Research Article

Diarrhoeagenic *E. coli* and Associated Virulence Factors on Bioko Island, Equatorial Guinea

Úrsula-Eva EñesoEfuá[1], Carlos Javier Ciria Gil[2], Agustín Benito Llanes[3], Fátima Patabobe[1], Pascual Erasmo Owono[1] and Silvia Herrera León[2]*

[1] Department of Microbiology, Polyclinic Dr. Loeri Comba- Malabo, Equatorial Guinea.
[2] Reference Laboratory for waterborne and foodborne diseases, National Center for Microbiology, Carlos III Health Institute, Madrid, Spain.
[3] National Center for Tropical Medicine. Carlos III Health Institute. Biomedical Network Research Center for Infectious Diseases (CIBERINFEC), ISCIII, Madrid, Spain.

Corresponding Author: Silvia Herrera León, Reference Laboratory for waterborne and foodborne diseases, National Center for Microbiology, Carlos III Health Institute, Madrid, Spain.

Abstract

Among the various aetiological agents causing acute diarrhoeal disease (ADD), the heterogeneous group of different pathotypes of *Escherichia coli* continues to be responsible for a high percentage of cases, especially in children under 5 years of age in middle and low-income countries in Latin America, Asia and Africa. The aim of the present study was to identify the main pathotypes of *E. coli* associated with ADD in patients in the two main hospitals on Bioko Island, Equatorial Guinea (November 2011-April 2012). A total of 716 clinical samples were analysed, 496 samples from patients with symptomatology compatible with ADD (symptomatic) and 220 from patients without symptomatology compatible with ADD (asymptomatic). Of the samples from symptomatic patients, 41.53% were positive for a diarrhoeagenic *E. coli* pathotype while only 1.36% of asymptomatic patients were positive for a diarrhoeagenic *E. coli* pathotype. The most affected age group was children aged 0-4 years (37%). The ECD groups identified in symptomatic patients were: 22.98% enteroaggregative *E. coli* (EAEC); 9.68% enterotoxigenic *E. coli* (ETEC); 6.45% enteropathogenic *E. coli* (EPEC) and 2.42% enteroinvasive *E. coli* (EIEC). In asymptomatic patients, only three cases of ECD due to ECEA (0.90%) and ECEP (0.45%) were detected. In our study no verotoxigenic *E. coli* pathotype was detected in a single case. The results suggest that EAEC, ETEC, EPEC and EIEC pathotypes are important aetiological agents of ADD on Bioko Island, Equatorial Guinea. The results highlight the need to develop appropriate diagnostic methods for this group of pathogens. Improved diagnostic capacity coupled with implementation of prevention and control measures could help reduce the number of ADD caused by DEC in Equatorial Guinea.

Keywords: diarrhoeagenic E. coli, acute diarrhoeal disease, pathotype, virulence genes.

Introduction

Bacteria are the second leading cause of acute diarrhoeal disease (ADD) worldwide and continue to be an important public health problem in low- and middle-income countries because of their high morbidity and mortality rates in under 5 years of age (1). Among the various bacteria causing ADD, the different diarrhoea-causing *E. coli* pathotypes are most common, with the EPEC pathotype being particularly important in children under 5 years of age, followed by *Salmonella spp*, *Shigella spp*, *Campylobacter spp* and *Vibrio cholerae* (2). Although most studies cite diarrhoeagenic *E. coli* (DEC) as a major cause of clinical diarrhoea in children in low and middle-income countries, they are not as commonly isolated in Equatorial Guinea because of the lack of appropriate diagnostic tests. This leads to a lack of knowledge about the epidemiological situation of DEC, which prevents an objective assessment of DEC prevalence rates in the country.

