Applied Microbiology: Theory & Technology
http://ojs.wiserpub.com/index.php/AMTT/

UNIVERSAL WISER PUBLISHER

Research Article

Antimicrobial Resistance of Diarrheagenic *E. coli* in the Ashanti Region of Ghana

Frank Awuah[1,2*], Mathew Glover Addo[1], Linda Aurelia Ofori[1]

[1]Theoretical and Applied Biology Department, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[2]Science Department, Abetifi Presbyterian College of Education, Box 19, Abetifi Kwahu, Ghana
Email: pasawuah@gmail.com

Received: 13 May 2023; **Revised:** 4 July 2023; **Accepted:** 17 July 2023

Abstract: This study aimed to investigate the antimicrobial susceptibility of diarrheagenic *E. coli* (DEC) in patients seeking medical attention at Ashanti Regional and St. Michael's Hospitals. A total of 502 people in all were included in the study. To collect the data, a structured questionnaire was used. Participants' stool samples were examined using a biochemical technique to separate *E. coli*, and a two multiplex Polymerase Chain Reaction (PCR) was performed to distinguish DEC from the *E. coli* isolates. Out of the 502 participants, 222 tested positive for DEC. The EUCAST 2022 Breakpoint for the Enterobacterales was utilized to determine the antibiotic susceptibility pattern for each isolate of DEC using the Kirby Bauer disc diffusion method (Bauer, 1966). In all, 83% of the DEC isolates were discovered to be Multi drug resistance (MDR) With an index of 81% > 0.2. The findings of this investigation indicate a high level of antimicrobial resistance to tetracycline, ampicillin, and cotrimoxazole. In the study area, meropenem is suggested for the empirical care of DEC infections. It is advised that both the community and the two healthcare settings routinely screen for antibiotic susceptibility.

***Keywords*:** antimicrobial resistance, diarrheagenic *E. coli*, multi drug resistance, multi drug resistance index, *E. coli*

1. Introduction

Since the time of ancient civilizations, diarrheal illness has been one of the oldest infectious diseases to be recorded [1]. Diarrheal illnesses are a recurring public health problem in poor countries [2]. In a study on diarrhea regarding children [3] observed that children's health continues to be at risk from diarrheal infections. Also looking it from the perspective of low- and middle-income nations, according to [4], children are at the highest risk of diarrhea, the disease is generally one of the leading causes of morbidity and deaths globally among all ages. In terms of this diarrheal threat, underdeveloped countries that are frequently not financially stable enough suffer the most. Diarrheal illnesses are a recurring public health problem in poor countries [5]. With 1 in 10 fatalities in children under the age of five being attributable to diarrhea in 2019, diarrhea is the third highest cause of mortality worldwide, with South Asia and sub-Saharan Africa bearing the brunt of the disease's impact on young children [6].

According to [7], who cited a UNICEF Ghana report from 2016 that said that over 300,000 children under the age of five were expected to have died from diarrhea in Ghana, the burden of the disease is extremely high. According to [8], the Ashanti area had Ghana's third-highest rate of diarrhea episodes in 2011, with an average of 2,218 per 100,000

DOI: https://doi.org/10.37256/amtt.4220233033

inhabitants.

Yandag et al. [9] proposed that diarrheal illnesses are common worldwide and that strains of diarrheagenic *E. coli* (DEC) are the main etiological agents, taking into account the many causative agents of diarrhea. Additionally, Canizalez-Roman et al. [10] reports that diarrheagenic *Escherichia coli* (DEC) is the most prevalent bacterial cause of pediatric diarrhea in various developing nations.

The major varieties of *E. coli* strains can cause diarrhea, each with its own specific pathogenic mechanism [11-14]. Therefore, finding a solution to the DEC's health problem is vital. The human challenge of DEC may be solved by relying on antimicrobials, which are medicines that either eradicate or restrict the growth of bacteria. Nevertheless, [15] attests that antibiotics should not be used in persistent diarrhea except for certain specific pathogens such as Shigella and Clostridium, which is known to cause persistent diarrhea can be treated with antibiotics. Patients with chronic diarrhea typically require some evaluation, but in some cases, a history and physical exam are enough to guide therapy [16]. It is necessary to switch to guided antibiotic therapy in tandem with the advancement of diagnostic clinical microbiology since such empirical antibiotic therapy increased the appearance and spread of antimicrobial resistance [17]. Antimicrobial resistance continues to pose a challenge and threatens global public health [18-19]. It makes the standard medical care offered to patients with a certain disease ineffective, which encourages the persistence and spread of infections [20-22].

When clinical isolates of DEC were analyzed, a substantial prevalence of antibiotic resistance was found [9, 23]. Antimicrobial resistance must therefore be of great concern to public health as it has the potential to negate all the efforts made by medical science over the years to fight the activities of pathogenic microorganisms.

The emergence of antimicrobial resistance if not addressed is likely to send public health to the darker days in which antimicrobials were not discovered. It is a well-known fact that antimicrobial resistance occurs in humans as a result of drugs through the transfer of energy in food from one organism to the other in the environment [24]. This can be explained by the overuse of antimicrobials in animal feed that has the side effect of promoting the rise and the spread of antimicrobial resistance although the original intention might be to maintain the health and productivity of livestock. Abdalla et al. [25] reported that food obtained from animal sources can act as a source of pathogenic *E. coli* which has developed resistance to antibiotics. This might transfer antimicrobial resistance and virulence genes to microbes in the gastrointestinal tract. This has the potential to threaten human health as it could subject the treatment of infections of *E. coli* causing diarrhea to difficulty.

