



Antibiotics Susceptibility Pattern and Plasmid Profile of *Escherichia coli* O157 Isolated from Fecal Samples of Apparently Healthy Children

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Authors' contributions

This work was carried out in collaboration between all authors. Author MKO designed the study. Author DEF wrote the protocol for the manuscript and performed the statistical analysis. Authors DEF and OO managed the analyses of the study. Author OO wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Escherichia coli O157:H7 is an enterohemorrhagic serotype of the bacterium *Escherichia coli*. It is a cause of severe colitis, bloody diarrhoea and Hemolytic uremic syndrome (HUS) which is associated with life threatening systemic manifestations. This study was designed to investigate the occurrence, antibiotics susceptibility pattern and plasmid profile of *E. coli* O157 from fecal samples of children. A total of 311 fecal samples were collected from apparently healthy children (111 males and 200 females) within the age range of 0-5 years at Mother and Child Hospital, Anchorite Daycare, Women's' Forum Daycare and FUTA Daycare in Akure, Ondo State, Nigeria. The fecal samples were screened using standard bacteriological and serological techniques. Twenty one (6.75%) of the samples were positive for *E. coli* O157. Antibiotics susceptibility testing was carried out using disk diffusion technique, many of the isolates displayed multi-drug resistance to the antibiotics employed in this study. The resistance pattern observed is as follows: Ceftriazone 19 (90.5%), Amoxycilin 18 (85.7%), Tetracycline 13 (61.9%), Gentamycin 11(52.4%), Cotrimoxazole 9 (42.9%), Augmentin 8 (25.8%), Ofloxacin 2 (9.5%), Ciprofloxacin 2 (9.5%) and Nitrofurantoin 2 (9.5%). Plasmid profiling of the strains revealed the presence of multiple plasmids

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ranging in sizes from 4.0 to 17.5 kb. Conclusively, since the mode of transmission of this pathogen is via the fecal-oral route and an exceptionally low dose of the organism is able to cause infection. It is therefore recommended that routine screening is carried out to determine the carrier rate of the organism, more public awareness should be carried out to educate the community on need for adherence to personal hygiene, environmental hygiene and good food handling practices to prevent an outbreak.

Keywords: *Escherichia coli* O157; antibiotics resistance; plasmid profile.

1. INTRODUCTION

Several studies have shown that infection with *E. coli* O157 is responsible for most cases of Hemolytic uremic Syndrome (HUS) which is a major cause of acute renal failure in children [1, 2,3], thrombotic thrombocytopenic purpura [4]; and a common cause of bloody and non bloody diarrhoea [5,6]. The organism evolved and acquired specific virulence factors which enables it to infect and colonize the human colon, usually without invasion of the blood stream [7].

Humans become infected by ingestion of contaminated food and water, or by direct contact with infected animals [8,9]. Transmission of *E. coli* O157: H7 infection through person-to-person contact, particularly in institutional settings like day care centres nursing homes and hospitals have been documented [10]. The spread of *E. coli* O157: H7 infection from person-to-person is a result of the low infectious dose of the pathogen [6,11]. Shiga toxin-producing *E. coli* (STEC) causes 2,801,000 acute illnesses annually [12]. Isolation of the pathogen from animals, food, clinical samples and environment has been reported in Nigeria [13,14,15,16].

Coupled with the dearth of data on the rate of occurrence of *E. coli* O157:H7 in Akure, and the consequences of infection with the organism being most severe in children. This study therefore aimed at determining the occurrence, resistance pattern and plasmid profile of *E. coli* from fecal samples of children.

2. MATERIALS AND METHODS

2.1 Study Population and Sample Collection

The study population consisted of three hundred and eleven (311) apparently healthy children made up of one hundred and eleven (111) males and two hundred (200) females within the age range of 0-5 years at Mother and Child Hospital Akure, Anchorites school, Women's Forum

Daycare Centre and Federal University of Technology Daycare Centre, Akure Ondo State.

The fecal samples were collected using sterile sample bottles and immediately transported to the Microbiology Postgraduate Research Laboratory, Federal University of Technology Akure for processing.

2.2 Ethical Approval

Ethical permit was given by Mother and Child Hospital, Akure Ondo State.

