Int.J.Curr.Microbiol.App.Sci (2022) *11*(01): 93-103

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 01 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1101.012

Antibiogram of Putative Bacterial Pathogens Isolated from Some Water Bodies in Rivers State, Nigeria

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Keywords

Antimicrobial Susceptibility, Human Infections, Ogbogoro, Rumuolumeni

Article Info

Received:
02 December 2021
Accepted:
30 December 2021
Available Online:
10 January 2022

A B S T R A C T

Exponential increase in the fouling of surface water bodies through discharge of untreated domestic and industrial effluents and direct defecation is posing a big treat to surface water quality. This study was aimed at determining the population of pathogenic bacteria in the selected surface water during dry and wet seasons with a view to determine their level of antimicrobial resistance and susceptibility. Water samples were collected from three tributes of the New Calabar River along Ogbogoro, Rumuolumeni and Mgbuodohia which are located in the Niger Delta region of southern Nigeria. The samples were collected during dry and wet season. The sampling locations for each of the tributaries were near dredging site, near human waste disposal site and 300 meters away from the river bank destinated as general point. Bacteria isolation was carried out using standard spread plate method. Isolates were identified based on the morphology, biochemical/molecular characterization of the isolates. The isolates were subjected to antimicrobial testing using the disk diffusion method. Bacterial isolates from the three tributes were identified to belong to the following genera: C*itrobacter*, *Enterobacter*, *Bacillus*, *Vibrio*, *Klebsilla*, *Pseudomonas*, *Proteus*, *Staphylococcus*, *Shigella*, *Salmonella*, *Serratia*, *Chromobacterium and Escherichia coli*. *E.coli* had the highest occurrence in the three water sources followed by *Staphylococcus aureus*. Antimicrobial susceptibility test for *E.coli*, *Salmonella*, *Shigella* and *Vibrio cholera* isolates was performed using twelve commonly prescribed antibiotics: Amoxicilin Clavulanete (30µg), Cefotaxine (25µg), Imipenem/Cilastatin (10/10µg), Ofloxacin (5µg), Gentamycin (10µg), Nalidixic Acid (30µg), Ampiclox (10µg), Cefexime (5µg), Levofloxacin (5µg), Nitrofurantoin (300µg), Cefuroxime (30µg) and Ceftriaxone Sulbactam (45µg). The antibiogram showed 100% susceptibility of all the tested isolates from the three water bodies to Ofloxacin and Levofloxacin. *E.coli* isolates recorded 100% resistance to Cefuroxime, Cefotaxime, Nitrofurantoin and Ampicolx. *Salmonella* isolates recorded 100% resistance to Augmentin and Ampiclox. *Shigella* isolates recorded 100% resistance to Augmentin, Nitrofurantoin and Ampiclox. *Vibrio cholerae* isolates recorded 100% resistance to Cefotaxime, Nalidixic acid and Nitrofurantoin. The result showed that susceptibility of the bacterial isolates tested was generally less for the older and more widely used antibiotics. It is concluded that Ofloxacin and Levofloxacin can be the antibiotics of choice in the treatment of infections caused by these bacteria.

Introduction

Water is an essential resource, necessary for the sustenance of life. For humans, it is necessary for domestic, recreational and economic purposes. However, the fouling of water bodies is posing a threat on the usefulness of this essential resource. Increase in industrialization, urbanization and of course human population is a treat to global water quality as large amount of household and industrial waste are channeled to surface water (Koshy and Nayar, 1999). The stress on the physicochemical and microbial quality of surface water calls for serious policy on water management and monitoring globally (Dahunsi *et al.*, 2014).

Some of the bacterial pathogens responsible for human infections associated with use of contaminated water get to the water bodies through fecal contamination. They include *Salmonella typhi and Salmonella paratyphi* associated with typhoid fever and paratyphoid fever respectively (Cheesbrough, 2005), *Shigella* species causing bacillary dysentery, *Vibrio cholera* associated with cholera and some specific strains of *Escherichia coli* causing gastroenteritis (Cheesbrough, 2005). These bacteria pathogens are of great public health importance as infections arising from them can result in outbreaks of epidemics.

The pollution of water bodies through indiscriminate dumping of refuse, open defecation, seepage from waste dump sites and surface runoffs have been considered as a source of spread of antibiotic resistant genes amongst bacteria associated with water borne infections (Frederico *et al.,* 2002). Indiscriminate use of antibiotics enhances selection pressure which increases the antimicrobial resistance of surviving bacteria.

