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Evaluation of Multi-Drug Resistant *E. coli* from Drinking Water Sources in a Tertiary Institution

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Authors' contributions

This work was carried out in collaboration among all authors. Author IPU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IAI and STA managed the analyses of the study. Author STA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Infectious diseases control of recent is a major health concern globally due to high increase in number of microorganisms that are resistant to conventional antimicrobial agents. This study aimed at ascertaining the microbiological quality and multiple antibiotic resistance profile of *E. coli* strains isolated from different sources of drinking water. A total of 136 water samples from different drinking water sources, including the storage tanks (the school and the commercial storage tank), sachet and bottle water were obtained from University of Nigeria Enugu Campus and analyzed. Standard microbiological techniques were employed for bacteria isolation, identification and antibiogram. From the water samples collected 25 *E. coli* strains were isolated. The school storage tanks account for 60% of the isolates, while bottled water showed no growth. 92% of the *E. coli* isolated showed resistance to the tested antibiotics. Resistant were higher with Augmentin (64%), Chloramphenicol (48%) and Streptomycin 11 (44%) while most were sensitive to Tarivid and Perfloxacine (100%). Isolates from school storage water sources showed the highest resistance to

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Augmentin (76.5%) while those from commercial storage water sources showed the highest resistance to Streptomycin (66.7%). Out of the 23 (92%) antibiotics resistant *E. coli* isolates 18 (78.3%) were multidrug resistance (MDR). The school storage water sources had the highest number of MDR *E. coli* 14 (77.8%) followed by the commercial storage water sources 4 (22.2%), but the sachet and bottled water had no MDR *E. coli*. In conclusion, drinking water may potentially contribute to the source of multidrug resistance *E. coli* in this community

Keywords: Infectious diseases; multidrug resistance; microorganisms; antibiotics.

1. INTRODUCTION

Water like air is the most essential commodity for the survival of living things. It is needed for daily life activities such as drinking, cooking and recreational activities [1]. Drinking water has been implicated as a medium for transporting microbial pathogens to majority of people and leading to various illnesses [2,3]. Evaluation of microbiological quality of drinking water lies to a greater extent on examination of indicator bacteria such as coliform (for example *E. coli*, and *Pseudomonas aeruginosa)*. *E. coli* being a member of the faecal coliform group is widely accepted as the better indicator of faecal pollution than other faecal coliforms. Reports abound indicating that most *E. coli* (over 95%) express β-D-glucuronidase (GUD) activity hence making this enzyme a sensitive and specific marker for *E. coli* detection and thus faecal pollution [4,5].

Faecal contamination of water is the pollution of water with disease-causing organism which inhabits the gastrointestinal tracts of mammals but with particular attention to human illness [6]. Leakages of human and animal wastes into pipe born water, improper sewage disposal system, discharge of improperly treated sewage are the predisposing factors to the contamination of water by fecal contaminant organism [7].

A microbial indicator called faecal coliforms, in particular, Escherichia coli (*E. coli.*) is used generally to accessed microbiological safety of drinking water. When *E. coli* is present in water, it is an indicator of faecal contamination and more so existence of other fecal bacterial pathogens [8]. World health organization reported that 80% of all diseases are attributed to unsafe source of water [9].

The presence of certain pathogenic features and virulence genes which are located in the transmissible genetic element that distinguish them from ordinary commensal strains, occasionally aid some strains of *E. coli* to emerge as pathogens[10]. Furthermore, study had revealed E. coli as a significant reservoir of genes coding in antimicrobial drug resistance hence is a useful marker when studying resistance in bacterial communities. Indiscriminate use of antibiotics in agriculture and their release from sewage works has rapidly increased the development of antibiotic-resistant strains in environmental water bodies, hence the need for accurate and regular monitoring [11].

Multi-drug resistance in drinking water sources is a serious issue all over the world [12,13,14,15, 16]. However, there is a rapid concern regarding the prevalence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in water environments [17,18]. Aquatic environment provides a convenient platform for the acquisition and dissemination of antibiotic resistance, since it is the main receptacle for pollution from agriculture, industry, or domestic life [19,20].

Assessment of pathogenic bacteria and antibiotic resistance in human drinking water sources is of great importance, considering the fact that water is directly related to human activity and health [21].

Globally multi-drug resistance is on the increase and hence a big threat in our society because resistant microorganisms often fail to respond to conventional treatment. In University of Nigeria Enugu Campus (UNEC), good sources of water have been challenged, students are highly dependent on commercial and school tankers for the supply of drinking water, while some depend on sachet water and few on bottled water. Therefore, there is a yearning need to access this various water sources for safe.

