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Plesiomonas shigelloides diarrhea in Enugu area of south eastern Nigeria: Incidence, clinical and epidemiological features

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Abstract: *The role of Plesiomonas shigelloides was determined in patients attending health centres and cottage hospitals in parts of Enugu State of Nigeria had diarrhoea. During the 12-month study (March 2012 – Feb 2013), P. shigelloides was isolated from 51 (7.2%) patients who had diarrhoea and none from 500 patients without diarrhoea (p<0.01) recruited from a match control group. The age and sex distribution of the P. shigelloides patients showed that the age group 19 – 31 months (16.7%) yielded the highest isolation for Plasiomonas shigelloides, while females 31 (60.8%) were higher than males 20 (39.2%), though the difference was not statistically significant (p>0,05). A seasonality distribution in favour of rainy season was also observed with the highest cases recruited in the periods June – August (54.9%) and September – October (23.5%). Only 2 (3.9%) were recruited between December and February. Thirty-seven (5.2%) patients had single infection with Plesimonas while 14 (2.0%) were co-infected with other bacterial pathogens. No parasitic agents were detected in the samples analysed. Twenty-seven (73%) of the patients with simple Plesimonas infection had watery diarrhoea; 24 (64.8%) had abdominal pain; 12 (32.4%) had fever, 7 (18.9 %) had visible mucus in stool, 6 (16.2%) had bloody diarrhoea while 5 (13.5%) were vomiting. Of the 5 children up to 5 years of age who had single infection, 3(60%) were clinically dehydrated. Of the 37 who had single infection, 8 (21.6%) had diarrhoea >/- 14 days. The antibiogram shows that the Plesimonas strains were highly resistant to the more available antibiotics in the area, with Ampicillin having a resistivity of 90.2%, Trimetoprim sulphamethoxazole (66.7%) and Tetracycline 51%, while the less available ones like Imipenem (2%), Aztreneonam and Cefotaxime (3.9%) each, and Laevofloxacillin (5.9%), had the least resistance. The findings may be of Public Health importance for creating awareness among physicians about the clinical profile and management strategy of P. shigelloides-diarrhoea in the area.*

I. Introduction:

Plesimonas shigelloides (formerly known as Aeromonas shigelloides) is an oxidase – positive, facultatively anaerobic Gram – negative bacillus found in water and soil [1]. It has emerged as a cause of enteric disease in humans [2, 3] especially following consumption of contaminated water and raw sea foods. It has also been isolated from a number of extra-intestinal sites [4-7]. Molecular studies have shown the organism is closely related to the members of the Enterobacteriaceae family and therefore been placed into the family in which it is the only oxidase-positive member [1]. The species named shigelloides is derived from the fact that many strains crossreact antigenically with shigella especially Shigella sonnei [1].

Outbreaks of diarrhoeal disease have been associated with contaminated water and oysters containing P. shigelloides of which the severity of the disease has been reduced by appropriate antibiotics therapy [7, 8]. The organism which is primarily a fresh water aquatic organism increases in isolation rates during the warmer months of the year [9] frequently so in tropical and subtropical areas of the world. In the south eastern part of Nigeria, little is known about diarrhoea associated with P. shigelloides. Only the 'traditional' organisms are sought after in our medical laboratories either due to ignorance or lack of facilities for the non-traditional ones.

Previous studies on this organism in the southern parts of Nigeria has largely concentrated in the south west and south south and not much has been done in the south east Nigeria.

In this paper, we report on the role of P. shigelloides in patients with and those without diarrhoea and comment on the epidemiological and clinical patterns as well as their antibiogram.

