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Full Length Research Article

PREVALENCE OF BACTERIAL GASTROENTERITIS IN CHILDREN ATTENDING DAYCARE CENTERS WITHIN KADUNA METROPOLIS

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ABSTRACT
This research was carried out on Prevalence of bacterial gastroenteritis among children attending daycare centers within Kaduna Metropolis. Children between the age of 6-24 months were studied in Chukun Local Government, Kaduna South Local Government and Kaduna North Local Government Areas of Kaduna State, Nigeria. Two Hundred and forty (240) stool specimens were collected from eight (8) Daycare centers, where thirty samples were collected from each centre. The samples collected were cultured on Eosin Ethylene blue agar, *Salmonella Shigella* agar, MacConkey and Nutrient agar. Out of the 240 stools samples collected, *E. coli 40%, Salmonella enteric 10%, Shigella sp 6%, proteus sp 8%, Klebsiella pneumonia 6%, Entrobacter sp 5%, Erwinina sp 3%, Citrobacter sp 7%, Yersinia sp 2%, Serriatia sp 3%, Pseudomonas aeroginosa 2%, probidencia sp 1%, Morginella morganii 1%, Alcaligenes sp 1%.* The bacteria isolated were found to be sensitive to most Ciprofloxacin, Gentamycin and Ofloxacin, Pefloxacin, Chloranphenicol, tarivid, Colistin sulfate. The result obtained revealed that there could be public health breach in the maintenance and management of the Daycare centres within Kaduna Metropolis.

Keywords: Prevalence, *Gastroenteritis,* Children, Daycare Centres

INTRODUCTION
Establishment of Daycare and Orphanage centres is increasing in number since it has become a business in Nigeria and in many countries of the world today. A case of gastroenteritis leading to the death of affected children has been reported (Scallan *et al.,* 2015). The most commonly infectious bacteria causing gastroenteritis are *Clostridium difficile, Shigella* species*, E. coli*, *Salmonella* species*, Campylobacter jejuni/coli, Vibrio cholerae* among others (Heidi *et al.,* 2005). The sanitary status in many of these institutions are over stretched and compromised. Oral communications revealed that number of deaths due to infectious gastroenteritis might have occurred in these facilities (Roux *et al.,* 2016).
Investigation showed that children who are admitted into the Daycare centres; most of these children are newly introduced to artificial feeding, having been withdrawn from breastfeeding which can predispose them to the infection. Recent investigations on infections of newly admitted children showed an increase in gastroenteritis cases during the first year which may persist from the first day of contact till the time medication is administered (Hellegie *et al*., 2016).
Scallan *et al. (*2015) also reported that death due to gastroenteritis has doubled from 7,000 to 17,000 in the United States between 1999 and 2007. Children ranging from few days to six years accounted for 83% deaths. *Clostridium difficile Shigella sp, Salmonella species, Campylobacter jejuni/coli, Vibrio cholerae* were the most common bacterial causing gastroenteritis associated deaths. Globally, children within the age of day one to six years have been reported to have gastroenteritis each year. Estimated number of 1.7 billion cases of gastroenteritis each year was reported, with 124 million clinic visitations, 9 million are said to be hospitalized, and 1.34 million deaths with more than 98% in the developing countries (Fischer *et al.,* 2012).
Most of the developing countries in the world today Nigeria inclusive are plagued with severe sanitary problems that predispose children to high rates of gastroenteritis which can lead to high morbidity and mortality rates. The clinical manifestations of gastroenteritis includes complications such as diarrhea, vomiting, abdominal pain, cramping and dehydration (Huang *et al* ., 2017).
Worldwide, about 3-5 billion cases of acute gastroenteritis and nearly 2 million deaths occur each year in children within the ages of few days and six years have been reported (Koslap -Petraco, 2006). Children in the united states are said to have experienced about 1.3-2.3 cases of gastroenteritis each year and the overall cases of gastroenteritis accounts for about 1.5 million out patients hospital visitations, 220,000 been hospitalized, the cost implications was more than 2 billion dollars each year in the united states alone (Dennehy, 2005).
Cases of acute gastroenteritis in children has changed a little over the past four decades, mortality has drastically changed from 4.6 million in 1970s to 3 million in 1980. The reason for this drastic decrease in the infections rate was due to the support from the international body through the supply of oral rehydration solution (ORS) as the choice treatment for gastroenteritis with the proportion of gastroenteritis case treated with ORS rising from 15% to 40% in 1993 (Kosek *et al.,* 2003). Nigeria and other under-developed countries in the tropics, gastroenteritis is one of the principal causes of morbidity and mortality among young. Gastroenteritis mortality rate specifically in children within the ages of few days to six years of age. In Africa it has been estimated to be one hundred and six per one thousand. (Olowe *et al*., 2003).
Kaduna State is located between Latitudes 10^{o} 34' and 7^{o} 20' East of the Greenwich meridian (Aziegbe, 2006) with the total population of 100,283817 as at 2006 population census (Gambo, 2014). Also located in the northern part of Nigeria with majority of its inhabitants depending on private boreholes, well, pipe borne water; some depends on river water supply for domestic and agricultural purposes. This may have been contaminated with faecal materials directly or indirectly. The level of hygiene of the

