

ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *E. coli* SEROTYPE O157:H7 IN ABUTH

ZARIA

Abstract

The emergence and re-emergence of new strains of microorganisms with high virulent traits and resistance to even new generation antibiotics is a significant limiting factor to patients' recovery in clinical settings. This has indeed created a lot of economic burdens and loss of productive activities 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. Of the 150 samples from diarrhea patients 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 result of the isolates to 14 antibiotic commonly prescribed at ABUTH showed that the isolates were 70% resistant to Ceftazidime, 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, good hygiene practices to prevent food/water born outbreak of diarrhea and development of fast diagnostic techniques for the detection and treatment of diarrhea, as the identified serotype has been implicated in several deaths around the globe.

Kew words: Food/water borne disease, *E. coli* O157:H7, Antibiotics, Zaria metropolis

INTRODUCTION

E. coli O157:H7 was first recognized as a cause of illness during 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 in United States in 2006 [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 requirement [9][10] [11][12]. *E. coli* O157: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 proposes to evaluate the presence of *E. coli* O157:H7 in Ahmadu Bello University Teaching Hospital (ABUTH) and also understand their antibiotic susceptibility profile.

METHODOLOGY

Ethical Consent

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

Sample Collection and Isolate Identification

Stratified convenient sampling method was used 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) [46]). The samples were evaluated for the presence of *E. coli* for the period of 6months (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°C for 24hrs as stock culture for further morphological identification using standard microbiology methods [13] [14] [15].

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 [16][17]. While *E. coli* serotype O157:H7 was identified using Sorbitol-MacConkey medium containing potassium tellurite and cefixime, as selective media for *E. coli* O157:H7 [18]. 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 [18].

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 [18] and interpreted by CLSI [19].

Determination of Multiple Antibiotic Resistance Index (MARI)

The MAR Index was determined according to the method of Krumperman [20] and Paul *et al* [21] by dividing the number of antibiotics to which the isolate is resistant to by the total number of antibiotic groups tested.

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

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. 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* O157:H7 (Figure 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).

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

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

By morphological, phenotypical and biochemical test (Figure 1), *E. coli* O157:H7 appeared colourless on CT-SMA (S₅₆) while other serotypes of *E. coli* showed pickish colour (U₇) on CT-SMA agar.



Figure 1: Morphological Characteristics of *E. coli* Serotype O157:H7 on Sorbitol MacConkey Agar.

Key: S₅₆ = Positive, U₇ = Negative, A = Greenish metallic sheen of *E. coli* on EMB agar, B = *Klebsiella* spp. on eosin methylene blue.

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 [23], Lagos and Ibadan [24], Benin [25], Borno and Adamawa [26], Zaria [27][28] and Kano [29] had implicated *E. coli* O157:H7 in stool of diarrhea patients resulting from eating unwashed vegetables, undercooked meat or other food sources. According to Josefa *et al.*, [30] the distribution of *E. coli* O157: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.*, [29]; the prevalence of *E. coli* O157:H7 in Kano was 53% in fresh beef and 25.3% in roasted beef. Schlundt, [31] 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 [32][33]. 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 [34][35]. But according to Umeh and Okpokwasili [36]; the prevalence of *E. coli* O157: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 fecal 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 [27][38][39]. 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][40].

The antibiotic susceptibility profile result of *E. coli* serotype O157:H7 (Figure 2) to fourteen (14) antibiotic commonly prescribed at ABUTH showed that the isolates were 70% resistant to Ceftazidime (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.