Continuous shedding of antibiotic resistant bacteria into water bodies through pollution is an important contributory factor to the spread of antibiotic resistance. The exponential increase in the emergence of antimicrobial resistance and spread of resistant bacteria as well as multidrug resistant bacteria has become a big challenge in the treatment and control of most common infections (WHO, 2018). For different bacteria the patterns of antibiotic resistance/susceptibility differs from one region to another (Talukdar *et al.,* 2005). This variance in antibiotic susceptibility pattern in different regions could be associated to the level of exposure of the environment to antimicrobial agents as well as intake of antibiotics by people in the regions involved.

Antibiotic resistance has emerged as a public health problem and global health challenge (CDC, 2017). There is therefore need to regulate the discharge of human waste, domestic waste and industrial effluents into water bodies. This study is aimed at determining the current level of antimicrobial resistance as well as susceptibility of these pathogens within the study area.

Materials and Methods

Study Area

The study area was New Calendar River that flows through; Ogbogoro, Rumuolumeni and Mgbuodohiain Rivers State, the Niger Delta region of Southern Nigeria. The New Calabar River has tributaries that lies between the coordinates latitude: 4.416667 and longitude 7.033333. Ogbogoro River GPS coordinates are latitude: 4.794381 and longitude: 6.976720.

Rumuolumeni River is located at coordinates 4.089361 and 6.928667. Mgbuodohia River GPS coordinates are latitude: 4.8188 and longitude 6.9664.The sampling locations in each of the rivers we're: near dredging site, near human waste disposal area and 300 meters away from the river bank, designed as General point.

Sampling Procedure

Water samples were collected within the first week of January and first week of March for the dry season samples and the third week of June and first

week of September for the wet season samples. Water collection was done twice in each season. Samples were collected from the three sampling locations from each of the tributaries. Pre-sterilized and pre-labelled bottles we're used collect the water samples and were transported to the laboratory in icebox. Analysis was done within two hours from the time of collection of the water samples.

Microbial Analysis

Bacterial isolation was carried out using selective and differential culture media and the spread plate method as described in Prescott *et al.,* (2005). Culture media used were *Salmonella - Shigella* (SSA) agar (Hi Media). Thiosulphate Citrate Bile Salt (TCBS) agar (Hi Media), Eosin methylene Blue (EMB) agar (Tm Media) and nutrient agar (Tm Media).

The SSA and TCBS agar plates were incubated at 37°C for 24-48 hours, while inoculated EMB agar plates were incubated at 44.5°C, after which the ensuring colonies were observed. Colonies typical of *Salmonella* sp., *Shiegella* sp., *Vibrio* sp. and *E.coli* were subcultured on nutrient agar plates for Gram stain and other identification tests. The identification test used include: indole, methylred, voges-proskeuer, citrate utilization, catalase, oxidase, urease motility and sugar fermentation. Molecular characterization of the isolates was also carried out using molecular based techniques.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out using the Kirby Bauer disc diffusion techniques as described in Adebayo-Tayo *et al.,* (2012). A 0.5 MacFarland's standard was employed in inoculum suspension preparations as recommended by the National Committee for Clinical Laboratory Standard (NCCLS) and Clinical and Laboratory Standards Institute (CLSI)(NCCLS, 2002; CLSI, 2012). Peptone water was prepared, five discrete colonies of the respective identified isolate were inoculated into 5ml of the broths and incubated at 35°C for 24 hours. After incubation, sterile normal saline was added to the broth culture until its turbidity matched the turbidity of 0.5 McFarland Standard. The diluted broth cultures having 0.5 McFarland turbidity, were then swab inoculated onto different Mueller-Hinton agar plates. Paper discs containing antibiotics were then aseptically placed on the swab inoculated agar plates. The plates were incubated at 37°C for 24 hours. Interpretation of result was done according to CLSI (2011).

