World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Antibiotic resistant bacteria isolated from Agulu lake in Anambra State of Nigeria West Africa

Ezendianefo Josephine Ngozi *

Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria, West Africa.

World Journal of Advanced Research and Reviews, 2022, 15(03), 155–160

Publication history: Received on 25 July 2022; revised on 01 September 2022; accepted on 03 September 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.15.3.0881

Abstract

This research investigated the antibiotic resistant bacteria isolated from Agulu Lake in Anambra state of Nigeria. Water experiment was carried out on four (4) water samples aseptically collected from different points in the Agulu Lake. The samples were analyzed for physicochemical features, Most Probable Number (MPN), Gram stain, microscopy, motility, biochemical traits and antibiotic susceptibility tests using standard microbiological instrumentation. The results were analyzed through inferential statistics. The bacterial isolates were characterized based on their Gram stain, microscopy, motility and biochemical properties. The degree of susceptibility of the isolates against conventional antibiotics was determined using antibiotics' impregnated discs assay. The physicochemical characteristics revealed the following average results: acidic pH of 6.0, temperature of 34 ^{0}C and turbid. The highest MPN value was recorded by water sample collected from site D while the lowest MPN value was found at site B of the stream. The results of Gram stain, microscopy, motility and biochemical characterization inferred the presence of *Pseudomonas* species and *Escherichia coli* in the water samples. The study confirmed that Agulu Lake in Anambra state of Nigeria contains bacteria that are resistant to most conventional antibiotics. So, there is a call for massive information dissemination to all for caution towards health promotion.

Keywords: Agulu Lake; Water analysis; Antibiotic resistance; Environmental bacteria; Anambra and Nigeria

1. Introduction

Antibiotic resistance is now a worldwide health crisis that could kill 10 million individuals by 2050 (1).

The World Health Organization has classified antimicrobial resistance as a widespread "serious threat that is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country" (2).

Antibiotic resistant bacteria and antibiotic resistant genes were recognized in China as at the year 2016 (3) and were found in Nigeria in 2022 (4).

This study is meant to link Agulu Lake in Anambra state of Nigeria to the global map of areas that have been investigated so far on the impact of this dreaded health hazards that is here with us termed drug resistance.

* Corresponding author: Ezendianefo Josephine Ngozi
Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria, West Africa.

1.1. Statement of Problem

Many researchers have found out that there are antibiotic resistant bacteria in waterways (5, 6) which pose health risks to the populace.

Aim of the Study

This study aims at investigating Agulu Lake in Anambra state of Nigeria for the presence of antibiotic resistant bacteria with a view to saving lives that are lost through treatment failures caused by drug resistance.

Objective of the Study

The objective of this study is the analysis of water samples collected from different sites of Agulu Lake in Anambra state of Nigeria in order to investigate the presence of antibiotic resistant bacteria.

1.2. Research Question

The following research question guided the study:

Are there antibiotic resistant bacteria in Agulu Lake in Anambra state of Nigeria?

1.2.1. Area of Study

This research was done in Agulu lake found Agulu which is located in Anaocha Local Government Area of Anambra State. Anambra state is one of the thirty-six (36) states of Nigeria and located in the South-Eastern region of the country.

The location of Anambra in Nigeria is 6°20'N 7°00'E, West Africa, the maps below depicts the lake and its location:

Source: https://simple.wikipedia.org/wiki/file

Figure 1 Agulu Lake

Source: http://www.tinkerandbell.com

Figure 2 Map of Nigeria depicting Anambra state

1.2.2. Scope of Study

The scope of this study was limited to the presence of antibiotic resistant bacteria from Agulu Lake in Anambra state of Nigeria in conjunction with other researches made by fellow scientists (3, 7).

Limitation of Study

Finance is the major limitation to this study- transportation and laboratory materials were money-intensive.

2. Materials and Methods

2.1. Sample Collection

Water samples were aseptically collected from four (4) different sites of Agulu Lake. The water samples were labeled properly (A to D) and then transported to the laboratory for analysis within 48 h. Aliquot of the samples were analyzed using standard microbiological procedures.

