not only limited to clinical uses presently; large amount of it is used in Agriculture, Food industries, and Aquaculture (Van Boeckel et al., 2015). The environmental spread of unused antibiotics and their incomplete metabolism in the environment has elicited a bacterial adaptation response to develop antibiotic resistance and genes (Purohit et al., 2017). Antibiotics resistance in wastewater, surface water and drinking water has been documented (Purohit et al., 2017). The problem of antibiotic resistant bacteria is a global phenomenon in drinking water, rivers, lakes, groundwater and waste water (Mulamattathil et al., 2014). Effects of water pollution are much on human health when it is further complicated with the spread of antibiotic resistant bacteria (Novo et al., 2013). This problem of antibiotic resistant is a major problem because infections or diseases caused by these bacteria might result in high cost of treatment and increased mortality (Lupan et al., 2017).

In the past, leachate of faecal contaminant in ground water, marine water, fresh water and portable water supplies has been determined traditionally by quantification of faecal coliform bacteria and by the determination of some inorganic compounds, such as ammonia and nitrogen (Tonny et al., 2004). However, faecal sterol such as coprostanol remains the most prominent human biomarkers indicator of faecal pollution (Bachtiar, 2002). These compound markers derivatives are coprostanol (5β-cholestan-3β-ol), which contains 40 to 60% of the aggregate sterols in human.

Therefore, this study was carried out to determine the concentrations of feacal sterols in the water samples as an indication of feacal contamination and prevalence of antibiotic resistant bacteria in the studied wells.

MATERIALS AND EXPERIMENTAL METHODS

Description of the study area

Oko is a densely populated rural residential/village area in Oyo State, Nigeria. The justification for selecting the study area was based on the high usage of pit latrine in the community. The elevation of the ground above sea level is above 400 m. The topography of the area is of gentle low land in the south, rising to the plateau by about 40 m. The town has an equatorial climate of dry and rainy seasons and relatively high humidity. The dry season begins from December to March while the rainy season starts from April and ends in October. Average daily temperature ranges from between 25°C (77.0°F) to 35° (95.0°F) almost throughout the year (Figure 1).

Sample collection

A total of 11 well were randomly chosen for this study, water samples were taken from the shallow hand-dug wells in the area following standard sampling procedures. Water samples were collected during the peaks of dry and rainy seasons. Water samples were collected using standard methods, water was collected into the sampling bottles, the caps were carefully replaced and the sample was transported in ice box to the laboratory for immediate analysis (Adejuwon et al., 2011). Table 1 shows the GPS coordination of the studied wells.

Bacteriological analysis

Samples were examined after collection according to standard methods for examination of water samples (APHA, 2012). Estimation of Total Heterotrophic Bacteria (THB) and Total Enterobacteriaceae Count (TEC) was determined with a little modification to conventional plate count techniques as described by Larry and James (2001). Nutrient Agar and Eosine Methylene Blue Agar were used for THB and TEC cultivation, respectively. The agar plates were incubated at 37°C for 24 to 48 h to enumerate the aerobe facultative bacteria and the faecal coliform. After incubation, the colonies that grew on the medium were counted and expressed as colony forming units (Cfu/100 ml) of the samples using previous methods (Guo et al., 2013; Hussain et al., 2013). Individual pure colonies were determined by morphological and biochemical techniques according to the methods described by Leonard et al. (2016). Microbial identification was performed using the keys provided in the Bergey's Manual of Determinative Bacteriology (Bergey et al., 1994).

Antimicrobial sensitivity test for the isolated microorganisms

Mueller-Hinton Agar procured from MICROMASTER, Maharashta, India and Antibiotics disks from Oxoid Company (UK) were used for this test. Diffusion technique as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2014) was used to test the sensitivity of organisms to the following antibiotics impregnated disks: Cefixime (5 µg); Cluverate/Augumentin (30 µg); Ceftazidine (30µg); Cefuroxime (30 µg); Ciprofloxacin (5 µg); Ofloxacin (5 µg); Nitrofurantin (300 µg); Gentamicin (10 µg); Erythromycin (15 µg); Novobiocin (5 µg) and Penicillin (10 µg). Results after 18 to 24 h of incubation were interpreted according to clinical breakpoints from CLSI (2014). Resistance values were recorded either as susceptible (S), intermediate (I), or resistant (R) (Jennifer, 2001).

Extraction of extractable organic matter (EOM)

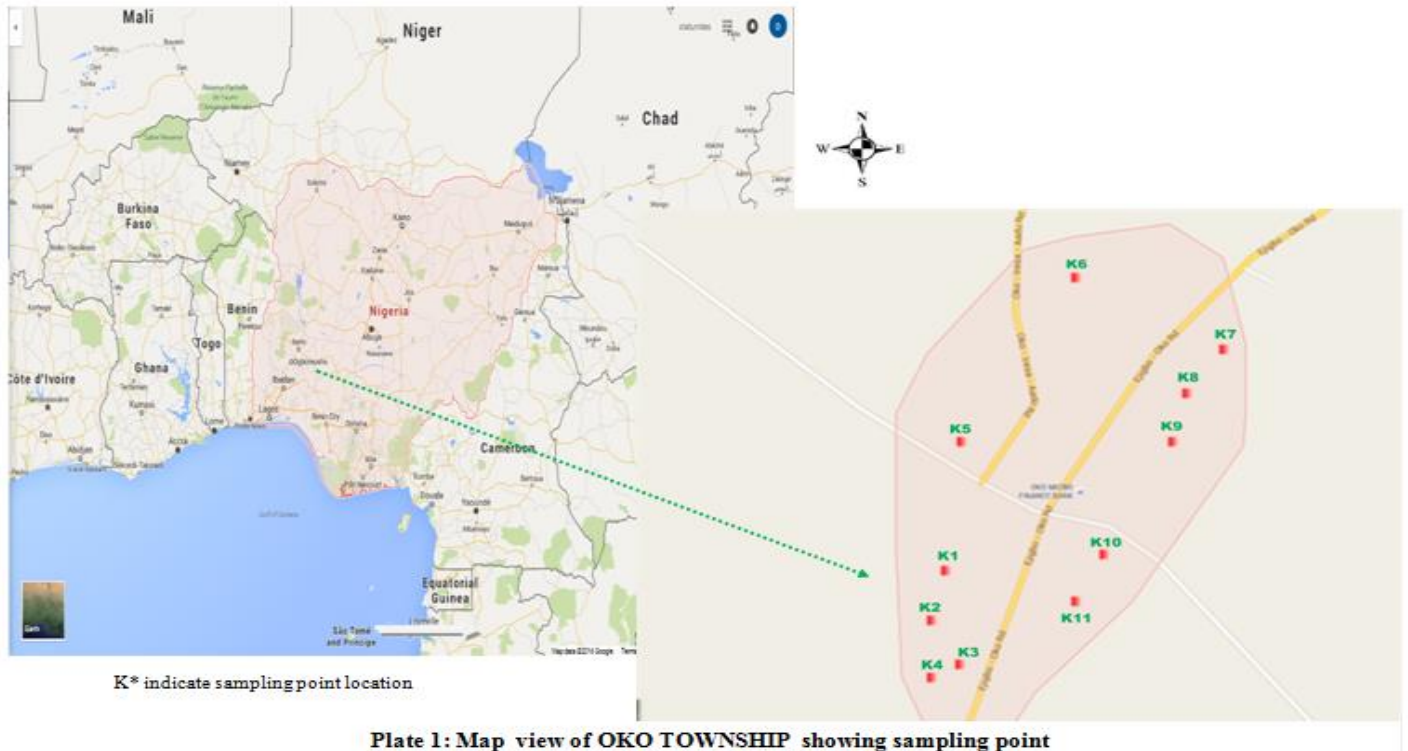Water samples (250 ml) was treated with 25 ml of dichloromethane

K* indicate sampling point location

Plate 1: Map view of OKO TOWNSHIP showing sampling point

Figure 1. Map view of OKO Township showing sampling location.

Table 1. GPS coordination of sampling sites.

