Bacteriological Quality and Antibiogram of Isolates Obtained from Creek Town River, Odukpani L.G.A., Cross River State, Nigeria

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Authors’ contributions

This work was carried out in collaboration between all authors. Author FCA designed the study, wrote the protocol and the final draft of the manuscript. Author JUO managed the laboratory analysis, wrote the first draft of the manuscript and carried out statistical analysis. Author RN managed the sample collection, preparation and literature searches. All the authors read and approved the final manuscript.

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ABSTRACT

The bacteriological quality and antibiogram of isolates from Creek Town River were investigated to determine the quality of the river water and sensitivity patterns of the bacterial isolates. The bacteriological assessment was studied using pour plate and membrane filtration techniques. There was significant difference (\(P=.05\)) in total heterotrophic bacterial count, \(E.\ coli\) and coliform counts. The total heterotrophic bacterial and total coliform counts were shown to be highest in the CTR3 (8.0\(x10^6\)cfu/ml) and CTR2 (3.0\(x10^5\)cfu/ml). The total \(E.\ coli\) counts ranged from 1.5\(x10^2\)cfu/ml to 5.8\(x10^2\)cfu/ml. Bacterial counts were higher than the acceptable limit of the WHO standards. The bacteria isolated and characterized included eleven (11) bacterial genera: Escherichia coli, Klebsiella spp., Pseudomonas spp., Bacillus spp., Enterobacter faecalis, Streptococcus spp.,...
Salmonella spp., Staphylococcus aureus, Proteus spp., Shigella spp. and Citrobacter spp. Out of these organisms, *Ent. faecalis, Staph. aureus, Klebsiella spp.*, *E. coli* and *Proteus* spp. are the most prevalent (100%) in the samples. The antibiogram result indicated that there was significant statistical variation (*P*=.05) in the sensitivity profile of the test organisms to antibiotics. Moreover, *E. coli* and *Proteus* spp. had the highest sensitivity of 90% while *S. aureus* had the lowest sensitivity of 30%. Result also revealed that chloramphenicol was the most effective antibiotic with 100% sensitivity, and there was significant difference (*P*=.05) in the effective of each antibiotic against test organisms. These findings indicated that the water from Creek Town River was not suitable for direct human consumption and it poses a serious threat to the health of the consumers. Water treatment is therefore recommended before it can be used for domestic purposes.

Keywords: Bacteriological quality; total heterotrophic bacteria; total *E. coli*; total coliform; creek town river; antibiotics; antibiogram.

1. INTRODUCTION

Water is a vital resource and a life support of all living organisms. Though, it occupies about 70% of the earth’s surface, yet a greater percentage of the world’s population, most especially in developing countries, live without access to safe water [1-3]. This is due to lack of infrastructure for the treatment of water and its eventual distribution for the populace. The multifarious uses of water for drinking, bathing, washing and cooking are well known. Water meant for human consumption should be free from pollution, safe and acceptable. The bacterial quality of water sources should not exceed the maximum limits specified in the water quality guidelines [4-6].

In Nigeria, lack of efficient water supply facilities has led to the prospecting of river water by individuals especially in rural areas for the provision of drinking water. The microbiological quality of river water sources in rural communities in the Cross River State, Nigeria has been reported to be poor, unsafe and not acceptable for human consumption [7,8]. Several bacterial enteropathogens namely *Campylobacter jejuni, Salmonella, Shigella, Plesiomonas, Aeromonas, Vibrio cholera* and *E. coli* were reported to have been isolated from the river water sources [9]. These enteric bacterial pathogens are variously incriminated in cases of diarrhoea, which accounts for a substantial degree of morbidity and mortality in different age groups worldwide [10]. Because of the easy contamination of river water and reports on alarming prevalence of various disease-causing microbes in this water source, there seems to be increased search for underground water by rural dwellers [11,9]. Domestic, industrial and agriculture waste should not be disposed of without treating [12].

