

Prevalence of Aeromonas Species Among Patients Attending General Hospital Owerri

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ABSTRACT

A total of two hundred (200) stool samples were collected from patients attending General Hospital Owerri and screened for the presence of Aeromonas species. Out of the two hundred (200) stool samples, one hundred and fifty (150) were collected from diarrheal patients while fifty (50) were collected from non-diarrheal patients. Aeromonas species were only isolated from diarrheal patients stool samples. The prevalence of Aeromonas species in diarrheal patients was 5.3%. Aeromonas species were found to be highly (100%) susceptible to ceftazidime, followed by cefotaxime (85%), then Augmentin (75%) and Gentamicin (65%), but highly (100%) resistant to Ampicillin and Tetracycline followed by cotrimoxazole (83%). This observation could probably indicate that Aeromonas as enteropathogen associated with diarrheal and should be considered amongst the causative agents of diarrheal.

KEY WORDS.

Prevalence, Aeromonas species, diarrheal patients, Owerri.

INTRODUCTION

Aeromonas Species are Gram-negative, non-spore-forming, rod-shaped, facultatively anaerobic bacteria that occur ubiquitously and autochthonously in aquatic environments¹. Historically, the *Aeromonas* genus has been placed in the family Vibrionaceae. There have been proposals to place it in its own family, the Aeromonadaceae. The aeromonads share many biochemical characteristics with members of the Enterobacteriaceae, from which they are primarily differentiated by being oxidase-positive. The genus includes at least 13 genospecies, among which are the mesophilic *A. hydrophila*, *A. caviae*, *A. sobria*, *A. veronii*, and *A. schubertii*, and the non-motile, psychrophilic *A. salmonicida*. Organisms from the genus *Aeromonas* are widely distributed in the aquatic environment, and its ability to produce diseases in different animal species is well established and documented in the scientific research². The potential of the *Aeromonas* species to cause diseases in humans has been studied, but only recently a major number of clinical cases have been confirmed and attributed to these organisms. *Aeromonas* are widely distributed in the aquatic environment, and are considered to be emerging organisms that can produce a series of virulence factors. The role of some *Aeromonas* species in rare but serious conditions including wound infections, necrosis, septicemia and meningitis is well documented. The role of *Aeromonas* in food and waterborne gastroenteritis remains hotly argued³. Members of four *Aeromonas* groups may cause gastroenteritis: *A. hydrophila*, *A. veronii* biovar *sobria*, *A.*

caviae and *A. trota*, *A. schubertii* and *A. jandaei* have also rarely been isolated from faeces. Some reported cases/outbreaks have implicated consumption of food contaminated with *Aeromonas*. Many potential virulence factors have been identified, and these may one day assist in the identification of virulent strains⁴. *Aeromonas* can grow at refrigeration temperatures and under both aerobic and anaerobic conditions, however they are easily destroyed when food is cooked. They do not form spores. Hence, it is the purpose of this research work to establish the prevalence of *Aeromonas* species in Imo State Nigeria.

MATERIALS AND METHODS

Study Area and Subjects

This study was carried out at General Hospital Owerri, Imo State, Nigeria. Patient examined during this study were from children's ward and emergency ward, patients from diarrhoeic clinic. Patients whose stool samples were watery were regarded as diarrhoeic. Both children and adults were used for diarrhoeic and healthy control. All the patients/samples used where those referred to the laboratory for investigation.

Sample collection

All stool samples for this study comprised of 150 stool samples from diarrheal clinic and 50 samples from apparently health individuals, picked across all ages as they were referred. All stool samples were collected in a wide month transparent sterile contains. All stool samples collected were properly labelled and recorded in a book set aside as a registers, this carried all the information of all the participant of this research. All of the samples were given serial numbers as they were received. These numbers were used for sample identification as the work proceeded.

Sample processing

All collected stool samples were processed as follows:

Macroscopy: The appearance of all the stool samples were taken into consideration if they were formed, semi formed or watery in nature. They were also examined to find out if the samples contained blood, pus, mucus or worms.

