Occurrence and pathogenicity of *Escherichia coli* serotypes from processed water in Gwagwalada, FCT, Nigeria

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**Abstract**

*Escherichia coli* (*E. coli*) are important commensal bacteria that occur in a variety of sources, including processed water. This study was carried out to determine the prevalence and pathogenicity potential of *E. coli* serotypes predominating processed water vended in Gwagwalada metropolis. A total of 240 samples consisting of 60 bottled water, 150 sachet water and 30 tap water were randomly sampled bi-monthly for six month period. The samples were analyzed biochemically for the presence of *E. coli* isolates. The overall prevalence rate recorded was 5.0%. Bottled and sachet water samples had zero prevalence rates. Serotyping was conducted and the isolates were grouped into 5 different serotypes consisting of O86, O44, O103: H2, O113 and O26: H11. Pathogenicity studies using Lethal dose assay (*LD₅₀*) was conducted on an isolate and the titre was 10³ µl/ml. Virulence factors were determined for all the isolates and the results revealed that 25.0% of the isolates produced verotoxins. Haemolysin production was indicated in 14.7% isolates, while cell surface hydrophobicity was found in 50.0%. Serum resistance was 33.3% and gelatinase production was 33.3%. Our results clearly demonstrated that the *E. coli* serotypes isolated from tap water in our environment have high pathogenic potentials. It is therefore suggested that rampant public health awareness campaign on the use and consumption of tap water needs to be instituted in our communities. Moreover water distribution systems in Gwagwalada needs public health intervention from Federal Capital Development Authority (FCDA) water board.

**Keywords:** *Escherichia coli*, Pathogenicity, Verotoxins, Haemolysin, Cell Surface hydrophobicity, Gelatinase.

**Introduction**

Processed water is any water that has been altered from its natural state for safety reasons. Some examples of processed water include bottled water, sachet water, tap water, dispensed water. Water has immense economic importance as it is needed for drinking, agricultural, industrial and recreational purposes. Water is the life wire of the body and a critical part of human diet. Clean drinking water is essential to humans and other life forms.

The need for having potable water is of great public health significance because of water-borne infections. Water contamination could be a major cause of illness and potential health problems may exist due to the microbial content of water. This is so because water is one of the vehicles for transmission of pathogenic organisms. Water contamination remains a problem of global concern, contributing to high morbidity and mortality rates from water and food borne diseases.

Water-borne diseases are caused by pathogenic microorganisms, which are directly transmitted when contaminated drinking water is consumed. Water-borne diseases can be caused by protozoa, virus or bacteria, helminthes.

It is the most preferred faecal pathogen used in assaying water quality is *E. coli* because it gives indication of faecal contamination. *E. coli* is the most common cause of food and water-borne human diarrhea in developing countries causing 80 thousand deaths out of 650 million cases per year especially infants. Some *E. coli* strains are harmless to human but pathogenic strains cause gastroenteritis, urinary tract infection, neonatal, meningitis and in rare cases hemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicaemia and gram-negative pneumonia.

Some strains of *E. coli* produce shiga toxins. These toxins are responsible for lethal acute bloody diarrhoea (haemolytic colitis and haemolytic-uremic syndrome) in humans. This often occurs when non-pathogenic *E. Coli* serotypes are genetically converted by the integration of genes into the host genome which code for the production of virulence factors. These virulence factors could be characterized using different parameters which include: verotoxins assay, cell surface hydrophobicity, serum resistance, haemolysins production, gelatinases production and extended spectrum beta-lactamas production since they play important role in determination of bacterial pathogenicity. Thus, our study was undertaken to determine the occurrence and determine the pathogenic potentials of *E. Coli* serotypes from processed water vended in Gwagwalada metropolis.