Water pollution is associated with its consequent health problems. Heavy rainfall and floods are related to extreme weather and creating different diseases for developed and developing countries [13]. Some pathogens are worldwide, some are found in well-defined area [14]. Most water borne diseases spreads from man to man [15]. Fecal pollution of water sources is the cause of many waterborne diseases and results in fecal-oral route of infection [16]. Health risk associated with polluted water includes different diseases such as respiratory disease, cancer, diarrhoeal disease, neurological disorder and cardiovascular disease [17]. Contaminated water has large negative effects in those women who are exposed to chemicals during pregnancy; it leads to the increased rate of low birth weight as a result fetal health is affected [18]. Nitrogenous chemicals are responsible for cancer and blue baby syndrome [19]. Mortality rate due to cancer is higher in rural areas than urban areas because urban inhabitants use treated water for drinking while rural inhabitants don’t have facility of treated water and drink unprocessed water. Poor people are more vulnerable to disease due to improper sanitation, hygiene and water supply [20]. Poor quality water affects and impairs crop production and infects our food which is hazardous for aquatic life and human life [21]. Pollutants distort the food chain [15] and heavy metals, especially iron affects the respiratory system of fishes. Metal contaminated water leads to hair loss, liver cirrhosis, and renal failure [22]. Water pollutants are harmful to sea weeds, mollusks, marine birds, fishes, crustaceans and other sea organisms that serve as food for humans. Insecticides like DDT were also found to be harmful for humans [23].

Isolation of pathogens from water sources connotes a serious public health risk for consumers. To further compound this problem,
enteric bacterial pathogens have been widely reported to demonstrate resistance to several antibiotics [24,2]. For example, in 1984, 82% of *Campylobacter* strains from Lagos, Nigeria, were sensitive to erythromycin, and 10 years later only 20.8% were sensitive [25]. Strains of *S. typhi* with multiple resistances to chloramphenicol, ampicillin and trimethoprim have led to several outbreaks [26]. Antibiotics shorten the duration of diarrhoea, decrease stool output and may mitigate complications [27].

In spite of the poor water quality in rural regions of Cross River State, there is paucity of data. Such data, if available in appreciable quantity will be useful in the empiric management of patients with diarrhoea in the regions because antibiograms vary with time and geographical region [3,28]. Since most of the water obtained from these sources is seldom treated because of the lack of awareness and erroneous perception that they are generally safe to drink, this study was therefore, aimed at evaluating the bacteriological attributes of water samples of Creek Town River in Odukpani L.G.A, Cross River State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study site is a community known as Creek Town, located in Odukpani L.G.A., Cross River State. The occupation of most dwellers of this community is predominantly fishing and farming. The town is located at an elevation of 99 meters above sea level with a population of 171,672 people. Its coordinates are 4° 48’ 60”N and 8° 16’ 60”E in DMS (degrees minutes seconds) or 4.9833 and 8.28333 (in decimal degrees). It was observed during sample collection that faeces are discharged into the Creek Town River by the rural dwellers. The river is used for bathing and washing of clothes, and sometimes drank by children during recreational activities. The river is surrounded by plant vegetation and decaying organic matters.

2.2 Sample Collection

Water samples were collected from Creek Town River (CTR), Odukpani Local Government Area. For the purpose of clarity and reference, the selected samples were coded CTR1, CTR2, CTR3, CTR4, CTR5 and CTR6. A total of six (6) samples (3 samples in the morning, 7.30 am, and 3 samples in the evening, 6.30 pm) were randomly and aseptically collected at different points of the river. The sampling points were between upstream and downstream with a distance of 50 metres apart. It was observed that anthropogenic activities are usually high in the morning and in the evening. That is the period when farmers and other dwellers (especially school children) visit the river for various activities. In the afternoon, human traffic to the river is usually low because farmers will be in their farmlands and school children will be in school leaving a negligible number of inhabitants who may be petty traders and those who work in the Local Government Council. In addition, during the afternoon, the water undergoes flocculation and filtration since it was flowing with a mild current. These indices informed the need to collect samples in the evening and morning periods. The water samples were collected in 1000 ml sterile plastic bottles and transported on ice to the Microbiology Laboratory, Cross River University of Technology, Calabar for microbiological analysis. The samples were analyzed within 6 hours after collection.

