Research Article

Preliminary Investigation of *Plesiomonas Shigelloides* and *Aeromonas* Species as Emerging Pathogens Found in Selected Rivers in Abakaliki, Ebonyi State.

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ABSTRACT

The recovery of Aeromonas species and *Plesiomonas shigelloides from stool samples of patients having gastroenteritis has been commonly reported. This occurrence is mostly seen in developing* countries, and less in developed countries. Inositol brilliant-green agar was used in the recovery of *Plesiomonas shigelloides* and Aeromonas species from fresh water samples, to characterize and determine their susceptibility profiles. A total of 30 isolates were recovered from the water samples, 17 of which is Aeromonas species and 13 *Plesiomonas shigelloides*, putting their prevalence in these fresh waters at 56.7% and 43.3% for *Aeromonas* and *Plesiomonas* respectively. They were further differentiated through biochemical tests, and their susceptibility to various antimicrobial agents was tested, which classified the isolates to be either susceptible or resistant. Aeromonas species was seen to be 58.82% susceptible and 41.17% resistant to the antibiotics used, while *Plesiomonas shigelloides* was 38.46 % susceptible and 61.53% resistant, making them highly resistant to the conventional antibiotics used. The ARI and MARI values for Aeromonas species are 0.7 and 7 while that for *Plesiomonas shigelloides* are 0.8 and 8 respectively. A previous report suggests that aside being aquatic organisms, they are also distributed widely in the environment. This is confirmed by this study, carried out in Abakaliki, Ebonyi State, where these organisms were isolated from fresh water samples.

Keywords: Antibiotics, *Plesiomonas shigelloides*, Gastroenteritis, Susceptibility

INTRODUCTION

Water is a transparent, tasteless, odourless, and nearly colourless chemical substance, which is the main constituent of the earth's streams, lakes, and oceans, and the fluids of most living organisms[12]. The health risks associated with the consumption of contaminated water are of great interest[13]. Marine ecosystem is recognized as a natural habitat of some pathogenic microorganisms. Pollution of water resources by microorganisms of faecal origin, sewages and effluents from industries is a current world-wide public health concern[10] and this places human population at risk of contracting water related diseases such as gastroenteritis, typhoid and cholera. *Plesiomonas shigelloides* and *Aeromonas* species are pathogenic microorganisms that can inhabit freshwaters contaminated with faecal wastes such as sewages. So many reports have been given on the isolation of *Aeromonas* and *Plesiomonas* from diarrheal cases in humans[9]. *Plesiomonas shigelliodes* and *Aeromonas* species are aquatic microorganisms, which were recognized recently as potential human and animal pathogens. While *Aeromonas* are Gram-negative, motile, facultative anaerobes, non-spore-forming bacilli and oxidase positive, within the family of Aeromonadaceae, *Plesiomonas shigelloides* are Gram negative, stationary, facultative anaerobes, are non-spore forming having cocci-bacilli shape, belonging to the family Enterobacteriaceae. They are oxidase negative, and just like *Aeromonas* species, can be transmitted through contaminated water and sea food sources. *Aeromonas* has been reported as the second cause of gastroenteritis in children, and the fifth in adults patients. Although, *P. shigelliodes* and *Aeromonas* species has been reported to be susceptible to broad-spectrum antimicrobials such as cephalosporins, quinolones, chloramphenicol,[7] their resistance to these antibiotics has also been reported. *P. shigelloides* has been identified as the cause of outbreak of diarrheal disease, and is known to play etiologic role in travellers' diarrhoea in paediatric and adult out patients[4].Open defeacation, discharging of untreated sewage and dumping of refuse into water bodies have been a regular practice in Abakaliki [14], hence the need to investigate the prevalence and the antibiotics susceptibility profile of *Plesiomonas shigelloides* and *Aeromonas* species in freshwater rivers in Abakaliki, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out in Abakaliki, Ebonyi State, South Eastern Nigeria. Ebonyi state is located between 6° 20N and latitude 8° 06°E. It shares boundaries with Enugu State to the West, Benue State to the North, Cross River

State to the East and Abia State to the South. The bimodal pattern of rainfall in Ebonyi State is seen from April-July and September-November, with a short break around august. The predominant vegetation is savannah, with mean annual rainfall of 1000mm-1500mm.The population census[3], estimated the population of people in Ebonyi State as 1,064,156 males and 1,112,791 female, totaling about 2.176 million people and are predominantly farmers[8].

