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Antibiotic Susceptibility Profile of *E. coli* Serotype 0157:H7 in ABUTH, Zaria, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JCI, JAO and JOE did the study design and wrote the protocol. Authors ROB, NCO, BOO and MK did the statistical analysis and literature searches while analyses of study was by authors ABT, MTS, AM and MSS. All authors read and approved the final manuscript.

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ABSTRACT

The emergence and re-emergence of new strains of microorganisms with high virulent traits and resistant to even new generation antibiotics are significant limiting factor to patients' recovery in clinical settings. This has indeed created a lot of economic burdens and loss of productive activities

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at work places especially in developing countries. This study was conducted to evaluate the presence of *E. coli* serotype O157:H7 in Zaria metropolis and the antibiotics resistance pattern of the isolates. Out of the 150 samples submitted for bacteriological diagnosis at the Medical Microbiology Laboratory of Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria, Nigeria, for the period of 6months (March - August, 2011), 60% of the isolates obtained were identified as *E. coli*. The incidence of *E. coli* serotype O157:H7 was 36.7% while the antibiotic susceptibility profile of the isolates to 14 antibiotic commonly prescribed at ABUTH showed that the isolates were 70% resistant to Ceftazidine, 60% to Tetracycline, Ampicillin-Sulbactam, Amoxicillin-Clavulanic acid, 40% to Amoxicillin, Cefuroxime, Nalidixic acid and Cefalexin, 30% to Nitrofurantoin, 20% to Ofloxacin, Chloramphenicol and Ciprofloxacin, 10% to Ceftriaxone and all the isolates were sensitive to Gentamicin. We conclude that there is an incidence of *E. coli* serotype O157:H7 with varying resistant pattern in Zaria metropolis which has influenced the results obtained from clinical samples in ABUTH. This calls for significant antibiotic surveillance and good hygiene practices to prevent food/water born outbreak of diarrhea associated with *E. coli* serotype O157:H7, as the identified serotype has been implicated in several deaths around the globe.

Keywords: Food/water borne disease; E. coli O157:H7; antibiotics; Zaria metropolis.

1. INTRODUCTION

E. coli O157:H7 was first recognized in an outbreak in 1982 traced to contaminated hamburgers [1]. Since then, most infections are believed to have erupted from eating undercooked ground beef and drinking contaminated water [1]. The pathogenicity of this strain is associated with the production of verotoxin (VT) or Shiga-like toxin that causes severe damage to the lining of the intestine, leading to diarrhea [2], and even a life-threatening complication such as hemolytic uremic syndrome [3]. It has been documented that diarrhea infection kills an estimated 1.8 million people each year [4], creating substantial economic and quality of life burden on the society by way of acute morbidity and chronic squeal [5,6]. Among children under five years in developing countries, the prevalence of diarrhea accounts for 17% of all deaths [7]. Food products associated with *E. coli* outbreaks include cucumber, raw ground beef, raw seed sprouts or spinach [8], raw milk, unpasteurized juice, unpasteurized cheese and foods contaminated by infected food workers via fecal-oral route [2]. According to the U.S. Food and Drug Administration, the fecal-oral cycle of transmission can be disrupted by cooking food properly, preventing cross-contamination, instituting barriers such as gloves for food workers, instituting health care policies for food industry employees to seek treatment when they are ill, use of potent and effective antibiotics, pasteurization of juice or dairy products and proper hand washing [9-12]. *E. coli* 0157:H7 is a serotype belonging to the group of enterohemorrhagic *E. coli* (EHEC), while other strains of *E. coli* that cause gastroenteritis in humans include: enteroaggregative (EAEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC) and diffuse adherent (DAEC) [3]. Although the use of antibiotics in diarrhea is not encouraged, the need to study the antibiotic susceptibility profile of *E. coli* O157:H7 is important in severe infection situation in order to proffer better treatment option to immunocompromised patients and elderly as antibiotic resistance is a growing global problem. This study therefore evaluates the presence of *E. coli* O157:H7 in Ahmadu Bello University Teaching Hospital (ABUTH) and also understand their antibiotic susceptibility profile.

