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Detection of multidrug resistant coliforms in drinking water samples in Madorawa community in Sokoto State, Nigeria

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ABSTRACT

Coliforms especially the faecal coliforms are well established indicators of water quality and their presence in drinking water sources is indicative of the presence of water –borne pathogens. This study was conducted to evaluate the occurrence of multiple antibiotic resistant (MAR) coliform in different drinking water sources in Madorawa Community in Sokoto State. Water was aseptically sampled from public wells, borehole sources and sachet water plants of the Sokoto State University. Samples were transported immediately to the laboratory in Sokoto state university for microbiological analysis such as isolation, enumeration, and identification of isolated bacteria using standard microbiological techniques. Isolates were screened for antibiotic susceptibility and multiple antibiotic resistance using the Kurby Bauer technique. *Escherichia coli* and *Citrobacter* species were isolated from bore hole water source, while *Klebsiella pneumoniae* and *Escherichia coli* from WW1, *Citrobacter* specie, *Escherichia coli* and *Klebsiella pneumoniae* were also isolated from WW2. The findings suggest that the sachet-water produced in Madorawa MDSW and Sokoto state university are free of contamination and safe for consumption, however, water samples from wells and boreholes in this study showed high coliform counts which indicate high level of contamination and a potential risk of water borne illnesses *Klebsiella pneumoniae* isolated from Well 1, showed resistance (25%) to three antibiotics with MAR index of 0.5. Four species of *Citrobacter* were screened and screened for MDR against six antibiotics and none of the species showed resistance to the antibiotics tested. The findings of the study showed that the quality of Sachet water in the study area was safe for consumption and in compliance with WHO recommended limits while only well water showed the presence of MDR coliform.

Keywords: Coliforms, Well, Borehole, Antibiotic Resistance, Mar Index, Madorawa, Sachet Water

Introduction

According to the World Health Organization (WHO), approximately 827,000 fatalities are related to the unhygienic condition of water, inadequate and improper sterilization, and insufficient hygiene practices of water which lead to waterborne illnesses in low- and middle-income countries (WHO, 2019). Water contamination is attributed to large population of microbial consortium in water and recorded as the leading factor in 80% of human diseases in developing countries (WHO, 1993). Water sources face various contamination hazards, including the presence of animal faeces, the practice of open defecation, and pollution from on-site sanitation facilities like pit latrines and septic systems, as well as urban runoff (Okullo *et al*., 2017).

Water contamination caused by fecal matter from domestic and human excreta presents a notable public health hazard that can transmit different waterborne diseases through the fecal-oral route (Ashbolt, 2004:Kostyla *et al*.,2015).

Water is important for all living being, and crucial for the formation and rejuvenation of cells, and irreplaceable in the sustenance of life on our planet (Ball, 2017). Accordingly, a consistent and abundant provision of water is imperative for the survival of biological entities. Surface water bodies remain the main source of drinking water, accounting for nearly half of global demand (WHO, 2016). It's disheartening that many regions throughout the globe including Nigeria encounter difficulties in maintaining the quality of their water supplies (Ashbolt *et al*., 2004).

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The scarcity of treated and microbiologically safe portable water has led to the rising demand and adoption of packaged drinking water famously known as pure water which is accepted as a safe option than raw water from untreated sources. Its processing ranging from production, packaging and handling makes it susceptible to microbial contamination. Adetunji and Ibrahim (2014) about 70% of Nigerians depend on this water sources for daily consumption due to its perceived safety and adoptability, however the safety of this sachet water is not guaranteed. Studies have revealed various points of contamination throughout the process right from the source which is mainly boreholes or wells Eke (2016).

Coliforms are one of the largest group of bacteria of relevance in faecally polluted water, this poses a major public health concern. They are also known as model organisms as their presence is indicative of the prevalence of other potential pathogenic organisms, and their presence also signals water contamination. Coliform densities vary geographically and seasonally which leads to the lack of unusually uniform regulatory guidelines regarding water potability which leads to ineffective detection of these model organisms.

