

Bacteriology of Diarrhoeic Infection among Primary School Pupils in Akoko South West Local Government Area in Ondo State, Nigeria

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Abstract

This study shows the bacteriological investigations of Diarrhoeal diseases among selected children who were between the ages of 0 – 14 years. Stool samples from primary school pupils in Akoko South West Local Government Area in Ondo State, Nigeria were used for this purpose. The organisms were isolated and identified using cultural and biochemical test. Isolates obtained were subjected to some antibiotic susceptibility testing. Out of total number of 120 samples examined only 40 (33.3%) of primary school pupils were found to have Diarrhoea associated with bacteria in which 17 (42.5%), 15 (42.5%), and 8(32.5%) were from 0-4 years, 5-9 years and 10-14 years respectively. The Bacteria species isolated include *Escherichia coli* were the most predominant microorganism. This is followed by *Staphylococcus aureus*, *Bacillus cereus* and *Vibrio cholerae* respectively. As at the time of the study, there was significance difference between male and female gender with the male having high incidence of 55.0%. Among the antimicrobials used sparfloxacin and cefuroxime was most effective against Gram negative and Gram positive bacteria respectively. Also, most of the children tested were found to have common illness with clinical manifestations of diarrhoea followed by dysentery, abdominal pain and fever which may be accompanied with vomiting. Severity of infections varies with individual.

Keywords: Bacteria; Children; Diarrhoeal diseases; Nigeria.

1. Introduction

Diarrhoea is a clinical condition manifested with passage of watery stool by infected patient. The most common cause is gastroenteritis. Diarrhoea is coined from the Greek word $\delta\iota\alpha$ *dia* "through" and $\rho\acute{\epsilon}\omega$ *rheo* "flow" meaning "flowing through". It is the condition of having three or more loose or liquid bowel movements per day [1]. Acute diarrhea is a common problem that usually lasts 1 or 2 days and goes away on its own. Diarrhoea lasting more than 2 days may be a sign of a more serious problem. Diarrhoea may cause dehydration, which means the body lacks enough fluid and electrolytes, chemicals in salts, including sodium, potassium, and chloride to function properly. Loose stools contain more fluid and electrolytes and weight more than solid stools [2].

People of all ages can get diarrhoea. In the United States, adults and young children by epidemiological statistics have an average of two episodes of acute diarrhoea each year. Nevertheless, commonly used epidemiological studies reported that those with some specificity have three or more loose stools, per any number of stools containing blood, in 24-hour period. The end of an episode will mean three diarrhoea-free days [3]. Diarrhoea remains a leading cause of morbidity and mortality in the world, predominantly affecting children in developing countries. During the 1990s, diarrhoea was estimated to cause 20 to 25per cent of mortality among children younger than 5 years in the developing world [1, 4]. Effective intervention, including correct case management (oral rehydration therapy, continued feeding and antibiotics in cases of dysentery), promotion of breastfeeding, and better weaning practices, has the potential to reduce the burden of diarrhoea disease substantially in the future [5].

Oral rehydration solutions (ORS) with modest amounts of salts and zinc tablets are the treatment of choice and have been estimated to have saved 50 million children in the past 25 years. In cases where ORS is not available, homemade solutions are often used. It is a common cause of death in developing countries and the second most

common cause of infant deaths worldwide. The loss of fluids through diarrhea can cause dehydration and electrolyte disturbances such as potassium deficiency or other salt imbalances. In 2009 diarrhea was estimated to have caused 1.1 million deaths in people aged 5 and above and 1.5 million deaths in children under the age of five (5) [6].

If a person drinks solutions with excessive sugar or excessive salt, these can draw water from the body into the bowel and cause osmotic diarrhoea. Osmotic diarrhea can also be the result of maldigestion (e.g., pancreatic disease or Coeliac disease), in which the nutrients are left in the lumen to pull in water. Or it can be caused by osmotic laxatives (which work to alleviate constipation by drawing water into the bowels). In most cases, osmotic effects during diarrhea stops when offending agent (e.g. milk, sorbitol) is stopped or eliminated gradually in body circulation [7].