The main pathotypes of DEC differ in the mechanisms they use to trigger the disease. Diagnostic methods target the genes encoding the different virulence factors involved in the pathogenicity mechanisms of each group (3). Thus, we have found five pathotypes of DEC that are of particular interest in the aetiology of ADD in humans: VTEC, verotoxigenic *E. coli*, which can cause haemolytic uremic syndrome and ulcerative colitis (3); EPEC, enteropathogenic *E. coli*, which are closely associated with watery diarrhoea in children under 5 years of age. Depending on whether or not they are carriers of the *bfp* gene, the product of which is an adhesin involved in intimal attachment to the enterocyte and in *A/E* injury, they are subdivided into typical and atypical EPEC, respectively (3); ETEC, enterotoxigenic *E. coli*, the main aetiological agent of travellers' diarrhoea. It is characterised by the production of at least one of the associated LT or ST enterotoxins (4); EIEC, enteroinvasive *E. coli*, whose pathogenesis and clinical presentation is identical to that produced by *Shigella spp*. (5); and EAEC, enteroaggregative *E. coli*, which is associated with persistent diarrhoea in both high-income and low- and middle-income countries and is, together with ETEC, another major aetiological agent of travellers' diarrhoea (3). Several studies in Africa have identified EAEC as the predominant pathotype, highlighting the importance of molecularly characterising the virulence factors of with the most commonly identified serotypes in order to optimise prevention and control measures to avoid deterioration of patient health and possible spread of the infectious agent (8,14,16).

The aim of this study was to identify the main DECs associated with acute diarrhoea, and the associated virulence factors, in different age groups in patients on Bioko Island, Equatorial Guinea, who attended hospital with symptoms consistent with ADD, focusing on children under 5 years of age, and its main objective is to serve as a baseline reference to evaluate improvements in the microbiological system, surveillance and case notification to adapt the public health response and reduce morbidity and mortality associated with DECs.

Material and Methods

Study area and population

The study area is located on the African island of Bioko, in Malabo, the capital of Equatorial Guinea, with 300,374 inhabitants (29), most of them under 15 years of age. The island is divided into two distinct regions, North Bioko and South Bioko.

Sampling, isolation and identification of diarrhoeagenic bacteria.

Between November 2011 and April 2012, a total of 716 clinical stool samples collected in sterile containers were analysed. Samples were obtained from 496 (62%) patients attending the Loeri Comba Polyclinic Hospital with a clinical picture consistent with ADD and, to a lesser extent from 220 (38%) patients attending Malabo Regional Hospital (Equatorial Guinea) without symptomatology compatible with ADD (asymptomatic). In both cases the majority age group was children under 5 years of age, with 402/496 and 158/220 respectively.

Samples were processed and analysed as follows:

Stool cultures for *E. coli* were performed on MacConkey agar (Oxoid, England) and TSA (Bio-Rad, France) at the PLC Hospital in Malabo, Bioko Island. The presence of *E. coli* was confirmed by complementary biochemical testing according to PLC-INSESO microbiology protocols (6): TSI (OXOID, England), Christensen's urea (OXOID, England), lysine decarboxylase (Liofilchem, Italy), Simons' citrate (Liofilchem, Italy), indole (Liofilchem, Italy), mannitol-motility (Liofilchem, Italy) and subsequently with commercial semiautomated biochemical methods 18R (Liofilchem, Italy) or Api 20-E (Biomerieux, France). At the National Centre for Microbiology (Madrid) the different pathotypes were detected and characterised by PCR amplification of specific virulence genes (7, 8). In ECEA-positive cases, additional genetic analyses were performed by amplifying the genes *aggR*, *sepA*, and pic, which are involved in biofilm formation and disease persistence, and the genes *astA* and pet, which encode toxins associated with acute diarrhoea and intestinal damage, respectively (9).

Informed consent and ethical considerations

The present study was approved by the appropriate hospital ethics committee of the PLC (INSESO) and the consent to work with children was given in writing by the parents/guardians of the children, after an informational interview, according to the criteria of the PLC Ethics Committee, which are in turn based on the criteria of the Helsinki Code of Ethics (3). Pte

Results and Discussion

Of the total samples tested from symptomatic patients (496), 95.96% (474) were positive for *E. coli* by conventional diagnostic tests in the PLC. Of these, 41.53% (206) were confirmed positive for any DEC pathotype. Of the asymptomatic patients (220), 1.36% (3) were positive for any DEC pathotype (Figure 1).

