Isolation and Characterization of Multi-drug Resistant Bacterial Species from Selected Water Sources in Izzi Area, Southeastern Nigeria

S.C. Onuoha

Abstract: This study assessed the bacteriological qualities of some selected surface waters in Izzi, between April and September 2016. Surface water samples were collected from four streams for bacteriological analysis. Bacteria species were identified using standard microbiological and biochemical techniques. Antibiotic susceptibility study was carried out using Kirby Bauer disc diffusion method. The result of the Mean heterotrophic bacteria count from the streams showed that Ngele-Amachi stream had 218 CFU/100 mL counts, Ebonyi River 282 CFU/100mL, Igbeagu stream 173 CFU/100mL and Ndi-Udara stream had 180 CFU/100mL. Out of the eighteen (18) isolates obtained, E. coli had the highest percentage occurrence (27.8%), S. aureus (22.2%), Klebsiella and Salmonella sp (16.7%), Shigella spp (11.1%), while Streptococcus spp had the least percentage occurrence (5.6%). The antibiotic susceptibility studies showed that large proportion of isolates were resistance to tetracycline, oxytetracycline, nalidixic acid, cephalothin and sulphonamides. The most effective antibiotic was azithromycin followed by imipenem. The presence of these multi-drug resistant strains in water samples could act as a vehicle in transferring antibiotic resistance. This emphasizes the need for proper treatment of water in the study area.

Key words: Antibiotic Resistance • Bacterial species • Izzi • Surface Water

INTRODUCTION

Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, meet the standard guidelines for taste, odour, appearance and chemical concentrations and must be available in adequate quantities for domestic purposes [1]. However, inadequate sanitation and persistent faecal contamination of water sources is responsible for a large percentage of people in both developed and developing countries not having access to microbiologically safe drinking water and suffering from diarrhoeal diseases [1]. Diarrhoeal diseases are responsible for approximately 2.5 million deaths annually in developing countries, affecting children younger than five years, especially those in areas devoid of access to potable water supply and sanitation [2, 3].

Diarrhea is one of the major causes of mortality in children under five years old in developing countries [4]. Many cases are not diagnosed, either because they are mild and self-limiting, in which the patient does not seek medical attention, or because, especially in developing countries, the medical and laboratory resources are not available [5]. Despite the various available microbiological techniques, about half of the cases of diarrhea have no defined etiology, which complicates the implementation of policies and strategies for mapping and monitoring endemic areas of the occurrence of such pathogens [6].

The development and spread of antibiotic resistance among bacteria is considered a universal threat to human, animal and environmental health. Numerous studies have demonstrated the importance of the environmental settings (e.g. water or soil) on the cycling of antibiotic resistance in nature, either because antibiotic resistance mechanisms can originate in environmental bacteria or because human and animal commensals and pathogen can contaminate the environment [7, 8]. As a microbial habitat, water may represent the origin of resistance genes, be an amplifier and/or reservoir of genes already acquired by human
pathogens and released as pollutants in the environment or act as a bioreactor, facilitating the interchange of resistance genes between pathogenic and non-pathogenic bacteria [9].

In view of the fact that there has been acute shortage of portable water in Izzi local government area of Ebonyi State, this work was aimed at sampling some surface water being used by the population for drinking and cooking purposes.

**MATERIALS AND METHODS**

**Description of Study Area:** Izzi is one of the Local Governments Area in Ebonyi State. There are several streams that are located within the area, with Ebonyi river running through the city and flow past the outskirts and adjoining villages. The supply of municipal water is completely lacking in the area and its adjoining towns and villages. Most of the inhabitants therefore resort to the use of hand dug wells and streams as sources of water for drinking and other domestic purposes. Probability of contamination of the streams is high especially during the rainy seasons, from wastewater contaminations from urban and rural run offs and agricultural activities as the streams flow through the city and its suburb.