Other forms of diarrhoea includes exudative diarrhea that occurs with the presence of blood and pus in the stool. This occurs with inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis, and other severe infections such as *E. coli* or other forms of food poisoning. It can also be caused by tuberculosis, colon cancer, and enteritis [1]. While dysentery diarrhea is said to occur if there is blood visible in the stools. It is not diarrhoea, but dysentery. The blood is trace of an invasion of bowel tissue. Dysentery is a symptom of many types of diseases including *Shigella* spp., *Entamoeba histolytica*, and *Salmonella* spp [8]. Diarrhoea may be accompanied by dehydration, cramping, abdominal pain, nausea, an urgent need to use the bathroom, or loss of bowel control. In addition, bacterial infections, can result in stooling accompanied with blood and mucus [5]. Immunization against Measles can reduce diarrhoea incidence and death. A vaccine for rotavirus also has the potential to reduce mortality. Micronutrients, especially vitamin A and zinc, have protective effects against diarrhoea. Recent studies have shown that zinc supplementation reduces the severity and duration of diarrhoea episodes and can also help prevent occurrence of diarrhoea disease. However, effective interventions focus on family and community practices and appropriate technologies [9, 10]. Although hand washing is often incorporated into child survival programs, hygiene improvement behaviors are usually supported by separate programs and other bureaucracies (the ministries of water resources, public works, agriculture, and environment, as well as health and education). This improves environmental health activities [11, 12].

Exclusive breastfeeding up to six months and continued breastfeeding thereafter provides significant protection. Collaboration between CDD, nutrition, and hygiene programs is important to prevent diarrhoea deaths [13]. Until diarrhoea subsides, avoiding caffeine and foods that are greasy, high in fiber, or sweet may lessen symptoms. These foods can aggravate diarrhoea. Some people also have problems digesting lactose during or after a bout of diarrhoea. Yogurt, which has less lactose than milk, is often better tolerated. Yogurt with active, live bacterial cultures may even help people recover from diarrhoea more quickly. Some children recovering from viral diarrhoea have problems digesting lactose for up to a month or more [14]. This study therefore aims at isolating and determines the incidence of diarrhoeic infection among Primary School Pupils in Akoko South West Local Government, Ondo State, Nigeria. Similarly, the antibiotics sensitivity profile of isolated diarrhoeic aetiological agents was determined.

2. Materials and Methods

Sample Collection: One hundred (100) stool samples were collected from primary pupils in various Health Centre and Primary School in Akoko South West Local Government Area, Ondo State, Nigeria. The sample were labelled with respective name, gender, age, and gastroenteritis symptoms such as diarrhoea, fever, dysentery (with mucus or blood) and abdominal pains experiences by pupils recently. And transported to the laboratory with transport medium immediately for analysis.

Study Area: This study was conducted at Akoko South West Local Government Area of Ondo State, Nigeria. The choice was based on the enormous increase in number of primary school in the areas and a need to assess activities that may lead to this diarrhoeic infection. The headquarters of Akoko South West Local Government Area is Oka. It has an area of 226 km² and a population of 229486 at 2006 census. The area is characterised by two distinct seasons which are the dry season and the wet season. The project research focus on seven communities which are: Akungba Akoko, Iwaro Oka Akoko, Ayegunle Oka, Oba Akoko, Supare Akoko, Oke Oka Akoko, Ikun Akoko, and Eti-Oro.

Macroscopic Examination

The colour, consistence, blood, mucus or pus or any parasitic in stool sample were sorted with the naked eye. Bristol stool chart were used in characterization of the macroscopic properties of stool sample. Macroscopic observation was recorded and noted.

Sterilization of Glass Wares and Culture Medium Used

All glass wares that were used in the course of this practical work were sterilized using hot air oven at 170°C for 2 hours and allowed to cool before use. Similarly, culture medium routinely used for the study include Nutrient Agar (NA), Gelose Sorbitol MacConkey Agar and Muller Hinton Agar. They were sterilized at 121°C for 15 mins in an autoclave before use.

Stool Dilution and Streak-Plate Method

A loop of the stool sample was picked using the inoculating loop and diluted with 5ml of sterilized distilled water inside the test-tube and stirred evenly to ensure even distribution. A loop of the diluted mixture were streaked on the solidified agar and incubated at 37°C for 24 hrs. The colonies growth on all the plates (both on nutrient agar and gelose sorbitol macConkey agar) were observed.