2. METHODOLOGY

2.1 Ethical Consent

Ethical approval for the collection of human samples was obtained from the ABUTH Ethical committee.

2.2 Sample Collection and Isolate Identification

Stratified convenient sampling method was used for sample collection in this study. One hundred and fifty (150) samples [Stool (50), urine (50) and blood (50)) were obtained from 50 patients (patients who had visited the toilet and discharged watery stool with or without blood for more than 3 time a day (diarrhea patients) [13]. The samples were evaluated for the presence of *E. coli* for the period of 6 months (March -

August, 2011), at the Medical Microbiology Laboratory of ABUTH, Shika, Zaria, Nigeria. Eosin methylen blue agar was used as the basic identifying media for *E. coli*. Isolates that showed greenish metallic sheen were inoculated onto nutrient agar and incubated at 37℃ for 24hrs as stock culture for further morphological identification using standard microbiology methods [14-16].

2.3 Biochemical Test and Principle

Biochemical tests for *E. coli*; indole, methyl red, Voges-Proskauer, citrate, urease, MacConkey, mannitol, reducing sugar (triple sugar iron test), were carried out [17,18]. While *E. coli* serotype 0157:H7 was identified using Sorbitol-MacConkey medium containing potassium tellurite and cefixime, as selective media for *E. coli* 0157:H7 [19]. Unlike typical *E. coli*, isolates of *E. coli* O157:H7 do not ferment sorbitol and are negative with the 4-methylumbelliferyl-beta-D-glucuronide (MUG) assay; therefore, these criteria are commonly used for selective isolation [19].

2.4 Antibiotic Susceptibility Test

The antibiotics prescribed at the ABUTH for the treatment of infections associated with *E. coli* were used for this test as described by Cheesbrough [19] and interpreted by CLSI [20].

2.5 Determination of Multiple Antibiotic Resistance Index (MARI)

The MAR Index was determined according to the method of Krumperman [21] and Paul et al. [22] by dividing the number of antibiotics to which the isolate is resistant to by the total number of antibiotic groups tested.

$$MAR\ Index = \frac{Number\ of\ antibiotics\ to\ which\ resistant}{Total\ number\ of\ antibiotics\ tested}$$

3. RESULTS AND DISCUSSION

Presumptive isolates that showed pink red colouration on MacConkey agar; do not grow on mannitol salt agar and subsequently produce greenish metallic sheen on eosin methylen blue agar were selected for biochemical characteristics [23]. Furthermore, isolates that were able to convert tryptophan to indole with the help of tryptophanase enzyme and maintained the methyl red colour (positive), produced negative result of Voges-Proskauer, citrate, cetrimide and urease tests were identified as *E. coli*. While organisms that showed colourless on further analysis using cefixime and potassium tellurite –sorbitol MacConkey agar (CT-SMA) were identified as *E. coli* 0157:H7 (Fig. 1).

Out of the 150 samples evaluated, 60% (90) of the isolates obtained were identified as *E. coli*. High percentages (66.7%) of the *E. coli* isolates were from stool, 20% from urine and 13.33% from blood (Table 1). The incidence of *E. coli* serotype O157:H7 in the total isolates was 36.67% (33/90) and this account for 55% (33/60) of stool sample isolates (Table 1).

By morphological and biochemical test (Fig. 1), *E. coli* O157:H7 appeared colourless on CT-SMA (S_{56}) while other serotypes of *E. coli* showed pickish colour (U_7) on CT-SMA agar.

