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Bacteriological agents of infantile diarrhea in Imo State, Nigeria

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Bacteriological agents of infantile diarrhea in Imo State, Nigeria were studied between September 2009 and September 2010. With wide mouth specimen bottles, stool samples were collected from six hundred diarrheic infants hospitalized in different hospitals, and health centers in Imo State were analysed using standard microbiological methods. The data was collected, sorted and analyzed using Statistical Package for Social Sciences (SPSS). Results revealed that out of the 600 infants; made up of 300 males and 300 females, 450 representing 75.0% of the total number were infected by different bacterial agents, while 250 representing 25.0% were not infected. Different bacterial genera and their percentage occurrences were observed as follows; *Salmonella* sp. (13.3%), *Shigella* sp. (18.8%), *Escherichia coli* (22.2%), *Campylobacter* sp. (10.0%), *Vibrio cholera* (15.5%), *Staphylococcus* sp. (8.8%) and *Klebsiella* sp. (11.1%). Result further showed that male infants were infected more than females with percentage levels of 55.5% (P<0.05) and 44.4%, respectively. Infants between the ages of 49-60 months had the highest prevalence of 25.5% (P<0.05), while those between 0 and 12 months were least infected with percentage level of 11.1% (P<0.05). Infants from Okigwe zone were observed to be infected more with percentage level of 37.7% (P<0.05), followed by those from Orlu zone (33.3%) and finally Owerri zone (28.8%). Children whose sources of drinking water are sachet water and bottled water had the highest and least percentage levels of infection of 27.7% (P<0.05) and 8.8%, respectively. From the findings, infantile diarrhea is highly prevalent in Imo State. There is therefore urgent need for public enlightenment on the public health implications, need for proper hygiene, as well as strategies for preventing and controlling the diseases.

Key words: Infantile diarrhea, children, prevalence, Imo State.

INTRODUCTION

World Health Organization (WHO, 2013, 2000) defined diarrhea as the passage of loose or liquid stools more frequently than is normal for the individual. Torres et al. (2001) also defined diarrhea as excessive and frequent evacuation of watery feaces usually indicating gastrointestinal distress or disorder of 3-7 days duration. It can also be defined as the rapid movement of fecal matter through the intestine resulting in poor adsorption of water, nutritive element and electrolytes into the intestine tissue, which therefore leads to the production of frequent watery stools (Torres et al., 2001). Diarrhea commonly results from gastroenteritis by bacterial toxin (Willey et al., 2006). In malnourished individuals, diarrhea can lead to severe dehydration and become life threatening without treatment (Valentiner et al., 2003). Diarrhea is a leading cause of childhood morbidity and mortality in the developing world (WHO, 1997). It causes 1.5-5.1 million infant deaths per year in the whole world (Bern, 1992) and in addition contribute to malnutrition and retarded physical growth and mental development (Begin, 1993). A research conducted in Southeast Asia and Africa proved that diarrhea is responsible for 8.5 and 7.7% of all deaths, respectively (WHO, 2000).

This work is aimed at determining the Bacteriological

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Figure 1. Map of Imo state showing study areas.

agents of infantile diarrhea in Imo State Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in the three senatorial zones of Imo State namely; Owerri, Orlu and Okigwe. Imo state which is located in South Eastern Nigeria lies between latitude 5°10′ and 5°51′ North and longitude 6°35′ and 7°28′ E and comprises of 27 local government areas (Figure 1). Imo State has two climatic seasons yearly namely: the dry season which starts from November to April and the rainy season which starts from May to October. The harmattan period usually occurs from December to February. The mean annual rainfall is between 1800 and 2500 mm/year. The average relative humidity is about 74% occurring mostly during the wet season. The vegetation is tropical rainforest with rubber and palm trees as the major resources. The people of the area are predominantly of the Igbo ethnic origin. Education is a thriving industry in Imo State. The inhabitants are mostly civil servants, traders with few artisans and subsistence farmers.

The sources of drinking water in the study area include streams, ponds (mostly used in dry season), boreholes, sachet water, bottled water and pipe borne water. Chemical treatment of water available appears to be the only method of water treatment. Generally, sewage disposal in the area is very poor. In some cases, fecal materials inadvertently contaminate these sources of drinking water including those used for recreational activities (Swimming pools). While in search of drinking water for their animals, nomadic herdsmen take their cattle to streams, ponds and pools created for water gushing out of broken underground water pipes, and are contaminated through breakage by runoff waters from the environment. Pit latrines are used for defecation in the study area. Water closet systems were present sometimes in poor sanitary condition because of inadequate supply of pipe borne water.