Identification of Bacterial Isolates

The diluted stool were used for streaking using streak-plate method for the isolation of bacteria. The inoculated plates of nutrient agar and gelose sorbitol macConkey agar were incubated at 37°C for 18-24 hours for bacteria. Colonies on the plates were randomly selected for subculture on sterile nutrient agar and gelose sorbitol macConkey agar for re-incubation. Different parameters were used to identify the isolates which include cultural characteristics and biochemical test such as Gram's stain reaction, Catalase test, Indole test, urease test, ornithine test, and fermentation of sugar.

Antibiotics Sensitivity Assay

The antibiotic sensitivity test was done using the modified Bauer-Kirby agar diffusion method [15]. Muller hinton agar was used for the antibiotic sensitivity assay. Solidified agar plates were inoculated with the isolates and antimicrobial Gram's negative disc that contained, sparfloxacin (10 µg), septrin (30µg), tarivid (10µg), chloramphenicol (30µg), amoxicillin (30µg), streptomycin (30 µg), ciprofloxacin (10 µg), gentamycin (10 µg), augmentin (30 µg), and pefloxacin (30µg). And also antimicrobial Gram's positive disc that contained cefuroxime (20 µg), rocephin (25 µg), septrin (30 µg), amoxicillin (30 µg), erythromycin (10 µg), pefloxacin (10 µg), gentamycin (10 µg), streptomycin (30 µg), ampiclox (30 µg) and ciprofloxacin (10 µg) was aseptically placed on the culture plates and incubated at 37°C for 24hrs. Zone of inhibition were measured after 24hrs of incubation [16, 17].

Result

Diversified types of pathogenic organism were obtained from stool sample sources studied in Akoko South West Local Government Area, Ondo State, Nigeria. Table 1 shows the macroscopic examination used to characterized the 120 samples collected and processed.

Considering the presence or absent of mucus, out of the 120 sample, 23 samples were mucus present with 2 samples from A location (Akungba Akoko), 3 samples from B location (Iwaro Oka Akoko), 3 samples from C location (Ayegunle Oka), 2 samples from D location (Oba Akoko), 3 samples from E location (Supare Akoko), 2 samples from F location (Oke Oka Akoko), 4 samples from G location (Ikun Akoko). Similarly, with consideration to the presence of visible parasite, out of the 120 sample, only one samples had visible parasite present which is from A location (Akungba Akoko)

TYPE 1 to **TYPE 6**, were isolated from different locations studied including D location (Oba Akoko), E location (Supare Akoko), F location (Oke Oka Akoko), G location (Ikun Akoko), and H location (Eti-Oro) (Table 1). 15 samples among the overall studied were **TYPE 7**, with 1 sample from A (Akungba Akoko), 2 samples from B location (Iwaro Oka Akoko), 1 sample from C location (Ayegunle Oka), 4 samples from D location (Oba Akoko), No samples from E location (Supare Akoko), 2 samples from F location (Oke Oka Akoko), 5 samples from G location (Ikun Akoko) and No samples from H location (Eti-Oro).

In Table 2, out of 120 samples collected and processed, 56 samples were from the primary school pupils with diarrhoea (46.7%), followed by dysentery (33), fever/ vomiting (18) and abdominal pain (13) with 27.5%, 15.0% and 10.8% respectively. While Table 3 shown 20 of the 40 bacterial isolates obtained from 120 stool samples collected were *Escherichia coli* with a frequency rate of 50.00%, other isolates obtained in this context includes *Escherichia coli* 0157:H7 (12), *Staphylococcus aureus* (5), *Bacillus cereus* (2) and *Vibrio cholerae* (1) representing 30.00%, 12.25%, 5.00% and 2.50% respectively. In Table 4, the 40 bacterial isolates obtained from 120 stool samples collected were made up of 5 distinct isolate from the various location of the study area. *Escherichia coli* and *Staphylococcus aureus* were predominant isolates obtained from this study sources. *Vibrio cholerae* occur only in B location (Iwaro Oka Akoko).