**Materials and Methods**

**Experimental Design/Sampling:** Based on the number of sample locations and different types of potable water (bottled, sachet and tap water) subjected to bacteriological analysis, randomized complete block design was adopted in this study. In this experimental plan, water samples were collected at random in triplicates. From each sample locations for a period of six months based on bi-monthly collections. Twenty...
different brands of bottled water and fifty different brands of sachet water were collected from stores on the streets of Gwagwalada. The tap water samples were collected at ten different designated locations in Gwagwalada metropolis.

Isolation and Biochemical Identification of E. coli

Laboratory analysis was undertaken at the laboratories of the Department of Microbiology, University of Abuja, and in the bacterial zoonoses Laboratory, Ahmadu Bello University, Zaria, Nigeria. The study was conducted from August 2014 to August, 2015.

Water samples were first filtered using membrane filter, then the filter paper was inoculated into MacConkey broth (Fluka Biochemical, Germany) and incubated at 37°C for 24 hours. A loopful of the MacConkey broth samples was spread onto plates of Eosin methylene blue (EMB, Oxoid, SA) prepared according to the manufacturer’s instruction and further incubated at 37°C for 24 hours.

Isolates that had green metallic sheen on EMB agar were further purified by picking discrete colonies and sub cultured onto fresh plates of EMB agar and incubated at 37°C for 24 hours. Colonies that had green metallic sheen on EMB agar were presumptively taken as E. coli and were characterized microscopically and biochemically and refrigerated on nutrient agar slants.(12)

Serotyping of E. coli Isolates

The serotypes of the E. coli isolates were determined using slide agglutination tests and test tube method.(5,7) Side agglutination tests were performed on the selected twelve presumptive single colonies using polyvalent E. coli antisera 2, 3 and 4 (Bioweb PTY, SA) prepared according to the manufacturer’s instructions. Determination of the O-antigen was carried out using heat-inactivated bacteria by the slide agglutination method while determination of H-antigen was carried out using the test tube method with the E. coli isolate cultured in liquid media. E. Coli ATCC 25922 was used as a positive control. Serotyped E. coli isolates were inoculated onto tryptic soy (TS) slants, incubated at 37°C for 24 hours and then stored at 4°C until it was used for further studies.

Toxigenicity Studies of the E. coli Isolates

LD50 Determination

The 50% lethal dose (LD50) test was determined using albino mice. The forty two albino mice used for this study were bought from the Laboratory Animal Unit of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, and Zaria, Nigeria. E. coli isolates were grown at 28°C for 24 hours on Brain Heart infusion broth (Fluka Biochemical, Germany) prepared according to the manufacturer’s instruction. The cells were then washed twice in sterile saline. The pellet was diluted in universal bottles with sterile saline to give concentration comparable to 2% Barium Chloride, the barium Chloride which gave concentration of 6 x 10^7 Cells/ml. Serial dilutions of 10^-1, 10^-2, 10^-3, 10^-4 and 10^-5 were made and dispensed in Bijou bottles by adding 1ml of the initial dilution to 9ml of the serial dilution. The 100µl of each dilution was injected into five groups of six 20-25g male and female albino mice intraperitoneally using a 23 gauge tuberculin syringe. The animals were then observed six hourly (non-specific deaths were observed for 1-2 hours post injection) for 96 hours. Viable counts of various dilutions were determined by the aerobic plate count method using plate count agar (International Diagnostics Group, UK) in triplicate which was then incubated at 37°C for 24 hours and the colonies counted. Average counts from the triplicate plates were calculated. The LD50 was then calculated.(2)

Determination of Virulence of E. coli Isolates

Haemolysin Production Test

E. coli isolates were screened for haemolysis on 5.0% sheep blood agar using standard method.(1,18) The 5.0% sheep blood agar was prepared by adding 5ml of washed sheep blood to 95ml of blood agar base. The E. coli isolates were picked from the nutrient agar slants and streaked on the surface of the medium and then incubated at 37°C for 24 hours. Production of β-haemolysis was indicated by the presence of clear zone around the point of E. coli growth.

