Antimicrobial-resistant in *Escherichia coli* isolated from different effluent locations within Ahmadu Bello University, Zaria, Nigeria

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**Abstract**

The safety of municipal water is increasingly becoming of concern globally. Agricultural activities, industrial and residential effluents and community waste are ways through which water sources are contaminated and resistant bacteria can be spread via effluents to municipal water. The study aimed to isolate and determine the distribution of antimicrobial drug-resistant *Escherichia coli* from different points of the University sewer system in April 2018. A total of 48 samples were collected twice weekly from the six randomly selected inspection chamber sites out of the 14 identified sites. The selected sites of the sewer were located in some hostels, markets and health service areas within the ABU main campus. The samples were processed by culturing on an EMB agar plate followed by biochemical characterization using conventional biochemical tests and Microbact 12E. An antimicrobial sensitivity test was also carried out using 13 different antibiotic discs. The results obtained revealed that the Community market had an isolation rate of 4(50%), while Sickbay had 3(37.5%) and Danfodiyo hostel with 2(25%). Multiple antimicrobial resistance index (MARI) was found to be 0.31 from four isolates (36%) of *E. coli* of which 3(75%) were sampled from Sickbay and 1(25%) from ABU Dam. Also, five isolates (45%) had MARI of 0.23, of which 2(40%) were sampled from Danfodiyo hostel, 1(20%) from Ribadu hostel and 2(40%) from Community market. The *E. coli* isolates were more resistant to Ampicillin, tetracycline and cephalothin. Other bacteria isolated were *Klebsiella ozaenae*, *Hapnea alvei* and *Morganella morganii* all with MARI of 0.31. There is a need for public health awareness on the effect of discharging antibiotic-resistant *E. coli* contaminated effluent into the environment and water bodies. Hence, the public health significance of recycling such water for domestic usage and agricultural purpose.

**Keywords:** Antimicrobial-resistant, ABU Zaria, Effluents, *E. coli*, Sewer
Introduction
Effluent is a treated or untreated wastewater/sewage that flows out of a treatment plant, sewer, or industrial outfall and is discharged into surface waters (USEPA, 2006). Urban effluent comprises mainly of water (99.9%) with relatively small concentrations of suspended and dissolved organic and inorganic solids. Substances present in effluents include fats, carbohydrates, lignin, soaps, synthetic detergents, proteins and their decomposition products, etc. Inorganic substances from domestic and industrial sources are also present in municipal effluents (Tilley et al., 2014).

In agricultural use of effluents, the contaminants of great public and environmental health concern are the pathogenic micro- and macro-organisms. These pathogens include viruses, bacteria, protozoa and helminths, which are found in different concentrations and will survive in the environment for long periods (WHO, 2006). Pathogenic bacteria are usually present in wastewater at much lower levels than the *E. coli* form group of bacteria, which are much easier to identify and enumerate. *Escherichia coli* are the most widely adopted indicator of faecal pollution and they can also be isolated and identified (Fricker, 2000).

*Escherichia coli* (*E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms (endotherms) (Tenaillon et al., 2010). *E. coli* is a dominant coliform in aquatic environments contaminated by antimicrobial-resistant bacteria originating from human and animal faeces. The *E. coli* in such environments are associated with antibiotic resistance genes that can be transferred to human and animal flora, where they can exert intense pressure for the spread of resistance (Adefisoye & Okoh, 2016).

