FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 6 No. 2, April, 2022, pp 169 - 174
DOI: https://doi.org/10.33003/fjs-2022-0602-936

ISOLATION, CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF DIARRHEAGENIC *ESCHERICHIA COLI* (DEC) AMONG CHILDREN ATTENDING SOME SELECTED HOSPITALS WITHIN KADUNA METROPOLIS

***[1]Alhaji, A. I. [1]Mulade Gospel Lelegha, [1]Ajibade, G. A., [2]Benjamin Okoro Innocent**

[1]Department of Biological Sciences, Faculty of Science, Nigerian Defence Academy Kaduna, P.M.B. 2109, Kaduna, Kaduna State, Nigeria.
[2]Nigerian Defence Academy Hospital, Kaduna

*Corresponding authors' email: aialhaji@gmail.com Phone: +2348034095282

ABSTRACT

Diarrhoea has been associated with significant morbidity and mortality, ranked the second cause of death in children aged 0 to 5 years. Bacteria, viruses, protozoa, and helminths have all been implicated in diarrhoea, however rotavirus and diarrhoeagenic *E. coli* (DEC) are the most common cause. This study aimed to determine the antibiotic susceptibility pattern of diarrheagenic *Escherichia coli* isolated from children 0-5 years attending selected hospitals in Kaduna metropolis. A total of 264 stool samples were collected from children attending four selected hospitals in Kaduna metropolis. Standard culture procedures and molecular techniques such as PCR and 16s rRNA were employed in isolation and characterization of diarrhoeagenic *E. coli* from the stool samples. The study established a prevalence of 24.2% for diarrheagenic *Escherichia coli*, and all the isolates demonstrated multiple antimicrobial resistance index (MARI) of 0.5 and above, and showed significant resistance against augmentin (100%), amoxacillin (100%), ampicillin-cloxacillin (ampiclox) (100%), erythromycin (100%), gentamycin (100%), cefoxitin (100%), cefuroxime (95%), ceftriazone (95%) and ceftaxidime (85%). The least (60%) resistance was observed against imipenem. The study concluded that antibiotics have not been very effective in the treatment of *E. coli*-related diarrhoea. The study, therefore, recommends the implementation of programmes geared towards good hygiene, good nutrition and good health.

Keywords: Diarrhoea, Diarrhoeagenic *E.coli* (DEC), Kaduna, Multidrug resistance (MDR)

INTRODUCTION

Diarrhoeal diseases are still one of the leading causes of morbidity and mortality among children under five years in developing countries (WHO, 2017). Diarrhoea is the second leading cause of death in children aged 0 to 5, killing nearly 525,000 children each year (WHO, 2017). Nigeria is one of the five countries with a high childhood diarrhoea rate, with an estimated 150,000 deaths each year (Kehinde & Umar, 2018). The condition is common in developing countries, especially in areas with poor sanitary standard such as open defecation that results in the contamination of water sources. In developing countries, other factors such as malnutrition can increase the risk of diarrhoea. These factors may result in a serious disease problem and negative economic consequences such as higher medical costs, lower quality of life, and a high mortality rate (Zhang et al., 2018).

Diarrhoea is caused by infectious organisms such as bacteria, viruses, protozoa, and helminths. These infectious agents are transmissible from person to person through faecal-oral route. However, the severity of disease depends on the portal of entry and size of inoculums necessary to induce illness (Zhang et al., 2018). The most common causes of diarrhoea are enteric infections; rotavirus and Diarrhoeagenic *E. coli* (DEC), with DEC, cited as the most important cause in developing countries (Agbla et al., 2018).