Verotoxin Production Test

This test was carried out using antibody based Douperth kit (Biotec laboratory, United Kingdom) prepared according to the manufacturer’s instruction.(3) E. coli isolates were pre cultured in 1ml casemaminic yeast extract (CAYE) broth (International Diagnostic Group, UK) prepared according to the manufacturer’s instruction and incubated at 37°C with rotation at 100 rpm for 24 hours. After incubation, 10µl (1 x 10^7 cells/ml) of the pre-cultured broth was inoculated into fresh CAYE broth and further incubated for 16 hours with rotation at 100 rpm at 37°C. The culture was centrifuged at 5000g for 5 minutes to separate the supernatant and cell pellets. The cell pellets were then washed three times with phosphate buffered saline (PBS, 5ml) and then suspended in 0.25ml 0.01m Tris-HCL (pH 7.5). 0.5ml distilled water containing 50µg/ml polymyxin B was added to the pellets and the suspension incubated at 37°C for 30 minutes. 200µl of the culture suspension was transferred onto the test device using a sterile Pasteur pipette and the result was read after 10 minutes. The appearance of red bands on the Vtx1 and Vtx2 bands indicated the presence of either one or both verotoxins.

Cell Surface Hydrophobicity Test

This test was carried out using salt aggregation method (SAT) (19, 17). 10µl loopful of E. coli suspension in 1ml of phosphate buffer (pH 6.8)
equivalent to $5 \times 10^9$ colonies/ml, was mixed with equal volumes of ammonium phosphate solution of different molarities (1.4M, 2.0M and 4.0M) on a glass slide. The suspensions were rotated carefully for 1 minute and then microscopically examined for agglutination. The highest dilution of ammonium sulphate solution giving a visible agglutination of E. coli was scored as the SAT value. E. coli suspension clumping at the lowest dilution (1.5M) was considered auto-aggregative while those with SAT values of ≥ 2M were considered hydrophobic.

**Gelatinase Production Test**

Gelatine agar (Fluka Biochemical, Germany) prepared according to the manufacturer’s instructions was incubated at 37°C for 24 hours. Thereafter, the plate was flooded with mercury chloride (HgCl$_2$) solution. The development of opacity in the medium and a zone of clearing around the E. coli colonies indicated positive result for the presence of gelatinase enzyme.\(^{(3)}\)

**Bactericidal Serum Resistance Assay**

E. coli isolates were grown on blood agar for 18 to 24 hours at 37°C. The cells were harvested and suspended in Hank’s Balanced Salt Solution (HBSS). 0.05ml of E. coli suspension and 0.05ml serum were mixed in a test tube and then incubated at 37°C for 180 minutes and the absorbance was read at 600nm. Viable count (%) was determined by calculating the differences in absorbance value before and after incubation. Resistance of E. coli isolates to serum bactericidal activity was expressed as the percentage of E. coli isolates that served after 180 minutes of incubation with serum in relation to the original count. E. coli isolates were termed serum sensitive if viable count dropped to 1% of initial value and resistant if viable count increased above 90% after 180 minutes of incubation.\(^{(2,6)}\)

**Statistical Analysis**

In line with the experimental plan used in this study, all the data was analyzed using the one-way analysis of variance to determine the differences between mean values of the three types of water samples obtained from the 10 different locations.\(^{(15,16)}\)

**Results**

Table 1 shows the prevalence of E. coli isolates in different sampling periods. There was no E. coli isolate from the bottled and sachet water samples during the first, second and third sampling periods. But E. coli was isolated in 12.0 (40.0%) of the tap water samples during the three sampling periods. E.coli was similarly isolated from 90.0% of well water samples (positive control) tested during the sampling periods. The overall prevalence rate of E.coli isolation was 5.0%.

Table 2 shows the results of biochemical tests conducted on the 12 E. coli isolates showed that E. coli isolates were Gram-negative rods. The isolates were also negative to Vogues Proskauer, citrate, oxidase, gelatine liquefaction, and triplesugar iron and deoxyribonuclease tests but were positive to indole, methyl red, and catalase and motility tests.

