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Isolation of Multidrug Resistant Bacteria from Commercial Bottled Water

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Abstract - Antibiotic resistance is one of the major problems in the world and as such most antibiotic-resistant bacteria particularly the multi drug resistant (MDR) bacteria, are associated with consumable water especially the commercially consumed water. The main objective of this study is to determine the presence of multidrug resistant bacteria in commercially produced water. Samples from twenty (20) commercial bottle water were collected from different water vendors and transported to the Microbiology Laboratory of Federal University of Technology, Minna. Inoculation of these samples was done by streaking on various media. Bacterial isolates were identified through Gram staining and various biochemical test. The identified bacterial isolates were subjected to antibiogram using disc diffusion method. The result revealed that sample D4 an E1 had the highest bacterial count ($0.02x10^2$). *Escherichia coli* had the highest predominance (33.3%) compared to other isolated bacteria. The study also revealed the presence of 50% of multidrug resistant *Staphylococcus* sp and 33.3% of multidrug resistant *E.coli* in commercial bottled water. Therefore, there is need for continuous surveillance of these bottled water to avoid the accumulation and growth of multidrug resistant bacteria in bottled water which when consumed, could cause infections that are difficult to curtail or control.

Keywords: Water; Bottled water; Bacteria; Multidrug resistant bacteria

Introduction

Water especially portable water is a necessity needed to sustain and maintain human life. Portable water is indispensable in human life (Mishra *et al*., 2018). Limited access to portable drinking water and inadequate sanitation have increased the percentage of humans suffering from water borne diseases such as diarrhea diseases, which is due to the presence of pathogenic microbes such as protozoans, viruses or bacteria, in both developed and developing countries (Onuoha, 2017; Mishra *et al*., 2018). The World Health Organization (WHO) estimates that 3.4 million people, die every year from water-related diseases (Abu and Wondikom, 2018) majorly caused by agents such as bacteria, of which many are resistant to various antibiotics.

Resistant microorganisms that withstand the effect of various antibiotics and are responsible for various diseases are basically a major threat to human health (Tawyabur *et al*., 2020). The hazards associated with such resistant microorganisms contaminating drinking water are aggravated by their abilities to resist destruction by antibiotics (Mulamattathil *et al*., 2014). The major risk for public health is that these multidrug-resistant genes may be transferred from microorganisms to humans through contaminated water causing infections that are difficult to treat by antibiotics (Abu and Wondikom, 2018).

Overtime consumable water especially those obtained from developing countries, have been exposed to certain contaminating agents and the accessibility of portable water by humans have proved difficult or almost impossible. This in turn has rendered most available drinking water as habitats of various pathogenic organisms, especially multidrug resistant bacteria. However, the consumption of multidrug resistant bacteria from contaminated water by most humans has led to various water borne diseases which are difficult to treat, prolonged stay of patients in the hospital, high morbidity which in turn leads to mortality (Chatterjee *et al*., 2020). Hence there is a need for adequate monitoring of water before it is consumed by the entire populace to ensure it is free from any microbial agents and this is the basis of this study.

Materials and Methods

1 Study Area

Samples were collected from various retail stores in Bosso Local Government area of Minna, North Central, Nigeria.

2 Collection of Samples

Samples were collected aseptically by swabbing the plastic bottles using a sterile swab stick. A total of twenty (20) bottled water cans, four (4) each from five (5) different companies was used. The samples were taken to the Microbiology Laboratory of Federal University of Technology, Minna for analysis.

Culture Media, Characterization and Identification of Isolates

Standard media such as Nutrient agar, MacConkey agar and *Salmonella- Shigella* agar were used to isolate various bacteria. Media to be used for this analysis were prepared according to manufacturer's instruction.

The isolated bacteria were identified via Gram staining and other conventional biochemical tests such as: Coagulase, Oxidase, Catalase, Citrate, Urease, Indole and Triple sugar test as described by Cheesbrough, (2010).

Sample	Bottle	Bacterial growth	Bacterial count
A	A1	-	-
	A2	-	-
	A3	-	-
	A4	-	-
B	B1	+	$0.01x10^2$
	B2	-	-
	B3	-	-
	B4	-	-
C	C1	-	-
	C2	-	-
	C3	-	-
	C4	-	-
D	D1	+	$0.01x10^2$
	D2	-	-
	D3	-	-
	D4	+	$0.02x10^2$
E	E1	+	$0.02x10^2$

Antibiotic susceptibility test was carried out and Multiple Antibiotic Resistance Index (MARI) of each isolate was determined using the formula first described by Krumperman (1983).