TABLE 1 contd

33	C3	Yellow	Absent	Absent	Absent	Absent	Type 4
34	C4	Yellow	Present*	Absent	Present*	Absent	Type 3
35	C5	Brown	Absent	Absent	Absent	Absent	Type 3
36	C6	Yellowish brown	Absent	Absent	Absent	Absent	Type 1
37	C7	Brown	Absent	Absent	Absent	Absent	Type 1
38	C8	Brown	Absent	Absent	Absent	Absent	Type 1
39	C9	Brown	Absent	Absent	Absent	Absent	Type 3
40	C10	Yellowish brown	Absent	Absent	Present*	Absent	Type 3
41	C11	Brown	Absent	Absent	Absent	Absent	Type 4
42	C12	Yellow	Absent	Absent	Absent	Absent	Type 5
43	C13	Yellowish brown	Absent	Present*	Absent	Absent	Type 2
44	C14	Brown	Absent	Absent	Present*	Absent	Type 1
45	C15	Magoon	Absent	Absent	Absent	Absent	Type 3
46	D1	Yellow	Present*	Absent	Absent	Absent	Type 1
47	D2	Brown	Absent	Absent	Absent	Absent	Type 3
48	D3	Brown	Absent	Absent	Absent	Absent	Type 4
49	D4	Brown	Absent	Absent	Absent	Absent	Type 5
50	D5	Brown	Absent	Absent	Absent	Absent	Type 6
51	D6	Brown	Present*	Absent	Absent	Absent	Type 6
52	D7	Brown	Absent	Absent	Absent	Absent	Type 2
53	D8	Brown	Absent	Absent	Absent	Absent	Type 7
54	D9	Brown	Absent	Absent	Absent	Absent	Type 7
55	D10	Brown	Absent	Absent	Absent	Absent	Type 2
56	D11	Brown	Absent	Absent	Absent	Absent	Type 1
57	D12	Brown	Absent	Absent	Absent	Absent	Type 2
58	D13	Brown	Absent	Absent	Absent	Absent	Type 2
59	D14	Yellow	Absent	Absent	Absent	Absent	Type 7
60	D15	Yellowish brown	Absent	Absent	Absent	Absent	Type 7
61	E1	Brown	Absent	Absent	Absent	Absent	Type 3
62	E2	Brown	Absent	Absent	Absent	Absent	Type 5
63	E3	Brown	Absent	Absent	Absent	Absent	Type 5
64	E4	Brown	Absent	Absent	Absent	Absent	Type 4
65	E5	Brown	Absent	Absent	Absent	Absent	Type 3

The incidence and frequency of diarrhoeal diseases (based on bacterial pathogens isolated) were higher in 0-4 year age groups of the primary school pupils with 17 isolates consisting 10 *E.coli*, 3 *Escherichia coli* 0157:H7, 3 *Staphylococcus aureus* and 1 *Vibrio cholerae* representing 42.5% followed by 5-9 (15 isolates consisting 5 *E. Coli*, 8 *Escherichia coli* 0157:H7, 1 *Staphylococcus aureus*, 1 *Bacillus cereus*), and 10-14 (8 isolates consisting 5 *E. Coli*, 1 *Escherichia coli* 0157:H7, 1 *Staphylococcus aureus*, 1 *Bacillus cereus*) age groups, representing 37.5% and 20.0% respectively as shown in Table 5. Table 6 conceptualizes that the 22 isolates obtained from male consisting of 13 *E.coli*, 4 *Escherichia coli* 0157:H7, 3 *Staphylococcus aureus*, 1 *Bacillus cereus* and 1 *Vibrio cholerae* with a frequency of 32.5%, 10.0%, 7.5%, 2.5% and 2.5% respectively. 18 isolates were obtained from female consisting of 7 *E.coli*, 8 *Escherichia coli* 0157:H7, 2 *Staphylococcus aureus*, 1 *Bacillus cereus* and No or Nil *Vibrio cholerae* with a frequency of 17.5%, 20.0%, 5.0%, 2.5% and 0.0% respectively.

TABLE 1 contd

66	E6	Yellow	Absent	Absent	Absent	Absent	Type 4
67	E7	Yellowish brown	Absent	Absent	Absent	Absent	Type 3
68	E8	Brown	Present*	Absent	Absent	Absent	Type 5
69	E9	Brown	Absent	Absent	Present*	Absent	Type 4