The major concern is the escalating prevalence of multidrug resistance in coliform which renders antibiotic ineffective therapy in effective. Antimicrobials are increasingly used in households, clinics veterinary and agricultural settings, sub-optimal concentrations of these antimicrobials are intentionally but regularly dispersed into the environments through seepages, sewages or runoffs thus substantially adding to the ever increasing pool of antibiotic resistant genes that the coliform readily stimulate and further propagate to pathogens. Severity of which is evidenced by the high multiple antibiotic resistance (MAR) shown by bacterial isolates. This study focuses on the detection of multidrug resistant (MDR) coliform bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter*s pp, in public well water, borehole water and sachet water sampled from Madorawa in Bodinga Local Government Area of Sokoto State. The importance of this study is to ascertain the quality of water in Madorawa, as the presence of enteric bacteria is associated with faecal contamination, especially *Escherichia coli* which is used as a standard in water analysis.

Materials and Methods

Sample Collection

Drinking water samples were aseptically collected from public well water, borehole water and from the Sokoto State University sachet water plant source in sterile 100ml sample bottles on weekly basis for three weeks. Collected samples were transported immediately to the laboratory for further processing and analysis. A total of fifteen samples was collected and three in each sample source was used for this study.

Determination of Total Coliform Counts in Water Samples

Total coliform was enumerated by multiple tube fermentation tests (APHA, 2005). Coliform count was determined using the three-tube assay of the Most Probable Number (MPN) technique. Presumptive coliform test was carried out using MacConkey broth. The first sets of three tubes were having a sterile 10ml Double Strength Lactose Broth (DSLB) and the second and third sets were having 10ml Single Strength Lactose Broth (SSLB).

All the tubes were sterilized. The three sets of the tubes received 10, 1 and 0.1 ml of water samples using sterile pipettes. The tubes were incubated at 37ºC for 24 hours for estimation of total coliforms for 24 hours and were examined for acid and gas production. Acid production was determined by colour change of the broth from reddish purple to yellow and gas production were checked for entrapment of gas in the Durham tube.

The MPN was then determined from the MPN table for the three set of tube (Dhawale & LaMaster, 2003).

Confirmatory Test for Coliforms

Confirmed test was carried out by transferring a loopful of culture from a positive tube from presumptive test into a tube of Brilliant Green Lactose Bile (BGLB) broth with Durham tubes. The tubes were incubated at 37ºC for 24 hours for total coliform and were observed for gas production (Adetunde, 2010).

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Completed Test for Coliforms

Completed test was carried out by streaking a loopful of broth from a positive tube onto Eosin Methylene Blue (EMB) agar plate for pure colonies.

The plates were incubated at 37ºC for 24 hours. Colonies developing on EMB agar were further identified. Colonies with green metallic sheen were confirmed to be coliform bacteria with rods shape (Adetunde & Glover, 2010).

Isolation and Identification of the bacterial Isolates

Macroscopic and Microscopic Examination

The macroscopic examination for physical morphology were performed based on the size, colour, texture, pigmentation, odour and consistency Microscopic and Biochemical Reactions The microscopic examination was carried out through Gram staining and biochemical tests was carried out according to Cheesbrough (2006) and Forbes *et al*. (2002).

Biochemical Tests

Indole, Methyl red, Voges Proskeur, and citrate utilization, tests were the confirmatory test carried out to identify all the isolates and results were then matched with the Bergey's manual of determinative bacteriology for confirmation (William, 1994).

Gram's Staining

Thin smear of the suspected coliforms isolates was made on a grease free slide and were fixed under burning flame. A crystal violet solution was applied to cover the smear for 30 seconds and washed using distilled water. Secondly, lugol`s iodine was applied to the surface for 30 seconds. Acetone was used to decolorize the stain and lastly Safranin was applied for one minute, which was washed and allowed to dry and view under oil immersion lens. Presences of purple cell indicate Gram-Positive and reddish -pink cells indicate Gram-Negative (Oyeleke & Manga, 2008).

Indole Test

One percent (1%) tryptophan broth in a test tube was inoculated with a bacterial colony. After incubation period of 37ºC for 48 hours, one millilitre (1 ml) of chloroform was added to the broth.

The test tube was shaken gently, then 2.0 ml of Kovac's reagent was added and this was also shaken gently and allowed to stand for 20 mins, after which each tube was observed for the formation of red colouration at the top layer, indicating a positive result (Oyeleke & Manga, 2008).