Molecular Characterisation of Multidrug Bacteria

The DNA of the resistant bacterial isolates were extracted using the protocol stated by (Trindade *et al*., 2007). This was followed by the detection of 16S rRNA gene using polymerase chain reaction (PCR) as described by (Frank *et al*., 2008). The PCR products were amplified using Agarose gel electrophoresis.

Each sequenced gene was uploaded in national center for biotechnology information (NCBI) - basic local alignment search tool (BLAST) (that the NCBI-gene bank) for sequence identification (Frank *et al*., 2008).

The genetic amplification of various resistant genes such as β-lactamase genes (*blaTEM, blaSHV, blaCTX-M)* and aminoglycoside resistance-associated genes (*aacC1* and *aacC2*).

3 Result and Discussion

Out of all the twenty (20) samples of bottle water, seven (7) samples of bottle water showed microbial growth.

Table 1: Bacterial count of various commercially consumed bottle water

E2	+	$0.01x10^2$
E3	+	$0.01x10^2$
E4	+	$0.01x10^2$

isolates code	Antibiotic Resistance Pattern	MAR I	Resistance Category
BW-2	S, AMX, GEN, APX, CH, LEV	0.8	MDR
BW-5	NA, GEN, AUG, CEP, PN	0.6	MDR

Key: - = No growth, + = growth occurred.

Escherichia coli was found to occur more than all the other organisms. The percentages of these organisms according to their predominance were *Escherichia coli* (33.3%), *Staphylococcus sp*. (22.2%), *Salmonella sp*. (22.2%), *Bacillus sp*. (22.2%).

Fifty percent (50%) of *Staphylococcus sp.* were resistant to Chloramphenicol, Aminoglycosides, Fluoroquinolones, and the Beta-lactam classes and thirty-three percent (33.3%) of *Escherichia coli* were resistant to Nalidixic Acid, Aminoglycosides and Beta-lactam classes as seen in Table 2.

Table 2: Frequency of Occurrence and Percentage of Multidrug Resistant Bacteria

Organisms	Frequency of occurrence	Percentage of occurrence (%)	Number of MDR bacteria	% of MDR bacteria
Staphylococcus sp.	2	22.2	1	50
Escherichia coli	3	33.3	1	33.3
Bacillus sp.	2	22.2	0	-
Salmonella sp.	2	22.2	0	-
Total	9	100	2	

Table 3: Resistant Pattern multiple antibiotics resistance index (MAR I) of various isolated bacteria from various commercialized bottle

Key:S=Streptomycin, AMX= Amoxicillin, GEN= Gentamycin, APX= Ampicillin, CH= Chloramphenicol, LEV= Levofloxacin, NA= Nalidixic acid, AUG= Augmentin, CEP= Ceporex

Amplification of the 16s rRNA region in the selected multidrug resistant bacterial isolates

Plate I presents the electrograph of various multidrug resistant bacterial isolates amplified gene (lane A and B). The DNA of the isolates amplified at 1500bp indicated pure bacteria isolates. Lane MK represents the molecular marker (ladder) (Plate I).

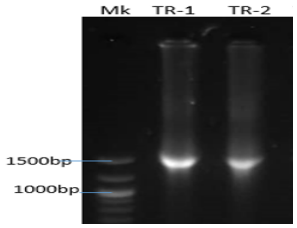

Plate I: Agarose Gel Electrophoresis Indicating the Positive Amplification of the 16s rRNA Gene Fragment used for Bacteria Identification

The presence of a 1500bp indicates positive amplification.
Key: TR1= BW-2; TR 2= BW-5

Molecular detection of various genes coding for various bacterial resistance

The molecular analysis of various bacterial resistance genes in two (2) bacterial isolates is shown in Plate II –VI. These results revealed electrographs of PCR products of various resistant genes.

Molecular detection of *blaTEM*-coding Genes

The electrograph (plate II-VI) shows that all the resistant bacteria, haboured *bla TEM, SHV, CTX-M, aacC1* and *aacC2* gene.