2.3 Methods of Isolation

The samples were inoculated by pour plate method onto freshly prepared Eosin Methylene Blue (EMB) agar plates and incubated at 37°C for 24 hours. Presumptive identification of *E. coli* was based on the characteristic green metallic sheen on the EMB agar plates, three to five representative colonies were streaked again on freshly prepared plates of EMB. These plates were then incubated aerobically at 37°C for 24 hours [17]. After incubation, the cultural and morphological characteristic of distinct, well isolated colonies were studied. These included the shape, size, elevation, edges, opacity, surface and colour. Representatives were picked per plate and stock cultures of pure isolates were labelled and stored accordingly at 4°C for further use.

2.3.1 Detection of hemorrhagic *Escherichia coli*

The culture media used for the detection of the bacterium was Sorbitol MacConkey (SMAC) agar, which was prepared according to the manufacturer's specification. The colonies were picked aseptically from the slants with an inoculating loop and streaked on Sorbitol MacConkey agar plates. These plates were then incubated aerobically at 37°C for 24 hours. After incubation, the colonies which were (non-sorbitol fermenting) pale brown/ colorless,

presumptively indicated the strain O157 [16]. The presumptive colonies were streaked again on SMAC agar to ensure purity, and were further confirmed using the agglutination test with O157 antiserum. The colonies that agglutinated when tested were considered to be *E. coli* O157: H7 colonies [16].

2.4 Biochemical Test

Furthermore, characterization was carried out with various biochemical tests. These tests included: catalase test, indole production test, citrate utilization test, methyl- red test, voges-proskauer test, sugar fermentation tests [17].

2.5 Antibiotic Susceptibility Testing

The agar diffusion disc was used according to the Kirby-Bauer technique [18]. Nutrient broth was prepared according to the manufacturer's instruction, poured into test tubes, corked and sterilized in an autoclave for 15 mins at 1.5 Pascal, at 121°C. After cooling, inoculums from the slants were introduced into the test tubes using inoculating loop and left for 3-4 hours. Sterile swab sticks were steeped into the broth and seeded on solidified Mueller Hinton agar. Sterile forceps was used to pick the disc, which contained ten antibiotics, and placed gently on the surface of the agar. These plates were incubated at 37°C for 24 hours in an incubator. The plates were observed for growth. The zones of inhibition were measured and compared with CLSI standard [19] to check if it was susceptible or resistant. The paper disk contains the following antibiotics; Augmentin (30 µg), Ofloxacin (5 µg), Gentamicin (20 µg), Ciprofloxacin (10 µg), Nitrofurantoin (300 µg), Cotrimoxazole (25 µg), Amoxicillin (30 µg), Tetracycline (25 µg), Pefloxacin (5 µg) and Ceftriazone (30 µg) (Oxoid).

2.6 Plasmid Profiling

2.6.1 Plasmid DNA extraction

Plasmid extraction was carried out using the method described by Akinjogunla and Enabulele [20]. Broth culture of the bacterial isolates was incubated at 37°C for 18 hours, after which it was introduced into Eppendorf tube and centrifuged at 12,000 rpm for 5 minutes, 100 µl TE buffer was added to the sediment and this was gently tapped. Thereafter, 200 µl Sodium Dodecyl Sulfate (SDS) was added to it and gently tapped, this was left at room temperature for 5 minutes.

300 µl of a 30% Sodium acetate solution (pH 4.8) was added to each isolate, then mixed vigorously and left at room temperature for 5 minutes. The isolates were later centrifuged at 12,000 rpm for 15 minutes and decanted, the supernatant was collected into fresh Eppendorf tube and incubated in ice for 30 minutes, after which 100% ethanol (-20°C) was added to precipitate DNA. This was later shaken vigorously and incubated for 1 hour in ice. After incubation, it was centrifuged at 12,000 rpm for 15 minutes and decanted. Plasmid DNA was dissolved in 100c TE buffer. Agarose gel was prepared by boiling 0.8 g of agarose powder in 100 ml of 0.5xTBE buffer, after boiling, the solution was allowed to cool and 10 µl ethidium bromide was added to the cooled agarose solution, this was poured into a casting tray with a comb placed across its rim to form wells. The gel was allowed to set for 30 minutes and the comb was removed, 20 µl of the plasmid DNA samples were then loaded into the wells. This was later mixed with 2 µl of bromo phenol blue. A DNA molecular weight marker was loaded into one of the wells, the agarose gel plates were placed in TBE buffer in electrophoresis horizontal tank at constant voltage of 60V for 1 hour 30 minutes. After electrophoresis, plasmid DNA bands were viewed by fluorescence of bound ethidium bromide under a short wave ultraviolet light trans-illuminator.