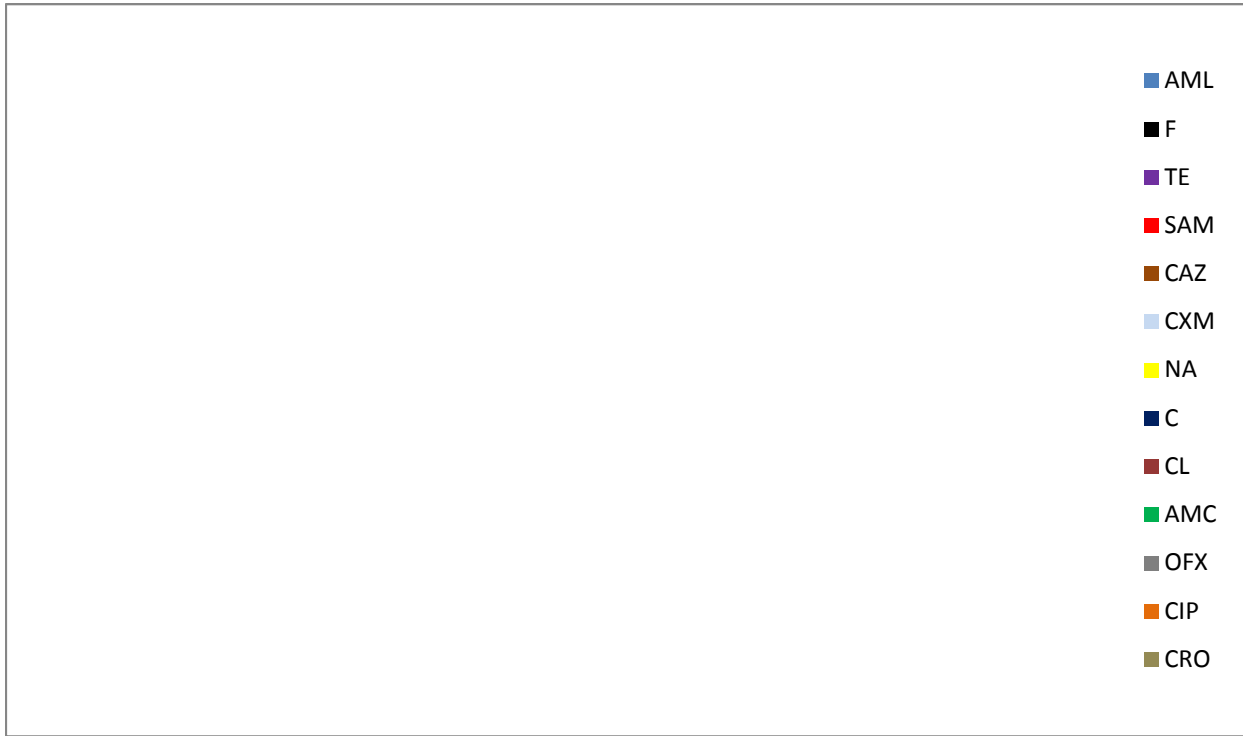


Figure 2: Percentages of resistance of *E. coli* O157:H7 to selected antibiotics

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

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.*, [28] and Umolu, *et al.*, [42] 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 [24]. On resistant level, Chijioke and Christian [43] 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 [21]. It also indicates that a large proportion of the bacterial isolates have been exposed to several antibiotics [47].

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	

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 [48].