2.3 Microbiological Analysis of Samples

2.3.1 Enumeration of Total Heterotrophic Bacterial (THB) counts, Total *E. coli* and coliform counts

Total heterotrophic bacterial count was determined using serial dilution and pour plate method as described by Cheesbrough [29]. Ten (10) fold serial dilutions of the samples were carried out and 1 ml from 10^-4^-10^-6 dilutions was dispensed into sterile Petri dishes containing molten Nutrient agar in triplicates. The plates were swirled to spread the inoculum evenly within the agar medium. The plates were incubated for 24 hours at 37°C temperature. Thereafter, the isolates are counted and recorded in colony forming units. One (1) milliliter of the dilutions was also cultured on McConkey agar (MCA) for determination of Total *E. coli* count (bright pink colonies). The plates were incubated at 37°C for 24 hours before enumeration. Membrane Filtration (MF) Technique was employed in the evaluation of total coliform count. Sterilized forceps were used to transfer sterilized membrane filter (47 mm diameter, with 0.45 µm pore size: Merck Millipore, USA) onto the porous plate of the membrane filtration unit with the grid side up and a sterile meshed funnel placed over the receptacle and locked in place. One hundred (100 ml) milliliter of the sample water were added.
to the membrane filtration unit using the funnel measure. After filtration, the filtrates were discarded and the funnel unlocked and removed. In each case, sterile forceps were then used to transfer the membrane filter and then rolled over the surface of a Petri dish containing Eosin Methylen blue agar (EMB) carefully making sure that air bubbles were not trapped between the filters and the medium. The mean total coliforms (blue-green colonies) were determined after incubation for 24 hours at 37°C. Aseptic standards were maintained throughout the experiment.

2.3.2 Isolation and identification of bacteria

The colonies were isolated and characterized using standard bacteriological procedures [29]. For the isolation of Salmonella and Shigella species, 1 ml water sample and 10⁻⁶ serial dilution were inoculated in 9 ml selenite-F-broth and incubated for 18-24 hrs at 37°C for enrichment. The enriched samples were plated on Salmonella-Shigella Agar (Oxoid) and incubated for 48 hrs at 37°C. Small colourless colonies were subcultured on nutrient agar slants and identified using methods described by Cowan and Steel [30]. For the identification of S. aureus, isolates were subcultured on Gelatin Mannitol Salt Agar (GMSA; Oxoid Limited, Basingstoke, UK) and incubated at 37°C for 24 hours after which growth was observed for white colonies, surrounded by yellow zone and tested coagulase positive. Other enterobacteria were identified by culturing samples on MacConkey agar (Oxoid) and characteristic colonies sub-cultured on nutrient agar slants for further biochemical tests. Non-enterobacterial species were identified by culturing on nutrient agar and subsequent biochemical tests carried out following standard bacteriological procedures [30].

2.4 Determination of Antiobogram of Isolates

Pure isolates were cultured for antibiotic susceptibility assessment using Kirby-Bauer disc diffusion method described by Jorgenson and Ferraro [31]. Pure bacterial inoculum (0.1 ml), which was standardized according to 0.5 McFarland turbidity standard (10⁶ cells equivalent) was spread on Mueller-Hinton agar plates, and antibiotic discs (Antibiotic Susceptibility Discs, Oxoid Ltd., England), were aseptically placed using sterile forceps and plates were incubated for 24hrs at 37°C. Zones of inhibition were measured and compared with standard values [32]. Antibiotics in the panel included Ciproflox (10 µg), Norfloxacin (10 µg), Gentamycin (10 µg), Amoxill (20 µg), Streptomycin (10 µg), Rafamycin (20 µg), Erythromycin (30 µg), Chloramphenicol (30 µg), Ampiclox (20 µg) and Levoflaxacin (20 µg) with concentrations as recommended by the National Committee for Clinical Laboratory Standard [32].

2.5 Statistical Analysis

Data generated from the experiment were subjected to Analysis of Variance (ANOVA) at 0.05 α level of significance using SPSS to determine the significant statistical difference in bacteriological counts, sensitivity pattern of the test organisms to antibiotics and the effectiveness of each antibiotic against bacterial isolates [33].

3. RESULTS AND DISCUSSION

3.1 Enumeration of Total Heterotrophic Bacterial (THB), Total E. coli and Coliform Counts

The results of total heterotrophic bacterial (THB), total E. coli and Coliform counts revealed a high level of bacterial load associated with the samples (Table 1). Creek Town River sample CTR3 had the highest THB count of 8.0x10⁶ cfu/ml while sample CTR1 had the least total heterotrophic bacterial counts (4.0x10⁶ cfu/ml). The results of the mean total E. coli count showed that 5.8x10⁵ cfu/ml (being the highest) count was obtained from CTR3 while the least count (1.5x10⁵ cfu/ml) was obtained from CTR6. Total coliform count obtained from the different samples ranged from 2.0x10⁵ cfu/ml to 3.0x10⁵ cfu/ml. According to the result, CTR2 had the highest number of coliform count (3.0x10⁵ cfu/ml). This was closely followed by count from CTR4, which had 2.8x10⁵ cfu/ml while CTR1 had the lowest count of 1.0x10⁵ cfu/ml.