The emergence of multidrug-resistant *E. coli*, has been observed in various countries over the past decades. With the increase in cephalosporins resistance, especially the parallel increasing frequency of multidrug-resistant *E. coli*, the treatment of *E. coli*-associated disease is becoming worrisome to expert. The predominant mechanism of resistance to β-lactam antibiotics in *E. coli* is the production of plasmid-borne extended-spectrum β-lactamases (ESBLs). Since the first report at the beginning of the 1980s, ESBL-producing organisms have become widespread throughout the world (Rawat & Nair, 2010). The ESBL genes are frequently encoded on transferable plasmids that encode resistance genes. Acquisition of such resistant genes by commensal or faecal isolates leads to multidrug resistant (MDR) pathogens. Multidrug-resistant (MDR) strains and strains that produce extended-spectrum-lactamases (ESBL) are also becoming more common in humans and animals (Zeighami et al., 2015). Due to the high prevalence of multidrug resistance, there is an urgent need for broad-based, local antimicrobial resistance surveillance and the development of successful approaches to reduce multidrug resistance in these pathogens (Olayinka et al., 2004). The presence of plasmids containing one or more resistance genes, each encoding a single antibiotic resistance phenotype, is most commonly associated with multiple antibiotic resistance (MARI) in bacteria (Nikaido, 2009).

Multiple antibiotic resistance (MAR) indexing has proven to be a reliable and cost-effective method of tracing bacteria sources. The ratio of the number of resistant antibiotics to which an organism is resistant to the total number of antibiotics to which the organism is exposed is calculated as the multiple antibiotic resistance index (Afunwa et. al., 2020). MAR index values greater than 0.2 indicate the high-risk source of contamination where antibiotics are frequently used.

This study was carried out to investigate the antibiotic susceptibility pattern, of Diarrhoeagenic *Escherichia coli* (DEC) isolated from children 0-5 years attending selected hospitals in Kaduna metropolis, in order to provide a proper basis for clinical treatment of DEC-based diarrhoea infections.

MATERIALS AND METHODS

Study area and Study Population

The study was conducted in Kaduna metropolis. Kaduna metropolis is the capital of Kaduna state. It comprises of two local government areas: Kaduna South and Kaduna North and also extends to Chikun and Igabi Local Government areas. A cross-sectional study was conducted among children between the ages of 0-5 years, presenting with diarrhoea in selected hospitals in Kaduna metropolis. Ethical approval for the study was obtained from Kaduna State Ministry of Health, Kaduna (MOH/ADM/744/VOL.1/939).

Sample Size

The sample size was determined using the formula of (Chioma et al., 2019), which is as follows: $N = Z^2Pq/L^2$ Where n is sample size, Z is the standard normal distribution at 95% confidence interval = 1.96, P is the prevalence rate, which is taken as 21.1% (Chioma et al., 2019), q is 1 –P, L is the allowable error, which is taken as 5% = 0.05 Therefore n = (1.96)2 x 0.217 x (1-0.217)/ (0.05)2n = 3.8416 x 0.217 x 0.783 / 0.0025n = 261. The sample size calculated is 261 samples. A total of two hundred and sixty-four samples were collected from the diarrhoeic children for this study.

Sample Collection and Processing

Stool samples were collected from children aged 5 and below visiting four selected hospital presenting with diarrhoea. Stool samples were collected using sterile stool containers and transferred to the microbiology laboratory immediately for laboratory analysis.

Preparation of Media

Media used in this study included, MacConkey Agar, Nutrient Agar and Mueller-Hinton Agar were prepared according to the manufacturer's instructions.

Inoculation of Faecal Sample into Culture Media

Approximately 10 µl volumes of a micropipette homogenate of faecal samples were inoculated directly onto MacConkey Agar and Sorbitol MacConkey agar (Chioma et al., 2019). The plates were incubated at 37°C, for 18 - 24 hrs. Lactose fermentation (pinkish colonies) on MacConkey Agar is presumptive for *E. coli.*

Characterization of Diarrheagenic *E. coli*

Bacterial isolates suspected to be *E.coli* were identified according to the standard microbiological procedures as described (Gillespie & Hawkey, 2006), which includes: microscopy (e.g. gram stain), culture techniques and biochemical characterization (such as Indole, methyl red, voges-proskauer, citrate, catalase, coagulase, motility, sugar fermentation tests (glucose, lactose), mannitol salt agar Hydrogen sulphide production (H_2S), urease reaction and blood agar).