Sample Collection

Sample used for this study were collected from the Rivers located in Abakaliki Metropolis, Ebonyi State. Five different sterile containers were immersed to a depth of about 5cm below the water surface and against water flow to collect different water samples from Onuebonyi River, Azuiyiokwu River, Ebia River, Ebonyi River and Iyiudele River. The samples were taken to Microbiology Laboratory at Alex-Ekwueme Federal University Ndufu-Alike Ikwo for analysis.

Isolation of Bacteria

1 ml of Sample collected was diluted with 9ml of distilled water in a test tube and used to perform a ten fold serial dilution. 0.1 ml of the serial diluted samples was plated out on the selective medium, inositol brilliant green agar and incubated for 24 hours at 37°C [5].

Bacterial Identification

The pure cultures were viewed macroscopically, microscopically and identified on the basis of morphology, colony characteristics, Grams reaction and biochemical tests[2].

Antibiogram Determination

The isolates were tested for antibiotic susceptibility, using Muller-Hinton agar, and applying Kirby-Bauer disc diffusion method[1]. The antibiotics tested for were gotten commercially from Optun Laboratories (Nigeria), and comprises: Ciporoflox 10mcg, Norfloxacin 10mcg, Gentamycin 10mcg, Amoxil 20mcg, Streptomycin 30mcg, Rifampicin 20mcg, Erythromycin 30mcg, Chloramphenicol 30mcg, Ampiclox 20mcg, Levofloxacin 20mcg. The isolates were cultured in nutrient broth, and incubated at a temperature of 37°C for 24 hours. The suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard, and 100 microliters of the adjusted suspension onto Muller-Hinton agar, by surface spreading (Merek, South Africa). Antibiotics discs were placed over the seeded plate, and incubated aerobically for 24 hours. Isolates were classified as susceptible or resistant.

RESULTS

Table 1: Showing the two bacteria organisms isolated from inositol brilliant-green agar

S/N	Name of isolates	Colour of colonies on BBG	Form	Motility
1	*Aeromonas. Species*	Colourless	Round	Swarmy/non-motile
2	*Plesiomonas shigelloides*	Pink	Round/Flat	Stationary

The two isolates (*Aeromonas* sp and *Plesiomonas shigelloides*) grew on inositol brilliant green agar with colourless and pinkish colonies respectively.

Table 2: Morphological characteristics of *Aeromonas* species and *Plesiomonas shigelloides* isolates

Isolate code	Morphology	Arrangement	Gram react ion	Indole test	Catalase test	Citrate test	MR	VP	Probable oranism
A	Colourless	Single	Cocci	-	+	+	_	+	*Aeromonas spp*
	Colourless	Single/short chain	Rod	-	+	-	+	-	Aeromonas spp
B	Pinkish	Short chain	Rod	-	++	-	-	+	Plesiomonas shigelloides
	Milky	Single	Rod	-	++	-	-	+	*Aeromonas spp*
C	Pinkish	Single/short chain	Rod	+	+	+	-	+	*Plesiomonas spp*
	Colourless	Single	Cocci	-	+	+	+	_	*Aeromonas spp*
D	Colourless	Short chain	Rod	-	+	-	-	+	*Aeromonas spp*
	Pinkish	Single	Cocci	-	+	-	-	+	*Plesiomonas spp*
E	Colourless	Single/short chain	Cocci	-	-	-	+	-	*Aeromonas spp*
	Colourless	single	Cocci	-	+	-	-	+	*Aeromonas spp*

These were the different microorganisms isolated from the different rivers. From this identification, the frequency at which *Aeromonas* sp was present in the different rivers was higher than *Plesiomonas shigelloides.*

Table 3: Frequency of isolates occurrence in each of the sample

SAMPLES	A	B	C	D	E	TOTAL	% PREVALENCE
Plesiomonas species	4	1	4	2	2	13	43.3%
Aeromonas species	3	2	7	2	1	17	56.7%

This table shows the frequency of occurrence of these organisms from the different rivers sampled. The prevalence of *Aeromonas* specie was 13.4% higher than *Plesiomonas* specie.