The presence of high THB, total E. coli and Coliform counts could be traced to anthropogenic activities, as the River is used for bathing, washing of clothes and other recreational activities indulged by children.

The high total E. coli and Coliform counts is indicative of faecal contamination. The WHO standard for heterotrophic bacteria in potable water states that the total heterotrophic bacteria count should not be more than 100 cfu/ml [34]. WHO [4] reported that the greatest risk to humans from water sanitary point of view is from
the bacterial quality of the water was poor and
not suitable for human use. This finding
agrees with reports on the physiochemical and
bacteriological analysis of water used for drinking
and swimming purposes in Abeokuta, Nigeria by
Shittu et al. [42]. The result is in tandem with that
obtained from various water sources around the
Venda region; South Africa [9]. It also
corroborates the findings of Akubuenyi et al. [43],
which reported high total heterotrophic bacterial
counts, *E. coli* and coliform counts from most
major sources of water for domestic uses in
Calabar metropolis. The poor quality of water
may cause a wide range of diseases, including
cholera, typhoid fever, hepatitis, dysentery,
giardiasis, and other gastrointestinal infections in
rural communities. This corroborates the findings
of Stanley et al. [44] that the water from the New
Calabar River has poor microbiological quality,
according to quality guidelines for drinking water.

### 3.2 Isolation and Identification of Bacterial Isolates

Bacterial isolates obtained from the water at
various sampling points in the study and their
prevalence is shown in Table 2. They include:
*Enterobacter faecalis* (100%), *Escherichia coli*
(100%), *Klebsiella* spp (100%), *Streptococcus*
spp (66.67%), *Shigella* spp. (100%), *Salmonella*
spp. (50%), *Staphylococcus aureus* (100%),
*Pseudomonas* spp. (66.67%), *Proteus* spp
(100%), *Bacillus* spp (66.67%) and *Citrobacter*
spp (33.33%). The result revealed that
*Enterobacter faecalis*, *Escherichia coli*,
*Staphylococcus aureus*, *Klebsiella* spp and
*Proteus* spp had the highest prevalence (100%
each) while *Citrobacter* spp had the least
prevalence (33.33%).

In a related study, the most isolated organisms
from water sources included *E. coli* (22.7%),
*Enterobacter aerogenes* (2.5%), *Salmonella*
spp. (13.3%), *Shigella* spp. (19.3%), *Proteus*
spp. (18.5%), *Klebsiella* spp. (19.3%), and
*P. aeruginosa* (4.2%) [28]. *Shigella* has been
identified as one of the most common pathogens
responsible for the outbreaks of diarrhoea in Italy [45]. Obi et al. [9] reported the presence of E. coli, Vibrio cholerae, Aeromonas hydrophila, Shigella, Plesiomonas, and Campylobacter spp. as the most common isolates in their study. In a related study, Akubuenyi et al. [43] recommended adequate treatment of water collected from major water sources in Calabar metropolis before consumption in order to epidemic of water related diseases. E. coli and Salmonella represents important water-borne pathogens. Enterohaemorrhagic E. coli have emerged as a serious gastrointestinal pathogen in many countries, albeit from consumption of contaminated meat [46]. Most of these organisms are known causative agents of outbreaks of diarrhoea in areas where there is lack of potable water. Salmonellae are frequently found in streams that receive sewages and industrial wastes and it is associated with salmonellosis [4]. The consumption of the river water poses potential health risk for humans.

### 3.3 Antibiogram of Isolates

The result of the antibiotic sensitivity testing of bacterial isolates is shown in Table 3. It revealed that E. coli and Proteus spp. had the highest percentage sensitivity of 90% each. This was closely followed by Bacillus spp., Enterobacter spp. and Citrobacter spp. (80%) respectively. Klebsiella spp. and Shigella spp. had 70% sensitivity each while Salmonella spp. and Pseudomonas spp. had 50% each. Streptococcus spp. had 40% sensitivity, and the least percentage sensitivity was obtained from Staphylococcus aureus with 30% sensitivity.

The result of percentage sensitivity, in terms of the effectiveness of each antibiotic against all the bacterial isolates, revealed that chloramphenicol was most effective with 100% sensitivity. Streptomycin was the next antibiotic with percentage sensitivity (91%). Ciprofloxacin had 82% sensitivity while gentamycin and ampiclox had 73% each. Erythromycin had 64% and amoxil had 57%. Norfloxacin had 55% while rafamycin had the least percentage sensitivity (45%).