It is therefore of paramount importance to examine the antimicrobial susceptibility of diarrheagenic *E. coli* in areas where much of such studies have not been conducted taking into consideration commonly used antibiotics. This is to say that for an effective treatment of diarrhea in general an antimicrobial susceptibility of the antimicrobial agents must be determined in order to evaluate the efficacy of the antimicrobial or the commonly used antibiotic in the case of bacterial related diarrhea. In spite of this not much is reported on the antimicrobial susceptibility of the diarrheagenic *E. coli* in this area of the current study.

In order to address the issue of diarrhea caused by *E. coli* and the threat of antibiotic resistance, which is currently a public health challenge, this study sought to examine the antimicrobial susceptibility of diarrheagenic *E. coli* in this area of the current study. As a result, it has helped to alert public health authorities to the need for intervention.

2. Materials and methods

2.1 *The research site and design*

This cross-sectional study was conducted at the Ashanti Regional and St, Michael's hospitals in the Ashanti Region of Ghana between July 2020 and July 2021. Asokwa Municipal Assembly is home to the Ashanti Regional Hospital, formerly known as the Kumasi South Hospital with coordinates 6.6513° N, 1.5864° W while St. Michael's Hospital is located in the Bosomtwe District with the coordinates, 6°33'32.9"N, 1°30'45.4"W. A total of 502 people who consented to the study were recruited as study participants.

2.2 *Sampling procedure*

A convenient sampling method was employed to recruit participants for this study. Hence, a total of 502 people

who consented to the study were recruited as study participants. From these patients who visited the aforementioned hospitals, a total of 502 stool samples, one per participant were taken. Out of the total individuals recruited into this study, 321 (63.94%) were females, while 181 (36.05%) were men. However,159 of these participants had diarrheal symptoms, while 343 people did not. The age range of patients was from 0-5 to ≥ 42 years.

2.3 *Isolation of E. coli from the faecal samples*

The stool samples were streaked on MacConkay agar (OXOID CM0007 MacCONKEY AGAR, Oxoid Ltd., United Kingdom). Bright pink-red colonies after an overnight culture were thought to contain bacteria like *E. coli*, which may digest lactose. Each sample was subcultured onto Cystine-Lactose Electrolyte Deficient Agar (CLED) with a single isolated colony, which was then incubated at 37 °C for 24 hours. Yellow colonies could potentially contain *E. coli*. A test tube containing tryptophan broth and an isolate from the CLED was incubated for 24 hours at 44 °C. The Kovacs' reagent was then added to the tryptophan broth positive tubes. Following the completion of successful indole and Eosin Methylene Blue (EMB) Agar assays, all isolates were determined to be *E. coli*. In order to extract the DNA, the isolates were then placed in sample tubes with Mueller-Hinton agar.

2.4 *Extracting DNA and identifying pathotypes*

Genomic DNA was extracted from the isolated bacterial cells using the boiling procedure, and the template was then exposed to multiplex PCR utilizing certain primers, as stated by [12]. To detect the genes of interest, which were the virulence markers, the study used two multiplex PCRs.

2.4.1 *Multiplex Polymerase Chain Reaction (PCR) Assay 1*

The detection of enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), and enterotoxigenic *E. coli* (ETEC) was the focus of PCR 1. A 25 µl Ben Taq mixture (Beneficial Bio, UK), 12 µl of nuclease-free water, 5 µl DNA template, and 1 µl each of the forward and reverse primers (hlyA for EHEC, CVD432 for EAEC, elt, and Stla for ETEC isolates) were used to carry out the optimized process. DNA samples containing the relevant virulence gene or genes were used as positive controls in each test. The negative control, however, was sterile distilled water.

Each test was conducted in accordance with the ideal cycling conditions shown below: 35 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 5 min were then performed.

2.4.2 *Multiplex Polymerase Chain Reaction (PCR) 2*

The PCR 2 employed eaeA and bfpA for isolates of EPEC and ial for isolates of EIC, despite the fact that the methodology was the same as the PCR 1. Additionally, PCR 2 needed 14 µl of nuclease-free water as opposed to PCR 1's 12 µl.

The results of the PCR reactions were subjected to gel electrophoresis in both instances (PCR 1 and PCR 2) using 1.5% (W/V) agarose gel in 120 mil of buffer solution. Ethidium bromide, an intercalating dye, was used to stain the gel, enabling the visualization of the DNA bands during UV photography.

2.5 *Antimicrobial susceptibility testing*

Antimicrobial susceptibility pattern for each *E. coli* isolate was determined by employing the Kirby Bauer disc diffusion method [26] using EUCAST 2022 Breakpoint for the Enterobacterales. The pure bacterial isolates earlier grown on the sterile nutrient agar were inoculated on the saline solution to make a suspension. The inoculum of turbidity standard was matched with McFarland 0.5 turbidity standard. Sterile cotton swab was dipped into the suspension forming a uniform lawn over the entire surface of the Mueller-Hinton agar. In all, the bacterial isolates were screened for susceptibility patterns against twelve (12) antimicrobial agents. These were as follows: Ampicillin (AMP; 10 µg), Ampicillin/Sulbactam (SAM; 20 µg), Cefuroxime (CXM; 30 µg), Cetriaxone (CRO; 30 µg), Ceftazidime (CAZ; 10 µg), Cefepime (FEF; 30 µg), Meropem (MEM; 10 µg), Tetracycline (TE; 3 µg), Gentamicin (GN; 10 µg), Ciprofloxacin (CIP; 5 µg), Cotrimaxazole (SXT; 25 µg), and Chloramphenicol (CL; 30 µg). With the aid of a ruler, the zone of inhibition

was determined to the nearest millimeter after an overnight incubation at a temperature of 37 °C . The classification of these measurements as sensitive (S), intermediate (I) and resistant (R) were done according to the EUCAST 2022 Clinical Breakpoint for the Enterobacterales.

2.6 *Data analysis*

Descriptive statistics were used to summarize the findings. Tables and charts were used to show frequencies where appropriate. To determine statistical difference, when necessary, the chi-square test as approved by [27] and [28] was performed. Statistics were judged to be significant for variables having a p value less than 0.05 at a 95% confidence level.

