ANTIBIOTIC SUSCEPTIBILITY PROFILE OF BACTERIA ISOLATED FROM DIFFERENT RECREATIONAL WATERS IN ABRAKA COMMUNITY, DELTA STATE, NIGERIA

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Abstract: The study was aimed at assessing the antibiotic susceptibility profile of bacteria isolated from recreational waters in Abraka community as well as the determination of physicochemical parameters and heavy metal contents of the waters. The sampled recreational waters were McCarthy, Rivotel and Mudi Beach, respectively. A total of thirty samples were collected and cultured using pour plate technique.Pure isolates obtained were subjected to susceptibility testing using commercial antibiotics disc by Kirby-Bauer method. Thirteen bacteria genera were isolated which included *Staphylococcus* sp., *Escherichia coli*, *Proteus* sp., *Shigella* sp., *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp., *Pseudomonas* sp., *Citrobacter* sp., *Enterobacter* sp., *Clostridium* sp., *Streptococcus* sp. and *Enterococcus* sp. The total bacterial count ranged from 1.94 ± 0.46 to $2.21 \pm 0.22 \times 10^3$cfu/ml for Rivotel and McCarthy beach respectively which showed a significant difference ($p< 0.001$) across sampling sites. Coliform counts ranged from 4.40 ± 0.33 to $5.94 \pm 0.24 \times 10^3$. Antibiotic test of Gram positive bacterial isolates showed that ofloxacin (88%) and pefloxacin (88%) were the most susceptible antibiotics while Augmentin (0%) was the least sensitive antibiotic. Whereas the Gram positive antibiotic disc showed high level of potency to the isolates with erythromycin having the highest activity while zinnacef (60%) and gentamycin (60%) were the least active antibiotics from the study. Generally, the high bacteria and coliform counts observed in the study suggest possible contamination of the water samples which could pose dangerous health effects to their users.

Keywords: Antibiotics, potability, recreational, susceptibility, water

Introduction

Water is a basic necessity of man for drinking, domestic and recreational purposes. It is often exposed to several kinds of pathogenic microbes and harmful chemical substances termed as contaminants, which when not properly removed or treated can cause various disease outbreaks (Cabral, 2010; Idibie *et al.*, 2018). Gastroenteritis is a common symptom of infection arising from consumption of contaminated waters especially among young children compared to adults, this is because children overplays and tend to swallow more water during swimming (Denno *et al.*, 2009; Lihan *et al.*, 2017).

Microbes, such as bacteria, protozoa and viruses are the primary source of microbiological contamination of water sources. The major routes of exposure to these pathogenic microbes in water bodies are either by contact, ingestion, or inhalation thereby resulting to detrimental health effects (USEPA, 2012). Also, in public health perspective, water quality enhancement and safety measures will boost the economy most especially in tourist zones. Proper management of these recreational waters will entail adequate surveillance of both the microbiological, physical and chemical qualities of the waters. These mechanisms are however not easy to be conducted due to microbial diversity, their various infectious form, as well as limited availability or unaffordable cost of non-culture based or molecular techniques within the country, Nigeria.

Furthermore, resistant of bacteria strains to antibiotics has been investigated over the years by many researchers on various water compartments such as surface water, ground water, sediments, waste water and soils. It becomes necessary to assess the presence of these pathogenic bacterial genera in the water bodies for antibiotic susceptibility pattern. Water bodies are usually colonized by bacterial and fungal strains of faecal origin reason they thrive and proliferate easily in water system (Denno *et al.*, 2009; Lihan *et al.*, 2017; Idibie *et al.*, 2018).

Although the recreational waters serve as tourist zone in Abraka community, the indigenes and its users are uninformed about the potential risk associated with pathogenic bacteria genera that may cause waterborne diseases. Also, just little study has been conducted on the issue of resistance of bacteria strains to many antibiotics from recreational waters in Abraka communities. The above findings was what led to the motivation to investigate the antibiotic susceptibility profile of bacteria genera's isolated from selected recreational waters in Abraka community, Delta State, Nigeria.

