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Research article

Risk assessment and photo-disinfection of antibiotic residues and antibiotic-resistant bacteria in water sources from Ede, Nigeria

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ABSTRACT

Environmental antibiotic residues (EARs) and antibiotic-resistant bacteria (ARB) are known to contribute to global antimicrobial resistance (AMR). This study investigated EAR levels in selected wells, river, abattoir wastewater, bottled water and sachet water from Ede, Nigeria. Ecological risk quotient (RQ) and health risk (Hazard quotient) of the levels of these EARs, ARB and multidrug-resistant bacteria (MDR) with their antibiotic resistance were calculated. Antibiotic residues detected included tetracycline-TET (14.2–135.8 μg/L), chloramphenicol-CHL (6.8–224.7 μg/L), metronidazole-MET (3.7–83.8 μg/L), sulfamethoxazole-SUL (0.56–18.6 μg/L), and ciprofloxacin-CIP (3.8–97 μg/L). Antibiotic residues in STW samples were below the detection limit while ampicillin was not detected in any of the water samples. Chloramphenicol posed the highest ecological risk to algae while infants were particularly at risks of ciprofloxacin and metronidazole resistance in various water sources. No health risk due to bottled water exposure is observed for any population group. The mean log10 bacteria count (cfu/mL) followed the trend abattoir (5.68) > river (5.67) > hand-dug well (5.53) > sachet (5.03) > bottled (4.83). The most occurring ARB in water samples are Bacillus spp (36.3 %) > Staphylococcus (27.5 %) and the most dominant MDR isolate is *Bacillus cereus*. All isolates exhibited 62.5, 100, 31.3, 77.5, 58.8 and 33.8 % resistance to AMP, MET, CIP, TET, CHL and SUL, respectively. Visible-light composite material (Cu/Zn-doped delaminated kaolinite) completely disinfected 12.5 and 15.8

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L of water containing Log_{10} 7.5 cfu/mL of ARB *Enterobacter* sp and *Bacillus* sp respectively with no regrowth in treated water after storage for three days. Levels of EAR in the water sources in this study are among the highest in aquatic systems worldwide and can potentially lead to community AMR. Usage, discharge and sales of antibiotics should be guided by policies while routine monitoring of drinking water sources should be encouraged to reduce the AMR burden in the region. The photocatalytic material used in this study for water disinfection offers a promising, cost-effective solution for mitigating AMR risks from drinking water.

1. Introduction

Globally, over seven hundred thousand people die yearly from antimicrobial-resistant infections and mortality is projected to reach an annual rate of >10,000,000 people by 2050 [1]. Antimicrobial resistance (AMR) is affirmed a "global public health concern" by the World Health Organization (WHO) [2] and environmental surveillance is a mitigation strategy proffered to curb AMR. This is because the environment, mainly soil and water serves as reservoirs for microorganisms including antibiotic-resistant bacteria (ARB) [3]. These ARB and multi-drug resistant bacteria (MDR) are not only common in hospitals and farmhouses but also other locations. The dissemination of biological contaminants of emerging concerns (CECs) like ARB, mobile genetic elements (MGEs), and antibiotic resistance genes (ARGs) is enhanced in water matrices more than any other environmental matrix. Ingesting water containing such CECs could result in serious health complications including antimicrobial resistance making treatment costly and life-threatening [4]. Surveillance of AR, ARB, ARGs and MRGs in the environment can provide valuable insights into the AMR burden and offer potential solutions to the AMR menace.

An environmental survey strategy is recommended for mitigating AMR and in achieving UN SDGs no. 3 and 6 [2] across the globe. It is thus necessary that a more practical approach should be adopted. However, environmental AMR surveys are few compared to AMR studies from human samples and this is worse with studies from Africa. Unlike Europe [5], North America [6] and Asia [7], a publication search covering the years 2014–2024 in January 2024 using the PubMed search engine for AR articles from Africa revealed only 59 research articles on ARs either in soil, air or water, with 34 articles from water alone and only 5 of the 59 from Nigeria. The scarcity of data on ARs in the African continent is due to a lack of funds and the non-availability of appropriate equipment with high sensitivity to detect AR levels in environmental samples [8]. Resultantly, even though ARB and MDR reports are common [9], a few of these studies consider evaluating a combination of both antibiotic residues and ARB in water consumed by most African communities [10] with none for treated packaged water from Africa [11]. This gap makes it difficult to associate these ARs with the ARB found in the environment and thus the inability to establish community AMR from a holistic and empirical view. Also, there are hardly any reports on AR that include risk assessments in studies fron Africa apart from recent reports by Addis et al and Bolujoko et al [12,13]. Even then, these studies did not reflect their association with the ARB and MDR found. To understand and ameliorate the AMR challenge, it is important to estimate the AR levels, and types of bacteria and compare the AR to the ARB found in potable water. The contributions from Africa on environmental AMR are paramount in contributing to a global overview of AMR.

Aside surveillance of ARs and ARB in environmental samples like in water, it is also critical to consider viable and sustainable treatment options for the remoal of these CECs (ARs and ARB) from water. Present treatment options employed in most African water municipalities cannot effectively remove most CECs in water and with increasing urbanization and industrialization, the high demand for potable water in most communities in Africa [14]. In response to the dwindling access to potable water, these communities now depend on water supply alternatives which include privately owned small-scale treatment facilities that package drinking water for sale in sachets and bottles. For their drinking and domestic use, others depend on surface water, boreholes and hand-dug wells which are susceptible to contamination by CECs including antibiotics-related contamination, depending on their proximity to pollution sources [15]. Unfortunately, groundwater sources are often erroneously considered 'clean' and not treated before drinking, while packaged water brands (sachet and bottled water) presumed to be safe are purchased by the populace as "pure water".

Even though conventional treatment methods (chlorination. ozonation and filtration) for the removal of pathogens in water are well known, they have their drawbacks including that these pathogens become resistant to the treatment technique, resulting in multi-resistant drug pathogens in the water. However, the use of photocatalysts for water disinfection is gaining attention and we have successfully mineralised (conversion of bacteria to CO_2 and H_2O) multi drug resistant *E. coli* and its genes in contaminated water with visible-light catalytic nanocomposite material in our previous study [4].

This study, therefore, investigates the levels and distribution of AR and the ecological and human risk assessment associated with their presence in water sources (River water, well water, abattoir wastewater, sachet water and bottled water) from Ede. We also determine their bacterial quality including ARB, antibiotic resistance (AST) and MDR testing. In this study, we also propose to disinfect water containing a gram-negative and a gram-positive ARB (*Enterobacter* sp, and *Bacillus cereus*) with the photocatalytic composite material used by Ugwuja et al [4]. Ede, Osun State in Southwestern Nigeria is the study location; it is one of the towns with a bank bordering the Osun River with a major municipal water treatment station situated in it. Osun River feeds the Ede water treatment station which in turn provide potable water to 70 % of the Osun state populace [16] including Ede and the surrounding towns. Though studies had earlier reported the levels of other contaminants in Ede water sources [17] yet no such studies have reported AR and its risk assessments.

It is expected that data obtained from this study will bridge the current data paucity in AMR studies from Africa, highlighting the concurrent presence of ARB and AR in the potable water samples from Nigeria. Information derived will be relevant for developing

sustainable policies and solutions to the malaise of antibacterial resistance in Nigeria and Africa at large.

2. Materials and methods

2.1. Sampling location and sites

The study area is located within Ede, a sub-urban town with a population of about 156,866 people [18]. Many of its residents possess low levels of education and lack awareness of the use and implications of antibiotics. Most of the Ede populace and economic activities centre around the Osun River and the market setting, poor sanitation conditions typify this vicinity and the community abattoir discharges its wastewater into the Osun River.

Sampling points were selected from the Osun River and groundwater (available boreholes and hand-dug wells) around the Ede market, some samples were also collected from the discharge points of the abattoir into the river. These water samples are used as potable water and for domestic uses including cooking. In addition, sachet and bottled water from the five most popular commercial brands sold in the locality and generally in Osun State were purchased and used for this study.

2.2. Sample Collection and Physiochemical Characterization

A total of 69 water samples, including 15 surface water, 12 groundwater, 12 abattoir wastewater, 15 sachet water, and 15 bottled water samples representing major potable water sources in the study area (Table S1) were collected. The samples for AR measurement were collected in amber glass bottles while samples for microbial and molecular samples were collected aseptically. Surface water samples were collected from five sites along River Osun and were labelled R1, R2, R3, R4, and R5 while the groundwater samples were collected from four available wells labelled W1, W2, W3, and W4. Samples from abattoir wastewater were collected from four points labelled A1, A2, A3, and A4. Additionally, sachet water (STW1, STW2, STW3, STW4, and STW5) and bottled water (B1, B2, B3, B4, and B5) samples were also analyzed collected, and all samples were collected in triplicates. Basic parameters such as pH, Total Dissolved Solids (TDS), and temperature of water samples were determined on-site with a HANNA meter (HI 9124N model). The water samples were promptly transported to the laboratory at 4 °C for further analysis.