2.2. Physicochemical Analysis

2.2.1. Determination of temperature

The temperature was determined using a centigrade thermometer as done by Ezendianefo (4).

2.2.2. Determination of pH

The pH of the water samples was determined using a pH meter. This was done in triplicate as described by Umeaku (6).

2.2.3. Procedure for Determining Turbidity

This study followed the method of Lavender (8).

2.2.4. Microbiological Analysis

Preparation of the media

All the media were prepared according to the specifications of the manufacturer.

Sterilization of materials

All the materials and prepared media were sterilized by autoclaving at 121 ^{0}C for 15 minutes and other glass wares were sterilized in the laboratory hot air oven at a temperature of 180 ^{0}C for 1 hr before use.

Dilution of the Samples

- Serial Dilution

A ten-fold serial dilution of the samples was carried out by adding 1 ml of water samples aseptically into test tubes containing 9 ml of sterile peptone water labeled 10^{-1} to 10^{-10} dilution, with the aid of sterile pipettes.

- Isolation Process

The most probable number (MPN) method of analysis was done as described by Umeaku (6).

- Kirby-Bauer Disc Diffusion

This followed the method described by Umeaku (6).

A total of seven (7) antibiotics that are mostly used in the treatment of human and animal diseases were employed in this study. The antibiotics and their needed concentrations are as follows: CLIN: Clindamycin (2 µg), GENT: Gentamycin (10 µg), CIP: Ciprofloxacin (5 µg), ERY: Erythromycin (15 µg), SULF: Sulfonamides (250 µg), AMP: Ampicillin (10 µg), TET: Tetracycline (30 µg).

3. Results

3.1. Physical Parameters of the Water Samples

Table 1 Physical parameters of the water samples

Sample	pH	Temperature (℃)	Turbidity
A	5.00	35.00	turbid
B	6.50	33.00	clear
C	6.50	36.00	turbid
D	6.00	32.00	turbid

3.2. Most Probable Number (MPN) Values of the Water Samples

Table 2 MPN values of the water samples

Sample	Positive tube	MPN/mL
A	5-3-2	110
B	4-3-0	27
C	5-2-1	70
D	5-4-1	170

3.3. Characteristics of the Isolates

Table 3 Characteristics of the bacterial isolates

Parameter	A	B	C	D
Catalase	+	+	+	+
Indole	+	_	_	_
Coagulase	_	_	_	_
Citrate	_	+	+	+
H2S	_	_	_	_
Methyl red	+	_	_	_
Voges Proskauer	_	_	_	_
Glucose	+	_	_	_
Maltose	_	_	_	_
Lactose	+	_	_	_
Sucrose	+	_	_	_
Inositol	_	_	_	_
Mannitol	+	+	+	+
Sorbitol	+	_	_	_
Gram stain Microscopy Motility Bacterium	_ Rods + *E. coli*	_ Rods + *Pseudomonas species*	_ Rods + *Pseudomonas species*	_ Rods + *Pseudomonas species*

+ means positive; - means negative

3.4. Susceptibility Testing of the Isolates

Diameter zones of inhibition of susceptible isolates against the conventional antibiotics

Table 4 The Results of the Antibiotic Susceptibility Test (mm)

	Antibiotics						
Isolate Identity	**CLIN**	**GENT**	**CIP**	**ERY**	**SULF**	**AMP**	**TET**
ISOLATE A	12	11	12	13	08	12	05
ISOLATE B	11	13	10	11	05	11	10
ISOLATE C	10	12	11	07	13	10	12
ISOLATE D	07	08	06	10	10	08	09

4. Discussion

The study was on the antibiotic susceptibility profiling of bacteria gotten from Agulu Lake in Anambra state of Nigeria. The result of the study revealed variation in the physicochemical parameters which could be attributed to environmental contamination due to human activities. Water samples from the lake were turbid which could further support the idea of contamination. This observation supports the finding of several researchers (5, 4) who reported that human activities are capable of influencing physicochemical parameters in water bodies.

The isolation of coliform bacteria from the sampled stream could be ascribed to fecal contamination. Most rural dwellers are frequently engaged in open defecation due to non-availability of toilet facilities, which serves as major source of water contamination by bacteria. These bacteria finally find their way into the water ways through majorly wind or flooding. Similar bacterial species were isolated by several researchers (6, 7).