Materials and Methods

Study area

The Study was conducted at McCarthy Beach, Rivotel Beach and Mudi Beach, in Abraka, Ethiopia East Local Government Area, Delta state. Abraka is located between 6 to 15˚C North and 5˚C East of Delta state, Nigeria.

Sample collection

A total number of thirty (30) water samples were collected from three (3) recreational waters (McCarthy Beach, Rivotel Beach and Mudi Beach) in Abraka, Delta state, Nigeria between February to September except the lockdown period of April and May 2020. Ten (10) each of the water samples were randomly collected from Mudi, MacCarty and Rivotel beaches. The samples were collected with the aid of sterile plastic containers after which they were transported to the microbiology laboratory.

Sample processing, isolation and identification

The analysis was carried out based on the principles of pour plate techniques. In this method, one ml of the various water samples were drawn with the aid of a pipette into appropriately labeled empty petri dishes and sterile molten agar was added after which they were shaken and to stand on the laboratory bench for uniformity. Each plate was then incubated at 37ºC for 24 h. Further subculture was carried out to obtain pure isolates. The allowed organisms were identified on the basis of Gram staining, motility, and relevant biochemical test (Cheesbrough, 2009).

Antimicrobial susceptibility test

The test for antibiotic susceptibility was performed using Kirby-Bauer disc diffusion technique. The identified isolates were emulsified into a peptone water solution, thereafter incubation took place for 18 h after which growth (turbidity) was observed. Further steps involve inoculation of the bacterial isolates with the aid of a sterile swab stick onto a freshly prepared and solidified nutrient agar plates. At 5mins of drying the plates, with the aid of a sterile forcep, the antibiotic discs were carefully placed on the surfaces of the agar plates. This was followed by incubation at 37ºC. The zones of inhibition were measured following the guidelines of the Clinical Laboratory Institute (CLSI, 2016).

Isolation of coliforms

The process of coliforms isolation was done using membrane filtration technique. 50ml size of a membrane filter funnel was used. The funnel was placed on top of a holder attached to a vacuum pump which permits the outflow of water via the sterile and porous membrane filter (0.45 µm). Thereafter, with the aid of a sterile forcep, the membrane filters were placed on the already prepared and solidified McConkey agar plates which were incubated after outflow of 100 ml of water sample.

Physicochemical parameters and heavy metals

Physicochemical properties and heavy metal composition of the various water samples was examined in the study at the Nigerian Institute for palm Oil research (NIFOR), following the World Health Organization Standard (WHO). The Temperature, pH, Conductivity, Total Dissolved solids, Total soluble solids, Total hardness, biological oxygen demand and alkalinity were determined at the point of collection using the HANNA combo (H198130) instrument attached to the electrodes to the various meters at the bottom of a sterile beaker containing the water samples. Other parameters such as iron, lead, and nickel were examined at the chemistry laboratory section of NIFOR. The Agilent (240) FS flame atomic-absorption spectrophotometer digested with 55% nitric acid was used to examine the heavy metal composition of the various water samples. All chemicals and reagents employed in the study was of laboratory standard and needs no further purification processes (APHA, 1998)

Statistical analysis

SPSS version 21.0 with the aid of Microsoft excel 2014 was used to analyze the data. Descriptive statistics and ANOVA were used. The values ($p < 0.05$) were noted as statistically significant (Ogbeibu, 2005)

Results and Discussion

Table 1 presents the total bacteria and coliform counts of the water samples. The counts ranged from 1.94 ± 0.46 to 2.21 ± 0.22 × 10^3 CFU/ml. This is far more than the recommended limit of 1.2 ×10^2cfu/ml of the WHO standard (2011). The results corroborate with the findings of Okonkwo *et al.* (2008) who worked on the microbiological and physiochemical analysis of different water samples used for various domestic purposes in Abeokuta, Ogun state and also in Ojota, Lagos state, Nigeria. However, it is important to point out that the bacterial diversity of McCarthy Beach (10 isolates) is relatively compared with Rivotel (6) and Budi Beach (6), respectively. This might be due to the presence of high organic matter or could be connected to high number of McCarthy Beach users (Benwo, 2006). The total coliform count ranging from 4.40 ± 0.33 to 5.94 ± 0.24× 10^3 cfu/ml for Rivotel and MacCarty beach, respectively. This is also not within the allowable limit (1.2 ×10^2cfu/ml) of the WHO. This therefore disagrees with the findings of Okonkwo (2008). The main sources of coliforms are from dead insects, rodents and faecal matter (Magnus, 2011).