2.2.1. Sample preparation for antibiotics in water samples

The collected water samples were filtered with a filter pore size of 0.2 μm using a membrane filtration apparatus. Water samples were spiked with 100 μL aliquots of 1.0 μg/mL internal standard mixture of ciprofloxacin (CIP), sulfamethoxazole (SUL), ampicillin (AMP), chloramphenicol (CHL), metronidazole (MET), and tetracycline (TET) to enhance the signal to noise ratio and aid quantification. The water samples were loaded on hydrophilic-lipophilic balance (HLB) solid-phase extraction (SPE) cartridges (300 mg sorbent). The water samples were loaded on hydrophilic-lipophilic balance (HLB) solid-phase extraction (SPE) cartridges (200 mg sorbent). The SPE cartridges were conditioned with 5 mL MeOH followed by 5 mL Milli-Q water. 200 mL portion of water samples were loaded onto SPE cartridges at a flow rate of 1.5 mL/min and allowed to dry in air. The dried SPE cartridges were washed with 5 mL Milli-Q water and then allowed to dry under vacuum for about 10 min. The sorbents were eluted with 5 mL of MeOH at a flow rate of 1 mL/min. The sorbents were eluted with 5 mL MeOH. The elution was done at a flow rate of 1 mL/min. The eluates were evaporated to dryness under a vacuum and then transferred into brown flat-cap HPLC vials for analysis. The reconstituted samples were analyzed for the presence of antibiotics using Agilent HPLC 1200 series (HPLC-DAD detector). Elution was performed using a mixture of MeOH and Millipore water (4 % acetic acid as a buffer) in a ratio of 70:30, at a flow rate of 0.3 mL/min. Separation was achieved with a Phenomenex Luna 4 × 250 μm reversed-phase column. 20 μL of each sample was injected for analysis. Quantification of antibiotic residue was achieved through the use of internal standards. The analysis was conducted in triplicates [19].

2.2.2. Quality assurance and quality control

Procedural blanks were carried out for each extraction to monitor for possible contamination from the solvents and materials used in the extraction processes. The concentrations found in procedural blanks were subtracted from the measured concentrations in the water samples. Methanol blank and midpoint calibration standard was injected after each batch analysis to check for drift in instrumental response and also for carry-over of target analytes from prior injections. Quantification of analytes was carried out using external standards, and a calibration curve was constructed by analyzing aqueous solutions containing the analytes ranging from a

Table 1
Linear range, regression coefficient, LODs, LOQs, and % Recovery of antibiotics in water samples.

Antibiotics	R_T (min)	R^2	Linear Range (μg/L)	LOD (μg/L)	LOQ (μg/L)	Recovery (%) ± SD
CIP	9	0.998	0.1–1000	5.22	14.52	113 ± 26.16
TET	10	0.932	0.05–500	22.64	105.36	103 ± 4.42
SUL	11	0.994	0.05–1000	28.01	85.13	83 ± 10.52
MET	12	0.999	0.05–2000	4.77	14.44	77 ± 20.52
CHL	13	0.997	0.05–2000	5.95	18.02	99 ± 35.74
AMP	15	0.985	0.05–1000	194.77	590.21	56 ± 6.02

R_T = retention time; LOD = Limit of Detection; LOQ = Limit of Quantification, SD = Standard Deviation, TET = Tetracycline; SUL= Sulfamethoxazole; MET = Metronidazole; CHL= Chloramphenicol; AMP= Ampicillin; CIP= Ciprofloxacin.

concentration of 10–1000 μg/L.

The limit of detection was calculated as three times the signal-to-noise ratio using the standard deviation of the seven-point calibration intercepts divided by the slope. The limit of quantification (LOQ) was calculated as ten times the ratio. The LOD was between 5.2 and 194.8 μg/L and the coefficients of determination (R^2) of calibration curves were >0.930. The coefficients of determination, LOD, and LOQ are presented in Table 1. The recovery of targeted analytes was up to 140 % [20].

2.2.3. Ecological risk assessment

The risk quotient (RQ) is commonly used as a ranking criterion to assess ecological risk arising from the presence of contaminants [21]. It is a ratio of the measured environmental concentration (MEC) to the predicted no-effect concentration (PNEC) which is frequently

estimated using acute or chronic toxicity data (LC_{50} or EC_{50}) [22], see equation (1). An RQ < 0.1 indicates a low risk to aquatic organisms, while 0.1 < RQ < 1 represents a medium risk, and RQ > 1 reveals a high ecological risk [21]. In this study, the PNEC values of each antibiotic for the algae, invertebrates, and fish were obtained from literature [23,24].

$$Risk\ Quotient(RQ) = \frac{MEC}{PNEC} \tag{1}$$

2.2.4. Human risk assessment

Human risk assessment primarily evaluates the risk of exposure and the impact of contaminants on the health of specific individuals. This is done by calculating the likelihood of harmful effects on the human body based on the health risk assessment theory of EPA [25]. In this study, the Monte Carlo approach was used to evaluate the non-carcinogenic risks of antibiotics in drinking water [5], see equations (2) and (3). The human risk assessment was computed using five age-group population categories namely; infants (<1 yr old), toddlers (1–3 yrs), children (4–11 yrs old), teenagers (12–20yrs) and adults (≥21 yrs old).

$$Estimated\ Daily\ intake\ (EDI) = \frac{C^{*}D}{BW} \tag{2}$$

$$Hazard\ Quotient\ (HQ) = \frac{EDI}{ADI} \tag{3}$$

Where C is the concentration of the analyte in water (μg/L), D is the daily water consumption rate (L/day), and BW is the body weight (kg). The maximum concentration of antibiotics in water samples was used to calculate the EDI. The daily water consumption rate (D), and body weight used for calculating the EDI were: infants (1 L/day, 9.2 kg), children (1.3 L/day, 31.8 kg), and adults (3.1 L/day, 80 kg) respectively. Acceptable Daily Intake (ADI) for Sulfamethoxazole (130 μg/kg/day), Ciprofloxacin (7.1 μg/kg/day), Tetracycline (170 μg/kg/day), Chloramphenicol (29 μg/kg/day) and Metronidazole (0.6 μg/kg/day), [26–28].

2.2.5. Screening for antibiotic resistant bacteria (ARB)

Triplicate samples were collected aseptically from the same point for microbiological analyses. These samples were serially diluted, and pure isolates were subjected to antibiotic-resistant assays. Standardized inoculum size (using 0.5 McFarland standard) of pure isolates were inoculated on Mueller Hinton agar (MHA) media plates, using sterile cotton swabs as described by Kristanto and Koven (2019) [29]. After the sample was streaked onto the MHA plates, the antibiotic discs of tetracycline (30 μg), ampicillin (30 μg), chloramphenicol (30 μg), metronidazole (50 μg), ciprofloxacin (10 μg), and sulfamethoxazole (30 μg) were placed using an automatic, hand-held disk dispenser. The plates were then incubated at 37◦C for 24 h. The zone of inhibition for each antibiotic was measured in millimetres with a standard laboratory caliper and the isolates were reported as either sensitive or resistant based on CLSI (CLSI M100-S26) [30] and EUCAST interpretations [31].

2.2.6. Multi-Antibiotic Resistance Index

Multi-Antibiotic Resistance Index (MARI) was determined using the mathematical expression in equation (4) [32]. Conventionally, isolates with 80 % MARI are considered multi-drug resistant (MDR isolates). Multi-antibiotic-resistant phenotypes of isolates were evaluated and mapped out accordingly (Tables S5 and S6).

$$MARI = \frac{Number\ of\ antibiotic\ classes\ to\ which\ isolate\ was\ resistant}{Total\ number\ of\ antibiotics\ tested\ in\ the\ study} \times 100 \tag{4}$$

2.3. DNA extraction and sequencing of MDR isolates

2.3.1. DNA extraction

DNA was extracted from the MDR isolates using Zymo Fungal/Bacterial Soil Microbe DNA MiniPrep kit (Zymo Research, California, USA) according to the manufacturer's instruction and suspended in a final volume of 100 μL. Sanger's sequencing of the 16S rRNA gene was used to identify the multidrug-resistant bacteria.

2.3.2. DNA sequencing

The DNA extracts from the MDR bacteria were subjected to Sanger's sequencing, based on the analysis of the results of the

amplification of the 16S rRNA gene using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TGACTGACT-GAGGCTACCTTGCGA-3′). The nucleotide sequences with 1400 bp were used for BLAST DNA homology searches in the NCBI DNA database (http://www.ncbi.nlm.nih.gov). The sequence results of MDR isolates from groundwater, surface water and abattoir wastewater are shown in Table S7 while similar MDR sequenced data for packaged water (sachet and bottled water) are shown in Table S8.

2.4. Removal of MDR Enterobacter sp, and Bacillus cereus from water

A fixed-bed setup was employed for photo-disinfection of water using prepared visible-light active delaminated clay material doped with Cu/Zn, which has been previously applied in disinfection of drinking water [4]. In brief, the composite materials prepared are Zn doped, Cu doped, and Cu/Zn doped clay composite materials from a combination of $ZnCl_2$, delaminated kaolinite, and *Carica papaya* seed biomass in the case of Zn-doped clay composite, $CuCl_2$, delaminated kaolinite and *Carica papaya* seed biomass in the case of Cu-doped clay composite, and a combination of the two metal salts (i.e. $ZnCl_2$ and $CuCl_2$), delaminated kaolinite and *Carica papaya* seed biomass in the case of Cu/Zn doped clay composite. Details of the preparation of this visible-light active composite material have been described in our previous report [4]. In the fixed-bed mode, a fixed weight (2.0 g) of each of the photocatalytic composite materials which were initially sterilized by dispersing in 70 % ethanol and dried to constant mass at 105 °C, were packed in a 400 mm × 10 mm transparent glass column. The transparent glass column used was initially dry-sterilized at 160 °C for 2 h and primed by running 400 mL of sterile water through the packed glass column containing the composite material. Suspensions of two frequently occurring, pathogenic, multidrug-resistant bacteria (*Enterobacter* sp, and *Bacillus cereus*) from this study were tested for effective simultaneous removal by these materials. McFarland standardized isolates were added into sterile distilled water and the solutions were passed through the column at a flow rate of 8 mL/min under natural solar light conditions. Samples for analysis were collected every 1 h and checked for inactivated bacteria using the pour plate method. A regrowth experiment was done to determine the efficacy of the treatment technique when the treated water is stored for some time. Section S1.0 in the supplementary information document contains details of the regrowth experiment.