Results and Discussion

Antimicrobial Percentage Susceptibility of *E.coli* isolates

Antimicrobial Percentage Susceptibility for *E.coli* isolates from the study 66 isolates (18 from Ogbogoro River, 25 from Rumuolumeni River and 23 from Mgbuodohia River) were tested against twelves (12) commonly used antibiotics which includes Cefuroxime(30ug), Amoxicillin Clavulanate (30µg), Cefotaxime (25µg), Imipenem/Cilastatin (10/10µg), Ofloxacin (5µg), Gentamicin (10µg), Nalidixic acid(30µg), Nitrofurantoin(300µg), Ceftriaxone Subactam (45µg), Ampiclox (10µg), Cefexime (5µg,) Levofloxacin (5µg). Fig.1 presents the antimicrobial percentage susceptibility of *E.coli* isolates from the three water bodies. *E.coli* isolates from the three water bodies recorded 0% susceptibility to Cefuroxime, Cefotaxime, Nitrofurantoin and Ampiclox. *E.coli* isolates from Ogbogoro River had 1% susceptibility to Augment in while *E.coli* isolates from Rumuolumeni and Mgbuodohia had 0% susceptibility to Augmentin. For Imipenem 80% susceptibility was observed in *E.coli* isolates from Ogbogoro River, 77.8% susceptibility for *E.coli* isolates from Rumuolumeni and 62% susceptibility for *E.coli* isolates from Mgbuodohia. For Ofloxacin and Levofloxacin 100% susceptibility was observed for *E.coli* isolates from the three water bodies. *E.coli* isolates from Ogbogoro River recorded 6% susceptibility to gentamycin but 0% susceptibility was observed for those from Rumuolumeni and

Mgbuodohia River. For Cefuroxime, 10% susceptibility was recorded for *E. coli* from Mgbuodohia and 0% susceptibility for *E. coli* isolates from Ogbogoro and Rumuolumeni River.

E.coli isolates from Ogbogoro River recorded 30% susceptibility to Ceftriaxone Sulbactam while *E.coli* isolates for Rumuolumeni River recorded 44.4% susceptibility and those from Mgbuodohia River recorded 50% susceptibility. *E.coli* isolates from Ogbogoro River had 60% susceptibility to Nalidixic acid, 44.4% was recorded from *E.coli* from Rumuolumeni while those from Mgbuodohia recorded 51% susceptibility.

Antimicrobial Percentage Susceptibility of *Salmonella* isolates.

Forty isolates of Salmonella from the three water bodies (10 from Ogbogoro, 18 from Rumuolumeni and 12 from Mgbuodohia) were tested against 12 commonly used antimicrobial. Fig2. Presents the antimicrobial percentage susceptibility of the Salmonella isolates from the three water bodies. *Salmonella* isolates from the three water bodies had 0% susceptibility to Amoxicillin Clauvulenate, Imipenem and Ampiclox. To Cefuroxime 10% susceptibility was observed for the isolates from Ogbogoro River while the isolate from Rumuolumeni and Mgbuodohia had 0% susceptibility. To Cefuroxime 35% susceptibility was recorded for the isolates from Ogbogoro River, 40% for isolates from Rumuolumeni River and 20% for isolates from Mgbuodohia River. *Salmonella* isolates from the three water bodies recorded 100% susceptibility to Ofloxacin and Levofloxacin. To Gentamycin, 20% susceptibility was recorded for *Salmonella* isolates from Ogbogoro River, 0% for isolates from Rumuolumeni River and 5% susceptibility for isolates from Mgbuodohia River. To Nalidixic acid *Salmonella* isolates from Ogbogoro River had 50% susceptibility, those from Rumuolumeni River showed 60% susceptibility while those from Mgbuodohia showed 72% to Nitrofurantoin *Salmonella* isolates from Ogbogoro River recorded 8% susceptibility while there was 0% susceptibility of the *Salmonella* isolates from Rumuolumeni and Mgbuodohia River. To Cefexime, *Salmonella* isolates from Ogbogoro River had 10% susceptibility while those from Rumuolumeni showed 0% susceptibility and those from Mgbuodohia River recorded 20% susceptibility.

Antimicrobial Percentage Susceptibility of *Shigella* isolates.

Thirty-two isolates of *Shigella* from the three water bodies (12 from Ogbogoro,10 from Rumuolumeni and 10 from Mgbuodohia) were tested against 12 commonly used antibiotic for susceptibility. The antibiotic include Cefuroxime (30µg), Amoxicillin Clavulanate (30µg), Cefotaxime (25µg), Imipenem/Cilastatin (10/10µg), Ofloxacin (5µg), Gentamicin(10µg), Nalidixic acid (30µg), Nitrofurantoin (300µg), Ceftriaxone Subactam (45µg), Ampiclox (10µg), Cefexime (5µg), Levofloxacin (5µg).