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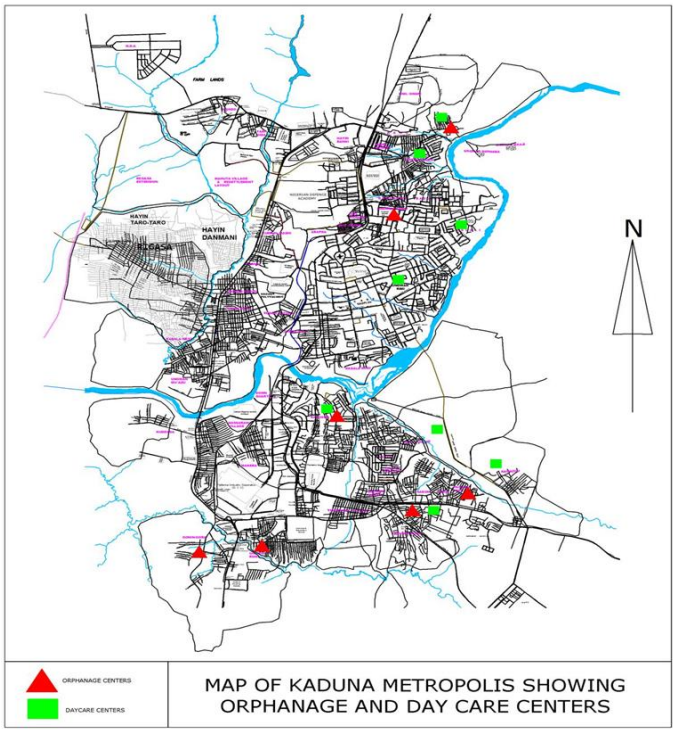
people of this area and their social economic status might likely predispose the inhabitants and most especially the infants to the risk of contracting the infection. Therefore, the aim of the study is to assess the association of pathogenic bacteria isolated from Daycares and orphanage centres within Kaduna metropolis and its public health implications.

MATERIALS AND METHOD

Study Site

The study was carried out within Kaduna metropolis which comprises of the following Local Government area (LGA): Chikun, Kaduna South and Kaduna North Local Government Areas of Kaduna State, Nigeria. The areas are located between Latitudes 10º 34' and 7º 20' East of the Greenwich meridian (Aziegbe, 2006) with the total population of 100,283817 as at 2006 population census (Gambo, 2014).