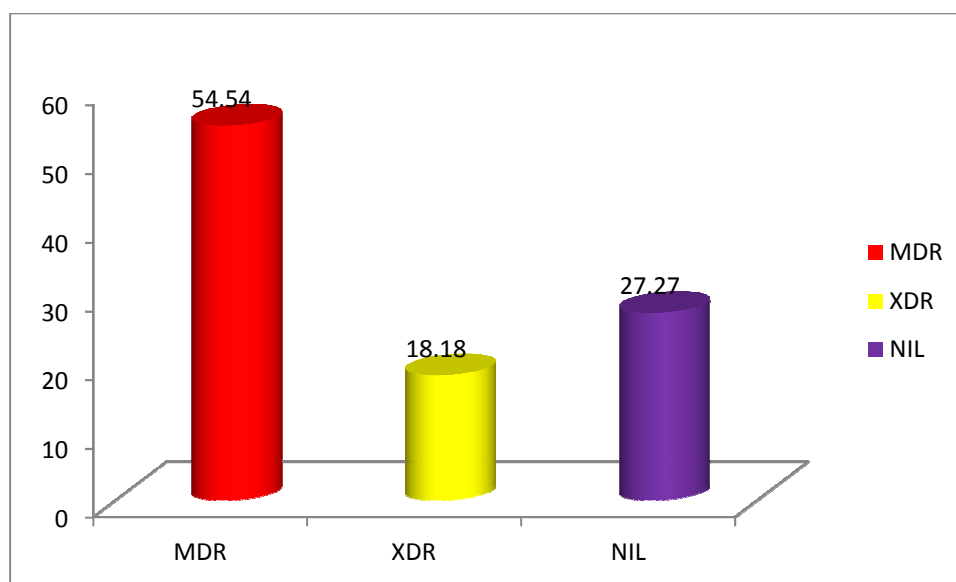


Figure 2: Categories of Antibiotic Resistance in *E. coli* isolates

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.*, [41] are prescribed for infections associated with *E. coli* in A.B.U Teaching Hospital Shika, Zaria.

The high multidrug resistance (54.5%) observed in this study might be attributed to the misuse of antibiotics in this location [44]. 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 [45]. 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 [45].

CONCLUSION

We conclude that there is an emergence of varying resistant pattern and virulent multidrug resistance isolate of *E. coli* serotype O157:H7 in Zaria metropolis which has influenced the results obtained from clinical samples in ABUTH. This calls for significant antibiotic surveillance and development of fast diagnostic techniques 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.

REFERENCES

1. United State Environmental Protection Agency. Water: Basic Information about Regulated Drinking Water Contaminants; Basic Information about *E. coli* O157:H7 in Drinking Water, 2012. <http://water.epa.gov/drink/contaminants/basicinformation/ecoli.cfm>
2. Food and Drug Administration (FDA). "Enterohemorrhagic *E. coli* O157:H7". Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. U. S. Department of Health and Human Services, 2009. www.hhs.gov.
3. Nataro JP, Kaper JB. "Diarrheagenic *Escherichia coli*". *Clinical Microbiology Review*. 1998; 11(1): 142–201.
4. World Health Organization. World Health Report 2005. Geneva: World Health Organization. 2005
5. Hazariwala A, Sanders Q, Hudson CR, Hofacre C, Thayer SG and Maurer JJ. Distribution of Staphylococci enterotoxin genes among *Staphylococcus aureus* isolates from poultry and humans with invasive staphylococcal disease. *Avian Diseases*. 2002; 46(1):132-136
6. Duff SB, Scott EA, Mafilios MS, Todd EC, Krilov LR, Geddes AM and Ackerman SJ. Cost-effectiveness of a targeted disinfection program in household kitchens to prevent food-borne illnesses in the United States, Canada and the United Kingdom. *Journal of food protection*. 2003; 2: 2103-2115.
7. United Nations. UN Statistical Division. Progress towards the Millennium Development Goals, 1990-2005, New York: The United Nations. 2006.
8. Faysal F, Ron CM and Jill JM. *E. coli* Outbreaks Affect Demand for Salad Vegetables. JEL Classifications: Q11, Q13. Choice magazine. 2006. <http://www.choicesmagazine.org/magazine/article.php?article=72>
9. Joanne MW, Linda MS and Christopher JW. Prescott, Harley and Klein's – Microbiology text books. 7th Edition. McGraw Hill Higher Education Publication. 2008. Pp. 835 - 974. Inc., 1221 Avenue of the Americas. New York.
10. Tauschek M, Gorrell R, Robins-Browne RM. "Identification of a protein secretory pathway for the secretion of heat-labile enterotoxin by an enterotoxigenic strain of *Escherichia coli*". *PNAS*. 2001. 99(10): 7066–7071.
11. Vogt R. L. and Dippold L. "*Escherichia coli* O157:H7 Outbreak Associated with Consumption of Ground Beef, June–July 2002". *Public Health Rep*. 2005. 120(2): 174–178
12. The Local Germany News in English (TLGNE) (2011). "Deadly *E. coli* found on bean sprouts". <http://www.thelocal.de/national/20110610-35583.html>
13. De Silva ZN, Cunha AS, Lins MC, Carneiro L, Almeida AC and Queiro ML. Isolation and serological identification of enteropathogenic *Escherichia coli* in pasteurized milk in Brazil. *Rev. Saude Publica*. 2001. 35(4): 375-379