3. Results

3.1 *E. coli isolates*

From the 502 stool samples that were gathered, the study was able to isolate 312 (62.15%, n/N = 312/502) *E. coli* isolates. It was observed that 71.15% (n/N = 222/312) of the 312 *E. coli* isolates tested positive for DEC with a prevalence of 44.22% (n/N = 222/502) among the study population. Statistically the difference between the *E. coli* isolates which tested positive for DEC and those 90 (28.85%, n/N = 90/312) which tested negative for DEC was significant ($p < 0.05$) . Also, there was statistical difference between the prevalence of DEC positive participants and the participants 55.78% (n/N = 280/502) who tested negative for DEC ($p < 0.05$).

Table 1. Hospital-related distribution of EC and DEC isolates detected by biochemical Test and PCR respectively

Hospital	Samples	EC	DEC	Prevalence of DEC (%)
Ashanti Regional Hospital	201	122	90	44.78 N = 201
St. Michael's Hospital	301	190	132	43.85 N = 301
Total	502	312	222	44.22 N = 502

EC = *E. coli,* DEC = *diarrheagenic E. coli*

Participants from Ashanti Regional Hospital had a DEC prevalence of 44.78% (n/N = 90/201), compared to 43.85% (n/N = 132/301) for St. Michael's Hospital. The DEC prevalence between the two hospitals did not statistically differ significantly ($P > 0.05$)). The antibiotic susceptibility test was then performed on the DEC isolates. Table 1 shows the results of *E. coli* (EC) and DEC positive isolates as obtained by the biochemical test and the PCR respectively while Figure 1 shows product of agarose gel electrophoresis after PCR amplification.

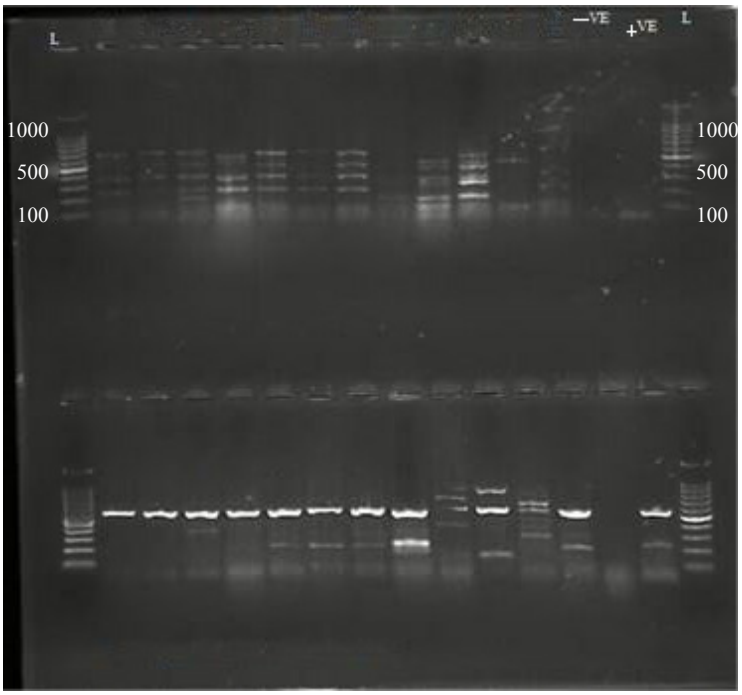

Figure 1. Agarose gel electrophoresis after PCR amplification of target genes of DEC isolates
L = ladder (100 bp molecular marker), VE = positive control, VE = negative control

3.2 *Antimicrobial susceptibility of diarrheagenic E. coli*

3.2.1 *Comparison of isolated E. coli's antibiotic sensitivity profiles*

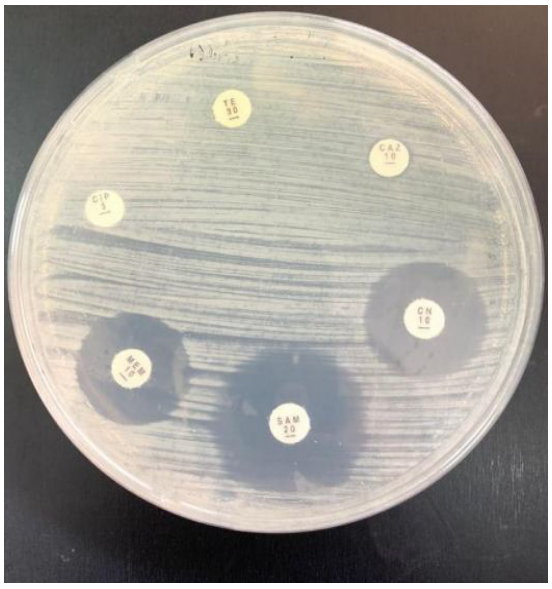

Figure 2. Photograph of the antimicrobial susceptibility study

A total of 12 antibiotics were used on the DEC isolates. DEC, with a prevalence of 44.22%, displayed rather significant antimicrobial resistance to rates to the majority of the drugs examined. Each of the 12 antimicrobial agents

experienced resistance against it by at least 9 (4.05%) of these 222 isolates. The isolates were mostly resistant to Ampicillin (AM) 84% (n/N = 195/222) followed by Cotrinoxazole (CXM) 82.43% (N/n = 183/222), tetracycline (TETRA) 81.98% (n/N = 182/222), Ciprofloxacin (CIPRO) 55.86% (n/N = 124/222), Cefuroxime (CXM) 54.50% (n/N = 121/222), Cefepime (FEP) 54.05% (n/N = 120/222), Ceftriaxone (CRO) 52.25% (n/N = 116/222), Ampicillin/Sulbactam (SAM)14.86% (n/N = 33/22), Chloramphenico (C) 13.06% (n/N = 29/222), Ceftazidime (CAZ) 9.46% (21/222), Gentamicin (CN) 4.05% (n/N = 9/222). However, all the 222 isolates which tested positive for diarrheagenic *E. coli* were sensitive to Meropenem.