Study population

The study was carried out from the month of September 2009 to the month of September 2010. Infants between the ages of 0 and 60 months who were hospitalized were enrolled in the study. Different hospitals and health centers were used as collection centers. Prior to the study/enrolment, clinical diagnosis of acute diarrhea was established by a pediatrician and medical personnel in hospitals where pediatricians are not available. In all, six hundred patients with acute diarrhea were sampled. Out of the six hundred patients examined, 300 (50.00%) were male while 300 (50.00%) were female.

Sampling technique

Hospitalized infants (0-60 months) with acute diarrhea and infants in motherless babies homes confirmed with symptoms of diarrhea were sampled. Before sample collection, a questionnaire was given to the parents or the person in charge of infants in motherless babies home to

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fill. The questionnaire contain such items as age, sex, local government area, is the child schooling, toilet facility, source of drinking water, parents hygiene level, etc. After the questionnaire has been duly completed, a small portion of each child's feace were obtained with plastic spoons and collected in sterile wide mouth plastic universal specimen container and were immediately transported to the laboratory in an insulated box for analysis. The samples were examined within an hour of collection.

Laboratory analysis

The samples (stool) were subjected to standard microbiological analysis followed by microscopic examination of the stool samples.

Stool microscopy

Smear of the stool were prepared on clean grease free glass slides by placing a drop of normal saline at the center of the glass slide; using applicator stick, small quantity of the stool was collected and added to the drop of normal saline on the glass slide followed by emulsification. After which the smear was carefully covered with a cover slip making sure that no air bubble was trapped. The preparation was then examined under the microscope using 10x and 40x objectives with iris diaphragm sufficiently closed to ensure good contrast.

Cultural analysis

Isolation of bacterial agents from specimen was done using cultural method as described by Chessbrough (2002), Suleman and Ibrahim (2002), Fawole and Oso (1998), Prescott et al. (2007) and Uwaezuoke (2006). The stool specimens were inoculated on sterile agar media in Petri dishes, using streaking method and then incubated at 37°C for 24 h. The incubation was done with the aid of the Petri dishes on the floor of incubator and the bottom containing the medium upper most to avoid condensation. After the incubation, colonies formed were described for morphological appearances and then colonies were picked and sub-cultured on nutrient agar in other to obtain a pure culture. Stock cultures of isolates were maintained on nutrient agar slant for further identification using various biochemical tests.

Identification of isolates

The isolates were identified using colonial and cellular characteristics, then biochemical properties. Biochemical test carried out include ureas test, citrate utilization test, oxidase test, indole test, methyl-red, Voges-Proskaurer test, coagulase test, sugar fermentation test and catalase test.

Antimicrobial susceptibility test

After isolation and identification, antimicrobial susceptibility test was carried out using the disk diffusion method as described by Cheesbrough (2000) and Prescott et al. (2007). The following antibiotics were employed for the sensitivity analysis; streptomycin, gentamycin, ciprofloxacin, tetracycline, chloramphenicol, siprosan and avicef. They were sourced from government approved pharmacies. The media (nutrient Agar) were inoculated evenly with the test organisms and control organisms. The antibiotic disc were then placed and incubated for 18-24 h at 37°C. After incubation, the diameter of zone of inhibition was measured.

Statistical analysis

The data was collected, sorted and analyzed using Statistical Package for Social Sciences (SPSS) version 20 (CDC, Atlanta, Georgia in USA). Frequency tables and charts were generated for relevant variables. Descriptive statistics (means and standard deviation) were used to summarize quantitative variables (age and parasitemia). Qualitative variables were summarized as proportions. The Chi-square test was used as appropriate to investigate associations between proportions while the Student t-test was used for continuous variables. All analysis was done at the 5% significant level.

RESULTS

Overall prevalence of infections in the study area

In this study, a total of six hundred (600) stool samples were examined. Out of the six hundred samples, 450 were infected while 250 were uninfected, giving a percentage infection to un-infection in the ratio of 75:25%. There was a significant high infection in the study area ($P<0.05$). The result is shown in Figure 2.