The Daycare and orphanage centres that were investigated during the study are located in Malalin Gabas, Ungwan Dosa, Ungwan Mai-Gero, Malali, and Gonin-Gora, Ungwan Sunday, Sabon Tasha, Ungwan Romi, Gonin-Gora, Barnawa and Ungwan Sarki . The ages of the children ranges between 6 to 24 months. The total number of staff from both centers is fifty (50) females and twenty (20) males; most of the staff is literate with an average number of them with formal education. Most of the Daycare buildings are single rooms, while others have three to four bedroom flats. The person in charge is called 'head of the school' he/or she make sure things are well taken care of. Staff should treat the children as their own, and if any child falls sick he or she should be taken to the nearby clinic immediately. Feeding in the Daycare centres requires that each child goes with his or her food from home. After closure, staff must sign out before leaving.



(Kadgis, 2019)

Ethnical Permit

Ethical approval for this study was obtained from the Kaduna State Ministry of Health and human services. The consent of the officials managing the affairs of the orphanage and Daycare centres were sought and their approvals obtained in writing.

Study Population

The study population included infants and young children between ages 6 and 24 months in some Daycare centres studied within Kaduna metropolis, Nigeria.

Sample size

The samples size was calculated using the standard statistical formula documented by Adamu *et al*. (2016). Prevalence rate of 9%, confidence level at 95% (standard value of 1.96) and margin of error at 5% (Standard value of 0.05).

Formula $N = \frac{Z2 \times P \times (1-P)}{D2}$

Where:

N= Number of samples
P= estimated proportion of the population= 25%
Z= Statistics for 95% confidence limit (1.96)
D= Allowed error in research

Sample Collection

The sample collection procedure of Chakraborty *et al*. (2015) was adopted. The general visual assessment of Daycare and orphanage centres was done. Each stool sample was collected in a clean sterile, wide mouthed, transparent plastic bottle and transported to Kaduna State University Microbiology Laboratory within an hour to avoid morphological changes and the death of the bacterial agents in the sample collected.

Macroscopic examination

Cheesbrough (2005) procedure of diagnosis of enteric pathogens was adopted. The stool samples were examined based on their visual consistency and appearance, indices such as forming, hard, loose, watery states, odour, bloody, colurs, mucous deposits were also observed and recorded.

Media preparation

All media preparations were done according to the manufacturer's instructions.

Laboratory Investigations

The specimens were processed according to the procedures used by Inabo *et al.* (2014) for the laboratory diagnosis of enteric pathogens.

Culture of the Stool Samples

Small Freshly collected stool sample was obtained by touching with a sterile wire loop, inoculated on already prepared Eosin Methylene blue agar and *Salmonella Shigella* agar plates simultaneously and incubated at 37°C for 24 h. After 24 h, the plates were observed for bacterial growth. Each different colony on each plate was picked using a sterile wire loop and sub-cultured on prepared MacConkey agar plates and incubated at 37°C for 24 h. After 24 h, the plates were observed for lactose fermentation and non-lactose fermentation. Each of these

different bacterial colonies was picked using a sterile wire loop and sub-cultured on a well labeled prepared nutrient agar slant in a specimen bottle and incubated at 37°C for 24 h. Thereafter, the slants were observed for growth and stored in a functioning refrigerator for further confirmatory tests that was carried out on the isolates.

Microscopy

Smear Preparation

Each slide was designated with a code for each bacterial isolate. The back of the slide site on which the colony of each bacterial isolate was smeared was demonstrated with a black coloured marker. Each air dried emulsion on the slide was fixed by passing through a bunsen burner flame three times.

Gram Staining

Inabo *et al*. (2014) Procedure for diagnosis of enteric pathogenic bacteria was adopted. Each slide with the heat fixed smear of the bacterial colony was placed on a staining rag and flooded with crystal violet and allowed to stand for one minute before rinsing with distilled water using wash bottle. This was followed by flooding each slide with Gram's iodine for another one minute before rising with distilled water using wash bottle. This was followed by adding few drops of 95% alcohol to each slide before washing each slide with distilled water immediately using wash bottle. Each slide was counter stained by flooding each slide with safranin and left to stand for 45 seconds before rising with distilled water using wash bottle. Then each slide was blotted and air dried on a stand.

