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Occurrence and Antimicrobial Susceptibility Patterns of *Aeromonas hydrophila* Isolates among Diarrhoeic Patients From University of Abuja Teaching Hospital, Nigeria

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The present study is the first to be conducted in order to determine the occurrence and antimicrobial susceptibility pattern of *Aeromonas hydrophila* strains from diarrheic patients attending University of Abuja Teaching Hospital in Nigeria. A total of 153 stool samples were collected from out patients attending University of Abuja Teaching Hospital (UATH). The diarrhoeic patients were screened for the presence of *Aeromonas hydrophila* using routine cultural methods and biochemical characterization. Our results did show that the overall isolation rate is (3. 92%). The prevalence per age group is presented showed that age group 26-30 years having the highest rate of (1.31%) of the total sample analysed. Age groups 11-15 and 16-20 years having the same prevalence rate of (0.65%) each, while the age groups d"5, 6-10 and >30 had no prevalence for *Aeromonas hydrophila.* The distribution of *A. hydrophila* infection among different sexes has shown that (2.22%) out of the six *A.hydrophila* were isolated from diarrheic stools collected from males, while (6.35%) were isolated from females. Statistical analysis showed that X^2 yates corrected)= 0.05 P= 0.596, OR=1.10 (0.50<OR<2.80).This indicated the level of association between age and rate of *Aeromonas* infection. Antimicrobial susceptibility patterns of *A. hydrophila* showed that the isolates were extremely (100%) resistant to Ampicillin, Cephalothin, Gentamicin, Streptomycin, Sulphatriazone, Tetracyclin and Cotrimoxazole. All the isolates are highly susceptible to Colistin and Ceftazidime (100%) followed by Augmentin (83%). They are moderately susceptible to Cefutoxime 50%). Earlier studies revealed resistance to Tetracycline and Cotrimoxazole. Our study confirmed that *Aeromonas hydrophila* as an important enteropathogen responsible for diarrhoea in humans in Gwagwalada. Diagnostic regime should involve screening of this organism alongside other microorganisms responsible for diarrhoeic symptoms in man and animals. This is the first report to involve *Aeromonas hydrophila* in human diarrhoea and sought regime for choice of antibiotics for the management of the infection.

Keywords: *Aeromonas*, diarrhoea, antibiotic, susceptibility testing, Occurrence.

Aeromonas hydrophila are Gram-negative, non-spore forming, rod-shaped facultative anaerobic bacilli. They are generally motile by polar flagella (Villari *et al*., 2003). They grow over a wide range of temperature 0-40^{0}C, with human (motile mesophilic) strains growing between 10-40^{0}C, with 30^{0}C as the optimum temperature, while the non-motile psychrophilic species grow at between 22-28^{0}C in soil, food and animal body (Cheesbrough, 2005). *Aeromonas* were classified in the family *Vibrionaceae* (Jawetz*et al.*, 2004). However, molecular genetic evidence (including

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16s rRNA catalog, 5srRNA sequence, and rRNA-DNA hybridation) suggests they are not closely related to *Vibrio* species.In the latest edition of Bergy's Manual of Systematic Bacteriology; therefore, they are classified as a separate family the *Aeromonadaceae* (Jawetz*et al.,* 2004). *Aeromonas* are ubiquitous in fresh and brackish waters. These organisms have also been isolated from a wide variety of sources including soil, sea foods and humans (Michael *et al*., 2000).

The concentration of *Aeromonas* varies with environment in which they are found. In clean rivers, lakes, and storages reservoirs or grocery store products, concentrations are typically around 102 Colony Forming Units (CFU)/ml. The concentration in ground water is generally less than one CFU/ml. Drinking water immediately leaving the treatment plant may contain between 0-102 CFU/ml, with potentially higher concentration in drinking water distribution systems, thereby attributing it to grow in biofilms (United State Environmental Protection Agency, USEPA, 2005). Higher densities of 108 CFU/ml can be found in waste waters, treated sewage and crude sewage (Holmes *et al.*, 1996). They are also found in sinks, drain pipes, household effluents and are often associated with biofilm. *Aeromonas hydrophila* have also been isolated from a variety of animal foods, including red meat (beef, pork and veal), poultry and poultry products, fish and shellfish (USEPA, 2005). It has been isolated with variable frequency from different foods being if raw, refrigerated or frozen food of animal origin (Ventura *et al.*, 1998).