2.5. Statistical analysis

The detection frequency and mean concentration ± standard deviation in triplicate of antibiotics were calculated using Microsoft Excel®. Analysis of variance, graphs were generated and drawn using GraphPad Prism 8.0.2® for Windows (GraphPad, Inc., San Diego, CA, USA) and OriginPro 9.1®

3. Results and discussion

3.1. Physicochemical Characterization

The physicochemical parameters of water samples collected in this study are summarized in Table S2. The pH of water samples was between 7.0 and 8.3, while the TDS and Electrical Conductivity (EC) were between 20 and 390 mg/L and 40–780 μS/cm, respectively. Also, the temperature of the water samples collected was between 31 and 33 °C. The pH and TDS values fall within and below the WHO permissible limit for clean drinking water respectively, while the temperature was slightly above the permissible limit of 30 °C

Table 2
Concentration (μg/L) of antibiotic residues in water samples.

Samples	SUL	CHL	MET	TET	AMP	CIP
B1	BDL	1.51 ± 0.01	0.62 ± 0.01	4.99 ± 0.00	BDL	1.82 ± 0.00
B2	BDL	6.82 ± 0.01	BDL	8.03 ± 0.01	BDL	1.70 ± 0.00
B3	BDL	0.85 ± 0.04	BDL	BDL	BDL	BDL
B4	0.56 ± 0.00	2.4 ± 0.10	3.65 ± 0.01	14.2 ± 0.03	BDL	3.78 ± 0.00
B5	BDL	0.50 ± 0.01	BDL	BDL	BDL	1.89 ± 0.01
W1	2.54 ± 0.02	27.8 ± 0.30	16.5 ± 0.20	23.7 ± 0.10	BDL	69.7 ± 0.00
W2	1.04 ± 0.01	49.8 ± 0.16	0.17 ± 0.00	16.2 ± 0.00	BDL	5.84 ± 0.05
W3	BDL	0.12 ± 0.03	5.21 ± 0.20	7.22 ± 0.01	BDL	7.05 ± 0.05
W4	0.67 ± 0.02	56.6 ± 0.30	BDL	14.6 ± 0.01	BDL	3.38 ± 0.00
R1	9.98 ± 0.10	BDL	10.5 ± 0.10	81.0 ± 0.01	BDL	BDL
R2	18.6 ± 0.30	BDL	10.0 ± 0.00	136 ± 0.00	BDL	BDL
R3	BDL	36.7 ± 0.10	78.8 ± 0.20	41.5 ± 0.00	BDL	26.4 ± 0.00
R4	1.49 ± 0.01	6.87 ± 0.10	33.8 ± 0.05	3.41 ± 0.01	BDL	1.10 ± 0.01
R5	4.23 ± 0.02	42.1 ± 0.10	79.7 ± 0.20	31.4 ± 0.01	BDL	72.6 ± 1.70
A1	0.98 ± 0.50	70.7 ± 0.50	17.5 ± 0.05	9.65 ± 0.00	BDL	9.71 ± 0.02
A2	3.39 ± 0.03	155 ± 2.20	75.7 ± 0.20	18.3 ± 0.00	BDL	45.8 ± 1.20
A3	2.27 ± 0.04	57.4 ± 1.10	23.7 ± 0.02	BDL	BDL	96.9 ± 2.50
A4	3.43 ± 0.20	225 ± 2.70	83.8 ± 0.10	3.45 ± 0.01	BDL	0.77 ± 0.00

Values below the limit of detection are represented as BDL. TET = Tetracycline; SUL= Sulfamethoxazole; MET = Metronidazole; CHL= Chloramphenicol; AMP= Ampicillin; CIP= Ciprofloxacin. B1-5 = Bottled water; W1-4 = Hand-dug well; R1-5 = River water; A1-4 = Abattoir wastewater.

(Table S2). The electrical conductivity for water samples from hand-dug wells had a mean value of 670 μS/cm which is above the WHO standard permissive limit of 400 μS/cm for drinking water [33]. This suggests that water from these hand-dug wells has dissolved ions.

3.2. Antibiotic chemical residues

Mean concentrations of antibiotic residues in water samples from various sampling sites are shown in Table 2. Expectedly, abattoir water provides some of the highest mean concentrations of antibiotic residues with bottled water providing the least mean concentration. The mean concentration values of antibiotic residues in bottled water samples are SUL = 0.56 ± 0.02 μg/L, CHL = 6.82 ± 0.01 μg/L, MET = 3.65 ± 0.007 μg/L, CIP = 3.78 ± 0.002 μg/L whereas abattoir wastewater samples gave higher mean concentrations for SUL (3.43 ± 0.2 μg/L), CHL (224.67 ± 2.7 μg/L), MET (83.75 ± 0.1 μg/L), TET (18.34 ± 0.001 μg/L) and CIP (96.98 ± 2.5 μg/L). These high concentrations of antibiotic residues in abattoir wastewater samples indicate the extensive use of these drugs in the prevention and treatment of diseases in livestock [34]. However, the Osun River water samples gave the highest mean values of 18.59 μg/L for SUL and 135.82 μg/L for TET antibiotic residues (Table 2). This could be due to a drug store nearby that releases expired drugs into the river, as well as the public dumping trash into the river, which contributes to their existence. The absence of antibiotic residues in sachet water, despite their presence in other water sources, may be attributed to potential degradation by sunlight due to the water's transparent packaging. Ampicillin in all samples were below detection limits (Table 2).

The average amount of SUL in the bottled, river and groundwater samples (rainy season) from Lagos State, Nigeria (3, 30, and 3 ng/L respectively) as earlier published [35] are far lower than what is reported for similar water samples in this study (0.56, 6.86, and 1.06 μg/L respectively) during the same season. Very few reports have looked into the presence of antibiotic residues in commercially packaged drinking water especially in many developing countries where proper treatment of packaged water is not enforced. These high concentrations of antibiotic residues in drinking water sources certainly create selective pressure that makes microbiota in both humans and the environment develop resistance to these drugs. For humans, this may lead, ultimately, to costly medical treatments and sometimes death.

In the other water samples (hand-dug well, river and abattoir), some antibiotics (SUL, CIP, CHL, MET, and TET) were detected in all samples except ampicillin (AMP), which was not detected in any of the water samples possibly because its level is well below the detection limit of the equipment.

In addition, there is a strong possibility that the concentration of antibiotics in river water in this study could be from the contribution of abattoir wastewater which is sent directly into the river water as well as the open dumping of refuse into the river which is frequently observed in the community.

The comparison of concentrations of antibiotic residues in various water samples from different countries (Table 3) indicates that water samples in this study currently contain some of the highest reported concentrations of antibiotic residues in aquatic systems globally. All levels of TET in this study ranging from 14.2 to 135.82 μg/L are higher than the level reported by Ref. [36] from river. The levels of CHL 6.82–42.01 μg/L are also higher than the reports of [37] (Dam) and [36] (0.0004 μg/L). CIP levels (3.78–72.62 μg/L) were lower than in hospital wastewater from Nigeria [24] but higher than reported in WWTP effluent from the USA (37). All SUL (0.56–18.59 μg/L) are higher than that reported by Refs. [5,36] (Rivers in China and Italy) as well as Aquaculture in Bangladesh. Only MET levels in river and abattoir water were higher than that reported in a Dam from Ghana [37]. The level of antibiotics earlier reported in drinking water from Nigeria is similar to this study.

Some of these antibiotic residues, specificlly CIP, TET, and SUL, are routinely added to animal feed to promote their growth and prevent or treat diseases in animals, especially in cattle breeding. The human activities in the area like waste discharge, the misuse and abuse of antibiotics by man, their agricultural use and the lack of policies guiding the access to antibiotics across the counter by the populace all contribute to the high levels of antibiotics recorded in these water sources, The direct or indirect ingestion of these water can aggravate antibiotic-resistance in the community, which is harmful to human health [40]. According to studies, antimicrobial agents are widely distributed and poorly regulated in several Nigerian states, as well as the ignorance/poor knowledge of the retailers selling these drugs to farmers, leads to drug inefficacy and, invariably, antibiotic resistance in man and his farm animals [41].

Table 3
Concentration of antibiotic residues in water sources from different countries (μg/L).