Antibiotic Susceptibility Testing

Antimicrobial resistance patterns of Diarrheagenic *E. coli* isolates were determined by the standard disc diffusion method of Kirby–Bauer as described by the (Clinical and Laboratory Standards Institute, 2012). The bacteria isolates were screened for resistance against 10 antibiotics belonging to different families of antimicrobials (Mast Diagnostics, United Kingdom).These included penicillins [ampicillin (10 µg), amoxacillin (25 µg) and augmentin 30µg]; cephalosporins [ceftriazone (30 µg), ceftazidime (30 µg), cefoxitin (30 µg) and cefuroxime (30 µg)]; carbapenems; [imipenem (10 µg)]; aminoglycosides [gentamicin (10 µg)]; macrolides [erythromycin (20µl)]. Pure isolates previously grown on sterile nutrient agar were inoculated on sterile physiological-buffered saline (PBS) solution (0.85% NaCl) to make up a bacterial suspension with a density equivalent to 0.5 McFarland standards. Sterile cotton swab-stick (Copan, Italy) was stroke into the suspension and spread uniformly onto the entire surface of the Mueller Hinton agar plates. Relevant antibiotic discs were placed on the surface of the inoculated plates using a disc dispenser (Mast Diagnostics, UK) and were incubated at 37°C for 18–24 hours.

After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimetre (mm) using a ruler on the underside of the plate. The interpretation of the measurement as sensitive, intermediate and resistant was made according to (Clinical Laboratory Standards Institute manual, 2012).

Multiple Antimicrobial Resistance Index (MARI)

The isolates which displayed resistance to three or more than three classes of antibiotics were designated as multi-drug resistant (MDR) bacteria. The Multiple Antibiotic Resistance (MAR) Index was calculated by using the formula as described by Afunwa et al. (2020) which is expressed as: MAR index = a/b, where "a" represents the number of antibiotics to which an individual isolate is resistant to and "b" is the sum of to which individual isolate was tested.

Molecular analysis

Pure colonies of bacterial isolates were placed into appropriately labelled Eppendorf tubes for DNA extraction. A commercially available kits (Accu prep Genomic DNA extraction kit) from Bioneer was used to extract DNA of the bacterial isolates according to the manufacturer's instructions. The sequences GGACTACAGGGTATCTAAT (16s Primer Forward) and AGAGTTTGATCCTGG (16s Primer reverse) were used to amplify the DNA of diarrhoeagenic *E. coli* (DEC) by PCR. The PCR amplicons were sequenced using a DNA sequence machine (ABI 3100). All the sequences were matched against nucleotide sequences present in GenBank using the BLAST of the NCBI program to identify the organism based on the most similar 16S rRNA gene.

Statistical Analysis

Results and data obtained from the study were entered into Microsoft Excel and analysed using statistical package for social sciences (SPSS) version 23. Chi-square analysis was used to determine association between the observed and expected frequencies and infection at 95% confidence interval and at 0.05 significant levels.

RESULTS

A total of 264 samples from four different hospitals were collected for the study. Eighty eight 88(33.3%) from hospital A, 60(22.7%) from hospital B, 35(13.3%) from hospital C and 81(30.7%) from hospital D. Sixty four (64) out of the 264 samples were positive for *E. coli* culture yielding a prevalence of 24.2%. The study established a significant difference in the distribution of *E. coli* in relation to hospitals. Hospital B, had the highest percentage prevalence of 26.7%, while Hospital D had a prevalence of 25.9%. The prevalence of *E. coli* infection on patients in Hospital A was 22.7%, and Hospital C (20%) **(**Table 1).

Table 2 show the antibiotic susceptibility pattern of *E. coli* isolated from diarrhoeic stool samples of children less than five years in Kaduna state. Isolates were most resistant

(100%) to Cefoxitin, Augmentin, Amoxacillin, Ampicillin-Cloxacillin (Ampiclox), Erythromycin and Gentamycin while the lowest rate of resistance was observed against Imipenem. Diarrheagenic *E. coli* was defined as multidrug resistant isolate when it was found non-susceptible to at least one agent in three or more different classes of antimicrobial agents.