Aeromonas hydrophila have been implicated in a variety of infections in humans such as gastroenteritis, wound infections (cellulites), septicaemia and diarrhoea. Occasionally, urinary tract infections, meningitis, and peritonitis (Michael *et al*., 2000). *Aeromonas* infections are typically acquired through routes such as ingestion of contaminated water and food, or through contact of the organisms with a break in the skin (Jawetz *et al.,* 2004). They are also implicated in colitis, meningitis, and are frequently isolated from wound infection sustained in aquatic environments (Krovacek *et al.,* 1992). They are also being implicated in respiratory infection (Janda and Abbot, 1998). In recent years, *Aeromonas hydrophila* has gained public health recognition as an emerging pathogen and plays an important role food poisoning associated with human gastroenteritis (Balaji *et al.,* 2004).

The presence of these organisms in human stools is significantly more often associated with diarrhoea than with the carrier state (Kandakai-Olukemi *et al.,* 2007). To the best of our knowledge, not much study has been carried out in order to ascertain the prevalence of *Aeromonas hydrophila* in outpatients suffering from diarrhoea especially in the areas covered by this study. The lack of defined diagnostic and treatment regime and definitive control strategy stimulated the antibiotic search involved in this study. The present research therefore is the first to be conducted in order to determine the occurrence and antimicrobial susceptibility pattern of *Aeromonas hydrophila* strains in diarrheic patients attending University of Abuja Teaching Hospital in Nigeria.

MATERIALS AND METHODS

Study Area

Federal Capital Territory (FCT), in central Nigeria was created in 1976 from parts of former Nasarawa, Niger, and Kogi States. It situated between latitude 8.25^0 and 9.20^0 north of the equator and longitude 6.45^0 and 7.39^0 east of Greenwich Meridian and located North of the confluence of rivers Niger and Benue. FCT is bordered by Niger state to the North, Kaduna state to Northwest, Nasarawa to the South-East and Kogi to the West with a land mass of approximately $7,315km^2$. FCT is currently made up of six Local Government Area Councils namely: Gwagwalada, Kuje, Bwari, Kwali, Abaji and Abuja city situated within the savannah region with moderate climate conditions (Anon, 2012). The study was carried out in University of Abuja Teaching Hospital, Gwagwalada, and Abuja.

Collection of Samples/ Sampling

The authorities of the teaching hospitals were approached with a letter of introduction seeking its consent to undertake the study. The criteria used to collect the samples after the ethical clearance from the Head of Microbiology Department included: consent of the authorities, availability of microbiological units in the hospital and willingness of the owners approached to cooperate.

One hundred and fifty three (153) human stool samples were conveniently collected from the Teaching Hospital with previous arrangement made with the doctors or matrons in hospital establishment. About 5 gm. or 5 ml of each faecal sample was submitted for the study. The samples were collected when freshly voided faecal specimens were submitted to the various laboratories for bacteriological or parasitological examination. The samples were scooped with a spatula and decanted, respectively into a labelled screw cap polystyrene bio freeze bottle and tightly screwed. Care was taken to avoid gross contamination with environmental material. A corresponding questionnaire was given to the appropriate caregiver. It was used to collect information on age, sex, type of food consumed, source of water for domestic use, presence of vomiting, fever and episodes of diarrhoea. The periods of sample collection lasted for about four months that is, October 1st2015 to 31st January 2016. The samples were collected mostly in the mornings and evenings each day from Mondays through Sundays.