Country	Matrix	TET	CHL	CIP	SUL	MET	Reference
China	River	0.0042	0.0004	0.0027	0.2596	–	[36]
Italy	River	–	–	0.019 ± 0.0164	0.0053 ± 0.0042	–	[5]
Bangladesh	Aquaculture	–	–	–	0.00671	–	[7]
Ghana	Dam	–	0.42 ± 0.29	–	–	22.23 ± 0	[37]
Egypt	Raw Industrial wastewater	–	–	–	–	–	[38]
Nigeria	Hospital wastewater influent	–	–	108	–	–	[24]
	Hospital wastewater effluent	–	–	107	–	–	
USA	WWTP effluents	–	–	0.26	2.9	–	[39]
Nigeria	River water	**135.82 ± 0.0005**	**42.10 ± 0.1**	**72.62 ± 1.7**	**18.59 ± 0.3**	**79.72 ± 0.2**	(this study)
Nigeria	Hand-dug well water	**23.67 ± 0.1**	**56.59 ± 0.3**	**69.71 ± 0**	**2.54 ± 0.02**	**16.54 ± 0.2**	(this study)
Nigeria	Abattoir wastewater	**18.34 ± 0.001**	**224.67 ± 2.7**	**96.98 ± 2.5**	**3.43 ± 0.2**	**83.75 ± 0.1**	(this study)
Nigeria	Bottled water	**14.2 ± 0.03**	**6.82 ± 0.01**	**3.78 ± 0.002**	**0.56 ± 0.002**	**3.65 ± 0.007**	(this study)

3.3. Risk assessments

The risk assessment of antibiotic residues in water in this study was done to determine the risk levels of the concentration of these antibiotics in the water samples to ecological life (Table 4) and their health risk to humans (Fig. 1). The potential ecological risks of SUL, CHL, MET, TET, and CIP were estimated. To provide greater illumination of the risks, the computed risk quotient (RQ) values were categorized into three risk profiles: low-risk <0.01–0.1, moderate risk 0.1–1, great risk >1. The RQ values for antibiotics used in

Table 4
Ecological risks assessment of various water types from Ede, Osun state, Nigeria.

Source of water	Organism	Antibiotics	RQ range (μg/L)	<0.1 (%)	0.1–1.0 (%)	>1.0 (%)
Bottled water	Algae	SUL	0–0.0033	100.0	0.0	0.0
		CHL	0.21–1.68	0.0	40	**60**
		MET	0–0.013	100.0	0.0	0.0
		TET	0–0.015	100.0	0.0	0.0
		CIP	0–0.0062	100.0	0.0	0.0
	Daphnia	SUL	0–0.0029	100.0	0.0	0.0
		CHL	0.0	0.0	0.0	0.0
		MET	0–0.011	100.0	0.0	0.0
		TET	0–0.14	80.0	20.0	0.0
		CIP	0–0038	100.0	0.0	0.0
	Fish	SUL	0–0011	100.0	0.0	0.0
		CHL	0.0	0.0	0.0	0.0
		MET	0–0.0041	100.0	0.0	0.0
		TET	0–0.0039	100.0	0.0	0.0
		CIP	0–0.015	100.0	0.0	0.0
Hand-dug well	Algae	SUL	0–0.014	100.0	0.0	0.0
		CHL	0.029–12.62	50.0	0.0	**50.0**
		MET	0–0.057	100.0	0.0	0.0
		TET	0.00039–0.026	100.0	0.0	0.0
		CIP	0.005–0.11	75.0	25.0	0.0
	Daphnia	SUL	0–0.013	100.0	0.0	0.0
		CHL	0.0	0.0	0.0	0.0
		MET	0–0.051	100.0	0.0	0.0
		TET	0.0036–0.24	25.0	75.0	0.0
		CIP	0.003–0.07	100.0	0.0	0.0
	Fish	SUL	0–0.0052	100.0	0.0	0.0
		CHL	0.0	0.0	0.0	0.0
		MET	0–0.019	100.0	0.0	0.0
		TET	0.0010–0.066	100.0	0.0	0.0
		CIP	0.011–0.27	75.0	25.0	0.0
River water	Algae	SUL	0–0.1	80.0	20.0	0.0
		CHL	0–17.37	40.0	0.0	**60.0**
		MET	0.034–0.27	60.0	40.0	0.0
		TET	0.0035–0.14	80.0	20.0	0.0
		CIP	0–0.11	80.0	20.0	0.0
	Daphnia	SUL	0–0.097	100.0	0.0	0.0
		CHL	0.0	0.0	0.0	0.0
		MET	0.011–0.24	40.0	60.0	0.0
		TET	0.034–1.36	40.0	40.0	20.0
		CIP	0–0.07	100.0	0.0	0.0
	Fish	SUL	0–0.038	100.0	0.0	0.0
		CHL	0.0	0.0	0.0	0.0
		MET	0.011–0.089	100.0	0.0	0.0
		TET	0.0094–0.37	60.0	40.0	0.0
		CIP	0–0.29	60.0	40.0	0.0
Abattoir wastewater	Algae	SUL	0.0057–0.020	100.0	0.0	0.0
		CHL	14.11–55.20	0.0	0.0	**100.0**
		MET	0.060–0.288	50.0	50.0	0.0
		TET	0–0.020	100.0	0.0	0.0
		CIP	0.0012–0.15	75.0	25.0	0.0
	Daphnia	SUL	0.0051–0.018	100.0	0.0	0.0
		CHL	0.0	0.0	0.0	0.0
		MET	0.054–0.261	50.0	50.0	0.0
		TET	0–0.18	75.0	25.0	0.0
		CIP	0.0007–0.097	100.0	0.0	0.0
	Fish	SUL	0.002–0.0088	100.0	0.0	0.0
		CHL	0.0	0.0	0.0	0.0
		MET	0.019–0.095	100.0	0.0	0.0
		TET	0–0.05	100.0	0.0	0.0
		CIP	0.0038–0.38	50.0	50.0	0.0

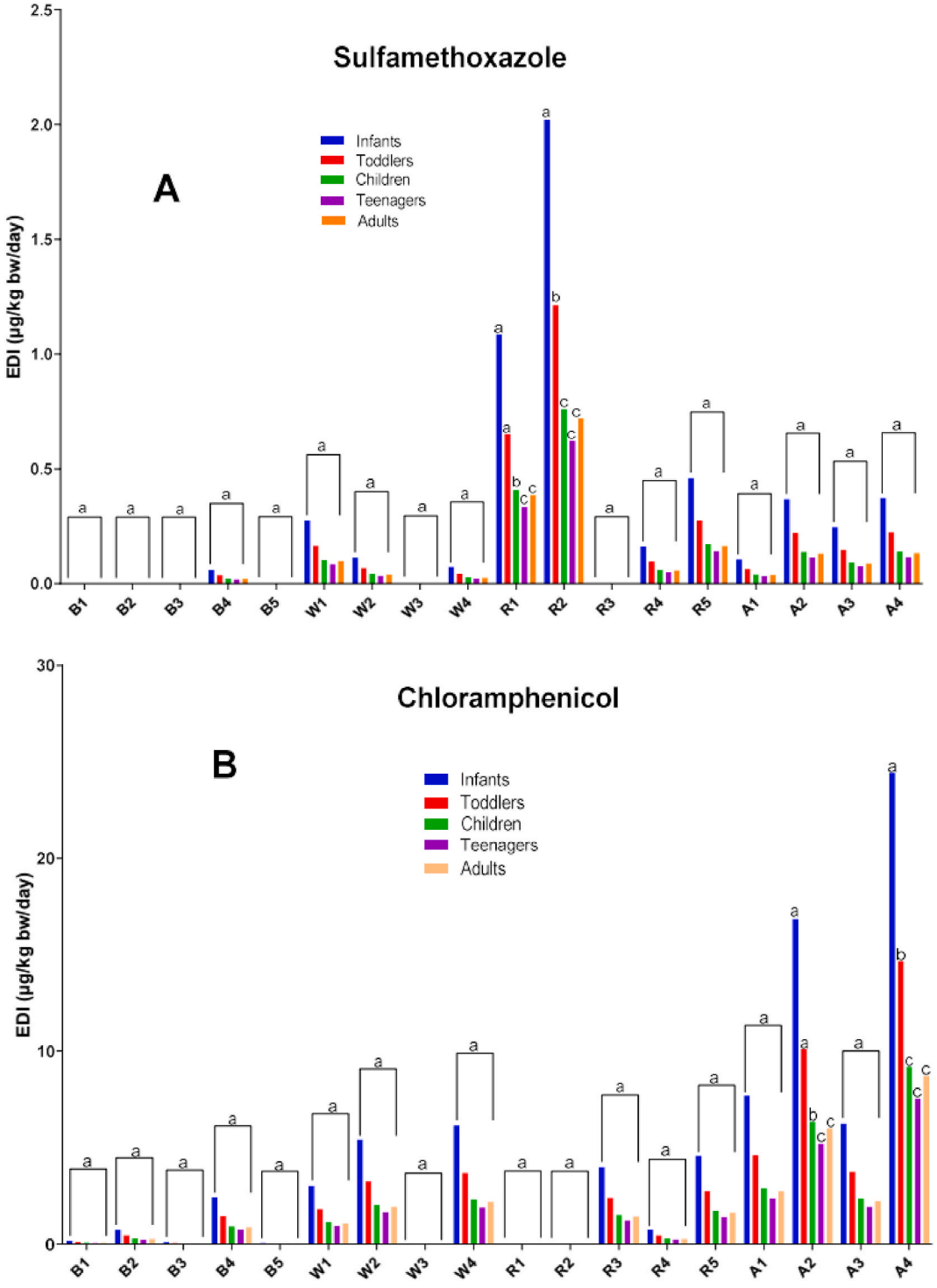

Fig. 1. Estimated Daily Intake (EDI) for (A) Sulfamethoxazole (B) Chloramphenicol (C) Metronidazole (D) Tetracycline (E) Ciprofloxacin in various water sample types calculated for the exposure groups in Ede, Osun State, Nigeria. Different letters (a–c) for age groups indicate significantly different means (Tukey's post hoc) multiple comparisons test ($p < 0.05$)