Table 1: Total mean bacterial and coliform counts of the beach water samples (×10^3 Cfu/ml)

Recreational waters	Mean bacterial count	Mean coliform count
McCarthy	2.21 ± 0.22	5.94 ± 0.24
Rivotel	1.94 ± 0.46	4.40 ± 0.33
Mudi	2.02 ± 0.15	4.87 ± 0.55

P-value P<0.05

Table 2 reveals that Thirteen (13) bacterial isolates were identified phenotypically from the various sampled recreational waters which include: *Staphylococcus* sp., *Escherichia coli*, *Proteus* sp., *Shigella* sp., *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp., *Pseudomonas* sp., *Citrobacter* sp., *Enterobacter* sp., *Clostridium* sp., *Streptococcus* sp. and *Enterococcus* sp. Similar organisms were reported by Oluyega *et al*. (2020) from the findings of antibiotic resistance pattern of bacteria isolated from recreational waters in Ado-Ekiti metropolis.

Table 2: Identification of bacterial isolates

Gram reaction Reaction	Shape	Oxidase	Catalase	In dole	Motility	Citrate	Glucose	Lactose	Sucrose	H_2S	Acid	Gas	Urease	Tentative bacteria Genera
+	Cocci	-	+	-	+	-	+	-	-	-	+	+	+	*Staphylococcus* sp.
-	Rod	-	+	+	+	+	+	+	+	-	+	+	-	*Escherichia coli.*
-	Rod	-	+	-	+	+	+	+	+	+	+	+	+	*Proteus* sp.
-	Rod	-	+	-	+	-	+	-	-	-	+	+	-	*Shigella*sp.
+	Rod	-	+	+	+	-	+	-	-	+	+	-	-	*Bacillus* sp.
-	Rod	+	+	-	+	+	+	-	-	-	-	+	+	*Klebsiella*sp.
-	Rod	-	+	-	+	-	+	-	-	+	+	+	-	*Salmonella* sp.
-	Rod	-	+	-	+	+	-	-	-	-	-	+	-	*Pseudomonas* sp.
-	Rod	-	+	-	+	+	+	+	+	+	+	+	+	*Citrobacter*sp.
-	Rod	-	+	-	+	+	+	+	+	-	+	+	-	*Enterobacter* sp
+	Rod	-	-	-	-	+	+	+	+	+	+	+	-	*Clostridium* sp
+	Cocci	-	-	-	-	-	+	+	+	-	+	-	-	*Streptococcus* sp.
+	Cocci	-	-	-	-	-	+	+	+	-	-	-	-	*Enterococcus* sp.