The objectives of this study are to ascertain the microbiological quality and multiple antibiotic resistance profile of *E. coli* strains isolated from different water sources, in order to determine their safety for human consumption and to provide updated antibiotic data for efficient treatment of patients

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at the University of Nigeria, Enugu campus (UNEC), Enugu State, South East of Nigeria. The sample was collected from all the hostels and canteens within the campus.

2.2 Sample Population

A total of 136 water samples were collected from all the 9 student hostels, comprising of 39 samples from commercial storage tanks and 39 from free water storage tanks usually supplied to students by the school, 15 bottled and 36 sachet water from major canteens easily accessed by students and 7 from the tankers that supplies water to the hostel (both commercial and school free water tankers).

2.3 Sample Collection

10 ml sterilized universal bottles were labeled and used to aseptically collect water from the various storage tanks at the student hostel while packaged water were purchased at selling points. The water from the storage tanks was first allowed to flow out for a while before the sample was collected; also care was taken to avoid bottles contact with the tips of the tap and all water samples collected were properly labeled. The sachet and bottled water were gotten from students' hostel canteens.

2.4 Bacteria Isolation and Identification

One ml of each of the samples was aseptically added into different sterile McCartney bottle containing 10 ml of freshly prepared MacConkey broth which was incubated at 37°C for 36-48 hours. From the bottles showing a color change (due to acid fermentation), a loopful of the broth was sub-cultured onto a freshly prepared well dried EMB (Eosin methylene blue) agar plate. The plates were incubated at 37°C overnight, the plates were read and colonies that are indicative of *E. coli* in EMB agar plate showed metallic sheen (green) color [22]. Mixed cultures were re-streaked for purity on plates of sterile EMB agar and biochemical identification were done on isolates.

2.5 Antibacterial Susceptibility Testing of *E. coli*

Susceptibility of the isolated *E. coli* pathogens were carried out by the modified Kirby-Bauer disc diffusion method, which has been standardized and evaluated by the methods of the National Committee for Clinical Laboratory Standards(NCCLS) [23]. The *E. coli* isolates grown overnight on Nutrient Agar were suspended in sterile normal saline (0.9% w/v NaCl) with the aid of a sterile wire loop until the turbidity was equivalent to 0.5 Mcfarland standards. Sterile swab sticks dipped into the standardized inocula were used to streak the entire surface of Mueller– Hinton agar plates. The *E. coli* isolates were tested against the following antibiotics: Tarivid(10µg), Ciprofloxacin (10µg), Saprofloxacin (10µg), Streptomycin (30µg), Gentamycin (10µg), Cotrimoxazole (30µg), Amoxicilin(30µg), Augmentin(30µg), Chloramphenicol(30µg), and Pefloxacin (30µg). The antibiotic disks were placed aseptically using sterile forceps and all the plates were incubated at 37°C for 24 hours [22]. Interpretation of result was according to NCCLS [23].

3. RESULTS

Results from Table 1 reveal the distribution of *E. coli* from 136 different drinking water samples analyzed in UNEC. A total of 25 (20.5%) *E. coli* was isolated. The highest number of *E. coli* was isolated from school storage tank water 15 (60%), followed by commercial storage tank water 6(4%), School tankers and sachet water 2 (8%) each. Bottle water and commercial tankers had no *E. coli* isolate 0 (0%).

Results from Table 2 show the antibiotic susceptibility profile of isolated *E. coli*, all the isolate were tested against 10 different antibiotics. *E. coli* were most resistant to Augmentin 16 (64%) followed by Chloramphenicol 12(48%), Streptomycin 11 (44%) and Cotrimoxazole 10 (40%), while it was more sensitive to Tarivid and Perfloxacin 25 (100%) each followed by Ciprofloxacin and Saprofloxacin 24 (96%) each

Results of Table 3 reveal the antibiotics resistant pattern of *E. coli* isolates from school storage water sources including the school tanker. The *E. coli* isolates showed highest resistance to Augmentin 13 (76.5%) followed by Chloramphenicol 10 (58.8%), while it was more sensitive to Tarivid and Perfloxacin 25 (100%) each.

Results of Table 4 show the antibiotics resistant pattern of *E. coli* isolates from commercial storage water sources including the commercial tankers. The *E. coli* isolate showed highest resistance to Streptomycin 4(66.7%) followed by Augmentin and Chloramphenicol 2 (33.3%) each but showed no resistance to Tarivid, Ciprofloxacin, Saprofloxacin, and Perfloxacin 0 (0%) each.