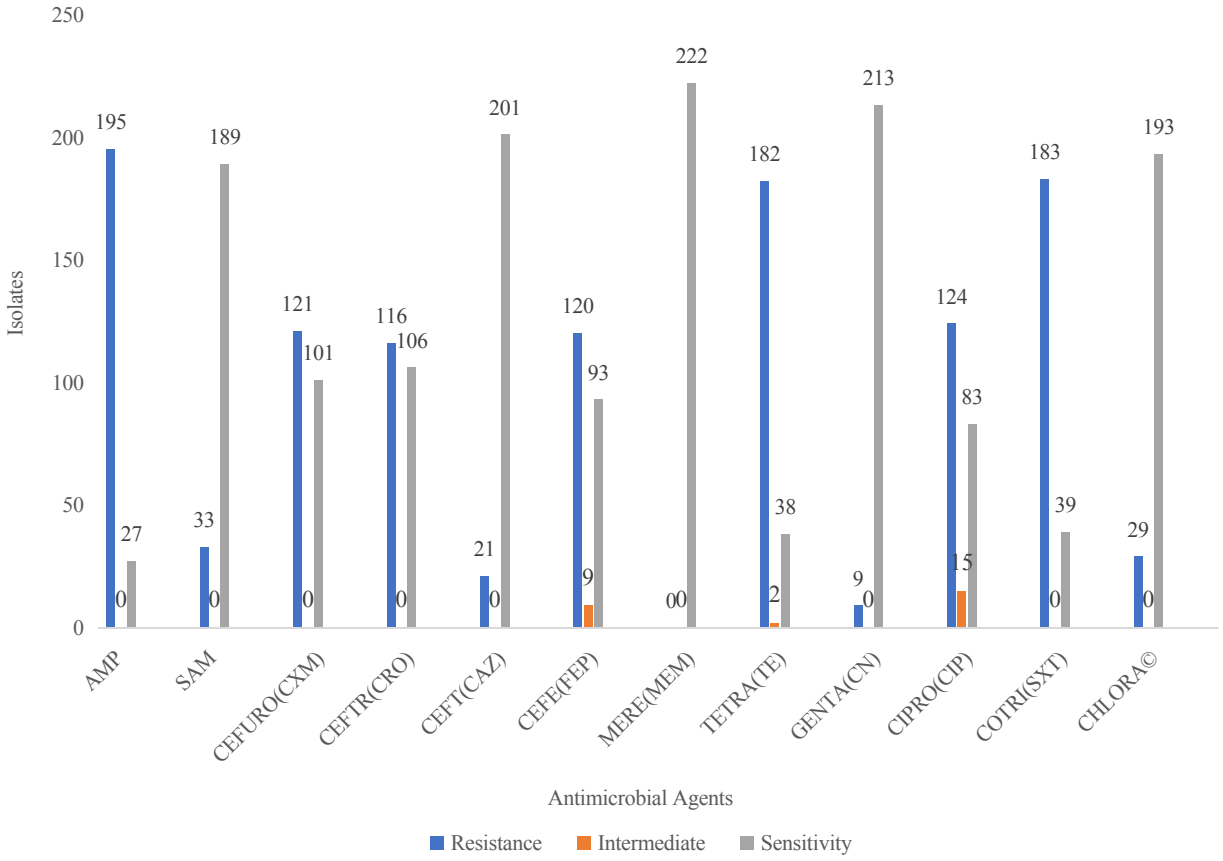

Figure 3. Antimicrobial susceptibility of diarrheagenic *E. coli*

Figure 3 illustrates the susceptibility pattern of diarrheagenic *E. coli* while Figure 2 shows a photograph of the antimicrobial test of this study.

3.2.2 *Multiple drug resistance (MDR)*

As shown below in Figure 4 overall, 83% (n/N = 184/222) of the DEC isolates were found to be MDR. Site specific multiple antimicrobial resistance of the 184 isolates were 56% and 44% for St. Michael's Hospitals and Ashanti regional hospital respectively. Site specific multiple drug resistance is shown in Figure 5.

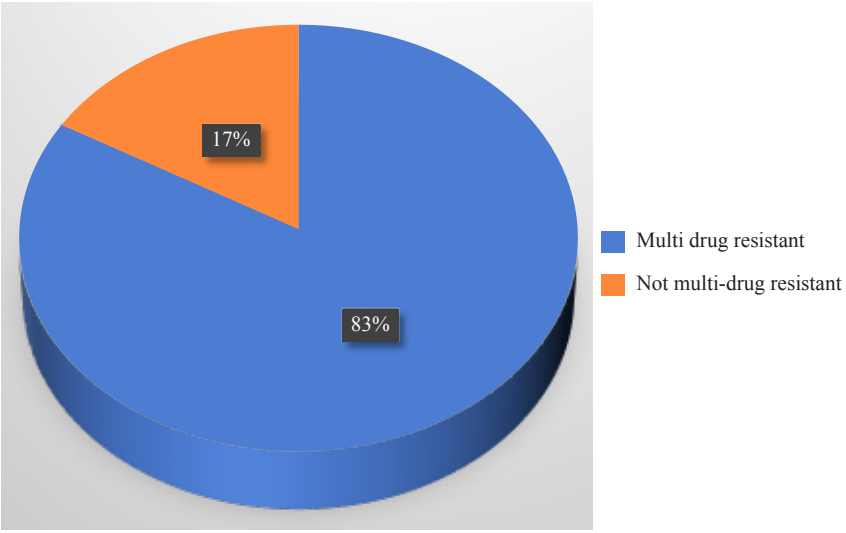

Figure 4. Proportion of diarrheagenic *E. coli* isolates which are multi drug resistant and those which are not.