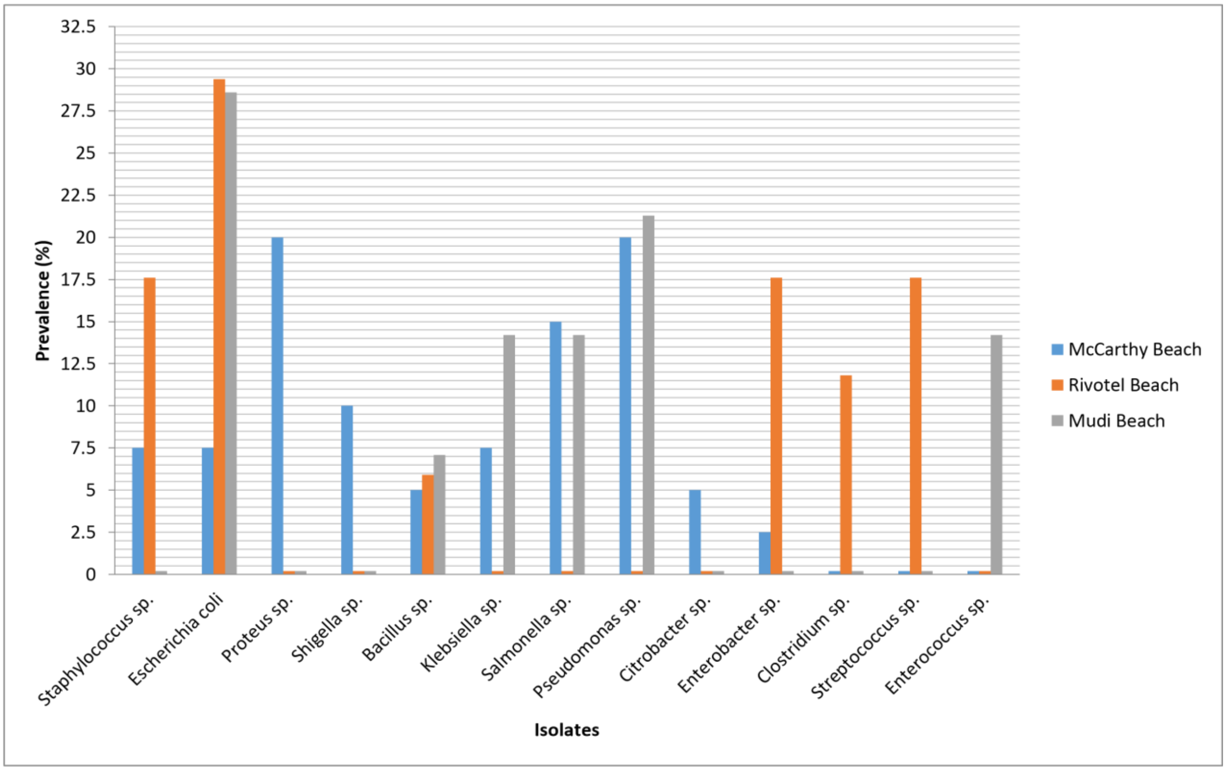

Fig. 1: Prevalence of bacteria isolates in all three (3) sampled recreational water

Figure 1 presents the prevalence of bacteria isolates from the sampled locations. The presence of *E. Coli*, is an indicator bacteria. The occurrence of these species may constitute a health hazard for the public use since swimmers or bathers may accidentally ingest the polluted waters in the course of swimming in those beaches, which in turn lead to serious water borne disease and infections such as cholera, typhoid and gastroenteritis. Similar study was conducted by Oluyega *et al.* (2020).

The result of antibiotic susceptibility profile of Gram negative bacteria isolates is presented in Table 3. It was observed that ofloxacin (88%) and pefloxacin (88%) were the most sensitive drugs followed by Gentamycin (75%) while augmentin (0%) appeared to be the least significant antibiotic when analyzed in the study. This report disagrees with the findings of Grigoryan *et al.* (2007) and Oluyege *et al.* (2020) who reported high level of resistance of pefloxacin and Gentamycin from their study. Thus, these drugs could represent a better drug of choice for the treatment of water borne diseases. On the other hand, results of the antibiotic susceptibility profile of the Gram positive isolates showed that virtually all treated antibiotics were sensitive to the isolates, with Erythromycin (100%) appearing to be the most sensitive drug followed by Ampiclox (80%), Ciprofloxacin (80%), Septrin (80%), Streptomycin (80%), while the least sensitive antibiotic were Zinnacef (60%) and Gentamycin (60%) . This study did not support the findings of Abas *et al.* (2019). Who carried out a study on the microbial quality and antibiotic sensitivity of bacteria isolates in Tuo Zaafi in the central business district of tamale. This could be attributed to different environmental factors or locations where these studies were being carried out.