Antibiotic resistance is the ability of organisms like bacteria and fungi to defeat the drugs designed to inhibit/kill them (CDC, 2010). Pollutants and antibiotic-resistant bacteria are constantly released as effluents into the environment which contribute to significant public health risk (McLellan et al., 2015). A major process of *E. coli* and other organisms becoming resistant is the selective pressure of antimicrobial use in human and animal medicine, as well as in aquaculture and agriculture (Kummerer, 2009). Wastewater is a hotspot of antibiotic-resistant gene exchange where environmental bacteria interact with microbes that originated from humans and other animal sources, through horizontal gene transfer (HGT) (Wackett, 2015). Opportunistic pathogens often have large and versatile genomes, prone to sharing genetic materials (Vanderlene et al., 2010). This peculiarity helps these organisms to colonize a more diverse set of environments. As consequence, aquatic ecosystems may become a threat to human health when they are affected by pollutants carrying resistant bacteria (Baquero et al., 2008). Antibiotic resistance, particularly MAR, is a major public health threat, and the presence of resistant organisms in environmental waters is an emerging concern in the world (Marti et al., 2013). The important role of the environment in the dissemination of antibiotic resistance is acknowledged, and the aquatic environment has been shown to act both as a natural reservoir and a channel for the spread of clinically relevant antibiotic resistance traits (Michael, et al., 2013; Canencia et al., 2016). The increasing spread of antimicrobial-resistant genes among environmental bacteria has led environmental scientist to consider Antimicrobial-resistant bacteria (ARB) and antimicrobial-resistant genes (ARGs) as emerging pollutants or contaminant in the natural environment (Pruden et al., 2013). Wastewater discharges from domestic sources affect the diversity of resistant bacteria (Czekalski et al., 2012). These impacts also shape the genetic pool of water bodies by increasing the abundance of antibiotic resistance genes within the habitats (Tacão et al., 2012). Developing countries like Nigeria have the highest concentrations of antibiotics and resistant bacteria in effluents released from hospitals and drug manufacturing sites (Bréchet et al., 2014). Effluents containing chemicals, antimicrobials and detergents or their residues are capable of exerting selective pressure to the microbial flora in the water and can select for resistant bacteria (Bennett, 2008; Schmitt et al., 2017). The study aimed to isolate and determine the antibiogram of *E. coli* and some *Enterobacteriaceae* from different effluent points of the university sewer system.

Materials and Methods

**Study area**
The study was conducted at Ahmadu Bello University main campus located in Zaria, Kaduna State of Nigeria on latitude 11° 03’ 60.00”N longitude 7°41’59.99”E, at an altitude of 550-700 meters. It is
about 13km from Zaria-city on the Sokoto road, 8km to Shika and 7km from Bassawa. The University covers a land area of about 7,000 hectares and comprises 17 Faculties, 106 Academic Departments, a Postgraduate School and a Distance Learning Centre. It also has 16 Research Institutes, 7 Centres of Excellence, a Microfinance Bank, Mini Refinery, University Health Service, Teaching Hospital, a School of Basic and Remedial Studies, a Demonstration Secondary School, a Primary School, 5 Guest houses, 3 Hotels and a Consultancy Outfit which provides a variety of services to the University and the wider society. It has a population of over 45,000 students and over 10,000 Academic and support staff.

The existing sanitary sewerage system (ESSS) at ABU Zaria is used for the conveyance and treatment of sewage (domestic wastewater) for the University's academic area. In the year 1962, it uses a trickling filter to treat the wastewater from the campus population, some of the halls of residence, their kitchen wastes were drained to an open ditch while the other buildings had separate and individual septic tanks to treat their wastewater. In 1972, a new "exigent plant model R" was installed and activated. Due to high load, in 1979 a waste stabilization pond (WSP) was designed and constructed as the first of its kind to be implemented in Nigeria. The ESSS is functional but evaluated to be 38% efficient, major challenges are poor maintenance of broken sewer pipes and low network coverage (Otun et al., 2009).

**Study design**

A cross-sectional study was conducted to sample three (Hostels, Markets and University Health Services) of the six (Hostels, University Health Services, Markets, Faculties/Departments, Residential Quarters and ABU Dam) major contributors of the ABU sewage network based on accessibility to inspection chambers.

The sampling sites identified were Undergraduate hostels (Suleiman, Danfodiyo, ICSA/Ramat, Ribadu, Amina), Postgraduate hostels (Sasakawa, Akenzua, Suleiman, Amina), Markets (community market, social centre, ICSA/Ramat), University Health Service (Sickbay) and ABU Dam. Six sites were selected from the fourteen identified sites by simple random sampling method (Horvitz & Thompson, 1952).

A total of 48 wastewater samples were collected at the inspection chambers of the six randomly selected locations, twice weekly for four weeks in April 2018. The collection was done every Monday and Wednesday which were the routine inspection days for the maintenance staff from the University Health Services, to have access to the inspection chambers. 10mls of the wastewater were collected at all the locations using a sterile syringe and deposited in sterile test tubes which were covered neatly and transported to the bacterial zoonoses laboratory of the Department of Veterinary Public Health and Preventive Medicine ABU Zaria.

Each sample was processed by making a 100-fold serial dilution, from which 1ml was taken and added to 9ml of Tryptone soya broth as enrichment and incubated for 24 hours. This was then plated on a selective media (EMB) and incubated for 24hr as first described by Levine (1918).