2.7 Statistical Analysis

Analysis of data was done using one way analysis of variance (ANOVA) and Means were compared by Duncan's Multiple Range Test at 95% confidence level using SPSS version 20.0. Differences were considered significant at $P < 0.05$.

3. RESULTS

3.1 Sample Distribution Based on Age, Gender, Appearance and Location

The sample distribution based on age and gender of children, location, appearance of feces; and the rate of occurrence of *E. coli* O157 are presented in Tables 1, 2, 3, 4 respectively. A total of 311 samples were collected, 111 (35.7%) from males and 200 (64.3%) from females within the age range of 0-5 years. It was observed that the higher the age, the lesser the watery and bloody stool; also highest number of feces were from children between 1-3 years followed by less than 1 year and then 3-5 years of age.

3.2 Bacteriological Findings and Identification of *E. coli* O157

Tables 5 and 6 show results of the colonial morphology and biochemical results of the isolates.

3.3 Susceptibility Patterns of Test Isolates to Commercial Antibiotics

The results of the susceptibility testing of the isolates to commercial antibiotics are presented in Figs. 1, 2, 3 and 4.

3.4 Plasmid Profile of Selected Multi-drug Resistant Isolates of *E. coli* O157

Plasmid profiles of 7 selected antibiotics resistant isolates of *E. coli* O157 was carried out. Plasmid sizes ranging from 4.0 to 17.5 kb were detected in the examined strains. Isolate in lane 8 had the highest number (4) of plasmids followed by isolates in lanes 3 and 5 with 3 plasmids, isolates in lanes 4 and 7 had 2 plasmids; while isolates in

lanes 2 and 6 had only one plasmid. Lane 1 represents the DNA ladder 1-10 kb. The plasmid sizes are as follows: lane 8 having size of 17.5, 15, 10 and 4 kb; lane 7: 15 kb, lane 6: 4 kb, lane 5: 17.5, 15 and 4 kb, lane 4: 10 and 4 kb, lane 3: 15, 10 and 4 kb and lane 2: 15 kb. The results are presented in Plate 1 and Table 7.

4. DISCUSSION

Out of the 311 children sampled, 21 (6.75%) were positive for *E. coli* O157:H7. Seven (6.31%) of the 111 males sampled were positive, while fourteen (7%) of the 200 females sampled were positive for the organism. This result is similar to the study of [21], who found that although all age groups were infected by the pathogen, but the highest percentage occurrence of infection was recorded among the age group of 0-9 years. The high infection rate among the children could be due to their uncontrolled and unguarded eating as well as water drinking habits as the pathogen is transmitted through the fecal-oral route [21].

Table 1. Sample distribution based on age and gender of children

Age	Males examined	Females examined	Total (%)
Less than 1	34	77	111 (35.7)
1-3	52	65	117 (37.6)
3-5	25	58	83 (26.7)
Total (%)	111 (35.69)	200 (64.31)	311 (100)

Table 2. Sample distribution based on location

Location	Number of samples (%)
Mother and Child Hospital	183 (58.8)
Anchorite Daycare	56 (18)
Womens' forum Daycare	39 (12.5)
FUTA Daycare	33 (10.6)
Total (%)	311 (100)

Table 3. Sample distribution based on appearance of feces

Age	Watery	Semi-solid	Bloody	Total
Less than 1	64	36	11	111 (35.7)
1-3	13	87	17	117 (37.6)
3-5	7	71	5	83 (26.7)
Total (%)	84 (27)	194 (62.4)	33 (10.6)	311 (100)

Table 4. Rate of occurrence of *E. coli* O157:H7 in fecal samples of children in Akure

Gender	Number of samples examined	Number positive (%)
Male	111	7 (6.31)
Female	200	14 (7)
Total	311	21 (6.75)

Table 5. Colonial morphology of O157:H7 isolated from fecal samples

Colonial morphology	EMB Agar	SMAC Agar
Pigmentation/ colour	Green metallic sheen	Colourless/ pale brown
Shape	Short rods	Short rods
Edge	Entire	Entire
Optical	Convex	Convex
Colony surface	Smooth	Smooth

Table 6. Biochemical results of O157: H7 isolated from feces

Biochemical test	Results
Gram stain	-ve
Catalase	+ve
Indole production	+ve
Methyl- red	+ve
Voges- proskauer	-ve
Citrate	-ve
Sugar fermentation	Lactose (AG), Glucose (AG), Sucrose (A) and Mannitol (A)