14. Ellis DI and Goodacre R. Detection, identification, and enumeration methods for spoilage and serological identification of enteropathogenic *Escherichia coli* in pasteurized milk in Brazil. *Rev. Saude Publica*. 2006. **35**(4): 375-379.
15. Chakraborty P and Nishith KP. Manual of practical microbiology and parasitology. New central book agency limited, 8/1 Chintamani Das Lane, Kolkata 700 009, West Bengal, India. 2008.
16. Harley JP and Prescott LM. Laboratory Exercises in Microbiology. Biochemical Activities of Bacteria: Carbohydrates; Fermentation And β -Galactosidase Activity. 5th Edition. The McGraw-Hill Companies. 2002.
17. Cowan ST and Steel KJ. "Manual for Identification of Medical Bacteria". Cambridge University Press. 3rd Edition. 1993. Pp. 199 – 241
18. Cheesbrough M. District laboratory practice in tropical countries (Part 11). Cambridge, University Press UK. 2000. Pp. 134-143.
19. Clinical Laboratory Standard Institute (CLSI). Performance standards of antimicrobial disc and dilution susceptibility tests for bacteria isolated from animal, approved standard. 3rd edition. 2006. 28(8)
20. Krumperman, PH. Multiple antibiotic indexing *Escherichia coli* to identifying risk sources of fecal contamination of foods. *Applied and Environmental Microbiology*. 1983. 46: 165-170.
21. Paul S, Bezbarauh RL, Roy MK and Ghosh AC. Multiple antibiotics resistance (MAR) index and its reversion in *Pseudomonas aeruginosa*. *Letters in Applied Microbiology*, 1997. 24:169-71
22. APEC. Drinking Water Contaminated with *Escherichia coli*. U.S., EPA Drinking Water Publication. 2011.
23. Aibinu IE, Peters RF, Amisu KO, Adesida SA, Ojo MO and Tolu O. Multidrug Resistance in *E. coli* O157 Strains and the Public Health Implication. *Journal for Animal Science*. 2007. **3**(3): 22-33.
24. Olatoye, IO. The incidence and antibiotics susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Nigeria. *African Journal of Biotechnology*. 2010. **9**(8): 1196-1199.
25. Jonathan OI and Afe OE. Determination of the Antibiotic Susceptibility Patterns of Local Isolates of *E. coli* O157:H7 from Edo State, Nigeria. *New York Science Journal*. 2012. **5**(10).
26. Moses AE, Egwu GO and Ameh JA. Antimicrobial Resistant pattern of *E. coli* O157 Isolated from Human, Cattle and Surface Water Samples in Northeast Nigeria. *J Vet Adv* 2012. **2**(5): 209-215