The susceptibility of *Shigella* isolates to the tested drugs is shown in fig. 3. *Shigella* isolates from the three water bodies recorded 0% susceptibility to Augmentin, Nitrofurantoin and Ampiclox. To Cefuroxime 30% susceptibility was recorded from the *Shigella* isolates from Ogbogoro River, 16.7% was recorded for those from Rumuolumeni River while those from Mgbuodohia showed 20% susceptibility. To Cefotaxime 20% susceptibility was observed from *Shigella* isolates from Ogbororo River, 16.7% from *Shigella* isolates from Rumuolumeni River and 0% susceptibility for those from Mgbuodohia River. *Shigella* isolates from Ogbogoro River recorded 11.4% susceptibility to Imipenem while 0% susceptibility was recorded for *Shigella* isolates from Rumuolumeni and Mgbuodohia River. But 100% susceptibility was observed in *Shigella* isolates from the three water bodies to Ofloxacin and Levofloxacin. To gentamycin 30% susceptibility was recorded for *Shigella* isolates from Ogbogoro River, 33.3% susceptibility was observed in those from Rumuolumeni while those from Mgbuodohia

recorded 50% susceptibility. To Nalidixic acid *Shigella* isolates from Ogbogoro River showed 0% susceptibility while those from Rumuolumeni River showed 16.7% susceptibility and those from Mgbuodohia recorded 10% susceptibility.

To Ceftrixone Sulbactam *Shigella* isolates from Ogbogoro River showed 16% susceptibility, those from Rumuolumeni River showed 16.7% susceptibility while those from Mgbuodohia showed 15% susceptibility.

Antimicrobial Percentage Susceptibility of *Vibrio cholerae* isolates

Forty-one isolates of *Vibrio cholera* from the three water bodies (13 from Ogbogoro, 15 from Rumuolumeni and 13 from Mgbuodohia) were tested against 12 commonly used antibiotics which include Cefuroxime 30ug, Amoxicillin Clavulanate (30µg), Cefotaxime (25µg), Imipenem/Cilastatin (10/10µg), Ofloxacin (5µg), Gentamicin (10µg), Nalidixic acid (30µg), Nitrofurantoin (300µg), Ceftriaxone Subactam (45µg), Ampiclox (10µg), Cefexime (5µg), Levofloxacin (5µg). Figure 4 present the susceptibility result. All the *Vibrio cholerae* isolates from the three water bodies had 0% susceptibility to Nalidixic acid, Nitrofurantoin and Ampiclox. To Cefuroxime 10% susceptibility was recorded for the *Vibrio cholerae* isolates, from Mgbuodohia River while those from Ogbogoro and Rumuolumeni recorded 0% susceptibility. For Augmentin 10% susceptibility was observed for isolates from Ogbogoro River while those from Rumuolumeni and Mgbuodohia recorded 0% susceptibility. To Imipenem, *Vibrio cholera* isolates from Ogbogoro showed 20% while the *Vibrio cholerae* isolates from Rumuolumeni showed 0% susceptibility and those from Mgbuodohia recorded 15% susceptibility. To Ofloxacin and Levofloxacin 100% susceptibility was observed in all the *Vibrio cholerae* isolates from the three water bodies. To gentamycin 35% susceptibility was recorded for the *Vibrio cholera* isolates from Ogbogoro River. Those from Rumuolumeni showed 22.2% susceptibility while those from Mgbuodohia had 10% susceptibility. To Ceftriaxone Sulbactam 50% susceptibility was observed in the *Vibrio cholerae* isolates from Ogbogoro River, those from Rumuolumeni had 11.1% susceptibility while those from Mgbuodohia had 36% susceptibility. To Cefexime *Vibrio cholerae* from Ogbogoro had 10% susceptibility, *Vibrio cholerae* isolates from Rumuolumeni had 11.1% susceptibility and those from Mgbuodohia had 15% susceptibility.

Antimicrobial resistance has been described as a product of antibiotic chemotherapy and human activities. However, there is also history of intrinsic resistance (Forsberge *et al.,* 2012) which is not associated to human activities. The extrinsic resistance is mainly driven by antibiotic selection pressure. Several studies have reported the pollution of water bodies due to environmental and human activities as a strong factor in the development of antibiotic resistance as well as spread of antibiotic resistance amongst bacteria in the aquatic environment (Abu and Egenonu, 2008; Ogan and Nwika, 1993 and Goni-Urriiza *et al.,* 2000). The Ogbogoro River, Rumuolumeni River and Mgbuodohia River are part of the tributaries of the New Calabar River that are exposed to heavy pollution due to human activities as well as industrial effluent discharge. The similarity in the susceptibility / resistance pattern of the isolates from the three different rivers in the study might be related to the fact that the communities around these water bodies have the same mode of life and livelihood such as open defecation into the river, swimming, fishing and laundry in the river. The river also serve as means of transport as well as source of water supply for drinking and other domestic use. However, the study observed slight difference in the incidence of antibiotic resistance within the different sources. Isolates from Ogbogoro River showed higher susceptibility compared to the isolates from Rumuolumeni and Mgbuodohia River. This slight variation in the susceptibility of the isolates to the tested antibiotics may be related to the impact on the bacteria due to the effect of human activities such as defecation into the river, laundry in the river, human and vehicular traffic as Ogbogoro

River was observed to be less exposed to these compared to the other two rivers in the study. Susceptibility of the isolates in the study was compared to the finding from other studies, the result is as presented below.