Stool Microscopy: Using saline and iodine with floatation technique. A drop of saline was placed on one end of a slide and a drop of iodine on the other end. Using a piece of stick or wire loop, small amount of fresh specimen was mixed (especially the part containing blood and mucus) with each drop. Each preparation was covered with cover glass. The preparations were examined using 10X and 40X objectives with the condenser iris sufficiently closed to give a good contrast. It was examined for parasites.

Culture: Each sample was immediately cultured on 10µ/ML of ampicillin sheep blood agar and incubated for 18-24hours at 37^{0c} in a canister with a candle light to provide the micro-aerophilic environment required by *Aeromonas* species. The second day, the 10µ/ml ampicillin sheep blood agar plates were checked for a grayish raised moist colony which is typical of *Aeromonas* species. Any colony resembling this was subcultured on a fresh CLEB (to have pure colonies of this organism for biochemical testing) and incubated at 37^{0c} overnight. The third day, the CLED purity plates confirmed to have pure growth of a single organism type were used to perform the biochemical tests described below. The stool sample was also inoculated on DCA and selenite f to look for other possible enteric pathogens.

BIOCHEMICAL TEST

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzymes will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium become alkaline as shown by a change in colour of the indicator to pink red.

Procedure

Inoculate heavily the test organism in a bijou bottle containing 3 ml sterile Christensen's modified urea broth. Incubate at 35-37^{0c} for 8-12 (preferably in a water bath for a quicker result). Look for a pink colour in the medium

Results

Pink colour ----- positive urease test

± different strains of *Aeromonas* gram negative enteric bacilli E.g *pleisiomonas* from other group of enterobacteriaceae.

Method

1. Moisten the strip with a drop of sterile water
2. Using a piece of stick or glass rod (not an oxidized wired loop) remove a colony of the test organism and rub it on the strips.
2. Look for a red-purple colour within 20 seconds.

Results

Blue-purple colour --- positive oxidase test (within 10 seconds)

No blue-purple colour --- negative Oxidase test (within 10 seconds)

c. Indole test

this was used to differentiate enterobacteriaceae that produce indole for example *E-Coli* from those that do not e.g *proteus species*

METHOD

1. Prepare a dense suspension of the test organism 0.25ml physiological saline in a small tube
2. Add 3 drops of Kovac's reagent and shake.
3. Wait 3 minutes before reading the indole reaction. Examine the colour of the surface layer.

Results

Red surface layer ----- positive indole test

Yellow surface layer ----- negative indole test

Different strains of *Aeromonas* give different results.

d. **Motility test**

Distilled H₂O motility test differentiate vibrio from *Aeromonas*

- A colony of growth from subculture agar plate (CLED) was mixed in a drop of distilled water on one end of a slide, on the other end a bacteria colony was mixed in a drop of peptone water (using the hanging drop method) the cover slips were used to cover the preparations and viewed at 10X and 40X objectives respectively.

Result:All vibrio species are immobilized in distilled water but remain motile in peptone water. While *Aeromonas* species remains motile in both distilled and peptone water.

e. **KIA (Kliggler Iron Agar):** KIA reactions are based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide.

-A yellow butt (acid production) and red- pink slope indicate the fermentation of glucose only. The slope is pink - red due to a reversion of the acid reaction under aerobic conditions. The reactions is seen with Salmonella and Shigella species and other enteric pathogens.

-Cracks and bubbles in the medium indicate gas production from glucose fermentation. Gas is produced by *S. Paratyphi* and some faecal commensals.

-A yellow slope and a yellow butt indicate the fermentation of lactose and possibly glucose. This occurs with *E. Coli* and other enterobacteria.

-A red pink slope and butt indicate no fermentation of glucose or lactose, this is seen with most strains of *P. Aeruginosa*.

-Blackening along the stab line or throughout the medium indicate hydrogen sulphide (H₂S) production, e.g *S typhi* produces a small amount of blackening whereas *S. Typhimurium* cause extensive blackening.

