

Prevalence of enteric pathogens among patients with gastrointestinal presentations in the Lagos University Teaching Hospital (LUTH), Idi-Araba Lagos Nigeria

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Abstract:

Background: Gastroenteritis is a major cause of ill health and premature death in developing countries. Prevalence study of enteric pathogens among patients with gastrointestinal presentations in Lagos was done in a Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos Nigeria.

Objective: The purpose of this study is to determine the prevalence of enteric pathogens thereby contributing to the existing data.

Methods: 150 stool samples were collected from patients with gastrointestinal presentations attending LUTH. Each specimen was cultured on media, isolates identified and the antibiotic susceptibilities of the isolates determined.

Results: 37.3% of the 150 patients were infected with bacteria. The isolates in order of prevalence include *Proteus* species 53.6%, *Pseudomonas* species 17.9%, *Aeromonas* species 14.3%, *Salmonella* species 10.7% and *Shigella* 3.6%. The isolates were sensitive to nalidixic acid, ceftriaxone, gentamycin and nitrofurantoin while isolates like *Salmonella* and *Shigella* species were resistant to ampicillin and *Proteus* and *Pseudomonas* species were resistant to ampicillin, ofloxacin and ciprofloxacin.

Conclusion: The prevalence of enteric pathogens among patients with gastrointestinal presentations in Lagos are not higher in LUTH than earlier reports from other parts of the developing world. This low rate record is due to public hygiene awareness and improvement in environmental sanitation exercise in Nigeria. Resistances to some of the antibiotics by the isolates were because antibiotics could have been obtained without prescription; therefore, retail availability of nalidixic acid should be controlled to prevent broad and indiscriminate use.

Keywords: enteric pathogens, diarrhoea, prevalence; gastrointestinal presentations,

I. Introduction

The burden of gastrointestinal illness in developing countries remain significantly high, despite a marked decrease in mortality rates from 4.6 million in 1980 to about 1.5 million in 1999 [1] [2]. Gastrointestinal illness is caused by a variety of different microbes and germs and causes a variety of symptoms such as diarrhoea, nausea, vomiting, abdominal pain, abdominal cramps, fever and sometimes headaches, rash and paralysis [3]. Some of these symptoms of gastrointestinal illness are symptoms associated with diarrhoea. Diarrhoea is a significant health problem worldwide, especially in the developing world where adequate sanitation facilities are lacking [4]. It is estimated that approximately 1.87 million children die from diarrhoea before reaching fifth birthday [5]. Globally, diarrhoea disease account for about a fifth of all deaths of children below five years of age, with an estimated 2.2 million deaths annually [6].

Enteric pathogens are transmitted by the faecal-oral route and foods ingested are major vehicles of this transmission. In developed countries, the enteric pathogens most commonly associated with food-borne disease are *Salmonella* species, *Staphylococcus aureus*, *Clostridium perfringens* and *Bacillus cereus* [7]. *Salmonella* also occurs in developing countries but it is not responsible for large fraction of episodes. *S. aureus*, *C. perfringens* and *B. cereus* almost certainly cause enteric illnesses in developing countries. Enteric pathogens can be found in at least half of the patients with endemic diarrhoea, with most of the agents being bacterial [8].

The most commonly found bacterial pathogens in children in developing countries are enterotoxigenic and enteropathogenic *Escherichia coli* and *Shigella* spp. They can be transmitted by means of contaminated food. Other bacteria pathogens, such as *Campylobacter jejuni* or vibrios also could be transmitted through contaminated food in developing countries. It is also likely that certain viral pathogens such as 27nm viruses like Norwalk virus and parasitic agents such as *Entamoeba histolytica*, *Giardia lamblia* or *Cryptosporidium* spp. are often ingested with food.

However bacterial pathogens are important causes of gastrointestinal illness particularly in developing countries where standards of personal and community hygiene are low [9]. The early identification and effective antimicrobial treatment of cases is an important step in management of infections [10]. Resistance to antibiotics is becoming more prevalent. The emergence of antimicrobial resistance is matter of concern especially in poor resource settings where the poor suffer most of the infections caused by enteric pathogens. Therefore, the aim of this study is to determine the prevalence of enteric pathogens among patients with gastrointestinal presentations, and also to ascertain the antibiotic susceptibility of the bacterial diarrhoeagenic agents isolated.