70	E10	Brown	Absent	Absent	Absent	Absent	Type 3
71	E11	Brown	Absent	Absent	Absent	Absent	Type 2
72	E12	Brown	Absent	Absent	Absent	Absent	Type 1
73	E13	Yellowish brown	Present*	Absent	Absent	Absent	Type 1
74	E14	Brown	Absent	Absent	Absent	Absent	Type 1
75	E15	Brown	Absent	Absent	Present*	Absent	Type 3
76	F1	Brown	Absent	Absent	Absent	Absent	Type 3
77	F2	Brown	Absent	Absent	Absent	Absent	Type 3
78	F3	Yellowish brown	Absent	Absent	Absent	Absent	Type 4
79	F4	Brown	Absent	Absent	Present*	Absent	Type 5
80	F5	Brown	Absent	Absent	Absent	Absent	Type 5
81	F6	Brown	Absent	Absent	Absent	Absent	Type 7
82	F7	Brown	Present*	Absent	Absent	Absent	Type 7
83	F8	Brown	Absent	Absent	Absent	Absent	Type 3
84	F9	Yellowish brown	Absent	Absent	Absent	Absent	Type 2
85	F10	Brown	Absent	Absent	Present*	Absent	Type 1
86	F11	Yellowish brown	Absent	Absent	Absent	Absent	Type 4
87	F12	Brown	Absent	Absent	Absent	Absent	Type 3
88	F13	Brown	Absent	Present*	Absent	Absent	Type 2
89	F14	Brown	Absent	Absent	Present*	Absent	Type 1

90	F15	Brown	Absent	Absent	Absent	Absent	Type 1
91	G1	Brown	Absent	Absent	Absent	Absent	Type 1
92	G2	Brown	Absent	Absent	Absent	Absent	Type 7
93	G3	Brown	Absent	Absent	Absent	Absent	Type 7
94	G4	Brown	Absent	Absent	Present*	Absent	Type 7
95	G5	Yellowish brown	Absent	Absent	Absent	Absent	Type 7
96	G6	Brown	Absent	Absent	Absent	Absent	Type 3
97	G7	Brown	Absent	Absent	Present*	Absent	Type 3
98	G8	Brown	Absent	Absent	Absent	Absent	Type 3
99	G9	Brown	Present*	Absent	Absent	Absent	Type 2
100	G10	Brown	Absent	Absent	Absent	Absent	Type 1
101	G11	Yellowish brown	Absent	Absent	Present*	Absent	Type 3
102	G12	Brown	Absent	Absent	Absent	Absent	Type 2
103	G13	Brown	Absent	Absent	Absent	Absent	Type 1
104	G14	Brown	Present*	Absent	Absent	Absent	Type 1
105	G15	Brown	Absent	Absent	Present*	Absent	Type 7
106	H1	Brown	Absent	Absent	Absent	Absent	Type 1
107	H2	Brown	Absent	Absent	Absent	Absent	Type 2
108	H3	Brown	Absent	Absent	Present*	Absent	Type 2
109	H4	Brown	Present*	Absent	Absent	Absent	Type 3
110	H5	Brown	Absent	Absent	Absent	Absent	Type 1
111	H6	Brown	Absent	Absent	Present*	Absent	Type 2
112	H7	Yellowish brown	Absent	Absent	Absent	Absent	Type 4
113	H8	Brown	Absent	Absent	Absent	Absent	Type 3
114	H9	Brown	Absent	Absent	Absent	Absent	Type 2
115	H10	Brown	Absent	Absent	Present*	Absent	Type 1
116	H11	Yellowish brown	Absent	Absent	Absent	Absent	Type 1
117	H12	Brown	Absent	Absent	Absent	Absent	Type 4
118	H13	Brown	Absent	Absent	Absent	Absent	Type 4
119	H14	Brown	Absent	Absent	Absent	Absent	Type 3
120	H15	Brown	Absent	Absent	Absent	Absent	Type 2



(Source: Amarenco, 2014)

Table 2: Distribution pattern of common illness among the primary school pupils in relation to age group

Age Groups (In Years)	Diarrhoea	Dysentery	Fever/Vomiting	Abdominal Pain	Total
0-4	10 (8.3)	9 (7.5)	8 (6.7)	3 (2.5)	30 (25)
5-9	34 (28.3)	20 (16.7)	8 (6.7)	6 (5.0)	68 (56.7)
10-14	12 (10.0)	4 (3.3)	2 (1.7)	4 (3.3)	22 (18.3)
Total	56 (46.7)	33 (27.5)	18 (15)	13 (10.8)	120 (100)

Table 3: Percentage occurrence and distribution of bacterial pathogen in the diarrhoeic infections

