

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 50 samples from diarrhea patients submitted for bacteriological diagnosis at the Medical Microbiology Laboratory of Ahmadu Bello University Teaching Hospital 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 *E. coli* serotype O157:H7 to fourteen (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 emergence of varying resistant pattern and virulent multidrug resistance isolates 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, 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 sequel [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]. Other strains of *E. coli* that cause gastroenteritis in humans include: enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC) [3], enterotoxigenic (ETEC), and diffuse adherent (DAEC). 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 ABUTH and also understand their antibiotic susceptibility profile.

METHODOLOGY

Ethical Consent

Ethical approval for the collection of human samples was obtained from the Ahmadu Bello University Teaching Hospital Ethical committee.

Sample Collection and Isolate Identification

Of the 50 samples (blood, urine and stool) of diarrhea patients for the period of 6months (March - August, 2011), submitted for bacteriological diagnosis at the Medical Microbiology Laboratory of Ahmadu Bello University Teaching Hospital Shika, Zaria, Nigeria, 30 isolates of *E. coli* were identified on eosin methylene blue and inoculated onto nutrient agar at 37°C for 24hrs incubation period for further morphological identification using standard microbiology methods [13] [14] [15].

Biochemical Test and Principle

Further biochemical tests for *E. coli* indole, methyl red, Voges-Proskauer, citrate, cetrimide, urease, catalase, oxidase, 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. Sorbitol-MacConkey agar has been used extensively to isolate this organism from clinical specimens. Hemorrhagic colitis agar, a selective and differential medium, is used in a direct plating method to isolate O157:H7 from foods. A third procedure uses Sorbitol-MacConkey medium containing potassium tellurite and cefixime. It includes an enrichment step and is a new method developed as a result of the recent foodborne outbreaks [18].

Antibiotic susceptibility test

The antibiotics prescribed at the Ahmadu Bello University, Teaching Hospital 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 (MAR) Index

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

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobe, non-sporulating, rod-like in shape and belongs to the family enterobacteriaceae, commonly found in the lower intestine of warm blooded eukaryotes. *E. coli* is commonly found in faeces, urine, sewage and contaminated water [22].

Of the 50 samples from blood, urine and stool of diarrhea patients submitted for bacteriological diagnosis at the Medical Microbiology Laboratory of Ahmadu Bello University Teaching Hospital Shika, Zaria, Nigeria, for the period of 6months (March - August, 2011), 60% (30/50) 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% (11/30) and this account for 55% (11/20) of stool sample isolates (Table 1).

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

Samples n= 50	No. Isolate	% from sample	<i>E. coli</i> O157:H7	Percentages
Stool	20	66.67	11	55
Urine	6	20	0	0
Blood	4	13.33	0	0

The morphological, phenotypical and biochemical characteristics of *E. coli* O157:H7 on sorbitol MacConkey agar are shown in Figure 1 and Table 2 respectively. *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 2: Morphological Characteristics of *E. coli* Serotype O157:H7 on Sorbitol MacConkey Agar.

Morphological and phenotypical characteristics of *E. coli* O157:H7 on sorbitol MacConkey agar. *E. coli* O157:H7 appeared colourless on CT-SMA (S_{56}) while other serotypes of *E. coli* showed pinkish colour (negative) on CT-SMA agar (U_7).

Key: S_{56} = Positive, U_7 = Negative, A = Greenish metallic sheen of *E. coli* on eosin methylene blue agar, B = *Klebsiella* spp. on eosin methylene blue

The result of the morphological and biochemical studies of *E. coli* isolates are shown in Table 1.

