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ISOLATION AND ANTIBIOTIC RESISTANCE PROFILE OF CLINICAL ISOLATES OF A. HYDROPHILA FROM A TERTIARY HOSPITAL IN NORTH EASTERN NIGERIA

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ABSTRACT: The prevalence and antibiotic resistance profile of *Aeromonas hydrophila* isolates from the diarrheic and non-diarrheic stool of patients attending the University of Maiduguri Teaching Hospital in Borno state; Northeastern Nigeria was studied. A total of eight (8) *A. hydrophila* were isolated from the diarrheic stool and characterized using standard biochemical tests and the Aerokey 11 identification scheme for *Aeromonas* spp. The antibiotic susceptibility test was performed using the disk diffusion method. All the 8 isolates were resistant to Ampicillin, Cephalothin, Augmentin, and Imipenem, while Seven (7) of the isolates were resistant to Cefazolin. Six (6) isolates were resistant to Gentamycin. Four isolates exhibited resistance to Tetracycline and Ciprofloxacin. However, all the eight (8) isolates were susceptible to Ceftazidime, while six (6) were susceptible to Tobramycin. Our result shows that *Aeromonas hydrophila* may be associated with diarrhea and should be considered as potential enteric pathogens in this geographical region.

KEYWORDS: Aeromonas, diarrhea, antibiotic, resistance profile.

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1. INTRODUCTION

The mesophilic Aeromonads are emerging as important opportunistic pathogens in humans with the potential to cause various types of diseases, which include extra-intestinal, systemic infections as well as gastrointestinal infections [1-4]. Among bacterial causes of diarrhea, *A. hydrophila* is increasingly accepted as a clinically significant enteric pathogen that is associated with

Bello et al RJLBPCS 2019 www.rjlbpcs.com Life Science Informatics Publications gastroenteritis in children, adults, and the elderly [5]. A. hydrophila is a bacteria that belong to the genus Aeromonas and family Aeromonadaceae [6]. The taxonomy of aeromonad has undergone several reviews due to lack of agreement between phenotypic and genotypic characteristics of species, and presently the genus has over 17 named species [3, 6]. It is gram-negative bacteria, oxidase positive, rods, and is widely distributed in nature and native to the aquatic environment worldwide [3]. They are widely isolated from clinical and environmental samples. Members of this genus are also found in foods, including fresh grocery produce, seafood, fresh and smoked fish, cheese, and milk [7-10]. The pathogenicity of the bacteria is associated with the production of toxins such as hemolysins, cytotoxins, enterotoxins, invasiveness, adherence, motility and lateral (polar) flagella [11-14]. A. hydrophila, A. caviae, and A. veronii biovar sobria are the most common species known to cause the majority of human infections and account for over 85% of all the clinical cases [2]. In Nigeria, like other developing countries, there is a paucity of information on Aeromonas related diarrheal diseases because Aeromonas is not among the bacteria that are routinely reported as etiological agents of diarrhea. However, some studies have been carried out to detect the presence of Aeromonas species in stool samples of patients in some parts of Nigeria, although, no published research is available on the presence of this important emerging pathogen in Maiduguri, Northeastern Nigeria. This study was therefore carried out to document the prevalence and antibiotic resistance pattern of Aeromonas hydrophila in stool samples of patients from Maiduguri, Nigeria.

2. MATERIALS AND METHODS

Study Area

The present study was conducted at the University of Maiduguri Teaching Hospital, Borno state, Northeastern Nigeria. It is one of the main referral centers for both state and local government specialist hospitals as well as many privately owned hospitals within the state. Hence it was thought suitable to use this center as a study site among other hospitals in the metropolis. The hospital is located in Maiduguri, which is the capital and biggest city of Borno state. Borno State occupies the significant parts of the Chad Basin and is located in the North Eastern part of Nigeria. The State borders the Republic of Niger to the North, Chad to the North East and Cameroon to the East. Based on the 2006 population census Maiduguri has a total population of 4,151,193 [15].

Collection of samples

A total of 231 samples comprising of 127 (diarrheic patients) and 104 (without diarrhea) samples, were routinely collected from patients attending the University of Maiduguri Teaching Hospital, Nigeria, between Jan-June, 2011. The samples were collected in Cary Blair transport media and transported to the laboratory within 4 hours of collection. A detailed history was obtained from patients via questionnaire regarding age, sex, and major symptoms.

Bacterial Isolation and presumptive identification

Stool samples were first inoculated onto alkaline peptone water (Oxoid, U. K) for enrichment and

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Figure 1: Growth of *Aeromonas species* on Rimler shotts media showing characteristic yellow colonies Speciation

Isolates were characterized to the species level based on biochemical tests according to the Aerokey II identification scheme for *Aeromonas* species [16]. These included bile aesculin hydrolysis, gas from glucose, acid from arabinose and sucrose, indole production, Voges-Proskauer reaction and resistance to cephalothin (30ug).

Antibiotic Testing

The *Aeromonas* isolates were tested for resistance to 10 antibiotics using the agar disc diffusion method. The antibiotics tested were Ampicillin (10ug), Gentamicin (10µg), Tetracycline (30µg), Cephalothin (30µg), Tobramycin 10µg), Ceftazidime (30µg), Augmentin (30µg), Imipenem (10µg) Cefazolin (10µg), Ciprofloxacin (5µg). Briefly, sterile Petri dishes of Mueller Hinton agar were prepared according to the manufacturer's specification. The sterile swab was dipped into an overnight culture of the isolates in Mueller Hinton broth that has been adjusted to 0.5 McFarland standards. The cultures were inoculated into the plates and left for about 10 minutes, and the antibiotics discs were aseptically picked and placed on the plates and incubated at 37°C for 24hrs. Zones of inhibition after incubation were observed and measured and the results were interpreted according to CLSI [17] standards.