Legend: AG = acid and gas produced, A = acid produced, -ve = negative reaction, +ve = positive reaction

The isolates exhibited the following susceptibility patterns to the antibiotics employed in this Study: Ofloxacin 18 (85.7%), Ciprofloxacin 19 (90.5%), Pefloxacin 18 (85.7%), Nitrofurantoin 18 (85.7%), Augmentin 13 (61.9%), and Cotrimoxazole 12 (57.1%); While showing the following intermediate susceptibility pattern: Gentamycin 9 (42.9%), Tetracycline 7 (33.3%), Ceftriaxone 4 (19.1%) and Amoxycilin 3 (14.3%); and exhibited the following resistance pattern: Ceftriaxone 19 (90.5%), Amoxycilin 18 (85.7%), Tetracycline 13 (61.9%), Gentamycin 11 (52.4%), Cotrimoxazole 9 (42.9%), Augmentin 8 (25.8%), Ofloxacin 2(9.5%), Ciprofloxacin 2(9.5%) and Nitrofurantoin 2(9.5%).

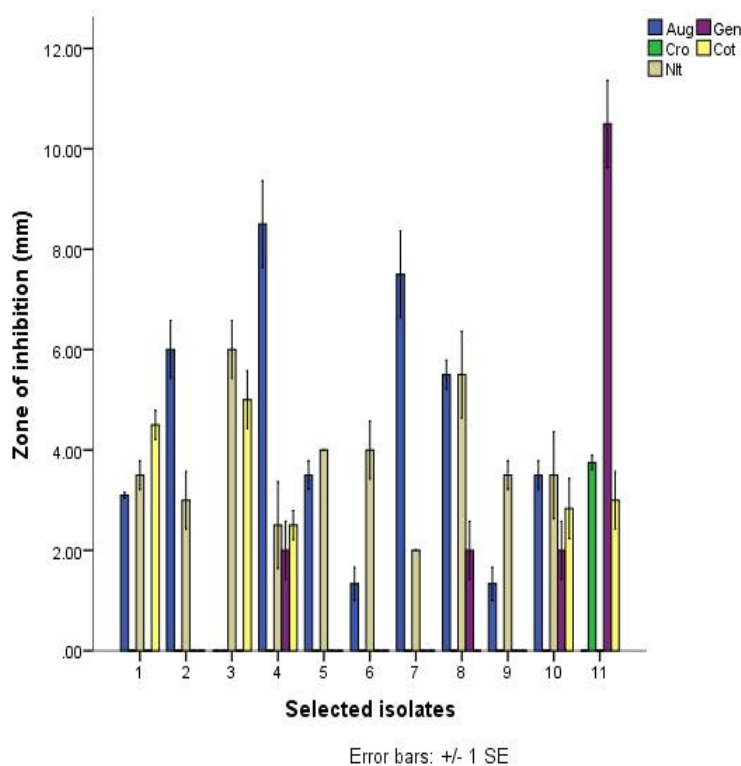


Fig. 1. Mean diameter of zones of inhibition (mm) of commercial antibiotics on test bacterium
 Legend: Aug= augmentin, Gen= gentamicin, Cro = Ceftriaxone, Cot= cotrimoxazole, Nit= nitrofurantoin