Results of Table 5 reveal the multidrug resistance pattern of *E. coli* isolate. Out of 23 antibiotics resistant *E. coli* isolated, 18 (78.3%) were multidrug resistance (MDR). The highest MDR *E. coli* was from the school storage water sources 14 (77.8%) followed by the commercial storage water sources 4 (22.2%) while no MDR *E. coli* were isolated in both sachet and bottle water analyzed.

4. DISCUSSION

Many strains of *E. coli* are known to be resistant to a wide range of antibiotics [24,25,26]. The findings from this study revealed existence of multiple drug-resistant (MDR) *E. coli* in some of the drinking water sources analyzed. A previous study has shown a high prevalence of MDR *E. coli* in the drinking water samples from Hydrabad [27].

Table 1. Frequency distribution of *E. coli* isolates from drinking water sources in UNEC

Water sources	No of sample analyzed	No of *E .coli* isolated	Percentage % Distribution
Free Storage Tank	39	15	60
Commercial Tankers	3	0	0
Comm. Storage Tank	39	6	24
School Tankers	4	2	28
Sachet Water	36	2	8
Bottle Water	15	0	0
TOTAL	136	25	100

Table 2. Antibiotics resistant pattern of *E. coli* isolates from the entire drinking water sample

Antibiotics	Disc Conc. (µg)	Resistance number (%)	Sensitivity number (%)
Tarivid	10	0	100
Ciprofloxacin	10	4	96
Saprofloxacin	30	4	96
Streptomycin	10	44	56
Gentamycin	10	36	64
Cotrimoxazole	30	40	60
Amoxicillin	30	32	68
Augmentin	30	64	36
Chloramphenicol	30	48	52
PERFLOXACIN	30	0	100

Table 3. Antibiotics resistant pattern of *E. coli* isolates from school storage water sources

Antibiotics	Disc conc. (µg)	Resistance number (%)	Sensitivity number (%)
Tarivid	10	0	100
Ciprofloxacin	10	5.9	94.1
Saprofloxacin	30	5.9	94.1
Streptomycin	10	41.2	58.8
Gentamycin	10	35.3	64.7
Cotrimoxazole	30	52,9	47.1
Amoxicillin	30	41.2	58.8
Augmentin	30	76.5	23.5
Chloramphenicol	30	58.8	41.2
Perfloxacin	30	0	100

Table 4. Antibiotics resistant pattern of *E. coli* isolates from commercial water sources

Antibiotics	Disc conc. (µg)	Resistance number (%)	Sensitivity number (%)
Tarivid	10	0	100
Ciprofloxacin	10	0	100
Saprofloxacin	30	0	100
Streptomycin	10	66.7	33.3
Gentamycin	10	50.0	50.0
Cotrimoxazole	30	16.7	83.3
Amoxicillin	30	16.7	83.3
Augmentin	30	33.3	66.7
Chloramphenicol	30	33.3	66.7
Perfloxacin	30	0	100

Table 5. Frequency distribution of multidrug resistance (MDR) pattern of the isolated *E. coli*

Water sources	Number of *E. coli* isolated	Number of MDR *E. coli*	Percentage MDR %
Free water	17.0	14.0	77.8
Commercial water	6.0	4.0	22.2
Sachet water	2.0	0.0	0.0
Bottle water	0.0	0.0	0.0
TOTAL	**25.0**	**18.0**	**100**

