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Prevalence of *aeromonas species* and *escherichia coli* in stool samples of primary school children in Ofada, Ogun state, Nigeria.

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Abstract Background: Diarrhoea is one of the main causes of mortality and morbidity in childhood. Bacterial diarrhoea is a common disorder. *Aeromonas species* and *Escherichia coli* (*E. coli*) are some of the aetiological agents associated with diarrhoea in children.

Objective: To determine the prevalence of *Aeromonas species* and *E. coli* in the stool samples of primary school children as well as the antibiotic sensitivity pattern of the isolates.

Methods: Stool samples were obtained from pupils (diarrhoeic and non-diarrhoeic) aged four to eighteen years in two primary schools in Ofada. The stool specimens were processed by standard microbiological methods and antibiotic susceptibility was determined using disc diffusion method.

Results: Out of 104 stool samples, *E. coli* was isolated from seven (6.73%) stool samples while three

stool samples yielded other pathogens such as *Salmonella species*, *Shigella species*, and *Proteus vulgaris*. *Aeromonas spp* was not isolated. Pathogenic *E. coli* was more prevalent among pupils aged 11 to 15 years. The susceptibility of *E. coli* to commonly used antibiotics (such as tetracycline and ampicillin) was $\leq 30\%$ compared to $\geq 70\%$ for Augmentin, ofloxacin and nalidixic acid. *E. coli* showed 100% susceptibility to only gentamicin.

Conclusion: *E. coli* was the predominant bacterial agent isolated from stool samples of school children in Ofada, Ogun State. Gentamicin may be recommended as an effective antibiotic agent against *E. coli* diarrhoeal diseases in the population studied.

Keywords: *Aeromonas*, antibiotics, children, diarrhoea, *Escherichia coli*.

Introduction

Diarrhoeal diseases constitute an important cause of childhood morbidity and mortality worldwide, most especially in the developing countries where sanitation is suboptimal.¹ The major factors predisposing to diarrhoeal diseases include limited access to good quality water, poor food hygiene and poor sanitation. The incidence of diarrhoeal diseases might have reduced partly because of improved public health measures, improved hygiene, improved case management and increased use of Oral Rehydration Therapy (ORT) as well as better understanding of the role of nutrition.² Contaminated water was hitherto, suspected to be a leading predisposing factor to diarrhoeal diseases. However, efforts at improving the quality of water and sanitation observed in other regions outside Africa, have not yielded significant reduction in diarrhoeal morbidity.³ This observa-

tion suggests that poor hygiene and the ingestion of contaminated food may be more important in the causation of diarrhoeal diseases. Some of the other important risk factors for diarrhoeal diseases include poverty, poor access to health care services, poor nutrition and overcrowding as obtains in day care centers.⁴ In most developing countries, the use of improved hygiene, improved sanitation and improved quality of water, may not be sufficient in the prevention of enteric illnesses. The widespread indiscriminate use of antibiotics also allows the pathogens to develop resistance to commonly used antibiotics such as tetracycline and ampicillin among others.

Aeromonas and *E. coli* are some of the aetiological agents associated with diarrhoea in children.⁵ Other agents include *Shigella*, *Salmonella*, *Campylobacter* and *Yersinia*. *Aeromonas species*, formerly believed to be an

opportunistic organism capable of infecting only immunodeficient individuals like children, have recently been associated with both gastrointestinal disease⁶ and extra-intestinal complications like the haemolytic-uraemic syndrome.⁷

Aeromonas diarrhoea is a mild, self-limiting infection. Isolation rates for *Aeromonas* range from less than 1% to more than 60% in diarrhoeic populations in various geographic locations.⁸ A study had also shown that a small percentage of diarrhoeal diseases in Nigeria can be attributed to *Aeromonas hydrophila*.⁹

The aim of the study was to determine the prevalence of *Aeromonas* species and *E. coli* in the stools of primary school children and to determine the antimicrobial susceptibility profiles of these pathogens in two population groups in Obafemi-Owode local government area, Ogun State, Nigeria.

Subjects and methods

Study population and sampling

The study population was drawn from randomly selected pupils in two primary schools (St. David Anglican Nursery and Basic School and Imedu Nla Nursery and Primary School) at Mowe, Obafemi-Owode Local Government Area, Ogun State, Nigeria. Consent for inclusion in the study was obtained from the pupils and their parents through the respective head teachers.