The multiple antimicrobial resistance indices of the isolates were found to be above the acceptable 0.2 threshold value. When the MAR indices of the isolates were calculated, all isolates (100%) were resistant to at least 5 of the 10 antibiotics screened having a MAR index of at least 0.5 (**Table 3**). *E.coli* presented hight multidrug resistance, showing a MAR index of 1.00.

Table 1. Distribution of *Escherichia coli* isolates to in relation to hospital.

Name of Hospital	Samples Collected	Culture Positive	Prevalence (%)	X2	p-value
Hospital A	88	20	22.7	192.0	0.000*
Hospital B	60	16	26.7		
Hospital C	35	7	20		
Hospital D	81	21	25.9		
Total	264	64	24.2		

X2 = chi-square, p-value<0.05, (*) = statistically significant

Table 2: Susceptibility patterns of *E. coli* isolates to different antimicrobials

Antibiotic	Concentration (µg)	Sensitivity patterns			
		NT	NS	NI	NR
Ceftriazone	30	64	3(5%)	0(0%)	61(95%)
Ceftazidime	30	64	0(0%)	10(15%)	54(85%)
Cefoxitin	30	64	0(0%)	0(0%)	64(100%)
Cefuroxime	30	64	0(0%)	3(5%)	61(95%)
Augmentin	30	64	0(0%)	0(0%)	64(100%)
Amoxacillin	25	64	0(%)	0(0%)	64(100%)
Ampicillin-Cloxacillin	10	64	0(%)	0(0%)	64(100%)
Imipenem	10	64	6(10%)	19(30%)	38(60%)
Erythromycin	20	64	0(0%)	0(0%)	64(100%)
Gentamycin	10	64	0(0%)	0(0%)	64(100%)

NT= Number tested, NR= Number resistant, NI= Number intermediate, NS=Number susceptible

Table 3: Showing Multiple Antibiotic Resistance (MAR) Index of Diarrhoeagenic *E. coli*

MARI Value	Percentage of Isolates (%)
0.1	0(0)
0.2	0(0)
0.3	0(0)
0.4	0(0)
0.5	0(0)
0.6	3(5%)
0.7	0(0)
0.8	22(35%)
0.9	29(45%)
1.0	10(15%)

Molecular Characterization of Diarrheagenic *E. coli*

Based on 16S rRNA gene sequencing, bacterial species were taxonomically confirmed as Diarrheagenic *E. coli* as shown in Plate 1.

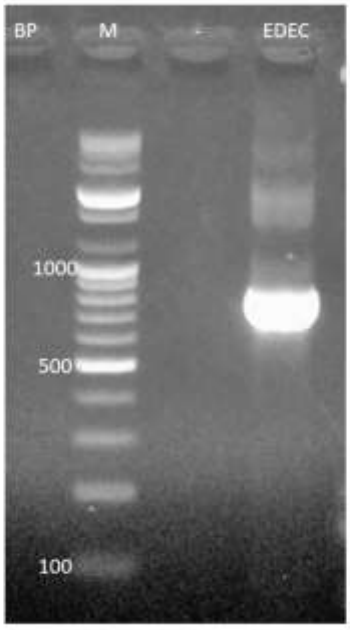

Plate 1: Agarose gel electrophoresis after PCR amplification of 16S rRNA gene from diarrhoeic stool isolates. (M) Molecular Marker of 100bp; (EDEC) Diarrhoeagenic *E. coli* (-ve) Negative control with no visible band.

DISCUSSION

A total of 264 diarrhoeal children aged 0-5 years participated in the study. Out of this number, 64 yielded cultures characterized as diarrheagenic *E. coli* giving a prevalence of 24.2 this corroborate with the findings of other studies (Chioma et al., 2019; Franzolin, 2015 and Mandomando et al., 2009) which demonstrated a prevalence of 21.7%, 25.2% and 22.6% respectively. David et al. (2019) reported that 20% of diarrhoeagenic *E. coli* isolated from children with diarrhea in Ebonyi state, Nigeria. Iseghohi et al. (2021) in a study carried out in Minna reported a prevalence of 37.7% of Diarrheagenic *E. coli* (DEC) obtained from stool samples evaluated. On the other hand, significant higher prevalence of 40% (Spano et al., 2017), 60% (Eseigbe et al. 2013) and 62.8% (Ifeanyi et al. 2010) were also reported in previous studies. David et al. (2020), reported the isolation of 54 out of 80 faecal samples of children, under five years, with 67.5% prevalence in a tertiary health centre in south-east Nigeria. Saka et al. (2019) also reported a 73.7% identification of *E. coli* from children with diarrhoea in Kano.