Susceptibility of *E.coli* Isolates

The present study recorded 100% susceptibility of *E.coli* isolates to Ofloxacin, this agrees with the findings of (Nsofor *et al.,* 2014) who reported 100% susceptibility of their *E.coli* isolates to Ofloxacin. Abu and Egenonu (2008) reported 70%susceptibility to Ofloxacin of *E.coli* isolated from other sites within the New Calabar in their study. On the contrary, Nrior *et al.,* (2020) reported 100% resistance to Ofloxacin from their study on well water in Ula-Ubie community, Rivers State, Nigeria. Omololu (2017) also reported reduced susceptibility of 28.5% of *E.coli* isolates to Ofloxacin. Frederick *et al.,* (2015) reported 91.07% susceptibility to Gentamycin against the 0.6% susceptibility observed in this study. One percent (1%) susceptibility of *E.coli* to Augmentin, observed in this study is in contrast to 100% susceptibility recorded by Nsofor *et al.,* (2014). The observed 100% susceptibility to Levofloxacin in the study disagrees with Akubuenyi *et al.,* (2018) which reported 50% susceptibility of *E.coli* isolates from their study to Levofloxacin. Also in contrast is the 6% susceptibility to gentamycin recorded in the study against 100% susceptibility reported by Nsofor *et al.,* (2014). The 50 – 60% susceptibility to Nalidixic acid reported in the present study agrees with the findings of Abu and Egenonu, (2008). The reduced susceptibility pattern of the *E.coli* isolates to the other tested antibiotics is similar to the findings from other researchers (Abu, and Egenonu, Namboodiri *et al.,* 2011; Omololu, 2017).