Isolates	Frequency (%)
<i>Other Escherichia coli strain</i>	20 (50.00)
<i>Escherichia coli 0:157:H7</i>	12 (30.00)
<i>Bacillus cereus</i>	2 (5.00)
<i>Vibrio cholerae</i>	1 (2.50)
<i>Staphylococcus aureus</i>	5 (12.50)
Total	40 (100)

Table 4: Overall incidences with location of bacterial pathogens in diarrhoeic infections

Towns	<i>E. coli</i>	<i>E. coli 0:157:H7</i>	<i>V. cholerae</i>	<i>S. aureus</i>	<i>B. cereus</i>	Total
A	3	1	Nil	1	Nil	5
B	2	2	1	Nil	Nil	5
C	2	2	Nil	Nil	1	5
D	1	4	Nil	Nil	Nil	5
E	2	2	Nil	1	Nil	5
F	3	Nil	Nil	2	Nil	5
G	3	1	Nil	1	Nil	5

H	4	Nil	Nil	Nil	1	5
Total	20	12	1	5	2	40

KEYS: A- Akungba Akoko; B- Iworo Oka Akoko; C- Ayegunle Oka; D- Oba Akoko.
E- Supare Akoko; F- Oke Oka Akoko; F- Ikun Akoko; G. - Oba Akoko; H - Eti- Oro

Table 5: Overall incidences with age distribution of associated bacterial pathogens

Isolates	Frequency (%)			
	No.	0-4	5-9	10-14
<i>Other Escherichia coli strain</i>	20(50.0)	10(25.0)	5(12.5)	5(12.5)
<i>Escherichia coli 0:15:H7</i>	12(30.00)	3(7.5)	8(20.0)	1(2.5)
<i>Bacillus cereus</i>	2(5.00)	0(0)	1(2.5)	1(2.5)
<i>Staphylococcus aureus</i>	5(12.5)	3(7.5)	1(2.5)	1(2.5)
<i>Vibrio cholerae</i>	1(2.5)	1(2.5)	Nil	Nil
Total	40(100.0)	17(42.5)	15(37.5)	8(20.0)

Table 6: Overall incidences of bacterial pathogens associated with diarrhoeic infection with sexes among primary school pupils in Akoko South West L.G.A, Ondo State, Nigeria.

Isolates	No.	Male	Female
<i>Other Escherichia coli strain</i>	20 (50.0)	13 (32.5)	7 (17.5)
<i>Escherichia coli 0:157:H7</i>	12 (30.00)	4 (10.0)	8 (20.0)
<i>Bacillus cereus</i>	2 (5.00)	1 (2.5)	1 (2.5)
<i>Staphylococcus aureus</i>	5 (12.5)	3 (7.5)	2 (5.0)
<i>Vibrio cholerae</i>	1 (2.5)	1 (2.5)	Nil (0.0)
Total	40 (100.0)	22 (55.0)	18 (45.0)

Table 7: Antibiotic susceptibility pattern of Gram's Negative micro organisms or (in mm) antibiotics and frequency (%) of isolates susceptible to the antimicrobial agent.

ISOLATES	PEF	OFX	S	SXT	CH	SP	CPX	AM	AU	CN
<i>E.oli</i>	22 (9.0%)	24 (8.7%)	24 (8.7%)	15 (6.0%)	10 (3.5%)	32 (10.0%)	Nil (0%)	28 (9.5%)	Nil (0%)	12 (3.8%)
<i>E.coli 0:157:D7</i>	16	Nil	37	11	13	30	18	21	10	25

	(6.0%)	(0%)	(13.0%)	(3.7%)	(4.0%)	(10.9%)	(6.3%)	(8.3%)	(3.5%)	(8.8%)
<i>V. cholerae</i>	Nil (0.0%)	22 (7.2%)	Nil (0.0%)	12 (3.7%)	Nil (0%)	28 (9.1%)	29 (10.7%)	19 (6.4%)	13 (3.8%)	28 (9.2%)

KEYS: SP----SPARFLOXACIN; SXT---SEPTRIN; OFX---TARIVID; CH----CHLORAMPHENICOL; AM---AMOXACILLIN ; S----STREPTOMYCIN; CPX---CIPROFLOXACIN; CN--GENTAMYCIN; AU---AUGMENTIN PEF---PEFLOXACIN

Table 8: Antibiotic susceptibility pattern of Gram's positive micro organisms (in mm) and frequency (%) of isolates susceptible to the antimicrobial agent.

