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# Isolation, Identification, Distribution and Antibiotic Profile of Bacteria Contaminants of Ebenyi River

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#### Authors' contributions

This work was carried out in collaboration between both authors. The Authors conceptualized, designed the study, experimented, did statistical analysis, wrote the protocol, and the manuscript. Both authors read and approved the final manuscript.

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#### ABSTRACT

Despite the abundance of water on earth, only a small fraction of water is readily available for the use of man. Even the small available fraction is not completely clean and safe for industrial and domestic uses. This study investigated the distribution of bacteria contaminants in Ebenyi River and their antibiotic resistance profile. Water samples were randomly collected from three points along each of the five communities that make up Eha-Amufu town through which River Ebenyi passes using 200 ml sterilised borosilicate glass bottles. Collected water samples were labeled and transported in an ice pack to the laboratory for analysis within 6 hours of sample collection. Bacteria isolation and characterisation were done using the culture method while antibiotic susceptibility was performed using the Kirby–Bauer method. A total of 59 species were identified, 25 (42.4%) were *Escherichia coli*, 12 (20.3%) were *Bacillus* sp, 12 (20.3%) were *Klebsiella* sp, and 10 (16.9%) were *Staphylococcus* sp. The results showed that Isu location had the highest concentration of bacteria species 15 (25.4%) while Umuhu locations had the least, 9 (15.3%). The isolated bacteria showed resistance to sulfamethoxazole-trimethoprim, penicillin, cephalosporins, tetracycline and

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aminoglycosides. It was therefore recommended that government at all levels, nongovernmental organizations or affluent and philanthropic individuals should immediately provide alternative sources of clean and safe water to the people of Eha-Amufu Community to help preserve the lives of the people. Any alternative source of water provided to the people must undergo sufficient filtration and exposure to ultraviolet radiation to take care of microorganisms present in the water.

Keywords: Bacteria; Ebenyi river; contaminants; water quality; pollutants; penicillin antibiotic.

#### **1. INTRODUCTION**

Water is a crucial resource for all living beings on earth, including humans. It guenches our thirst, irrigates our crops, and sustains our ecosystems. Yet, we face a daunting challenge: water contamination [1-3]. Contaminants such as heavy metals, pesticides, microplastics, synthetic dyes, and polycyclic aromatic hydrocarbons (PAHs) are produced in large quantities due to industrial and agricultural development [4]. These harmful substances, along with pathogenic microorganisms, including bacteria, viruses, parasites, and protists, pose a significant threat to human health and the environment especially in the developing world where waterborne diseases caused by contaminated water mostly affect children under the age of five in underdeveloped countries, especially in Asia and Africa [5]. Water contamination is a pressing issue in developing regions, and it poses a significant threat to public health. Poor sanitation, food sources, and hygiene account for some 1.7 million deaths a year worldwide, with nine out of 10 of these deaths occurring in children [2,6,7]. unacceptable. Disease-causing This is organisms (pathogens) transmitted via drinking water are predominantly of faecal origin [8,9]. Bacterial contamination of drinking water is a major contributor to water-borne diseases in rural areas of most developing countries where water sources are communally shared and exposed to multiple faecal-oral transmission pathways in their neighbourhood boundaries [10-12]. The World Health Organization (WHO) estimates that diarrheal disease due to exposure to unsafe drinking water, inadequate sanitation and hygiene practices contribute to more than 25% of the reported global environmental burden of the disease [13].