Citrate Utilization Test

The inoculum was aseptically picked from the centre of a discreet colony and streaked back and forth onto the surface of freshly prepared slant of Simmon citrate medium on a test tube. The cultured test tube was thereafter incubated for 48 hours at 37ºC. Tubes were observed for the development of deep-blue colouration after 48hrs of incubation (Oyeleke & Manga, 2008).

Methyl Red-Voges-Proskauker (MR-VP) Test

Five millilitres (5 ml) of MRVP broth were inoculated with the test organism with a syringe and incubated for 48 hours at 37^0C after which, one millilitre (1 ml) of the broth was transferred into a small test tube. Three drops (3 drops) of methyl red were added to the tubes and shaken gently. A red colour on the addition of the methyl red indicator signified a positive test while yellow colour signified a negative test. To the rest of the broth in the original tube, five (5) drops of 4% potassium hydroxide (KOH) was added, followed by fifteen (15) drops of 5% α-naphtol in ethanol. The test tube (sealed with cotton plug) was shaken and placed in a sloping position. The development of a red colour starting from the liquid-air interface within 1 hour indicated a VP positive result while no colour change indicated VP negative (Oyeleke & Manga, 2008).

Antibiotic susceptibility

Antibiotic susceptibility studies of coliforms isolated from water samples namely *E. coli, Citrobacter and Klebsiella* spp. to different classes of antibiotics was performed by disc diffusion method. Sterile Mueller-Hinton agar was prepared and a 0.5 MacFarland equivalent standard of the test organisms was streaked on the surface of the agar and allowed for 20 min to pre-diffuse. The following antibiotics disc, Amoxicillin (30µg), Gentamicin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (30µg), Septrin (30 µg), Augmentin (10 µg) streptomycin (30 µg), Perfloxacin (30 µg), Ofloxacin (10 µg), Specifloxacin (10 µg, were placed on the surface of agar plates with a sterile forceps. These were incubated at 35°C for 18 - 24 h, after which the inhibition zone diameter in (mm) was taken and interpreted using CLSI standard (Bauer *et al.,* 1966).

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Results

The results of the cultural and biochemical characteristics of coliform bacteria isolated from different water sources in Madorawa Community in Sokoto State are presented in Table 1. While results of other microbiological analysis are presented in Tables 2 to 6.

Table 1: Cultural and biochemical characteristics of coliform bacteria isolated from different water sources in Madorawa Community in Sokoto State

Isolate code	Colonial morphology on EMB	Gram reaction	TSI	Indole	Methyl Red	Voges Proskaur	Citrate	Urease	Oxidase	Inference
W 1-T1-1	Red colony	Gram-ve rods	A/A_{+G}	-	-	+	+	+	-	*K. pneumoniae*
W 2-T1-1	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
W 2-T1-2	Red colony	Gram-ve rods	A/A_{+G}	-	-	+	+	+	-	*K. pneumoniae*
W 2-T1-3	Red colony	Gram-ve rods	A/A_{+G}	-	+	-	+	+	-	*Citrobacter* sp
BH T1-1	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
BH T1-2	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
BH T2-1	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
BH T2-2	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
BH T2-3	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
BH T2-4	Red colony	Gram-ve rods	A/A_{+G}	-	+	-	+	+	-	*Citrobacter* sp
BH T3-1	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
BH T3-2	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
W1 T2-1	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
W1 T2-2	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
W1 T2-3	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
W1 T2-4	GMS	Gram-ve rods	A/A_{+G}	+	+	-	-	-	-	*E. coli*
W2 T3-1	Red colony	Gram-ve rods	A/A_{+G}	-	+	-	+	+	-	*Citrobacter* sp
W2 T3-2	Red colony	Gram-ve rods	A/A_{+G}	-	+	-	+	+	-	*Citrobacter* sp
W1 T3-1	Red colony	Gram-ve rods	A/A_{+G}	-	-	+	+	+	-	*K. pneumoniae*
W1 T3-2	Red colony	Gram-ve rods	A/A_{+G}	-	-	+	+	+	-	*K. pneumoniae*
W2 T2-1	Red colony	Gram-ve rods	A/A_{+G}	-	-	+	+	+	-	*K. pneumoniae*
W2 T2-2	Red colony	Gram-ve rods	A/A_{+G}	-	-	+	+	+	-	*K. pneumoniae*
W2 -T1-1	Red colony	Gram-ve rods	A/A_{+G}	-	-	+	+	+	-	*K. pneumoniae*
W2 -T1-2	Red colony	Gram-ve rods	A/A_{+G}	-	-	+	+	+	-	*K. pneumoniae*

Key: GMS = Green metallic sheen.