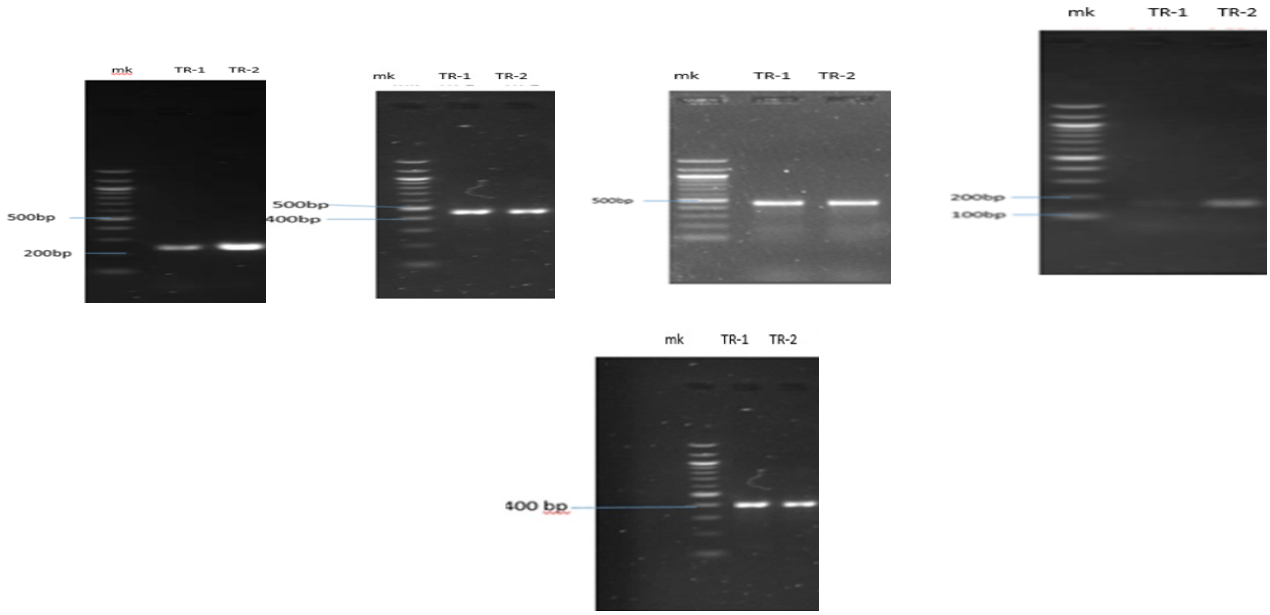

Plate II- VI: Agarose Gel Electrophoresis of the PCR Products of *blaTEM, SHV, CTX-M, aacC1* and *aacC2* Gene in Selected Bacteria Isolates

Lane 1: 1.5 Mb pair of the genomic DNA ladder

Lane 2: Isolate TR- 1shows a *blaTEM* , *SHV, CTX-M, aacC1 and aacC2* band with a gene size of 237bp, 470bp, 470bp, 169bp and 400bp

Lane 2: Isolate TR- 2 shows a *blaTEM* , *SHV, CTX-M, aacC1 and aacC2* band with a gene size of 237bp, 470bp, 470bp, 169bp and 400bp

4 Discussion

This study revealed that sample D4 and E1 had the highest bacterial count ($0.02x10^2$). This could be based on certain factors such as; poor treatment of water from its source before it was dispensed into the various commercial bottles, poor compliance to poor hygiene standards of the producers, poor packaging standards of the water and the expiration of packaged bottle water which facilitates the growth and development of bacterial contaminants in the bottles of commercially produced water, which leads to epidemics such as typhoid fever, dysentery and cholera after direct consumption by the consumers. This finding agrees with Adesanya *et al.* (2017), Odonkor and Addo, (2018), Mishra *et al*. (2018), Onuoha (2017).

Escherichia coli had the highest predominance (33.3%) compared with the other isolated bacteria. This could be based on the fact that most sources of these commercially produced bottled water were contaminated from fecal matters of both man and animal sources. The presence of *Escherichia coli* as an indicator in a water body depicts that the water body was recently contaminated with various fecal contaminants, which were deposited directly into the water bodies or through various

environmental means such as wind or rain. The presence of *Escherichia coli* found in some of the plastic bottles containing consumable water samples may spell health hazards such as diarrhoeal diseases which account for some of the health breakdown and mortality among adults and children which agrees with Odonkor and Addo, (2018).

In various environmental settings, MARI value below or equal to 0.2 implies low exposure to antibiotics, whereas, MARI value greater than 0.2 implies high level exposure to antibiotics (Akande and Onyedibe, 2019). In this study, the high multiple antibiotic resistance indices among the isolates implies that the environment, particularly, the water bodies are major inhabitants of microorganisms especially the resistant pathogens, which enhance transfer of resistant gene among susceptible pathogens that are also co-inhabitants with these resistant pathogens. This is a major risk to public health, based on the fact that this process practice has led to high acquisition of antimicrobial resistance in various pathogens within the community settings and its environs (Akande and Onyedibe, 2019).

The molecular analysis revealed that all the resistant organisms harboured genes coding for resistance to the aminoglycosides and betalactam antibiotics. This therefore implies that such pathogens harbour genes resistant to multiple antibiotics in the study area. Similarly, such pathogens are also said to harbour genes coding for betalactamase, which helps to degrade betalactam drugs in the study area and this in turn is a major threat to the general public.