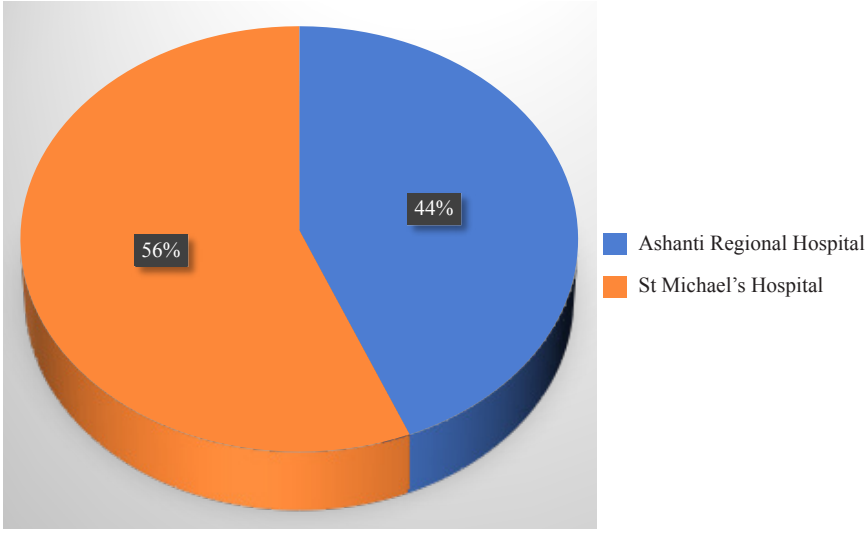

Figure 5. Site specific multiple drug resistant *E. coli* isolates in relation to hospital

3.2.3 *Multiple Antibiotic Resistance (MAR) index*

When the multidrug resistance of the DEC isolates was calculated, 81% were found to have an index of > 0.2. The results indicate that the lowest MAR index of 0.0 was recorded by the 5.0% (n/N = 11/222) of the *E. coli* isolates with the highest MAR index of 0.9 experienced by 0.5% (n/N = 1/222) of the isolates. The MAR index with the greatest number of isolates, 34.7% (n/N = 77/222) was 0.6, and the MAR index with the second-highest number of isolates 23.9% (n/N = 53/222) was 0.3. Table 2 shows the MAR indices of the 222 *E. coli* isolates of this study.

Table 2. MAR indices of Diarrheagenic *E. coli*

MAR Index	No of isolates (%)
0	11 (5.0)
0.1	18 (8.1)
0.2	13 (5.9)
0.3	53 (23.9)
0.4	9 (4.1)
0.5	23 (10.4)
0.6	77 (34.7)
0.7	14 (6.3)
0.8	3 (1.4)
0.9	1 (0.5)
1	0 (0)

Total number of DEC isolates n = 222

4. Discussion

4.1 *Antibiotic sensitivity profiles of isolated E. coli.*

The development of multidrug-resistant bacteria continues to pose a serious danger to medicine and is one of the key obstacles to the prevention and treatment of microbial illnesses [29]. Meanwhile, diarrheal diseases are common with worldwide distribution, and *diarrheagenic Escherichia coli* (DEC) strains are the main causative agents [9]. Not much is reported on the antimicrobial susceptibility of DEC in this study area. Therefore, this study has examined the antibiotic susceptibility profiles of *E. coli* that causes diarrhea in people who visit the chosen hospitals in the area.

This study found that DEC has a high level of overall antimicrobial resistance. At least 9 (4.05%) of these 222 isolates showed resistance to each of the 12 antimicrobial drugs. The majority of the isolates were resistant to ampicillin (195; 87.84%), followed by cotrimoxazole (183; 82.43%), tetracycline (182; 81.98%). This result is comparable to that of [30], who found that 89% of all the *E. coli* isolates were ampicillin-resistant and 83% were tetracycline-resistant. In their study, [31] reported 93.3% DEC isolates were resistant to cotrimoxazole and none of them exhibited resistance to ciprofloxacin. However, this study has recorded a decrease in the DEC resistance (82.43%) to cotrimoxazole and an increase in the resistance (55.86%) to ciprofloxacin. This difference might be attributed to increase and a decrease abuse of ciprofloxacin and cotrimoxazole respectively. The discrepancies in antimicrobial resistance might be due to the difference in which these antibiotics are reasonably priced and accessible at the market [32]. The abuse of antibiotics in the treatment of infectious disorders and the usage of antibiotics as a preventative measure may be to blame for this conclusion about the resistance of the DEC isolates to antibiotics in this study. This is supported by the fact that the majority of the isolates were resistant to commonly used antibiotics such ampicillin, cotrimoxazole and tetracycline. In some nations, these are the antibiotics that are frequently administered to treat enteritis brought on by bacteria and hence are commonly abused [33].

However, Meropenem was detected to be effective against all 222 isolates that tested positive for diarrheagenic *E. coli*. This finding is in conformity with [34] who observed that only Meropenem and the other Carbapenems were

effective against DEC. The effectiveness of Meropenem as an antimicrobial agent can be explained that meropenem, like other carbapenems stops the formation of the bacterial cell wall which inhibits growth and results in cell death [35]. Carbapenem-resistant enterobacterales (CRE) frequently produce ambler class. Meropenem, a carbapenem antibiotic, therefore has an effective antimicrobial property against DEC.

