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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF ENTEROTOXIGENIC *E. COLI* ISOLATED FROM DIARRHOIEC SAMPLES OF UNDER FIVE CHILDREN IN REMO, OGUN STATE, NIGERIA

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ABSTRACT

A total of 195 diarrhoiec samples were collected from the children whose parents gave their consent to participate in selected hospitals in Remo, Ogun State. *Escherichia coli* was isolated from the samples using standard Microbiological and Molecular techniques. Characterization of enterotoxigenic *Escherichia coli* was determine using Molecular methods. Out of 195 diarrhoiec samples analyzed in this study, 84(43.1%) were positive for *Escherichia coli*. Out of the 84 *Escherichia coli* isolated, 82 (97.6%) were confirmed to be positive by the molecular method. Only 15 (17.9%) were enterotoxigenic (ETEC) while others 69 (82.1%) were non-pathogenic.

KEYWORDS: Enterotoxigenic *E.coli*, Diarrhoea, Remo.

INTRODUCTION

Diarrhoea accounts for 11 percent of all under-five deaths worldwide (Black et al., 2010) and claims the lives of 1.8 million children per year worldwide annually (WHO, 2012). In developing countries diarrhoea has the highest mortality rate in young children (Liu et al., 2015). According to UNICEF, (2011) the recent mortality estimates among children under five years old show that just five countries account for 50 percent of global mortalities. Of those five, Nigeria accounts for 11 percent of all deaths. Report from Liu et al., (2015) states that diarrhoea can be prevented, yet responsible for nine percent of all deaths among under age five children worldwide. The causes of diarrhoea include a wide range of viruses, bacteria and parasites, among the bacteria pathogens, *Escherichia coli* play an important role (Peleg et al., 2005).

MATERIALS AND METHODS

The study was carried out in Remo Land comprising of Sagamu, Ikenne and Remo North Local Government Areas of Ogun State, Nigeria, between July 2016 and June 2017.The study was approved by Olabisi Onabanjo University Teaching Hospital Health Research Ethics Committee under the authorization number: NHREC/08/10/2012. The sample size calculation was determined as described by Daniel, (2009). A total of 195 diarrhoiec samples were collected from the children that meet the criteria and whose parents gave their consent to participate in selected hospitals in Remo, Ogun State.

Collection and processing of specimens

The stool samples were collected in a sterile sample bottles directly from the children with the assistance of the laboratory staff and health workers in the study site who were educated on how to collect the samples aseptically, the samples were transported to the laboratory for analysis within 30 minutes after collection. Samples were collected for a period of twelve months from children under the age of five (5) years with incidents of diarrhoea as presented by the parents and confirmed by the clinician. The samples were processed in Microbiology Laboratory, Babcock University, Ilisan Remo, Ogun State, Nigeria.

Isolation and identification of *E.coli*

Using a sterile standard wire loop, a loop full of stool sample was streaked on sterile Mac Conkey agar plates and incubated for 24 hour at 37 ºC. All Lactose fermenting colonies were sub cultured on Eosin Methylene Blue (EMB) agar and incubated overnight at 37^{o}C. Colonies that exhibited green metallic sheen colour, characteristic of *E. coli* were sub cultured on Nutrient agar plates and incubated at 37 ºC for 24 hour, Biochemical tests such as Indole, Methl red -Voges-Proskauer (MR-VP), Citrate tests and sugar fermentation test were performed on the colonies. The pure colonies

identified as *E. coli* were inoculated on Nutrient agar slant and were stored in the refrigerator at 4°C.

Molecular Identification of *E. coli*

DNA extraction

The stock cultures of *E. coli* were cultured on Nutrient agar to reactivate them, incubated at 37°C for 18-24 hours**.** The resuscitated isolates were inoculated in Tryptone soy broth (TSB) and incubated at 37°C overnight, centrifuged at 5000xg for 10 minutes. The supernatant was discarded and the pellet was re-suspended in 150uL of sterilized distilled water. The suspension was subjected to boiling at 100°C in water bath (Uniscope, Surgifriend, England) for 10 minutes, and centrifuged at 5000xg for 30 seconds. The supernatant was then transferred into fresh Eppendorf tubes before it was stored at 4 °C. Aliquots of 5uL of template DNA for Polymerase Chain Reaction (Martins et al., 2015).