Conclusion and Recommendation

Samples of various commercially produced bottle water were observed to be contaminated with various bacterial isolates. The bacterial with the highest predominance was *Escherichia coli* (33.3%), followed by *Staphylococcus* sp. (22.2%), *Bacillus* sp. (22.2%) and *Salmonella* sp. (22.2%). This result also revealed the presence of multidrug resistant organisms among *Escherichia coli* and *Staphylococcus* sp., thus there is an eminent need for Government and Food and water safety agencies in developing countries, to continuously employ adequate surveillance on various water bodies in the environment. To ensure that most water packaged in commercial bottles and are made available for public consumption is completely free from microorganisms, particularly resistant organisms, which when consumed along the bottle water could be a major threat to the general public thereby resulting to infections difficult to curtail or control.

References

[1] G. O. Abu and A. C., Wondikom. Isolation, characterization and antibiotics resistance profile studies of bacteria from an excavated pond in Port-Harcourt Metropolis, Nigeria. *Journal of Applied Sciences and Environmental Management*, vol 22, no (8), pp. 1177-1184, 2018

[2] K. M. Adesanya, K.O. Adekoya, L. A. Ogunkanmi and B.O. Oboh . Identification and Characterization of Bacteria Isolated from Pipe-borne Water Samples in Selected Areas of Lagos Using Api kits and RAPD-PCR. *Egyptian Academic Journal of Biological Sciences*, vol 9, no 1, pp.85-92, 2017.

A. Adesoji, A. Ogunjobi, I. Olatoye, and D. Douglas. Prevalence of tetracycline resistance genes among multi-drug-resistant bacteria from selected water distribution systems in Southwestern Nigeria. *Annals of Clinical Microbiology and Antimicrobials*, vol 14, no 1, pp. 1-8, 2015.

[3] Akande, and K.I. Onyedibe . First report of enteropathogenic and enteroinvasive *Escherichia coli* with multiple antibiotic resistance indices from African catfish (*Clarias glariepinus*) in Nigeria. *African Journal of Clinical and Experimental Microbiology,* vol 20, no 2, pp. 95 – 103, 2019.

[4] J. Ahmed, L.P. Wong, Y.P. Chua, and N. Channa. Drinking Water Quality Mapping Using Water Quality Index and Geospatial Analysis in Primary Schools of Pakistan. *Water*, vol 12, no 12, pp. 3382,2020

[5] R. Chatterjee, D. Singh, S. Tripathi, A. Chauhan, M.L. Aggarwal, and A.Varma. Isolation and characterization of multiple drug resistant human enteric pathogens from sewage water of Delhi. *Nature environment and pollution technology*, vol 20, no 2, pp.569-578, 2021

[6] J.A. Frank, C.I. Reich, S. Sharma, J.S. Weisbaum, B.A. Wilson and G.J. Olsen. Critical evaluation of two primers commonly used for amplification of bacterial16S rRNA genes. *Applied and Environment Microbiology*, vol 74, no 8, pp.2461-2470,2008

[7] P.H. Krumperman. (1983). Multiple antibiotic resistance indexing of Escherichia coli to identify high risk sources of faecal contamination of foods. *Appl. Environ. Microbiol*., vol 46, no 1, 165–170,1983

[8] S.G. Mulamattathil, C. Bezuidenhout, M. Mbewe, and C.N. Ateba, C. N. Isolation of environmental bacteria from surface and drinking water in Mafikeng, South Africa, and characterization using their antibiotic resistance profiles. *Journal of Pathogens*, vol 2, pp.1-4, 2014

[9] M. Mishra, A.P. Arukha, A.K. Patel, N. Behera, K.T. Mohanta, and D, Yadav. Multi-drug resistant coliform: Water sanitary standards and health hazards. *Frontiers of pharmacology*, vol 9, pp. 311, 2018

[10] S.C. Onuoha. Isolation and characterization of multidrug resistant bacterial species from selected water sources in Izzi Area, Southeastern Nigeria. *World Applied Sciences Journal,* vol 35, no 1, pp. 27-32, 2017.

[11] S.T. Odonkor, and K.K. Addo. Prevalence of multidrug-resistant *Escherichia coli* isolated from drinking water sources. *International Journal of Microbiology*, vol 2, pp.18, 2018.

[12] M. Tawyabur, M. S. Islam, M. A. Sobur, M.J. Hossain, M.M. Mahmud, S. Paul, H. T. Muhammad, M.H. Ashour, and T. Rahman. Isolation and characterization of multidrug-resistant *Escherichia coli* and *Salmonella spp*. from healthy and diseased turkeys. *Antibiotics*, 9(11), 770, 2020

[13] L.C. Trindade, E. Marques, D.B. Lopes and M. A. S. V. Ferreira. Development of a molecular method for detection and identification of *Xantomonas campestris* pv. *Viticola. Summa phytopathologica*, vol 33, no1, pp. 16-23, 2007