DEC pathotypes were identified in all study age groups, with the highest percentage of cases positive for EAEC, ETEC, EPEC and EIEC in children aged 0-4 years, with a prevalence of 73.3% (151/206) (Figure 2). Regarding gender distribution, 107 positive cases were male and 99 were female.
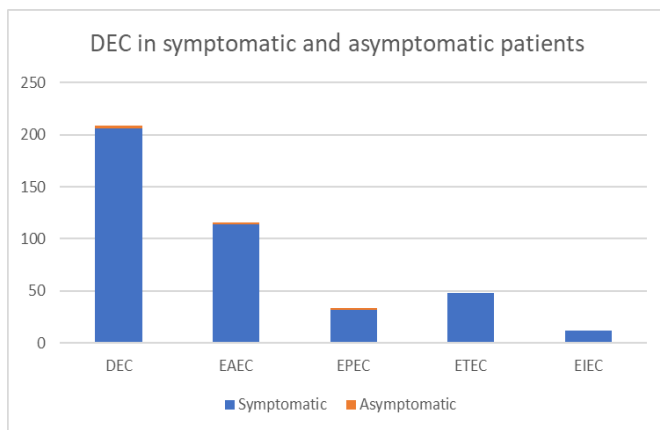

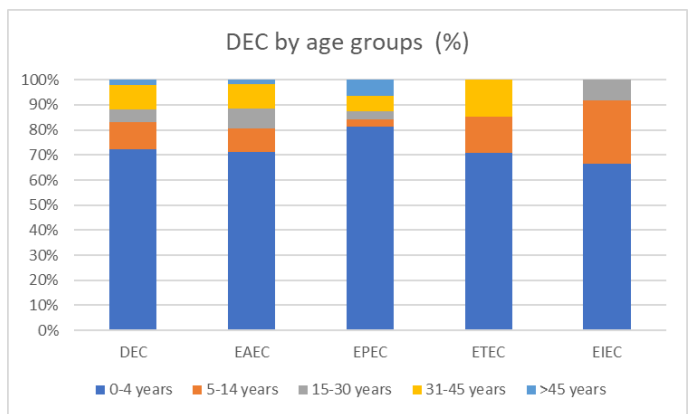*Figure 1- Incidence of diarrhoeagenic E. coli in cases of patients presenting to hospital with and without diarrhoea.*

Figure 2- Distribution of DEC in different age groups.

Genetic variability of *E. coli* isolates.

The 114 EAEC-positive isolates were screened for the presence of plasmid *pCVD432,* with the 0-4 years age group having the highest proportion of 81 cases (71.05%).

In addition, other virulence factors were detected in this group:

In 26 of the 81 isolates (32.09%) a single one out of the four genes examined was amplified by PCR. The gene most frequently detected in 12 isolates (14.8%) was the one associated with the *pet* toxin. The remaining genes were detected as follows: *aggR* in six isolates (7.40%); *pic* in three isolates (3.7%); *astA* in three isolates (3.7%) and *sepA* in two isolates (2.4%).

Combinations of two or more different virulence genes were detected in 55 of the EAEC-positive isolates (67.90%), as follows:

In 26 isolates (32.09%) 2 different virulence genes were determined in combination as follows: *aggR-pet* 19 (23.45%), *aggR-pic* 1 (1.23%), *pet-pic* 1(1.23%), *sepA-pet* 2 (2.46%), *astA-pet* 1(1.23), *sepA-aggR* 1 (1.23%) and *astA-aggR* 1 (1.23%). The most prevalent combination was *aggR-pet*.

In 24 isolates (29.6%), the presence of three combined virulence genes was determined as follows: *astA-sepA-pet* in 5 (6.17%), *astA-pet-aggR* in 7 (8.6%), *sepA-pet-pic* in 5 (6.17%), *sepA-aggR-pic* in 1 (1.23%), *astA-pet-pic* in 1 (1.23%), *astA-aggR-pic* in 1 (1.23%), *pet-aggR-pic* in 3 (3.7%) and *sepA-pet-aggR* 1 (1.23%). The most prevalent combination was *astA-pet-aggR*.

In five isolates (6.17%), 4 virulence genes were found in combination as follows: *sepA-pet-aggR-pic* in 3 (3.7%), *astA-sepA-pet-pic* in 1 (1.23%) and *astA-sepA-pet-aggR* in 1 (1.23%).

In the EPEC-positive cases, attention was paid to the presence of the *bfp* gene, which was present in 15 isolates (typical EPEC) and absent in 9 (atypical EPEC).