II. Materials And Methods

The study population: During March 2012 to February 2013, serial case-control study were conducted in 8 communities in 4 LGAs of Enugu State of south east Nigeria to assess the aetiological agents of diarrhoeal episode in the area as pertaining to P. shigeloides. Briefly each community was visited 3-4 times on a rotating basis during which cases of diarrhoea were identified by a visit to medical centres, private hospitals/clinics, General Hospitals, cottage hospitals and Health Centres. These were people reporting to these places for complaints of =/> 3 loose stools in a 24 hour period. Faecal samples were also collected from 500 age/sex match control apparently healthy persons seen within the same areas either visiting for other reasons like staying with wards on admission, selling in the hospitals or as workers, or from nearby houses during a canvas visit. These controls had no symptoms of diarrhea during the past 7days and were not on any antibiotics. Faecal samples were collected from each candidate – 713 cases and 500 controls in sterile 50ml ice-cream and plastic cartons distributed to them. Candidates/controls were advised on how to collect the samples which were kept in a cooler/box with ice and taken to the medical microbiology laboratory of the College of Medicine of University of Nigeria, Enugu Campus, for studies.

Demographic information such as age/sex etc and clinical data of the candidate were also recorded with the aid of the medical doctors and nurses covering the health institutions. Questionnaires were also distributed to patients or wards, which also aided with vital information.

Stool culture and identification of isolates

Stool specimens collected were cultured onto Bismuth sulphate, Deoxychocolate citrate lactose-sucrose, Hektoen enteric, (efsulodin Irgasan novobion, Sorbitol MacConkey and Compylobacter selective, MacConkey and sheep blood Agars. Specimens were also cultured on to inositol-brillant green-bile salts agar which has been suggested as a selective differential medium for the detection of P. shigelloides [10].

The media were screened after 24 hour incubation at 37^{o}C for potential pathogens in accordance with standard procedures [11]. Blood agar cultures were screened by flooding the plates with oxidase reagent and selecting oxidase-positive colours for identification. Whitish or pinkish colours on inositol – brilliant green bile salts agar were subcultured to blood agar and tested for oxidase. Potential pathogens were identified by classical biochemical and with serological configuration. P. shigelloides were identified by being oxidase positive, indole, lysine, arginine and ornithine positive and for being negative for Hydrogen sulphide, urease, arabinose, sucrose, mannitol, voges – Poskaver and DNAase tests [12]. P. shogelloides isolates were also tested for antimicrobial sensitivity using standard disk diffusion method [13].

Statistical analysis was done using Chi square tests.

III. Results

A total of 712 respondents with acute diarrhea from March 2012 to February 2013 were involved with the study. There were 348 males and 364 females, ranging in age from 3 months to 74 years. P. shigelloides was isolated from 51 (7.2%) patients with acute diarrhea and accounted for 20.9% of total bacterial isolates. The P. shigelloides isolation ranked third among bacterial pathogens in descending order. The top two were salmonella spp. 64 (9.0%) and E. coli (EFEC), 59 (8.3%) (Tables 1&2)

Patients with P. shigelloides infection ranged in age from 3 months to 72 years. More than half, 28 (54.3%) patients were 0 -28 years old. There was no satisfactory significant difference between males (20) and females (31) with respect to the incidence to P. shigelloides and also with age (p>0.05) (Tables 2&3).

Sensitivity pattern showed that P. shigelloides was isolated mostly in the periods of rain (rainy season) (early rains to late rains) with a peak season between June-August and September-November though not statistically significant (p>0.05) (Table 4).

Of the 51 patients infected with P. shigelloides, single infections were observed in 37 patients (72.5%) y and co-infections with other pathogens were reported in 14 patients (27.5%) though not significant)p>0.05.(Table 2).

The other pathogens isolated were salmonella spp. (64 9.0%), E. coli EFEC 57 (8.3%), 8 (1.15%). Aeromonas hydrophils 44 (6.2%), Compylobacter spp 13 (1.8%), Yersina spp 3, vibro cholera. con 01 and shigella spp each 2 (0.28%), the least being Enterohaemorrhagic E. coli (EHEC) with only 1 isolate (0.14%)(Table 1).