4.2 *Multi drug resistance (MDR)*

This analysis discovered MDR of DEC for the first time in the research area. The study found that 83% of the *E. coli* isolates were MDR (Table 2). This is higher than the 76.51% of the *E. coli* isolates which exhibited MDR as reported by the [36]. The practice of unqualified clinicians prescribing drugs to patients has the potential to promote MDR resistance as observed by this study [37]. In this case there might be abuse of the drugs either by overuse or lower than what is required to treat the disease. Antibiotic usage and overuse in human medicine have led to the development of MDR *E. coli* in human feces as well and these procedures have caused MDR *E. coli* to coexist in these important human intestinal disease reservoirs [38-40]. This can be explained in that when *E. coli* is exposed to the drugs, they are triggered to evolve adaptations through mutation to develop feature to enable the organism to withstand the antibiotics [41]. In a similar vein, organisms exposed to antibiotics through human or animal feed are stimulated to undergo mutations that render them immune to routinely used antimicrobial substances [38]. This study demonstrates this pattern, with a high rate of resistance frequently observed.

4.3 *Multiple antibiotic resistance index of DEC*

Multiple antibiotic resistance index, according to [42], is crucial because it helps microbiologists to assess health risks and gauge the prevalence of antibiotic resistance. When treating infected patients with antibiotics that are frequently used, MAR index analysis has been utilized to distinguish isolates from various sources [36]. In comparison to other ways of locating the source of bacteria, it is inexpensive, quick, simple to use, and doesn't call for expensive equipment or specialized training [43].

This study discovered multidrug resistance index regarding people attending the two institutions considered. This research found that a greater percentage (81%) of *E. coli* isolates had MAR indices greater than 0.2 (Table 2). It can be inferred that greater proportion of the participants from whom the isolates were obtained either originated from locations where antibiotics are commonly used or the organism had previously been exposed to the commonly used antimicrobial medications. This can be explained by the fact that sources of contamination with MAR index > 0.2, according to [43], are those where antibiotics are often used. This suggests that the isolates are coming from areas where there are greater dangers of developing antibiotic resistance. Most of this multidrug resistant *E. coli* is acquired from the participants' diverse communities from where they attend these hospitals because the majority of the isolates were found from individuals in the outpatient department with very few of them coming from the inpatient department.

5. Conclusion

Generally, this study indicated high resistance of *DEC* against most of the commonly used antibiotics. The findings of this investigation indicate a very high level of antimicrobial resistance to ampicillin (87.84%), cotrimoxazole (82.43%) and tetracycline (81.98%) which is comparable with other studies. This study's very high MAR index of DEC (81%) above 0.2 indicates that the isolates are from sources with a high risk of antibiotic resistance.

For the empirical management of DEC infections in the research area, meropenem is advised. It is recommended to frequently screen for antibiotic susceptibility in both the community and the two healthcare settings.

Conflict of interest

The authors declare no competing financial interest.

References

[1] Lim ML, Wallace MR. Infectious diarrhea in history. *Infectious Disease Clinics of North America*. 2004; 18(2): 261-274. Available from: https://doi.org/10.1016/j.idc.2004.01.006 [Accessed 20th June 2023].

[2] UNICEF. *Under-five mortality*. UNICEF; 2023. Available from: https://data.unicef.org/topic/child-survival/under-five-mortality/ [Accessed 20th April 2023].

[3] Powell H, Liang Y, Neuzil KM, Jamka LP, Nasrin D, Sow SO, et al. A description of the statistical methods for the Vaccine Impact on Diarrhea in Africa (VIDA) study. *Clinical Infectious Diseases*. 2023; 76(Suppl 1): S5-S11. Available from: https://doi.org/10.1093/cid/ciac968 [Accessed 12th June 2023].

[4] Selendy JM, Selendy H, Farmer P, Fawzi W. *Water and Sanitation-Related Diseases and the Changing Environment: Challenges, Interventions, and Preventive Measures*. John Wiley & Sons, Inc.; 2019. Available from: https://doi.org/10.1002/9781119415961 [Accessed 11th January 2022].

[5] Birhan TA, Bitew BD, Dagne H, Amare DE, Azanaw J, Genet M, et al. Prevalence of diarrheal disease and associated factors among under-five children in flood-prone settlements of Northwest Ethiopia: A cross-sectional community-based study. *Frontiers in Pediatrics*. 2023; 11: 1056129. Available from: https://doi.org/10.3389/fped.2023.1056129 [Accessed 11th January 2022].

[6] Paulson KR, Kamath AM, Alam T, Bienhoff K, Abady GG, Abbas J, et al. Global, regional, and national progress towards Sustainable Development Goal 3.2 for neonatal and child health: All-cause and cause-specific mortality findings from the Global Burden of Disease Study 2019. *The Lancet*. 2021; 398(10303): 870-905. Available from: https://doi.org/10.1016/S0140-6736(21)01207-1 [Accessed 11th January 2022].

[7] Tetteh J, Takramah WK, Ayanore MA, Ayanore AA, Bisung E, Alamu J. Trends for diarrhea morbidity in the Jasikan District of Ghana: estimates from district level diarrhea surveillance data, 2012-2016. *Journal of Tropical Medicine*. 2018; 2018: 4863607. Available from: https://doi.org/10.1155/2018/4863607 [Accessed 11th January 2022].

[8] Peprah NY, Ameme DK, Sackey S, Nyarko KM, Gyasi A, Afari E. Pattern of diarrheal diseases in Atwima Nwabiagya District-Ghana, 2009-2013. *The Pan African Medical Journal*. 2016; 25(Suppl 1): 15. Available from: https://doi.org/10.11604/pamj.supp.2016.25.1.6207 [Accessed 12th January 2022].

[9] Yandag M, Tsend-Ayush A, Gunregjav N, Erdenebayar O, Byambadorj B, Juniichiro N, et al. Detection and antibiotic resistance of diarrheagenic Escherichia coli from patients with diarrhea in Ulaanbaatar, Mongolia. *The Journal of Infection in Developing Countries*. 2023; 17(2): 202-209. Available from: https://doi.org/10.3855/jidc.17256 [Accessed 11th January 2022].