In the ETEC-positive cases, the enterotoxin carried by the isolates was identified. In 14 isolates, the *estA* gene encoding thermostable enterotoxin (TE) was detected, and in 11 isolates the *eltA* gene coding for thermolabile enterotoxin (TL) was detected. In seven isolates, the presence of both was determined.

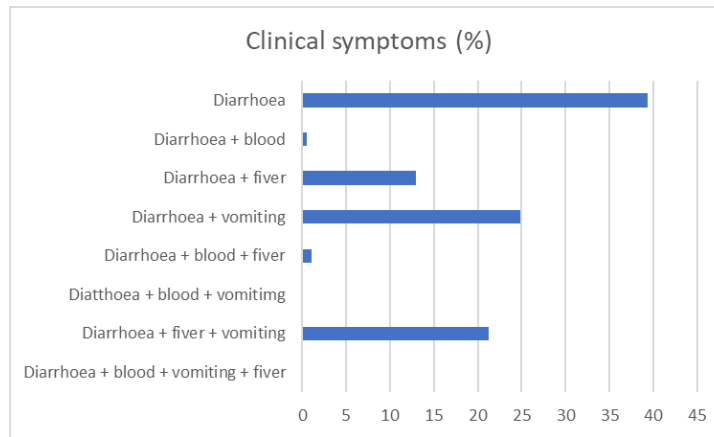The verotoxigenic *E. coli* pathotype (VTEC) was not detected in any case.

Figure 3- *Clinical picture in symptomatic patients.*

The aim of the present work was to study the prevalence of DEC in patients with ADD on Bioko Island, Equatorial Guinea. Four of the five most common pathotypes of DEC were detected in the study. EAEC was the most prevalent in a total of 116 isolates from symptomatic (114) and asymptomatic (2) individuals, predominantly aged 0-4 years (71.05%) which differs from previous reports from West Africa, where EPEC and ETEC are the most common pathotypes associated with childhood diarrhoea and EAEC is present in only 2% of cases, and in children under 3 years of age (10). In Venezuela, a study was conducted in which this pathotype was associated with cases of ADD in children up to 2 months of age, but not in children over this age (11). There are increasing reports linking this pathotype to cases of diarrhoea in children in low-income countries (12,13). Okeke and Bounkonkou (14,15) note in their studies that EAEC pathotypes found in neighbouring Nigeria, Ghana and Burkina Faso are more closely related genetically and evolutionarily than those found in Equatorial Guinea or Gabon, which are located on the Gulf of Guinea and share a border. In Gabon, where EAEC is also an important diarrhoeal pathogen in children under 5 years of age, the virulence factors detected in most cases are those associated with the *pet* and *astA* genes (16), which are associated with acute episodes and intestinal damage. These results consistent with those obtained by us. The *aggR* gene, which is responsible for disease persistence in most cases, is the second most common in our study (17, 18, 19, 20, 9). This difference could be mainly due to the different geographical, climatic and socio-economic conditions in these countries. In our case, ETEC was the second most frequently detected pathotype in the 0 to 4 years age group, which is consistent with previous studies in other parts of the world, where ETEC and EPEC are the most common DECs in children under 5 years of age, highlighting the importance of this pathotype in this age group. It should be noted that the mechanisms regulating LT or ST enterotoxin expression have not yet been elucidated and we cannot explain why LT-ETEC is more common in South America, whereas ST-ETEC is more common in Equatorial Guinea (Africa) (30). The third pathotype detected with the highest proportion was EPEC, which contradicts reports from other regions of the world where this pathotype is most commonly associated with childhood diarrhoea in children under 5 years of age (17, 21). Considering the subdivision of EPEC into typical and atypical strains, depending on whether they are carriers of *bfp* or not, our study found a higher percentage of typical strains, which are an important cause of child morbidity and mortality in low-income countries. These findings are consistent with those of other authors (22), although in some recent studies an increasing number of cases of ADD are caused by atypical EPEC (23). The least isolated pathotype in our study was EIEC. In most studies, VTEC is the most commonly detected aetiological agent in diarrhoeal cases, but it was not detected in any case in our study. This could be due to the fact that the main reservoir for VTEC is cattle and there is little industrial livestock production in Equatorial Guinea, as well as good preparation of meat and vegetables, typical of the food culture of the Guinean population and the low or non-existent consumption of milk or fresh dairy products in the country, which could also play a role (24). Of note, mixed DEC infections were found in 20.3% of the samples. Of these, a high percentage were coinfected with a combination of EAEC-EPEC pathotypes (10). It is difficult to determine the specific DEC responsible for the different symptoms that make up the clinical picture in the cases of coinfection detected in our study. However, some authors suggest that the combination of different toxins and virulence genes between DECs of the same type (intrapathotypic), or in combination with other DECs (interpathotypic), would lead to a greater synergistic effect (25). In our study, EAEC seems to be the main aetiological agent responsible for most of the described clinical pictures, whereas the other pathotypes contribute to a higher virulence of EDD, in mixed infections.

