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

Mailafia Samuel[1,*], Emelogu Chukwudi[2], Mohammed Balarabe Rabiu[3]

[1]Associate professor Dept. of Veterinary Microbiology,[2]Researcher, Dept. of Microbiology,[3]Senior Lecturer, Dept. of Veterinary Parasitology and Entomology, University of Abuja, Nigeria

***Corresponding Author:**
Email:smailafia@gmail.com

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 potentialsof *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 5different serotypes consisting of O86, O44, O103: H2, O113 and O26: H11.Pathogenicity studies using Lethal dose assay (LD_{50}) was conducted on an isolate and the titre was 10^{-3}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 in14.7% 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 thatrampant 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.(6) 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.(13)

The need for having potable water is of great public health significance because of water-borne infections.(4) 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.(17) Water contamination remains a problem of global concern, contributing to high morbidity and mortality rates from water and food borne diseases.(5) 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.(11)

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.(9) 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.(12)

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.(8) 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.(10) 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- lactamases production since they play important role in determination of bacterial pathogenicity.(7) 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.(17) 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 37^0C for 24 hours. Aloopful 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 37^0C 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 37^0C 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.[3,7] 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 37^0C for 24 hours and then stored at 4^0C until it was used for further studies

Toxigenicity Studies of the *E. coli* Isolates

LD_{50} Determination

The 50% lethal dose (LD_{50}) 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 28^0C 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 pallet 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 37^0C for 24 hours and the colonies counted. Average counts from the triplicate plates were calculated. The LD_{50} 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 37^0C 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 caseminacid yeast extract (CAYE) broth (International Diagnostic Group, UK) prepared according to the manufacturer's instruction and incubated at 37^0C 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 37^0C. 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 37^0C 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 x 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 37^0C 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 37^0C. 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 37^0C 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: O86, O44, O103: H2, 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* inoculums 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.

Fig. 1 is a bar chart showing the virulence factors of *E. coli* isolates from processed water. From the bar chart, cell surface hydrophobicity test has the highest percentage isolates positive for virulence factors (50.0) seconded by haemolysin production test (41.7) while verotoxins production test has the least (25.0). Similarly, bacterial serum resistance assay and gelatinase production test have marginal of 33.3% isolates positive for virulence factors.

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

Water samples	No. of samples collected	No. of positive samples	% of positive samples
Bottled	60	-	-
Sachet	150	-	-
Tap	30	12	40.0
Total	240	12	5.0

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

E. coli serotypes	Number of *E. coli* isolates
O86	1
O44	4
O103:H2	2
O113	3
O26:H11	2

Total	12

Table 3: The 50 Percent Lethal Dose (LD_{50}) Estimate Values

Bacterial Dilutions	Mortality Ratio	Died	Survived	Accumulated values			
				Died (D)	Survived (S)	Mortality Ratio	Percentage = (D) x 100 (D+S)
10^{-1}	6/6	6	0	15	0	15/15	100
10^{-2}	5/6	5	1	9	1	9/10	90
10^{-3}	3/6	3	3	4	4	4/8	50
10^{-4}	1/6	1	5	1	9	1/10	10
10^{-5}	0/6	0	6	0	15	0/15	0

Using Karber's method of estimation of LD_{50} (Cruickshank *et al.*, 1975)

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

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

$$\text{Log } LD_{50}\text{titre} = 0.5 - 1.0 - 2.5$$

$$\text{Log } LD_{50}\text{titre} = -3.0$$

$$LD_{50} = 10^{-3}$$

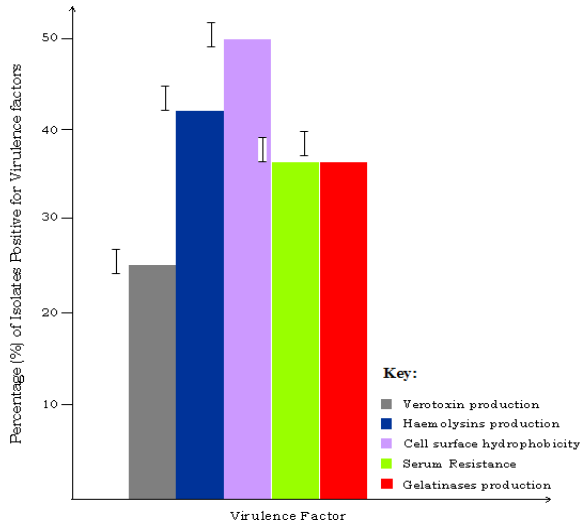

Fig. 1: Virulence Factors of *E. coli* Isolates from processed water

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[5] 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[4] 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 10^9 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, LD_{50} represents a convenient means of determining virulence. The LD_{50}titre of 10^{-3} µl/ml encountered in this study was consistent with previous findings [8] which reported that most clinical isolates had LD_{50} of $1.55x10^{8-9}$ 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 cytotonic 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.

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