ANTIBIOTIC SUSCEPTIBILITY PATTERN OF SOME ENTERIC BACTERIA ISOLATED FROM DIARRHEA STOOL OF PATIENTS ATTENDING UMARU SANDA GENERAL HOSPITAL (USHGH), BIDA, NIGERIA.

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Abstract
The study was carried out to determine the antibiotic susceptibility pattern of some enteric bacteria isolated from stool of diarrhea patients attending Umaru Sanda General Hospital, Bida, Niger State from August to September 2015. A total of 50 samples were collected from diarrheal cases. The samples were analyzed using Nutrient agar, MacConkey agar, Salmonella-Shigella agar and Eosin Methylene blue agar. The isolates were Gram stained and identified using biochemical tests. Antibiotic susceptibility test was carried out on each of the isolates using disc diffusion method. E. coli 15(37.5%), Klebsiella sp 5(12%), Shigella sp 13(32%) and Salmonon sp 7(17.5%) were common enteric bacteria isolated from diarrhea stool in the study area. The disc diffusion antibiotic susceptibility test illustrated an increased resistance to Amoxicillin and Ampicillin by all strains of bacteria except Klebsiella.sp. All the tested strains of bacteria were susceptible to Ciprofloxacin and Tetracycline. The present study revealed that Ciprofloxacin, and Tetracycline can be used as drug of choice for the treatment of diarrheal due to enterobacteriacaea infection. Statistical analysis revealed that Age, Sex, Area of domicile and Occupation are all statistically significant (P<0.05). The rate of isolation of the strains of bacteria was highest among age group 1-5 years. The high prevalence rate among ages 1-5 years, could be ascribed to indiscriminate ingestion of contaminated food or water, vulnerability in poor sanitary environment and less developed state of immunity that are associated with this age groups. monitoring and regulating the distribution and use of antibiotic by government may be necessary to reduce the resistance to drugs.

Keywords: Antibiotics, Susceptible, Pattern, Bacteria, Diarrhea.

INTRODUCTION
Generally, diarrhea can be defined as a health condition which involves the passing out of unusually loose or liquid stool. In other words, it is the passing out of three or more loose watery stool and/or one bloody loosed stool for a period of 24-hour (Guarrant et al., 2001). Also, medically diarrhea is defined in terms of frequency, consistency and either weight or volume of faeces. Liquid stool with frequency of more than three times per day and weighing more than 200gram in total is referred to as diarrhea (Forbes, 2003). More so, according to Word Health Organization (1991), diarrhea is defined as decrease in the consistency of the stool from that which is normal for the patient. However, diarrhea is a symptom of underlying pathology which can impact upon quality of life and also life threatening. It is an infection responsible for considerable morbidity and mortality especially in developing country like Nigeria. World Health Organization (WHO) in 1991 reported that diarrhea disease accounts for about 1.5 million deaths among diarrhea patient in the world annually. Also, according to Ifeanyi et al. (2010), due to low socioeconomic status and poor hygienic condition of the people, intestinal parasitic and bacterial infections constitute a major cause of morbidity and mortality which leads to several epidemics each year (Guarrant et al., 2001).

Basically, diarrhea is of acute or chronic types which may be infectious or non-infectious. While, acute diarrhea is a condition which last for a period of less than four weeks, usually self-limiting and majorly caused by infections (either virus, parasite or bacterium) and can spread quickly from person-to-person, especially in day care centers. Chronic diarrhea on the other hand is a condition persisting for a period of greater than four weeks and can be caused by toxins (such as certain types of food poisoning), chronic diseases (such as cystic fibrosis) or antibiotics (such as Ampicillin) and which does not spread from person-to-person (Schiller, 2006). Diarrhea may be caused by infectious enteric pathogenic organisms, including viruses, bacteria and parasite which are transmitted from the stool of an infected individual to the mouth of another, termed fecal-oral transmission. Some are well known, others are recently discovered or emerging new agents and presumably many are yet to be identified. They differ in the routes from the stool to the mouth and in the number of organisms needed to cause infection and illness. Certain entero pathogens are adapted to infect animals and pose no threat to animals, and others are adapted to humans and do not infect animals. However, the majority, are not adapted to a specific host and can either infect humans or domestic animals, thus facilitating transmission of these organisms to humans (Mandomando et al., 2007). The most common enteric pathogens include, among others, Rotavirus, Salmonella sp, Escherichia coli, Shigella sp, Yersinia, Campylobacter jejuni, Giardia lamblia, Cryptosporidium pavum and Entamoeba histolytica (Besson et al., 2009).