In sub-Saharan Africa, with deteriorating environments attributed to high levels of open defecation, drinking water sources remain vulnerable to faecal contamination [14,15]. The issue of water quality is an increasingly pressing concern in developing nations. Drinking water sources are under threat from a range of natural and man-made factors, resulting in negative impacts on the health of individuals, families, communities, and the wider nation [7,13,16,17]. drinking water The contamination of is responsible for a significant number of cases of morbidity and mortality from waterborne diseases, including but not limited to typhoid. cholera, dysentery, and hepatitis, as well as various protozoal and helminthic infestations [13]. In Nigeria, for instance, the under-5 mortality rate due to diarrheal diseases is estimated to be as high as 13.5% [18]. While access to drinking water has improved considerably in recent years, safe drinking water remains inaccessible to approximately one billion people, and adequate sanitation is not available to more than 2.5 billion people worldwide [17,19,20]. In Nigeria, specifically, it is estimated that 41% of the population, or 160 million people, lack access to safe drinking water, with rural areas being more affected than urban areas [21]. Reports from the Water Supply and Sanitation Baseline Study (WSSBS) and the UNICEF/WHO Joint Monitoring Program (JMP) indicate that Nigeria has not met the Millennium Development Goal (MDG) target of 75% coverage for safe drinking water. This can be attributed to a range of challenges, including increased population urbanization. industrialization. density, inadequate and inequitable distribution of surface and groundwater supplies, and the global threat of climate change [22,23]. When potable water is not available in sufficient quantity and quality for household consumption, people are often compelled to use contaminated water from less hygienic sources, resulting in a range of waterborne diseases and outbreaks. This underscores the critical importance of ensuring access to clean and safe drinking water for all communities, both in Nigeria and around the world [2,17,24].

In Isu-uzo Local government of Enugu State, the Ebenyi River transverses some communities in Eha-Amufu which include Agumede, Amede, Ihenyi, Mgbuji and Umuhu and remains the major source of water supply to residents of the communities especially in the dry season [10]. It is sparingly hard water with earth colour. In the rainy season, it receives a lot of runoff containing dissolved and suspended materials from the catchment communities. The ground in Eha-Amufu is embedded with hard clav soil and rocks and is thus semipermeable to water to some extent. This explains why borehole water is nonexistent in the communities leaving hand-dug wells as the only alternative source of water to Ebenyi River in the communities. Most of the well also hard and unsuitable water is for consumption and laundry. Despite the state of Ebenyi River containing some pollutants, a large population of the communities drink from it, bathe and wash with it, thus exposing themselves to the risk of contracting diseases [10]. The open defecation practised by some people in the communities, the deliberate dumping of waste by the people into the river and the runoffs that load the river with pollutants in the rainy seasons may continue to contaminate the river with various organic and inorganic substances containing pathogenic organisms capable of constituting health risks to the people who make use of the river for drinking and other domestic purposes [13,20]. The inhabitants of the communities engage in agricultural and agro-allied activities and some of the people cultivate close to the river and make use of an off-season irrigation system to grow the crops in the dry season. They make a significant contribution in loading the river with pollutants such as fertilizers or manure, pesticides and herbicides during the dry season [5.6,25.26]. For now, possibly out of ignorance or a carefree attitude, the inhabitants of the communities feel unconcerned about the level of pollution that is taking place in the river. The nonchalant attitude is a cause for concern as the continuous consumption and domestic use of the river water may expose people to the risk of contracting some waterborne diseases [27,28].

There is a dearth of information on Ebenyi River bacterial contaminants and their antibiotic resistance hence, this study investigated the distribution of bacteria contaminants of the Ebenyi River and their antibiotic resistance profile. Specifically, the study ascertained the bacteria distribution at different locations along the Ebenyi River and determined the antibiotic resistance profile of the isolated bacteria in the Ebenyi River of Eha-Amufu in the Isi-Uzo Local Government Area, Enugu State, Nigeria.

#### 2. MATERIALS AND METHODS

#### 2.1 Study Area

The study was carried out at Eha-Amufu in the Isi-Uzo Local Government Area, Enugu State,

Nigeria. The location coordinates for Eha-Amufu are latitude 6° 39' 32.94" N and longitude 7° 45' 34.60" E. The elevation of the area is 109 meters above sea level and it is traversed by the Ebenvi River (ER) and its tributaries. The residents of Eha-Amufu primarily rural farmers. are fishermen, artisans and traders who belong to the Igbo speaking tribe. The area experiences two distinct seasons: the dry season from November to April or May, and the rainy season from May to October. The average temperature Eha-Amufu is 27.0°C and in average precipitation is 1669 mm. The sunrise occurs at 06:17:35 and sunset at 18:08:08 during daylight hours.