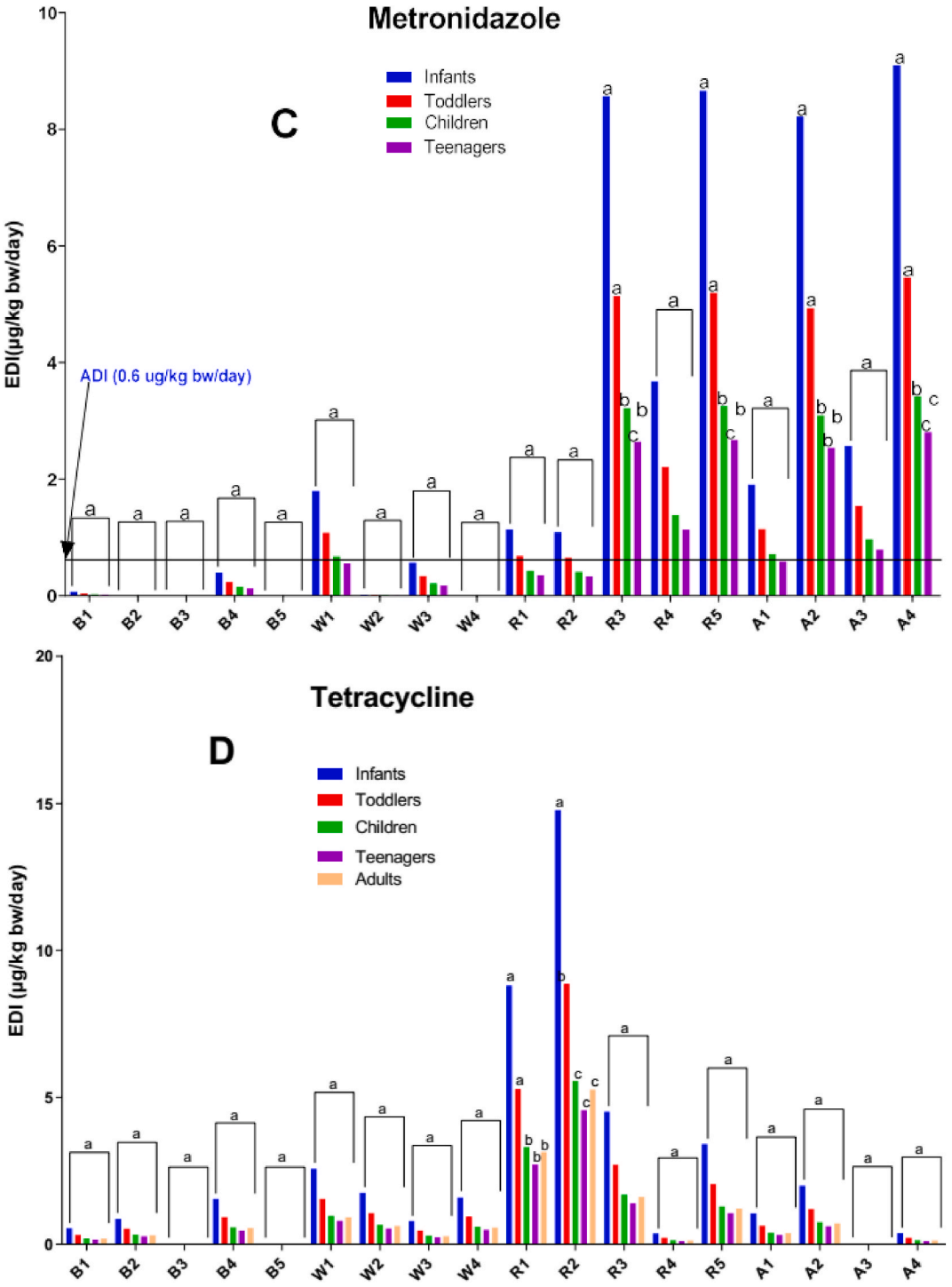

Fig. 1. (*continued*).

this study were mainly at the lower risk level with some moderate risks for all ecological life studied (Algae, Daphnia, and Fish). The only exceptions are CHL and TET (RQ > 1.0). CHL is of major risk to algae life in 60 %, 50 %, 60 % and 100 % of the bottled, well, river and abattoir water samples, respectively (Table 4). This major risk to algae may signify an advantage, as algal blooms with their resultant release of cyanotoxins are undesirable in drinking water but the presence of CHL in drinking water is detrimental to human health because long-term ingestion may lead to antibiotic resistance that may eventually lead to death [42].

Tetracycline in water samples from hand-dug wells has a maximum concentration of 23.7 µg/L (Table 3), indicating a low-risks

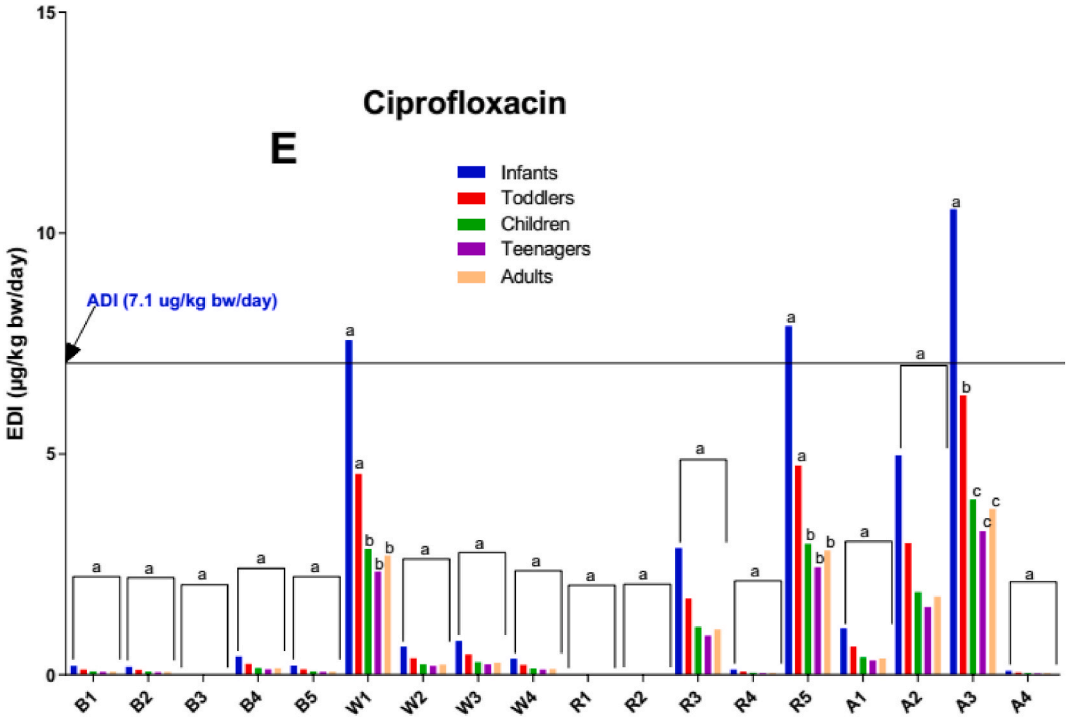

Fig. 1. (*continued*).

effect on Daphnia than Algae and Fish. In addition, there is an indication of a major risk of TET in 20 % of river samples to Daphnia spp. (Table 4). Likewise, the maximum concentrations of metronidazole (abattoir wastewater = 83.8 µg/L; river water = 79.7 µg/L, well water = 16.5 µg/L; bottled water = 3.7 µg/L) have significantly low-risks impact on Algae and Daphnia especially in river water.

Also, in considering the human risks involved in ingesting these antibiotics, the estimated daily intake (EDI) and hazard quotient (HQ) of these antibiotics in drinking water sources were calculated for various exposure groups. The EDI for the investigated water sources from Ede, Osun State Nigeria was compared with the Acceptable Daily Intake (ADI) for Sulfamethoxazole (130 µg/kg/day), Ciprofloxacin (7.1 µg/kg/day), Tetracycline (170 µg/kg/day), Chloramphenicol (29 µg/kg/day) and Metronidazole (0.6 µg/kg/day) from drinking water [26–28]. The EDI values for all the investigated antibiotic residues in this study were significantly ($p < 0.05$) less than their ADI values (Fig. 1A and B and D), except for Metronidazole in 44.4 % (Fig. 1C) of the water samples across all the investigated age groups. Likewise, the Ciprofloxacin EDI values for W1, R5 and A3 in the Infant age group only were greater than the ADI (Fig. 1E). Values higher than ADI values indicate a high risk of these antibiotic residues causing toxicity in humans if ingested. Although humans do not drink directly from the abattoir discharge point (A1-A4), the wastewater discharged into the river is a major concern. Furthermore, W1 is adjacent to the abattoir's discharge point, and water from this well is used to wash slain cow and chunks of beef intended for public sale. These antibiotics may be get absorbed into beef tissues before sale [43].

Furthermore, the results of the post hoc analyses (Tukey's test) showed that the computed EDI values for sulfamethoxazole and tetracycline in R2 were significantly different ($p < 0.05$) between the infant and toddler age groups with infant having a higher value (Fig. 1). This result showed that the consumption of water from R2 may lead to the accumulation of sulfamethoxazole and tetracycline residues more in infants. However, the EDI values for sulfamethoxazole (R1), metronidazole (R3), tetracycline (R1) and ciprofloxacin (R5 and W1) were not significantly different ($p < 0.05$) for the same exposure groups (Infant and Toddler), indicating similarity in consequences and impacts. Additionally, the non-significant difference ($p < 0.05$) in the EDI values for all studied antibiotics residue in all bottled water samples across the investigated age groups showed similarity in source and impact, with tetracycline residues having the highest potential (Fig. 1D). This result suggests that tetracycline are more abundant in raw water sources utilize for the production of these studied bottled water. The health risk assessment and human quotients have implications not just on the toxicity to these population groups but also on promoting the emergence and selection of resistant commensal bacteria in these population groups which gives rise to antibiotic resistance [26]. Besides, infants have a higher health risk when exposed to these antibiotics but this reduces with the age of the population group (Fig. 1A–E).