Overall prevalence of bacterial isolated in the study area

In the study, seven different bacterial genera were isolated, which include *Salmonella* sp., *Shigella* sp., *Escherichia* coli were isolated in one hundred (100) samples, *Campylobacter* sp. were isolated in forty five (45) samples, *Vibrio cholera* isolated in seventy (70) samples, *Staphylococcus* sp. were isolated in forty (40)

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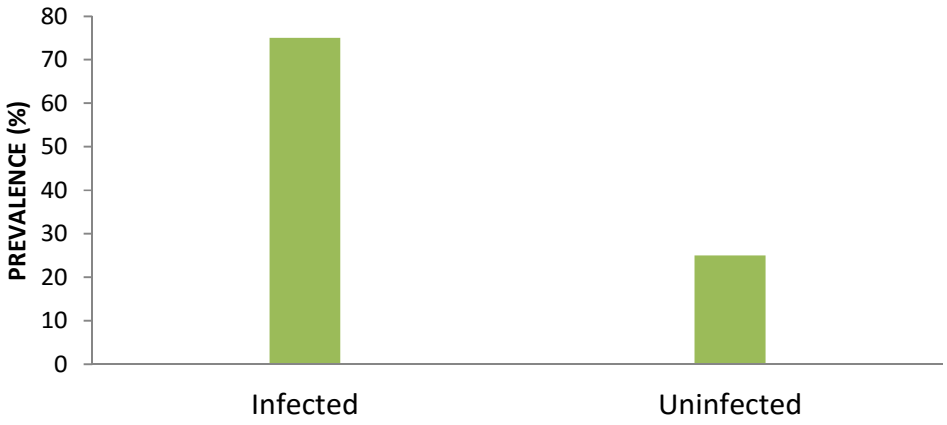

Figure 2. Overall prevalence of infection in the study area.

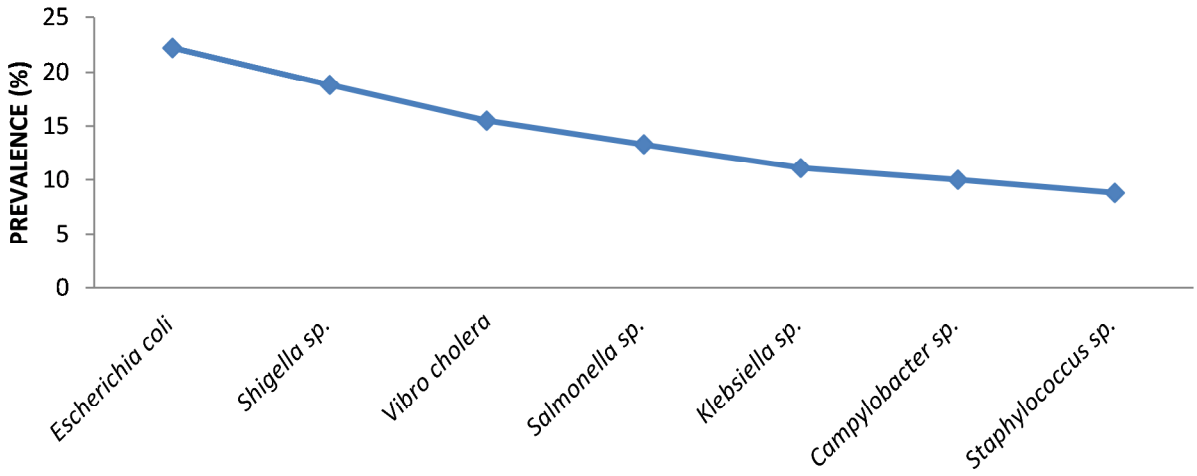

Figure 3. Overall prevalence of bacterial isolates in the study area.

samples and *Klebsiella* sp. were isolated in fifty (50) samples. The result is shown in Figure 3. Of these bacterial isolates, statistical analysis ($P<0.05$) showed that *E. coli* have the highest prevalence of 22.2%. This is followed by *Shigella* sp. with 18.8%, *V. cholera* with 15.5%, *Salmonella* sp. with 13.3%, *Klebsiella* sp. with 11.1%, *Campylobacter* sp. with 10.0% and *Staphylococcus* sp. with 8.8%. The result is shown in Figure 3.

Overall sex related prevalence of infection in the study area

Out of the six hundred children examined, three hundred were males while three hundred were females. Out of the three hundred males examined, one hundred and fifty of them were infected while out of the total of three hundred females examined; two hundred were infected. This gives a percentage prevalence of 55.5 and 44.5% of males and females, respectively. Statistical analysis ($P<0.05$) shows that there were higher infection rate in male compared to female infants. The result is shown in Figure 4.