Microscopy viewing

Oil immersion was applied on each bacterial stained smeared slide and viewed under the microscope using the oil immersion objective lens (×100).

Biochemical Tests

Mortality Test by Agar-stab Method

The semi-solid agar for the characterization of the bacterial isolate was prepared according to the method described by Islam (2014). The Semi-solid nutrient agar was prepared by dissolving 12 grams of Nutrient Agar powder into 1000 ml of distilled water and heated to dissolve the medium completely. Before distributing into 10ml test tubes and sterilized by autoclaving at 121^{o}C for 15minutes and allowed to gel. This was followed by inoculating each pure bacterial isolate into each test tube using a sterile straight wire to stab. This was also followed by incubating the inoculated test tubes aerobically at 37^{o}C for 48 h. Before observing for growth along the line of stab or in diffused state or diffused.

Indole Test

Islam *et al.* (2014) procedure was adopted. Each pure culture of bacterial isolate was inoculated into 5ml indole peptone water and incubated aerobically for 37^{o}C in water bath for 24 hours. This was followed by adding 5 drops of kovac's reagent into the broth culture and was shaking gently and the reaction was observed. The formations of red ring above the peptone water within 1minute indicated positive indole production, while yellow colour retention indicated negative indole production.

Citrate Utilization Test

Islam *et al.* (2014) procedure was adopted. Each of the pure culture of the bacterial isolates was inoculated on Simmon's citrate agar slants in universals bottle and incubated aerobically at 37^{o}C for 30 hours. Then the slants were observed for the development of deep blue colour which indicated positive citrates utilization test.

Urease Test

Islam *et al.* (2014) procedure was adopted. Each pure culture of the bacterial isolate was inoculated on urea agar slant in a bijou bottle and was incubated for 24 hours. Each of the incubated bijou bottle was observe for the development of red pink colour which indicated positive urease test.

Methyl Red Test

Manga *et al.* (2008) procedure was adopted. Exactly 5ml of Methyl red broth was inoculated and incubated for 55 hours at 37^{o}C. Then each of the pure culture of the bacterial isolate was introduced after 48 hours incubation. Then 1ml of the broth culture was transferred to sterile test-tube and 3 drops of the methyl red was added. The development of red colour on the addition of the methyl red indicator signifies a positive methyl red test. While yellow colour signifies negative test.

Voges-Proskauer Test

Manga *et al.* (2008) procedure was adopted. Exactly 2ml portion of each of the pure culture of bacterial broth cultures in the original tube, 5 drops of 40% potassium hydroxide (KOH) was added, followed by 15 drops of 5% naphthol in ethanol. The tubes were shaken and the cap of the tubes were loosened and placed in a sloping position. The tubes were observed for the development of red colour starting from the liquid air interface within an hour which indicated Voges-Proskauer positive, while no colour change indicated negative test.

Triple sugar

Islam *et al.* (2014) procedure was adopted. Exactly 64.42 grams was dissolved in 1000 ml of distilled water. The media was heated to dissolve completely. The homogenates were distributed into test tubes and Sterilized by maintaining at 115°Cfor 30 minutes. The medium was allowed to set in sloped form with a butt about 2.5cm long. Each pure culture bacteria isolate was introduced into the already prepared triple sugar agar by the means of stabbing using a sterile straight wire before incubating at 37^{0}C for 24 hours. The colour change of each test tube was observed for sugar fermentation, gas production, H_2S production.

Antibiotic Susceptibility Testing

Manga *et al.* (2008) procedure was adopted. Pure culture of bacterial isolates was subjected to antibacterial agents using Mueller Hinton agar plate. Each antibacterial disk was aseptically picked using a sterile forceps and placed on the center of already prepared Mueller Hinton agar plate. The plates were incubated at 37^{o}C for 24 hours. The antibiotic used are: OFX=Tarivid 10µg , S= Streptomycin 30µg, SXT= Septrin 30µg, CH= Chloranphenicol 30µg, SP= Sparfloxacin 10µg, CPX= Ciprofloxacin 10µg, Am= Amoxacillin30µg, Au= Augmentin 30µg, CN= Gentamycin 10µg, PEF= Pefloxacin30µg, AK= Amikacin 3 0µg, MEM= Meropenem 10µg, CT= Colistin sulphate 10µg.