The potential health risks linked to extended exposure to these antibiotics through drinking water are depicted in (Fig. 2) and HQ > 1 is regarded as a high health risk [44]. Apart from infants who are at high risk of exposure to CIP in W1, R5 and A3 water when ingested (Fig. 2A), infants and toddlers are at high risk of MET when W1, R1-R5 and A1-A4 are consumed and all other age groups are at high risk of MET when W1, R3-R5 and A1-A4 are consumed (Fig. 2A–E).

Note that there is no health risk (EDI and HQ) due to exposure to these antibiotics in bottled water unlike the other natural water sources, this is likely because of the normal treatment regime that the bottled water is taken through before it is packed and sold to the

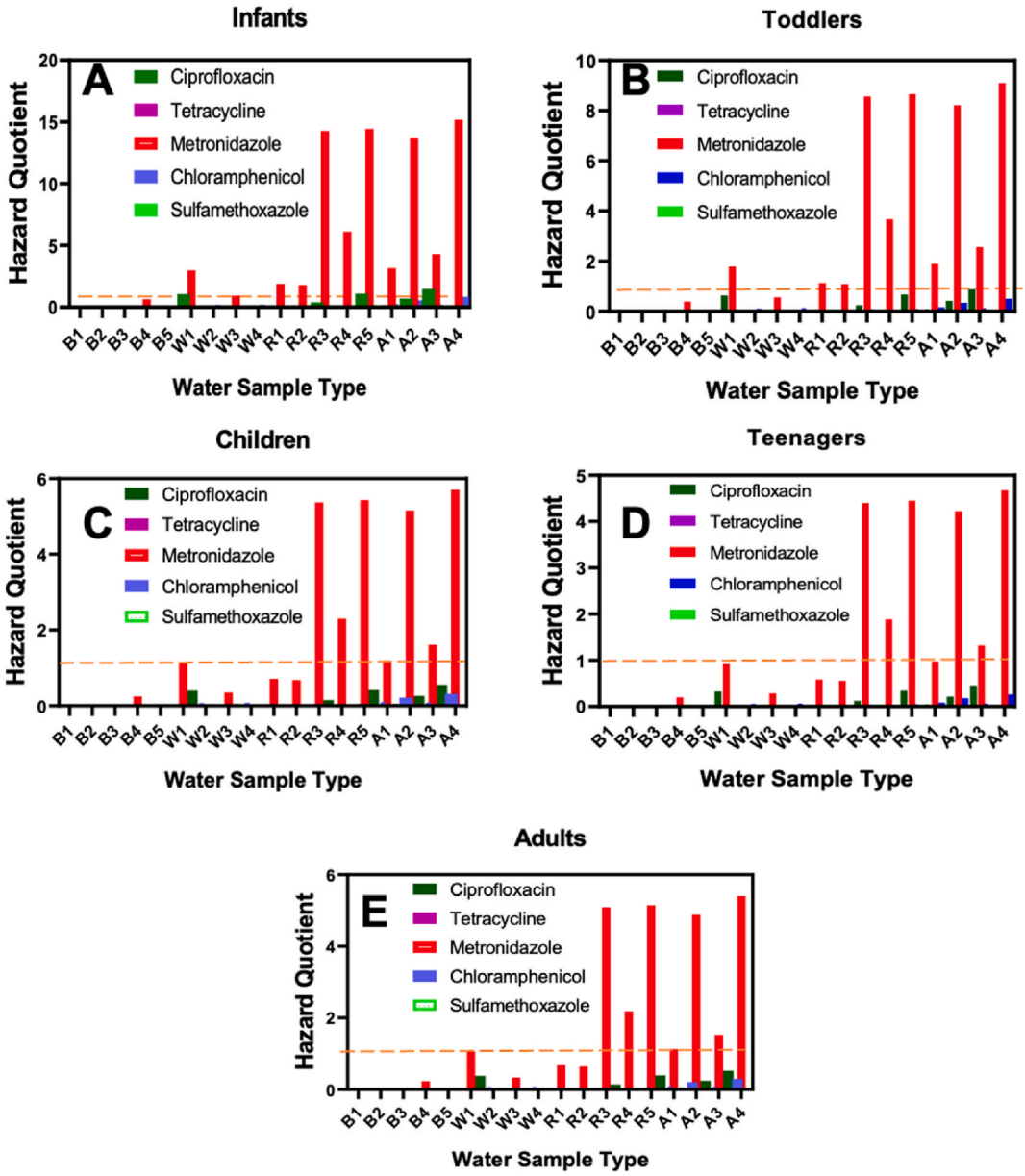

Fig. 2. Hazard Quotient (HQ) calculated for the exposure groups (A) Infants, (B) Toddlers, (C) Children, (D) Teenagers (E) Adults in various water sample types in Ede, Osun State, Nigeria. Dotted lines in figures represent HQ Threshold Limit of 1.0.

public. However, despite the treatment process, there are limited levels of exposure due to the residual amount of antibiotics in the bottled water as well as in hand-dug wells, except W1 (Fig. 2). These limited levels may pose very low health risks but consistent exposure over a long time may trigger some level of antibiotic resistance in those who ingest them [26]. It is also noteworthy that W1, R3-R5 and all abattoir water could cause health issues across the age group considered in this study. Uncertainty still exists as to the appropriate evaluation of risk assessment for antibiotic chemical residues due to certain factors not yet considered. These factors include the absence of data on the gastrointestinal absorption of daily intake of different antibiotic residuals in drinking water [26].

3.4. Antibiotic resistant bacteria

A total of 80 bacteria were the distinct pure isolates initially screened on tetracycline-supplemented nutrient agar. Thirty (30) of such isolates were from surface, ground and abattoir water (Table S3) samples while Fifty (50) of the isolates were from packaged (sachet and bottled) water samples (Table S4). Using Bergey's Manual of Determinative Bacteriology, the isolates subjected to various biochemical tests indicate the identities of suspected genera of bacteria (Tables S3 and S4). Fig. 3 shows the bacteria count from

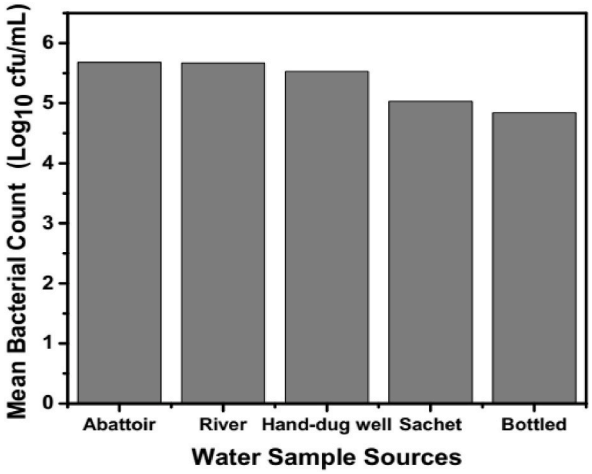

Fig. 3. Mean bacterial count for water samples in Ede, Osun state, Nigeria.

samples from each site and suggests that the bacteria count range is between 4.3 and 5.83 log10 cfu/mL. The mean log10 bacteria count (cfu/mL) followed the trend abattoir (5.68) > river (5.67) > hand-dug well (5.53) > sachet (5.03) > bottled (4.83). The total mean bacterial count values for packaged water samples (sachet and bottled water) are expectedly lower than other water types with bottled water having the better quality followed by sachet water, this can be due to the treatment they undergo before packaging. This is in addition to the fact that bottled water and their corresponding sachet water samples, each set of two brands, did not show any heterotrophic plate count (B4 and B5/STW4 and STW5).

The Log_{10} mean bacteria count difference between the remaining packaged water samples and other water samples is ca. 1.0. This suggests that whatever treatment procedure was used for the packaged water samples did not cause a sufficient difference between the quality of packaged and the untreated naturally occurring water. However, the Log_{10} mean bacteria counts for sachet and bottled water in this study are similar to the mean total bacterial count for bottled (Log_{10} 4.69 cfu/mL) and sachet (Log_{10} 5.67 cfu/mL) water samples from Ado Ekiti in Nigeria [45]. Bacterial plate count alone can hardly be used as a standard to determine the quality of drinking water but can be a pointer to possible contamination especially when the samples are monitored repeatedly over time. In this case, the treated water samples having some levels of bacterial count might indicate no treatment or inadequate treatment processes, since some other brands were equivalently free from bacteria when analysed. This could be because the bottled water samplesd isare of different brands/products with different treatment options. There is a need for constant monitoring by the relevant agencies responsible for quality checks of these packaged water products in the region/nation.