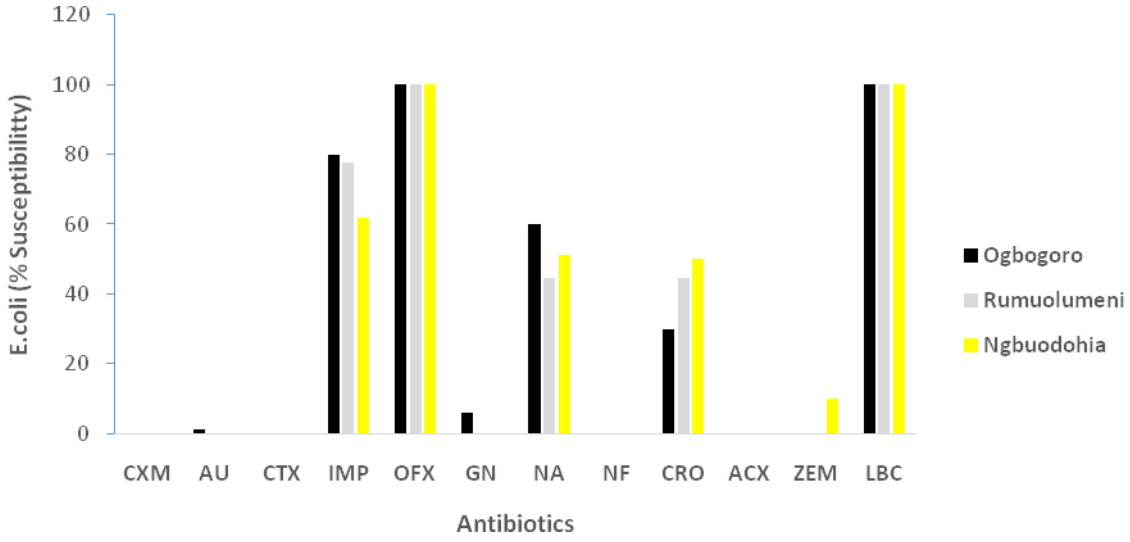
Susceptibility of *Salmonella* Isolates

Susceptibility of *Salmonella* isolates to the tested antibiotics; high susceptibility rate of 100% of the *Salmonella* isolates to Ofloxacin agrees with the findings of Aremu *et al.,* (2011). The 100% susceptibility to Levofloxacin recorded in the present study disagrees with the reported 50% susceptibility by Akubuenyi *et al.,* (2013). Susceptibility of 20 – 25% of *Salmonella* isolate in the study is in contrast with Adzitey *et al.,* (2016) which reported 70.59% susceptibility. Adzitey *et al.,* (2016) also reported 73.53% susceptibility to Augmentin contrary to the 0% susceptibility observed in the study. Akubuenyi *et al.,* also recorded high susceptibility rate of 90% of their *Salmonella* isolates to Ampiclox against 0% susceptibility observed in the present study. Susceptibility rate of 50 – 70% to Nalidixic acid recorded in this study agrees slightly with the 100% reported by Baoguang *et al.,* (2014) from their study on surface water in USA. Asseja and Girma (2019) reported 100% susceptibility of their *Salmonella* isolates to Ceftriaxone while the present study recorded low susceptibility rate of 20 – 25%.

Susceptibility of *Shigella* Isolates

High level susceptibility of *Shigella* isolates to Ofloxacin and Levofloxacin (100%) was observed in this study. Also observed was reduced susceptibility of the *Shigella* isolates to gentamycin. In this study isolates from Ogbogoro river had the least gentamycin susceptibility level of 30%, Rumuolumeni River 33.5% and 50% susceptibility from isolates from Mgbuodohia River. Similar levels of susceptibility to gentamicin was reported by Demissie *et al.,* (2014); which recorded 41.7% resistance from their study and Mulatu *et al.,* (2014) which reported 27.3% resistance to gentamycin. This finding suggests the emergence of gentamycin resistant *Shigella* in the regions covering the study area. Augmentin, Nitrofurantoin and Ampiclox recorded 0% percent susceptibility, this is in contrast to the findings of Temu *et al.,* (2007), reduced susceptibility was also observed to Cefuroxime, Cefotaxime, Imipenem, Nalidixic acid, Ceftriaxone and Cefexime. On the contrary, Assefa and Girma (2019) reported 100% susceptibility to Ceftriaxone. Temu *et al.,* (2007) reported 100% susceptibility of *Shigella* isolates from their study in Tanzania to Nalidixic acid.

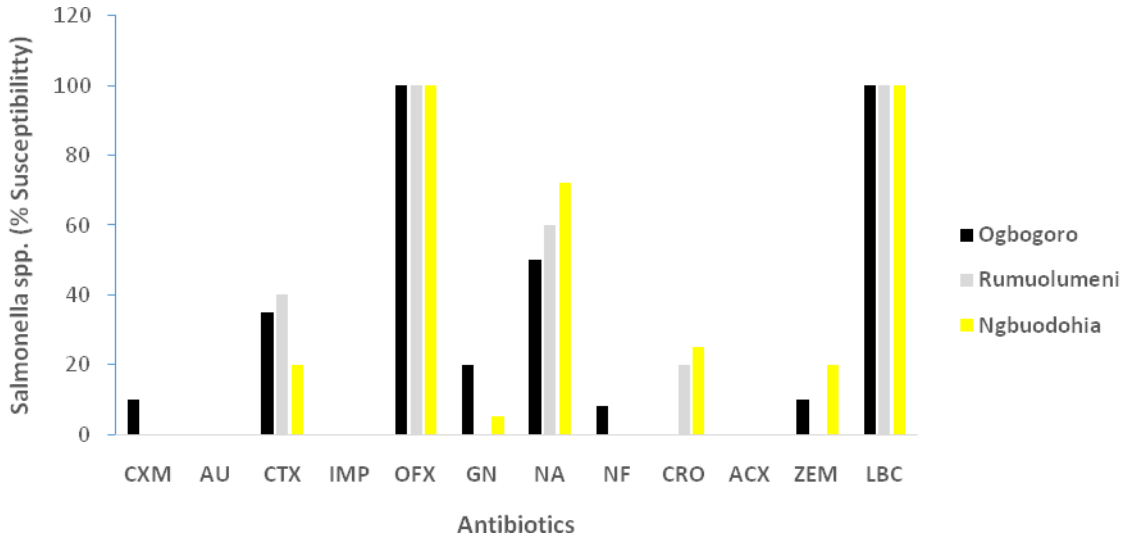
Fig.1 Susceptibility of *E.coli* isolates



Key:

