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ISOLATION OF *AEROMONAS* SPECIES FROM CHILDREN WITH AND WITHOUT DIARRHOEA IN JOS, NIGERIA.

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ABSTRACT

An investigation on the prevalence and antibiogram of *Aeromonas* species among children in Jos was conducted. The samples analysed included a total of 104 (52 diarrhoeal and 52 non – diarrhoea) stool samples collected from Vom Christian and Plateau Specialists Hospital in Jos. *Aeromonas* isolates were identified using standard biochemical tests. Of the total number examined, 6 (5.7%) were positive for *Aeromonas* species, 2 (3.9%) from diarrhoeal and 4 (7.7%) from non diarrhoeal samples (P>0.05). All isolates were identified as *Aeromonas hydrophilia*. The highest number of isolates 3 (10.7%) were recovered from the group 7-12 months. No isolates were recovered from exclusively breast fed children while the highest number 4 (9.8%) was found in children fed with breast milk and formula. The isolates were found to be very sensitive to ciprofloxacin, but resistant to penicillin.

INTRODUCTION

Diarrhoeal diseases constitute major childhood mortality and morbidity world wide especially in developing countries (1). Estimates show that diarrhoeal diseases cause nearly 5 million deaths annually in children under 5 years old in developing countries. Traditional aetiologic agents of diarrhoea include *Entamoeba histolytica, Giardia lamblia, Salmonella species, Shigella* species and *Vibrio cholerae* (2). However, other agents as *Campylobacter, Yersinia, Aeromonas, Plesiomonas* and Crytosporidium have also been implicated in gastrointestinal diseases and are often referred to as new agents of diarrhoea (3,4).

Of growing importance in recent times is *Aeromonas* which affects all age groups but is said to be most common in children under 5 years, the elderly and the immunocompromised (5).

Aeromonas species are gram-negative bacilli of the Aeromonadaceae family. These motile bacteria are involved in both intestinal and extraintestinal human infections (6) with clinical manifestations ranging from skin and soft tissue infection, bacteremia, to gastroenteritis (7). However, acute watery diarrhoea with a short duration is the most common clinical feature(8).

The first reported association of *Aeromonas* with gastrointestinal disease was in 1958 in Jamaica(9), since then numerous reports have appeared from several countries including Italy, England, Australia and the United States regarding the isolation of *Aeromonas* from faeces of patients with diarrhoea (10,11).

In Nigeria Obi *et al* (12) identified *Aeromonas* species and *Plesiomonas shigelloides* as bacterial of diarrhoea in urban and rural areas. *Aeromonas* have also been found in cases of acute diarrhoea and asymptomatic infections in Nigerian school children (13).

Reported frequency of isolation from symptomatic (diarrhoeic) as compared with asymptomatic (non-diarrhoeic) cases varies considerably, with some studies showing no significant difference in isolation rates (14, 15).

This study was therefore undertaken to examine the prevalence of *Aeromonas* species among children with and without diarrhoea and to identify the antibiogram of recovered isolates.

MATERIALS AND METHODS

Samples

The samples analysed in this study included a total of one hundred and four (52 diarrhoeal and 52 non-diarrhoeal) stool specimens collected from Vom Christian and Plateau Specialist Hospital in Jos.

Stool samples were collected from patients in clean, transparent wide-mouthed bottles. Information was also obtained from each subject regarding age, sex, major symptoms (diarrhoea, vomiting and fever) duration of disease, source of water and feeding pattern.

Processing of Specimens

The specimens were processed according to guidelines provided by Cheesbrough(16) for the laboratory diagnosis of enteric pathogens. These include, macrosoopy, microscropy, gram stain, motility testing, culture, biochemical testing and antimicrobial sensitivity testing.

Specimens were inoculated into the medium of Agger *et al* (5) for the isolation of *Aeromonas* species (5% sheep blood agar containing 30μg/ml ampicillin). The inoculated plates were then incubated aerobically at 37°C for 24 hours. Resultant colonies were identified using biochemical tests.

Biochemical testing

Isolates that were beta haemolytic on sheep blood agar and gram –negative bacilli were identified as *Aeromonas* species using the following standard tests; oxidase test, indole test, urease test, citrate utilization test and test to determine motility after distilled water and peptone water subcultures. All tests were done using the methods described by Collee and Miles (17) and Porter and Duguid (18).

Characterization of Species

Isolates were characterized to the species level based on seven biochemical tests as described by Carnahan *et al* (19). These included aesculin hydrolysis, gas from glucose, acid from arabinose, indole production, acid from sucrose, Voges-Proskauer reaction and resistance to cephalothin (30μg).