Overall age related prevalence of infection in the study area

In this study, children between the ages of 0-60 months were examined. Out of the 70 children in 0-12 months examined, 50 of them were infected. Of the children between the ages of 13-24 months, a total of one hundred of them were examined and ninety of them were infected. Of the children between the ages of 25-36 months, 100 of them were examined and 85 of them were found infected. Of the children between the ages of

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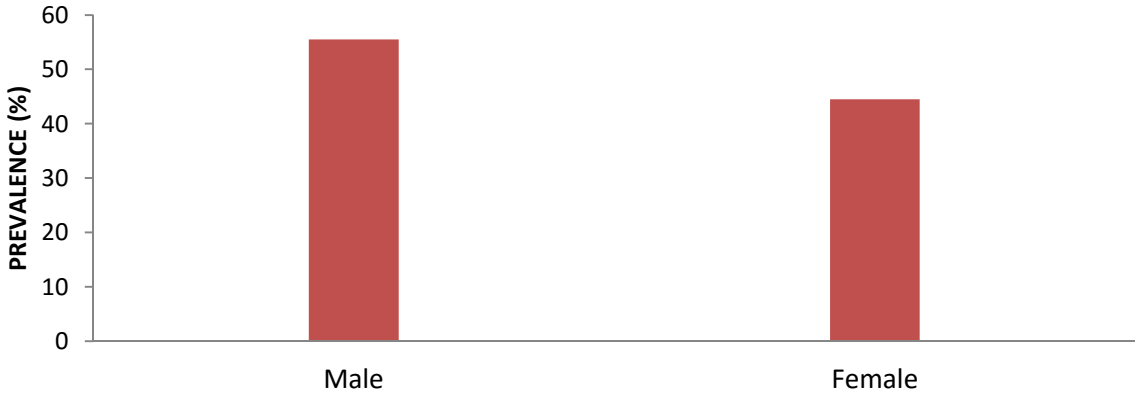

Figure 4. Overall sex related prevalence of infection in the study area.

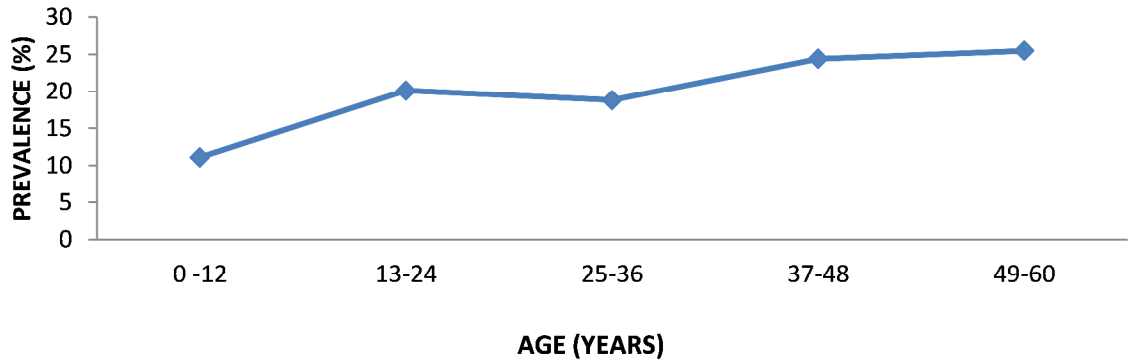

Figure 5. Overall age related prevalence of infection in the study area.

37-48 months, 150 of them were examined and 110 of them were infected, while of the children between the ages of 49-60 months, 180 of them were examined and 115 of them were infected. The children between the ages of 49-60 months had the highest percentage prevalence of 25.5% followed by children between the ages of 37-48 months with 24.4% prevalence, followed by children between the ages of 13-24 months with 20.1% prevalence, followed by children between the ages of 25-36 months with 18.8% prevalence and children between the ages of 0-12 months that has the least prevalence of 11.1%. Statistical analysis reviewed that infection is dependent ($P<0.05$) on the age group. The result is shown in Figure 5.

