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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *AEROMONAS HYDROPHILA* AMONG PATIENTS PRESENTED WITH DIARRHEA ATTENDING TWO TEACHING HOSPITALS IN NORTHERN, NIGERIA

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ABSTRACT

A total of one hundred and twenty eight (128) stool samples were collected from patients attending Ahmadu Bello University Teaching Hospital Zaria and Aminu Kano Teaching Hospital Kano, and screened for the presence of Aeromonas hydrophila infection. Out of the one hundred and twenty eight samples analyzed 4 (3.12%) were found positive for Aeromonas hydrophila infection. Antibioticsusceptibility testing of Aeromonas hydrophila isolated showed that all the 4 (100%) isolates were sensitive to Colistin and Ceftazidime, 3 (75%) to Augmentin and 2(50%) to Gentamicin and Cefuroxine. All the isolates (100%) were however resistant to Cotrimoxazole, Tetracycline, Sulphatriad, Streptomycin, Cephalothin and Ampicilin. This study confirmed that Aeromonas hydrophila as a sole enteropathogen could be responsible for diarrhea and should be considered amongst the causative agents of diarrhea.

Key words: Aeromonas, diarrhoea, antibiotic, susceptibility testing, ABUTH, AKTH.

INTRODUCTION

Aeromonas hydrophila are Gram-negative, non–spore forming, rod-shaped facultative anaerobic bacilli. They are generally motile by polar flagella (Baron and Finegold, 1990; Villari *et al.,* 2003). They grow over a wide range of temperature 0-40^{0}C, with human (motile mesophilic) strains growing at 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 (Jatau and Yakubu, 2004; Cheesbough, 2005).

Until recently, *Aeromonas* were classified in the family Vibrionaceae (Jawetz *et al*., 2004). However, molecular genetic evidence (including 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 (Sylvia *et al.,* 2004; Jawetz *et al*., 2007). *Aeromonas* are ubiquitous in fresh and brackish waters (Jawetz *et al.,* 2004). These organisms have also been isolated from a wide variety of sources including soil, sea food and human (Bishara, 1984; Michael *et al.,* 2000). The concentration of *Aeromonas* varies with environment in which they are found. In clean rivers, lakes, and storages reservoirs, concentrations are typically around 10^{2}cfu/ml. The concentration in ground water is generally less than 1 cfu/ml. Drinking water immediately leaving the treatment plant may contain between 0-10^2 cfu/ml, with potentially higher concentration in drinking water distribution systems, attributed to growth in Biofilms (Payment *et al.,* 1988; United State Environmental Protection Agency, 2005). Higher densities of 10^{8}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 and household effluent (Araujo *et al.,* 1991). *Aeromonas* species have been isolated from a variety of foods, including red meat (beef, pork and lambs) poultry produce, fish and shellfish (USEPA, 2005). *Aeromonas* species have been implicated in a variety of infections in humans such as gastroenteritic, wound infections (cellulites), speticaemia, and occasionally others including urinary tract infection, meningitis, and peritonitis (Michael,1991). *Aeromonas* infections are typically acquired through two routes, ingestion of contaminated water or food, or through contact of the organisms with a break in the skin (Jawetz *et al*., 2004). Diseases associated with *Aeromonas* are intestinal and extra-intestinal. 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 (Bottarelli and Ossiprendi, 1999). Although food poisoning potential has not been reported, the association with human gastroententis strongly suggests that *A. hydrophila* plays a significant role in food borne diseases (Balaji *et al*., 2004).

The presence of these organisms in stools is significantly more often associated with diarrhea than with carrier state (Agger *et al.,* 1985; Aslani and Alikhani, 2004; Jawetz *et al.,* 2007; Kandakai-Olukemi *et al.,* 2007). *Aeromonas hydrophila* can be isolated with variable frequency from different foods (raw, refrigerated or frozen) of animal origin (Ventura *et al,* 1998). Some preservative techniques seem ineffective in inhibiting the replication of *A. hydrophila,* which can multiply although at slow rate in products which are refrigerated and vacuum packed or packaged in modified atmosphere. The organism can also replicate at low pH (4.5) or at high sodium chloride (NaCl) concentration (up to 5%) in the environment (Bottarelli and Ossipnendi, 1999). The isolation of *A. hydrophila* from chlorinated water has been reported and it is less sensitive to chlorine compared to the coliforms (Chamorey *et al.,* 1999). The presence study was therefore conducted with the aim of isolation and determining the antimicrobial susceptibility pattern of *Aeromonas hydrophila in diarrheic stools* of patients attending Aminu Kano Teaching Hospital, Kano and Ahmadu bello University Teaching Hospital, Zaria.
The presence study was therefore

MATERIALS AND METHODS

Study Area

The study area covered Zaria metropolis and Kano metropolis. Zaria is located on longitude 8^0 and latitude 9^0 in Kaduna State Northern part of Nigeria. Kano is located on longitude 10° and latitude 11° in Kano state Northern part of Nigeria. Samples were collected from patients presented with gastroenteritis attending Ahmadu Bello University Teaching Hospital Shika, Zaria and Aminu Kano Teaching Hospital, Kano.

Collection of Samples

Stool samples were collected from patients attending Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria and Aminu Kano Teaching Hospital (AKTH) Kano. The diarrhea was defined on the basis of frequency of defecation per 24 hours and the form of the stool. Samples were collected in wide mouth screw capped bottles and transported to the laboratory in an insulated icebox with ice packs as described by Cheesbrough (2005). Information was also obtained from the patients regarding age, sex, major symptoms (diarrhea, vomiting and fever) and duration of disease. All samples were analysed within 8 hours of collection.