Statistical Analysis

All the data was analysis using the Statistical Package for Social Sciences (SPSS) program, version

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3. RESULTS AND DISCUSSION

A total of 231 diarrhea and non-diarrhea stool samples were analyzed for the presence of Aeromonas species among patients with age range between 0 to 65 yrs. The Aerokey11 identification scheme [16] identified all the isolates as A. hydrophila. A. hydrophila was isolated from 3.5% (8/231) of stool samples from patients with diarrhea while none were isolated from the stool samples collected from patients without diarrhea with highly statistical significant difference between diarrheal and non-diarrheal patients at (P<0.05) as shown in Table 1. Aeromonas hydrophila were isolated from 5(4.8%) of the 105 stool samples collected from males and 3(2.4%) of the 126 samples collected from females, but no statistical significant difference was observed between sex and the prevalence of the bacteria (p>0.05) (Table 2). Table 3 showed that the age group 21-36 years have the highest prevalence rate of 5 (2.16%) of the total sample analyzed, followed by age group 37yrs and above with 3 (1.30%). Aeromonas was not isolated from the age groups <5 and 6-20 yrs. Also, no statistical significant relationship was observed between age of the patients and the presence of the bacterial (P> 0.05) (Table 3). All the 8 isolates were resistant to Ampicillin, Cephalothin, Augmentin, and Imipenem, while Seven (7) of the isolates were resistant to Cefazolin. Six (6) isolates were resistant to Gentamycin. Four isolates exhibited resistance to Tetracycline and Ciprofloxacin. However, all the eight (8) isolates were susceptible to Ceftazidime, while six (6) were susceptible to Tobramycin as shown in Table 4.

Patients	Number tested	Number positive (%)
Diarrheic	127	8(6.3)
Non diarrheic	104	0
Total	231	8(3.5)

Table 1: Prevalence of Aeromonas hydrophila among diarrheic and non-diarrheic patients

(X = 5.0328, df = 1, p-value = 0.02487)

Table 2: Sex-related prevalence of Aeromonas hydrophila among patients

105	5(2.16)
126	3(1.30)
231	8(3.5)
	126 231

(X= 0.3895, df = 1, p-value = 0.5325)

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Age Group(Yrs.)	No. of samples	No. of positive for	Percentage Prevalence (%)
		A. hydrophila	
< 5	43	0	0
6-20	47	0	0
21-36	94	5	2.16
≥ 37	47	3	1.30
TOTAL	231	8	3.5

 Table 3: Age-related prevalence of Aeromonas hydrophila among patients

(X = 5.3956, df = 3, p-value = 0.145)

Table 4: Antibiotic Resistance Pattern of Aeromonas hydrophila

S No.	Antibiotics	No. Of Isolates Resistant
1	Ampicillin	8
2	Cephalothin	8
3	Augmentin	8
4	Imipenam	8
5	Cefazolin	7
6	Gentamycin	6
7	Tetracycline	4
8	Ciprofloxacin	4
9	Tobramycin	2
10	Ceftazidime	0

Diarrheal diseases associated with *Aeromonas* spp have been reported by several researchers worldwide [18-21]. In this study, *Aeromonas hydrophila* was the only species isolated and is from the diarrheic stool samples. Several studies have shown a similar preponderance of the species [22-25]. The prevalence rate of 3.5% from diarrheic stool samples in the present study is consistent with that of Rogo *et al.* [25] who reported a prevalence of 3.12% in 128 patients attending Ahmadu Bello University Teaching Hospital (ABUZTH) and Aminu Kano Teaching Hospital (AKTH) but less than the 13% reported by Nzeakor and Okafor [26] in Nigeria and 12.8% reported by Samie *et al.* [27] in South Africa. Differences in the prevalence rate of *Aeromonas* species from countries or regions have been attributed to differences in the sampling period, geographical location, origin of samples and methodology for analysis [28]. Although it has been reported that *Aeromonas species* related gastroenteritis is more prevalent in children and the elderly [29]. The present study shows that *Aeromonas hydrophila* was not isolated from the age groups that are below 20 years. Literature regarding antimicrobial resistance for *A. hydrophila* differs among researchers. According to Janda

Bello et al RJLBPCS 2019 www.rjlbpcs.com Life Science Informatics Publications and Abbort [3], drug resistance may vary significantly due to individual species, geographic locales, or environmental selection pressures. In the present study, all the isolates (8) were resistant to Ampicillin and Cephalothin. This is consistent with some previous studies [30-31]. However, in contrast to Rogo *et al.* [25] who reported only a few species were resistance to Augmentin in their study, although, Imipenem has been reported to be effective for treatment of *Aeromonas* infection [32], in the present study all the isolates were resistant to Imipenem. Four isolates of *A. hydrophila* in this study were resistant to cefazolin and gentamycin but susceptible to ciprofloxacin and tetracycline. Resistance to commonly used antibiotics has been attributed to subtherapeutic and extensive usage of these antibiotics [25]. The limitation of this study is that stool samples positive for *Aeromonas species* were not tested for the presence of other enteric bacteria.

4. CONCLUSION

The results of this study have shown that *Aeromonas hydrophila* may be associated with diarrhea and should be considered as potential enteric pathogens in this geographical region.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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