In the present study, Diarrheagenic *E. coli* demonstrated a much higher resistance rate against augmentin, amoxacillin and ampicillin-cloxacillin (ampiclox) (100%). This study indicated that E. coli isolates presented high resistance rate to ampicillin. This finding is in agreement with reports from Bangladesh, Thailand, Iran and Kenya (Loha et al. 2021; Wilunda and Panza, 2009; Alizade, 2018 and Sang et al., 2011). A study by Adesoji et al. (2020) also reported a 100% resistance to ampicillin and high resistance of 80% to amoxacillin, in Katsina, North-west Nigeria. This substantiates the fact that; these penicillins used in treating a broader range of bacterial infections are the most administered antibiotic in children, thus suggesting an elevated use of this drug, and consequently resulting in high resistance rates and a severe threat to public health (Fair and Tor, 2014). The level of resistance observed in this study for ceftriazone, ceftazidime, cefoxitin and cefuroxime corroborates with results from several studies by other authors who demonstrated high rates of resistance of *E. coli* against these third generation cephalosporins. Ugwu et al. (2017) reported 100% resistance to cefuroxime; 91% for ceftazidime and ceftriazone, while, Zhou et al. (2018) reported 59% and 35.2% for cefuroxime and ceftazidime, respectively. One explanation for this could be the widespread use of these antibiotics in the treatment of diseases associated with Gram- negative bacteria, especially in children under two years of age with acute infectious diarrhea (Bruzzese et al. 2018; Sulaiman et al. 2020). Castro et al. (2019), in a study reported that the *E. coli* isolates were more sensitive to cefuroxime, ceftriaxone and this was in contrast to the level of sensitivity observed in this study. These antibiotics tested are widely used to treat diarrhoea because of their ready availability (Fair and Tor, 2014). The isolates were, however, least resistant to imipenem (60%), indicating that imipenem may still be the most effective antimicrobial against the isolates. Similar findings by Mahmoud et al. (2020) reported a 33% resistance of *Escherichia coli* isolates to imipenem, with the detection of several carbapenemases resistant genes. However, Beshiru et al. (2022) reported a much higher sensitivity (98%) for imipenem against DEC isolated from ready to eat foods sold in Yenagoa, Nigeria. Another report, Adesoji et al. (2020) reported that the *E. coli* isolates were more sensitive to ceftriaxone than imipenem, and this disagrees with the level of sensitivity of imipenem observed in this study. The low sensitivity recorded (10%) in this study, corroborates with several research globally, indicating the steady rise in carbapenem resistant Enterobacteriaceae (CRE), which could be as a result of increased use of last resort/reserved antibiotics such as Carbapenems.

Multiple antibiotic resistance index helps analyse health risks, as well as to check the extent of antibiotic resistance (Joseph et al., 2017). MAR index analysis has been used to differentiate isolates from different sources using antibiotics that are commonly used in the treatment of infectious cases. According to Thenmozhi et al. (2014), MARI values higher than 0.2 indicates existence from high risk contaminated sources with frequent use of antibiotics. In this study, all the isolate of Diarrheagenic *E. coli* demonstrated a MAR index of 0.6 and above which should be a great cause of concern to

health providers. In principle, these findings reveal inappropriate use of antimicrobials in the region which poses a significant therapeutic setback and consequently, public health burden. Similar results were obtained in a study conducted in Oyo, southwest, Nigeria (Ayandele et al., 2019). The high prevalence of multiple antibiotic resistance obtained in this study may be because *E. coli* acts as a reservoir for resistance available to enteric pathogens (Akingbade et al., 2013) or may be due to the fact that antimicrobial resistance in *E. coli* has increased worldwide and its susceptibility patterns show substantial geographic differences and variations (Rodrigo, 2020).