Age and sex related prevalence of infection in the study area

Of the children between the ages of 0-12 months, 70 were examined out of which 50 of them were infected. Out of this, 24 of them were males while 25 were females giving a prevalence of 5.3 and 5.7%, respectively. Of the children between the ages of 13-24 months, 100 of them were examined, out of which 90 were infected, comprising of 50 males and 40 females, giving a prevalence of 11.1 and 8.8%, respectively. Of the children between the ages of 25-36 months, 85 of them were infected out of 100 examined, 45 of them were male while 40 were female, giving a prevalence of 10.0 and 8.8%, respectively. For children between the ages of 37-48 months, out of the 150 examined, 110 were infected, out of this, 71 were males while 39 were females, which gives a percentage prevalence of 15.7 and 8.6%, respectively. For children between the ages of 49-60 months, 115 of them were infected, out of the 180 examined, 60 were male while 55 were female which gives percentage prevalence of 13.3 and 12.2%, respectively. The result is shown in Figure 6.

Prevalence of infection according to zones in the study area

In the study area, there were three zones, namely Owerri zone, Okigwe zone and Orlu zone. Of these zones, 200 children were examined from each zone. Out of the 200 infants examined in Owerri zone, 130 of them were

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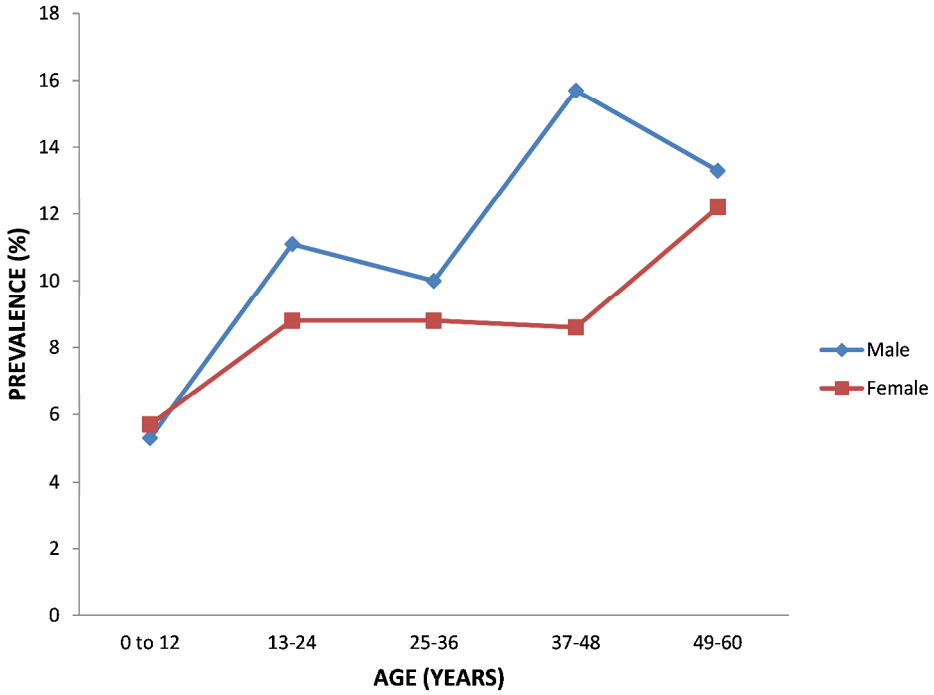

Figure 6. Age and sex related prevalence of infection in the study area.

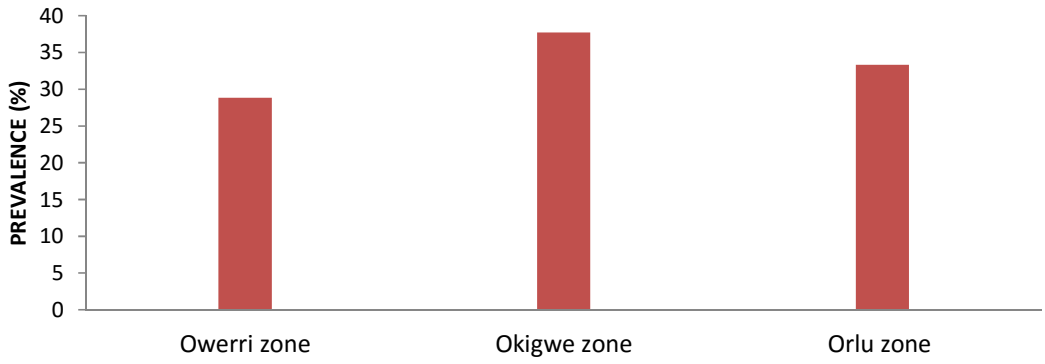

Figure 7. Prevalence of infection according to zones in the study area.

infected with a percentage prevalence of 28.8%. Of the two hundred children examined in Okigwe zone, 170 were infected while of the 200 children examined in Orlu zone; 150 of them were infected. Okigwe zone have the highest percentage prevalence of the infection with 37.7% followed by Orlu zone with 33.3% prevalence and Owerri zone with 28.8% prevalence. The result is shown in Figure 7.