Antimicrobial Suseptibility Testing

Standardized single disc method for susceptibility testing for *Aeromonas* was used. The method was standardized by correlation of zone diameters with minimal inhibitory concentrations (MIC) of the antibiotics tested:

Antibiotic (Disc Concentration)	Antibiotic Make	Brand Name	Family Name
Ampicillin (10µg)	Juhel	Ampicillin	Penicillin
Gentamicin (10µg)	Generic	Gentamicin	Aminoglycoside
Tetracycline (30µg)	Juhel	Tetracycline	Tetracycline
Cotrimoxazole (25µg)	GSK	Septtrin	Solphonamide
Ceftazidime (30µg)	GSK	Fortum	Cephalosporin
Augmentin (30µg)	GSK	Augmentin	Penicillin
Cefotaxime (30µg)	May and Baker	Claforan	Cephalosporin

PROCEDURE

-A sterile wire loop was used to pick 3-5 colonies of the test organism with similar appearances and emulsified in 3-4ml of peptone water.

-A sterile swab stick was dipped into the organism suspension; excess fluid was removed by pressing and rotation the swab stick against the side of the tube above the level of the suspension

-The swab was used to inoculate the test organism suspension onto an already prepared nutrient agar plate by streaking the swab evenly over the surface of the medium

-The plate was covered with dish lid and allowed for the surface of the agar to dry for 3-5 minutes

-A sterile forcep was used used to place the appropriate antimicrobial disc, evenly distributed on the inoculated plate.

-Within 30 minutes of applying the disc, the plates were inverted and incubated aerobically at 35⁰C for 16-18hours.

-The plates were examined after overnight incubation.

-The diameter of each zone of inhibition wasw measured in millimetres (mm) using a meter ruler.

Result

The organism was reported as Sensitive, Intermediate or Resistant based on the diameter of zone of inhibition below.

Antibiotic (Disc Concentration)	Diameter of zone of inhibition (mm)		
	Susceptible(S)	Intermediate(I)	Resistant(R)
Ampicillin (10µg)	17	14 – 16	13
Gentamicin (10µg)	15	13 – 14	12
Tetracycline (30µg)	19	15 – 18	14
Cotrimoxazole (25µg)	16	11 – 18	10
Ceftazidime (30µg)	18	15 – 17	14
Augmentin (30µg)	18	14 - 17	13
Cefotaxime (30µg)	23	15 - 22	14

Statistical analysis

Statistical analysis was done using the chi-square test.

Results analysis

A total of 200 stool samples were collected and analysed. Out of this number, 8(4%) was found to contain *Aeromonas* (Table 4.1). this table also shows the prevalence of the subject aged 11-15 were infected giving also percentage of 9.5% while those at the age brackets (6-10years) and (21-25years) had infestation of (0%) and (0%) respectively. However, this was not statistically significant using ($X^2 = 4.897$; $P > 0.05$).

Table 4.2 shows prevalence of *Aeromonas* species among study participants by gender. More female subjects (4.5%). There was no significant ($X^2 = 0.66$; $P > 0.05$) difference in the prevalence of *Aeromonas* species among study participants by gender.

Table 4.3 is based on the prevalence of *Aeromonas* species among study participants by diarrheal disease. *Aeromonas* species were only isolated from diarrheal patient's faecal samples. The prevalence of *Aeromonas* species in diarrheal patients was (5.3%). There was no statistical significant ($X^2 = 2.356$; $P > 0.05$) difference in the prevalence of *Aeromonas* species irrespective of diarrheal disease.

The prevalence of some enteric pathogens among study participants is presented in Table 4.4. salmonella paratyphi A was isolated from (8.5%) diarrhoieci participants while salmonella typhi was isolated for (5%) of the participants and *Aeromonas* species from (4%). *Aeromonas* species amongst others, were the third most prevalent enteric pathogen among the study participants.

Table 4.5 shows antibiotic susceptibility pattern of *Aedromonas* species. All the *Aeromonas* isolated were susceptible to Ceftazidime. (85%) were susceptible to Cefotaxime (75%) to Augmentin and (65%) to Gentamicin. All the *Aeromonas* isolated (100%) were resistant to tetracycline. While (83%) were resistant to contrimoxazole.

Table 4.1: Prevalence of *Aeromonas* species among study participants by age distribution.