Determination of the Sample Size:

Using formula: $n = \frac{Pq}{(E/Z)^2}$

Where: p = prevalence obtained from a previous study¹⁰ = 15%

q = 100 - p = 100 - 15 = 85

E = Error margin of 5% ; CI = 1.96 ; Z = Standard normal distribution at 95%

n = desired sample size

Therefore: n = 195

Sampling technique: 250 sterile universal sampling bottles were randomly distributed to consenting pupils irrespective of their health status. Relatively equal number of males and females were selected in each class and school. Overall, a total of 104 pupils (53 females and 51 males) returned stool samples for the study.

Media: The culture media used in this study included MacConkey agar, Kliggler Ion agar, Simon Citrate agar, Motility agar, Nutrient agar, Mueller Hinton agar and Peptone water.

Bacterial isolation and identification:

Each stool sample was enriched in peptone water and sub-cultured on MacConkey agar. This was then incubated at 37°C for 18-24 hours. The culture plates were

observed for growth and distinctive cultural characteristics. The resultant colonies were identified using standard biochemical tests.¹¹ Specifically, Gram-negative isolates were identified using different biochemical tests such as oxidase test, indole test, urease test, citrate utilization test, motility test, gas production and sugar fermentation test. All the tests were conducted using standard laboratory methods.^{12,13}

Antimicrobial susceptibility testing

Sensitivity of isolates to antimicrobial agents was determined on Mueller-Hinton agar plates using the disc diffusion method.¹² Interpretation of results was done by measuring the zone sizes on the agar plates. Zone diameter above or equal to 12mm defined susceptibility to the respective antibiotic while zone diameter below 12mm defined resistance to the respective antibiotic.¹⁴ All the isolates were tested for sensitivity to the following antibiotics: ampicillin (10µg), tetracycline (10µg), gentamicin (10µg), cotrimoxazole (25µg), streptomycin (10µg), nalidixic acid (30µg), nitrofurantoin (200µg) and colistin (25µg), all of Abtek Biologicals Ltd, UK.

Results

Aeromonas spp. was not isolated from the 104 stool samples analyzed. The isolates included *E. coli* from seven (6.73%) stool samples as well as *Salmonella* species, (Table 1). There was no considerable difference in the total number of isolates from diarrhoeic (watery or mucoid) and non-diarrhoeic (formed or semi-formed) stool samples. Watery stools yielded the highest number of *E. coli* isolates (Table 2). In addition, *E. coli* was predominantly found in both sexes between 11 and 15 years of age (Figure 1).

Table 1: Enteropathogens identified in stool samples

Pathogen	School A (n=45)	School B (n=59)	Total	% n=104		
	Freq	%	Freq	%		
<i>E. coli</i>	4	8.8	3	5.1	7	6.7
<i>Shigella</i>	1	2.2	-	-	1	0.9
<i>Salmonella</i>	-	-	1	1.6	1	0.9
<i>Proteus vulgaris</i>	-	-	1	1.6	1	0.9
<i>Aeromonas</i>	-	-	-	-	-	-
Total					10	9.6

School A -St. David Anglican Nursery and Basic school, School B - Imedu Nla Nursery and Primary School.

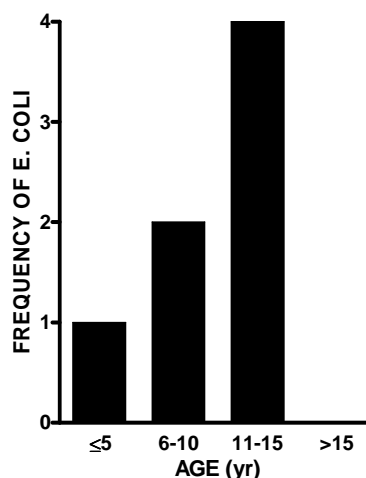
Table 2: Appearance of stool Samples and Frequency of Isolation of *E.coli*

	School A	School B	
	No. of Isolates	No. of Isolates	Total No Isolates
Appearance of Stool			
Watery Stool	1	2	3
Watery and Mucoïd Stool Formed	-	-	-
Semi-formed	2	-	2
	1	1	2
Total No. of Samples	4	3	7

School A - St. David Anglican Nursery and Basic School,
School B - Imedu Nla Nursery and Primary School.