Processing of Samples

The samples were transported to the National Agency for Food and Drug Administration and Control (NAFDAC) reference laboratory Kaduna for examination. The stool samples were picked with the aid of sterilized cotton swabs and were dipped into the prepared tetrathionate broth. It was incubated at 37^0C for 24 hours. If not used immediately, it was sent to the Artificial Insemination Unit Laboratory of the Nigerian Agricultural Research and Liaison Service (NEARLS), and was stored in liquid nitrogen (-196^0C) until ready for culture and isolation. The conventional culture and isolation method used for the detection of *Aeromonas hydrophila* as described by Cowan and Steel (1974) were used. This involves enrichment, selective plating, detection of colonies, preliminary identification and complete biochemical identification.

Cultivation and Laboratory Identification

The isolation of *Aeromonas hydrophila* was by the methods adopted by Jatau and Yakubu (2004); Jawetz *et al.,* (2004). One gram (1g) of each sample was briefly emulsified in 3 ml of sterile 0.85% (w/v) saline and subsequently vortexed under safety carbine for 30 seconds. Organic debris was allowed to settle down for five minutes. Wet mounts were prepared and examined microscopically with X10 objective followed by X40. Stools with protozoan parasites or worms were excluded from the study. The samples were pre enriched in alkaline peptone water (Oxoid, PH 9.0) and sub-cultured after incubation at 37^0C for 6 hours onto MacConkey agar (Oxiod) and Sheep blood agar (5% sheep blood) supplemented with 10mg/l ampicillin (SBAA), flowed by incubation at 37^0C for 24hrs. Ampicillin-resistant â-hemolytic colonies that appeared greyish white, stippled and translucent on SBAA and colonies which failed to ferment lactose on MacConkey agar were Gram stained and Gram negative rods isolated and stored on nutrient agar (Oxoid) slants as presumptive *A. hydrophila.* Non-lactose fermenting colonies were picked and tested for production of oxidase. Oxidase positive organisms were purified by re-streaking on nutrient agar slants and pure colonies inoculated onto the surface of prepared nutrient agar slants. They were incubated for 24 hrs at 37^0C and stored in the refrigerator for further biochemical tests.

Biochemical Characterization of the Isolates

Ampicillin-resistant â-haemolytic colonies on SBAA and Non-lactose fermenting colonies on MacConkey agar were subjected to indole, methyl red, Vogues proskauer, citrate IMVIC test, and also inoculated on Kligler Iron Agar (KIA) slants (Oxoids). Those that gave ++++ IMVIC reactions and K/AG (glucose and gas positive, lactose negative) reactions were tested for cytochrome C oxidase activity by Kovac's method (Cowan and Steel, 1994). Oxidase-positive colonies were examined for amylase activity on Starch-Ampicillin agar (Jatau and Yakubu, 2004). The isolates were further tested for hydrolysis of aesculin and acid production from arabinose The isolates were further tested for resistance to 150µg 0/129 Vibrio static agent (2, 4-diamio-6, 7-diisoprophylpteridine). Owing to the reported increased incidence of pteridine resistant *Vibrio cholera*, all identified *A. hydrophila* were examined for motility in distilled water (Cheesbrough, 2005), and confirmed according to the methods of Cowan and Steel (1994) and McFaddin (2000). The isolates were stored on nutrient agar slants (Oxoid) for further tests.

Antimicrobial Susceptibility Testing

Kirby-Bauer National Committee for Clinical and Laboratory Standard (NCCLS, 2002) modified disc diffusion technique was used to examine the antimicrobial susceptibility of the isolates. The antibiotic multiple disc (Abtek Biologicals Ltd-LotHJ03/P) used comprised of Ampicillin (10µg), Cotrimoxazole (25µg), Gentamicin (10µg), Streptomycin (20µg)Tetracyline (25µg), Cephalothin (5µg), Colistin (25µg), Sulphathiazone (200µg), Cefutoxime (30µg), Ceftazidime (30µg), Augmentin (Amoxycillin and Clauvulanic acid) (30µg).