Table 3 shows results of the determination of E. coli serotypes. It revealed that the twelve E. coli isolates were grouped into five different serotypes namely: O103, O44, O113, and O26: H11. E. coli serotype O44 had four isolates while O86 had only one isolate. Three isolates belong to E. coli O113 serotype while two serotypes, O103:H2 and O26:H11 had two isolates each.

Table 4 demonstrated the pathogenicity of the isolates using albino mice. The $10^1$ bacterial dilution recorded the highest mortality of 6 while the $10^5$ bacterial dilution recorded zero mortality. In the overall, five groups of six albino mice inoculated with $10^1$, $10^2$, $10^3$, $10^4$ and $10^5$ dilutions of E. coli inoculum recorded 50 percent mortality. But a group of six mice inoculated with E. coli O157 (positive control) recorded 83 percent mortality while another group of six mice inoculated with sterile saline solution (negative control) recorded zero mortality. The LD$_{50}$ titre value was estimated $10^3$.

Table 5 results demonstrated that the virulence factors were present in the E. coli isolates. From the results, 25.0% of the isolates were positive to verotoxins production (both Vtx1 and Vtx2), 41.7% were positive to haemolysin production, 50.0% were positive to cell surface hydrophobicity, 33.3% were positive to bactericidal serum resistance assay and 33.3% were positive to gelatinase production.

**Table 1: Prevalence of E. coli Isolates from Water Samples**

<table>
<thead>
<tr>
<th>Water samples</th>
<th>No. of samples collected</th>
<th>No. of positive samples</th>
<th>% of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottled</td>
<td>60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sachet</td>
<td>150</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tap</td>
<td>30</td>
<td>12</td>
<td>40.0</td>
</tr>
<tr>
<td>Total</td>
<td>240</td>
<td>12</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**Table 2: Number of E. coli Isolates in Different Serotypes**

<table>
<thead>
<tr>
<th>E. coli serotypes</th>
<th>Number of E. coli isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>O86</td>
<td>1</td>
</tr>
<tr>
<td>O44</td>
<td>4</td>
</tr>
<tr>
<td>O103:H2</td>
<td>2</td>
</tr>
<tr>
<td>O113</td>
<td>3</td>
</tr>
<tr>
<td>O26:H11</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 3: The 50 Percent Lethal Dose (LD₅₀) Estimate Values

<table>
<thead>
<tr>
<th>Bacterial Dilutions</th>
<th>Mortality Ratio</th>
<th>Died</th>
<th>Survived</th>
<th>Accumulated values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Died (D)</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>6/6</td>
<td>6</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>10⁻²</td>
<td>5/6</td>
<td>5</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>10⁻³</td>
<td>3/6</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>1/6</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>0/6</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Using Karber’s method of estimation of LD₅₀ (Cruickshank et al., 1975)

\[
\log LD_{50}\text{titre} = \left\{ \frac{0.5 + \log \text{of greatest bacterial conc. used}}{\text{sum of % dead animals}} \right\} - \frac{\text{sum of % dead animals}}{100}
\]

\[
\log LD_{50}\text{titre} = 0.5 + (-1.0) - \left\{ \frac{100+90+50+10+0}{100\log LD_{50}\text{titre}} \right\}
\]

\[
\log LD_{50}\text{titre} = 0.5 - 1.0 - 2.5
\]

\[
LD_{50} = 10^{-3}
\]

Fig. 1: Virulence Factors of E. coli Isolates from processed water

Discussion

The results of this study conducted at the Department of Microbiology, University of Abuja documented the occurrence of pathogenic strains of E. coli in tap water samples consumed in Gwagwalada. The prevalence rate of E. coli in processed water samples investigated in this study was about 40%. This agreed with a previous study undertaken(13) in which a similar prevalence rate was reported while investigating E. coli isolates in some public water sources in Gusau municipal in north-western Nigeria.