Table 3: Antibiotic susceptibility profile of Gram negative bacteria isolates

Isolates	OFX	S	SXT	CH	SP	AM	AU	CN	PEF	CPX
Escherichia sp.	20(S)	15(S)	14 (R)	19(S)	18(S)	12(R)	12(R)	12(R)	20(S)	24(S)
Proteus sp.	24(S)	14(R)	14(R)	0(R)	0(R)	14(R)	0(R)	0(R)	26(S)	20(S)
*Shigella*sp.	14(R)	22(S)	22(S)	0(R)	20(S)	0(R)	0(R)	10(R)	19(S)	20(S)
*Klebsiella*sp.	19(S)	14(R)	14(R)	8(R)	20(S)	0(R)	0(R)	16(S)	20(S)	10(R)
Salmonella sp.	20(S)	12(R)	20(S)	0(R)	22(S)	18(S)	0(R)	10(R)	12(R)	26(S)
Pseudomonas sp.	20(S)	0(R)	0(R)	0(R)	4(R)	12(R)	0(R)	8(R)	24(S)	10(R)
*Citrobacter*sp.	22(S)	14(R)	10(R)	0(R)	0(R)	14(R)	0(R)	14(R)	22(S)	16(S)
Enterobacter sp.	22(S)	16(S)	14(R)	20(S)	18(S)	10(R)	11(R)	11(R)	19(S)	24(S)
	88%	38%	25%	25%	50%	13%	0%	13%	88%	75%

CH = Chloramphenicol (30ug), SP = Sparfloxacin (30ug), AM = moxacillin (30ug), AU = Augmentin (10ug), CN = Gentamycin (30ug), PEF = Pefloxacin (30ug), OFX = Tarivid (10ug), S = Streptomycin (30ug), CPX = Ciprofloxacin (30ug) and SXT = Septrin (30ug); S=Sensitive, R= Resistance

Table 4: Antibiotic susceptibility profile of Gram positive bacteria isolates

Isolates	APX	Z	AM	R	CPX	S	SXT	E	PEF	CN
Streptococcus sp.	17(S)	15(S)	19(S)	17(S)	14(R)	15(S)	19(S)	22(S)	18(S)	14(R)
Staphylococcus sp.	20(S)	14(R)	24(S)	20(S)	28(S)	24(S)	22(S)	20(S)	22(S)	24(S)
Bacillus sp.	12(R)	20(S)	14(R)	20(S)	30(S)	30(S)	20(S)	22(S)	20(S)	20(S)
Enterococcus sp.	20(S)	14(R)	18(S)	17(S)	22(S)	20(S)	17(S)	24(S)	20(S)	18(S)
	80%	60%	80%	80%	80%	80%	80%	100%	80%	60%

PEF= Pefloxacin (10 ug), CN=Gentamycin(10 ug), APX=Ampiclox (30 ug), Z=Zinnacef (20 ug), AM=Amoxicillin (30 ug), R=Rocephin (25 ug), CPX=Ciprofloxacin(10ug), S=Streptomycin(30 ug), SXT=Septrin(30ug), E=Erythromycin(10 ug), S= Sensitive, R=Resistance

Table 5: Physicochemical properties of the recreational water samples

Location	Temperature	pH	EC µS/cm	TDS mg/l	TSSmg/l	THmg/l	DOmg/l	BOD mg/l	Alkalinitymg/l
McCarty	27.7 ± 0.42	7.0 ± 0.24	81.7 ± 0.24	0.34 ± 0.45	16.2 ± 0.12	23.2± 0.55	2.4 ± 0.11	4.5 ± 0.22	1.5 ±0.31
Rivotel	27.3 ± 0.38	7.0 ± 0.56	86.5 ±0.33	0.42± 0.16	17.0± 0.15	25.5± 0.26	20.5±0.20	4.8 ± 0.34	1.5 ± 0.44
Mudi	27.5 ± 0.24	7.1 ± 0.24	83.4±0.28	0.37± 0.32	17.2 ± 0.34	22.4 ±0.27	17.4 ± 0.22	4.38 ± 0.56	1.4 ± 0.22
WHO Standard	30-35	6.5-9.0	400	1	30	60	73	43	200