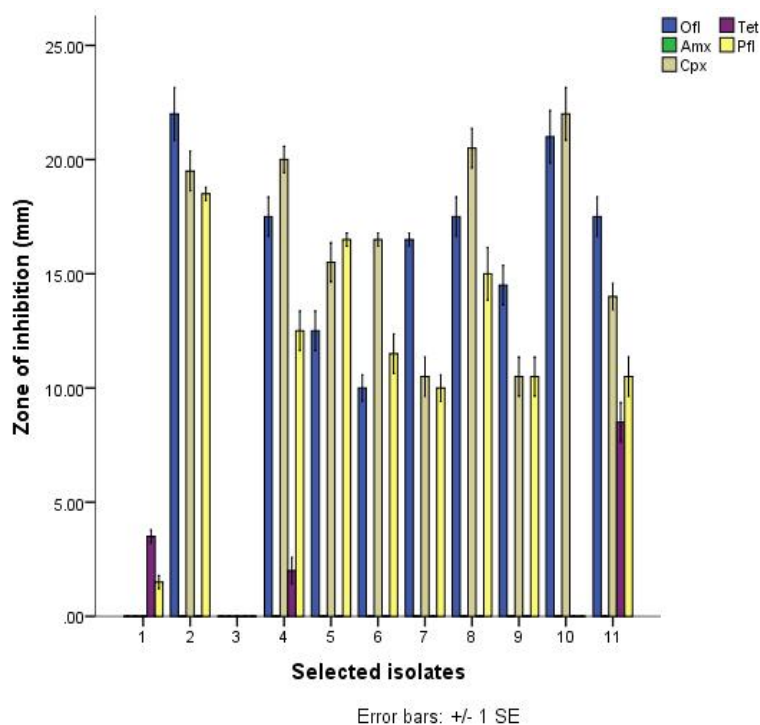


Fig. 2. Mean diameter of zones of inhibition (mm) of commercial antibiotics on test bacterium
 Legend: Ofl= ofloxacin, Tet= tetracycline, Amx= amoxicillin, Pfl= pefloxacin, Cpx= ciprofloxacin

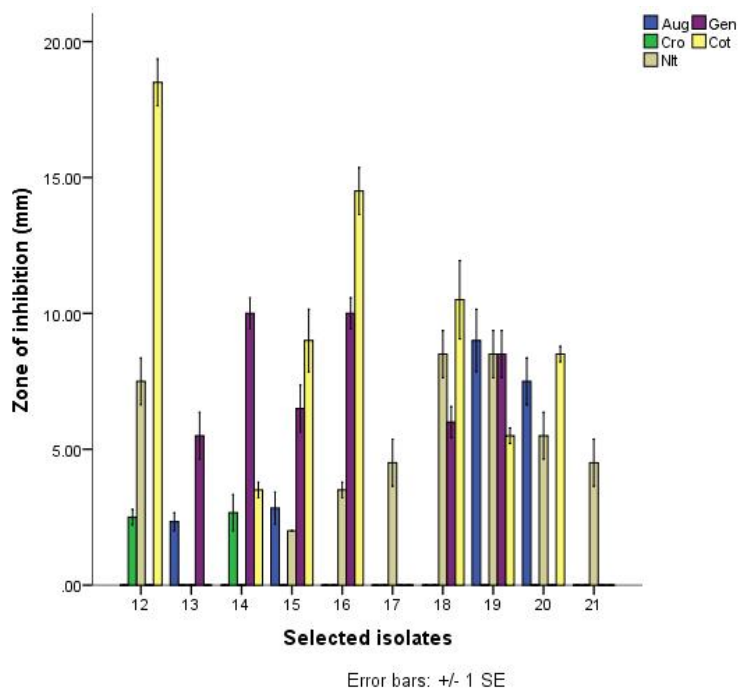


Fig. 3. Mean diameter of zones of inhibition (mm) of commercial antibiotics on test bacterium
 Legend: Aug= augmentin, Gen= gentamicin, Cot= cotrimoxazole, Nit= nitrofurantoin, Cro= Ceftriaxone

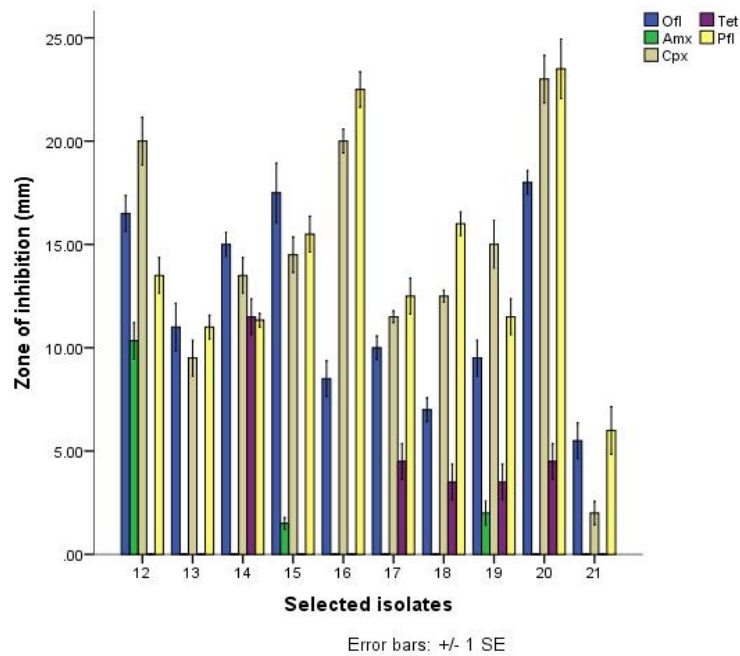


Fig. 4. Mean diameter of zones of inhibition (mm) of commercial antibiotics on test bacterium
 Legend: Ofl= ofloxacin, Tet= tetracycline, Amx= amoxicillin, Pfl= pefloxacin, Cpx= ciprofloxacin