II. Materials and Methods

2.1 Study Area

The study was carried out in Lagos Nigeria. Lagos, the former capital of Nigeria is Nigeria's commercial nerve centre. It lies on Latitude 6° 30N and 3° 25E. It is about 78 kilometers South of Abeokuta and 100 kilometers Southeast of Ijebuode. It lies by the Gulf of Guinea and harbours the boundary of Atlantic ocean. With an averagely hot humid and pertinent rainfall, it occupies an area of 79 square kilometers with an estimated population of 13.8 million people

The study site was Lagos University Teaching Hospital Idi-araba, (LUTH) Lagos Nigeria which is located at Lagos Mainland.

2.2 Study Design and Sample Collection

The study design was cross-sectional. Stool specimen were collected from patients from 0-55 years with gastrointestinal presentations from May, 2008 to August, 2008 at Lagos University Teaching Hospital, Idi-araba, Lagos Nigeria. Out of 150 samples, 94 were from male patients while 56 were from female patients. Age, sex and clinical history of each patient was recorded and each patient was instructed on the precautionary measures to adopt for specimen collection so that specimens will not be contaminated. The specimens were properly labeled with identification number, date of collection, age and sex of the patients. Visual examination of the stool specimens were carried out on each of the stool specimen in Medical Microbiology and Parasitology Laboratory in LUTH. Microscopic examination of the stool specimen involved the following parameters like its consistency (formed, unformed (soft) or liquid), its colour (white, yellow, brown or black) and the presence of any abnormal components (e.g. mucous or blood).

2.3 Culture and bacteria isolation

Stool specimen were cultured as soon as possible after arrival in the laboratory. A faecal suspension by suspending approximately 1g of the stool specimen in a tube containing 1ml of sterile saline was made. If the stool specimen was liquid, normal saline does not need to be added. This was followed by inoculation of high selectivity media of Xylose-lysine-deoxycholate (XLD) agar and Salmonella – Shigella (ss) agar with three loopful of the faecal suspension and that of low selectivity, MacConkey agar with one loopful of the faecal suspension. The stool specimens were also inoculated on Blood agar media. Also, three or more loopfuls of faecal suspension were inoculated to the enrichment broths and incubated at appropriate time and temperature. Enrichment was commonly used for the isolation of *Salmonella* species and *Vibrio cholera* from stool specimens. Selenite F broth was used for the enrichment of *Salmonella* species at 37° C for 18 hours while alkaline peptone water for enrichment of *V. cholera* for 6 – 8 hours. The incubation of the enrichment media, subculture of colonies was done by streaking a loopful of broth culture on XLD and SSA for isolation *Salmonella* species and on Thiosulfate Citrate Bile Salts Sucrose (TCBS) medium for isolation *V. cholera* and incubated at 35° - 37° C for 18 – 24 hours.

2.4 Identification of Isolates

Biochemical identification of isolates were performed using Motility Indole Urea (MIU) medium, Voges Proskauer test, Ciliate utilization test, oxidase test and peptone water. *Salmonella* isolates were further identified by serotyping based on slide agglutination using the antiserum against the somatic O antigen and flagellar H antigens,

2.5 Antimicrobial susceptibility testing of identified isolates

Antimicrobial susceptibility testing was performed for Nalidixic acid, Ciprofloxacin, Ampicillin Gentamicin, Chloramphenicol, Ceftriaxone and Nitrofurantoin by disk diffusion method as recommended by the [11].

III. Result

Of the one hundred and fifty (150) stools tested, 56 (37.3%) yielded bacterial isolates as shown in Table 1. Out of 60 patients with diarrhoea and 90 patients without diarrhoea. 25 (41.7%) and 31 (34.4%) were infected with bacteria respectively. The frequencies with which the bacteria were isolated from individuals with or without diarrhoea are presented in Table 2. For patients with diarrhoea, *Aeromonas Salmonella* and *Shigella* were significantly more frequently isolated from them while *Proteus* and *Pseudomonas* were more frequently isolated from non-diarrhoeic patients. Single isolates were isolated from most stool samples positive for diarrhoea. The age and sex distribution for all enteric flora in patients with diarrhoea are shown in Table 3.

The sensitivity pattern of the five bacteria genera - *Aeromonas*, *Salmonella*, *Shigella*, *Proteus* and *Pseudomonas* to eight antibiotics is shown in Table 4. *Salmonella*, *Shigella* were susceptible to almost all the antibiotics used but were resistant to ampicillin. *Aeromonas* also was sensitive to nalidixic acid, gentamycin, ceftriaxone and nitrofurantoin while resistant to ampicillin too. *Proteus* and *Pseudomonas* were resistant to ampicillin, ceftriaxone (*Proteus* was intermediate), ofloxacin and ciprofloxacin. *Proteus* and *Pseudomonas* were susceptible to chloramphenicol, nalidixic acid, gentamycin and nitrofurantoin.