**Biochemical test**

Colonies growing on nutrient agar slants were subjected to further biochemical tests namely; Simmon citrate, Urea, Triple Iron Sugar (TSI), Sulfate, Indole, Motility (SIM) and Methyl Red (MR), Vogesproskeur (VP). Various reactions of the tests such as colour change, motility and gas formation were used to interpret results as either positive or negative after 24-hour incubation. These tests were carried out as described by Khandaghi et al., 2010. Positive isolates were further characterized biochemically using Microbact 12E (MB1130A+, Oxoid, UK) according to the manufacturer's instruction for further confirmation of *E. coli* due to its higher degree of accuracy in comparison to conventional biochemical tests (Mugg & Hill, 1981). It uses 4 digit codes that were obtained from the culture and was fed into the computer identification software; which gave the probable identity of the organism tested in percentage score. The Microbact software recommends a 75% cut-off point for probable identification. All tests that gave less than 75% were not accepted as *E. coli*.

**Antibiotic susceptibility test**

Antibiotic susceptibility tests were performed on all the isolates to determine their antibiotic-resistant profiles using the disc diffusion method developed by Kirby – Bauer and standardized by the World Health Organization as modified by Sozmen et al. (2011). Commercially available antimicrobial disks from Oxoid, the UK used were; nitrofurantoin (F300ug), gentamicin (CN10ug), trimethoprim (W5ug), ampicillin (AMP10ug), amoxicillin/clavulanic acid (AMC30ug), chloramphenicol (C30ug), cephalothin (KF30ug), ciprofloxacin (CIP5ug), tetracycline (TE30ug), cefoxitin (FOX30ug), nalidixic
acid (NA30ug), sulphamethoxazole/trimethoprim (SXT25ug) and Amikacin (AK30ug). The bacteria were inoculated on tryptone soy broth and incubated at 37°C for 24hrs. The turbidity was adjusted to 0.5 McFarland standards (Eduardo et al., 2018) and a sterile swab stick was eluted into the overnight culture broth and any excess moisture were expressed by pressing the swab against the side of the tube. The surfaces of the Mueller – Hinton agar was also swabbed completely and then turned at 90° and repeat the swabbing process until the entire circumference of the plate was covered. The media was allowed to dry for about 5 min before placing antibiotic discs using an antibiotic dispenser. Then each disc was lightly touched with a sterile inoculating loop to make sure it is in good contact with the agar surface, and then incubated upside down at 37°C overnight. A transparent plastic metric ruler was used across the zone of inhibition (ZI), at the widest diameter to measure from edges of the zones in millimetres. Results were reported for resistance only (CLSI, 2014). Multiple antimicrobial resistance indexes were determined by dividing number of antimicrobials to which the organism was resistant by the total number of antimicrobials to which the organism was exposed.

**Results**

**Distribution of *E. coli* based on sample locations**

From a total of 48 samples collected from 6 locations, 32 samples were positive for *E. coli* on EMB (Figure 1). The positive samples from EMB were subjected to Biochemical tests, 22 were positive. From the 22 biochemical positive samples (Figure 2), 11 were positive on microbat. Samples from the community market had the highest isolation rate 50% (4/8), followed by samples from sickbay with an isolation rate of 37.5% (3/8). Ribadu hostel and ABU. Dam had an isolation rate of 12.5% (1/8), while Danfodiyo hostels had an isolation rate 25% (2/8) (Table 1).

**Susceptibility of *E. coli* isolates to antimicrobials**

The eleven isolated *E. coli* were subjected to in-vitro activities of 13 antimicrobials (Table 2). Four isolates had the highest multiple antimicrobial resistance index (MARI) of 0.31, while 5 isolates had MARI of 0.23. Only isolate 1 and 2 were resistant against Trimethoprim (W5) and Sulphamethoxazole/Trimethoprim (SXT25) (Figure 3).