Code	Co-ordinates of well (GPS) location	Elevation (m)	Distance of well from pit latrine (m)	Co-ordinates of pit latrine (GPS) location
K1	7° 57' 7'' North 4° 20' 25'' East	389.50	30	7° 57' 7'' North 4° 20' 24'' East
K2	7° 57' 9'' North 4° 20' 25'' East	393.57	9	7° 57' 8'' North 4° 20' 24'' East
K3	7° 56' 58'' North 4° 20' 19'' East	377.50	10	7° 56' 57'' North 4° 20' 19'' East
K4	7° 56' 55'' North 4° 20' 17'' East	376.00	18	7° 56' 54'' North 4° 20' 17'' East
K5	7° 57' 21'' North 4° 20' 28'' East	399.00	20	7° 57' 21'' North 4° 20' 17'' East
K6	7° 57' 33'' North 4° 20' 43'' East	410.00	8	7° 57' 32'' North 4° 20' 43'' East
K7	7° 57' 27'' North 4° 20' 44'' East	410.00	11	7° 57' 26'' North 4° 20' 43'' East
K8	7° 57' 24'' North 4° 20' 44'' East	391.00	16	7° 57' 25'' North 4° 20' 44'' East
K9	7° 57' 27'' North 4° 20' 48'' East	394.50	12	7° 57' 26'' North 4° 20' 48'' East
K10	7° 57' 18'' North 4° 20' 37'' East	388.00	30	7° 57' 18'' North 4° 20' 36'' East
K11	7° 57' 18'' North 4° 20' 36'' East	390.00	11	7° 57' 18'' North 4° 20' 37'' East

Figure 2. The diagrams of wells and pits in close proximity in the studied area.

followed by rigorous shaking in separating funnel, the separating funnel was left to settle down on a retort stand for 4 or 5 h and the organic layer was separated from the inorganic content (Radwan et al., 2009). Final evaporation of the organic layer was carried out under a vacuum. Sterile foil paper was used to seal the conical flask containing the EOM to prevent interference of other organic matter. The dried extract was then dissolved in 25 ml of n-hexane and package for chromatographic analyses.

Chromatographic techniques (Fractionation) and spectrophotometry analysis

Modified method of Radwan et al. (2009) was used to separate the sterol from EOM through a column chromatographic techniques, the silica gel and alumina used were first activated and packed at ratio 3:6 inches. The dichloromethinated Extractable Organic Matter ware subjected to column chromatography on silica gel (at the top) and alumina (bottom). The column was eluted with (i) n-hexane (25 ml), (ii) 25 ml mixture of Dichloromethane and n-hexane (3:2) and (iii) 25 ml of methanol. The first fraction which is the saturated compound contained the aliphatic hydrocarbons, the second fraction which is the aromatic extract contained the polycyclic aromatic quantified using a UV– visible spectrophotometer.

Statistical analysis

The Statistical Package for Social Scientist (SPSS) 16.0 model was used in undertaking the statistical analysis. The t-test analysis of mean was used to establish the significant differences that exist between the microbial quality of well water between the dry and rainy season at P<0.05. Pearson correlation was used to determine the relationship between concentration of faecal sterol, distance of well from pit latrine and growth of Enterobacteriaceae.

RESULTS AND DISCUSSION

Table 1 shows the distance of the well water to the pit

latrines, only 13.3% of the studied wells conformed to the standard set by WHO. According to the WHO standard of 2007, 30 m is the approved standard distance in citing a well in close proximity to a pit latrine.

Figure 2a to c showed examples of three sampling sites used in this study; the arrows are pointing to the wells and the pit latrines. The pictures showed that some of these latrines are in close proximity to the wells.

The mean Total Heterotrophic Bacteria Count and Total Enterobacteriaceae Count of the samples were shown in Table 2. K5 had the highest Total heterotrophic count of 8.58×10^5 Cfu/100 mL, while the least count of 1.14×10^5 Cfu/100mL was recorded for K8 during the dry season. The highest count of 11.6×10^5 Cfu/100 mL and least count of 0.54×10^5 Cfu/100 mL was observed in K9 and K10 respectively during the rainy season. The statistical analysis showed that there is no significant difference for all the values obtained during the two seasons. During the dry season, the highest total enterobacteriaceae count was observed in K3 to be 4.62×10^5 Cfu/100mL, followed by K4 (2.61×10^5 Cfu/100 mL) and K8 (2.37×10^5 Cfu/100 mL), while K5 had the least count of 1.18×10^5 Cfu/100 mL. The highest enterobacteriaceae count was found in K7, with count of 14.1×10^5 Cfu/100 mL followed by K6 and K5 with counts of 13.6×10^5 Cfu/100 mL and 13.3×10^5 Cfu/100 mL respectively, while the lowest count of 4.58×10^5 Cfu/100mL was found in K1 (Table 2).

A significant difference was noted to have accompanied the changes in season (p > 0.05) when results obtained during dry season was compared with that of rainy season for the Enterobacteriaceae count. Generally, highest counts for heterotrophic bacteria and total Enterobacteriaceae were observed during rainy season. Result obtained from Total Enterobacteriaceae count revealed that all wells were heavily contaminated

Table 2. Mean of total heterotrophic bacteria and total Enterobacteriaceae counts.

Sample	Growth of THB Cfu/100 ml		Growth of TEC Cfu/100 ml	
	Dry season (Cfu/100ml×10⁵)	Rainy Season (Cfu/100ml×10⁵)	Dry season (Cfu/100ml×10⁵)	Rainy season (Cfu 100/ml×10⁵)
K1	3.30^f	4.20^d	2.16^g	4.58^a
K2	3.91^h	6.06^g	1.73^e	9.09^c
K3	5.53^j	5.55^f	4.62^k	12.30^g
K4	3.83^g	6.07^g	2.61^j	12.42^h
K5	8.58^k	7.06^h	1.18^a	13.30^i
K6	4.74^i	7.06^h	1.67^d	13.61^j
K7	2.28^d	4.31^e	1.61^c	14.10^k
K8	1.14^a	3.19^c	2.37^i	9.60^e
K9	1.63^b	11.61^i	1.82^f	9.93^f
K10	2.13^c	0.54^a	2.21^h	7.03^b
K11	2.38^e	2.69^b	1.42^b	9.11^d

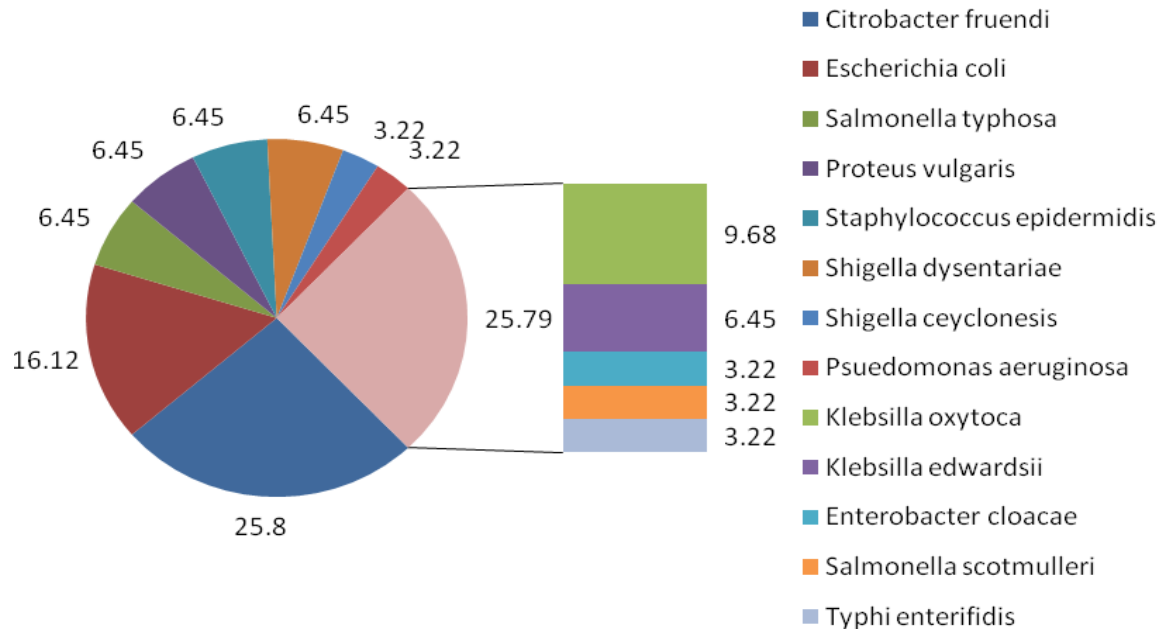K* = Sampling point; Values are calculated in colony forming unit per 100 mL.

Figure 3. Frequency of occurrence of the identified microorganisms during rainy season (Percentage).

and did not meet up with the WHO (2007) standard that stated that coliforms or feacal coliforms should not be detectable in any 100 ml of drinking water. High counts of heterotrophic bacterial counts and total coliforms in various groundwater wells cited near pit latrines have been reported (Adelekan, 2010; Akinbile and Yusoff, 2011). Kiptum and Ndanbuki (2012) also reported an increase in the coliforms counts in the rainy season compared to dry season in which counts of less than 100Cfu/mL was observed, while Howard et al. (2003) also reported that rain fall can lead to heavy microbial

contamination. It has been reported that feacal matter, domestic and wildlife animals are the natural reservoirs of many bacteria belonging to the family Enterobacteriaceae and they can be found in the groundwater either directly or through vectors via cross contamination of feaces with ground water (Ateba and Maribeng, 2011). Microbial contamination has been reported to be the greatest health risk that is associated with drinking water (Cabral, 2010).