**Sample Collection and Procession:** Water samples were collected from four (4) different sources namely: Ngele-Amachi Stream, Ebonyi River, Igbeagu Pond and Ndi-Udara Pond. The water was collected in the morning (Between 8 - 11:59 am) in a sterile container which was drawn directly from the water. The samples were labeled properly and transported on ice to the laboratory for analysis. Aliquots of the samples were used for selective isolation of targeted bacteria based on standard microbiological procedures [10].

**Isolation, Purification and Characterization of Planktonic Bacteria:** For all samples, three volumes of 100 mL were filtered through 0.45 @m pore sized filter (Whatman Laboratory Division, Maidstone, England) using a water pump (Model Sartorius 16824). The membrane was then placed on the plate containing nutrient media ensuring that no air bubble were trapped the plate were incubated at 37°C for 24 hours to observe growth, after which the numbers of colony was counted using colony counter chamber machine and recorded. The colonies were then sub-cultured into appropriate selective media such as Salmonella-Shigella Agar (SSA) for the isolation of *Salmonella* and *Shigella* spp., Mannitol Salt Agar (MSA) for the isolation of Staphylococci, Eosin Methylene Blue (EMB) and MacConKey Agar (MA) for the isolation of *E. coli* and other Enterobacteriaceae. The agar plates were incubated at 37°C for 18-24 hours. All the bacterial isolates were identified based on their colony morphology, Gram’s reaction, catalase test, motility test and biochemical tests such as oxidase test, citrate test, indole test, Methylred-VogesProskauer (MRVP) test, urease test, hydrogen sulphide production test and sugar fermentation.

**Disc Diffusion Susceptibility Test:** Bacteria isolates were subjected to in-vitro susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute [11]. In brief, by taking pure isolated colony, bacterial suspension was adjusted to 0.5McFarland turbidity standards. The diluted bacterial suspension was then transferred to Mueller-Hinton agar plate using a sterile cotton swab and the plate was seeded uniformly by rubbing the swab against the entire agar surface and plates were left on room temperature for 30 min. Then antibiotic disks were applied to the surface of the inoculated plates using sterile forceps. The plates were then incubated aerobically at 37 °C for 24 h. Finally, the zone of inhibition was measured including the disk diameter. The susceptible and resistant categories were assigned on the basis of the critical points recommended by the CLSI and according to the manufacturer’s leaflet attached to them. The standard antibiotic discs (Oxoid, England) and their concentrations used against the isolates were streptomycin (10 µg), gentamicin (30µg), tetracycline (20µg), azithromycin (15 µg), erythromycin (10 µg), penicillin G (10unit), imipenem (10µg), oxytetracycline (30µg), cefotaxime (30 µg), cephalothin (30µg), nalidixic acid (30 µg), sulphonamides (300 µg), norfloxacin (10µg), cefuroxime sodium (30 µg) and cefpirome (30 µg). These antibiotics were chosen because they are either used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them.

**Multiple Antibiotic Resistance Index (MARI):** MARI values of isolated bacteria against the antibiotics used were computed. MARI is a tool that helps in analyzing health risks and checking antibiotic resistance in a given area. The value of MARI is 0.20 and it differentiates the low risk (<0.20) from the high risk (>0.20). It is calculated by dividing the aggregate resistance of total isolates of an organism to all antibiotics by the product of the total number of antibiotics used and the number of isolates of an organism from the sample site. i.e. $x/(y.z)$, where $x$
represents the aggregate resistance of total isolates of an organism to all antibiotics, y represents the total number of antibiotics used and z represents the number of isolates of an organism from the sample site. This formula was used since the MARI was being calculated from a sample site (Environmental sampling) where many isolates were obtained according to the method of Riaz et al. [12].

RESULTS

Total Bacterial Count: The results of the mean heterotrophic count of the bacteria isolated from the different sources are presented in Table 1. The mean values of the colony count for each source were computed and presented. From the results, the number of colonies per 100 mL for the four (4) water sources ranged from 173-282 CFU/100 mL. The highest number of colonies was seen in the water obtained from the Ebonyi river (282CFU/100 mL) followed by Ngele Amachi stream (218 CFU/100mL) and the least was from Igbeagu pond (162CFU/100 mL).