Table 1: Biochemical Identification of *E. coli* O157:H7

Lab No.	MA	MN	EMB	I	MR/VP	C	CR	H ₂ S/TSI	UR	O/CA	Organism	CT-SMA
S_8	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
S_{16}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
S_{11}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
S_{39}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
S_{52}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
S_{35}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
S_{43}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
S_{44}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
B_{46}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
B_5	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
B_{18}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
B_{27}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
S_{41}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
S_{42}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
S_{40}	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+

136	S ₄₇	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
137	S ₄₈	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
138	S ₄₅	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
139	U ₇	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
140	U ₃₁	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
141	U ₂₀	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
142	U ₃₈	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
143	S ₃₇	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
144	U ₅₀	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
145	U ₅₆	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
146	S ₅₇	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-
147	S ₄₆	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
148	S ₅₄	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
149	S ₅₆	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	+
150	S ₁₇	Pink red	-	+	+	+/-	-	-	-/+	-	-/+	<i>E. coli</i>	-

151 Keys: MA= MacConkey Agar, MN = Mannito salt agar, EMB = Eosin methylene blue, I =
 152 indole test, MR= Methyl red, VP = Voges-Proskauer, C= Citrate agar, CR = Cetrimide agar, TSI
 153 = Triple sugar iron, UR = urease agar, O = oxidase, CA = Catalase test, CT-SMA = Cexifime
 154 and potassium tellurite –sorbitol MacConkey agar, G = Gas in glucose, - = Negative result, + =
 155 Positive result, S = Isolate from stool, U = urine, B = blood
 156

157 The calls for critical examination of the ways in which food and water are treated within Zaria,
 158 Nigeria is imperative, because previous studies, in Lagos [23], Lagos and Ibadan [24], Benin
 159 [25], Borno and Adamawa [26], Zaria [27][28] and Kano [29] has implicated *E. coli* O157:H7 in
 160 stool of diarrhea patents resulting from eating unwashed vegetables, undercooked meat or other
 161 food sources. According to Josefa *et al.*, [30] the distribution of *E. coli* O157:H7 disease are
 162 foodborne 183 (52%), unknown 74 (21%), person-to-person 50 (14%), waterborne 31 (9%),
 163 animal contact 11 (3%), and laboratory-related 1 (0.3%). But according to Dahiru, *et al.*, [29];
 164 the prevalence rate of *E. coli* serotype O157:H7 in Kano was 53% in fresh beef and 25.3% in
 165 roasted beef. This concurs with the work of Schlundt, [31] that consumption of inadequately
 166 cooked beef might pose a serious risk of infection. However, *E. coli* serotype O157:H7 illness
 167 has been reported not to be in outbreak proportions in Nigeria since 1994 [32][33]. Available
 168 information indicates that the carriage of *E. coli* serotype O157:H7 in cattle was an important
 169 factor in the emergence of this pathogen in Africa [34][35]. According to Umeh and
 170 Okpokwasili [36]; the prevalence of *E. coli* O157:H7 in livestock is extrapolated as 20% in cattle,
 171 12.5% in sheep, 7.5% in goat, 5% in chicken and 2.5% in pig faecal samples and increased
 172 shedding is usually observed during the months of December- March. However, illness in reared
 173 animals has frequently been linked to contaminated irrigation water, with animal faeces from
 174 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 3) 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 3).