Figure 3 shows the occurrence of bacterial isolates during rainy season in the well water samples, a total of
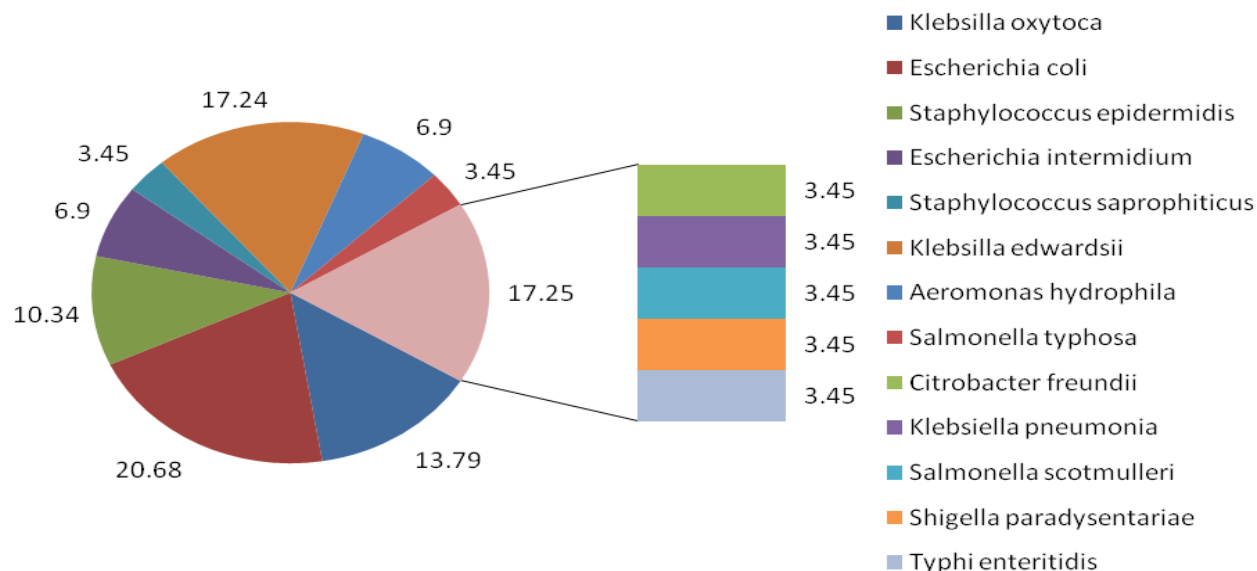
Figure 4. Frequency of occurrence of the identified microorganisms during dry season (Percentage).

thirty-one bacteria were isolated with *Citrobacter freundi* having the highest percentage of occurrence of 25.80 %, while four bacterial isolates namely; *Shigella ceyclonesis*, *Enterobacter cloacae*, *Salmonella scotmulleri* and *Typhi enterifidis* had the least percentage of occurrence of 3.22%, during rainy season. Twenty-nine bacteria were isolated during the dry season, but *Escherichia coli* had the highest percentage of occurrence (20.68%), while seven bacteria showed the least percentage of occur-rences of 3.4%, these isolates include, *Staphylococcus saprophiticus*, *Salmonella typhosa*, *Salmonella typhosa*, *Citrobacter freundi*, Klebsiella *pneumonia*, *Salmonella scotmulleri*, *Shigella paradysentariae* and *Typhi enterifidis* (Figure 4).

The Enterobacteriaceae are large family of Gram-negative bacteria that includes many harmless symbiots, many of the familiar pathogens such as *Salmonella*, *Escherichia coli*, *Yersina pestis*, *Klebsiella* and *Shigella*. Other disease causing bacteria in this family include *Proteus, Enterobacter, Serratia*, *Citrobacter* and others (Environmental protection Agency, 2002). Tairu et al. (2015) also reported 90% of *E. coli*, 89% *Staphylococcus* species, 72% of *Streptococcus* species, 56% of *Bacillus* species, 38% of *Pseudomonas* species, 23% of *Enterococcus* species present in the well water sampled around pit latrines in Igboora community, Nigeria. Bacterial isolates that include *E. coli*, *Salmonella typhilis*, *Streptococcus faecalis* and *Proteus* sp were also isolated from shallow wells in Makurdi (Isikwue et al., 2011). Many feacal or thermotolerant coliforms have been isolated from many wells in close proximity to pit latrines from many countries all over the world especially in the developing countries (Nwachukwu and Otokunefor, 2006; Kamanula et al., 2014). The increase in the numbers of

well water contaminated with feacal coliforms is a cause for alarm all over the world (WHO, 2008; EPA, 2009). Some of these isolated bacteria have been associated with several infections and some are opportunistic pathogen responsible for a wide range of acute and chronic infections (Adenodi et al., 2014).

Antibiotic resistance pattern of the isolated microorganisms

A total number of thirty one (31) organisms were isolated during rainy season and they were subjected to antibiotics sensitivity testing as shown in Figure 5. All the isolates showed 100% resistant to Cefuroxime, followed by Augumentin in which 30 isolates (96.77%) were resistant to it , while Ofloxacin had the least number of organisms that were resistant to it (11(35.48%)).. All isolates were found to be multi-antibiotic resistant (resistant to ≥3 antibiotics).

Twenty nine bacteria were isolated in the dry season, but all these isolates showed 100% resistant to three antibiotics; Augumentin, Ceftazidine and Cefuroxime (Figure 6). All isolates were also resistant to three or more antibiotics and they are therefore regarded to as multi drugs resistant isolates. Resistance by majority of the isolates to antibiotics in these wells can be attributed to wrong prescription and indiscriminate uses of antibiotics. Previous work on ground water in northern California found widespread occurrence of resistance to antibiotics among their isolates (Li et al., 2014).