CONCLUSION

The dangers of diarrhoeal diseases in children under five years of age have long been established, to this end, the following conclusion has been drawn from this study. High levels of multidrug resistant Diarrheagenic *E. coli* were isolated from the study population further confirming *E. coli* as an active causative agent of diarrhoeal diseases in Kaduna state, Nigeria. This study concluded that the diarrhoeic isolates presented multidrug resistance as many commonly prescribed antibiotics were no longer effective against it. This indicates the often unnecessary and uninformed use of these drugs in the treatment of most infantile diarrhoea cases. A significant number of the diarrhoeic isolates had a MAR index > 0.2 indicating previous exposure to antibiotics.

REFERENCES

Adesoji, A. T., & Liadi, A. M. (2020). Antibiogram studies of *Escherichia coli* and *Salmonella* species isolated from diarrheal patients attending Malam Mande General Hospital dutsin-ma, Katsina state, Nigeria. *Pan African Medical Journal*, *37*.

Afunwa, R.A., Ezeanyinka, J., Afunwa, E.C., Udeh, A.S., Oli, A.N. and Unachukwu, M. (2020). Multiple Antibiotic Resistant Index of Gram-Negative Bacteria from Bird Droppings in Two Commercial Poultries in Enugu, Nigeria. *Open Journal of Medical Microbiology, 10*(4).

Agbla, J. M. M., Capo-Chichi, A., Agbankpé, A. J., Dougnon, T. V., Yadouleton, A. W. M., Houngbégnon, O., Glele-Kakai, C., Armah, G. E., and Bankolé, H. (2018). Epidemiological Survey of Rotaviruses Responsible for Infantile Diarrhea by the Immunomolecular Technique in Cotonou (Benin, West Africa). *International Journal of Microbiology*.

Akingbade, O.A., Akinjinmi, A.A., Olasunkanmi, O.I., Okerentugba, P.O., Onajobi, B.I. and Okonko, I.O. (2013). Bacterial organisms isolated from children with diarrhoea in Abeokuta, Nigeria. *Stem Cell Journal, 4*(4), 5- 9.
Alizade, H. (2018). *Escherichia coli* in Iran: An Overview of Antibiotic Resistance: A Review Article. *Iranian Journal of Public Health, 47*(1), 1–12.

Ayandele, A. A., Ajala, O. O., Oyekemi, S. A., and Awotunde, M. O. (2019). Microbiological evaluation and antimicrobial resistant pattern of bacteria isolated from surface drinking water sources in Ogbomoso, Oyo State, Nigeria. *Nigerian Journal of Biotechnology*, *36*(1), 17.

Beshiru A, Okoh AI, Igbinosa, E.O. (2022) Processed ready-to-eat (RTE) foods sold in Yenagoa Nigeria were colonized by diarrheagenic *Escherichia coli* which constitute a probable hazard to human health. PLoS ONE 17(4): e0266059. https://doi.org/10.1371/journal.pone.0266059

Bruzzese, E., Giannattasio, A., & Guarino, A. (2018). Antibiotic treatment of acute gastroenteritis in children. *F1000Research*, *7:* 193. https://doi.org/10.12688/f1000research.12328.1

Castro, A. M., Santos-Balbuena, H. F., García-García, A. E., & Arzate-Barbosa, P. (2019). Association of diarrheagenic *Escherichia* coli with virotypes and sensitivity to antimicrobials in children of the Mexico City. *Revista Medica del Hospital General de Mexico*, *82*(2).

Cheesbrough, M. (2009). *District laboratory practice in tropical countries, Part 2*. Cambridge University Press.

Chioma, R. O., Mzungu, I., and Orpin, J. B. (2019). Antibiogram of Diarrhoeagenic *Escherichia* coli Patients under Five Years Attending Selected Hospitals in Kaduna State, Nigeria. *Journal of Advances in Microbiology*, *14*(4), 1–11.