Each isolate was grown overnight on nutrient agar to obtain isolated colonies. Isolated colonies were transferred to a test tube of sterile saline (0.8% W/V NaCl) and vortexed thoroughly until the turbidity compared to the same with 0.5 McFarland turbidity standards (1x108cells/ml). Within 15 minutes after standardizing the inoculum, a sterile cotton swab was dipped into the inoculum and excess liquid was removed by pressing the swab firmly against the inside wall of the tube just above the fluid level. The swab was used to streak the entire surface of Mueller –Hinton agar (Oxiod) plates. The plates were allowed to stand for 5 minutes. Antibiotics discs were aseptically placed firmly on the surface of the inoculated agar plates using sterile forceps, and the plates were incubated at 37^0C for 24 hours. Diameters of zone of inhibition were measured and isolates were characterized as susceptible or resistant according to NCCLS (2002) interpretation chart.

Table 1. Prevalence of *Aeromonas hydrophila* in Various Age groups of Diarrhoeic Humans

Age (years)	No. of samples positive	Age (years) samples positive	Age (years) percentage positive
≥ 5	84	0	0
6 – 10	10	0	0
11 – 15	6	1	0.65
16 – 20	6	1	0.65
21 – 25	7	0	0
26 – 30	20	2	1.31
> 30	20	2	1.31
Total	153	6	3.92

RESULTS

Out of the one hundred and fifty three (153) diarrheic stool samples analysed, 6(3. 92%) were found to be positive for *Aeromonas hydrophila*. The prevalence per age group is presented in Table1. Showed that age group 26-30 years having the highest rate of 2 (1.31%) of the total sample analysed. Age groups 11-15 and 16-20 having the same prevalence rate of 1 (0.65%) each, while the age groups d”5, 6-10 and >30 had no prevalence for *Aeromonas hydrophila*. The distribution of *A. hydrophila* infection among different sexes is shown in Table2. 2 (2.22%) out of the six *A. hydrophila* were isolated from diarrheic stools collected from males, while the remaining 4 (6.35%) were isolated from samples collected from females. Table 3 presents the antimicrobial susceptibility patterns of *Aeromonas hydrophila* to eleven antibiotics tested against the isolates. Out of the six *Aeromonas hydrophila* isolates, two showed varying susceptibility to Cefotaxime and Amoxycillin/ Clauvulanic acid. Colistin, and ceftaxidim, were susceptible to the entire four (6) isolates. However, all the (6) isolates were resistant

Table 2. Prevalence of *Aeromonas hydrophila* infection in various human sex groups

Sex	No of samples Collected	No of positive for *A. hydrophila*	Percentage Prevalence
Male	90	2	2.22
Female	63	2	6.35
Total	153	6	3.92

X^2(yates corrected)= 0.05 P= 0.596, OR=1.10(0.50<OR<2.80)

to cephalothin, Ampicillin, streptomycin, sulphatriazone, tetracycline, and cotrimoxazole. Table 4-5 shows the existence of high level of multiple drug resistance among the strains particularly to cephalothin, streptomycin, sulphatriazone, tetracycline, ampicillin and cotrimoxazole.

DISCUSSION

The recovery of six strains of *Aeromonas hydrophila* from human faecal samples indicates the suitability of this medium for field isolation of members of the genus *Aeromonas*. The research conducted also documented the occurrence of *A. hydrophila* in humans in the Gwagwalada area. There are several bases for a diagnosis of aeromoniasis, but the definitive one is isolation of the organism (Nzeako *et al.*, 2002). The identification of *Aeromonas* strains from humans as revealed by this study are therefore of public health significance. Most previous reports on the investigation of aeromoniasis among human population were based on clinical signs and there have not been much effort to isolate and characterized aeromonae from human population in Nigeria since reviews of literature from various parts of the country have revealed that aeromoniasis widely distributed. There is a pattern of low, medium and high prevalence in specific areas and prevalence also varies among animal species in the same area (Abbey *et al.*, 2004). The confirmatory diagnosis of aeromoniasis is based on isolation and identification of the organism from the infected host. The isolation and subsequent characterization of *Aeromonas hydrophila* from humans is therefore very significant.