Diarrhea disease is a major cause of morbidity worldwide. It is one of the leading causes of death globally. Worldwide, according to Koloff et al. (2013), one in ten infant mortality occurring in sub-Saharan African countries annually results from diarrheal disease during the first 5 years of life which results in 800,000 fatalities globally (Wanke et al., 2013b). Also, Wanke et al. (2013b) in another research reported that in developed countries, acute diarrhea results in over 200,000 hospitalization and 1.5 million patients visiting doctors. The main etiology of diarrhea in developed countries is related to a wide range of bacteria-Salmonella, Shigella sp, Campylobacter, Aeromonas sp, Escherichia coli and as well as Vibro cholerae and Yersinia sp, enteroparasites and viruses (Vargas et al., 2004). However, in developing countries, Shigella sp, Salmonella sp and Vibrios have been reported to be more predominating etiological bacterial agents of diarrhea (Kumar and Subitha, 2012). In Africa, the diarrhea-specific mortality of under-5-aged children has been...
estimated at about 106 per 1000 (Olowe et al., 2003). Diarrhea diseases are the cause of almost three million deaths annually in Nigeria mainly among children younger than five years of age. Available reports in Nigeria indicate that more than 315,000 deaths of under-5-aged children are recorded annually due to diarrhea epidemics (Ifeanyi et al., 2010).

Bacterial infections however, have posed a major public health challenge worldwide with over 200 million cases of diseases such as diarrhea resulting due to enteric organisms. Enteric bacteria causes mild to severe form of intestinal tract infections such as bacillary dysentery characterized with watery or bloody diarrhea. These organisms are transmitted through ingestion of contaminated food and water. These infections are common in developing countries where there is lack of hygienic portable water supply and proper sewage disposal system (Breener et al., 2005). Some of the bacteria pathogen of diarrhea diseases include the most commonly implicated in the endemic form of diarrhea worldwide are strains of Escherichia coli, Campylobacter sp, Yersinia sp, Shigella sp, Salmonella sp, Vibrio sp (Iruka, 2003).

**METHODOLOGY**

**Study area**

The study was conducted in Bida local government area of Niger state between the periods of Augusts to September, 2015 using diarrhea out patients attending the hospital. The study population was diarrhea patients attending the outpatient (OPD) of Umaru Sanda General Hospital, Bida Niger state, Nigeria. The study approval was obtained from the ethical committee of the hospital. A single fecal specimen was collected from each patient with diarrhea on presenting at the hospital. The relevant clinical data were recorded in a proforma. Stools of patients with the presence of blood or mucous were excluded from the study.

**Research design**

A case control study was conducted between Augusts to September, 2015. All patients who sought care at the hospital within the period were screened for diarrhea. Healthy control persons (no history of diarrhea in the previous seven days) were randomly selected from the neighborhood in which the case resided using the demographic surveillance system (DSS) within 14 days of presentation of the index case. After informing the patient’s representatives of the objectives of the study and approval of the ethical committee of the hospital, stool container and instruction was given to the patients for sample collection. Case samples were collected within 24 hours of registration of the diarrheal episode and control samples within 14 days after case enrolment. Once collected, samples were kept in cool box until processed and history of taking antibiotic in the previous four hours was recorded (Sacarlal et al., 2009).

**Sample collection**

A total of 50 fecal samples were each collected from the patients into sterile, transparent, wide mouthed containers. A total of 45 samples were collected from diarrheal cases and 5 specimens from non-diarrheal patients which served as control. The name, age and sex of the patients were properly and clearly labeled on the universal containers. The fecal specimens were processed according to the guidelines provided by Cheesbrough (2006), for the laboratory diagnosis of enteric pathogens.