Antimicrobial Susceptibility Testing

Sensitivity of isolates to antimicrobial agents was determined on Mueller-Hinton agar plates using the disc diffusion method of Scott (20). From a pure culture of the isolate to be tested a uniform streak was made on the agar plate. The antibiotic (Antec Diagnostics, UK) discs were placed on the plates and incubated at 37^0C overnight. Interpretation of results was done using the zone sizes. Zones of inhibition of ≥ 18mm were considered sensitive while 13-17mm were considered intermediate and <13mm were considered resistant. All isolates were tested for sensitivity to the following antibiotics, ciprofloxacin (5mcg) cotrimoxazole (25mcg) streptomycin (10mcg), gentamycin (10mcg), erythromycin (5mcg), tetracycline (10mcg) penicillin (5mcg) peflacine (10mcg) and tarivid (10mcg).

Statistical Analysis

The data obtained were subjected to the chi-squared test using a probability of P=0.05 as the level of significance.

RESULTS

A total of 104 (54 diarrhoeal and 52 non-diarrhoeal) stool samples were examined. The age range of the patients was 0-72 months. Of the total number of specimen examined, 6 (5.7%) were positive for *Aeromonas* spp. 2 (3.9%) of *Aeromonas* spp were recovered from diarrhoeal stool specimens while 4(7.7%) from non-diarrhoeal samples (Table 1). The difference is not statistically significant (P>0.05). All the isolates were found to be *Aeromonas hydrophila.*

The highest numbers of isolates 3(10.7%) were recovered form the age group 7-12 months. The age brackets 13-18 months, 19-24 months and 67-72 months had 1 isolate each. No isolates were recovered from age group 0-6 months and from 25-66 months (Table 2). The difference is not statistically significance (P>0.05).

Macroscopic examination of the specimens showed that 36 were watery 13 mucoid, 3 blood stained, 40 soft-formed and 12 hard-formed. The soft-formed specimens yielded the highest number of isolates 3 (7.5%), watery samples 2(5.6%) and, hard-formed 1(8.3%). The blood stained and mucoid specimens yielded no isolates (Table 3). This difference is not statistically significant (P>0.05).

Table 4 shows the prevalence of *Aeromonas* spp in relation to the feeding pattern of the children. The highest number of isolates was found in children fed with breast milk and formular 4 (9.8%) followed by formular and family diet 2(4.7%). No isolates were recovered from exclusively breast fed children. This result is not statistically significant.

Table 5 shows the in-vitro susceptibility pattern of the isolates. Six (100.0%) of the isolates were sentive to ciprofloxacin, 5 (83.33%) to gentamycin, peflacine and tarivid, 4 (66.67%) to erythromycin and streptomycin, 3 (50.0%) to tetracycline and cotrimoxazole. All isolates were resistant to penicillin.

Table 1: Prevalence of *Aeromonas* species among symptomatic and asymptomatic patients.

Patients	No. of Specimens Examined	No. (%) Positive
Symptomatic (with diarrhoea)	52	2(3.9)
A symptomatic (without diarrhoea)	52	4(7.7)
Total	**104**	**6(5.8)**

$\chi^2 = 1.2$ df = 1 P>0.05

Table 2: Prevalence of *Aeromonas* species isolated in relation to age and sex:

Age Group (Months)	No of Specimens Collected		No (%) Positive		
	Male	Female	Male	Female	Total
0-6	5	14	0(0.0)	0(0.0)	0(0.0)
7-12	17	11	2(7.1)	1(3.6)	3(10.7)
13-18	10	5	1(6.7)	0(0.0)	1(6.7)
19-24	8	5	1(7.7)	0(0.0)	1(7.7)
25-30	5	2	0(0.0)	0(0.0)	0(0.0)
31-36	3	1	0(0.0)	0(0.0)	0(0.0)
37-42	3	2	0(0.0)	0(0.0)	0(0.0)
43-48	2	1	0(0.0)	0(0.0)	0(0.0)
49-54	1	0	0(0.0)	0(0.0)	0(0.0)
55-60	2	1	0(0.0)	0(0.0)	0(0.0)
61-66	2	1	0(0.0)	0(0.0)	0(0.0)
67-72	1	2	0(0.0)	1(33.3)	1(33.3)
Total	59	45	4(3.9)	2(1.9)	6(5.8)

$\chi^2 = 21.35,$ df = 11 P>0.05

Table 3: Types of samples treated and the number (%) of *Aeromonas* species isolated.