A significant and apparently visible relationship was observed between the resistance pattern of Cephalosporin (Cefixime, Ceftazidime, Cefuroxime) and
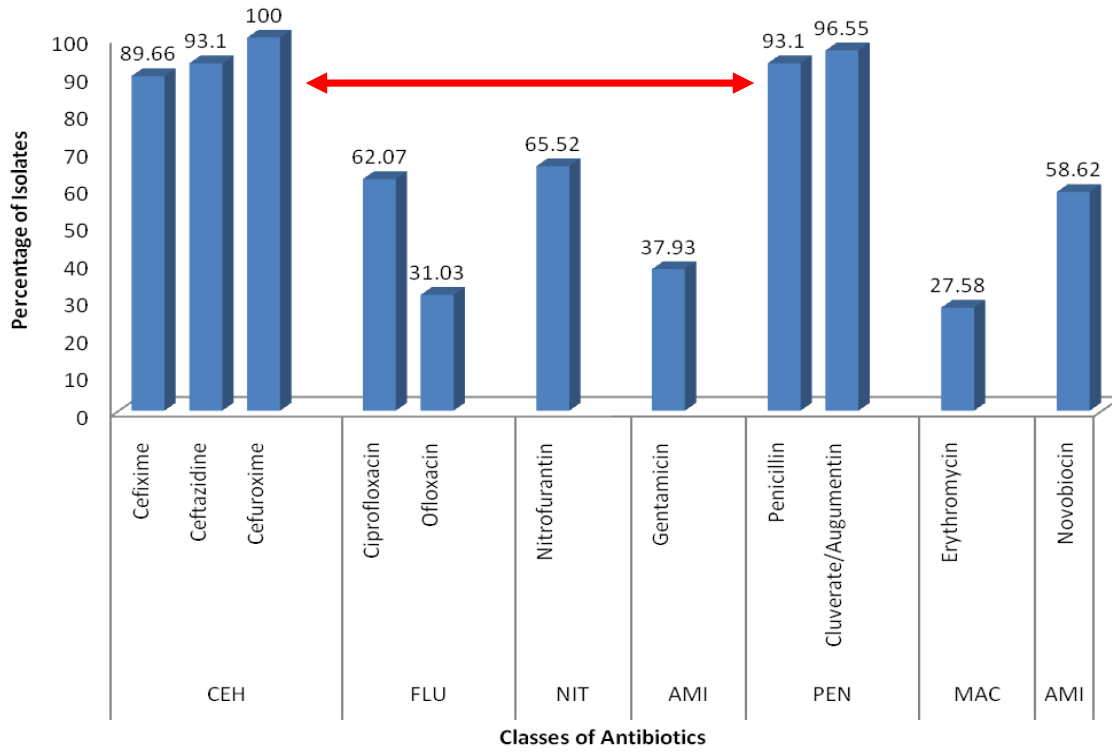
Figure 5. Percentage of resistant isolates during rainy season. CEP, Cephalosporin; PEN, Penicillin; FLU, Fluoroquinolones; MAC, Macrolides; NIT, Nitrofurans; AMI, Aminocoumanin; AMI- Aminoglycoside.
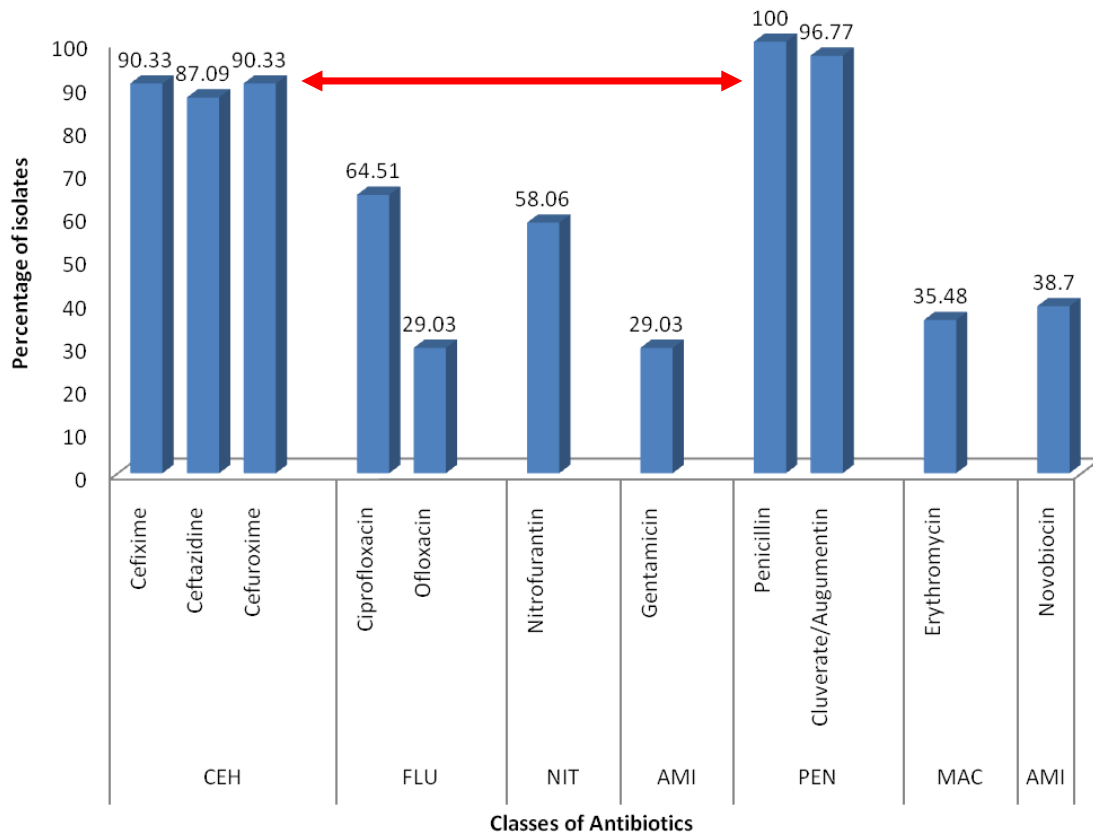

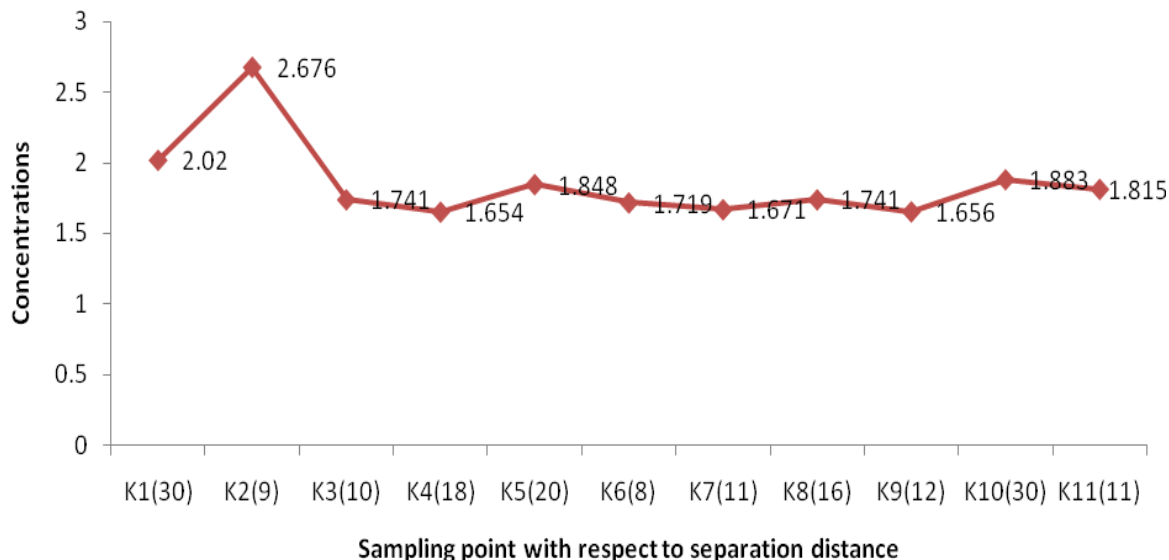**Figure 6.** Percentage of resistant isolates during dry season.

Figure 7. Concentration of the faecal sterol. K* = Sampling locations with reference to distance in meters.

Penicillin (Cluverate/ Augumentin and Penicillin) classes of antibiotics within the two season, The Frequency of isolates that are resistant to cephalosporin corroborate with the resistance pattern of Penicillins isolates. A common myth is that about 10% of patients with a penicillin allergy history will experience an allergic reaction if administered a cephalosporin. Flynn (2013) established this relationship to the chemical instability of the common B-lactam nucleus, the minor differences in chemical structures between the analogues, and the complex and relatively fast degradation of the compounds in aqueous solutions, however, it is also worthy to note that, both classes attacks the cell by inhibiting the synthesis of the cell wall. These two classes of antibiotics belong to the classes of most prescribed antibiotics, evidence from these research indicated that when such antibiotics are prescribed to patient or victims within the study location, the prescriptions might not be effective as expected.

Ayandiran et al. (2014) reported a 40 to 100% resistance to antimicrobial agents by the isolates used in their study. It was observed that Ofloxacin which is a broad spectrum drug was the most effective antibiotic among those tested during the rainy and dry seasons having the least resistance of 11(35.48%) and 12(41.37%) respectively, other fairly potent antibiotic during rainy season was Gentamicin (48.28 and 38.71%). Jiang et al. (2013) also reported the prevalence of antibiotic resistant bacteria from Huangpu river and drinking water sources in their study.

Occurrence of feacal sterol in the studied wells

This study showed that the correlation of absorbance and concentration of sterol shows a concerned variation and detectable concentrations of coprostanol were recorded in all water samples analysed, with values ranging from 1.654 to 2.676 abs (Figure 7). The optimum wavelength for the faecal sterol was observed at 226 nm. The maximum concentration was observed in K2 (2.676) with the well situated at 9 m away from Pit latrine while minimum was found to be K4 with 1.654 concentrations at a distance of 18 m. Overall, mean concentration was found to be 1.857. It was also noted that K1 had a concentration of 2.025 at a distance of 30 m. Gerardo et al. (2000) observed the highest spectra at 458 nm while Tonny et al. (2004) optimum wavelength of coprostanol was observed in ethanol at 250 nm. Previous studies had proposed that values greater than 1.000 abs to be an indication of faecal contamination. Obuseng et al. (2013) also reported that values greater than 1.5 should be considered as an indication of human derived faecal source. Therefore, the microbial contamination of these well waters is due to feacal concentration.

Relationship between different variables used in this work

A Pearson correlation coefficient was used to calculate the relationship between concentration of faecal sterol, distance of well from pit latrine and growth of Enterobacteriaceae (Table 5) for both the dry and rainy seasons, a non-significant weak negative relationship was found between growth of Enterobacteriaceae for dry season and distance of well from pit latrine (r (20) = -0.131, $p > 0.05$), while a weak positive, non-significant relationship was found between distance of well from latrine and the growth of Enterobacteriaceae for rainy

Table 5. Relationship between the concentration of sterol, distance of well from pit latrine and growth of enterobacteriaceae during the dry and rainy seasons.