ISOLATE S	SXT	E	PEF	CN	APX	Z	AM	R	CPX	SP
<i>S. aureus</i>	Nil (0.0%)	18 (6.4%)	Nil (0.0%)	31 (10.0%)	28 (9.3%)	30 (10.9%)	13 (4.1%)	14 (4.3%)	18 (6.3%)	20 (6.5%)
<i>B. cereus</i>	23 (6.9%)	20 (6.5%)	Nil (0.0%)	12 (3.7%)	Nil (0.0%)	20 (6.5%)	12 (3.7%)	14 (4.0%)	12 (3.7%)	Nil (0.0%)

KEYS: Z-----ZINNACEF; SP--SPARFLOXACIN; R----ROCEPHIN ; SXT---SEPTRIN; AM---AMOXACILLIN; E---ERTHROMYCIN; PEF---PEFLOXACIN; CN --GENTAMYCIN; APX---AMPICLOX; CPX--CIPROFLOXACIN

3. Discussion

Sample of stool obtained from school children in Akoko South West Local Government Area, Ondo State, Nigeria were characterized for their diarrhoeic aetiologic agent during the study. Changes in stool (faeces) colour are often harmless and reflect dietary influences. The normal brown colour of the stool occurs due to the presence of bilirubin are formed as a breakdown product of haemoglobin (from red blood cells) in the liver and is secreted into the bile, which enters the intestines. if the intestinal contents travel at a normal speed, chemical changes in bilirubin produce stool that is light to dark brown. There are variety of reasons why stool may be yellow, it can be due to the intestine's inability to digest fat because of malabsorption such as in celiac diseases and cystic fibrosis or because of pancreas is unable to manufacture adequate digestive enzymes.

Yellow colour can be caused by gastrointestinal infection caused by Giardiasis, a protozoan infection that can cause significant diarrhoea. It can also be due to bacteria infection too. Yellowish- brown is deviation from the normal brown colour and yellow colour which has a diagnostic features of the both colour. Some of these features are clarified by Alkizim *et al.*, [19]. The bristol stool chart help in the characterization of marcoscopic nature of the stool sample as described in the result. However ,it remains in use as a research tool to evaluate the effectiveness of treatments for various disease of the bowel, as well as clinical communication. Diarrhoea was the most common illness recorded by children with Diarrhoeal diseases, this could probably be because parents take their children to the hospitals immediately they found out that their children were passing watery stool. These corroborates with the findings of Arora [20] and Gallies [21].

E.coli has the highest incidence among primary pupils with 32 isolates from the 40 isolates obtained, which consist of 20 other *E.coli* strain and 12 Enterohaemorrhagic *E.coli* O:157:H7 with a frequency of 50% and 30% respectively. This is consistent with previous reports on diarrheic infections [22, 23]. *E.coli* occurrence were higher in Eti-Oro, high in Akungba Akoko, Oke Oka Akoko, and Ikun Akoko, low in Iwaro Oka Akoko, Supare Akoko, Ayegunle Oka and Oba Akoko. *Escherichia coli* O157:H7 occurrence were higher in Oba Akoko with 4 isolates, high in Iwaro Oka Akoko, Supare Akoko and Ayegunle Oka with 2 isolates, low in Akungba Akoko and Ikun Akoko and Nil in Oke Oka Akoko and Ikun Akoko. *Staphylococcus aureus* occurrence were higher in Oke Oka Akoko, high in Supare Akoko, Ikun Akoko and Akungba Akoko and nil in others. *Bacillus cereus* occurrence were higher in Ayegunle Oka and Eti-oro with 1 isolates each and others Nil. *Vibro cholerae* with occurrence only in Iwaro Oka Akoko others Nil in other location. Areas found to high frequency of microorganism, are suspected to be areas which are associated with poor hygiene and feeding habits.

In this study, primary school pupils with the age group of 0-4 yrs were mostly infected with Diarrhoeal diseases, followed by 5-9 and 10-14 respectively. Previous study in Nigeria, shows that diarrhoea diseases are still the major cause of dysentery in children aged 0-9 months and many older children are hospitalized almost immediately after onset of disease [24]. This can attributed to the facts that the parent of these pupils can only monitor them during non-school hours but after that they can't be able to check mate their hygiene condition. In addition, many hormones, growth factors and bioactive substances present in the maternal organs are now known to pass into the colostrums often exceeding concentration occurring in maternal plasma. As baby is growing, the concentration of breast milk reduces, also the concentration of most essential factors reduce [25]. Complimentary to this, male children were more infected than the female. Generally in the society today female are assumed to be hygiene than the male. Male are regarded to be stubborn.