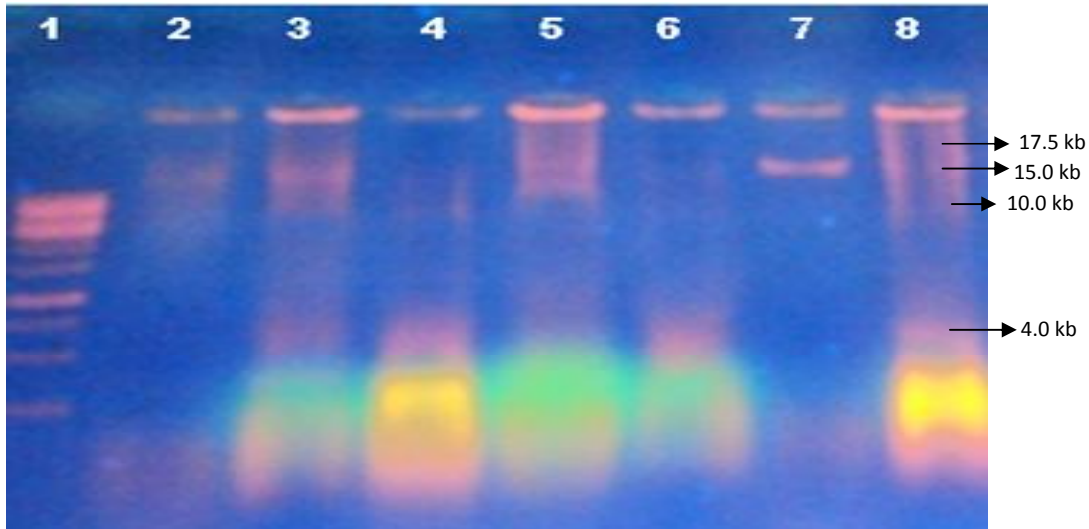


Plate 1. Electrophoretic pattern of plasmid profiling of selected multidrug resistant *E. coli* O157 Isolates from fecal samples
 Legend: kb: kilobase pair

Resistance of bacteria to antibiotics has been attributed to the abuse and misuse of antibiotics, as well as the acquisition of mobile genetic elements coding for antibiotics resistance [22]. This correlates with the works of [23] who reported the resistance of *Escherichia coli* to Tetracycline, Amoxicillin and Cotrimoxazole, and [16] who reported multi drug resistance in *E. coli* O157 isolated from water and diarrhoeal stool

specimen. The high resistance of the test isolates to Ceftriaxone and Amoxicillin could be attributed to the fact that the cell wall of Gram negative bacteria has a minor component of peptidoglycan polymer thereby making them less susceptible to β -lactam antibiotics which act by inhibiting the synthesis of bacterial cell wall.

Table 7. Profile of Plasmid from the Isolated *E. coli* O157:H7

Isolates	Number of plasmids
Lane 1	1-10 kb (DNA ladder)
Lane 2	1
Lane 3	3
Lane 4	2
Lane 5	3
Lane 6	1
Lane 7	2
Lane 8	4

The presence of multiple plasmids observed in some of the isolates in this study could probably be responsible for the resistance observed. Plasmids have been shown to carry genes for antibiotics resistance, a single plasmid can carry genes for resistance to multiple antibiotics [24]. This is in coherence with the work of [16] who also reported multiple plasmids in *E. coli* O157 isolates from water and fecal samples.

5. CONCLUSION

This study revealed that the rate of occurrence of *E. coli* O157: H7 among the study population was 6.75% and that the isolates were resistant to many of the conventional antibiotics employed and possess multiple plasmids. An exceptionally low dose of this organism is able to cause infection and once introduced into a closed group or institutional setting, it can spread by person-to-person transmission and reach levels of public health concern. It is therefore recommended that routine screening is carried out to determine the carrier rate of the organism, more public awareness should be carried out to educate the community on need for adherence to personal hygiene, environmental hygiene and good food handling practices to prevent outbreak of infections due to this organism.

CONSENT

Authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this paper and accompanying images.

ETHICAL APPROVAL

Authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical

standards laid down in the 1964 declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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