Regarding the prevalence of DEC in symptomatic and asymptomatic patients, it appears that the aetiology of the disease in patients with diarrhoea is more closely associated with the presence of a diarrhoeagenic *E. coli* pathotype. While it is true that asymptomatic patients may be colonised with a particular pathotype of DEC and act as a reservoir for the pathogen by spreading it through their faeces (26), our study did not find a high prevalence of DEC in this group. This suggests that these individuals are neither a reservoir nor involved in the transmission of the disease on the island (26).

A close association was found between ADD, DEC and age range, with the 0-4 years age group being the most affected by the disease, although this population was overrepresented in our study, as most cases of ADD were from the infant population. This could be due to the fact that mothers are more likely to seek medical attention when their child is ill, whereas adults usually wait for symptoms to resolve and only seek medical attention when symptoms become more severe, as in most cases they resolve on their own. It is important to properly diagnose bacterial-related ADDs in the paediatric population, especially those caused by VTEC, although this group does not seem to contribute to ADD cases in our study, as they can develop into haemolytic uraemic syndrome, renal dysfunction and ulcerative colitis, chronic conditions that affect the patient's quality of life for the rest of his or her life. Several studies highlight the importance of adapting DEC surveillance systems in low- and middle-income countries to improve public health systems and prevent the spread and progression of the disease to disabling stages (27, 28).

We would like to add that, at the time of the study, Equatorial Guinea was a country in an early stage of development thanks to oil production. It has since become a middle-income country where investments have been made to improve health conditions but ADDs due to DEC are still significant. Malnutrition, consumption of food processed or stored in unsanitary conditions and poor personal hygiene are factors that promote person-to-person transmission of diarrhoea of infectious aetiology. The close association between cases of acute diarrhoea and the use of unsafe household water stored in unsanitary conditions and the use of water contaminated with human faeces from rivers, septic tanks, or latrines, should prompt future surveillance and studies (31).

Study Limitations

It is important to also consider the possibility that there are individuals with symptomatic ADD and that the diarrhoea is caused by another aetiological agent such as a virus or parasite, but in our case we did not perform comparative studies or differential aetiological diagnosis to fully confirm that the diarrhoea was associated with DEC. In the present work, only the identification of *E. coli* pathotypes in diarrhoeal samples was performed, and we were not able to confirm in parallel by negative coproparasitological and coproviral tests that the diarrhoea was caused only by the identified DEC.

The wide range of risk factors associated with ADDs in children under 5 years of age has not been fully explored.

Conclusions

The most common pathotypes of DEC associated with ADD detected in our study were EAEC, ETEC, EPEC and EIEC, respectively.

The results demonstrate the importance of DEC in ADD and highlight the need to develop appropriate diagnostic methods for this group of pathogens. Improved diagnostic capacity combined with the application of appropriate prevention and control measures and the creation of sanitation and water treatment infrastructures, could help to reduce ADD caused by ECD.

Conflict of Interest

None of the authors of this paper has a conflict of interest.

References

1. Croxen M.A., Law R.J., Scholz R., Keeney K.M., Wlodarska M., Finlay B.B. Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev. 2013;26(4):822–880.

2. O'Ryan M, Prado V, Pickering LK. A millennium update on pediatric diarrheal illness in the developing world. Semin PediatrInfectDis. 2005; 16(2):125–136. [PubMed: 15825143]).