A total of 500 non diarrhoeal candidates were also enrolled in the study. There were 280 males and 220 females, with ages ranging from > 1year to 70 years. P. shigelloides strains were not isolated from the non diarrhoeal cases. (Table 1) However the co-infected cases were recorded as follows : diarrhoeal 59 versus non diarrhoeal 12 for EFEC, Yesinia spp 8 vs 1 vibro 2 vs 0 yrs spp, salmonella spp 64 vs 10, aeromonas spp 44 vs 9, campylobacter 13 vs 2. Antibacterial sensitivity pattern shows that of the 51 isolates, 90.2% were resistant to Ampicillin, Trimethoprim/ sulphamethoxazole, 66.7%, followed by 50.5% Tetracycline, 49.0% for Amikacin, Gentamicin 43.1% and 29.4% for Ampicillin/Clavulanic acid among the high ones, Ciprofloxacillin 15.9%.

However, less than 10% were resistant to Piperacillin, Cefuroxime, Cefoxitin, Cefuzotin and Laevofloxacillin while less than 5% were resistant for aztreonan, cefotaxine, and imipenem. None of the isolates was completely sensitive to any of the drugs tested. (Table 6)

The Clinical symptoms show that watery diarrhoea and abdominal pain were the highest observable symptoms among those patients with single infection with P.shigelloides, 73% and 64.8% respectively. Their counterparts with co-infection with P.shigelloides and other pathogens were 64.3% and 71.4% respectively for 2 symptoms. These are followed by fever in 32.4% of single infections and 42.9% of co-infection. Visible mucus in stool was detected in 18.9% and 21.4% of single and co-infections respectively, bloody diarrhea was observed in 16.2% and 21.4% of single and co-infected cases respectively while vomiting was recorded in 13.5% and 14.3% of single and co-infected cases respectively.

Clinically significant dehydration was observed only in 4 children up to 5 yrs of age with 37.5% and 33.3% recorded in the 2 groups respectively(single vs co-infection . The differences in the number of these symptoms were not statistically significant (p>0.05) for both groups. (Table 5)

Table 1 Total Bacterial pathogens isolated from the diarrhoeal patients sampled

Bacteria isolated	Diarrhoeal n=712	Non-diarrhoeal N=500	p
Single infection with P. shigelloides	37 (5.2%)	0	0.040
Coinfection of P. Shigelloides and any other bacterial agent	14 (1.96%)	0	0.304
Total P. shigelloides infection	51(7.2%)	0	0.005
Escherichia coli (EPEC)	59 (8.3%)	12 (2.4%)	0.040
Enterohaemorrhagic E. coli	1 (0.14%)	0	0.470
Salmonella spp	64 (9.0%)	10 (2%)	0.006
Aeromonas spp	44 (6.2%)	09 (1.8%)	0.040
Vibrio cholera non01	2 (0.28%)	0	0470
Compylobacter jejuni	13 (1.82%)	2 (0.4%)	0.304
Yesinia enterocolibia	8 (1.12%)	1 (0.2%)	0.304
Total	244 (34.3%)	34 (6.8%)	0.001

% of Plesimonas among 244 is (20.9%)

*P. shigelloides isolation rate was statistically significantly different in patients with acute diarrhea (p<0.05) when compared with without diarrhea.

Table 2 Distribution of positive cases according to age No positive for Plesimonas shigelloides

Age of patients (years)	No sampled	Single infection	Co-infection with other organisms	% Total
0 - 5	111	5	3	8 (72)
6 - 18	103	3	2	5 (4 – 7)
19 - 31	90	11	4	15(16.7)
32 - 44	126	7	2	9 (7.1)
45 - 57	162	6	3	9 (5.6)
58 -70	74	3	0	3 (4.1)
>70	42	2	0	2(4.8)
Total	712	37 (5.2)	14 (2.0)	51 (7.2)

(37/51 72.5%) 44/51= 27.5% p>0.05

Table 3 Distribution according to sex

Age (years)	No.	Sam-	pled	No.	Posi-	tive
	M	F	T	M	F	T
0-5	62	49	111	5	3	8
6-18	47	60	107	2	3	5
19-31	50	40	90	4	11	15
32-44	49	77	126	4	5	9
45-57	88	74	162	4	5	9
58-70	29	45	74	0	3	3
>70	23	19	42	1	1	2 P>0.05
	348	364	712	20	31	51
	(48.9%)	(51.1%)		(39.2%)	(60.8%)	M is20/712=(2.8%) F is 31/712 = (4.4%)

(Period of Research- March 1 2012- February 2013).