CXM =Cefuroxime,	AUG = Amoxicillin Clavulanate,	CTX = Cefotaxine,
CRO = Ceftriaxone Sulbactam,	NF = Nitrofurantoin,	LBC = Levofloxacin,
NA = Nalidixic acid	IMP = Imipenem/Cilastatin	ZEM = Cefexime,
OFX = Ofloxacin,	ACX = Ampiclox	GN = Gentamycin

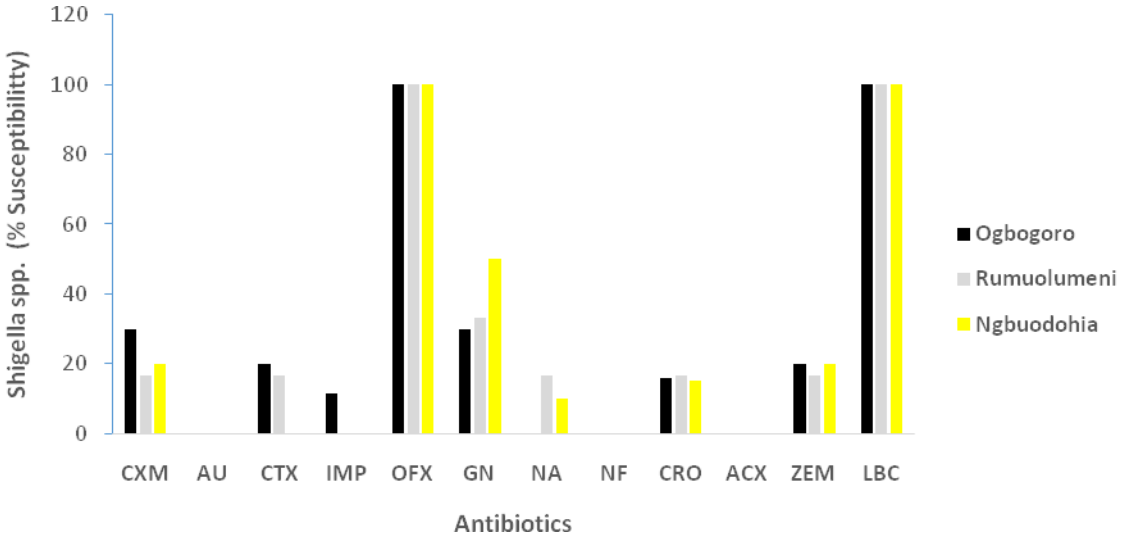
Fig.2 Susceptibility of the *Salmonella* isolates



Key:

CXM = Cefuroxime,	AUG = Amoxicillin Clavulanate,	CTX = Cefotaxine,
CRO = Ceftriaxone Sulbactam,	NF = Nitrofurantoin,	LBC = Levofloxcin,
NA = Nalidixic acid	IMP = Imipenem/Cilastatin	ZEM = Cefexime,
OFX = Ofloxacin,	ACX = Ampiclox	GN = Gentamycin

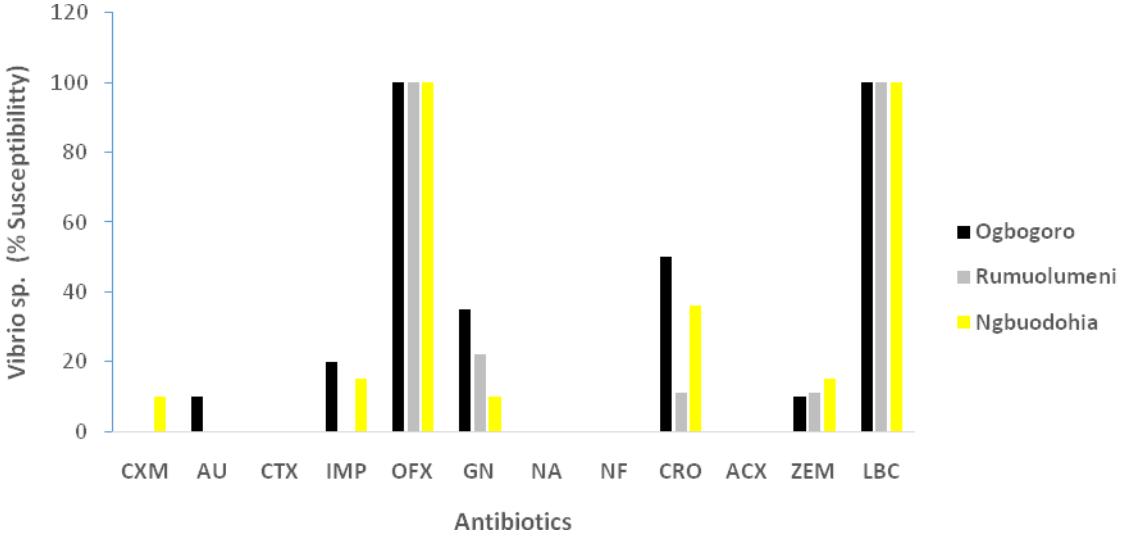
Fig.3 Susceptibility of the *Shigella* isolates