The significant percentage (36.67%) of *E. coli* serotype O157:H7 observed in this study calls for critical examination of the ways in which food and water are treated within Zaria, Nigeria. Previous studies, in Lagos [24], Lagos and Ibadan [25], Benin [26], Borno and Adamawa [27], Zaria [28,29] and Kano [30] had implicated *E. coli* 0157:H7 in stool of diarrhea patients resulting from eating unwashed vegetables, undercooked meat or other food sources. According to Josefa et al. [31] the distribution of *E. coli* 0157:H7 disease was observed to be 52% (183) in foodborne, unknown 21% (74), person-to-person 14% (50), waterborne 9% (31), animal contact 3% (11), and laboratory-related 0.3% (1). While according to Dahiru, et al. [30]; the prevalence of *E. coli* O157:H7 in Kano was 53% in fresh beef and 25.3% in roasted beef. Schlundt, [32] also acknowledged that consumption of inadequately cooked beef could pose a serious risk of infection. However, *E. coli* serotype O157:H7 illness has been reported not to be in outbreak proportions in Nigeria since 1994 [33,34]. Available information indicates that the carriage of *E. coli* serotype O157:H7 in cattle was an important factor in the emergence of this pathogen in Africa [35,36]. But according to Umeh and Okpokwasili [37]; the prevalence of *E. coli* 0157:H7 in livestock is extrapolated as 20% in cattle, 12.5% in sheep, 7.5% in goat, 5% in chicken and 2.5% in pig feacal samples and increased shedding is usually observed during the months of December- March. However, illness in reared animals has frequently been linked to contaminated irrigation water, with animal faeces from discharged sewage effluent or surface run off [38-40].

Table 1. Incidence of *E. coli* O157:H7 in ABUTH, Shika, Zaria

Samples (n= 150)	No. Isolate	% from sample	*E. coli* O157:H7	Percentages from identified *E. coli* isolate
Stool (50)	60	66.67	33	36.67
Urine (50)	18	20	0	0
Blood (50)	12	13.33	0	0

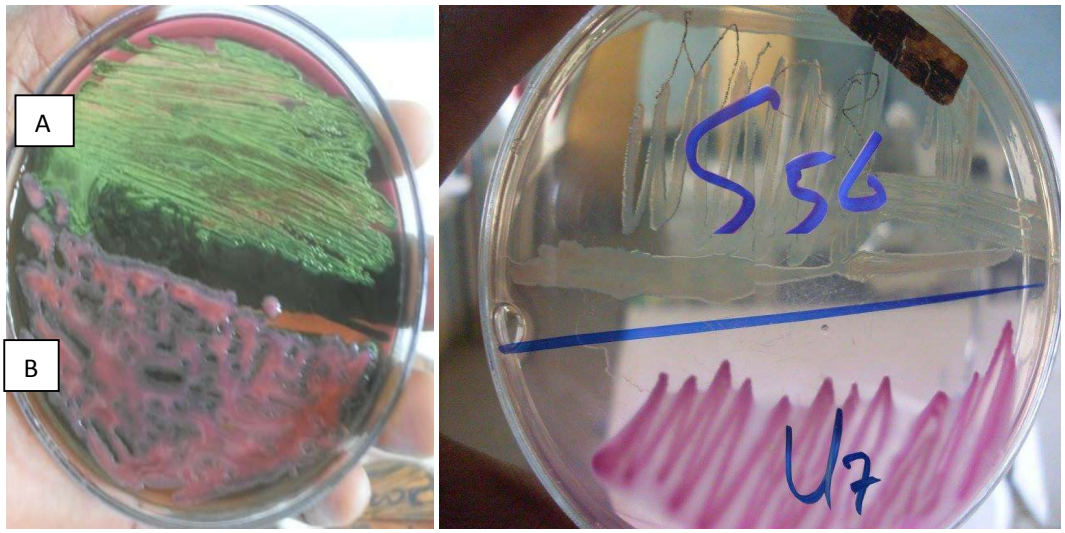

Fig. 1. Morphological characteristics of *E. coli* serotype O157:H7 on Sorbitol MacConkey Agar
Key: S_{56} = Positive, U_7= Negative, A = Greenish metallic sheen of E. coli on EMB agar, B = Klebsiella spp. on eosin methylene blue.

In Nigeria, as in most developing countries, surface waters which constitute an important source of water for domestic and agricultural purposes are vulnerable to faecal pollution [39-41].