Sex-related prevalence of infection according to zones in the study area

In the study area, out of the 200 children examined in Owerri zone, 17.0% of the infected children were male while 11.7% were female. In Okigwe zone, of the infected children, 20.0% of them were male, while 17.7% were female, while in Orlu zone out of the 200 children examined, 18.3% were male while 15.0% were female. The result is shown in Figure 8.

Prevalence of infection according to sources of drinking water

In the study area, five major sources of drinking water were considered which include bottled water, sachet water, borehole water, pipe borne water and streams. Of

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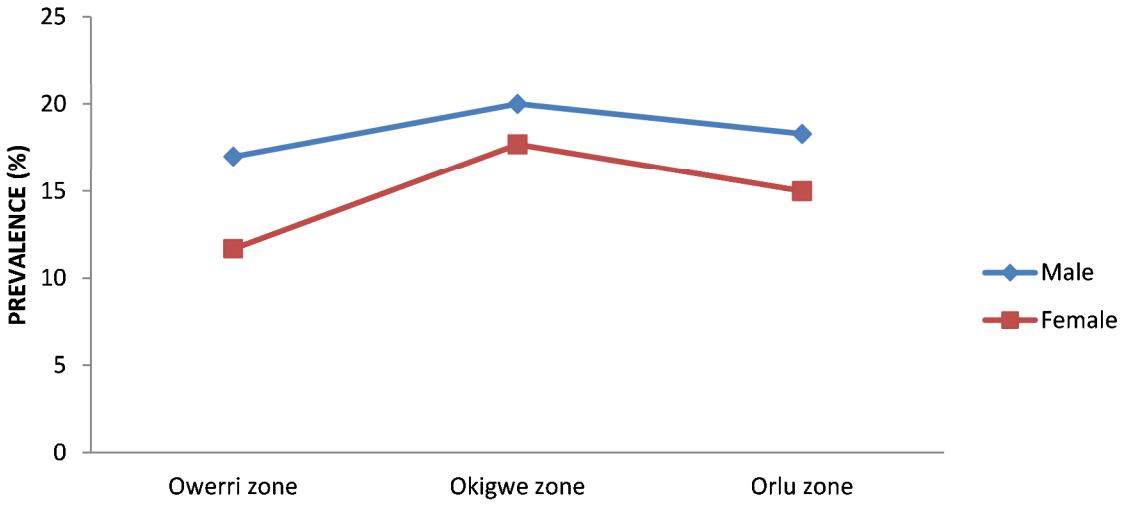

Figure 8. Sex-related prevalence of infection according to zones in the study area.

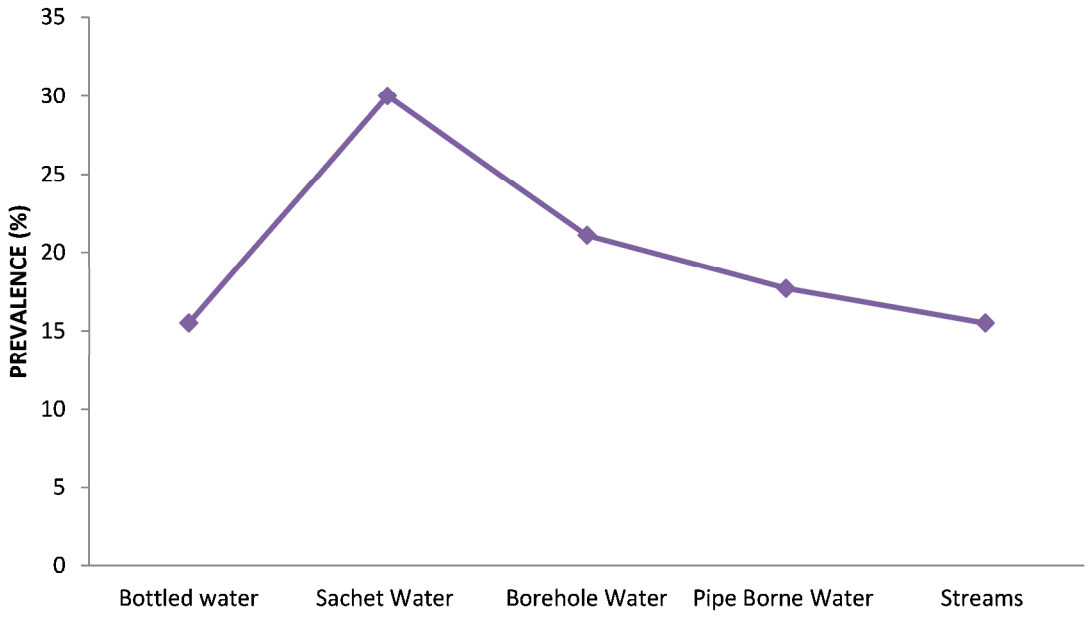

Figure 9. Prevalence of infection according to sources of drinking water.