Table 2: Occurrence of coliform bacteria in different water sources in Madorawa Community in Sokoto State

Water source	Coliforms	Isolated and Identified bacteria
Borehole	Present	*E. coli* and *Citrobacter* sp.
Well-1	Present	*K. pneumoniae* and *E. coli*
Well-2	Present	*Citrobacter* spp., *E. coli, K. pneumoniae*
SSU Sachet water	Absent	Nil
MDSW	Absent	Nil

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Table 3: Antibiotic susceptibility pattern of *Citrobacter* species isolated from water sources in Madorawa Community in Sokoto State

Antibiotics (Potency) *n*=4	Susceptible(S) Number (%)	Intermediate (I) Number (%)	Resistant (R) Number (%)
Septrin SXT (30 µg)	4(100.0)	0(0.0)	0(0.0)
Chloramphenicol CH (30 µg)	4(100.0)	0(0.0)	0(0.0)
Sparfloxacin SP (10 µg)	4(100.0)	0(0.0)	0(0.0)
Ciprofloxacin CPX (30 µg)	4(100.0)	0(0.0)	0(0.0)
Amoxacillin AM (30 µg)	4(100.0)	0(0.0)	0(0.0)
Augmentin AU (10 µg)	4(100.0)	0(0.0)	0(0.0)
Gentamycin,CN (30 µg)	4(100.0)	0(0.0)	0(0.0)
Perffloxacin PEF (30 µg)	4(100.0)	0(0.0)	0(0.0)
Ofloxacin OFX (10 µg)	4(100.0)	0(0.0)	0(0.0)
Streptomycin S (30 µg)	1(25.0)	3(75.0)	0(0.0)

Table 4: Antibiotic susceptibility pattern of *Escherichia coli* isolated from water sources in Madorawa Community in Sokoto State

Antibiotics (Potency) *n*=12	Susceptible(S) Number (%)	Intermediate (I) Number (%)	Resistant (R) Number (%)
Septrin SXT (30 µg)	12(100.0)	0(0.0)	0(0.0)
Chloramphenicol CH (30 µg)	12(100.0)	0(0.0)	0(0.0)
Sparfloxacin SP (10 µg)	12(100.0)	0(0.0)	0(0.0)
Ciprofloxacin CPX (30 µg)	12(100.0)	0(0.0)	0(0.0)
Amoxacillin AM (30 µg)	12(100.0)	0(0.0)	0(0.0)
Augmentin AU (10 µg)	12(100.0)	0(0.0)	0(0.0)
Gentamycin,CN (30 µg)	12(100.0)	0(0.0)	0(0.0)
Perffloxacin PEF (30 µg)	12(100.0)	0(0.0)	0(0.0)
Ofloxacin OFX (10 µg)	12(100.0)	0(0.0)	0(0.0)
Streptomycin S (30 µg)	11(91.7)	1(8.3)	0(0.0)

Table 5: Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from water sources in Madorawa Community in Sokoto State

Antibiotics (Potency) *n*=8	Susceptible(S) Number (%)	Intermediate (I) Number (%)	Resistant (R) Number (%)
Septrin SXT (30 µg)	6(75.0)	0(0.0)	2(25.0)
Chloramphenicol CH (30 µg)	8(100.0)	0(0.0)	0(0.0)
Sparfloxacin SP (10 µg)	8(100.0)	0(0.0)	0(0.0)
Ciprofloxacin CPX (30 µg)	8(100.0)	0(0.0)	0(0.0)
Amoxacillin AM (30 µg)	6(75.0)	0(0.0)	2(25.0)
Augmentin AU (10 µg)	6(75.0)	0(0.0)	2(25.0)
Gentamycin,CN (30 µg)	6(75.0)	0(0.0)	2(25.0)
Perffloxacin PEF (30 µg)	8(100.0)	0(0.0)	0(0.0)
Ofloxacin OFX (10 µg)	8(100.0)	0(0.0)	0(0.0)
Streptomycin S (30 µg)	6(75.0)	0(0.0)	2(25.0)