#### 2.2 Water Sample Collection

With 200 ml sterilized borosilicate glass bottles, water samples were collected from three different points along each of the five communities that make up Eha-Amufu Town through which River Ebenyi passes. The communities include Isu, Ihenyi, Amede, Mgbuji, and Umuhu. At each community, samples were collected randomly from three different spaced spots. Collection of water samples was done for a period of three months. The water samples were labeled and transported in a pack of ice to the laboratory for analysis within 6 hours of collection. The ice helped to slow down the multiplication of microorganisms in the water samples.

#### 2.3 Bacteriological Analyses: Bacterial isolation, Identification and Enumeration

Bacteria isolation and characterization were conducted using a selection of culture media, including MacConkey agar, Simon's citrate agar and well as nutrient agar, kligler iron agar, peptone water and urea medium. In the laboratory, each water sample, in a volume of 10 µl, was aseptically transferred onto individual MacConkey agar plates. These plates were then uniformly spread using a sterile glass spreader. To ensure comprehensive analysis, each water sample was duplicated. The incubation of the MacConkey agar plates took place at a temperature of 37°C for a period of 24 hours. Then, a meticulous examination of the plates was performed, and the isolated bacteria species were enumerated based on their colonial morphology, coloration, and texture. Diverse colonies were subjected to sub-culturing to achieve the isolation of distinct colonies.

Distinct colonies obtained from the agar were further investigated to determine their capacity to ferment lactose. Colonies displaying non-lactose fermenting characteristics were further subjected to additional inoculation onto deoxycholate citrate agar (DCA) and were incubated overnight at 37°C. To facilitate detailed identification, a representative colony from each unique colony type undergo Gram staining and was subjected to a battery of biochemical tests, adhering to the guidelines outlined in the WHO Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World (WHO/CDS/CSR/RMD/ 2003.6).

#### 2.4 Viable Cell Count

The number of colonies was determined by converting the number of colonies per 10  $\mu$ l to colony forming unit per 1000  $\mu$ l (CFU/ml).

#### 2.5 Antibiotic Sensitivity Testing

Each of the bacterial isolates was subjected to antibiotic susceptibility testing using the Kirby-Bauer method as modified by the Clinical and Laboratory Standards Institute (CLSI) [29]. Isolates grown overnight on nutrient agar were suspended in sterile normal saline (0.9% w/v NaCl) using a sterile wire loop until the turbidity was equal to 0.5 McFarland standards. Sterile non-toxic cotton swabs dipped into the standardized inoculum were used to streak the entire surface of Mueller-Hinton agar plates. Gram-positive bacteria were tested against antibiotics such as: ampicillin (10  $\mu$ g), cloxacillin (10  $\mu$ g), erythromycin (15  $\mu$ g), tetracycline (30  $\mu$ g), cotrimoxazole (25  $\mu$ g), cefuroxime (30  $\mu$ g), penicillin gentamicin (10 μg), (10 IU), ciprofloxacin  $(5 \mu g)$ , augmentin (30 μg), vancomycin (30  $\mu$ g), and meropenem (25  $\mu$ g). Gram-negative bacteria were tested against antibiotics such as ampicillin (10  $\mu$ g), tetracycline (30  $\mu$ g), cotrimoxazole (25  $\mu$ g), cefuroxime (30  $\mu$ g), chloramphenicol (30  $\mu$ g), ceftriaxone (25  $\mu$ q), cefotaxime (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), amikacin (30  $\mu$ g), vancomycin (30  $\mu$ g), and meropenem (25  $\mu$ g). Antibiotic disks were aseptically placed using sterile forceps, and all plates were incubated at 37°C for 24 hrs. The results were interpreted using CLSI [29] The susceptibility testing was repeated for each isolate to ensure that the results obtained were consistent.

The classes of antibiotics used in this study and the codes that represent them are as follows; Sulfamethoxazole-trimethoprim (SXT), Cephalosporins (CTX, CRO, CEP, CT, CXM), Penicillins (TZP), Tetracyclines (TE), Aminoglycosides (AMC) and Carbapenems (IPN).