P-value significant at> 0.05

Results obtained for temperature analysis showed that there were significant differences P < 0.05 observed for the temperatures across the sampled recreational waters. However, the temperature across the sampling sites were below the minimum stipulated limit of (30-35), set up by WHO (2011). Thus, the waters are for relaxation and other recreational activities. This could be due to the regular flow of water from other channels. The pH of water has to do with acid-base equilibrium and in nature; water is being regulated by the carbon dioxide - bicarbonate - carbonate equilibrium. The pH of the sampled locations were within the allowable limit of 6.50 - 8.50 set up by the WHO, (2011). The pH ranged from 7.0 ± 0.24 to 7.1 ± 0.24 mg/l. This therefore suggests a good condition of the recreational waters. Also, the waters is said to be safe for relaxation. The pH also had great influence on the bacteria diversity in the water due to the neutrality of the water. Ogbeifun *et al.* (2019) reported similar findings in respect to borehole water in Benin City, Edo state. The electrical conductivity ranged from 81.7 ± 0.24 to 86.5 ±0.33 µS/cm. These values were below the recommended limit of 400 µ/cm by WHO (2011). This reveals that there is low dissolved inorganic salt in the water table. Similar report on conductivity was carried out in Edo state by Iyasele and Idiata (2011). Total dissolved solids total soluble solids were also found to be within the recommended limit by WHO (2011). This also contributes to the level of bacteria population in the water body. The low values of the Total Dissolved Solids corroborates with the report of Ogbeifun *et al.* (2019). Dissolved oxygen indicates aerobic and anaerobic nature of biological activities in the water bodies. The low dissolved oxygen had great influence in the proliferation of aerobic bacteria observed in this study, which agrees with the report of Olaiya *et al.* (2016) who worked on the physiochemical and microbial risk assessment of some selected abbatoir operations in Etsako East and Central, Edo state, Nigeria. BOD reported in this study fell below the recommended limit of 40, set up by WHO (2011). The BOD across the sampling sites ranged from 4.38±0.56 to 4.80± 0.34 mg/l. The BOD observed might be due to the presence of decaying organic matter observed in the beach waters and run offs from the neighbouring communities, Efe *et al.* (2005). The level of alkalinity observed in this study was low compared with the recommended standard of 200 set by WHO (2011), this could be the reason why we have a neutral pH of the recreational waters hence, favourable proliferation of bacteria species (Nduka *et al.,* 2008).

Table 6: Concentrations of metal ions (mg/l) in recreational water by sampling sites (M±SE)

	Iron (Fe)	Lead (Pb)	Nickel (Ni)
McCarty Beach	0.01± 0.33	0.001± 0.22	0.001 ± 0.36
Rivotel Beach	0.02 ± 0.26	0.001 ± 0.18	0.001 ± 0.14
Mudi Beach	0.02±0.14	0.001 ±0.44	0.001±0.65
WHO Limit	0.03	0.01	0.01

Analysis of the heavy metal ions included iron (Fe), lead (Pb) and nickel (Ni). It was observed that all the heavy metal ions studied were less than the recommended limit across the various sampling stations. This therefore shows that the recreational waters are free from toxic substances that may cause dangerous health effects to its users. This report agrees with the work conducted by Odiana and Edosomwan (2019). WHO reported low levels of heavy metals from his study in Edo State. The findings in this study corroborates previous report on heavy metals in Abraka. The heavy metals studied were within permitted limit except for nickel and cadmium which were above WHO limit (Adomi and Morka, 2020).

Conclusion

Results obtained from the study revealed the values of the physiochemical and heavy metals content to be below the recommended limits by WHO, which therefore means that the water free of toxic chemical substances. Although, the study recorded high levels of total bacteria and coliform counts across the various sampled sites which therefore renders the water unsafe for its users. However, the antibiotic susceptibility study provided useful information in the search for safe and efficient antibiotics for the treatment of water borne diseases and infections.

Conflict of Interest

The authors declare that there is no conflict of interest reported in this work.

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