Prevalence of Bacterial Species: A total of six (6) bacterial species were isolated and identified. They included Escherichia coli, Salmonella spp., Staphylococcus aureus, Shigella spp., Klebsiella spp, and Streptococcus spp. The frequency of each organism isolated varied between the different waters as shown in Table 2. E. coli and Staphylococcus spp. had the highest occurrence of 27.8 and 22.2 % respectively from the water samples. It was followed by Klebsiella spp and Salmonella spp with prevalence of 16.7% each, while the least prevalence of 5.6% was observed among Streptococcus sp. (Table 2).

Antibiotic Resistance/Susceptibility Patterns of Bacterial Isolates: The results revealed that large proportions of isolates were resistant to tetracycline, oxytetracycline, nalidixic acid, cephalothin and sulphonamides. For Gram negative isolates, E. coli was completely resistant to penicillin, erythromycin, tetracycline, oxytetracycline and nalidixic acid while, Klebsiella spp was completely resistant to eight of the antibiotics: nalidixic acid, imipenem, penicillin, streptomycin, cefotaxime, cefuroxime sodium, sulphonamides and cepirome. In general multiple antibiotic resistances were found to be more prominent among Salmonella spp, Streptococcus spp, Staphylococcus spp, Shigella spp. and Klebsiella spp. (Table 3).

Multiple antibiotic resistance indices (MARI): The result from the MARI studies showed that all the organisms had MARI values >0.20, with the highest value exhibited by Streptococcus spp. (0.81), followed by S. aureus, Klebsiella, Shigella and Salmonella sp. (0.50) respectively. The least MARI value was seen in E. coli (0.31) (Table 4).

Table 1: Total viable count (CFU/mL) of bacterial isolates

<table>
<thead>
<tr>
<th>S/N</th>
<th>Sample point/location</th>
<th>colony count (CFU/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NgeleAmachi Stream</td>
<td>218</td>
</tr>
<tr>
<td>2</td>
<td>Ebonyi River</td>
<td>282</td>
</tr>
<tr>
<td>3</td>
<td>Igbeagu Pond</td>
<td>173</td>
</tr>
<tr>
<td>4</td>
<td>Ndi-udara pond</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>213.30</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of Bacterial isolates from four water sources in Izzi

<table>
<thead>
<tr>
<th>S/N</th>
<th>Isolates identified</th>
<th>Igbeagu</th>
<th>Ebonyi</th>
<th>Ndiudara</th>
<th>NgeleTotal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli</td>
<td>2(40%)</td>
<td>1(20%)</td>
<td>2(40%)</td>
<td>0(0)</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella spp.</td>
<td>1(33.3%)</td>
<td>0(0%)</td>
<td>1(33.3%)</td>
<td>1(33.3%)</td>
</tr>
<tr>
<td>3</td>
<td>S. aureus</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
</tr>
<tr>
<td>4</td>
<td>Shigella spp.</td>
<td>1(50%)</td>
<td>0(0%)</td>
<td>1(50%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>5</td>
<td>Klebsiella spp.</td>
<td>1(33.3%)</td>
<td>1(33.3%)</td>
<td>0(0%)</td>
<td>1(33.3%)</td>
</tr>
<tr>
<td>6</td>
<td>Streptococcus spp.</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>1(100%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6(33.3%)</td>
<td>3(16.7%)</td>
<td>6(33.3%)</td>
<td>3(16.7%)</td>
</tr>
</tbody>
</table>
**DISCUSSION**

Safe drinking water is a fundamental right and if contaminated with opportunistic pathogenic environmental bacteria, it may have health implication. In rural communities, untreated surface water from rivers, dams and stream is directly used for drinking and other domestic purposes. In view of the prevailing problem of portable water in Izzi area of Ebonyi State, the study was investigated to sample some of the surface water used by the population for domestic and drinking purposes. The sampled waters contained some bacteria of public health importance such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Shigella* spp., *Klebsiella* spp and *Streptococcus* spp etc., the isolated strains were multidrug resistant towards some common antibiotics in use.