#### 3. RESULTS

#### 3.1 Bacteria Distribution at Five Different Sample Locations

Table 1 shows the distribution of bacteria isolates among the five different sampled locations of the river. A total of 59 species of bacteria were isolated and identified from 120 water samples collected from 5 different river locations. Sampling was performed at 3 different points per location for 3 months. Out of the 59 species identified, 25 (42.4%) were Escherichia coli, 12 (20.3%) were Bacillus sp, 12 (20.3%) were Klebsiella sp, and 10 (16.9%) were Staphylococcus sp. At location 1 (Isu), 15 (25.4%) species of bacteria were isolated, of which 7 (46.7%) were Escherichia coli, 4 (26.7%) were Klebsiella sp. 2 (13.3%) were Bacillus sp. and 2 (13.3%) were Staphylococcus sp. At location 2 (Ihenye), 13 (22.0%) species of bacteria were isolated, of which 5 (38.5%) were Escherichia coli, 3 (23.1%) were Klebsiella sp, 2 (15.4%) were Bacillus sp, and 3 (23.1%) were Staphylococcus sp. At location 3 (Amede), 12 (20.3%) species of bacteria were isolated, of which 4 (33.3%) were Escherichia coli, 3 (25%) were Klebsiella sp, 3 (25%) were Bacillus sp, and 2 (16.7%) were Staphylococcus sp. At location 4 (Mgbuji), 10 (16.9%) species of bacteria were isolated, of which 5 (50%) were Escherichia coli, 1 (10%) was Klebsiella sp, 3 (30%) were Bacillus sp, and 1 (10%) was Staphylococcus sp. At location 5 (Umuhu), 9 (15.3%) species of bacteria were isolated, of which 3 (33.3%) were Escherichia coli, 1 (11.1%) was Klebsiella sp, 2 (22.2%) were Bacillus sp. and 2 (22.2%) were Staphylococcus sp.

## 3.2 The Antibiotic Resistance Profile of the Isolated Bacteria

Table 2 shows the bacterial resistance of various isolates. Out of the 13 bacterial isolates tested, 4 were found to be resistant to sulfamethoxazole-trimethoprim (SXT) in Isu, 3 in Ihenyi, 3 in Amede, 2 in Mgbuji, and 1 in Umuhu. Similarly, 8 isolates were discovered to be resistant to penicillin (TZP), with 3 from Isu, 2 from Ihenyi,

| Location | E. coli   | Klebsiella sp | Bacillus sp | Staphylo. Sp | Total     |
|----------|-----------|---------------|-------------|--------------|-----------|
| lsu      | 7(46.7%)  | 4(26.7%)      | 2(13.3%)    | 2(13.3%)     | 15(25.4%) |
| lhenyi   | 5(38.5%)  | 3(23.1%)      | 2(15.4%)    | 3(23.1%)     | 13(22.0%) |
| Amede    | 4(33.3%)  | 3(25%)        | 3(25%)      | 2(16.7%)     | 12(20.3%) |
| Mgbuji   | 5(50%)    | 1(10%)        | 3(30%)      | 1(10%)       | 10(16.9%) |
| Umuhu    | 3(33.3%)  | 1(11.1%)      | 2(22.2%)    | 2(22.2%)     | 9(15.3%)  |
| Total    | 25(42.4%) | 12(20.3%)     | 12(20.3%)   | 10(16.9%)    | 59(99.9%) |