Correlation coefficient		Growth of Enterobacteriaceae during dry season	Growth of Enterobacteriaceae during rainy season	Distance of well from pit latrine	Concentration of sterol
Growth on EMB for Dry season	Pearson correlation	1	0.677**	-0.131	-0.121
	Sig. (2-tailed)		0.001	0.563	0.591
Growth of Enterobacteriaceae during rainy season	Pearson correlation	0.677**	1	0.089	0.053
	Sig. (2-tailed)	0 .001		0.694	0.815
Distance of well from pit latrine	Pearson correlation	-0.131	0.089	1	0.012
	Sig. (2-tailed)	0.563	0.694		0.957
Concentration of sterol	Pearson correlation	-0.121	0.053	0.012	1
	Sig. (2-tailed)	0.591	0.815	0.957	

Correlation Significant at the 0.01 Level (2- tailed).

season (r (20) = 0.089, p > 0.05), both indicating non-significant linear relationships between distance of well from latrine and growth of Enterobacteriaceae. A weak positive relationship which is not significant was also found between distance of well from pit latrine and concentration of the faecal sterol (r (20) = 0.012, p > 0.05), indicating a non-significant linear relationship between distance of well from pit latrine and concentration of the faecal sterol (Table 3). Possible explanation for this is that the contaminants are of faecal sources and this also suggests that even when a well is reasonable separated from pit latrine structures, they are still prone to faecal contaminant. Muruka et al. (2012) reported in their study that a significant association existed between distances from dug wells to nearest pit latrine and that this association between level of dug-well faecal contamination and distance to nearest pit latrines was indirect. Studies have shown that sometimes no association might exist between dug-well water

bacteriological quality and the distance of dug-wells to nearest pit latrines and they attributed this lack of association to many possible confounders, e.g. hygiene behavior, geomorphology of the area and the presence of other contamination sources. Other factors that can affect contamination of well water include; the environment where the well is cited, level of hygiene in terms of the use of drawers and the population of people using the well could be considered as other sources of contamination apart from pit latrines (Tairu et al., 2015). Studies have shown that until all these measures to control other sources of contamination are put in place, the role of latrines in the contamination of well water could not be quantified.

Conclusion

The study has revealed that well waters that are located in close proximity to sanitary pit latrines in Oko Township were highly vulnerable to

bacteriological and finger print pollution. Despite the facts that both isolated organisms and sterol analysis indicated the contaminant to be of feacal origin, yet, a weak positive, non-significant statistical relationship was found between distance of well from latrine and the growth of Enterobacteriaceae organisms during rainy and dry season. This implies that safety of ground water are not guaranteed by distance but by consideration of hydrological, geomorphologic characteristics, hygienic conditions of the well, abstracting container and cross contamination from the well users, a justification from the fact that soil texture and topography constitute the rate at which ground water could be contaminated. The prevalence of multi antibiotic resistant bacteria from the studied well water is also high and this is a concern to human health. Therefore, the findings from this study will serve as a preliminary investigation on how to prevent the outbreak of waterborne diseases through ingestion of these multi-antibiotic resistant bacteria.

CONFLICT OF INTEREST

The authors declared that there was no conflict of interest whatsoever throughout the period of this research.

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Determinants of antimicrobial use in diarrhoea management among under-five children in Zomba, Malawi

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Diarrhoea is one of the most common infections that under-five children suffer from across the world. Antibiotics remains one of the common measures used to treat the infection, which has led to increased usage; nevertheless, the extent to which an individual is prompted to use antibiotics in managing diarrhoea in the Malawian setting is not clearly known. This study was aimed at establishing the factors that prompt mothers/caregivers to use antibiotics in treating childhood diarrhoea. Women/caregivers with under-five children who suffered from diarrhoea two weeks before the study were recruited for the interviews (n=269). One-on-one interviews were conducted using a structured questionnaire and two focus group discussions were carried out to generate qualitative data. Quantitative data was entered in CSPro version 7 and exported to STATA version 12 for analysis. The study established that caregivers use antibiotics in managing diarrhoea due to long distances to the health facility (OR 1.93, 95% CI: 1.03-3.65; P<0.04), unavailability of drugs at the facility (OR 1.12, 95% CI: 0.60-2.10; P<.71), quality of medical care at the health facility (OR 1.11, 95% CI: 0.57-2.15; P<.75) and previous knowledge of the diarrhoea condition (OR 0.90, 95% CI: 0.49-1.66; P<.74). As a result of these findings, Government of Malawi should consider widening access to health facilities to its citizens such that citizens are encouraged to always visit the healthy facilities. Healthy professionals should always encourage caregivers to refrain from engaging in self-medicational behavior as it has devastating effects on increased drug usage which contributes to anti-microbial resistance.

Key words: Diarrhoea, under five children, anti-microbial, resistance, self-medication, over the counter, determinants, prescription.

INTRODUCTION

Diarrhoea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual (Le Doare et al., 2015). Diarrhoeal disease is the second important cause of under-five mortality, and is liable for death of around 760,000 children every year (Carvajal-Vélez et al., 2016). Despite the fact that diarrhoea related mortality has reduced over the years in developing countries (including Malawi), morbidity as a result of diarrhoeal disease continues to remain the main cause of misery among young children (Alam et al., 2011; Munos et al., 2010). Across the globe, diarrhoea is one of the most common infections that children below the age of five suffer from (Akinnibosun and Nwafor, 2015).

Infectious diseases (including diarrhoea) are a leading cause of illness and death throughout the world. The enormous diversity of microbes combined with their ability to advance and adapt to changing environments, populations, practices, and technologies produce ongoing threats to health and repeatedly challenges researcher's noble efforts of preventing and controlling infectious diseases (Awad and Aboud, 2015). Since children are still developing immunological systems and among the parents there is a poor sense of hygiene, these children are prone to many infections, the most common among them being diarrhoea and Acute Respiratory Infection (ARI). Diarrhoeal diseases in under-five children are a source of anxiety, misery and loss of time to their parents, as compared to their older siblings in which the course of disease is mild (Unicef/WHO, 2004).

Diarrhoeal disease can be prevented by simple measures like personal hygiene and environmental sanitation; nonetheless, antibiotic therapy remains one of the common measures used to curb the disease despite the presence of less invasive solutions such as the Oral Rehydration Salts which are key remedies in treating childhood diarrhoea (Padhy et al., 2017; Mahapatra et al., 2015; Gwimile et al., 2012). This key role of antibiotics has contributed to high usage of antibiotics in management of diarrhoea which is contributing to increased cases of antimicrobial resistance (Padhy et al., 2017; Alghadeer et al., 2018). Oral Rehydration Salts (ORS) and Zinc supplementation during diarrhoea illness has been shown to reduce the duration and severity of diarrhoea episodes in many countries which in the long run reduces morbidity and mortality among under-five children (Carvajal-Vélez et al., 2016). Communities need to be encouraged to use ORS in management of diarrhoea related illnesses which has been proved to be very effective in counteracting rotavirus which in the long run will reduce overuse and misuse of antibiotics.

In a study conducted in Thailand where there was low antibiotic usage in treating diarrhoea, the decision not to use antibiotics was driven by medical professionals (Carvajal-Vélez et al., 2016). In other similar studies conducted across the globe, the decision to use antimicrobials was triggered by mother's/caregiver's knowledge of the previous medical condition without visiting a healthcare facility (Gera et al., 2016; Ocan et al., 2014a; Chalovich and Eisenberg, 2013). Other researchers established that the use of antibiotics was influenced by quality of care at the facility, attitude of the medical personnel, availability of drugs at the facility, education level and marital status of the mother (Yadesa et al., 2015; Shafie et al., 2018). It is against this background that there was a need to establish the determinants of antimicrobial use towards management of diarrhoeal disease among under-five children in urban areas of Zomba District, which will be more specific to the Malawian setting.

METHODS

Study population

Women/caregivers having under-five children who suffered from diarrhoea two weeks before the study were recruited. Mothers/caregivers whose child did not suffer from diarrhoea two weeks before the study were excluded. For the purpose of this study, a caregiver is someone who "feeds and watches over the child, gives the child affection, communicates with the child, and responds to the child's needs. If the child is sick, the caregiver is usually the person who takes the child to a health-care provider (WHO, 2012)".

Study site

The study was conducted at four health facilities that are located in Zomba City which include; Sazi, Matawale, Police and City Clinic (Figure 1). One-on-one interviews were used to generate quantitative data and two focus group discussions were done to generate qualitative data. In some cases, the mothers/caregivers were followed in areas where an outreach clinic was conducted. The individual interviews were done on a one-on-one basis in an isolated environment to ensure that other eligible participants were not swayed by any bias.