The antibiotic susceptibility testing is vital in the treatment and management of bacterial diseases. Unfortunately, organisms have been shown to develop resistance against commonly-used antibiotics [47]. Multiple drug resistance was exhibited by Staphylococcus aureus and Streptococcus sp in this study. These findings are similar to those obtained by Obi et al. [9] who showed that bacterial isolates demonstrated multiple drug resistance to antibiotics. In the present study, it was found that susceptibility to amoxil, lovofloxacin, ampiclox is low. These antibiotics have been reported to be potent against a wide range of pathogens [9]. In a study in India, there was no evidence of resistance against this antibiotic, particularly among Shigella isolates from stool samples [48]. The high resistance exhibited by S. aureus could be due to the overuse of these antibiotics in the hospital environment and in the general population, leading to adaptive resistance. In a study of the isolates from river samples in the Limpopo province, the resistance level varied from 8% to 15% depending on the organisms tested, with lower resistance among Shigella sp. (8%) [49]. Profiles of resistance to about 20 antibiotics at a time were noted by Wasfy et al. [48] in Egypt where more than 25% of bacteria were resistant to three or more antibiotics. In a study in Nigeria,
Table 3. Antibiogram of bacterial species isolated from Creek Town River

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Antibiotics and their concentrations (mcg) and zones of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPX (10)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>18 (S)</td>
</tr>
<tr>
<td>Enterobacter faecalis</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>22 (S)</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>21 (S)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>18 (S)</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>19 (S)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>10 (R)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>22 (S)</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>11 (I)</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>23 (S)</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>19 (S)</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td></td>
</tr>
<tr>
<td>CPX</td>
<td>20 (S)</td>
</tr>
<tr>
<td>% Sensitivity</td>
<td>82</td>
</tr>
</tbody>
</table>

**Note:**
- CPX = Ciproflox; NB = Norfloxacin; CN = Gentamycin; AML = Amoxil; S = Streptomycin; RD = Rarfamycin; E = Erythromycin; CH = Chloramphenicol; APX = Ampiclox; LEV = Levofloxacin; R = Resistant; S = Sensitive; I = Intermediate; spp. = Species; Conc. = Concentration; Sal. = Salmonella; mcg = Micrograms; mm = Minimeter.
Oluyege et al. [28] found that over 10% of bacteria were resistant to four or more antibiotics, and antibiotic resistance was the highest in members of the genera; Enterobacter, Pseudomonas, and Proteus. The findings of the present study suggests that the water samples collected from Creek Town river were heavily contaminated with potential bacterial pathogens that have evolved resistance to one antibiotic or the other. This poses a serious public-health threat, as children and other dwellers used the water daily. Hence, the river is unfit for drinking purposes.

The effectiveness of each antibiotic showed that all enteric bacterial isolates were markedly sensitive to chloramphenicol (100%), while streptomycin and ciproflox showed 91% and 82%, respectively. These antibiotics could therefore be effective against enteric infections. The susceptibilities of these antibiotics are in agreement with reports of other investigators [47, 50]. It should be noted that susceptibility of bacteria to antibiotics is not static and resistance may be due to antibiotic abuse, antibiotic overuse or may be chromosomally or plasmid mediated [51]. Antibiotic usage must therefore be carefully regulated and monitored.

3.4 Statistical Analysis

Analysis of variance (ANOVA) on total heterotrophic bacterial (THB) count showed that there was significant difference (P=.05) among the six samples. ANOVA on total coliform and faecal coliform counts were significant with P=.05 level of significance. There was significant difference (P=.05) in total heterotrophic bacterial count and also between E. coli and coliform counts. There was significant difference (P=.05) in sensitivity patterns of organisms to the different antibiotics. ANOVA also revealed that there is significant statistical difference (P=.05) in the effectiveness of each drug against test organisms.

4. CONCLUSION

Creek Town River contains total heterotrophic bacterial, E. coli and Coliform counts that exceed WHO acceptable limits. The high faecal coliform count showed that the river was contaminated with human excrement. Some of the bacterial isolates identified from the water samples were known pathogenic bacteria. Appropriate water treatment or safe potable water sources should be provided in the area to improve the welfare of the riverine dwellers. There is need to educate the villagers on how to handle and locally treat water for domestic use. The government should evolve sanitation programmes and propagate these through environmental education throughout the communities in the river catchments to prevent pollution of water bodies and consequent transmission of water-related diseases. Control of human activities which could prevent faeces and refuse from entering water body is the key to avoiding bacterial contamination of the river water.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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