27. Chigor VN, Umoh VJ and Smith SI. Occurrence of *Escherichia coli* O157 in a river used for fresh produce irrigation in Nigeria. *African Journal of Biotechnology*. 2010. **9**(2): 178-182. ISSN 1684-5315.
28. Bello M, Lawan MK, Kwaga JKP and Raji MA. Assessment of carcass contamination with *E. coli* O157 before and after washing with water at abattoirs in Nigeria. *International Journal of Food Microbiology*. 2011. **150** (2011) 184-186
29. Dahiru M, Uraih N, Enabulele SA and Shamsudden U. Prevalence of *Escherichia coli* O157:H7 in fresh and roasted beef in Kano city, Nigeria. *BAJOPAS*. 2008. **1**(1): 39-42.
30. Josefa M. Rangel, Phyllis H. Sparling, Collen Crowe, Patricia M. Griffin, and David L. Swerdlow. Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982-2002. CDC. 2011. 11(4). http://wwwnc.cdc.gov/eid/article/11/4/04-0739_article.htm
31. Schlundt, J. Emerging Food Borne Pathogens. *Biomedical Journal of Environmental Science*. 2001. **14**(1-2): 44-52.
32. Ogunsanya TL, Rotimi VO, Adenuga A. Study of the aetiological agents of childhood diarrhoea in Lagos, Nigeria. *Journal of Medical Microbiology*. 1994. 40:10-14.
33. Olorunshola ID, Smith SI, Coker AO. Prevalence of EHEC O157:H7 in patients with diarrhea in Lagos, Nigeria. *Acta Pathologica Microbiologica et Immunologica Scandinavica* (APMIS). 2000. 108: 761-763.
34. Effler P, Isaacson M, Arntzen L, Heenan R, Canter P, Barrett T, *et al.* Factors contributing to the emergence of *Escherichia coli* O157: H7 in Africa. *Emerging Infectious Diseases*. 2001. 7: 812-819
35. Renter DG, Sargeant JM, Oberst RD, Samadpour M. Diversity, Frequency and persistence of *Escherichia coli* O157: H7 in Cow-Calf farms. *Applied Environmental Microbiology*. 2003. 69: 542-547.
36. Umeh SI and Okpokwasili GSC. Seasonal Prevalence of *Escherichia coli* O157: H7 in Ruminants and Nonruminants and the Antimicrobial Resistance Profile of the Organisms from Different Sources. *Nigerian Journal of Microbiology*. 2009. **23**(1): 1852 - 1858
37. Chalmers RM, Aird A and Bolton FJ. Waterborne *Escherichia coli* O157. *Journal of Applied Microbiology*. 2000. 88: 124-132.
38. Payment P and Riley SM. Resolving the global burden of gastrointestinal illness: a call to action. A Report from the American Academy of Microbiology. American Academy of Microbiology. Washington DC., USA. 2002. Pp. 1-25.
39. Umoh VJ, Okafo C and Galadima M. Contamination of vegetables cultivated on land irrigated with urban wastewater in Zaria and Kaduna, Nigeria. *Nigeria Journal of Parasitology*. 2001. 22: 95-104.
40. Okafo CN, Umoh VJ, Galadima M. Occurrence of pathogens on vegetables harvested from soils irrigated with contaminated streams. *Science Total Environ*. 2003. 311: 49-56.
41. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an

- international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbial Infection*. 2012. 18: 268–281
42. Umolu PI, Omigie O, Tاتفeng Y, Omorogbe FI, Aisabokhale F and Ugbo dagah OP. Antimicrobial Susceptibility and Plasmid Profiles of *Escherichia coli* Isolates Obtained. *Journal of American Science*. 2006. 2(4).
43. Chijioke AN and Christian UI. Antibiotic resistance profile of *Escherichia coli* isolated from apparently healthy domestic livestock in South-East Nigeria. *Journal of Cell and Animal Biology*. 2012. 6(8):129-135.
44. Ibezim EC. Microbial Resistance to Antibiotics. *African Journal of Biotechnology*. 2005. 4(13):1606-1611
45. Iruka NO, Adebayo L and Robert E. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. *Emerging infectious diseases*. 1999. 5(1):18-27
46. W.H.O (2013). Diarrhoeal disease. www.who.int/mediacentre/factsheet/fs330
47. Christopher A.J, Hora S and Ali Z (2013). Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of *Escherichia coli* isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly-India. *Annals of Tropical Medicine and Public Health*. 6(3): 285-289.
48. Orozova, P., Chikova, V., Kolarova, V., Nenova, R., Konovska, M. and Najdenski, H. (2008). Antibiotic resistance of potentially pathogenic *Aeromonas* strains. *Trakia Journal of Sciences*, 6(1): 71-78.