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Table 6 : Multiple antibiotic resistant (MAR) index of coliform bacteria isolated from different water sources in Madorawa Community in Sokoto State

Pathogen	Number of isolates screened	Classes of antibiotics tested	MDR Number (%)	Classes of antibiotic resistant to	MAR Index
Citrobacter species	4	6	0(0.0)	0	0
Escherichia coli	12	6	0(0.0)	0	0
K. pneumoniae	8	6	2(25.0)*	3	0.5

*Source = Well-1

Discussion

The coliform isolated from the water samples in the study sites were *K. pneumoniae* with 8(33.3%) with the highest recorded from the WW1 (well waterv1). *Escherichia coli* recorded highest among all the coliforms reported from the study site with 12(50%) followed by *Citrobacter* spp with 4(16.7%) occurrence. *Citrobacter* species recorded the lowest from the study site with. 2(8.33%). Occurrence with the highest from the WW2 and one from the BH (table5) were recorded. This is in conformity with the report of Asionye *et al*. (2023) whose report identified the following bacterial flora associated with the borehole and surface water samples were: *Bacillus* sp., *Escherichia* sp., *Staphylococcus* sp., *Streptococcus* sp., *Shigella* sp., *Proteus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Vibrio* sp. and *Micrococcus* sp. *Escherichia coli* is an indicator of feacal pollution. *Shigella* and *Salmonella* are enteric organisms responsible for Shigellosis and Salmonellosis. Furthermore, Eboh *et al*. (2017) corroborated our study in which the organisms namely *Escherichia* sp., *Enterobacter* sp., *Alcaligenes* sp., *Klebsiella* sp., *Staphylococcus* sp., *Bacillus* sp., *Proteus* sp., *Micrococcus* sp., *Serratia* sp., *Acinetobacter* sp., *Alcaligenes* sp. and *Pseudomonas* sp. were obtained from groundwater at Ukwuani LGA in Delta State. A previous study by Allamin *et al*. (2015) identified a high concentration of faecal coliforms in groundwater in the Kaduna metropolis. *Escherichia coli, Salmonella* sp., *Vibrio*, and *Enterobacter faecalis* have been implicated in cases of gastroenteritis. *Salmonella* sp. can lead to either typhoidal or non-typhoidal salmonellosis with varying degrees of fatalities (WHO, 2018). Basic source-tracking approaches can indicate the possible trajectory for the contamination of the well water.

Analysis of susceptibility pattern of *Citrobacter* species isolated from this study indicates that, all are susceptible to the antibiotics tested (100%) except for Septrin in which it shows exclusive resistant (75%).*Escherichi coli* was also (100%) susceptible to all the antibiotics tested except against Septrin with 83% resistance while susceptibility to *Klebsiella pneumoniae* differs with 100% against chloramphenicol as shown in Tables 3 - 5.

Four species of *Citrobacter* were screened for MDR against six antibiotics and none of the species shows resistance to all the antibiotics in the study. Twelve species of *E. coli* were also tested against six antibiotics and shows no resistance to all the antibiotics as seen in table 6. Eight isolates of *Klebsiella pneumoniae* in this study showed resistance (25%) to only three antibiotics with MAR index of 0.5%. This value recorded indicates that there could be repeated exposure of antibiotics in the study area, it agrees with the report of Sara *et al.* (2024) in assessing resistance pattern of *Klebsiella* in surface water from northern Portugal, also contrary with the report of Afiukwa *et al*. (2018) in the assessment of the presence of antibiotic resistance coliform in sachet water.

Conclusion

The assessment of Sachet water quality in the study area showed that they are safe for consumption and in compliance with WHO guidelines. However, the total coliform count was zero in all the sachet water samples, while the well water and bore hole water sources had total coliform counts that exceeded the WHO limit for treated water but the values were within the WHO recommended limits for untreated drinking water. The analysis of the borehole water and well water in the study area showed that they are not safe for consumption as they harbour coliforms with multiple resistances to different classes of antibiotics.

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