[10] Canizalez-Roman A, Velazquez-Roman J, Valdez-Flores MA, Flores-Villaseñor H, Vidal JE, Muro-Amador S, et al. Detection of antimicrobial-resistance diarrheagenic Escherichia coli strains in surface water used to irrigate food products in the northwest of Mexico. *International Journal of Food Microbiology*. 2019; 304: 1-10. Available from: https://doi.org/10.1016/j.ijfoodmicro.2019.05.017 [Accessed 12th January 2022].

[11] Prah I, Ayibieke A, Nguyen TT, Iguchi A, Mahazu S, Sato W, et al. Virulence profiles of diarrheagenic escherichia coli isolated from the western region of Ghana. *Japanese Journal of Infectious Diseases*. 2021; 74(2): 115-121. Available from: https://doi.org/10.7883/yoken.JJID.2020.356 [Accessed 12th January 2022].

[12] Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic Escherichia coli by multiplex PCR. *Indian Journal of Medical Microbiology*. 2012; 30(3): 279-284. Available from: https://doi.org/10.4103/0255-0857.99485 [Accessed 12th January 2022].

[13] Aranda KR, Fagundes-Neto U, Scaletsky IC. Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic Escherichia coli and Shigella spp. *Journal of Clinical Microbiology*. 2004; 42(12): 5849-5853. Available from: https://doi.org/10.1128/JCM.42.12.5849-5853.2004 [Accessed 13th January 2022].

[14] Nataro JP, Kaper JB. Diarrheagenic escherichia coli. *Clinical Microbiology Reviews*. 1998; 11(1): 142-201. Available from: https://journals.asm.org/doi/full/10.1128/cmr.11.1.142 [Accessed 12th January 2022].

[15] Das JK, Ali A, Salam RA, Bhutta ZA. Antibiotics for the treatment of Cholera, Shigella and Cryptosporidiumin children. *BMC Public Health*. 2013; 13(3): 1-9. Available from: https://bmcpublichealth.biomedcentral.com/counter/pdf/10.1186/1471-2458-13-S3-S10.pdf [Accessed 12th January 2022].

[16] Schiller LR, Pardi DS, Sellin JH. Chronic diarrhea: Diagnosis and management. *Clinical Gastroenterology and Hepatology*. 2017; 15(2): 182-193. Available from: https://sci-hub.hkvisa.net/10.1016/j.cgh.2016.07.028 [Accessed 12th January 2022].

[17] Shim H. Three innovations of next-generation antibiotics: Evolvability, specificity, and non-immunogenicity. *Antibiotics*. 2023; 12(2): 204.

[18] Tariq KA. Antimicrobial resistance, climate change, and public health. *Climate Change And Microbial Diversity.*

1st ed. Apple Academic Press; 2022. p.109-141.
[19] Woods M, McAlister JA, Geddes-McAlister J. A one health approach to overcoming fungal disease and antifungal resistance. *WIREs Mechanisms of Disease*. 2023; 15(14): e1610. Available from: https://wires.onlinelibrary.wiley.com/doi/full/10.1002/wsbm.1610 [Accessed 12th June 2023].
[20] Chiș AA, Rus LL, Morgovan C, Arseniu AM, Frum A, Vonica-Țincu AL, et al. Microbial resistance to antibiotics and effective antibiotherapy. *Biomedicines*. 2022; 10(5): 1121. Available from: https://doi.org/10.3390/biomedicines10051121 [Accessed 12th January 2023].
[21] Wozniak TM, Cuningham W, Ledingham K, McCulloch K. Contribution of socio-economic factors in the spread of antimicrobial resistant infections in Australian primary healthcare clinics. *Journal of Global Antimicrobial Resistance*. 2022; 30: 294-301. Available from: https://doi.org/10.1016/j.jgar.2022.06.005 [Accessed 12th January 2023].
[22] Vallabhaneni S, Jackson BR, Chiller TM. Candida auris: an emerging antimicrobial resistance threat. *Annals of Internal Medicine*. 2019; 171(6): 432-433. Available from: https://doi.org/10.7326/M19-2205 [Accessed 12th January 2022].
[23] Iguchi A, Takemura T, Ogura Y, Nguyen TT, Kikuchi T, Okuno M, et al. Genomic characterization of endemic diarrheagenic Escherichia coli and Escherichia albertii from infants with diarrhea in Vietnam. *PLOS Neglected Tropical Diseases*. 2023; 17(4): e0011259. Available from: https://doi.org/10.1371/journal.pntd.0011259 [Accessed 12th January 2022].
[24] Rhodes J, Abdolrasouli A, Dunne K, Sewell TR, Zhang Y, Ballard E, et al. Population genomics confirms acquisition of drug-resistant Aspergillus fumigatus infection by humans from the environment. *Nature Microbiology*. 2022; 7(5): 663-674. Available from: https://doi.org/10.1038/s41564-022-01091-2 [Accessed 12th August 2022].
[25] Abdalla SE, Abia AL, Amoako DG, Perrett K, Bester LA, Essack SY. Food animals as reservoirs and potential sources of multidrug-resistant diarrheagenic E. coli pathotypes: Focus on intensive pig farming in South Africa. *Onderstepoort Journal of Veterinary Research*. 2022; 89(1): 1963. Available from: https://doi.org/10.4102/ojvr.v89i1.1963 [Accessed 12th August 2022].
[26] Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*. 2009; 15: 55-63. Available from: https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-DiffusionSusceptibility-Test-Protocol-pdf.pdf [Accessed 12th August 2022].
[27] Richardson JT. The analysis of 2 × 2 contingency tables-Yet again. *Statistics in Medicine*. 2011; 30(8): 890. Available from: https://sci-hub.hkvisa.net/https://doi.org/10.1002/sim.4116 [Accessed 12th August 2022].
[28] Campbell I. Chi-squared and Fisher-Irwin tests of two-by-two tables with small sample recommendations. *Statistics in Medicine*. 2007; 26(19): 3661-3675. Available from: https://doi.org/10.1002/sim.2832 [Accessed 12th August 2022].
[29] Msolo L, Iweriebor BC, Okoh AI. Antimicrobial resistance profiles of diarrheagenic E. coli (DEC) and Salmonella species recovered from diarrheal patients in selected rural communities of the amathole district municipality, Eastern Cape Province, South Africa. *Infection and Drug Resistance*. 2020; 13: 4615-4626. Available from: https://doi.org/10.2147/IDR.S269219: 4615-26 [Accessed 11th January 2022].
[30] Heidary M, Momtaz H, Madani M. Characterization of diarrheagenic antimicrobial resistant Escherichia coli isolated from pediatric patients in Tehran, Iran. *Iranian Red Crescent Medical Journal*. 2014; 16(4): e12329. Available from: https://doi.org/10.5812/ircmj.12329 [Accessed 11th January 2022].
[31] Moyo SJ, Gro N, Matee MI, Kitundu J, Myrmel H, Mylvaganam H, et al. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. *BMC Pediatrics*. 2011; 11: 1-6. Available from: https://link.springer.com/article/10.1186/1471-2431-11-19 [Accessed 11th January 2022].
[32] Adu-Poku M, Addo MG. Antimicrobial susceptibility profile of urinary tract pathogenic infections in diabetic patients attending a health facility in Kumasi, Ghana. *International Journal of Pathogen Research*. 2020; 4(2): 37-45. Available from: Antimicrobial-Susceptibility-Profile-of-Urinary-Tract-Pathogenic-Infections-in-Diabetic-Patients-Attending-a-Health-Facility-in-Kumasi-Ghana.pdf [Accessed 11th January 2022].
[33] Okeke IN, Lamikanra A, Edelman R. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. *Emerging Infectious Diseases*. 1999; 5(1): 18. Available from: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=0e4b6d2b61d5e4f551798b5b446f8302e4d27595 [Accessed 11th January 2022].
[34] Liu S, Zhou Y, Niu X, Wang T, Li J, Liu Z, et al. Magnolol restores the activity of meropenem against NDM-1-producing Escherichia coli by inhibiting the activity of metallo-beta-lactamase. *Cell Death Discovery*. 2018; 4(1): 28. Available from: https://www.nature.com/articles/s41420-018-0029-6 [Accessed 11th January 2022].