PCR amplification for TEcol gene

PCR reaction was carried out using the Solis Biodyne 5X FIREPol Blend Master mix, the concentrated 5X FIREPol Blend Master mix was diluted with sterile distilled water to 1X. The Blend Master mix contains: 2 unit of FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 2.0 mM $MgCl_2$, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne). PCR was performed in 20 µL of a reaction mixture. Therefore, 4 µL master mix, 0.5 µL each of forward and reverse primers (10pMol) (BIOMERS, Germany) as in Table 3.1, 5µL of the extracted DNA and sterile distilled water was used to make up the reaction mixture (Åsa et al., 2015 & Christopher et al., 2019).

Amplification was conducted in a Peltier thermal cycler PTC 100 (MJ Research Series) for an initial activation at 95°C for 15 minutes followed by 35 amplification cycles of 30 seconds for denaturation at 95°C; 1 minute of Annealing at 56°C and 1 minute 30 Seconds of elongation at 72°C. This was followed by a final elongation step of 10 minutes at 72°C. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder was used as DNA molecular weight standard.

Table 1: Primers used for confirmation of *Escherichia coli.*

S/N	Primers	Sequence(5^1-3^1)	Annealing Temperature(°C)	BASE PAIR(b_p)
1.	TEcol-F	TGGGAAGCGAAAATCCTC	58	1258
	TEcol-R	CAGTTACAGGTAGACTTCTG		

Nolonwabo, et al., (2014).

Identification of enterotoxigenic *E. coli* by multiplex PCR

PCR amplification for enterotoxigenic genes.

A multiplex PCR reaction was carried out using the Solis Biodyne 5X FIREPol Blend Master mix, the concentrated 5X FIREPol Blend Master mix was diluted with sterile distilled water to 1X. The Blend Master mix contains: 2 unit of FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 2.0 mM $MgCl_2$, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne). PCR was performed in 20 µl of a reaction mixture. Therefore, 4 µl master mix, 0.5 µl each of forward and reverse primers (10pMol) (BIOMERS, Germany), 5µl of the extracted DNA and sterile distilled water was used to make up the reaction mixture (Åsa *et al*., 2015 & Christopher *et al*., 2019).

Amplification was conducted in a Peltier thermal cycler PTC 100 (MJ Research Series) for an initial activation at 95°C for 5 minutes followed by 35 amplification cycles of 30 seconds for denaturation at 95°C; 1 minute of Annealing at 55°C and 1 minute 30 Seconds of elongation at 72°C. This was followed by a final elongation step of 10 minutes at 72°C. The amplification product was separated on a 2.0% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder was used as DNA molecular weight standard.

Table 3.2: Primers used in the detection of virulence genes of *Escherichia coli*.

Pathotype	Target	Name	Primer (5^1-3^1)	Sequence PCR product size
ETEC	LT	LT-F	GCA CAC GGA GCT CCT CAG TC	218
		LT-R	TCC TTC ATC CTT TCA ATG GCT TT	
	St 11	St11-F	AAA GGA GAG CTT CGT CAC ATT TT	129
		St11-R	AAT GTC CGT CTT GCG TTA GGA C	

Nolonwabo, et al., (2014).

RESULTS AND DISCUSSION

Age and gender distribution of diarrhoea cases in study population

The age distribution of the children with highest cases of diarrhoea in this study showed that they were mainly infants (less than or equal to 12 months) in which 54 (27.6%) were boys and 20 (10.2%) were girls as presented in Figure 4.1. This corresponds to the result of Thielman and Guerrant (2004); Avisek et al., (2015); Ayuk et al., (2015); Magbagbeola et al., (2017);

Mohammed et al., (2018) and Thiagarajah et al., (2018) reported that the rates of diarrhea were highest for infants 6-11 months of age, remained at a high level among 1-year-old children and decreased when children got older. A decrease in number of cases among older children is due to the acquisition of immunity over the time which resist against infection agent. The results of this study is also in accordance with reports from Bahrain (Krishnamurthy, 1990), Saudi Arabia (Qadri et al., 1990 and Albert et al., 1995) which showed that 50%, 43.4% and 53.6% respectively of the children hospitalized with diarrhoea were below 1 year of age also support the results. The age-specific differences suggest that infants apart from having immature immune systems may be exposed to contaminated formula of milk, foods or environment or may have not been protected completely by maternal antibodies in breast feeding (Akbar, 2008). Furthermore, results of this study revealed that number of males 127 (65.1%) with diarrhoea were higher than in females 68 (34.8%) (Figure 1). This agrees with report of (Ogunlesi et al., 2006; Ogbu et al., 2008; Ohaeri & Odukaesieme, 2011) and is consistent with results of other researchers Ishiyama et al., (2001); Abdullahi et al. (2010), Anasari (2012), Ifeoma et al., (2017); Angela et al., (2018) which reported that male children were more infected than female children but in contrast to Gupta et al., (2015); John et al., (2016) and Bui et al., (2018).