The overall prevalence rate of 3.92 % of *Aeromonas hydrophila* found in this study is lower than the rate of 10.2 % found in Japan (Arai *et al*., 1980). It was also higher than the 3.8 % isolation rate reported by Kwaga *et al*. (1988) in Zaria. Conversely, higher prevalence rate of isolation is a

Table 3. Antimicrobial susceptibility patterns of*Aeromonas hydrophila* of six isolates

Antibiotics	No of Isolates Susceptible (%)	No of Isolates Resistant (%)
Ampicilin	0 (00	6(100)
Cephalothin	0 (00)	6(100)
Colistin	6 (100)	0(00)
Gentamicin	3 (50)	3(50)
Streptomycin	0 (00)	4(100)
Sulphatriazone	0(00)	4(100)
Tetracycline	0(00)	4(100)
Cotrimoxazole	0(00)	4(100)
Ceftazidime	6(100)	0(00)
Cefutoxime	3(50)	3(50)
Augmentin	5(83)	1(17)

N= 4. N-total number of *Aeromonas hydrophila* tested. Values in ( ) are percentages

Table 4. *Aeromonas hydrophila* Isolates and Their Resistance Patterns

Isolate	No. of brands	Resistance patterns[a]	% Resistance[b]
A. hydrophila	5	AMP,FLX,ERY,PEN	
A. hydrophila	6	AMP,FLX,ERY,PEN,CRX	
A. hydrophila	6	AMP,FLX,ERY,PEN,CRX,COT	
A. hydrophila	4	AMP,FLX,ERY,PEN,CRX,COT	
A. hydrophila	3	AMP,FLX,ERY,PEN,CRX,COT,TET	
A. hydrophila	3	AMP,FLX,ERY,PEN,CRX,COT	
A. hydrophila	2	AMP,FLX,ERY,PEN,CRX	
A. hydrophila	1	AMP,FLX,ERY,PEN,CRX,CIT,TET	
A. hydrophila	1	AMP,FLX,PEN,CRX,CIT	
A. hydrophila	1	AMP,FLX,ERY,PEN,CRX	
A. hydrophila	1	AMP,FLX,ERY,PEN,CRX	
A. hydrophila	1	AMP,FLX,ERY,PEN,CRX,COT,TET	

[a]Resistance patterns constructed from the antibiogram; antibiotic codes as defined under methodology. [b]Percentage abstained from the antibiogram

Table 5. Percentage resistance of *Aeromonas hydrophila* to different antibiotics

Antibiotic (pg/disk)	No. of strains resistant to antibiotics	%Resistance to antibiotic
Ampicillin (10)	6	100.0
Cephalothin(5)	6	100.0
Colistin(15)	6	00.0
Gentamycin (5)	6	100.0
Streptomycin(30)	6	100.0
Sulphatiazole(25)	6	100.0
Tetracycline (10)	6	100.0

Cotrimoxazole (10)Ceftazidime (5)Cefotoxime (10)Amoxycillin/Clauvulanic acid (10) 6666 100.00.0050.017.0

signal to public health authorities that aeromoniasis is on the increase. Appropriate measures need to be put in place to control and prevent this disease. Our results clearly showed the existence possible seriousness of these disease humans in Gwagwalada. The high prevalence of *Aeromonas* species reported in this study warns on public food safety problem in Nigeria. In most parts of Nigeria, people handle fish and poultry products with bare hands and sometimes consume contaminated water which may promote the spread of the disease. This suggests that *Aeromonas hydrophila* infection indicated in our study might be food borne or water borne rather than air borne in origin. Other factors responsible for the outbreak may include geographical location, seasons of the year, health status or hygiene of the individual and methods of isolation.