The zone of inhibition was measured using a metre ruler across the zone created by the diffusing antibiotics across the circumference created by the disk. The average of each antibiotic disk was calculated and documented. The calculated value was compared with standard CLSI (2017).

Results

Table 1 Age distribution and bacterial isolation rates obtained from the Daycare centres

Daycare Centres	Age Ranges (Months)	No. of Samples	No. of positive Samples	Bacterial Isolation Rates (%)
1	12-24	30	02	6.6
2	12-18	30	04	13.3
3	12-21	30	21	70.0
4	12-16	30	13	43.3
5	13-24	30	10	33.3
6	12-23	30	08	26.6
7	14-22	30	02	6.6
8	12-24	30	03	10.0
Total			Mean	24.54

Table 2: Biochemical characterization of pure bacterial isolates obtained from children in Daycare centres.

Daycare Centres	positive samples	V.P	Citrate	TSI	Urease	Indole	Mortality	H_2S	Gram's Reactions	Probable Bacteria
1	1	-	+	Alk /A	-	+	+	+	-	*Salmonella sp*
2	1	-	+	Alk /A	-	+	+	-	-	*Shigella sp*
3	2	-	-	V	+	-	-	+	-	*Morganella sp*
4	2	+	+	-	-	-	+	-	-	*Cronobacter sp*
5	1	-	+	-+	d	d	d	-	-	*Citrobacter sp*
6	1	+	-	-	-	+	+	d	-	*Erwinia sp*
7	1	+	+	+	+	+	-	-	-	*Klebsiella sp*
8	1	D	d	-	+	-	+	+	-	*Proteus sp*

Key: + = positive, - = negative. Alk= Alkaline, A= Acidity, VP = Voges proskaver Citrate= Citrate utilization test TSI= Triple sugar ions

Table 3: Biochemical characterization of the pure bacterial isolates obtained from Orphanage and Daycare centres

Daycare Centres	positive samples	V.P	Citrate	TSI	Urease	Indole	Mortality	Gram's Reactions	Probable Bacteria
1	1	-	-	-	-	d	-	-	*Shigella sp*
2	3	-	-	A/A	-	+	+	-	*E.coli sp*
3	19	-	+	+	+	-	-	-	*Alcaligenes sp*
4	11	-	+	+	D	-	+	-	*Entrobacter sp*
5	9	-	+	A/A	-	-	-	-	*Pseudomonas sp*
6	8	+	-	d	-	-	+	-	*Serratia sp*
7	1	-	+	-	-	+	-	-	*Providencia sp*
8	2	-	-	-	+	-	-	-	*Yersinia sp*

Key: + = positive, - = negative, A/A=Alkaline/Alkaline VP = Voges proskaver Citrate= Citrate utilization test TSI= Triple sugar ions, d= 25-70% positive, V= strain instability

Table 4: Antibiotic susceptibility profile of pure bacterial isolates from Orphanage and Daycare centres

centre	Samples	OFX	S	SXT	CH		CPX	AM	AU	CN	PEF	AK3	MEM	CT
1	02	S	S	S	S	S	S	S	S	S	S	S	S	S
2	04	S	S	S	S	S	S	S	S	S	S	S	S	S
3	21	S	S	S	S	S	S	S	IN	S	S	S	S	S
4	13	S	S	S	S	S	S	S	S	S	S	S	IN	S
5	10	S	S	S	S	S	S	S	S	S	S	S	S	S
6	08	S	S	S	S	S	S	R	S	S	S	S	S	S
7	02	S	S	S	S	S	S	S	S	S	S	S	S	S
8	03	S	S	S	S	S	S	S	S	S	S	S	S	S