Clinical and Laboratory Standards Institute. (2012). *Performance standards for antimicrobial disk susceptibility tests: Approved standard - Eleventh edition*, 32 (1).

David, E. E., Yameen, M. A., Igwenyi, I. O., Okafor, A. C., Obeten, U. N., Obasi, D. O., … David, C. N. (2020). The frequency of virulent genes and antimicrobial resistance patterns of Diarrhoeagenic *Escherichia* coli isolated from stools of children presenting with diarrhoea in a tertiary hospital in Abakaliki, Nigeria. 6(2), 147–152. https://doi.org/10.14202/IJOH.2020.147-152

David, E., Yameen, M. A., Igwenyi, I. O., Iroha, I. R., Nzelibe, H. C., Shuaibu, M. N., ... and Onwe, O. S. (2019). The frequency of fluoroquinolone resistant enterotoxigenic *Escherichia* coli from stools of diarrheic children in Ebonyi State, Nigeria.

Eseigbe, E.E., Iriah, S., Ayuba, G.I., Ibok, S., Adama, S.J. and Eseigbe, P. (2013). Bacterial isolates from the stools of children aged less than 5 years with acute diarrhoea in Kaduna, Northwestern Nigeria. *Annals of Tropical Medicine and Public Health, 6*(4), 452-455.

Fair, R. J., and Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in Medicinal Chemistry*, *6*, 25–64.

Franzolin, Marcia Regina et al. (2005). Prevalence of Diarrhoeagenic *Escherichia coli* in children with diarrhea in Salvador, Bahia, and Brazil.Memórias *do Instituto Oswaldo Cruz [online]*. 2005, v. 100, n. 4 [Accessed 3 November 2021] 359-363.

Gillespie, S. H., and Hawkey, P. M. (2006). Principles and Practice of Clinical Bacteriology: Second Edition. *Principles and Practice of Clinical Bacteriology*

Ifeanyi, C. C., Isu, N. I. R., Akpa, A. C., and Ikeneche, N. (2010). Enteric Bacteria Pathogens Associated With Diarrhoea of Children in the Federal Capital Territory Abuja , Nigeria. *New York Science Journal*, *3*(1), 62–69.

Iseghohi, F., Galadima, M. and Kuta, F.A. (2021). *Escherichia coli* Encoding Extended-Spectrum Beta-Lactamases Isolated from Diarrheic Patients in Minna,

Nigeria. *The International Journal of Biotechnology, 10(2), 52 - 68.*
Joseph, A.A. Odimayo, M.S. and Olokoba, L.B. Olokoba, A.B. & Popoola, G.O. (2017). Multiple Antibiotic Resistance Index of *Escherichiacoli* Isolates in a Tertiary Hospital in South-West Nigeria. *Medical Journal of Zambia*, *44*(4), 225-232.

Kehinde, A., and Umar, U. (2018). Combating diarrhoea in Nigeria: the way forward. *Journal of Microbiology & Experimentation*, *6*(4). https://doi.org/10.15406/jmen.2018.06.00213

Loha, A., Rahman, M. M., Biswas, A., Mou, M. H., Rifat, T. A., Choudhury, M. M., Jebin, F. M., Alarm, F. M., Sarker, P. K., Tariquzzaman, M., Husain, M. A., Noman, M. A., and Haroon, R. M. (2021). Antibiotic resistance studies of *Escherichia* coli and Salmonella species isolated from diarrheal patients. African Journal of Biological Sciences, 3(3), 122. https://doi.org/10.33472/afjbs.3.3.2021.122-130

Mandomando, I., Jaintilal, D., Pons, M. J., Vallès, X., Espasa, M., Mensa, L., Sigaúque, B., Sanz, S., Sacarlal, J., Macete, E., Abacassamo, F., Alonso, P. L., & Ruiz, J. (2009). Antimicrobial susceptibility and mechanisms of resistance in Shigella and Salmonella isolates from children under five years of age with diarrhea in rural Mozambique. *Antimicrobial Agents and Chemotherapy*, *53*(6), 2450–2454.