The distribution of the *Aeromonas hydrophila* from different age groups and sexes showed that females dissipated higher prevalence (6.35%) than males (2.22%) while the very young age groups had no infection. Age groups of 16-25 years had prevalence of (0.65%) and those within the range age of 26- above 30 years showed prevalence of (0.31%).Statistical analysis showed that X^2yates corrected)= 0.05 P= 0.596, OR=1.10 (0.50<OR<2.80).This indicated the level of association between age, sex and rate of *Aeromonas* infection. The implications of the findings is that *Aeromonas hydrophila* is common in children than adults may be attributed to the fact that children have comparably lower post exposure immunity and are more adventurous than adults. This may expose them to niches and surfaces that are easily contaminated. The habit of indiscriminate picking and putting up objects in the mouth by children also encourages transmission of the infection.

The fact that World Health Organization (WHO) report on infectious diseases recently declared that antibiotic resistance poses a severe threat to human health, and that the problem is growing globally (WHO, 2002). Thus monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control. In addition, selections of antibiotic patterns are sometimes useful as characteristics for species identification, especially for clinical isolates (Jawetz*et al*., 2007). Antimicrobial susceptibility patterns of *A.hydrophila* showed that the isolates were extremely (100%) resistant to Ampicillin, Cephalothin, Gentamicine, Streptomycin, Sulphatriazone, Tetracyclin and Cotrimoxazole. All the isolates are highly susceptible to Colistin and Ceftazidime (100%) followed by Augmentin (83%). They are moderately susceptible to Cefutoxime 50%). Earlier studies by Subaskumar *et al*. (2006) revealed resistance to Tetracycline and Cotrimoxazole and susceptibility for colistin, gentamicin and ceftazidime. This corroborates well with the findings indicated in our studies. The presence of antibiotic resistant *Aeromonas hydrophila* in diarrhoeic patients in Gwagwalada is of immense public health significance because of the dangers in promoting multiple antibiotic resistances through the colonization of the gastrointestinal tract and conjugal transfer of antibiotic resistance to the normal flora leading to increase in multi- drug antibiotic resistant strains of the bacteria. The apparent resistance of *A. hydrophila* to antibiotics may be a result of indiscriminate or sub therapeutic use of antibiotics. Multiple drug resistance among *Aeromonas hyrophila* has been reported from many parts of the world (Sinha *et al*., 2004). Multiple drug resistance occurred more in *A.hydrophila* than other species of *Aeromonas* and that isolates from humans and animals could be more resistant to antibiotics (Sinha *et al*., 2004). The prevalence of drug resistant strains of *Aeromonas* possess great challenge to clinicians and the consumption of animal food and food products like meat, milk and

eggs containing this antibiotics may be inappropriate and may require new and mostly expensive antibiotics. Our findings is the first to expose the presence of antibiotic resistant strains of *Aeromonas hydrophila* in diarrhoeic patients with its public health implications and recommends that stringent measures be taken to prevent their occurrence. This study may form basis for *Aeromonas* research especially in the investigation of risk factors associated with aeromoniasis, search for new drug discovery and control of the disease in humans and animal population and in the environment. On this light, routine diagnostic regime in our hospitals should involve screening of this organism alongside other microorganisms responsible for diarrhoeic symptoms in man and animals.

CONCLUSSION

The objectives of our research have been greatly achieved. The overall prevalence of *A. hydrophila* indicated in our study was 3.92% and prevalence was higher for females (6.35%) than males (2.22%). The isolates also developed multi drug resistance to commonly used antimicrobial agents. In view of the high level of multiple drug resistance observed in our study, regulations should be enforced governing the handling and sales of antibiotics to avoid indiscriminate use of drugs which may enhance the development of resistant mutants. Enlightenment of the public as regards to personal hygiene of individuals, use of antibiotics in animals, water and the environment is highly recommended.

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