1: Bacteria isolated from children diarrhoiec stool samples

Bacteria	Frequency (n=195)	Percentage
Escherichia coli	84	43.1
Non *Escherichia coli*	85	43.6
Without growth	26	13.3
Total	195	100.0

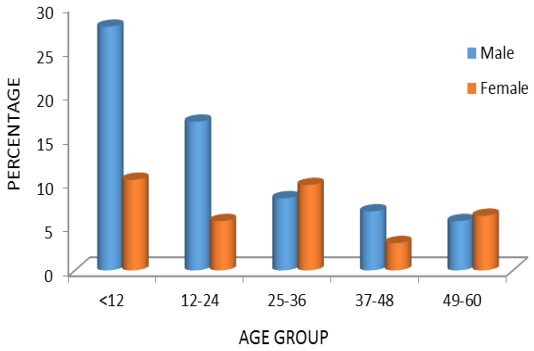

Figure1: Age and gender distribution of diarrhoea cases in study population.

Isolation, Phenotypic and Genotypic characteristics of *Escherichia coli* isolates from children diarrhoeic stool samples

Out of the 195 diarrhoeic samples analyzed in this study, 84 (43.1%) of the samples were positive for *Escherichia coli* as presented in Table 1 and 82 (97.6%) were confirmed positive by Molecular characterization (Figure 2). The prevalence of *Escherichia coli* from this study is lower than 89.4% reported from similar study in Benin Teaching Hospital, Benin, Nigeria; 83.1% reported in Abakaliki, South eastern Nigeria (Ogbu et al., 2008); 62.8% obtained in a study carried out in the Federal Capital Territory Abuja, Nigeria by Ifeanyi et al (2010) among children presented with diarrhea. Furthermore the prevelance is also lower than 61.76% recorded by Babu et al (2009) in Rajah Hospital, Annamalainagar, Tamil Nadu, in India and also lower compared with other studies carried out in Iran (Alikhani et al, 2006; Jafari et al, 2009; Al-Zogibi and Onizan Godian, 2015;). In contrast, it is higher than 34% recorded by Sule et al (2011) in Kaduna, another Northern State in Nigeria. Clarence et al (2007) had 41.3% prevalence of *Escherichia coli* in a study conducted among children with diarrhoea, attending Madonna University Teaching Hospital (MUTH) Elele, in Rivers State, in South South, Nigeria while Olanipekun, (1996) documented 26% prevalence among children with diarrhoea attending Jos University Teaching Hospital, Jos in Plateau State, in North east, Nigeria. It is reasonable to attribute the differences in prevalence of *Escherichia coli* to region, countries and geographical area.

REFERENCES

1. Black, R.E., Cousens, S., Johnson, H.L., Lawn, J.E., Rudan, I. & Bassani, D.G. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*, 2010.
2. World Health Organization, Diarrhoeal Diseases. (2012). Available at: http://www.who.int/mediacentre/factsheets/fs330/en/index.html
3. Liu, L., Oza, S. & Hogan, D. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet*, 2015; 385: 430-440.
4. UNICEF. Levels and trends in child mortality. In: Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation, 2011.
5. Daniel, W. W. Biostatistics: A foundation for analysis in the health sciences. Hoboken, NJ: J. Wiley & Sons New York, NY., 2009.
6. Martins, F. H., Guth, B. E. C. Piazza, R. M., Leão, S. C., Ludovico, A., Ludovico, M. S. Dahbi, G., Marzoa, J., Mora, A., Blanco, J., Pelayo, J. S. Diversity of Shiga toxin-producing *Escherichia coli* in sheep flocks of Paraná State, southern Brazil. *Veterinary Microbiology, Barcelona*, 2015; 175(1): 150-156.
7. Åsa, S., Leila, S., Daniel, N. & Joshua, T. "Detection of major diarrheagenic bacterial pathogens by multiplex PCR panels", Microbiological Research, 2015; 27-9.
8. Christopher Osita Eze, Dinebari Philip Berebon, Thadeus Harrison Gugu, Chukwuemeka Sylvester

Nworu, Charles Okechukwu Esimone. "Effects of *Lactobacillus* spp. isolated from the sap of palm tree Elaeis guineensis (palm wine) on cellular and innate immunity", *African Journal of Microbiology Research*, 2019; 61: 830–836.