In identifying the bacteria in water samples collected, it is observed that the suspected bacteria genera in this study cut across some pathogenic (*Enterobacter, Bacillus, Acinetobacter, Aerococcus, Brevibacillus*), non-pathogenic (*Lactobacillus and Paenibacillus*) as well as opportunistic (*Corynebacterium, Staphylococcus, Micrococcus, Arthrobacter, Streptococcus*) bacteria classes. Of these genera, species like *Staphylococcus* sp., *Enterobacter* sp, *Bacillus* sp., *Acinetobacter* sp., and *Brevibacillus* sp., were found to be multidrug resistant with respect to antibiotics (Tables S5 and S6). From Fig. 4, it is observed that *Staphylococcus* and *Bacillus* genera of bacteria were more frequently detected in all water samples types. *Neisseria* species, identified as gram-negative diplococci with the adjacent sides flattening, were found in the hand-dug wells, river and abattoir water samples. *Neisseria* species were also isolated from drinking water in a study from Gezira, Sudan [46]. Expectedly, abattoir wastewater, river water samples, and hand-dug well water samples all contain a rich diversity of ARB with river water samples providing the most diverse bacteria (Fig. 4). The most frequently detected bacteria in this study were

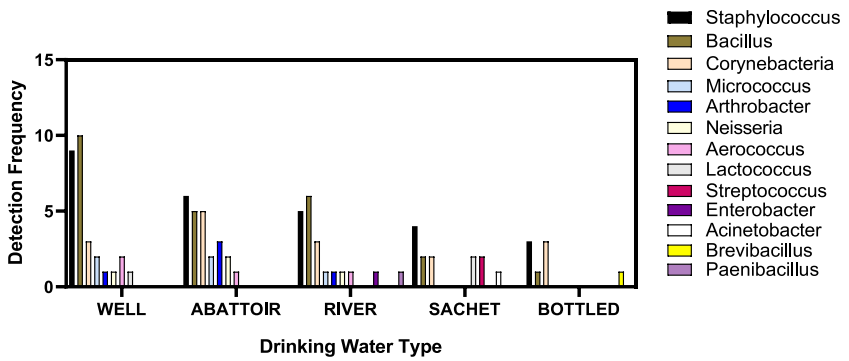

Fig. 4. Frequency of bacteria detected in water samples.

those belonging to the *Staphylococcus, Bacillus* and *Corynebacteria* genera (Fig. 4) and were found in all water sample types. Bacteria from these genera were reported to be present in commercially sold sachet drinking water in Ghana [47] and Ikpoba River water in Nigeria [48]. Although *Corynebacteria* was present in all the water samples from the different sources, it was detected more in water samples from hand-dug wells in this study (Fig. 4). The MDR *Acinobacter* and MDR *Brevibacillus* spp were detected only in sachet and bottled water samples respectively. It is important to state that the hand-dug wells are sources of drinking water for people living in both rural and urban settings in Ede. These wells are hardly covered and *Corynebacteria* sp which originate from humans, may have been introduced into water from these wells through constant contact with humans.

The majority of the bacteria detected from packaged water samples sold in Ede community belong to the genus *Staphylococcus* which gives credence to previous reports from studies on commercial sachet drinking water from Nigeria [49]. These bacteria were also found in river water samples. The presence of MDR Staphylococcus sp. in these samples may signify anthropogenic contamination coming from bathing or other human activities since these bacteria are part of the human microflora.

3.5. Multidrug-resistant isolates

The resistance of the bacteria isolates to six antibiotics (Tetracycline, Ampicillin, Chloramphenicol, Metronidazole, Ciprofloxacin, and Sulfamethoxazole) by the MDR bacteria isolates from samples is shown in Tables S5 and S6. All MDR isolates were defined as bacterial isolates resistant to $\geq$80 % of antibiotics used in this study. It is worthy of note that of the 80 isolates from the samples, 30 % (24/80) are MDR. Suspected members of the Bacillus genera were the most predominant species in the natural as well as packaged water samples, others include *Acinetobacter* spp, *Enterobacter* spp, and *Staphylococcus* spp. To define the specie(s) of these MDR bacteria, 16S rRNA gene sequencing was carried out on the various bacteria isolates (Tables S7 and S8). From Table S8, the resistance of all MDR to antibiotics used in the study showed percentage resistance as AMP 95.8 % (23/24), TET 95.8 % (23/24), CHL 66.67 % (16/24), SUL 71.16 % (19/24), MET 100 % (24/24).

Among the MDR isolates sequenced from hand-dug wells, abattoir and river water, two were of the genera Staphylococcus (*S. haemolyticus*) and Enterobacter (*E. ludwigii*), while the other eight were of the Bacillus genera. The MDR isolates from the packaged water were from the genera Bacillus (*B. cereus, B. sanguinis, B. tequilensis, B. subtilis*), Acinetobacter (*A. baumanni*), and Brevibacillus (*B. invocatus*) and Staphylococcus (*S. haemolyticus*). The *S. haemolyticus* found in this study is a well-known opportunistic pathogen that can cause meningitis, prosthetic joint infections, and skin, or soft tissue infections. *S. haemolyticus* strains with known resistance to one or more antibiotics, including penicillin, tetracycline, macrolides, quinolones, aminoglycosides, and glycopeptides have been reported. Its multi-drug resistance and ability to form biofilms make it a severe public health threat. *S. haemolyticus* is also known to transfer ARGs in water sources [50].

The presence of pathogenic *Enterobacter ludwigii* in one of the river samples (R4) is not a surprise because this bacteria species is ubiquitous and is known to be present in the intestinal tracts of animals. This may account for its wide distribution in water and sewage since human waste and livestock waste from abattoirs are indeed dumped into this river. *E. ludwigii* is also an opportunistic pathogen. It is a nosocomial pathogen responsible for various infections, including bacteremia, lower respiratory tract infections, skin and soft-tissue infections, and urinary tract infections [51]. The wide range of therapeutic drugs used for the treatment of infections caused by these bacteria could be the reason for their being multi-drug resistant.

Bacillus spp. are present in all types of water samples in this study. The prevalence of *Bacillus cereus* in packaged water samples is previously reported by Dahunsi et al., 2014 [52]. This is a pathogenic microorganism that causes meningitis, septicemia, and abscesses in humans. It has also been linked to eye infections, pneumonia, and meningitis [53]. In this study, it showed the most resistance to antibiotics. This strong resistance has been reported in previous studies from Nigeria [54]. The presence of MDR *Bacillus cereus* in packaged water samples implies that drinking water from this region of the State in Nigeria is not safe and would thus need to be well treated before release to the public. Bacilli are spore formers so this could be the reason for their wide presence across sample types and sites. They are also thermoduric hence boiling water may not eliminate *Bacillus* spores, particularly in water sources like dug wells or rivers. There is a need for additional research to evaluate the long-term effectiveness of photo-disinfection, especially on biofilms.

Although *Acinetobacter baumannii* is predominantly associated with nosocomial infections found in hospital environments, it was also found in packaged water samples in this study. This bacterium (*A. baumannii*) is a rapidly emerging pathogen that is known to cause infections like bacteraemia, pneumonia, meningitis, urinary tract infections, and wound infections [55].

Brevibacillus sp found in bottled water in this study is multidrug-resistant (MDR) and are environmental microorganism that is rarely implicated as human pathogens, although some species have been identified as opportunistic pathogens. These spore-forming bacteria can withstand heat treatments, which is a common method for treating drinking water before use in several developing countries. *Brevibacillus* spp are known to produce biofilm which makes them more persistent in drinking water [56] Their presence in the packaged treated water could cause opportunistic infections in immune-compromised patients or children. Although *Brevibacillus* spp is identified in the water matrix [57], this is the first time this bacteria will be isolated from commercially sold packaged drinking water in Africa.

Acinetobacter species are ubiquitous in aquatic habitats and several of them are pathogenic in nature. The *Acinetobacter baumannii* found in bacteria isolates from sachet water samples in this study is MDR. It is an emerging human pathogen whose prevalence in the aquatic environment raises concerns in public health [58]. There are previous reports of multidrug-resistant *Acinetobacter baumannii* in drinking water. This species of bacteria can cause infection in the blood, urinary tract, and lungs. Interestingly, they can also be asymptomatic in humans, usually present in open wounds and respiratory secretions [59].

3.5.1. Antibiotic-resistant bacteria and their resistance to antibiotics

The high concentrations of TET, CHL, AMP, MET and CIP found in the various water sources from Ede, Osun State connote their influence on the antibiotic resistance exhibited by ARB and MDR isolates found in the study. The most abundant ARB is Bacillus spp (with 36.3 % occurrence) > Staphylococcus (with 27.5 % occurrence), they show % resistance ranging between 33.7-83.3 % and 33.7–100 %, respectively to the antibiotics in this study (Tables S9 and S10). *Bacillus cereus* was the most dominant MDR isolate with two of them showing 100 % resistance and the other 18 showing 83.3 % resistance. The high resistance could be derived from the corresponding high levels of these EAR levels in the study.

It is important to note that the highest resistance of all MDR was to MET (100 %) followed by AMP and TET (both 95.83 %), and by SUL (75 %) (Table S6), while the least resistant were CIP and CHL (66.67 %). The 100 % resistance of MET is expected as MET is known to be resistant in aerobic bacteria hence, they are ineffective in the treatment of such infections [60]. Furthermore, this high resistance to MET from our study is in conformance with the literature review on antibiotic resistance from Sub-Saharan Africa, implying that environmental bacteria isolates are more resistant to metronidazole [10] even though MDR isolates from our study are equally resistant to AMP and TET.

The 30 isolates from wells, river and abattoir samples showed 100 % resistance to MET but exhibited 66.7, 23.33, 83.3, 80 and 56.67 % resistance against AMP, CIP, TET, CHL and SUL, respectively (Table S9). Similarly, the remaining 50 isolates showed 100 % resistance to MET but exhibited 60, 38, 74, 46 and 20 % resistance to AMP, MET, CIP, TET, CHL and SUL, respectively (Table S10). In both cases, the 100 % resistance is attributed to the non-effectiveness of MET to aerobic isolates as earlier stated. All isolates exhibited 62.5, 100, 31.25, 77.5, 58.75 and 33.75 %resistance to AMP, MET, CIP, TET, CHL and SUL, respectively (Table S10). Thus, apart from the 100 % resistance to MET, the isolates are more resistant to TET (77.5 %), and the Ede community might face the health risk of possible resistance to TET.