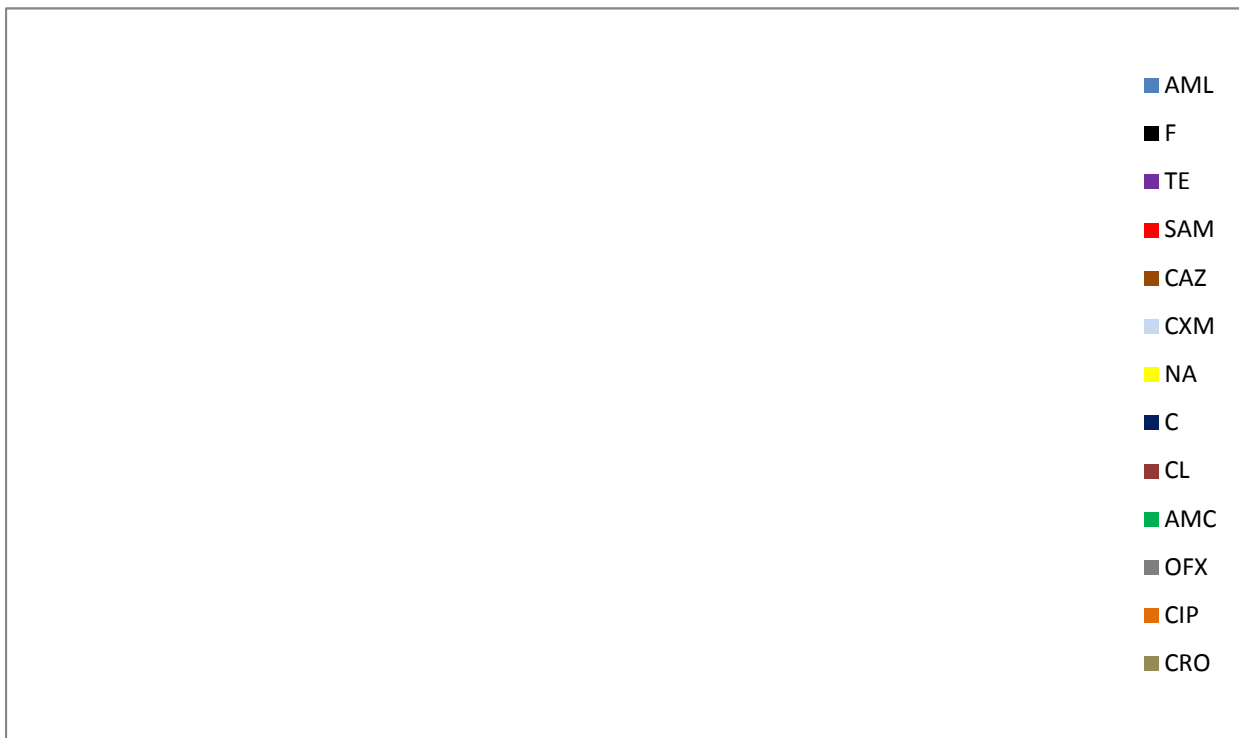


Figure 3: Percentages of resistance of *E. coli* O157:H7 to selected antibiotics

Keys: S = Sensitive, I = Intermediate, R= Resistance, 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).

The isolates also showed varying resistance pattern with 63% (7/11) resistant to more than 3 antibiotics commonly prescribed for *E. coli* associated infection/diseases (Table 3). At ≥ 0.3 MARI, all the *E. coli* isolates showed a total of 55.78% MARI (Table 3), while only serotype O157:H7 showed a MARI of 45.45% (Table 3) respectively.

Table 3: Resistant Pattern and MARI in Percentages of Isolated *E. coli* O157:H7

Lab code	Resistance Pattern	No of Antibiotic Resistance	MARI (n/14)
S ₈	AML	1	0.1
S ₁₆	F, TE, SAM	3	0.2
S ₄₆	TE, AMC, SAM	2	0.1
S ₅₂	CXM, TE, NA, AML, SAM, C, CL, AMC,	8	0.6
S ₄₄	CAZ, F, CXM,AMC,SAM, C, CL	7	0.5
S ₁₁	CAZ, SAM	2	0.1
S ₅₆	CAZ, NA, TE, AMC, CL	5	0.4
S ₄₀	CAZ,	1	0.1
	CAZ, CL, AMC, CRO, OFX, CXM, NA,	11	0.8
S ₄₅	TE, AML, CIP, SAM		
S ₃₇	CAZ, CXM, TE, AMC, SAM, AML,	6	0.4
S ₅₄	NIL	0	0

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)

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

In line with the International Expert Proposal for Interim Standard Definitions for acquired resistance [41], the resistance pattern of the isolates showed that 54.5% of the isolates were multidrug resistant, 18.2% showed extensively drug-resistant, while 27.7% were neither MDR nor XDR (NIL).