Distribution According to seasonality Table 4

Period	No. Tested	No.Positive %
March – May 2012	158	9 (17.6)
June – August 2012	245	28 (54.9)
Sept- Nov 2012	202	12 (23.5)
Dec – February 2013	107	2 (3.9)
Total	712	51 P>0.05

Table 5Clinical Characteristics Distribution

*

Symptoms	+distribution in single n=37	Co-infection n=14	p
Watery Diarrhoea	27 (73%)	9 (64.3%)	0.384
Bloody diarrhoea	6 (16.2%)	3 (21.4%)	0.636
Visible mucus in stool	7 (18.9%)	3 (21.4%)	0.636
Fever	12 (32.4%)	6 (42.9%)	0.365
Vomiting	5 (13.5%)	2 (14.3%)	0.636
Abdominal pain	24 (64.8%)	10 (71.4%)	0.180 p>0.05
Clinically significant dehydration	Of the 5 children up to 5 years of age, 3 (60%) were clinically significantly dehydrated	Of 3 children up to 5 yrs of age 1 (33.3%)Was clinically significantly dehydrated	For all
			*Symptoms of patients with single P. shigelloides infections different from the symptoms of co-infected patients, however the differences were not significant (p>0.05)

P>0.05

Table 6

Antibiotics resistance pattern of Plesiomonas shigelloides isolates in Enugu State

Antibiotics Tested	% Resistance n=51 (No. resistant)
Ampicillin	46 (90.2%)
Piperacillin	5 (9.8%)
Aztreonam	2 (3.9%)
Cefotaxime	2 (3.9%)
Gentamicin	22 (43.1%)
Imipenem	1 (2%)
Cefoxitin	4 (7.8%)
Trimethoprim/ sulphamethoxazole	34 (66.7%)
Laevofloxacillin	3 (5.9%)
Tetracycline	26 (51%)
Cefuroxine	4 (7.8%)
Amoxicillin / clavulanic acid	15 (29.4%)
Cefazolin	4 (7.8%)
Ciprofloxacillin	8 (15.7%)

IV. Discussion

Plesiomonas shigelloides has been implicated as an aetiological agent in sporadic cases and outbreaks of diarrhea in various parts of the world (2&3).

Sources of this organism in this part of the world is not fully known but water sources seem to be the most likely as the water sources in Enugu have some doubtful quality. Pipe-borne waters are grossly lacking or non-functional and so citizens depend largely on streams, ponds and rivers while many resort to hand-dug well waters which are all liable to contamination by filth littered in many areas, indiscriminate defeacation, and animals freely moving around the vicinities and littering the entire area. Such unwholesome materials could be washed into the water sources during showers of rains or even directly by people washing and bathing in the waters.

P. shigelloides has been associated with water sources in many studies globally including USA and China from fresh water fish, dogs and cats [1,14 & 15]. Outbreaks of P. shigelloides diarrhoea were attributed to contaminated water in Japan, among fresh water bathers, in Amsterdam and from consumption of fresh – water fish in Zaire , oysters and shellfish in the United States [16-18].

In the current series, there was an increase in the number of positivity in the periods of peak rainfall , June- August with 54.9%, followed by September- November (23.5%) and March- May with 17.6% period of no rains, December to February (3.9%) recorded the least isolation rate in the study period. Seasonal variation with an increase in August through October was recorded in the isolation rates of this organism in Hong Kong, Japan and Bangladesh and a similar variation pattern was also observed in the numbers of organisms detectable from surface waters [16-19]. A study in South South Nigeria by Eko and Utsalo also supported rainy season preponderance as observed in this work [20].

Plesiomonas shigelloides was not isolated from any of the non diarrhoea (control) cases. This finding appears to be common in several previous studies both from south west and south south Nigeria and elsewhere [15, 20, 21]. Generally patients who had P.shigelloides isolated from their stool had diarrhoea. This probably points to the fact that the organism is not a part of the intestinal flora of man.