Nikaido H. (2009). Multidrug resistance in bacteria. *Annual review of biochemistry*, *78*, 119-146.

Olayinka, B., Olonitola, O., Olayinka, A., & Agada, E. (2004). Antibiotic Susceptibility Pattern and Multiple Antibiotic Resistance Index of *Pseudomonasaeruginosa* Urine Isolates From a University Teaching Hospital. *African Journal of Clinical and Experimental Microbiology*, *5*(2), 198–202.

Rawat, D., & Nair, D. (2010). Extended-spectrum β-lactamases in Gram Negative Bacteria. *Journal of global infectious diseases*, *2*(3), 263–274. https://doi.org/10.4103/0974-777X.68531

Rodrigo, M. G.-M. E.-L. (2020). *Antimicrobial Resistance in Escherichia coli* (p. Ch. 9). IntechOpen. https://doi.org/10.5772/intechopen.93115

Saka, H. K., Dabo, N. T., Muhammad, B., García-Soto, S., Ugarte-Ruiz, M., & Alvarez, J. (2019). Diarrheagenic *Escherichia coli* Pathotypes from Children Younger Than 5 Years in Kano State, Nigeria. *Frontiers in public health*, *7*, 348. https://doi.org/10.3389/fpubh.2019.00348

Sang, W. K., Oundo, V., & Schnabe, l.D. (2012). Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhoea in four provinces of Kenya. *The Journal of Infection in Developing Countries*, *6(07): 572-578.* https://doi.org/10.3855/jidc.2196

Spano, L. C., da Cunha, K. F., Monfardini, M. V., de Cássia Bergamaschi Fonseca, R., and Scaletsky, I. C. A. (2017). High prevalence of diarrheagenic *Escherichia* coli carrying toxin-encoding genes isolated from children and adults in southeastern Brazil. *BMC Infectious Diseases*, *17*(1), 1–10.

Sulaiman, M. A., Muhammad , H., Muhammad Sani, A., Ibrahim, A., Hussain, I. M., and Anchau, Z. G. (2020). Evaluation of the impact of plasmid curing on antibiotic resistance on some clinical isolates of escherichia coli. *Fudma journal of sciences*, *4*(3), 323 - 327.

Thenmozhi, S., Moorthy, K., Sureshkumar, B. T., and Suresh, M. (2014). Antibiotic Resistance Mechanism of ESBL Producing Enterobacteriaceae in Clinical Field: A Review. *International Journal of Pure and Applied Bioscience*, *2*(3), 207–226.

Ugwu, M. C., Edeani, G. I., Ejikeugwu, C. P., Okezie, U., & Ejiofor, S. O. (2017). Antibiotic susceptibility profile of *Escherichia* coli and Salmonella causing childhood diarrhoea in Awka Municipality, South-Eastern Nigeria. *Clin Microbiol*, *6(277)*, 2.

Wilunda, C., & Panza, A. (2009). Factors associated with diarrhea among children less than 5 years old in Thailand: a secondary analysis of Thailand multiple indicator cluster survey 2006. *Journal of Health Research*, *23: 17-22.*

World Health Organization. (2017, May 7). "Diarrhoeal disease" Retrieved from https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease

Zeighami, H., Haghi, F., Hajiahmadi, F., Kashefiyeh, M., and Memariani, M. (2015). Multi-drug-resistant enterotoxigenic and enterohemorrhagic *Escherichia* coli isolated from children with diarrhea. *Journal of Chemotherapy*, *27*(3), 152–155.

Zhang, S. X., Zhou, Y. M., Tian, L. G., Chen, J. X., Tinoco-Torres, R., Serrano, E., Li, S. Z., Chen, S. H., Ai, L., Chen, J. H., Xia, S., Lu, Y., Lv, S., Teng, X. J., Xu, W., Gu, W. P., Gong, S. T., Zhou, X. N., Geng, L. L., and Hu, W. (2018). Antibiotic resistance and molecular characterization of diarrheagenic *Escherichia* coli and non-typhoidal *Salmonella* strains isolated from infections in Southwest China. *Infectious Diseases of Poverty*, *7*(1), 1–11.