Sampling

The sample size was 264 which was derived using the following formula:

$$n = \{z^2 (1-p) p\}/e^2$$

Where; n= required sample size; Z= level of probability that the true prevalence lies within the chosen confidence interval at 95%; P= prevalence of diarrhoea (22%) according to MDHS, 2016; e= level of precision required/margin error at 5% (standard value of 0.05). So if P = 0.22; e = + or -5% Z = 1.96 = $1.96^2 (1-0.22)0.22/0.05^2$ = 263.687 ⟹ 264 mothers

A total of 269 (which slightly above the required sample size) mothers/caregivers were interviewed during one-on-one interactions.

Ethical consideration

Zomba City Council and Zomba District Health Office provided a signed approval to conduct the study in the district. Furthermore, ethical approval was sought from College of Medicine Research and Ethics Committee (COMREC) and a signed consent was sought from study participants before the interview.

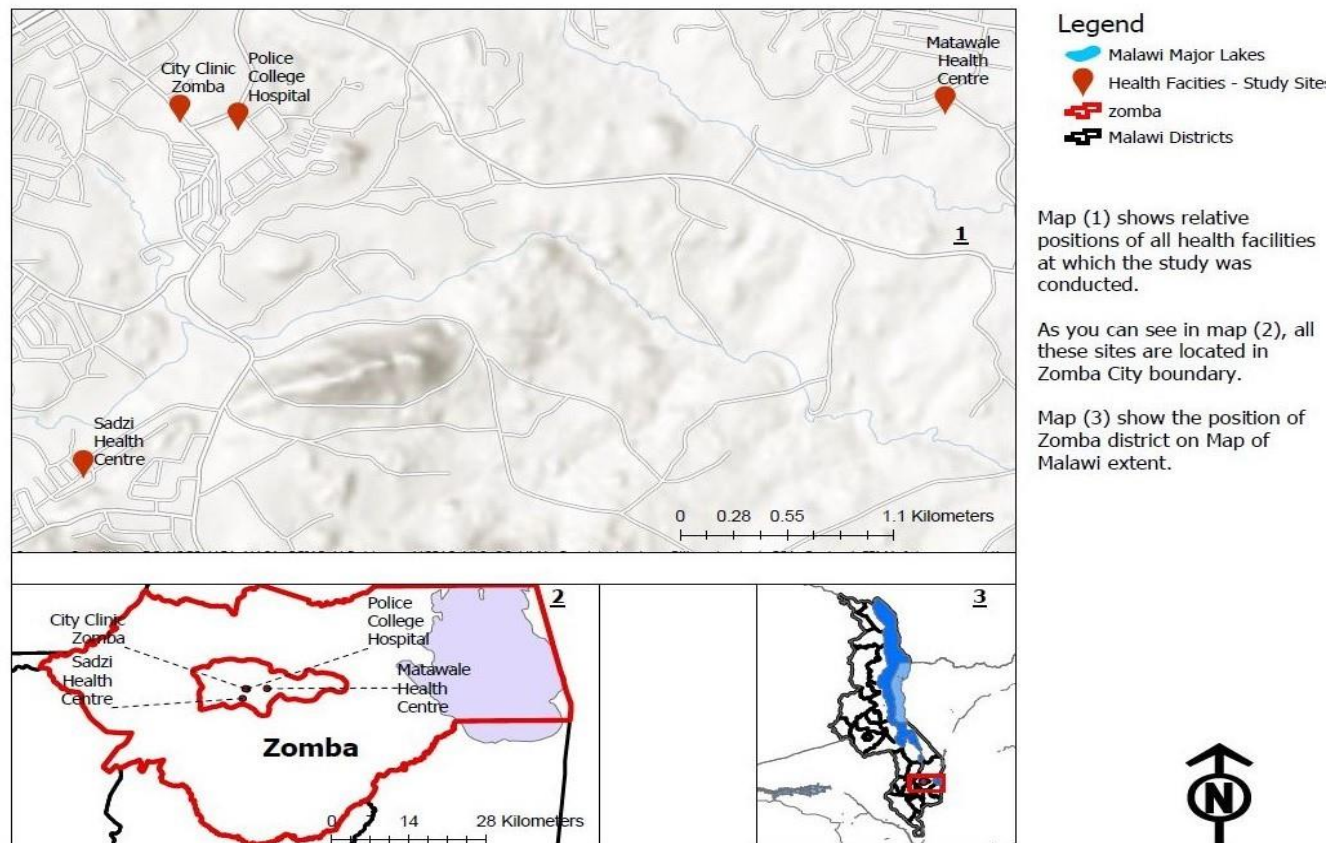*Corresponding author. E-mail: bdieckens@gmail.com.

Figure 1. Map of Zomba district showing health facilities where the study was conducted.

Data analysis

Quantitative data

Data which was collected using a questionnaire was entered in CSPro version 7.0 and exported to STATA version 12 for analysis. Research Assistants were adequately trained to capture correct data and the principal investigator checked the quality of data each day and addressed all inconsistencies before passing it on to the Data Entry Clerk. The data was protected with a password to ensure that it was not altered which may affect its validity. After data entry which was entered in (CSPro which has built in checks for accuracy and validity) the principle investigator cleaned the data before analysis. Descriptive statistics were conducted in order to summarize the data set quantitatively. Multi-variate logistic regression analysis was used to establish with certainty the determinants of antimicrobial use towards management of diarrhoeal diseases.

Qualitative data

This data was generated through Focus Group Discussions (FGDs) using a checklist protocol and analysed by following the Health Belief Model (HBM). An FGD is a guided conversation or a series of interviews with a small group of 6 to 12 people who are conversant with the topic under study (Masadeh, 2012). The qualitative data was transcribed verbatim and transcriptions were re-read to get general sense of the information and reflect on the overall meaning.

A list of commonly emerged topics was made. The main topics mentioned were allocated numbers and organised into appropriate words, phrases and sentences. Main themes were generated and presented in a narrative form. The themes were analysed according to the objectives of the study using a Health Belief Model.

The conceptual framework of HBM

This study used the HBM as a conceptual framework to analyse themes on hygine and sanitation practices, diarrhoea case management and determaints of antibiotic use. The fundamental principle behind HBM states that behaviour is influenced by individual beliefs or perceptions about the disease and measures available to reduce occurence of such a disease (Tarkang and Zotor, 2015). As shown in Figure 1, the HBM is rooted among four constructs which are perceived susceptibility, perceived seriousness, perceived benefits and perceived threats/barriers. Over the years, the HBM has been modified to cover other two principles which are motivating factors to take an action and self-efficacy. In this study, the HBM was used to deduce factors that influence mothers/ caregivers to use antibiotics in treating childhood diarrhoea.

RESULTS

Eighty eight percent of the study participants indicated that they wash hands at least four critical times, 75% of

Table 1. Hygiene and sanitation practices.

Parameter	Category	[n(%)]
Occasions where hand-washing is practiced	At least four times	237(88)
	Less than four times	32(12)
Hand-washing demonstration	Demonstrated correctly	202(75)
	Did not demonstrate correctly	67(25)
	Nappy	126(47)
Child defecation	Used the Diaper	11(4)
	In his clothes	27(10)
	Defecated in the house	5(2)
	Defecated outside the house	46(17)
	Defecated in the common toilet	51(19)
Disposal of stool	In the toilet	129(48)
	Washed	100(37)
	Outside the house	40(15)
Type of toliet facility	Flash toilet	16(6)
	Pit latrine	253(94)

Table 2. Access to hygine and sanitation information.

From whom do you access information about hand washing?	Where did you receive the information?		
	At the hospital [n(%)]	Under-5 clinic [n(%)]	Home visits [n(%)]
Medical staff (nurse, clinician)	71(48.86)	66(45.21)	9(6.16)
HSA	26(21.67)	79(65.83)	15(12.50)
Extension worker	0(0.00)	2(66.67)	1(33.33)

the respondents demonstrated correctly when asked to wash hands, 47% of the study participants defecated in the nappy while 17% defecated outside the house. In terms of disposal of stool, 47% of the study participants used the toilet with 15% disposing outside the house. Ninety four had properly constructed pit latrines (Table 1).

In relation to access to hygiene and sanitation information, 49% of the study participants obtain information from the medical staff who are found at the hospital whereas 45% obtain such information from medical staff found at under-five clinic. Sixty-six of the study participants obtain hygine and sanitation information from Health Surveillance Assistants who are usually found at under-five clinics (Table 2)

As shown in Figure 2, 71% of the study participants used ORS with only 0.2% using herbs, 10% used antibiotics to treat diarhoea infection.