The antibiotic susceptibility profile result of *E. coli* serotype O157:H7 (Fig. 2) to fourteen (14) antibiotic commonly prescribed at ABUTH showed that the isolates were 70% resistant to Ceftazidine (CAZ), 60% to Tetracycline (TE), Ampicillin-Sulbactam (SAM), Amoxicillin-Clavulanic acid (AMC), 40% to Amoxicillin (AML), Cefuroxime (CXM), Nalidixic acid (NA) and Cefalexin (CL), 30% to Nitrofurantoin (F), 20% to Ofloxacin (OFX), Chloramphenicol (C), and Ciprofloxacin (CIP), 10% to Ceftriaxone (CRO) and all the isolates were sensitive to Gentamicin.

This study observed that *E. coli* serotype O157:H7 isolated within Zaria metropolis express significant antibiotics resistances but some antibiotics were still found to be effective. Our observation on some of the effective antibiotics concur with the reports of Bell et al. [29] and Umolu et al. [43] in Lagos which showed that antibiotics such as Nitrofurantoin, Ofloxacin, Ceftriaxone and Ciprofloxacin have high activity against *E. coli* while antibiotics like Gentamicin are effective than newer antibiotics because of limited use [25]. On resistant level, Chijioke and Christian [44] work in Enugu also supported that *E. coli* isolates showed resistance rates of 85% to Ampicillin, 22.5% to Ceftriaxone, 16.3% to Nalidixic acid and 12.5% to Gentamicin.

Further observations showed that the isolates have varying resistance pattern with 66.7% resistant to more than 3 antibiotics commonly prescribed for *E. coli* associated infection while at ≥0.3 MARI, isolates had 54.6% MARI (Table 2). This suggests that the isolates originated from a high risk source of contamination where antibiotics are often used [22]. It also indicates that a large proportion of the bacterial isolates have been exposed to several antibiotics [45].

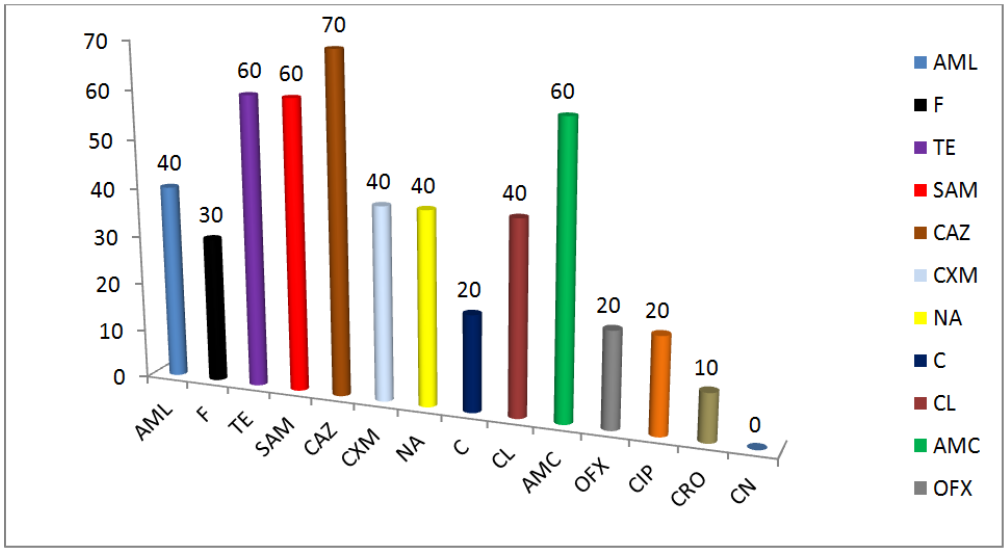

Fig. 2. Percentages of resistance of *E. coli* O157:H7 to selected antibiotics

Keys: Ampicillin-Sulbactam (SAM), Amoxicillin (AML), Amoxicillin-Clavulanic acid (AMC), Cefalexin (CL), Ceftriaxone (CRO), Ceftazidine (CAZ), Cefuroxime (CXM), Ciprofloxacin (CIP), Nalidixic acid (NA), Ofloxacin (OFX), Gentamicin (CN), Tetracycline (TE), Chloramphenicol (C), Nitrofurantoin (F).