3. Nataro JP, Kaper JB, 1998. Diarrheagenic Escherichia coli. Manual of Clin MicrobiolRev 11: 142–201.

4. Anderson JD, Bagamian KH, Muhib F, Amaya MP, Laytner LA, Wierzba T, Rheingans R. Burden of enterotoxigenic Escherichia coli and shigella non-fatal diarrhoeal infections in 79 low-income and lower middle-income countries: a modelling analysis. Lancet Glob Health. 2019;7(3):e321–22. doi: 10.1016/s2214-109x(18)30483-2.

5. M. J. C. van den Beld& F. A. G. Reubsaet. Differentiation between Shigella, enteroinvasive Escherichia coli (EIEC) and noninvasive Escherichia coli. Eur J Clin MicrobiolInfectDis (2012) 31:899–904. DOI 10.1007/s10096-011-1395-7.

6. Protocolos de trabajo Sección de Microbiología, Laboratorio Policlínico Loeri Comba (Guinea Ecuatorial), basados en García LS, Isenberg HD, editor in chief. Clinical Microbiology Procedures Handbook, 3th ed.:American Society for Microbiology; Washington DC, 2007.

7. AssètaKagambèga, OutiMartikainen, TaruLienemann, Anja Siitonen, Alfred S. Traoré, Nicolas Barro, KaisaHaukka. Diarrheagenic Escherichia coli detected by 16-plex PCR in raw meat and beef intestines sold at local markets in Ouagadougou, Burkina Faso.2011.

8. Mostafa Mohamed M. Ali, Zienat Kamel Mohamed, John D. Klena, Salwa Fouad Ahmed, Tarek A. A. Moussa, and Khalifa Sifaw Ghenghesh, 2012. Molecular Characterization of Diarrheagenic Escherichiacoli from Libya.

9. Hebbelstrup Jensen B, Poulsen A, Hebbelstrup Rye Rasmussen S, Struve C, Engberg JH, Friis-Møller A, Boisen N, Jønsson R, Petersen RF, Petersen AM and Krogfelt KA (2017). Genetic Virulence Profile of Enteroaggregative Escherichia coli Strains Isolated from Danish Children with Either Acute or Persistent Diarrhea. Front. Cell. Infect. Microbiol. 7:230. doi: 10.3389/fcimb.2017.00230

10. Diarrheagenic Escherichia coli pathotypes isolated from children with diarrhea in the Federal Capital Territory Abuja, Nigeria.

11. González R, Díaz C, Mariño M, Cloralt R, Pequeneze M, Pérez-Schael I (1997). Age-specific prevalence of Escherichia coli with localized and aggregative adherence in Venezuelan infants with diarrhea. J Clin Microbiol 35: 1103-1107.

12. Jalaluddin S, de Mol P, Hemelhof N, Bauma D, Brasseur P, Hennart R, Lomoyo E, Rowe B, Butzler J (1998). Isolation and characterization of enteroaggregative Escherichia coli (EAggEC) by genotypic and phenotypic markers, isolated from diarrheal children in Congo. Clin MicrobiolInfect 4:213-219.45.

13. Nataro J, Steiner T, Guerrant R (1998). Enteroaggregative Escherichia coli. Emerg Infect Dis 4: 251-261.

14. Iruka N. Okeke Adebayo Lamikanra John Czeczulin Filip Dubovsky James B. KaperJames P. Nataro. 2000. Dis 181: 252-260 Hyg 84: 886–891.Heterogeneous Virulence of Enteroaggregative Escherichia coli Strains Isolated from Children in Southwest Nigeria.

15. Bonkoungou IJ, Lienemann T, Martikainen O, Dembelé R, Sanou I, Traoré AS, Siitonen A, Barro N, Haukka K. Diarrhoeagenic Escherichia coli detected by 16-plex PCR in children with and without diarrhoea in Burkina Faso. Clin MicrobiolInfect. 2012 Sep;18(9):901-6. doi: 10.1111/j.1469-0691.2011.03675.x. Epub 2011 Oct 10.

16. Presterl Elisabeth, Zwick RZ, Reichmann S, Aichelburg A, Winkler S, Kremsner PG, Graninger W, 2003. Frequency and virulence properties of diarrheagenic Escherichia coli in children with diarrhea in Gabon. Am J TropMedHyg 69: 406–410.