Antimicrobial Susceptibility Patterns

Table 3 shows the antimicrobial susceptibility pattern of the four bacterial species tested: *E. coli*, *Salmonella species*, *Shigella species* and *Proteus vulgaris*, against eight antibiotics. The isolates showed the least susceptibility to tetracycline and ampicillin. The overall susceptibility of the isolates to these antibiotics was less than or equal to 30%. All the isolates showed 100% susceptibility to gentamicin. Generally, the isolates showed overall susceptibility of 90% to ofloxacin and nalidixic acid. Augmentin and nitrofurantoin were also effective against most bacterial pathogens except *Salmonella species*.

Fig 1: Fig 1: Bar chart showing the relationship between the frequency of isolation of *Escherichia coli* and the age distribution of the pupils.

Cotrimoxazole was only effective on *Salmonella species* and *Proteus vulgaris* and the overall susceptibility of the isolates to cotrimoxazole was 40%. Resistance to more than two antibiotics was observed in relation to four (57.1%) *E. coli species*, one (100%) *Shigella species* and one (100%) *Salmonella species*.

Table 3: Percentage antimicrobial susceptibility patterns of enteric bacteria from sampled population.

Organism	OFL	AUG	COT	NIT	NAL	GEN	AMX	TET
Escherichia coli (n=7)	6 (85.7)	5 (71.4)	3 (42.9)	4 (57.1)	6 (85.7)	7 (100)	2 (28.6)	1 (14.3)
Salmonella (n=1)	1 (100)	0	0	0	1 (100)	1 (100)	0	0
Shigella (n=1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	0
Proteus vulgaris (n=1)	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	0
Overall Susceptibility (n=10)	9 (90)	7 (70)	4 (40)	6 (60)	9 (90)	10 (100)	3 (30)	1 (10)

TET = Tetracycline (10µg), GEN = Gentamicin (10µg), COT = Cotrimoxazole (25µg), NAL = Nalidixic acid (30µg), NIT = Nitrofurantoin (200µg), OFL = Ofloxacin (5µg), AUG = Augmentin (30 µg), AMX=Ampicillin (10µg).

n = number of isolate

Discussion

Aeromonas was not isolated in the present study despite the use of standard microbiological procedures. Indeed, *Aeromonas* has been suggested to be rarely associated with diarrhoeal diseases in Nigeria.⁶ However, this claim contrasted with other Nigerian reports from Nassarawa State⁹ and in Edo State.¹⁵ The non-occurrence of *Aeromonas* species in the present report may be attributed to the fact that the study was conducted during the dry season (December through February). This observation was

similar to the reports from an earlier study which was similarly conducted during the dry season.⁹ *Aeromonas* infection is water-borne, thus, its frequency had been reported to be higher during the wet season (May through October).

This study showed that *E. coli* infection was most frequent among children aged eleven to fifteen compared to other age groups. This observation agreed with reports from Nsukka suggesting that school-age children were likely to consume contaminated water and food. In contrast, another report had shown that the highest prevalence of *E. coli* infections occurred in the younger age groups.¹⁶

The susceptibility pattern of *E. coli* isolates showed that, among the commonly used antibiotics, gentamicin was the only one that the isolates were still largely sensitive to, unlike other reports that recorded resistance to gentamicin. In addition, ofloxacin, Augmentin and nalidixic acid could serve as alternatives to gentamicin in the treatment of bacterial diarrhoeal diseases in the study area.^{16,17} The high resistance of *E. coli* species to tetracycline and ampicillin in the study area calls for a large-scale detailed research works on antibiotic susceptibility pattern of stool pathogens in the locality. In addition, emphasis must be placed on preventive measures such as improved hygiene rather than increasing reliance on antibiotic therapy as it obtains in some countries.

The development of new antibiotics may offer a short term solution to the problem of antibiotic resistance but more cost-effective measures like health education and other preventive measures should be encouraged.

Contribution of authors

Most research materials were provided by the Redeemer's University while the remaining were sourced by the researchers.

Conflict of interest : None

Funding: None

Conclusion

This study shows that *E. coli*, rather than *Aeromonas*, was the predominant bacterial agent associated with diarrhoeal diseases among school pupils in Obafemi-Owode Local Government Area, Ogun State.

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