Age (Years)	Number Examined	Number (%) Positive
5	55	1 (1.8)
6 – 10	18	0 (0)
11 – 15	21	2 (9.5)
16 – 20	14	1 (7.1)
21 – 25	17	0 (0)
26 – 30	28	2 (7.1)
> 30	47	2 (4.3)
Total	200	8 (4)

Table 4.2: Prevalence of *Aeromonas* species among study participants by gender

Gender	Number Examined	Number (%) Positive
Male	89	3 (3.4)
Female	111	5 (4.5)
Total	200	8 (4)

Table 4.3: Prevalence of *Aeromonas* species among study participants with diarrhea

Disease Status	Number Examined	Number (%) Positive
Non-diarrheal Patients	50	0 (0)
Diarrheal Patients	150	8 (5.3)
Total	200	8 (4)

Table 4.4: Prevalence of some enteric pathogens among study participants

Pathogens	Number Examined	Number (%) Positive
Salmonella Paratyphi A	200	17 (8.5)
Salmonella typhi	200	10 (5)
Aeromonas Species	200	8(4)
Enteropathogenic EColi	200	6(3)
Shigella	200	4(2)
Other Salmonella Species	200	4(2)

Table 4.5: Antibiotic susceptibility pattern of *Aeromonas* species

Antibiotic	No (% Susceptibility) (S)	No (% Resistance) (R)
Ampicillin		no (0) no (100)
Gentamicin		no (65) no (35)
Tetracycline		no (0) no (100)
Cotrimoxazole		no (17) no (83)
Ceftazidime		no (100) no (0)
Augmentin		no (75) no (25)
Cefotaxime		no (85) no (15)

DISCUSSION

Aeromonas are widely distributed in the aquatic environment, and are considered to be emerging organisms that can produce a series of virulence factors^{5,6}. *Aeromonas* species are the most common organisms reported in infections of burns exposed to contaminated water. The detection of potentially pathogenic *Aeromonas* strains may represent a potential risk for human health, supporting the idea that further studies on the microbiological quality^{7,8,9}. Owing to its capability to adapt to several types of aquatic environments, *Aeromonas* species ubiquity has been one of the reasons for the development of research aiming to assess the real distribution of these

organisms, as well as its survival and virulence factors, and possible sources and routes of transmission of water should include specific searches for this bacterium¹⁰. Despite the existence of detailed case reports and epidemiological case control investigations, the role of *Aeromonas* as the etiological agent of bacterial diarrhea has been questioned and debated several times¹¹. However, it is well accepted that if *Aeromonas* can cause different infections like cellulitis, meningitis, pneumonia, wound infections and more in healthy humans, it can also have the capacity to produce diarrhea¹². In several reported studies throughout the world, *Aeromonas* species have been isolated at a rate of 0.6 to 7.2% in patients with diarrhea, predominantly in infants and children¹³.

A total of 200 stool samples (150 diarrhoeic and 50 non-diarrhoeic) were investigated for the prevalence of *Aeromonas* species. The 4% prevalence was from diarrhoeic participants. This result is in line with that of Altwegg et al.¹⁴, implicating *Aeromonas* in diarrhoeic disease. This work differs from that done by Aravena et al¹⁵ who had a prevalence of 42% from diarrhoeic participants. The reduce prevalence could be due to improvement in personal hygiene as suggested by Ballal *et al.*,¹⁶.

This work also revealed the presence of other enteropathogens such as *S. Typhi*, *S. Paratyphi A*, and *Shigella*. *Salmonella paratyphi A* had the highest prevalence of 8.5% than other enteric pathogens and these agree with the work of Razzolini et al¹⁷ with *Salmonella* prevalence of 11%. The susceptibility pattern of *Aeromonas* to some antibiotics showed that it was resistant to ampicillin and tetracycline. This could be largely due to drug abuse of such antibiotics that are cheap and dispensed by patent medicine stores¹⁸. Coftazidine had 100% susceptibility followed by cefotaxime and Augmentin. The mode of action of this drugs, high cost and route of administration can explain the high susceptibility. These results tend to agree with studies reported by Villarruel-Lo´ *et al*¹⁹.

Therefore this study reveals the prevalence of *Aeromonas* in diarrheal samples thereby implicating *Aeromonas* in diarrheal disease. It was more prevalence in children than adult.

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