17. Jenkins C, Chart H, Willshaw G, Cheasty T, Smith HR. Genotyping of enteroaggregative Escherichia coli and identification of target genes for the detection of both typical and atypical strains. DiagnMicrobiol Infect Dis. 2006;55:13-9.

18. Sarantuya J, Nishi J, Wakimoto N, Erdene S, Nataro JP, Sheikh J, y al. Typical enteroaggregative Escherichia coli is the most prevalent pathotype among E. coli strains causing diarrhea in Mongolian children. J Clin Microbiol.2004.

19. Harrington SM, Sheikh J, Henderson IR, Ruiz-Perez F, Cohen PS, Nataro JP. The Pic protease of enteroaggregative Escherichia coli promotes intestinal colonization and growth in the presence of mucin, Infect Immun , 2009, vol. 77 (pg. 2465-2473).

20. Cennimo D, Abbas A, Huang DB, Chiang T. The prevalence and virulence characteristics of enteroaggregative Escherichia coli at an urgent-care clinic in the USA: a case-control study.j. med microbiol. 2009 Apr;58(Pt 4):403-7.

21. Mora FX, Avilés-Reyes RX, Guerrero-Latorre L, Fernández-Moreira E. Atypical enteropathogenic Escherichia coli (aEPEC) in children under five years old with diarrhea in Quito (Ecuador). 2016 Sep;19(3):157-160. doi:10.2436/20.1501.01.273. https://www.ncbi.nlm.nih.gov/pubmed/28494085.

22. Varela y cols.Escherichiacoli enteropatógeno clásico (EPEC) asociado a casos de diarrea en niños usuarios del Hospital Pereira Rossell.RevMedUrug 2007; 23: 153-163.

23. Theresa J. Ochoa and Carmen A. Contreras Enteropathogenic E. coli (EPEC) infection in children. Oct. 2011, 24(5), 478-483.

24. La estructura económica de Guinea Ecuatorial. Análisis y configuración. Javier Morillas Gómez.

25. De Sousa, C. P., and J. D. Dubreuil. 2001. Distribution and expression of the astA gene (EAST1 toxin) in Escherichia coli and Salmonella. Int. J. Med. Microbiol. 291:15-20. [PubMed].

26. Channah Rock and BereniseRivera, La calidad del agua, mE.coli y su salud. College of Agriculture and Life Sciences, University of Arizona. AZ1624S 2014. Edited by University of Arizona. https://extension.arizona.edu/sites/extension.arizona.edu/files/pubs/az1624s.

27. Oscar G. Gómez-Duarte. Enfermedad diarreica aguda por Escherichiacolienteropatógenas en Colombia. Rev Chilena Infectol 2014; 31 (5): 577-586.

28. AyaLakshmi SS, DhiviyaPrabaa MS, DhivyaMurugan, ShaliniAnandan and BalajiVeeraraghavan. Real- time multiplex PCR assay reveals the increased prevalence of Campylobacter spp and diarrhoeagenic Escherichia coli in humans from Vellore, South India. Journal of Medical Microbiology 2022;71:001478. DOI 10.1099/jmm.0.001478.

29. Instituto Nacional de Estadística (INEGE). Anuario estadístico de Guinea Ecuatorial-2021. Distribución de la población de Guinea Ecuatorial, año censal 2015, pág. 65

30. ÅsaSjöling et al. In vivo expression of the heat stable (*estA*) and heat labile (*eltB*) toxin genes of enterotoxigenic *Escherichia coli* (ETEC). Microbes and infections. Volume 8, Issues 12–13, October 2006, Pages 2797-2802.

31. World Health Organization. (2018). Guías para la calidad del agua de consumo humano: cuarta edición que incorpora la primera adenda, 4a ed + 1a adenda. World Health Organization.

Citation: EñesoEfuá UE, Gil CJC, Llanes AB, Patabobe F, Owono PE, León SH. Diarrhoeagenic *E. coli* and Associated Virulence Factors on Bioko Island, Equatorial Guinea. *SVOA Microbiology 2023,* 4:1, 20-26.