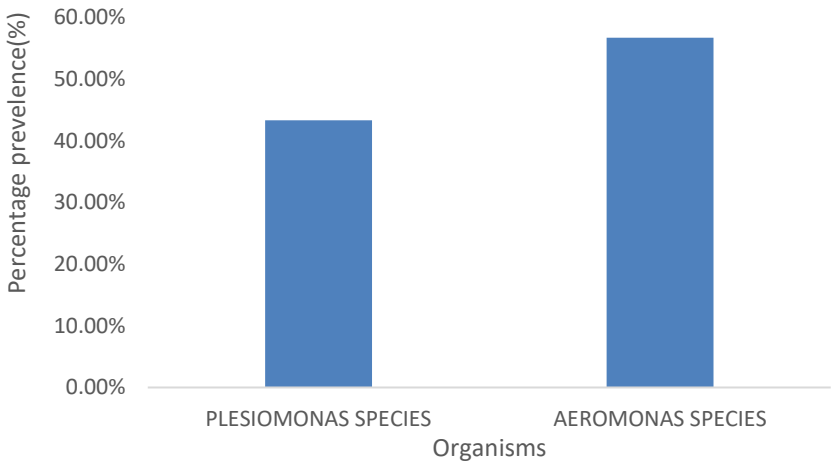

Figure 1 : Percentage prevalence of the isolates

Graphical representation of the prevalence of the two isolates showed that all the rivers sampled and isolated had the significant presence of Aeromonas species which cumulatively gave a highest value in *Aeromonas* specie.

Table 4: Percentage susceptibility and resistance profiles of the isolates

Isolates	Susceptible Frequency/Percentage %	Resistant Frequency/Percentage
Aeromonas species	10(58.82)	7(41.17)
Plesiomonas shigelloides	5(38.46)	8(61.53)

In *Aeromonas* specie the percentage of susceptible is higher than resistance whereas in *Plesiomonas shigelloides* susceptible is less than resistance.

Table 5: Antibiotic resistant index (ARI) and multiple antibiotic resistant index (MARI) for *Aeromonas* species and *Plesiomonas shigelloides*.

Isolates	Antibiotic resistance index (ARI)	Multiple antibiotic resistance index (MARI)
Aeromonas species	0.7	7
Plesiomonas shigelloides	0.8	8

The antibiotics resistance index (ARI) and multiple antibiotic resistance index (MARI) in *Plesiomonas shigelloides* are higher than Aeromonas species. This indicates a higher form of resistant strains in *Plesiomonas shigelloides.*

DISCUSSION

The dangers associated with coming in contact with waters containing these pathogens should be further studied. Humans and animals have been reported to harbour the enteric pathogens *Plesiomonas shigelloides* and *Aeromonas species*, in some parts of the world such as in the Nordic countries. The presence of these pathogens in Abakaliki has not been studied before; therefore, this is the very first study reporting the presence of these enteric pathogens in fresh waters, in Abakaliki metropolis, South East, Nigeria. These further draws attention to considering fresh waters as a risk factor for diseases, since they are prone to contamination by faecal materials. *P. shigelloides* and *Aeromonas* species have been termed microbes of tropical and subtropical regions. Nevertheless, they have global distribution, following the results obtained from this study, and hence should be modified. So many outbreaks have been reported in countries such as Sweden, but their aetiology has not been identified[15]. Five of the water samples used

for this study contained culturable *Aeromonas* and *plesiomonas species.* This report agrees with the report given by Swedish Institute for Infectious disease control, given in 1996, which explains that cases experienced as a result of waterborne infections, did not arise from a specific source. A selective media was used for this study, and this gave the reason why they were not counted. The prevalence of these organisms was also found to be 56.7% and 43.3% for *Aeromonas* and *Plesiomonas* species, respectively (Table 4). From the epidemiological and Ecological aspects, this study is very crucial. *Aeromonas* and *Plesiomonas species* not only occur in fresh waters in Abakaliki, it is also present in polar habitats. The current study assessed the sensitivity of conventional antibiotics, and found that the isolates are resistant to the some of the antimicrobial agents used. *Aeromonas species* was seen to be 58.82% susceptible and 41.17% resistant to the antibiotics, whereas *Plesiomonas shigelloides* was seen to be 38.46% susceptible and 61.53% resistant (Table 5). These shows that *Plesiomonas* is highly resistant to the antibiotics tested mostly Ampiclox, levofloxacin and others, although, erythromycin and Rifampicin has inhibitory effects on the isolates. The presence of isolates that are resistant to more than three antibiotics, indicates multiple antibiotic resistances, and highlights the potential risk to successful treatment against infectious diseases associated with the consumption of faecal contaminated water.

CONCLUSION

Aeromonas species and *Plesiomonas shigelloides* are microorganisms, which are usually found in the environment. They are usually recovered from the stool samples of patients having gastroenteritis, who must have come in contact with water contaminated by faecal samples. A previous study suggests that *Aeromonas* is usually implicated in cases of persistent diarrhoea whereas *Plesiomonas* may be the cause for mild dysentery. Summarily, this research work has increased the previous knowledge of *Plesiomonas shigelloides* and *Aeromonas species*, by giving insights into its distribution, its biochemical characteristics and the possibility of playing a significant role in Zoonotic diseases.

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