**Isolation of Enteric Bacteria from Stool**

The stool samples were inoculated aerobically on sterile Nutrient agar plate, MacConkey agar, Eosin methylene blue agar plate and Salmonella-Shigella agar plates and incubated aerobically at 37°C for 24 hours. All media were prepared according to manufacturer’s specifications. After the incubation, each of the pre-enrichment broth was sub-cultured onto Nutrient agar, MacConkey agar and Salmonella-Shigella agar for 24 hours at 37°C. Pure, sub-culture isolates were subjected to series of biochemical tests that includes, Gram stain, Catalase, Citrate utilization, Indole production, Methyl red, Voges Proskauer and Sugar fermentation.

**Antibiotic Susceptibility Testing**

Nutrient agar was prepared and dispensed into sterile Petri dishes, and was allowed to solidify. The isolates which were diluted to a standard of 0.5 McFarland’s standard (approximately 1x10⁸ to 2x10⁸ colony forming units per ml) were then streaked on the surface of the solidified nutrient agar using sterile swab sticks. Commercially prepared antibiotic disks, each of which has been pre-impregnated with a standard concentration of a particular antibiotic, were then picked using sterile forceps and placed on the surface of the agar already streaked with the test isolate, and then lightly pressed with the forceps. The plates were then incubated at 37°C for 24 hours. Zones of inhibition were measured to the nearest millimeters (mm) (Cheesbrough, 2002). The percentage susceptibility was determined by dividing the number of isolates susceptible to a particular antibiotic by the total number of isolates considered and then multiply by one hundred (100).

**Statistical analysis**

Statistical analysis was carried out using Chi-square test of association between socio-demographic factors of the patients and the occurrence of enteric bacteria in the patients.

**RESULTS**

Table 1 shows some enteric bacteria isolated from diarrhea stool of Patients

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Total Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td><em>Salmonella</em> sp</td>
<td>5</td>
<td>12.0</td>
</tr>
<tr>
<td><em>Klebsiella</em> sp</td>
<td>13</td>
<td>32.0</td>
</tr>
<tr>
<td><em>Shigella</em> sp</td>
<td>7</td>
<td>17.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>40</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 1 shows some enteric bacteria isolated from diarrhea stool of patients, the total number and the corresponding percentages of the isolates. Out of the 40 isolates obtained from the 45 total diarrheal samples investigated, *Escherichia coli* ranked highest with fifteen (15) isolates (37.5%), while *Shigella* sp least occurred with seven (7) isolates (17.5%). *Klebsiella* spand *E. coli* isolates were found to occur among all age groups isolates and there were 13 *Klebsiella* sp isolates (32.5%) which was distributed among all age groups.

Table (2) shows the percentage susceptibility of the enteric bacteria to some antibiotics. Highest activity against *Shigella* sp was shown by Tetracycline, followed by Ciprofloxacin. High resistance was shown by *Shigella* sp to Ampicillin and Amoxicillin with 85.8%. Ciprofloxacin, Tetracycline and Septrin were found to be equally active against all the isolates. Almost all the isolates showed resistance to other antibiotics except *Klebsiella* sp that was susceptible to Amoxicillin and Ampicillin.
Table 2: Percentage susceptibility of the enteric bacteria (Gram negative) isolates to some antibiotics.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. coli / Shigella sp. Salmonella sp.</th>
<th>Shigella sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Total</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=15</td>
<td>n=13</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>80.0 (20.0)</td>
<td>76.0 (23.1)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>76.9 (22.1)</td>
<td>61.5 (30.4)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>26.5 (72.1)</td>
<td>36.4 (61.6)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>13.3 (18.7)</td>
<td>60.2 (30.0)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>53.3 (46.6)</td>
<td>50.0 (46.2)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>20.0 (80.0)</td>
<td>92.0 (7.7)</td>
</tr>
</tbody>
</table>

The figures in parenthesis are percentages of resistance exhibited by the bacterial isolates to the antibiotics.

Fig (1) Occurrence of Enteric Bacteria by age of Patients
Chi-square (x²) test of association to assess the association between the different variables indicated that the presence of the enteric bacteria in the diarrhea stool of the patients has an association with the ages of patients as shown by the distribution in the curve. (p<0.05) (Fig.1).

Fig (2) Occurrence of Enteric Bacteria by Gender of the Patients
Chi-square (x²) test of association to assess the association between the different variables indicated that the presence of the enteric bacteria in the diarrhea stool of the patients has no association with the sex of patients as shown by the distribution in the curve. (p>0.05) (Fig.2).