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**Figure 1**: *E. coli* colony growth with greenish metallic sheen on EMB  
**Figure 2**: Some biochemical test result from colonies with greenish metallic sheen on EMB

| Location            | No. of samples | No. positive on EMB | No. positive biochemical | No. positive on Microbact | Isolation rate (%) |
|---------------------|----------------|---------------------|--------------------------|---------------------------|-------------------|
| Danfodiyo hostel    | 8              | 4                   | 3                        | 2                         | 25.0              |
| Ribadu hostel       | 8              | 6                   | 3                        | 1                         | 12.5              |
| Sasakawa hostel     | 8              | 4                   | 3                        | 0                         | 0.0               |
| Community market    | 8              | 7                   | 6                        | 4                         | 50.0              |
| Sickbay             | 8              | 7                   | 4                        | 3                         | 37.5              |
| ABU dam             | 8              | 4                   | 3                        | 1                         | 12.5              |
| Total               | 48             | 32                  | 22                       | 11                        | 100.0             |

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Table 2: Multiple antimicrobial resistance (MAR) from isolated *E. coli* within Ahmadu Bello University, Samaru, Nigeria

| Isolate | Resistance Profile | MAR |
|---------|-------------------|-----|
| 1       | W5, AMP10, KF30, SXT25 | 0.31 |
| 2       | W5, AMP10, KF30, SXT25 | 0.31 |
| 3       | AMP, AMC           | 0.2  |
| 4       | AMP10, C30, TE30   | 0.23 |
| 5       | AMP, TE30          | 0.2  |
| 6       | AMP, TE30, AK30    | 0.23 |
| 7       | AMP, TE30, AK30    | 0.23 |
| 8       | AMP, TE30, NA, AK30| 0.31 |
| 9       | CN10, KF30, CIP5   | 0.23 |
| 10      | C30, KF30, CIP5, FOX30 | 0.31 |
| 11      | AMP, AMC, TE       | 0.23 |

Note: Nitrofurantoin (F300), Gentamicin (CN10), Trimethoprim (W5), Ampicillin (AMP10), Amoxicillin/Clavulanic acid (AMC30), Chloramphenicol (C30), Cephalothin (KF30), Ciprofloxacin (CIP5), Tetracycline (TE30), Cefoxitin (FOX30), Nalidixic acid (NA30), Sulphamethoxazole/Trimethoprim (SXT25) and Amikacin (AK30)

Table 3: Other Microbes isolated from samples within Ahmadu Bello University, Samaru, Nigeria

| S/N | Sample ID | Isolate               |
|-----|-----------|-----------------------|
| 1   | D1        | *Klebsiella ozaenae*  |
| 2   | D2        | *Hapnea alvei*        |
| 3   | S5        | *Klebsiella ozaenae*  |
| 4   | SB        | *Morganella morganii* |
| 5   | CM        | *Morganella morganii* |

D1: Danfodiyo hostel sample 1; D2: Danfodiyo hostel sample 2; CM: Community Market; SB: Sickbay and S5: Suleiman hostel sample 5

Other gram negatives isolated from the effluent samples

Other Gram-negative microbes isolated includes *Klebsiella ozaenae* (2), *Morganella morganii* (2) and *Hapnea alvei* (1) (Table 3).

Susceptibility of other Gram-negatives isolated to antimicrobials

The five isolates were subjected to 13 panels of antimicrobials as shown in Table 4. Three of the isolates were resistant to four antibiotics, while the two remaining isolates were resistant to three antibiotics. The multiple antimicrobial resistance index (MARI) for the two *Klebsiella ozaenae* (sample D1 and S5) were found to be 0.31 and 0.23 respectively. *Hapnea alvei* (sample D2) had MARI of 0.31 while that for the two *Morganella morganii* (sample SB and CM) were 0.23 and 0.31 respectively.

Discussion

The isolation rate of *E. coli* in the present study was at 23%, which is relatively low when compared to a previous study within Zaria by Chigor *et al.* (2010) which had 42.1%. This difference may be due to
Multiple antimicrobial resistance (MAR) from isolated microbes within Ahmadu Bello University, Samaru, Nigeria

| Isolate | Resistance Profile | MAR |
|---------|--------------------|-----|
| D1      | W5, AMP10, KF30, SXT25 | 0.31 |
| D2      | W5, AMP10, KF30, SXT25 | 0.31 |
| S5      | F300, CN10, AMP10 | 0.23 |
| SB      | AMP10, AMC30, TE30, | 0.23 |
| CM      | AMP10, KF30, TE30, SXT25 | 0.31 |

MAR index—Multiple antimicrobial resistance index = No. resistance/Total number of antimicrobials