9. Nolonwabo, Nontongana., Timothy, Sibanda., Elvis, Ngwenya. & Anthony, I. Okoh. A. Prevalence and Antibiogram Profiling of *Escherichia coli* Pathotypes Isolated from the Kat River and the Fort Beaufort Abstraction Water. *International Journal of Environmental Research and Public Health*, 2014; 11: 82138227. doi:10.3390/ijerph110808213
10. Thielman, N. M., & Guerrant, R. L. Acute Infectious Diarrhoea. *The England Journal of Medicine*, 2004; 350(1): 38-47.
11. Avisek, Gupta., Gautam, Sarker., Arup, Jyoti. Rout., Tanushree, Mondal. & Ranabir, Pal. Risk Correlates of Diarrhea in Children Under 5 Years of Age in Slums of Bankura, West Bengal. *Journal of Global Infectious Diseases*, 2015; 7(1): 23–29. doi: 10.4103/0974-777X.150887, PMCID: PMC4338445.
12. Ayuk, Betrand. Tambe., Leonie, Dapi. Nzefa. & Nchang, Allo. Nicoline. Childhood Diarrhea Determinants in Sub-Saharan Africa: A Cross Sectional Study of Tiko- Cameroon Challenges, 2015; 6: 229-243. doi: 10.3390/challe6020229.
13. Magbagbeola, David. Dairo., Tosin, Faisal. Ibrahim. & Adetokunbo, Taophic. Salawu. Prevalence and determinants of diarrhoea among infants in selected primary health centres in Kaduna north local government area. *Nigeria Pan African Medical Journal*, 2017; 28: 109.
14. Mohamed, Lemine., Cheikh, Brahim. Ahmed., Abdellahi, Weddih., Mohammed, Benhafid., Mohamed, Abdellahi. Bollahi, Mariem, Sidatt, Khattry., Makhalla, Ali. H, Mokdad Jorg. Heukelbach. & Abdelkarim, Filali-Maltouf. Hospitalizations and Deaths Associated with Diarrhea and Respiratory Diseases among Children Aged 0–5 Years in a Referral Hospital of Mauritania. *Tropical Medicine* and *Infectious Disease*, 2018; 3(3): 103.
15. Thiagarajah, Jay R., Daniel S. Kamin, Sari Acra, Jeffrey D. Goldsmith, Joseph T. Roland, Wayne I. Lencer, Aleixo M. Muise, James R. Goldenring, Yaron Avitzur & Martín G. Martín. PediCODE Consortium Advances in Evaluation of Chronic Diarrhea in Infants, 2018; 12.
16. Krishnamurthy, S.N. Epidemiology of acute gastro enteritis in children in Defense Force Hospital, Bahrain. *Bahrain Medical Bulletin*, 1990; 12(1): 69-72.
17. Qadri, M.H., Al-Gham. M.H. &M. Imadulhaq, di. Acute diarrheal disease in children under five years of age in the eastern province of Saudi Arabia. *Annal of Saudi Medicine*, 1990; 10(3): 280-284.
18. Albert, M. J., Faruque, S. M., Faruque, A. S., Neogi, P. K. Ansaruzzaman, M. Bhuiyan, N. A. Alam, K. & Akbar, M. S. Controlled study of *Escherichia coli* diarrheal infections in Bangladeshi children. *Journal of Clinical Microbiology*, 1995; 33: 973–97.
19. Akbar, S.A., A study of *Escherichia coli* Isolated from children with diarrhea in Kalar, M.S. Thesis, College of Education-Kalar, University of Sulaimani, Sulaimaniah, Iraq, 2008.
20. Ogunlesi, T., Okeniyi, J., Oseni, S., Oyelami, O., Njokanma, F. & Dedeke, O. Parasitic etiology of childhood diarrhoea. *Indian Journal of Pediatric*, 2006; 73: 1081-1084.
21. Ogbu, O., Aguumadu, N., Uneke, C. J. &Amadi, E. S. Aetiology of acute infantile diarrhoea in South Eastern Nigeria: An assessment of microbiological and antibiotic sensitivity profile. *The Internet Journal of Third World Medicine*, 2008; 7(1): 2.
22. Ohaeri, C. C., &Odukasieme, O. P. Survey of geohelminthiasis among primary school children in Umuahia metropolis of Abia State, Nigeria. *International Journal of Environmental Health and Human Development*, 2011; 11(1): 41-45.
23. Ishiyama, S., Ono, K., & Rai, S. K. Study of enteropathogen and its predisposing factors in suburban public school children in Kathmandu, Nepal. *Nepal Medical College Journal*, 2001; 3: 5-9.
24. Abdullahi, M., Oloniola, S.O. & Inabo, I.H. Isolation of bacteria associated with diarrhoea among children attending some hospitals in Kano Metropolis, Kano State, Nigeria. *Bayero Journal of Pure and Applied Sciences*, 2010; 3(1): 10–15.
25. Anasari, S., Bahadur, S., Parajuli, K., & Paudyal, R. P. A. Pattern of acute parasitic diarrhoea in children under five years of age in Kathmandu, Nepal. *Open Journal of Medical Microbiology*, 2012; 2: 95-100.
26. Angela, F., Jarman, Sara. E., Long, Sarah. E., Robertson, Sabiha., Nasrin, Nur. Haque., Alam, Alyson. J. & McGregor, Adam. C. Levine. Sex and Gender Differences in Acute Pediatric Diarrhea: A Secondary Analysis of the DHAKA Study. *Journal of Epidemiology and Global Health*, 2018; 8(1-2): 42–47.
27. Gupta, A., Sarker, G., Rout, A. J., Mondal, T. & Pal, R. "Risk correlates of diarrhea in children under 5 years of age in slums of Bankura, West Bengal," *Journal of Global Infectious Diseases*, 2015; 7(1): 23–29.
28. John. T., Wisdom. K., Takramah. M. Amogre, A., Augustine. A. A., Elijah, B. & Josiah, A. Trends for Diarrhea Morbidity in the Jasikan District of Ghana: Estimates from District Level Diarrhea Surveillance Data, 2016; 2012–2016.
29. Bui, H.V. The most common causes of and risk factors for diarrhea among children less than five years of age admitted to Dong Anh Hospital, Hanoi. *Journal of Tropical Medicine*, Article ID 4863607, 2018; 10.
30. Ifeanyi, C.I.C., Bassey, B.E., Ikeneche, N.F. & Al-