The overall prevalence rate of 5.0% as obtained in this study was lower than 36.0% reported(14) while studying the prevalence of bacteria in packaged sachet water sold in Nnewi, South-eastern Nigeria. The prevalence rate of E. coli observed from this study implies that pathogenic E. coli is not totally eradicated in water in Nigeria. This finding calls for increased awareness campaign and improvement of the necessary sanitary measures already instituted in our water distribution systems. The low prevalence rate may also be due to high compliance to strict rules of hygiene by processed water producers in Gwagwalada metropolis. The absence of E. coli in both bottled and sachet water samples is in contrast with previous studies reported in which E. coli was frequently isolated from bottled and sachet water. \(^{(14,17)}\) It is possible that adequate precautionary measures were maintained or adhered to right from the time of processing, packaging and distribution of these water samples. The high prevalence E. coli in bottled and sachet water as reported by previous researchers may be attributed to poor hygienic practices which often mare water handling processes in Nigeria. The likely reason for high preponderance of E. coli in tap water could be attributed to high concentration of biofilms in pipe walls which provides carbon source for microbial biosynthesis and enabling environment for commensal activities, resulting possible proliferation of bacterial flora in pipe walls. \(^{(11)}\)

The occurrence of E. coli in tap water could be an indicator of faecal contamination via exogenous sources. It is possible that large numbers of the organisms up to about approximately 10³ per gram E. coli of could be washed via leaking pipes to contaminate tap water. \(^{(9)}\) The presence of E. coli in tap water is a significant public health issue because E. coli can cause water borne infections characterized by poor water quality. Therefore tap water in the environment covered by this study should be properly treated before consumption. The relevant authorities should be informed to repair all broken and leaking pipes and replace worn out parts which could be sources of microbial contamination. \(^{(18)}\)

The results from the biochemical characterization of the E. coli bacteria agreed with previous workers\(^{(12)}\) whose colonies were isolated from urine of asymptomatic students in Bayelsa State, Nigeria. Serotyping revealed five E. coli serotypes this concurs with previous studies.\(^{(3,14)}\) 10 serotypes out of 69 non O157:H7 E. coli isolates investigated while studying
virulence factors and antibiotic susceptibility among verotoxic nonO157:H7 Escherichia coli isolates obtained from water and waste water samples in Cape Town, South Africa.

The E. coli isolates investigated in this study had the required dose of toxins to kill 50 percent of the tested animals (mice). This implied high pathogenicity of the isolates. For most organisms, LD50 represents a convenient means of determining virulence. The LD50 of 10^3 µl/ml encountered in this study was consistent with previous findings (8) which reported that most clinical isolates had LD50 of 1.5x10^5 CFU. This finding is in tandem with previous works. (15)

This study also revealed the presence of virulence factors such as haemolysin, cell surface hydrophobicity production, verotoxin or shigatoxin production, gelatinase production, and other relevant toxins produced by E.coli that are responsible for the bacterial pathogenicity. Our findings is in agreement with the virulence markers in the various E. coli serotypes as previously reported. (15,18) There is possibility that these virulence factors may be responsible for cytotoxic and cytotoxic enterotoxigenic genes which are responsible for lethality, enterotoxigenicity and hemolysis associated with E.coli infections.

Conclusion and Recommendations

Processed water consumed in Gwagwalada metropolis like bottled and sachet water were likely to be free from faecal contamination except tap water. However, E. coli isolates from tap water samples were non O157:H7 pathogenic strains. The virulence factors produced by E. coli could be a signal for public health authorities that presence of potential human pathogens associated with infections such as diarrhoea, urinary tract infections and gastrointestinal diseases. Regulatory agencies in Nigeria should routinely evaluate the microbial quality of tap water to ensure compliance to quality standards. It is also suggested that rampant public health awareness campaign on the use and consumption of tap water needs to be instituted in our communities. Also, water distribution systems in Gwagwalada needs public health intervention from Federal Capital Development Authority (FCDA) water board.

References