Microbact 12E has been more sensitive and specific than conventional biochemical characterization tests (Vithanage et al., 2014). This study reveals Community market had the highest isolation rate (50%), followed by sickbay and Danfodiy hostel with 37% and 25% respectively. The high rate of isolation at the community market may be due to the wide range of human activities (such as restaurants, vegetables and meat/fish stalls, laundry services, etc.) and the dynamics of the human population. Human activities play a role in the increased spread and contamination of the environment with antibiotic-resistant bacteria and genes (Pruden et al., 2006). Also, animal and human microbiota serve as a reservoir for ARGs. Bacteria passing through the intestinal tract can acquire antibiotic resistance via conjugation and end up in human and animal faeces (Salyers et al., 2004). Another observation was the stagnant nature of the effluent discharge channels, compared to other sample locations. It has been established that stagnant water is a large mixture pool that creates a suitable environment for bacteria and other microorganisms to proliferate (Zlatanović et al., 2017).

The result of the present study indicates resistant E. coli in effluents from different locations within the university main campus. ARBs are more resistant to water treatment than their non-resistant counterparts; this prolongs their survival in the environment and spread through recycled water. Antibiotic-resistant virulent E. coli strain persisted longer than the less resistant non-virulent E. coli strain upon solar radiation, despite both being of the same genus and species (Al Jassim et al., 2017). This calls for a significant public health concern considering they all aggregate at the campus sewage system and the water reused after treatment.

An important finding from the present study was that majority of the E. coli isolates had multiple drug resistance (MDR). MDR is the resistance to all the tested antibiotics in at least two of the following 3 classes; Lactams, Aminoglycosides and Quinolones (Doyle et al., 2015). This study shows a majority of the effluent isolates were resistant to Ampicillin followed by Tetracycline and Cephalothin. This pattern was similar to previous studies that show resistance towards β-lactam, tetracycline, and cephalosporins (Olukosi et al., 2016).

Upon MAR index analysis in the present study, it reveals that 82% (9/11) of the E. coli isolates had MARI values greater than 0.2. This finding is similar to that of Chigor et al. (2010), which recorded MARI of 80% from the aquatic isolates in surface water sources in Zaria. The result is low in comparison to Edward et al. (2020), which recorded 99.8% E. coli with MARI >0.2 from abattoir wastewater in Abia State. A MAR index of 0.2 is used to distinguish between low and high-risk contamination sources (Krumperman, 1983). In Nigeria, poor drug quality is another contributory factor to multiple drug resistance through sub-inhibitory selective pressure. The poor-quality drugs may be due to poor preparation, low or no active content and poor storage (Okeke & Sosa, 2003). This indicates a possible significant exposure of E. coli in the environment to antibiotics, leading to selection pressure. A previous study reveals that multiple drugs resistance in bacteria is associated with plasmids that have several resistance determinants (Baker et al., 2018).

It has been noted that the spread of antibiotic-resistant bacteria in the environment may result to increase resistance in certain human infectious diseases (Titilawo et al., 2015). The present study also established the occurrence of other Gram-negative isolates from the effluent samples. The isolates were more resistant to ampicillin, followed by cephalothin and sulphonamethoxazole/trimethoprim. The isolates were more resistant to ampicillin, followed by cephalothin and sulphonamethoxazole/trimethoprim. Non-pathogenic ARB can serve as a reservoir with the potential to transfer ARGs to non-antibiotic resistant pathogens (Ashbolt et al., 2013), hence contributing to the spread of ARB. Klebsiella spp. is amongst the most common causes of a variety of community-acquired and hospital-acquired infections. Klebsiella
ozaenae is a subtype of *K. pneumonia* and is known to cause chronic inflammatory disease of the upper respiratory tract (Falkow & Mekalanos, 1990). In conclusion, effluents discharged from sickbay, Danfodiyo hostel, Ribadu hostel, Community market and ABU Dam in the campus serves as a shelter for antibiotic-resistant *E. coli* and other enterobacteriaceae as revealed by the study. The MAR index values are of great public health significance, hence the need to regularly evaluate the presence and quantity of ARB in reclaimed water. The contamination of water bodies with such effluents will render them unsafe for irrigation and domestic use. Several studies have established that conventional sewage treatment plants do not entirely remove ARB and ARG from treated effluents. To minimize the potential risk of effluent to public health and the environment, the sewage system should be properly maintained and channelled to a functional facility to ensure wastewater can be safe for recycle and agricultural use.

**Conflicts of Interest**
The authors declare no conflict of interest.

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