3.1 Tables

TABLE 1. Organisms isolated from stool samples

Organisms	Male Patients (n=30)		Female Patients (n=326)	
		%		%
<i>Salmonella species</i>	4	13.3	2	7.7
<i>Shigella species</i>	2	6.7	0	0.0
<i>Aeromonas species</i>	2	6.7	6	23.1
<i>Proteus species</i>	18	60.0	12	46.2
<i>Pseudomonas species</i>	4	13.3	4	23.1
Total number of isolates	30	100	26	100

TABLE 2. Frequency of isolation of Gram-negative bacteria from stool specimens of patients with and without diarrhoea.

Bacteria	Diarrhoea (n=60)		No Bacteria (n=326)	
		%		%
<i>Salmonella species</i>	5	8.3	1	1.1
<i>Shigella species</i>	2	3.3	0	0.0
<i>Aeromonas species</i>	6	10.0	2	2.2
<i>Proteus species</i>	10	16.7	20	22.2
<i>Pseudomonas species</i>	2	3.3	8	8.9
Total number of isolates	25	41.7	31	34.4
No Bacteria found	35	58.3	59	65.5
Total number of isolates	60	100.0	90	100.0

TABLE 3. Age and sex distribution of enteric isolates from diarrhoea patients

Bacteria	Number Found	Sex				Age Group			
		Male		Female		0-10 years	11-20 years	21-30 years	> 30 years
			%		%				
<i>Salmonella species</i>	5	4	80.0	1	20.0	0	4	1	0
<i>Shigella species</i>	2	2	100.0	0	0.0	2	0	0	0
<i>Aeromonas species</i>	6	2	33.3	4	66.7	0	2	1	3
<i>Proteus species</i>	10	6	60.0	4	40.0	1	2	2	5
<i>Pseudomonas species</i>	2	1	50.0	1	50.0	0	0	1	1
Total number of isolates	25	15	60.0	10.0	40.0	3	8	5	9

TABLE 4. Sensitivity pattern of the isolates

Most probable organism	Ampicillin	Ceftriaxone	Nitrofurantoin	Nalidixic acid	Gentamycin	Ofloxacin	Ciprofloxacin	Chloramphenicol
<i>Salmonella species</i>	R	S	S	S	S	S	S	S
<i>Shigella species</i>	R	S	S	S	S	S	S	S
<i>Aeromonas species</i>	R	S	S	S	S	S	I	S
<i>Proteus species</i>	R	I	S	S	S	R	R	S
<i>Pseudomonas species</i>	R	R	S	S	S	R	R	S

Key:

Sensitivity	S
Resistance	R
Intermediate	I

IV. Discussion

Salmonella organisms are found in virtually all animals including poultry, reptiles, livestock, rodents and domestic animals from where contamination of food chain occurs through faeces. Man is eventually infected by faecal oral route [12]. Unlike the genus *Salmonella*, *Shigella* is transmitted by faecal oral route, primarily by people with contaminated hands and less commonly by water or food [12]. *Aeromonads*, though more abundant in water and sewage than either *Salmonella* or *Shigella* is rarely associated with human infections in Nigeria [13]. On the other hand, infection due to *Salmonella* and *Shigella* are common since the organisms abound in some contaminated foods eaten locally [12]. Though *Salmonella* and *Shigella* are associated with gastroenteritis [14]; [15]; [16] that of *aeromonads* are disputed. [17]; [18]. [18] postulated that there is convincing evidence that *Aeromonas hydrophillia* and other *aeromonads* cause gastroenteritis but doubted whether some of the strains isolated from faeces were involved in diarrhoeal disease.

In this study, the highest prevalence of *Aeromonas* were found in ages between 11 and >30 years, while in *Salmonella*, the ages were between 11 and 30 years; and in *Shigella*, the ages were between 0 – 10 years. These findings agree with [19] who found the highest prevalence in patients between 6 and 16 years for *Aeromonas* and *Salmonella* but contrasts with the study conducted by [20] who found highest prevalence of *Aeromonas* in patients below the age of five.