The rate of isolation of P.shigelloides from the faecal specimens of out-patients with acute diarrhoea in this study was 7.2% (accounting for 20.9% of all bacterial isolates) which was lower than the of 11.4% reported in rural communities of north western remote area of Thailand, 2001-2002 [3] but higher than 2.9% from southeast China, 1.4% in Calabar , 0.68% in Lagos Nigeria and similar to 7.7% in rural areas of Ekpoma, Edo state Nigeria [15, 20 – 22]. Development of technology in terms of media requirements and techniques could account for the observed discrepancy in Nigeria since all the earlier works were done in the early nineties when there were limited choices of media. These results also indicate that the distribution of P.shigelloides differs greatly between regions and time period. In our study also, no P.shigelloides was isolated from the non diarrhoeal patients. In a Japanese study, P.shigelloides was isolated from <0.12% of asymptomatic individuals, 0% in southeast China and 0% also from the previous Nigerian studies. These results imply that P.shigelloides is not an indigenous organism of the human gastro-intestinal tract. The fact that P.shigelloides isolation rate in patients with acute diarrhoea was much higher than in the control group implies that P.shigelloides may be a diarrhoea causing pathogen in patients in Enugu state of East Central Nigeria. However, in Nigeria generally, pathogen detection in patients with diarrhea only involves tests for 'traditional' bacteria: Shigella, salmonella, E. coli, Vibrio cholera etc at present. The non conventional organisms like Plesiomonas shigelloides are rarely sought after due to lack of knowledge, lack of facilities or complete ignorance. This study therefore suggests the necessity to test for more pathogens including P.shigelloides in patients with diarrhoea in future routine clinical practice. In our study, total P.shigelloides accounted for the 3rd in a decreasing order of frequency (7.0%) after salmonella spp (9.0%) and enteropathogenic E. coli (EPEC) (8.3%) respectively. The antibiotic susceptibility pattern of our isolates were typical of the patterns reported by others [15, 18], except that P.shigelloides appeared to have increased resistivity due to indiscriminate use and abuse of antibiotics typical of the developing nations of the world like Nigeria where these drugs are available for patients' purchase with or without appropriate prescription and most of these drugs are substandard despite all efforts to control this practice by the authorities. It is therefore not surprising that commoner antibiotics have been unfortunately bastardized and have shown high resistivity pattern.

The current series shows a female preponderance in the positivity of P.shigelloides diarrhoea when compared with their male counterparts though this was not statistically significant (P>0.05), but this is likely to be due to the females being more associated with water in streams and rivers during their daily chores like washing clothes, fetching water, washing bitter leaves, cassava and other –related duties. This was also supported by the work of Obi et al [21] in Southwest Nigeria (Edo State). In terms of age relationships, the age group 19-31 yrs was more incriminated and this may also be linked to the same reason as this is the most active group involved in water related activities. Some studies elsewhere have shown that the organism was to affect all age groups and both males and females [8], and consistently associated with the consumption of sea food and untreated water [8].

The clinical features of Plesiomonas in the home of the uncontrolled studies suggested that the organism causes either secretory or invasive diarrhoea. Our findings and the results of some other studies [8, 19, 23] indicate that most patients that have plesiomonas infections have an acute illness with abdominal pain and colitis (as shown with pains and bloody and mucoid stool). These findings suggest that invasive rather than secretory diarrhoea is more commonly associated with plesiomonas infection in the area of study. We also found that 8(21.6%) of the 37 cases with single infection had symptoms lasting more than 2 weeks duration which was longer than that seen in patients infected with other organism. The isolation of co-pathogens in patients with P.shigelloides was relatively common in our study. This phenomenon has been reported by other researchers elsewhere [6, 15].

V. Conclusion

In summary, the present findings support the role of P. shigelloides as a gastrointestinal pathogen and indicate that the organism may be a more common cause of diarrhoea than is currently recognized in the area. Study patients with plesiomonas diarrhoea had acute illness. Many had abdominal pains and watery diarrhoea. It is therefore necessary to routinely test for this pathogen in future clinical work as many studies have associated it with extra-intestinal diseases or infections.

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