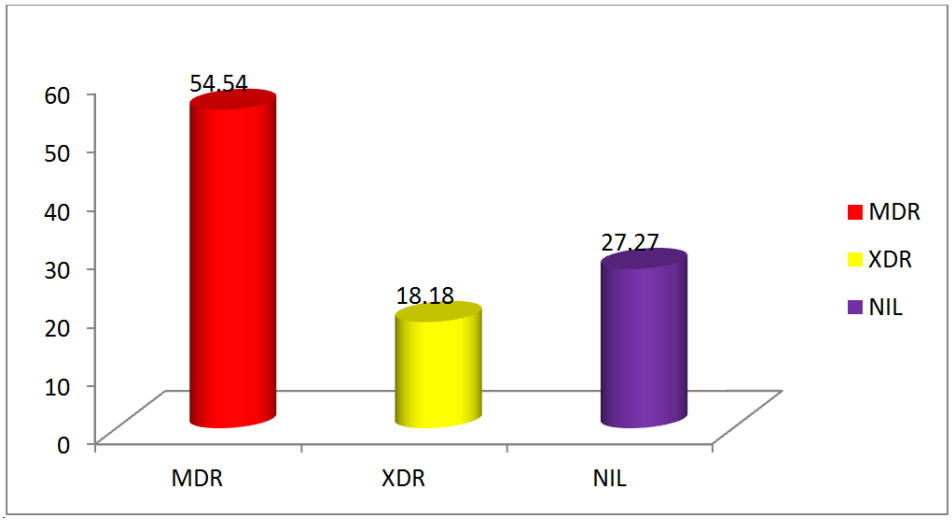

Fig. 3. Categories of antibiotic resistance in *E. coli* isolates

MDR: Multidrug-resistant, XDR: Extensively drug-resistant NIL: Neither MDR nor XDR
MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories.
XDR: non-susceptible to ≥1 agent in all but ≥2 categories.
PDR: non-susceptible to all antimicrobial agents listed. PDR was not considered because not all the antibiotics contained in the proposal of Magiorakos et al.*, [42] are prescribed for infections associated with E. coli in A.B.U Teaching Hospital Shika, Zaria.*

3.1 Determination of the Different Categories of Antibiotic Resistance in *E. coli* isolates in ABUTH, Shika

The resistance pattern of the isolates showed that 54.5% of the isolates were MDR and 18.2% showed XDR. This could be attributed to a combination of microbial characteristics such as selective pressure on antimicrobial usage, societal irrational use of antibiotics and technological changes that enhance the transmission of drug resistant organisms [46].

Table 2. MARI percentages of isolated *E. coli* O157:H7

S/N	MARI	No of isolate	Percentage (%)	
1	0.0	0	0	
2	0.1	5	15.2	
3	0.2	6	18.2	
4	0.3	7	21.2	Resistant to more than 3 antibiotics
5	0.4	0	0	
6	0.5	2	6	
7	0.6	3	9.1	
8	0.7	4	12.1	
9	0.8	1	3	
10	0.9	5	15.2	
11	1	0	0	

The high multidrug resistance (54.5%) observed in this study might be attributed to the misuse of antibiotics in this location [47]. It should be understood that irrespective of the efficacy and specificity of any antibiotic, broad spectrum antibiotics are sometimes given in place of narrow spectrum antibiotics as a substitute for culturing and sensitivity tests. This encourages the risk of selection of antibiotic-resistant mutants [48]. This situation is made worse by patients not completing their course of medication, probably as a result of ignorance or poor financial level creating an environment of antibiotic misuse and resistance [46].

4. CONCLUSION

We conclude that there is an incidence of *E. coli* serotype O157:H7 in ABUTH with varying resistant pattern. This calls for significant antibiotic surveillance for the detection and treatment of diarrhea *E. coli* associated diseases as this identified serotype has been implicated in several deaths around the globe. Also, encouragement of good hygiene practices to prevent food/water borne outbreaks of diarrhea is advocated.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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