the children whose source of drinking water is bottled water, 100 of them were examined and 40 were infected. Among those whose source of drinking water is sachet water, 130 were examined and 125 were infected. 120 children, whose source of drinking water is bore hold water, were examined and 100 of them were infected. Among children whose source of drinking water is pipe borne water, 140 of them were examined and 85 were infected, while those whose source of drinking water is streams, 110 of them were examined and 100 were infected. Statistical analysis ($P<0.05$) reviewed that there is a difference in the Infection with different source of drinking water. The prevalence of infection with respect to sources of drinking water have those with bottled water to be 15.5%, sachet water with 30%, bore hole water with 21.1%, pipe borne water with 17.7%, while those having streams as source of drinking water have 15.5%. The result is shown in Figure 9.

Antibiotic sensitivity pattern of bacterial isolates

Results presented in Table 1 indicated that among the antibiotics used which include ciprofloxacin, gentamycin,

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Table 1. Antibiotics sensitivity pattern of bacterial isolates.

	Zone of inhibition (mm)						
ISOLATES	**CPX**	**CN**	**STR**	**TET**	**CHL**	**SIP**	**AVF**
Salmonella sp.	18.5	12.0	R	10.0	15.0	R	R
Shigella sp.	16.0	13.5	R	14.0	9.5	R	R
Escherichia coli	17.0	17.5	10.5	13.0	R	R	R
Campylobacter sp.	13.5	13.0	R	11.5	R	14.0	R
Vibrio cholera	16.5	14.5	11.5	16.5	16.0	13.0	12.0
Staphylococcus sp.	R	12.0	10.5	R	10.5	11.5	11.0
Klebsiella sp.	R	R	13.5	10.5	R	9.5	12.0

R, Resistant; CPX, Cirpofloxacin; CN, Gentamycin; STR, Streptomycin; TET, Tetracycline; CHL, Chloramphenicol; SIP, Siprosan; AVF, Avicef.

streptomycin, tetracycline, chloramphenicol, siprosan and avicef, *Salmonella* sp. and *Shigella* sp. were resistant to Streptomycin, Siprosan and Avicef; however, they were sensitive to Ciprofloxacin. *E. coli* was also observed to be more sensitive to gentamycin followed by Ciprofloxacin but was resistant to Chloramphenicol; Siprosan was equally observed to be resistant to Streptomycin, Chloramphenicol and Avicef. *V. cholera* was observed to be sensitive to all the antibiotics though was observed to be more sensitive to Ciprofloxacin and Teteracycline. *Staphylococcus* sp. was resistant to Ciprofloxacin and Tetracycline. *Staphylococcus* sp. was resistant to Ciprofloxacin and Tetracycline but was more sensitive to Gentamycin. *Klebsiella* sp. was observed to be resistant to ciprofloxacin, gentamycin and Chloramphenicol; however, was more sensitive to Streptomycin.

DISCUSSION

The findings of this study showed that bacterial agents of infantile diarrhea are similar in most parts of the world, but the incidence rates varies from place to place. The overall prevalence of bacterial organisms observed in this study (75.0%) was in line with the report in Abakiliki by Ogbonnaya (2008) who reported a prevalence of 76.6%. The slight difference in the percentage may be attributed to the sample size; whereas, the present study examined 600 samples. The previous researcher examined 150 samples. This study also revealed that the following organisms; *E. coli* (22.2%), *Salmonella* sp. (13.3%), *Shigella* sp. (18.8%), *Campylobacter sp.* (10.0%), *V. cholera* (15.5%), *Staphylococcus sp.* (8.8%) and *Klebieslla sp.* (11.1%) are associated with diarrhea observed among infants in the study area. However, *E. coli* is observed to be the commonest bacterial pathogen ($P<0.05$). This was in conformity with Olowe (2003) who reported a high prevalence of *E. coli* (43%). Also, research conducted in Guinea Bissau by Valentiner et al. (2003) and Sao Paulo Brazil by Ethelberg et al. (2006) agreed with this finding. All these previous researchers also isolated other organism such as *Aermonas* sp. and *Yersina* sp. in addition to the ones isolated in this present research. The increase in the species of organisms may be due to locality, socio-economic reasons and the ecological niche in which each species finds itself at the onset of the epidemics.