Isolation of *Aeromonas hydrophila*

The isolation of *Aeromonas hydrophila* was by the methods of Nzeako *et al.* (2002) and Jatau and Yakubu (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 hrs 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 β-hemolytic colonies that appeared grayish 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.*

Biochemical Characterization of the Isolates

Ampicillin-resistant β-hemolytic colonies on SBAA and Non-lactose fermenting colonies on MacConkey agar were subjected to indole, methyl red, Voges-proskauer, citrate IMVIC test, and also inculated 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 Kovace method (Cowan, 1993). 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 (McFaddin, 2000). 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* (Ramamurthy *et al.,* 1992), all identified *A. hydrophila* were examined for motility in distilled water (Cheesbough, 2005), and confirmed according to the methods of Cowan (1993) 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, 2000; WHO, 2002) modified disc diffusion technique was used to examine the antimicrobial susceptibility of the isolates. The antibiotic multiple disc (Abtek Biologicals Ltd-Lot-HJ03/P) used comprised of Ampicillin (10µg), Contrimorazole (25µg), Gentamicin (10µg), Tetracyline (25µg), Cephalothin (5µg), Colistin (25µg), Sulphatriad (200µg), Cefurexine (30µg), Ceftazidine (30µg), Augmentin (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 ($1x10^8$cells/ml). Within 15 minute after standardizing the inoculum, a sterile cotton wool 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.

RESULTS

Out of the one hundred and twenty eight (128) diarrheic stool samples analyzed, four (3. 12%) were found to be positive for *Aeromonas hydrophila*. The prevalence per age group is presented in Table 1. The prevalence per age group as shown in Table 1 showed that age group 26-30 years having the highest rate of 2 (1.56%) of the total sample analyzed. Age groups 11-15 and 16-20 having the same prevalence rate of 1 (0.78%) each, with the age groups ≤ 5, 6-10 and >30 had no prevalence for *Aeromonas hydrophila* out of the total samples analyzed. The distribution of *A. hydrophila* infection among different sexes is shown in table 2. Two (2) 1.56% out of the four *A.hydrophila* were isolated from diarrheic stools collected from males, while the remaining two (1.56%) were isolated from samples collected from females. Table 3 presents the antimicrobial susceptibility patterns of *Aeromonas hydrophila* to various drugs tested against the isolates. Out of the four (4) *Aeromonas hydrophila* isolates, two (1.56%) were susceptible to Gentamicin and Cefuroxime, three (2.34%) were susceptible to the entire four (4) isolates. However, all the four (4) isolates were resistant to cephalothin, streptomycin, sulphatriad, tetracycline, amphicillin and cotrimoxazole. Generally, there is high level of multiple drug resistance among the strains particularly to cephalothin, streptomycin, sulphatriad, tetracycline, ampicillin and cotrimoxazole.

Table 1: Prevalence of *Aeromonas hydrophila* infection in various ages Groups

Age (years)	No of samples	No of positive for *A.hydrophila*	Percentage Prevalence (%)
≤ 5	46	0	0
6 – 10	10	0	0
11 – 15	6	1	0.78
16 – 20	6	1	0.78
21 – 25	7	0	0
26 – 30	20	2	1.56
> 30	33	0	0
Total	**128**	**4**	**3.12**

Table 2: Distribution of *Aeromonas hydrophila* From Positive Stool Samples by Sex

Sex	No of samples	No of positive for *A. hydrophila*	Percentage Prevalence
Male	68	2	1.56
Female	60	2	1.56
Total	**128**	**4**	**3.12**

Table 3: Antibiotic Susceptibility Pattern of *Aeromonas hydrophila*

Antibiotics	No of Isolates Susceptible (%)	No of Isolates Resistant (%)
Ampicilin	0 (00)	4(100)
Cephalothin	0 (00)	4(100)
Colistin	4 (100)	0(00)
Gentamicin	2 (50)	2(50)
Streptomycin	0 (00)	4(100)
Sulphatriad	0(00)	4(100)
Tetracycline	0(00)	4(100)
Cotrimoxazole	0(00)	4(100)
Ceftazidime	4(100)	0(00)
Cefutoxime	2(50)	2(50)
Augmentin	3(75)	1(25)

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

DISCUSSION AND CONCLUSION

The World Health Organization (WHO) report on infectious diseases in 2000 declared that antibiotic resistance poses a severe threat to human health, and that the problem is growing globally. 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).

The antimicrobial susceptibility patterns of *A.hydrophila* showed that the isolates were extremely resistant to Ampicillin, Cephalothin, Streptomycin, Sulphatriad, Tetracyclin and Cotrimoxazole (100%). All the isolates are highly susceptible to Colistin and Ceftazidime (100%) followed by Augmentin (75%). They are moderately susceptible to Gentamicin and Cefuroxime (50%). Earlier studies revealed resistance to Tetracycline and Cotrimoxazole (Subaskumar *et al*., 2006).

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 spp* has been reported from many parts of the world (Ko *et al.,* 1996; Sinha *et al.,* 2004). Multiple drug resistance occurred more in *A. hydrophila* than other species of *Aeromonas* and that isolates from humans and animals are more resistant to antibiotics (Sinha *et al.,* 2004).

High prevalence of multiple drug resistance amongst the *Aeromonas hydrophia* isolates was noticed. However, the study did not investigate viral etiologic agents of diarrhea. In view of the high level of multiple drug resistance shown by *A. hydrophila* in this study, regulations should be enforced governing the handling and sales of antibiotics to avoid indiscriminate use of drugs that could lead to sub therapeutic dosage thereby enhancing the development of resistant mutants. Enlightenment of the public as regards to personal hygiene of individuals, foods, water and the environment is highly recommended.

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