Table 1. Distribution of the bacteria isolates in the five different locations along River Ebenyi

| Table 2. Distribution of resistance to antibiotic agents among isolates from the 5 different |
|--|
| locations  |

|       | lsu | lhenyi | Amede | Mgbuji | Umuhu | Total |
|-------|-----|--------|-------|--------|-------|-------|
| SXT   | 4   | 3      | 3     | 2      | 1     | 13    |
| TZP   | 3   | 2      | 1     | 1      | 1     | 8     |
| CTX   | 1   | 0      | 1     | 1      | 1     | 4     |
| TE    | 2   | 2      | 2     | 1      | 0     | 7     |
| CRO   | 1   | 1      | 1     | 1      | 1     | 5     |
| IPN   | 1   | 1      | 1     | 1      | 2     | 6     |
| CEP   | 0   | 1      | 0     | 0      | 1     | 2     |
| CT    | 1   | 1      | 1     | 1      | 0     | 4     |
| AMC   | 1   | 1      | 1     | 1      | 1     | 5     |
| CXM   | 1   | 1      | 1     | 1      | 1     | 5     |
| Total | 15  | 13     | 12    | 10     | 9     | 59    |

and 1 each from Amede, Mgbuji and Umuhu. The findings also revealed that 4 isolates were resistant to the CTX brand of cephalosporin, and 7 isolates were resistant to tetracycline (TE). Worse still, 5 isolates were resistant to the CRO brand of cephalosporins, 6 were resistant to carbapenem (IPN), and 2 were resistant to CEP brand of cephalosporins. Furthermore, 4 isolates were found to be resistant to CT brand of cephalosporins, and 5 isolates were resistant to both aminoglycosides (AMC) and CXM brand of cephalosporins.

#### 4. DISCUSSION

Findings on the distribution of isolated bacteria along the five different river locations where water samples were collected revealed that the most prevalent isolated bacteria species in the water samples was Escherichia coli (42.4%) of all the isolates. This observation collaborates with previous reports [7,11,25,30] who in their various studies indicated that E. coli was the most important microbial contaminant of rivers and other water bodies. Other important microbes observed such as Bacillus species, Klebsiella and Staphylococcus species were less (20.3%, 20.3%) in occurrence and 16.9% their respectively) though prevalence underscored the importance of water sanitation and water treatment before consumption [2,17,20]. The current study further showed that the distribution of isolates varied among the different river locations in Eha-Amufu Town and suggests that bacterial contamination is widespread with uneven distribution along River Ebenvi River system. This observation could be attributed to the fact that the communities that are located very close to the river carry out more polluting activities within and around the river including washing clothes in the river, dry season farming along the riverbank, direct defecation into the river or by the riverbank [31,32]. Runoff from rain washes most of the waste generated in these communities into the river. More pollution and contamination were noticed along the river for communities living very close to the river than the communities living a little afar [10].

It was also found that some bacteria were resistant to different antibiotic agents at different locations along River Ebenyi. In some cases, some of the bacteria species showed multiple resistance to antibiotic agents which has some important health implications for the inhabitants living in the study area. The current finding from this study is in line with the findings of [30] who reported that the resistant abilities of bacteria species could be explained by the fact that some chemical substances probably from agro-allied sources such as fertilizers, pesticides, and herbicides as well as other sources find their way into the river [33]. Encounter of these bacteria species with some of these chemicals and the ability to survive in the presence of these chemicals might have contributed to conferring resistance ability to the bacteria species [34]. These results indicate a worrying trend of bacterial resistance to antibiotics in Isu, Ihenyi, Amede, Mgbuji, and Umuhu, which can potentially lead to severe health consequences for the people in these locations.

#### 5. CONCLUSION

The current study involved the analysis of water samples collected from different locations along River Ebenvi. The findings of the research indicated the presence of a significant number of bacteria species in the water samples, with Escherichia coli being the predominant species. In addition to this, several other bacterial species were found in the river with varving concentrations, including Klebsiella species. Bacillus species, and Staphylococcus species. One of the most concerning observations from this study is the resistance shown by some of the bacteria species to the antibiotic agents they were exposed to. It was also found that some of the bacteria species demonstrated multiple resistance to the antibacterial agents. This highlights the need for further research and a more proactive approach to addressing the issue of antibiotic resistance in the environment.

#### 6. RECOMMENDATION

The government must take immediate action to provide alternative sources of clean water to the community to ensure their health and well-being. We must prioritize the health of the people of Eha-Amufu and provide them with access to clean and safe water. Non-governmental organizations and affluent individuals can also play a significant role in supporting the community by providing alternative sources of clean water.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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