Types of Stool	No. Examined	No. (%) Positive
Watery	36	2(5.6)
Mucoid	13	0(0.0)
Blood stained	3	0(0.0)
Soft formed	40	3(7.5)
Hard formed	12	1(8.3)
Total	**104**	**6(5.8)**

$\chi^2 = 132$, df = 4 P>0.05

Table 4: Prevalence of *Aeromonas* species in Relation to the type of Feeding

Type of Feeding	No. of Patient Tested	No. (% positive)
Breast milk	20	0(0.0)
Breast milk & formular	41	4(9.8)
Formular & family diet	43	2(4.7)
Total	**104**	**6(5.8)**

$\chi^2 = 60.17$ df = 2 P>0.05

Table 5: Antibiotic susceptibility pattern of *Aeromonas* species isolated

Anitibiotics	Concentration (mcg)	No. of Isolate tested	No (%) sensitive
Ciprofloxacin	5	6	6(100)
Cotrimoxazole	25	6	3(50)
Erythromycin	5	6	4(66.66)
Gentamycin	10	6	5(83.33)
Penicillin	5	6	6(0)

Peflacine	10	6	5(83.33)
Streptomycin	10	6	4(66.66)
Tarivid	10	6	5(83.33)
Tetracycline	10	6	3(50)

DISCUSSION

A total of 104 stool samples were analysed in this study in which the prevalence rate for *Aeromonas* spp was 5.8%. This result is similar to the 5% prevalence rate documented by Obi *et al.*, (12) for urban population in Edo, Lagos and Cross River States of Nigeria.

There was a slightly higher isolation rate from children without diarrhoea (7.7%) compare to those with diarrhoea (3.9%). However this result is not statistically significant and agrees with findings from some researchers (Pitaragis *et al.*, (14) and Figura *et al.*, (15).

No *Aeromonas* spp was isolated from infant below 6 months. In contrast the highest isolation rates were found in infants 7-12 months. This result correlates with the findings of Abraham *et al* (21) and Regua *et al.*, (22). They both observed that the highest incidence of gastroenteritis in children was found within the age range of 7-12 months where weaning practices begin in many parts of the world (Nigeria inclusive).

The finding indicates that breast milk confers considerable protection to children as positive cases were not reported in children below 7 months whose mothers practice exclusive breast feeding.

The protective role of breast milk against diarrhoeal bacterial aetiologic agents has been reported previously (23, 24).

Data obtained in this study from the parents indicate that all the children below 6 months were exclusively breast fed whereas those 7-12 months had their breast feeding interrupted with mixed feeding or stopped completely.

Another probable reason for the increase incidence of gastroenteritis around 7-12 of age months might be due to faulty weaning practices and poor hygiene in preparing food.

The low isolation rate in asymptomatic children older than age 12 months might be attributed to immunity developed by the older children who may have come in contact with the effective agent through exposure to the organism from ingestion of contaminated food, water etc once solid foods have been introduced in their diet.

When considering the age / sex factor distribution, the incidence of *Aeromonas* spp was found to be higher in males (6.8%) than in females (4.4%). This finding may be related to the number of male and female children from who samples were collected. I.e. more samples were collected from males than females. However, this result is not statistically

significant and no sex preference has been reported.

Macroscopic examinations of the stool samples showed that a higher number of *Aeromonas* spp was isolated from soft-formed and watery stools than other types of stool. This agrees with reports in the literature which showed that acute watery diarrhoea is the most common clinical feature. This result is not statistically significant.

The result of in-vitro antibiotic sensitive test showed 100% sensitivity to ciprofloxacin and more than 80% sensitivity to peflacine, tarivid and gentamicin. Cirpoflaxacin, therefore is the drug of choice, when treating *Aeromonas* infections from this study. This presents cause for concern since it is expensive. Conventional and cheaper drugs like (cotrimoxazole, tetracycline, streptomycin and erythromycin) showed marked reduced in vitro susceptibility. This may be due to indiscriminate usage or an antibiotic (drug abuse) which has resulted in multiple drug resistance of many microorganisms in Nigeria (25). In addition, other enteric bacteria isolated in Jos have also been found to be resistance to these antibiotics (26, 27).

All isolates identified were found to be *Aeromonas hydrophilia.* This *Aeromonas* spp has been associated with many cases of diarrhoea (5). Other common enteric pathogens like *Salmonella, Shegella* and *Escherichia coli* were not sought for in this study therefore it can not be concluded that the *Aeromonas* spp isolated were the actual cause or the diarrhoea in this study.

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