From Table 3, occupation of the respondents had a p-value of 0.007, Education level had a p-value of 0.08 and marital status had a p-value of 0.70.

As shown in the Table 4, the odds that mothers/ caregivers will not go to the health facility because of lack of drugs at the facility are 1.12 times higher than that they will go to the health facility. The odds of not going to the health facility due to long distances covered are 1.93 times higher compared to the odds of going. The odds of not going to the health facility given that they have previous knowledge of the diarrhoea condition are 10% (1-0.9×100) lower than the odds of going. Finally, yet importantly, the chances that mothers/caregivers will not go to the facility because of poor quality of services are 1.11 times higher than the chances that they will go.

DISCUSSION

Hygiene and sanitation practices

In this study, there was an increase in diarrhoea episodes among children older that 6 months of age (22% as compared to 12% in >6 months of age). Malawians who follow exclusive breast feeding programs from birth to six months were shown to have low episodes of diarrhoea.

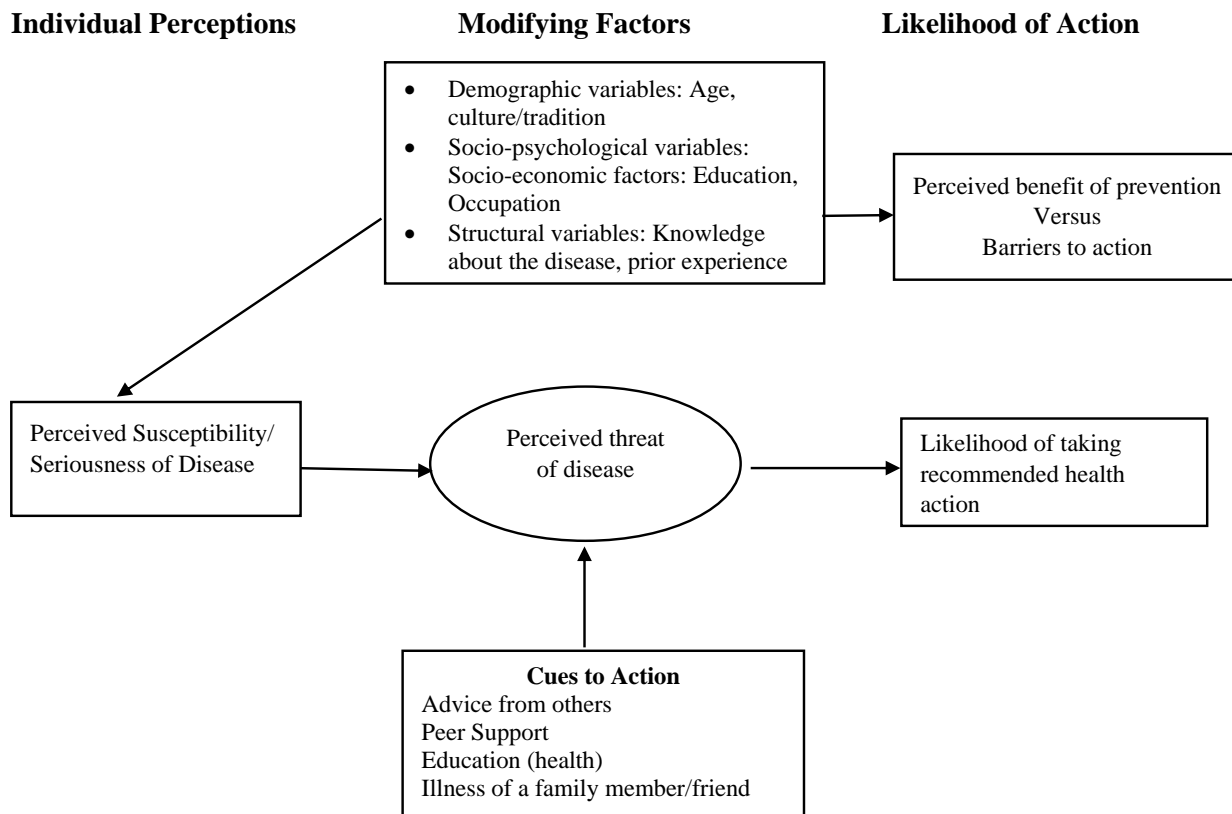This therefore implicates complementary food as a

Figure 2. Conceptual Model of HBM. Adapted from Tarkang and Zotor (Shafie et al., 2018).

Table 3. Relationship between demographic characteristics (occupation, marital status, education level) and the motive behind keeping diarrhoea drugs at home.

Source	Partial SS	df	MS	F	Prob > F
Model	140.792	12	11.733	56.67	0.000
Education level	2.003	5	0.401	1.93	0.089
Marital status	0.451	4	0.113	0.54	0.703
Occupation	3.666	3	1.222	5.90	0.007

R^2=0.73.

Table 4. Multivariate logistic regression on factors that influence use of antibiotics in treating childhood diarrhoea.

Predictor	Odds ratio	Std. error	z	P>z	95% CI
No drugs at the facility	1.126	0.358	0.37	0.71	0.60-2.10
Long distance to the healthy facility	1.939	0.625	2.05	0.04	1.03-3.65
Knowledge of the medical condition	0.901	0.281	0.33	0.74	0.49-1.66
Quality of care at the facility	1.110	0.374	0.31	0.75	0.57-2.15
Constant	1.639	.408	1.99	0.047	1.01-2.67

possible source of infection. In addition, the child begins to walk which poses a risk of environmental contamination through consumption of non-food items. The declining levels of maternally acquired antibodies may cause increase in diarrhoea espisodes aswell. Relatedly, in the Malawi Demographic and Healthy Survey (2015-2016)

report, children older than six months had increased episodes of diarrhoea than their younger siblings (ICF/NSO, 2017). Since the majority of study participants (82%) in this study were between the ages of 15-34 years, interventions related to hygine and sanitation should target this age category to minimise a situation where complementary foods become a source of contamination and infection. In addition, this age group should be targetted for use of ORS campaign unlike using antibiotics to treat diarhoea thereby reducing cases of antibiotic overuse and misuse.

Seventy-six percent of the respondents demonstrated hand-washing practices correctly which implies that the knowledge of hand-washing with soap among the research subjects is generally high. Nonetheless, the remaining 24% is a cause of concern as it poses the potential risk of diarrhoea infection among under-five children which may endanger their general well-being. This situation calls for scale-up of interventions related to water and sanitation hygiene to ensure that the hygiene and sanitation practices are up to standard. Relatedly, the DHS 2015-2016 report found out that the prevalence rate of diarhoea in urban areas is 24%, which is consistent with the findings of this study that 24% of the study participants did not practice sound hygiene and sanitation measures (NSO, 2015).

In terms of access to hygine and sanitation information by mothers/caregivers, the results of cross-tabulation established that Health survalance Assistance (HSA) just like nurses and clinicians are very instrumental in providing such information and that HSAs are very critical at all three levels that is; facility level, underfive clinics as well as during home visits (Table 2). This information is critical in the context of this study in the sense that if the households are well informed about dangers of poor hygiene and sanitation practices in relation to their child's health, they will be committed to minimise pathways for pathogens causing diarrhoea.

This will help in winning the fight against diarhoea and its associated illnesses. The crucial role played by these HSAs in the provision of primary health care was also emphasised by Masangwi et al (2016) when establishing care-seeking behaviours among mothers/caregivers in southern part of Malawi during diarrhoea (Masangwi et al., 2016).

Management of diarrhoea cases

In relation to managing the diarrhoeal disease, the respondents indicated that Oral Rehydration Salts is mostly used to manage diarrhoea either by prescription from the health professional or through self-medication; which entails buying from drug selling points. Nevertheless, ORS on its own cannot control stomach bugs such that the need to take it concurrently with antibiotics especially during persistent diarrhoea cannot

be overemphasised (Carter et al., 2015). Relatedly, 69% of the respondents indicated that ORS is a common remedy which is used to manage the diarrhoea condition. This agrees with what Masangwi et al (2016) who established that 68% households in southern part of Malawi uses ORS in situations where their child suffers from diarhoea (Masangwi et al., 2016).

Nonetheless, Flagile and Cotrimoxale were among the common antibiotics that were used by the respondents and mostly such drugs were bought from the shops which contibuted to wrong prescription (Figure 3). According to the Health Belief Model (HBM), the perceived benefits of taking the drugs without prescription outweighed the potential cost of drug resistance in the near future as a result of undiscriminate use of antibiotics. Notwithstanding, this might be due to the potential lack of knowledge on the dangers of self-medication without prescription such that a cue to action by a well informed family member can be a recipe for behaviour change.