Fig (3) Occurrence of Enteric Bacteria by Area of Domicile of Patients
Chi-square (x²) test of association to assess the association between the different variables indicated that the presence of the enteric bacteria in the diarrhea stool of the patients has an association with the area of domicile patients as shown by the distribution in the curve. (p<0.05) (Fig.3).

Fig (4) Occurrence of Enteric Bacteria by Occupation of the Patients
Chi-square (x²) test of association to assess the association between the different variables considered and the occurrence of enteric bacteria in diarrhea stool indicated that there is an association as shown by the distribution in the above curve (p<0.05) (Fig.4).

DISCUSSION
Diarrhea due to enteric bacterial diseases is a major cause of morbidity and mortality especially in children in most countries in Africa including Nigeria (Adegunloye, 2005). From the total of 45 diarrhea stool samples investigated in this study, 37.5% of the E. coli isolates were obtained. This is lower than 54% prevalence from a total of 150 fecal samples investigated by (Ifenayi et al., 2010). To further support the finding in this study, Clarance et al. (2007) had 41.3% prevalence of E. coli in a research conducted among children with diarrhea, attending Madonna University Teaching Hospital, Elele, in Rivers State, South-South, Nigeria. Olanipekun (2014), documented a lower value of 26% prevalence. However, prevalence of 34% recorded by Sule et al. (2001) in Kaduna, also a Northern State in Nigeria is consistent with the prevalence of 37.5% recorded in this work. The variation between the Nigerian cities might be attributed to differences in climatic weather conditions,
infrastructure and socioeconomic indices. *Escherichia coli* had the highest prevalence among the four enteric bacterial species identified in this research work followed by *Klebsiella* sp. This agrees with the reports of Clarke (2001). Also, the report of this study agrees with the statement of Podewils et al. (2004). The prevalence rate of *Klebsiella* species related to their age and sex was recorded in which 13 (32.5%) *Klebsiella* sp isolates were found across the various age groups, but were more prevalent between the ages 1-30 years. The 32.5% prevalence *Klebsiella* sp recorded in this work is higher than the 24(8.7%) prevalence obtained from a study conducted by (Ifeanyi et al., 2010). The prevalence of *Shigella* sp was recorded to be 7(17.5%) of the 40 total isolate obtained. In comparison, the result obtain in this work is higher than the 29(4.2%) prevalence values obtained by Mamatha et al. (2014). In the case of *Salmonella* sp, 5(12.5%) prevalence was obtained from the total of 40 isolates recorded. This was less than the rate obtained by Podewils et al. (2004). The reason for the high prevalence rate of the enteric bacterial pathogen among the ages 1-10 years, was as a result of indiscriminate ingestion of contaminated food items or water, vulnerability in poor sanitary environment, immature state of acquired immunity that are associated these age groups.

Although, there are geographical variations in the spectrum of enteric bacteria associated with diarrhea, *E. coli* and *Klebsiella* sp were isolated in high prevalence rate, *Shigella* and *Salmonella* were moderately prevalent. There are conflicting reports about *Salmonella enterica* bacteria associated with diarrhea (Seung-Hak et al., 2006). Notwithstanding, the prevalence of *Salmonella* species in this work agrees with the report by Ogwu et al. (2008). The (12.5%), *Salmonella* prevalence in this work is higher than the report value by Ifeanyi et al. (2010). Bacterial isolation age-wise diminished between the ages of 21-40 years and is in agreement with the report by Ifeanyi et al. (2010). Also, the seasonal variation in the incidence of the enteric bacteria in this work is in agreement with the report of Al-gallas et al. (2007).

The evaluation of the antibiotic susceptibility of the isolates (*E. coli, Klebsiella* sp, *Shigella dysenteriae* and *Salmonella* sp.), revealed that all the strains of enteric bacterial isolates were highly susceptible to Ciprofloxacin and Tetracycline, but show high resistance to Amoxicillin and Ampicillin, except for *Klebsiella* sp. This is in agreement with the findings of Akingbade et al. (2014), as well as (Livermore et al., 2005; Yang et al., 2009), and is in contrary to the report by (Usien et al., 2009). This means that these antibiotics are effective in the treatment of diarrhea due to enteric bacteria.

**Reference**


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