Gallas N. Molecular characterization and antibiotic resistance of *Salmonella* in children with acute gastroenteritis in Abuja, Nigeria. *Journal of Infection in Developing Countries*, 2014; 8: 712–719. pmid:24916869

31. Babu-Uma, Kesani. P., Saddayappan, R., Kannaiyan, K. & Yelavarthi, L. S. Antibiotic Sensitivity and Plasmid Profiles of *Escherichia coli* Isolated from Pediatric Diarrhoea. *Journal of Global Infectious Diseases*, 2009; 2: 107–110.
32. Alikhani, M.Y., Mirsalehian, A. & Aslani, M.M. Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhoea. *Journal of Medical Microbiology*, 2006; 55: 1159-1163.
33. Jafari, F., Hamidian, M., Rezadehbakhshi, M., Doyle, M., Salmanzadeh-Ahrabi, S. & Derakhshan, F. Prevalence and antimicrobial resistance of diarrhoeagenic *Escherichia coli* and *Shigella* species associated with acute diarrhoea in Tehran, Iran. Canadian *Journal* of *Infectious Diseases and Medical Microbiology*, 2009; 8: 712–719.
34. Al-Zogibi, Onizan Godian. "Molecular Characterization of Shiga-Toxigenic *Escherichia coli* Associated with Food Collected from Riyadh, Saudi Arabia King Abdulaziz University: Scientific Publishing Centre, 2015; 72.
35. Sule, E.I., Aliyu, A.M. & Abdula ziz, B.M. Isolation of diarrhegenic bacteria in children attending some selected hospitals within Kaduna metropolis, Kaduna state, Nigeria. *Continental Journal of Applied Sciences*, 2011; 6(1): 1-6.