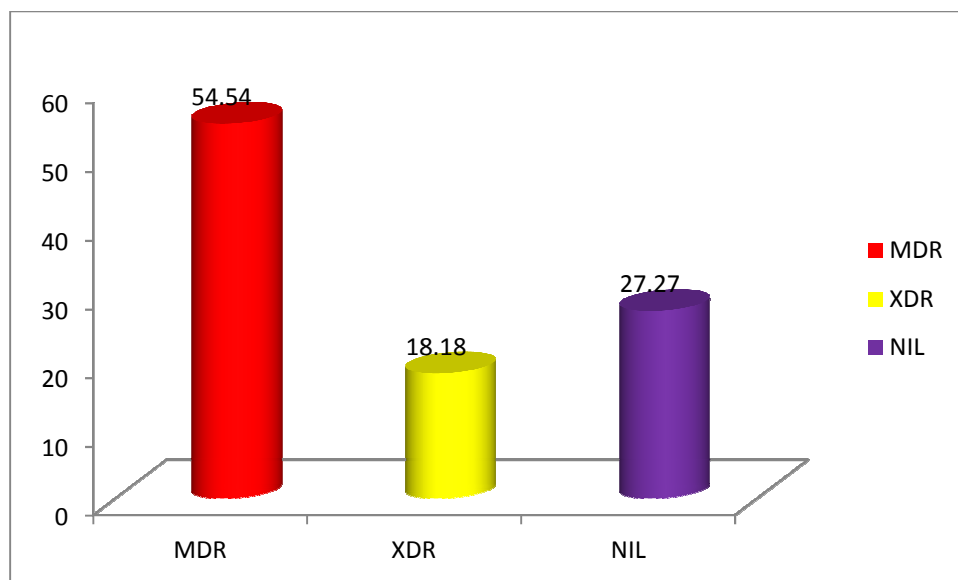


Figure 4: Categories of Antibiotic Resistance in *E. coli* isolates

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.

These express a significant number of antibiotics resistances within Zaria metropolis and of serotype O157:H7. The reports of Bell, *et al.*, [28] and Umolu, *et al.*, [42] in Lagos reported that *E. coli* showed maximum sensitivity to Ceftriaxone and Ciprofloxacin (mean score of 85%) while antibiotics like Gentamicin are effective than newer antibiotics because of limited use [24] with Nitrofurantoin and Ofloxacin showing the least resistance levels of 6% and 19% respectively to isolates of *E. coli*. Chijioke and Christian [43] who worked in Enugu using *E. coli* from livestock also supported that *E. coli* isolates showed resistance rates of 85% to Ampicillin; 22.5% to Ceftriaxone; 18.8% to Cefoxitin; 16.3% to Nalidixic acid and 12.5% to Gentamicin. But the variation in rate of resistance of microorganism to antibiotic might be a function of misuse of antibiotics in such 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, with the consequent risk of selection of antibiotic-resistant mutants. 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 Queuro 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 faecal 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 Rilely 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.

- 350 39. Umoh VJ, Okafo C and Galadima M. Contamination of vegetables cultivated on land
351 irrigated with urban wastewater in Zaria and Kaduna, Nigeria. *Nigeria Journal of*
352 *Parasitology*. 2001. 22: 95-104.
- 353 40. Okafo CN, Umoh VJ, Galadima M. Occurrence of pathogens on vegetables harvested
354 from soils irrigated with contaminated streams. *Science Total Environ*. 2003. 311: 49-56.
- 355 41. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al.*.
356 Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an
357 international expert proposal for interim standard definitions for acquired resistance.
358 *Clinical Microbial Infection*. 2012. 18: 268–281
- 359 42. Umolu PI, Omigie O, Tاتفeng Y, Omorogbe FI, Aisabokhale F and Ugbodagah OP.
360 Antimicrobial Susceptibility and Plasmid Profiles of *Escherichia coli* Isolates Obtained.
361 *Journal of American Science*. 2006. 2(4).
- 362 43. Chijioke AN and Christian UI. Antibiotic resistance profile of *Escherichia coli* isolated
363 from apparently healthy domestic livestock in South-East Nigeria. *Journal of Cell and*
364 *Animal Biology*. 2012. 6(8):129-135.
- 365 44. Ibezim EC. Microbial Resistance to Antibiotics. *African Journal of Biotechnology*. 2005.
366 4(13):1606-1611
- 367 45. Iruka NO, Adebayo L and Robert E. Socioeconomic and behavioral factors leading to
368 acquired bacterial resistance to antibiotics in developing countries. *Emerging infectious*
369 *diseases*. 1999. 5(1):18-27