The isolated bacteria were all resistant to all the seven (7) conventional antibiotics employed in the study which authenticates the findings of other researchers.

The isolation of antibiotic resistant genes in all the bacterial isolates confirms the source of resistant exhibited by the strains. Plasmid can mediate antibiotic resistance by several mechanisms. Some mechanisms are very popular among Gram negative bacteria. *E. coli,* which produces 1 of the 3 enzymes (β-lactamase) that are responsible for antibiotic alteration and degradation which render the antibiotics inactive. This enzyme is coded by both chromosome and plasmid. This observation agrees with the finding of several researchers (4, 9, 10, 3).

5. Conclusion

This study has authenticated the fact that antibiotic resistance has now been at an alarming increase in our environment cum water ways which poses great danger to human health. The most fearful fact of the growing antibiotic resistance is that the resistance is transferable. So, if the resistant bacteria are allowed to spread in the environment, there is a huge feasibility of transferring the phenotype to other bacteria. Antibiotics are used indiscriminately in Nigeria, West Africa due to lack of proper regulation and surveillance. This study will serve as a base that would recommend necessary initiatives to monitor and limit the applications of antibiotics, as well as proper surveillance following standardized protocol throughout the nation and beyond.

Compliance with ethical standards

Acknowledgments

I appreciate my mentor and friend by name Prof CN Umeaku of the Department of Microbiology in Chukwuemeka Odumegwu Ojukwu University Uli Anambra state Nigeria.

References

[1] United Nations Report. Antibiotics found global rivers exceed safe levels-study finds. *Eco Watch.* 2019; 20.

[2] WHO. Antimicrobial resistance: global report on surveillance. *The World Health Organization.* 2014.

[3] Zhang S, Pang S, Wang P, Wang C, Han N, Liu B, Han B, Li Y, Anim-Larbi K. Antibiotic concentration and antibiotic-resistant bacteria in two shallow urban lakes after stormwater event. *Environ Sci. Pollut.* 2016; 56-79.

[4] Ezendianefo JN. Antibiotic susceptibility profiling of bacteria isolated from Eze Stream in Nkpologwu Aguata found in Anambra state of Nigeria, West Africa. *Journal of Advance Research in Social and Humanities.* 2022; 8(8): 1-5.

[5] Zheng J, Gao R, Wei Y, Chen T, Fan J, Zhou Z, Makimilua TB, Jiao Y, Chen H. High-throughput profiling and analysis of antibiotic resistance genes in East Tiaoxi River, China. *Environ. Pollut.* 2017; 648- 654.

[6] Umeaku CN, Ezendianefo JN, Chris-Umeaku CI, Dimejesi SA. Occurrence of Antibiotic Resistant Bacteria and Antibiotic Resistant Genes in Rivers found in Anambra state, Nigeria. *GSC Biological and Pharmaceutical Sciences.* 2022; 304-316.

[7] Abu GO, Wondikom A. Isolation, Characterization and Antibiotic Resistance Profile Studies of Bacteria from an excavated Pond in Port Harcourt Metropolis, Nigeria. *J. Appl. Sci. Environ. Manage.* 2017; 22(8): 1177-1184.

[8] Lavender S, Beaugrand G, Outram N, Barlow N, Crotty D, Evans J, Kirby R. Seafarer citizen scientist ocean transparency data as a resource for phytoplankton and climate research. *PLOS ONE*. 2017; 12(12).

[9] Fu J, Yang D, Jin M, Liu W, Zhao X, Li C, Zhao T, Wang J, Gao Z, Shen Z, Qiu Z, Li JW. Aquatic animals promote antibiotic resistance gene dissemination in water via conjugation: role of different regions within the zebra fish intestinal microbiota. *Mol. Ecol.* 2017; 19: 26.

[10] Jiang H, Zhou R, Yang Y, Chen B, Cheng Z, Zhang M, Li J, Zhang G, Zou S. Characterizing the antibiotic resistance genes in a river catchment: influence of anthropogenic activities. *J. Environ. Sci.* 2017.