[35] Al-Shaer MH, Rubido E, Cherabuddi K, Venugopalan V, Klinker K, Peloquin C. Early therapeutic monitoring of β-lactams and associated therapy outcomes in critically ill patients. *Journal of Antimicrobial Chemotherapy*. 2020; 75(12): 3644-3651. Available from: https://academic.oup.com/jac/article/75/12/3644/5903832 [Accessed 11th January 2022].
[36] Niranjan V, Malini A. Antimicrobial resistance pattern in Escherichia coli causing urinary tract infection among inpatients. *The Indian Journal of Medical Research*. 2014; 139(6): 945. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4165009/ [Accessed 11th January 2022].
[37] Chokshi A, Sifri Z, Cennimo D, Horng H. Global contributors to antibiotic resistance. *Journal of Global Infectious Diseases*. 2019; 11(1): 36. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6380099/ [Accessed 11th January 2022].
[38] Joseph AA, Odimayo MS, Olokoba LB, Olokoba AB, Popoola GO. Multiple antibiotic resistance iIndex of EscherichiaColi isolates in a tertiary hospital in south-west Nigeria. *Medical Journal of Zambia*. 2017; 44(4): 225-32. Available from: Multiple-Antibiotic-Resistance-Index-of-EscherichiaColi-Isolates-in-a-Tertiary-Hospital-in-South-West-Nigeria.pdf (researchgate.net) [Accessed 11th January 2022].
[39] Novick RP. The development and spread of antibiotic-resistant bacteria as a consequence of feeding antibiotics to livestock. *Annals of the New York Academy of Sciences*. 1981; 368(1): 23-60. Available from: https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.1981.tb15430.x [Accessed 10th April 2022].
[40] Isenberg HD, Berkman JI. The role of drug-resistant and drug-selected bacteria in nosocomial disease. *Annals of the New York Academy of Sciences*. 1971; 182(1): 52-58. Available from: https://nyaspubs.onlinelibrary.wiley.com/doi/epdf/10.1111/j.1749-6632.1971.tb30642 [Accessed 10th April 2022].
[41] Van den Bergh B, Michiels JE, Wenseleers T, Windels EM, Boer PV, Kestemont D, et al. Frequency of antibiotic application drives rapid evolutionary adaptation of Escherichia coli persistence. *Nature Microbiology*. 2016; 1(5): 1-7. Available from: https://www.biw.kuleuven.be/dtp/cmpg/spi/pdf/2016-NM-VandenBergh.pdf [Accessed 10th April 2022].
[42] Riaz S, Faisal M, Hasnain S. Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum β-lactamase (ESBL) producing Escherichia coli and Klebsiella species in Pakistan. *African Journal of Biotechnology*. 2011; 10(33): 6325-6331. Available from: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=c8bbae8e25f9a29ac78aaefc009f741564e4d043 [Accessed 10th April 2022].
[43] Osundiya OO, Oladele RO, Oduyebo OO. Multiple antibiotic resistance (MAR) indices of Pseudomonas and Klebsiella species isolates in Lagos University Teaching Hospital. *African Journal of Clinical and Experimental Microbiology*. 2013; 14(3): 164-168. Available from: https://afrjcem.org/wp-content/uploads/2019/03/Multiple-Antibiotic-Resistance-MAR-indices-of-Pseudomonas-and-Klebsiella-species-isolates-in-Lagos-University-Teaching-Hospital.pdf [Accessed 10th April 2022].