Key: S=Sensitive (20-30mm), IN= Intermediate (12-20mm) , R= Resistant(6-12mm), OFX = tarivid, S = streptomycin, SXT = septrin, CH = chloranphenicol, SP = Sparfloxacin, CPX = ciprofloxacin, AM = amoxicillin, AU = augmentin, CN = gentamycin, PEF = pefloxacin, AK3 = amikacin, MEN = meropenem, CT = colixti sulphate

DISCUSSION

The study was carried out within Kaduna metropolis which comprises the following Local Government areas (LGA) : Chikun, Kaduna South and Kaduna North Local Government Areas of Kaduna State, Nigeria. The areas are located between Latitudes 10^{o} 34' and 7^{o} 20' East of the Greenwich meridian (Aziegbe, 2006,) with the total population of 100,283817 as at 2006 population census Gambo (2014).

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The total of 240 stool samples were collected from eight (8) study areas across Chikun, Kaduna South and Kaduna North Local Government Areas of Kaduna State, however 30 stool samples were sampled from each centre. Out of two hundred and forty stool samples collected in the Daycare centres, sixty three (63) 30.9% stool samples were positive with different bacterial species namely: *Escherichia coli* 60 %, *Samonella* sp 25%, *Shigella* sp 15%. *E. coli* had the highest prevalence followed by *Salmonella* and *Shigella* sp. The high prevalence of bacterial infection is in agreement with the work of Mohammed *et al*. (2018) who reported high prevalence of Gram negative bacteria from stool samples. This finding is not in line with the work of Lydia *et al*. (2018). The high rate of the diseases may be attributed to age, warm climate and poor environmental sanitation, hygiene, low

standard of living and educational background. From the study centre, centre three had the highest infection rates with 21(70%) followed the fourth centre 13(43.3%) and then the fifth centre 10(33.3%) and the other centres have low prevalence rates with 8(26.6%), 4(13.3%), 2(6.6%) and 2(6.6%) respectively The isolates were subjected to conventional antibiotics to check the antibiogram. The zones of inhibition were measured in milliliter using meter rule. The zones were interpreted using Clinical Laboratory Standard Institute (CLSI, 2017) chart, where some isolates were susceptible, intermediates and resistant to the antimicrobial agents. Most of the bacteria isolated were found sensitive to Ciprofloxacin, Gentamycin, Ofloxacin, Pefloxacin, Chloranphenicol, Tarivid, and Colistin Sulphate. The antibacterial susceptibility profile is confirmed with CLSI (2017). The susceptibility of the isolates to the antibiotics may be as a result lack of resistant plasmid in the bacterial cell wall, because most of the bacterial strain that are multi-drugs resistant posses resistant plasmid, synthesize enzyme or have coding genes that confer resistant to the antibiotics (Piddock ,2006).

From the study, the risk factors of the infection were also assessed and the environmental factor was discovered to be the most predominant, where most of these Day care premises were substandard, this may contributed to the high prevalence of the infection. Therefore, this study provides new data on bacterial infection from the various study centres.

Conclusion

From this study, 240 stool samples were investigated and 63 stool samples were positive. High prevalence of the bacterial infection was recorded in Day care within Kaduna metropolis. The bacterial isolates isolated from the samples includes *E. coli, Salmonella and Shigella, Alcaligenes sp ,Enterobacter sp, Pseudomonas sp, Serratia sp ,Providencia sp, Yesinia sp*. Antimicrobial agents exerted zone of inhibition against some isolated bacteria while others were resistant

Recommendation

The Sanitary condition and sizes of these centres should be improved to meet the standard prescribe by the Allison and Government. The staff responsible for the children feeding should increase their personal hygiene.Management of the Daycare and Orphanage centres within Kaduna metropolis should ensure strict compliance to the rules of hygiene practices.There is need for trained management and operational officers.The need for government to have a periodic supervisory team to visit these centers to ensure compliance and standard guiding principle are maintain**.**The parent should ensure that the food prepared is hygienically prepared and package**.**

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