The antibiotic susceptibility test shows multiple resistance by the microbial isoaltes from sample sources. Based on zone of inhibition and percentage of Gram's negative organism susceptibility as shown in figure 6, *E. coli* were highly susceptible to sparfloxacin, susceptible to pefloxacin, tarivid, streptomycin, and amoxacillin, but less sensitive to gentamycin, chloramphenicol and septrin and resistant to ciprofloxacin and augmentin. *Escherichia coli* 0157:H7 were highly susceptible to streptomycin and sparfloxacin, susceptible to amoxacillin and gentamycin, less sensitive to augmentin, chloramphenicol, septrin, and pefloxacin and resistant to tarivid. *Vibro cholerae* were highly sensitive to tarivid, sparfloxacin, ciprofloxacin, and gentamycin, sensitive to septrin and augmentin and resistant to pefloxacin, streptomycin and chloramphenicol.

Based on zone of inhibition and percentage of Gram's positive organism susceptibility as shown in figure 6, *Bacillus cereus* were highly susceptible to septrin, erthromycin and zinnacef, less sensitive to gentamycin, amoxacillin, rocephin, and ciprofloxacin and resistant to pefloxacin, ampiclox and streptomycin. *Staphylococcus aureus* were highly susceptible to gentamycin and zinnacef, susceptible to ampiclox and streptomycin, less sensitive to amoxacillin, rocephin, ciprofloxacin and erthromycin and resistant to septrin and pefloxacin.

Results of the antimicrobial sensitivity demonstrated that sparfloxacin and zinnacef was the most effective antimicrobials for Gram's negative and Gram's positive respectively while chloramphenicol and augmentin was the least susceptible antimicrobial agent to Gram's negative and pefloxacin was the least susceptible antimicrobial agent to Gram's positive which were found in the present study. Often the high cost of an antibiotic, results in an incomplete course being purchased, sufficient only to alleviate symptoms [26]. In addition, these children are frequently exposed to the hospital environment where the organisms have high rate of colonization and are transferred mostly to them by the health workers that deliver health services to them or some time when interacting with infected children at home, or at day care centre etc. [27]. The control of Diarrhoeal diseases is not only on early and appropriate treatment, but increasing fluids intake, UNICEF glucose-based, cereal-based oral hydration should be encouraged [22].

In conclusion, Bacteriological investigations of Diarrhoeal diseases were carried out among 120 primary school pupils in akoko south west children who were between the ages of 0 –14 years. Out of those number only a total of 40 (33.3%) of children were found to have Diarrhoeal diseases in which male were infected than female primary school pupils. The Bacterial species encountered include *Escherichia coli* which was most common among children, followed by *Staphylococcus aureus*, *Bacillus cereus* and *vibro cholerae* respectively. Sparfloxacin and Zinnacef was the most effective antimicrobial agent for Gram's negative and Gram's positive organisms respectively during the study.

There appear to be quite marked differences in the relative importance of Diarrhoeal diseases in different parts of Nigeria. Similarly, it is less of a scourge than in some other parts of the world. However, it is major attendance at health facilities, the second or third most common cause of admission to many of the hospitals in the Nigeria causing significant and often preventable death. Diarrhoeal diseases among children are believed to be very common in children, and can minimize or uncommon in a situation where personal hygiene quality of drinking water, quick isolation and treatment of infected cases. This research shows a high incidence of Diarrhoeal diseases among children of age group 0-4 in age distribution and also male in sex distribution mainly because of low idealogy about personal and environmental hygiene which could be from the parent part and also playing nature of male gender of primary school pupils. Parents are strongly advice to educate their children about measures to maintain personal and environmental hygiene at early or tender age.

The government should however endeavor to provide potable water to the community. Improving the sanitary awareness through basic health education and careful surveillance and monitoring incidence and spread of Diarrhoeal diseases, will go a long way to reduce the disease burden in children. The approach of oral dehydration therapy given to children by mother must be taught to reduce the debilitating effect of Diarrhoeal diseases [28].

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