3.6. Photo-disinfection of water

Water contaminated with Log_{10} 7.5 cfu/mL of multi-drug resistant *Enterobacter* sp and *Bacillus cereus* respectively, were passed through 2 g of the respective visible-light active metal-doped delaminated kaolinite clay composites in a fixed-bed setup. The breakthrough times (time for the first colony of bacteria to be found in treated water) were: 900 min (15 h) for Zn doped, 1380 min (23 h) for Cu doped and 1560 min and (26 h) for Cu/Zn doped composite materials (Fig. 5A–C). However, with water containing multi-drug resistant *Bacillus cereus* (Fig. 5D–F) it was; 1380 min (23 h) for Zn doped, 1920 min (32 h) for Cu doped, and 1980 min (33 h) for Cu/Zn doped composite materials. The volumes of bacteria-loaded water treated by these composites are: for *Enterobacter* sp Zn doped (7.2 L), Cu doped (11 L), Cu/Zn doped (12.5 L) and for *Bacillus cereus* loaded water are Zn doped (11.04 L), Cu doped (15.4 L), Cu/Zn doped (15.84 L).

The trend for the efficiencies of the metal-doped delaminated clay composites for the removal of MDR *Enterobacter* sp and MDR *Bacillus cereus* in water is Zn-doped < Cu-doped < Cu/Zn-doped (Fig. 5). However, the efficiencies of the composite materials are higher for the latter than for the former bacteria specie. However, when compared with their photo-disinfection efficiencies for multimetal-MDR *E. coli* previously reported [4], Cu/Zn doped clay composite shows reduced capacity from 36 h (MDR *E. coli*) to 26 h (MDR *Enterobacter* sp). On the other hand, the efficiency of Zn doped clay composite increased from 11 h (MDR *E. coli*) to 15 h (MDR *Enterobacter* sp), while there was a small difference (~3 h) between the breakthrough times for the disinfection of MDR *E. coli* and MDR *Bacillus cereus* (this study) when Cu/Zn doped clay composite was used.

The reason for the reduced efficiency of Cu/Zn doped in this study could be attributed to the fact that MDR *Enterobacter* sp are aerobics, that release catalase capable of converting the reactive oxygen species (ROS) released by the composite material, into CO_2 and H_2O [61]. Cumulatively, this negatively impacts the photocatalytic process by reducing the amount of ROS available for the photodegradation of the bacteria. On the other hand, *E. coli* is a facultative anaerobic bacterium that lacks enzymes that could transform ROS to less reactive species. However, the slight increase in photocatalytic efficiency exhibited by the delaminated Zn-doped clay composite could be as a result of the MDR *Enterobacter* sp not being resistant to metal. Although, *Bacillus cereus*, being a Gram-positive bacterium, is capable of forming biofilms at solid-liquid interfaces [62]. It is likely able to produce very little resistance to ROS released by Cu/Zn doped composite.

The results from the regrowth studies indicate that the bacteria (MDR *Enterobacter* and *B. cereus*) did not show any sign of growth in culture media after 3 days (72 h). This suggests that the photo-disinfected water is safe to drink (in terms of its ARB content) and remains safe when stored for some time.

In any case, a further study on the mechanism of these delaminated clay composites in inactivating these microbes, the mineralizing process, and metal toxicity from by-products of the mineralization of these microbes (especially for MDR *Bacillus cereus* which is not a metal-resistant bacteria) [4], would be worthwhile.

4. Conclusion

This study shows that there are antibiotic residues as well as antibiotic resistance bacteria and MDR bacteria in water sources (river, abattoir, hand-dug wells, sachet, and bottled water) from Ede, Osun State Nigeria. The concentrations of antibiotic residues: ciprofloxacin (CIP), sulfamethoxazole (SUL), chloramphenicol (CHL), metronidazole (MET), and tetracycline (TET) in water samples collected in this study are higher than those previously reported from other countries. Multi-drug resistant bacteria in the *Staphylococcus* and *Bacillus* genera were commonly found in water samples collected in this study including treated packaged water. The presence of the ARB in these water samples signifies that the drinking water sources are not of the best quality and thus not suitable as

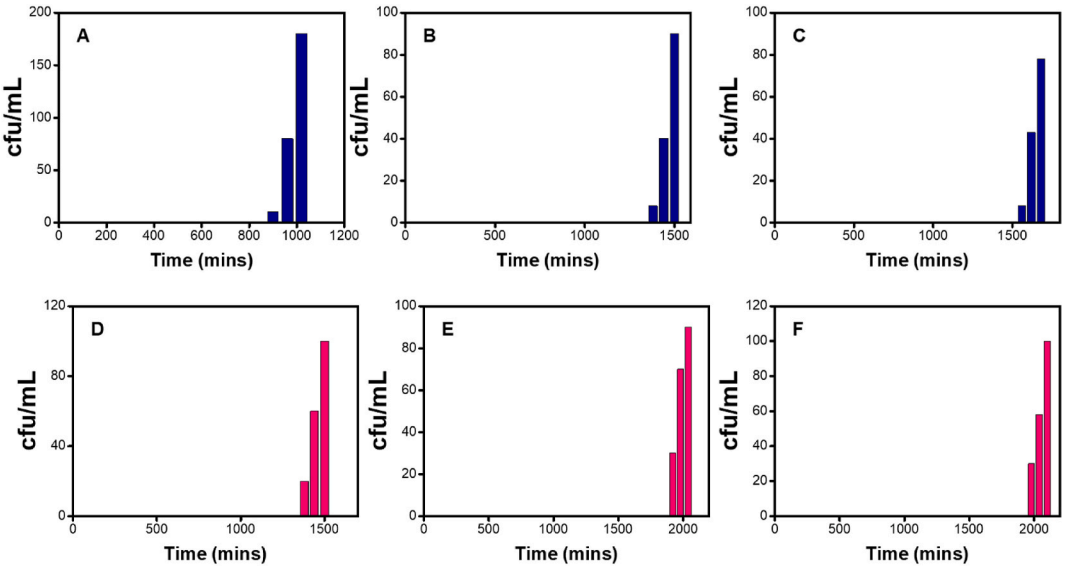

Fig. 5. Breakthrough plots for visible-light disinfection of MDR *Enterobacter* sp in water using (A) Zn-doped (B) Cu-doped (C) Cu/Zn-doped *d*-kaolinite clay composites; of MDR *Bacillus cereus* in water using (D) Zn-doped (E) Cu-doped (F) Cu/Zn-doped *d*-kaolinite clay composites (*d* = delaminated).

potable water. They will require further treatment procedures to reduce the bacteria load and antibiotic residues. There is a tendency of community antibiotic resistance to MET, AMP, TET, CIP and CHL in this study area if they continue to consume water from these sources. Treatment of water of these sources is recommended. Although the photo-disinfection technique we employed in the removal of these ARBs from water in this study, using Cu/Zn-doped delaminated clay composite, was effective, there is a need to improve the photoactivity time of the composites, determine their true mechanism of photo-disinfection, and enhance the reuse capacity of these composites.

To achieve the SDG report by 2030 and an effort to bridge the information gap from some countries on SDG progress reports, there are mandates by UN for annual reports emanating from national statistical systems and collated at regional levels. However, 'information and communication technology' with an 'open data' approach which involves data released by government agencies, non-profit organizations and researchers is encouraged to give a better overall picture [2]. Findings from this study on AR levels with their ecological and health risks as well as reports on the types of multidrug-resistant bacteria contribute to this scarce information on AMR monitoring in the region.

Furthermore, antibiotic residues, ARB and MDR bacteria recorded in some packaged waters are indicative of inappropriate treatment options used by the producers. To ensure proper treatment, regulatory bodies should step up efforts to monitor the water treatment plants of packaged water producers and the water products for the presence of these contaminants of emerging concerns (CECs) to avoid or ameliorate community antibiotic resistance. Government policies on antibiotic use, discharge and sales should also be reviewed and enacted and routine monitoring of natural drinking water sources,should also be encouraged to reduce the AMR burden in the region. Ultimately, the responsibility of providing potable water should be championed by Governments on the continent, the technology in municipal water works should also be updated to meet up with present-day challenges like the treatment of CECs. The use of our material for such treatment can be exploited.

CRediT authorship contribution statement

Gloria O. Taylor: Writing – original draft, Investigation, Formal analysis, Data curation. **Aemere Ogunlaja:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Olumide D. Olukanni:** Writing – review & editing, Supervision, Conceptualization. **Oluwatosin M. Awopetu:** Investigation, Data curation. **Frances Okodua:** Investigation, Formal analysis. **Daniel O. Godson:** Investigation, Formal analysis. **Afolarin Otusile:** Investigation, Formal analysis. **Daniella Ekpe:** Investigation, Formal analysis. **Esther Deguenon:** Writing – review & editing, Funding acquisition. **Kevin M. Sintondji:** Writing – review & editing, Funding acquisition. **Victorien Dougnon:** Writing – review & editing, Funding acquisition. **Olumuyiwa O. Ogunlaja:** Supervision, Investigation, Conceptualization. **Chidinma G. Olorunnisola:** Writing – original draft, Investigation. **Martins O. Omorogie:** Writing – review & editing. **Moses O. Alfred:** Writing – original draft, Investigation, Formal analysis. **Emmanuel I. Unuabonah:** Writing – review & editing, Resources, Funding acquisition, Conceptualization.

Data availability statement

Data will be made available on request.

Ethics declaration

Review and/or approval by an ethics committee as well as informed consent was not required for this study because this article did not involve any direct experimentation/studies on humans.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e41538.

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