Key:

CXM = Cefuroxime,	AUG = Amoxicillin Clavulanate,	CTX = Cefotaxine,
CRO = Ceftriaxone Sulbactam,	NF = Nitrofurantoin,	LBC = Levofloxacin,
NA = Nalidixic acid	IMP = Imipenem/Cilastatin	ZEM = Cefexime,
OFX = Ofloxacin,	ACX = Ampiclox	GN = Gentamycin

Fig.4 Susceptibility of the *Vibrio cholerae* isolates



Key:

CXM =Cefuroxime,	AUG = Amoxicillin Clavulanate,	CTX = Cefotaxine,
CRO = Ceftriaxone Sulbactam,	NF = Nitrofurantoin,	LBC = Levofloxcin,
NA = Nalidixic acid	IMP = Imipenem/Cilastatin	ZEM = Cefexime,
OFX = Ofloxacin,	ACX = Ampiclox	GN = Gentamycin

Susceptibility of *Vibrio cholerae* Isolates

The antibiogram pattern in this study showed high level of *Vibrio cholerae* susceptibility of 100% to Ofloxacin and Levofloxacin. On the contrary, Abu and Egeonu (2008) reported lower level of *Vibrio cholerae* susceptibility of 70% to Ofloxacin from their study on water samples from the New Calabar River. However, several levels of reduced susceptibility was observed to most of the first line drugs for *Vibrio cholerae*. Total resistance of 0% susceptibility was observed to Cefuroxime, Ampiclox, Nalidixic acid and Nitrofurantoin. This is in variation to the report from Abu and Egeonu (2008) which recorded 50% susceptibility of Vibrio isolates from other tributaries of the New Calabar River to Nalidixic acid. Comparing this to reports from outside Nigeria, high susceptibility of 96% to Nalidixic acid was reported from Haiti by Baron *et al.,* (2016). Mpho *et al.,* (2020) from study on surface water in South, Africa reported 50% susceptibility to Nalidixic acid. Also in contrast to the 0% susceptibility of the *Vibrio cholerae* isolates in this study to Nitrofurantoin is report of 100% susceptibility form Sarker *et al.,* (2019).

The reduced susceptibility of 10 – 22.2% of the *Vibrio* isolates in this study to gentamycin disagrees with high susceptibility of 100% reported by Sarker *et al.,* (2019). Oromadike and Ogunbanwo (2015) from a study in Nigeria also reported high *Vibrio cholerae* susceptibility to gentamycin.

The present study observed reduced susceptibility of the isolates generally to most of the first line antibiotics and this represents alarming rates in antimicrobial resistance. Ofloxacin and Levofloxacin were highly effective as the study recorded 100% percent susceptibility of all the isolates to these two antibiotics. Use of these two antibiotics by humans as well as in farm, animals and poultries within the study region should be strongly regulated. Development of resistance to these antibiotics will result to a serious public health challenge should there be an outbreak of water borne disease in these regions. It will be useful for the government to organize drug information programmes for the people in the study regions as well as the patent medicine shops which are the common sources through which the people acquire these antibiotics.

The antibiogram patterns showed that all the isolates from the three tributaries of the New Calabar River that were tested in the study were resistant to two or more of the drugs tested. Thus, they can be classified as multidrug resistant.

Recommendations

From the findings it is essential to recommend discouragement of discharge of industrial and domestic effluents into water bodies. Only properly treated effluents should be discharged and it should be regulated. The above measures are critical for effective reduction of the burden of water born infections and antimicrobial resistance. Also it is necessary to improve the sanitary condition of the host communities of the water bodies.

In the case of outbreak of waterborne infection around the study areas, Levofloxacin and Ofloxacin should be considered for emergency prescription.

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How to cite this article:

David Suberu Gold Ezienyi, D. N. Ogbonna and Peekate, L. P. 2022. Antibiogram of Putative Bacterial Pathogens Isolated from Some Water Bodies in Rivers State, Nigeria. *Int.J.Curr.Microbiol.App.Sci.* 11(01): 93-103. doi: https://doi.org/10.20546/ijcmas.2022.1101.012