Results from this study also revealed a trend in the occurrence of *Aeromonas* organisms in females than in males. This may be attributable to the fact that the organism are environmental water bacteria because females engage more in domestic activities than males and have more frequent contacts with the water sources like well water. [21] found a clear association between the drinking of untreated water and the occurrence of chronic gastroenteritis in adults and acute gastroenteritis in children due to *Aeromonas* species.

Moreover, the prevalence rate of *Salmonella* (10.7%) and *Shigella* (3.6%) in this study is considerable when compared with that from China. [22] recorded a prevalence rate of *Salmonella* (12.0%) and *Shigella* (2.0%). The reason for this disparity may be attributable to differences in study design, patients' selection, differing environmental conditions and behavioural pattern of people in those regions.

Isolation of *Aeromonas* from patients with diarrhoea have not been described as a health problem. They may however represent serious health problem in infants and the immunocompromised [23], [24] Their presence therefore in clinical specimens deserve urgent attention. The role of *Proteus* species and *Pseudomonas* species as gastrointestinal pathogens have not been well established. They may however be important in transferring resistant traits to other bacteria in intestine.

Drug susceptibility of *Salmonella* and *Shigella* isolates showed that they were resistant to ampicillin, which is in agreement with the findings of other researchers in Nigeria [25], [26] and also in agreement with studies done in Kenya [27] and in Uganda [28]. In this study, *Salmonella* and *Shigella* were sensitive to the following antibiotics: ceftriaxone, gentamycin, ciprofloxacin, chloramphenicol, and nitrofurantoin.

Ceftriaxone is commonly used to treat *Salmonella* and *Shigella* infections particularly invasive infections caused by *Salmonella* because of its favourable pharmacokinetic properties and the low prevalence of resistance. Sensitive to ceftriaxone by the isolates is in agreement with the work done at Ibadan Nigeria [29].

The sensitivity to gentamycin in this study is similar to a study done in Nigeria [30]. Also, the sensitivity to nalidixic acid by *Salmonella* isolates in this work is not consistent with studies conducted in Republic of Ireland [31] and in Owerri South Eastern Nigeria [32]. Ciprofloxacin showed a good antimicrobial activity against *Salmonella* and *Shigella* isolates. Results from this study also agree with those of [33] in Lagos and [34] from central part of Ethiopia which showed a comparable sensitivity to ciprofloxacin. The effectiveness of this drug may be because it is not widely used in countries like Nigeria, Ethiopia and other African countries [35]. *Shigella* isolates showed that they were resistant to ampicillin but sensitive to nalidixic acid, ofloxacin, ciprofloxacin, chloramphenicol, gentamycin and nitrofurantoin which is in agreement with the findings of [36]. In this study also, *Aeromonas* species were found to be resistant to ampicillin and intermediate resistance to ciprofloxacin, but were sensitive to gentamycin, nalidixic acid and nitrofurantoin. This result agrees with the work carried out by [37].

In this study, there was no resistance to nalidixic acid and ciprofloxacin except in *Proteus* and *Pseudomonas* where the organisms were resistant to ofloxacin and ciprofloxacin. Although in areas where nalidixic acid has been introduced as drug of choice to treat presumptive shigellosis, a marked increase in corresponding resistance has been observed [38], [39], [40]. The ease with which antibiotics can be obtained without prescription may add further to selective pressure [41]. Although nalidixic acid is an attractive choice of treating bloody diarrhoea where antimicrobial resistant limits other options, it should be used ideally only for illnesses most likely caused by *Shigella* or where *Shigella* infection could result in greater morbidity and increased risk of death [eg persons with acquired immune deficiency syndrome (AIDS)]. Retail availability of nalidixic acid should be controlled to prevent broad and indiscriminate use.

The limitation of this study was the inability to investigate whether *Campylobacter* was one of the etiological agents of diarrhoea in patients due to lack of reagents and appropriate isolation media for *Campylobacter*.

V. Conclusion

From the findings of this study, we therefore infer that the prevalence of enteric pathogens amongst patients with gastrointestinal presentations in Lagos are not higher in Lagos University Teaching Hospital (LUTH) than earlier reported from other parts of the developing world. This low rate record in this study may be due to ongoing public awareness campaigns and resultant improvement in environmental sanitation exercise put in place by the government of Nigeria at last Saturdays of each month and also that of the Lagos State government every Thursday of the week. Another reason could be that, since some of the samples under study were patients coming to clinics (outpatients) as a result of their illness, some of them would have been on chemotherapy.

In conclusion, this study showed that the prevalence of enteric pathogens among patients with gastrointestinal presentations in Lagos still represent a major health problem.

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