The bacteriological count of the water samples ranged from 173 – 282CFU/100 mL (Table 1). These values indicate very high microbial load and can be attributed to the poor sanitary and hygienic environment of the waters. The high counts obtained underline the unsuitability of these water sources for consumption purposes. Also none of the sources met the European Community and USEPA standards of maximum 100 HPC/ml of drinking and recreational waters. High bacteria counts obtained could be attributed to faecal contamination by domestic sewage consistently disposed into the streams by villagers living along the streams. Located along the streams were horticultural gardens; the runoffs of which flow directly into the stream. The runoffs contained high level organic pollutants from the fertilizer which is locally produced from cow dung. As the inhabitants of several communities operate free range system in rearing domestic animals which roam the communities daily in search of food and water, there is a high probability of faecal coliform bacteria contamination [13]. The animals thereby indiscriminately contaminate the stream environments with their droppings.

The study demonstrated the occurrence of various species of bacteria with most of them belonging to the family Enterobacteriaceae. The isolated strains were *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Shigella* spp., *Klebsiella* spp and *Streptococcus* spp. (Table 2). The presence of faecal coliform like *E. coli* and *Klebsiella* spp in some samples is an indication of recent pollution by sewage. The high coliforms level recorded in this work could be attributed to poor refuse and sewage disposal system, mainly open air disposal method practiced in this area. These organisms may harbor potential pathogens and the presence of pathogenic organisms that can pose severe health risks to consumers in general and immunocompromised individuals in particular [14]. Majority of the pathogens have been indicated in previous studies [15-17].

The result of the antibiotic study revealed the presence of multi-drug resistance among the isolated strains. Large proportions of isolates were resistant to tetracycline, oxytetracycline, nalidixic acid, cephalothin and sulphonamides. While a few of the isolates were susceptible to azithromycin, cefpirome, imipenem and gentamicin (Table 3). The prevalence of intrinsic multi-resistance common antimicrobial agents has been documented [18-22]. Many faecal bacteria isolates in this study showed resistance patterns indicative of acquired resistance, such as multiple resistances to antibiotics in different groups and resistance patterns indicative of extended spectrum beta lactamase production. Resistance to multiple antibiotics can lead to occurrence of newly emerging resistant bacteria which may be transmitted to consumers causing infections that are difficult to treat. The relatively high resistance of bacterial pathogens to antibiotics in this study agrees with the findings of Rakic-Martinez *et al.* [23] who reported the prevalence of MAR bacteria in...
wastewater. The observed high frequency of bacterial resistance may not only result in the therapeutic failure in the river fauna population, but also endanger the health of the people who make use of the water coupled with the possibility of plasmid transfer of resistance to human pathogenic bacteria [24].

The result of the multiple antibiotic resistance index showed that *Escherichia coli* had the least resistance index (0.31) which could be the result of efficacy of tested antibiotics against them.

**CONCLUSIONS**

This research study has investigated the bacterial profile and antimicrobial resistance pattern of bacteria isolated from selected surface waters in Izzi. The study demonstrated the occurrence of various bacteria species such as *E. coli*, *Shigellasp*, *Salmonellasp*, *Klebsiellasp*, *Streptococcus* spp and *Staphylococcus* spp which was an indication that the waters were contaminated with pathogenic bacteria. Antibiotic resistance in bacteria is a serious problem facing society today and one of the reasons responsible for this problem is overuse/misuse of antibiotics in humans, in view of the consequences associated with it, there should be a proper programme to monitor antimicrobial usage and resistance in bacteria from surface and drinking waters sources in the study area.

**REFERENCES**