In terms of the ability of the respondents to notice diarrhoea condition and take an action, 42% of the participants took at least 24 hours which is very detrimental to the health well-being of the child. This delay in medication provides a leeway for faster becterial multiplication which prolongs the time taken for the child to recover from diarrhoea and may affect its survival. This implies that their ability to detect diarrhoeal disease which in turn affects their response rate is low, such that some mothers/caregivers percieve diarrhoea as mild medical condition which does not warrant seeking medical care (Le et al., 2011; Ayalew, 2017).

During the focus group discussion, there was an indication by some mothers/caregivers (around 30%) that opening of bowels is one of the normal body processes that happens to every child espacially when the child is developing teeth which does not require any medical intervention. "*Since time immemorial, children open bowels periodically especially when they are developing teeth as such there is no need for us mothers to go to the hospital to seek medication. This is my third child and the trend remains the same" (R8).* According to the HBM, a person's perceived seriousness of the disease determines the course of action to take such that it was not surprising to note that mothers/caregivers took time to take an action since they regarded diarrhoeal disease as not serious. This augurs well with what Merga and Alemayehu (2015) established that 71% of the mothers associated diarrhoea with the teething process in children such that there is no need to seek any help during diarhoea episode.

Determinants of antimicrobial use in diarrhoea management

This study found out a number of factors are responsible for use of antibiotics in managing diarrhoeal diseases that
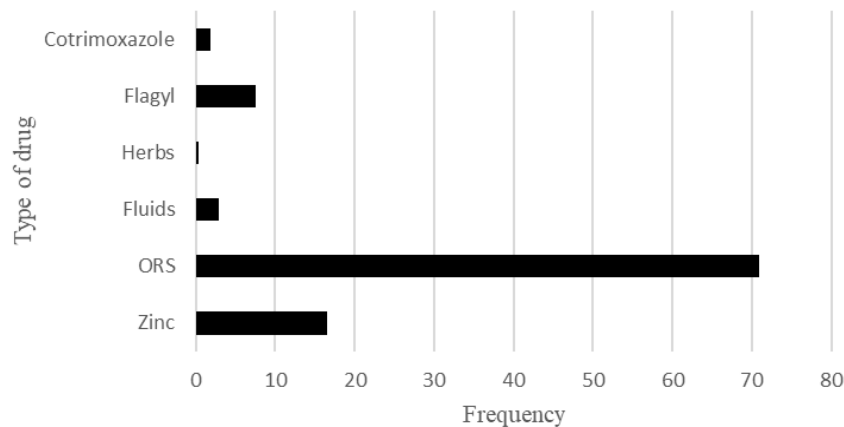
Figure 3. Type of drug used to treat diarhoea.

were established during one-one-interviews and FGDs. These factors are shortage of drugs at the health facility, previous knowledge of the medical condition, long distance to the health facility, quality of care at the health facility and transportation related challenges.

Furthermore, the study assessed the effect of demographic characteristics which include occupation, marital status and education level on use of antibiotics. On this, a linear regression analysis was computed and that an R^2 value of 73% was found implying that only 27% of the effect independent variables cannot be explained by the dependent variable leading to a statistically significant relationship. Occupation was found to affect self-medication behaviour much more than other demographic factors with a p-value of 0.007 which is less than 0.005 hence signficant at 95% confidence interval (Table 3). This implies that employed women were more likely to engage in the purchasing of drugs over the counter than their unemployed counterparts which can be attributed to their high purchasing power.

The shortage of drugs at a health facility was another factor responsible for haphazard use of antibiotics to treat under-five diarrhoea in this study. As shown by the results of multivariate logistic regression above (Table 4), the likelihood that a mother/caregiver will engage in self-medication as a result of shortage of drugs at the facility was 1.12 greater than that of not engaging. This implies that mothers/caregivers were likely not going to visit the health facility despite observing sickness on their child knowing fully that they will not find any medication at the healthy facility. As a result, alternative ways of acquiring drugs like purchasing over the counter which is a form of self-medication were invetible.

Long distances to the health facility is another factor that significantly influenced caregivers' behavior of using antibiotics withiout prescription. As observed in the logistic regression model above (Table 4), the likelihood that mothers/caregivers will not visit the health facility as a result of long distance was 1.93 higher than that of going to the clinic. This implies that mothers/caregiver's will likely not visit the clinic despite their child being presented with diarhoea. In addition, a p-value of 0.04 (Table 4) which is less than 0.05 is signficant at 95% confidence interval confirming that indeed long distance is critical towards illogical use of antibiotics among the study participants. This augers well with responses that were given by the participants when asked "How long does it take for them to travel to the healthy facility" of which 47% indicated having to walk more than one hour to the health facility. This is in support with what other researchers established in similiar studies that mothers/caregivers who had to travel more than 10KM to seek medical care mostly resort to self-medication behaviour (Masangwi et al., 2016; Ocan et al., 2014b).

The study participants during the FGD cited lack of money for transport as one of the factors behind irrational use of antibiotics without prescription. This lack of money for transport was exacerabated by long distances that the mothers/caregivers have to cover in order to reach the health posts. "*The distance to the health facility is long so we find it difficult to carry our baby and walk there. This force us to use other methods of treating diarrhoea other than going to the health facility like buying from shops"(R2).* As per the HBM, this transportation issue is a perceived barrier which may hinder a positive health behaviour of always visiting the clinic and may promote usage of drugs bought from shops contributing to antibiotic resistance.

Another factor responsible for use of antibiotics in the face of childhood diarrhoea is knowledge of the medical condition by the mother/caregiver. As seen in Table 4, the likelihood that mothers/caregivers will engage in self-medication behaviour due to their knowledge of the medical condition was 10% (1-0.9x100) lower than that of going. Despite this being the case, the results of the FGD and findings from other researchers revealed that previous knowledge of the diarrhoea condition which was obtained from the health facility, pharmacist and from

family and friends had a greater influence on the ability of the mothers/caregivers to administer drugs on their own (Masangwi et al., 2016; Mahapatra et al., 2015).

As per the Health Belief Model, a cue to action by mothers/caregivers influenced by the medical personnel, family and friends contributed to use of antibiotics without prescription which may contribute to antibiotic resistance. Other researchers established that these drugs are mostly bought from drug-retail outlets under the prescription of a pharmasist or a friend (Shafie et al., 2018; Ayalew, 2017; Bachrach and Gardner, 2002). These findings are in agreement with available published literature which indicated that intentions for self-medication with antibiotics result from the need to save money and the desire to act promptly to treat suspected or confirmed bacterial infections (Carvajal-Vélez et al., 2016; Chalovich and Eisenberg, 2013; Le et al., 2011). This knowledge of the condition is obtained from the health facility after the initial visit and knowledge of the exact medication that was provided (Viberg et al., 2010; Alghadeer et al., 2018).

Another determinant of antibiotic use is the quality of care rendered by health facility staff. As observed in Table 4, the likelihood that mothers/caregivers will engage in irrational use of antibiotics due to the nature of service rendered to them whenever they visit the facility was 1.11 higher than that of not engaging. This implies that study participants were less likely to visit the health facility in a situation where the quality of service rendered to them during their previous visit was poor. The quality of care at the health facility is associated with the expectations of the caregivers. Caregivers who receive antibiotics regard health facilities as more caring and responsive than those that do not provide antibiotics. This situation force medical practioners to provide broad-spectrum antibiotics even without proper diagnosis and justification contributing to drug resistant (Sharma et al., 2015).

Policy makers developing cost effective local interventions should pay attention to issues related to shortages of drugs at the health facility, previous knowledge of the medical condition, long distances to the health facility, quality of care at the health facility and transportation challenges if the battle against antibiotic resistance is to be won in Malawi.

CONCLUSION AND RECOMMENDATIONS

These findings add new knowledge on the determinants of antimicrobial use in diarrhoea management in the Malawian setting. Based on the findings of this study, investment in relieving distances traveled by mothers/caregivers in need to medical care is key to improving the quality of primary health care. Deliberate efforts by the Government of Malawi (GOM) such as the promotion of closely monitored mobile clinics and the construction of more health care posts in areas with critical distance challenges can go a long way in challenging the status quo.

Policy makers should focus on promoting interventions that lessen indiscriminate use of antibiotics to treat diarhoea among households by encouraging local councils through decentralisation to include by-laws stopping the malpractice as well as targetted mass awareness campaigns. More also, communities should be encouraged to use ORS and Zinc suppliments as they are critical towards reducing rotavirus which mostly cause childhood diarrhoea. There is a strong need to improve accessibility of medical facilities accross the nation to ensure that the general citizenry irrespective of social-economic status are motivated to visit the government-owned health facilities.

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CONFLICT OF INTERESTS

The researchers had no conflicts of interest.

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