Observation from the overall sex-related prevalence in Figure 4 suggests that male children have the higher occurrence ($P<0.05$) of these bacterial agents more than females ($P<0.05$). Reports from other developing regions like Enugu (Njoku-Obi and Anozie, 1984; Anozie and Antai, 1987) and Akure (Adegunloye, 2006) also indicate that males are more infected than females. Similarly, research conducted in Lagos State University Teaching Hospital, Idi-Araba, by Ogusanya (1994) agreed with this finding. Reasons for this trend of result could be probably due to the fact that males are more active and play around more; hence, more chances of being infected by the bacterial agents.

The result obtained from age related prevalence in Figure 5 suggests that children between 3-4 and 4-5 years stand a greater risk of contracting diarrhea infection with percentage prevalence of 24.4 and 25.5% ($P< 0.05$), respectively. This is in agreement with other researchers such as Anozie and Antai (1987), Adegunloye (2005) and Udo (1995). It is speculated that feeding habits might be a probable factor responsible for the high rate obtained from these age range. During this period in life, most infants move about; on their own and can pick contaminated materials. Moreover, they can suck their contaminated hands, hence more chances of contacting the aetiologic agents of diarrhea.

From the result in Figure 6, there were more infections in older male and female infants than the younger ones ($P<0.05$). Moreover, male infants were also observed to be more infected than the females. This trend of results can also be attributed to the same reasons for results in Figures 4 and 5.

The study established that there is a high prevalence of infantile diarrhea in the three zones of Imo State, namely; Owerri, Okigwe and Orlu with prevalence rates of 28.8,

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37.7 and 33.3%, respectively. The highest and higher prevalence in Okigwe and Orlu ($P<0.05$), respectively can be attributed to the more rural nature of these zones. In addition, it is possible that there may be poor sanitation in these zones and also lack of portable water supply.

Prevalence of infection based on the sources of drinking water presented in Figure 9 revealed that infants whose water intake is from bottled water, sachet water, borehole water, pipe borne water and streams have prevalence rates of 8.8, 27.7, 22.2, 18.8 and 22.2%, respectively. The highest prevalence ($P<0.05$) in infants that take sachet water indicates that such water is not well treated by the producers before being taken to the market for sale. The high prevalence among infants that take pipe borne water is indicated of improper treatment of such water.

Antibiotics susceptibility patterns of the bacterial isolates presented in Table 1 indicates that only *V. Cholera* among all the isolates showed 100% susceptibility to all the antibiotics. The inability of other tests isolates to exhibit 100% susceptibility to all the antibiotics could be attributed to uncontrollable usage of antibiotics and the common practice of self-medication leading to drug abuse. This possibly could cause the organism to develop resistance to some of the antibiotics used.

Conclusion and Recommendation

Bacteriological agents of infantile diarrhea in Imo State were studied. From the study, there is a high prevalence of the disease in the study area. Genera of bacterial associated with the disease in the study area include *Salmonella* sp., *Shigella* sp., *E. coli, Campylobacter sp., V. cholera, Staphylococcus* sp. and *Klebsiella sp.* Among all these microorganisms, *E. coli* had the highest prevalence rate. It was equally observed that male infants were more infected than the females. Infants between 4-5 years of age were infected more. Infection was observed to be highest in Okigwe zone. Infants whose source of drinking water is sachet water were observed to be infected more. Antibiotics susceptibility test revealed that some of the isolates were resistant to some of the antibiotics used. In view of the above, it is therefore recommended that: there should be good sanitation practices in the area of study; there should be adequate health education in our schools and homes; children suspected to have direct contact with animal and human feaces, dust and wastes should be bathed immediately with clean water and soap; there should be proper breast feeding of babies to boost their immune system; water must be boiled properly before usage; infants should be guided and should not be allowed to move about freely as this may expose them to sources of the infecting organisms. It is equally recommended that Nigeria Society for Microbiologists and the Nigerian Medical Association should embark on a massive public enlightenment program through the mass media on the dangers of drug abuse.

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