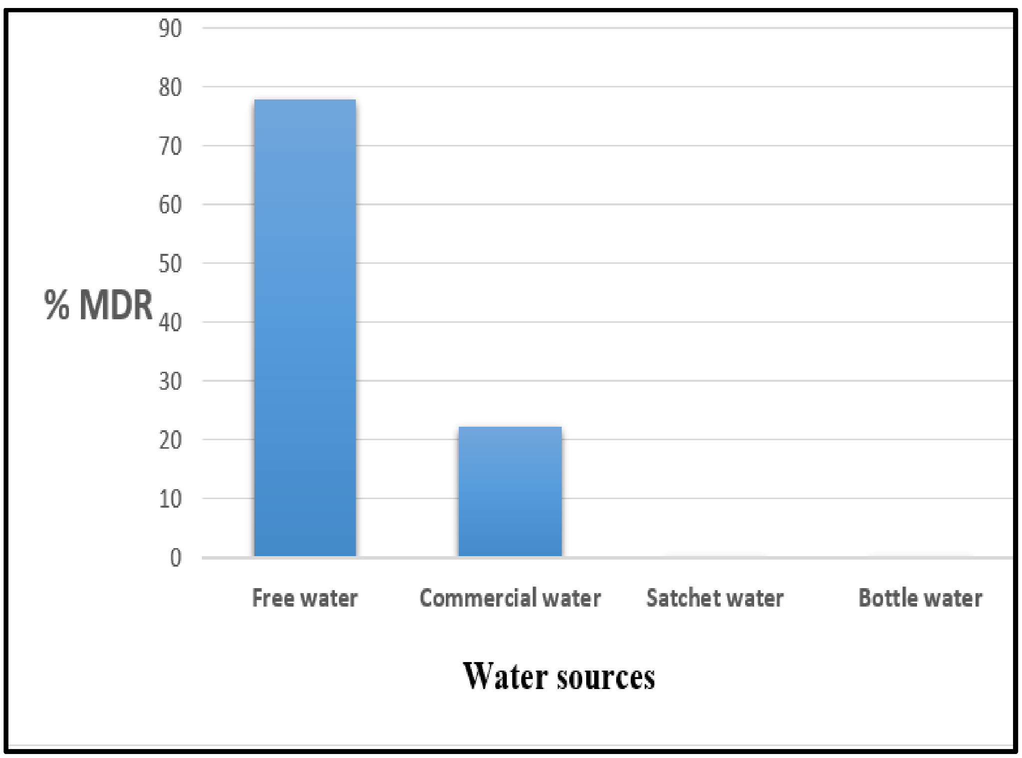

Fig. 1. Percentage distribution of multidrug resistance (MDR) *E. coli* from the various drinking water sources in UNEC student hostel

Out of 136 water samples' cultured, 25 samples yielded growth of *E. coli.* In a similar study done in Kaduna State, Nigeria, a total of 167 water sources for drinking were analysed 17 yielded growth of *E. coli* [28].The result of the present study was also consistent with the work of Larson *et al*. (2019), who also isolated *E. coli* from water sample in rural Andean Households in Cajamarca, Peru [29].

From the 25 *E. coli* isolated, the school storage tank water account for 60% thus had the highest number, this was followed by the commercial storage tank water 6 (24%) while there was no organism isolated from bottled water. In a documented report from Tamale Metropolis of Ghana, bottled water analysed for the presence of *E. coli* also showed no growth [30]. However, a contradictory report was noted in a study on bottled water from Isfahan, Iran [31].

The work of Chen et al., (2017) on the prevalence of multidrug resistant *E. coli* in drinking water sources in Hangzhou city shows a high prevalence of antibiotics resistant *E. coli* [32]. This is in line with the result of the present study, which showed resistance to the tested antibiotics (92%)Our finding showed that *E. coli* isolated were most resistant to Augmentin 16(64%) and chloramphenicol 12(48%). This is in line with works of Omololu-Aso *et al*. (2017) were *E.coli* isolated was most resistant to Augmentin and chloramphenicol (100%) [33] also this study is in line with the work of Chen et al. (2017) on the prevalence of multidrug-resistant *E.coli* in drinking water where *E.coli* isolates showed resistant to chloramphenicol (19%) [32]. The *E.coli* isolated from the commercial water sources showed the highest resistance to streptomycin 4 (66.7%). This is in line with the work of Aasmae *et al*., (2019) where the *E. coli* isolated showed high resistance to streptomycin [34].

Our finding showed a high prevalence of multidrug-resistant *E. coli* in drinking water sources in UNEC 18 (72%), this was in agreement with the work done by Okafor *et al.* (2019) on multidrug resistance *E. coli* isolated from drinking water from parts of Kaduna South, Kaduna State, the highest resistance was observed in Tetracyclin (80%) while all the isolates (20%) showed resistance to 7 antibiotics while 3 (30%) were resistant to 3 antibiotics respectively [28].

The highest multidrug resistance *E. coli* was from school storage water sources 14 (77.8%) followed by commercial water sources 4(22%). No MDR *E. coli* was isolated from both sachet and bottle water. This is not in line with the works of Momtaz et al. (2013) in the detection of *E. coli* in tap and packaged water in Isfahan, Iran, where *E. coli* was present in both sachet and bottle water [31].

The high prevalence of MDR *E. coli* in storage drinking water tanks could be attributed to the main sources of this water, because MDR *E. coli* was isolated from the main tankers that supply this water to the various school storage tanks. It could also be due to poor sewage system in UNEC because there is a lot of leaking sewages in UNEC campus and poor sanitation.

5. CONCLUSION

This study shows that drinking water sources in UNEC most especially the school storage water sources were generally of low quality and are reservoirs of Multidrug resistant *E. coli.* This could bring about a possibility of transfer of antibiotics resistance to other microbial organisms and this is a great risk to the health of students and the public at large.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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