International Journal of Biology Sciences
www.biologyjournal.net
Online ISSN: 2664-9934, Print ISSN: 2664-9926
Received: 30-05-2021; Accepted: 16-06-2021; Published: 29-06-2021
Volume 3, Issue 1, 2021, Page No. 30-36

Search for *E.coli* o157:h7 in stool samples from diahrreagenic patients attending dutsin-ma general hospital, Katsina state, north-western Nigeria

Khalifa Jamil Saleh[1*], Doyinsola Marvellous Alakiu[2], Abubakar Sunusi Adam[2]
[1] Department of Microbiology, Faculty of Life Sciences, Federal University Dutsin-Ma, Katsina, Nigeria
[2] Microbiology Department, Faculty of Life Sciences, Federal University Dutsin-Ma, Nigeria

Abstract
Escherichia coli O157 is pathogenic strain of E. coli that is known to cause diarrhea leading to fluid loss, and other severe complications like hemolytic uremic syndrome. This work was therefore aimed at isolating E. coli O157 from human stool with the set objectives of identifying the risk factors associated with diarrhea and determining the biochemical characteristic of E. coli isolates. A total of 30 stool samples were collected from patients with age ranging from zero to thirty (0-30) years, positive E. coli showed that only zero to five years age range of the respondents had the highest positive 10(38.5%).Stool specimens collected from patients were inoculated onto MacConkey and pink and colorless colonies on the media were sub cultured into Eosin methylene blue and Salmonella Shigella agar. The presumptive E. coli isolates that appeared as green metallic sheen on Eosin Methylene Blue agar were picked and confirmed biochemically as E. coli using biochemical test procedure. The confirmed E. coli isolates were then cultured on Rhamnose Sorbitol MacConkey Agar supplemented with Cefixime medium (Oxoid SR 172) and the isolates appeared colorless on CR- SMAC. 26 (72.22%) of the 30 samples yielded E. coli and 8(22.22%) of yielded positive salmonella spp and 2(5.6%) also yield positive Shigellaspp.The prevalence rate of 2 (7.7%) was recorded for Escherichia coli O157:H7. The presence of Enterohaemorhagic E. coli O157:H7 in Dutsin-ma town is no longer in doubt. We advocate that a more intense and well planned public enlightenment be mounted by our sanitary health officials, while cases of gastroenteritis with bloody or non-bloody diarrhea be properly investigated bacteriologically.

Keywords: diarrhea, risk factors, public health threat, escherichia coli o157, dutsin-ma town

Introduction

Enteric pathogens are gastrointestinal organisms known to cause gastrointestinal infection. Gastrointestinal infection also known as gastroenteritis is any infection caused by Viruses, Bacteria or Parasites and is characterized by excessive watery diarrhea and stomach pain. Acute diarrhea is a second most common cause of infant deaths worldwide and is a common cause of mortality in developing countries (Victoria *et al.*, 2008).It is estimated that 1.3 billion episodes of diarrhea occur in children below five years of age with about 760,000 deaths occurring yearly (WHO 2013). *Escherichia coli* is a common in habitant of the human and animal gut, but canal so be found in the physical environment such as; water, soil and vegetation and are thus referred to as being ubiquitous. Many Escherichia coli strains are usually not harmful and act as commensalism in the intestine of warm blooded animals, but some few strains have been found to cause mild to severe disease in man (WHO 2013).

*Escherichia coli*is the pathogen most commonly associated with endemic forms of childhood diarrhea (Huillan*et al.*, 1991). Diarrhea is a leading cause of morbidity and mortality among children in developing countries (Guerrant*et al.*, 1990; WHO 2005). Among the adult populace, diarrhea diseases can lead to drastic loss in man-hours, thus depleting state and national income (WHO 2011)

.

E. coli O157:H7isaGram negativebacillus (rod) bacteriuminthe *Entero bacteria ceae* family. The etiologic agent *E.coli* O157:H7 have several transmissions that can be spread around to animals and humans. In humans this serotype of E.coli "is transmitted to humans primarily through consumption of contaminated foods, (WHO 2011) mainly undercooked meat like raw beef and milk. With contaminated food products, it can also cause "cross---contamination during food preparation. (WHO 2011). Known contaminated foods that have caused outbreaks of infections besides beef are fruits and vegetables" Where by contamination maybe due to contact with feces from domestic or wild animals at some stage during cultivation or handling. (WHO, 2011). People that visit farms and come into direct contact with farm animals that carry the pathogen can also become infected. Another mode of human transmission is person to person contact through the oral–fecal route is "infected people do not wash their hands after using the toilet. (DHI New York 2006).

E. coli have many reservoirs, cattle and small "domesticated ruminants constitute a primary animal reservoir of *E.coli* O157:H7 (USN 2011) like poultry.

The bacterium can also be in vegetables, surface water, fruit and dairy products. Animal manure can also harbor the pathogen where it can contaminate water supply of both

humans and domesticated animals.
E. coli O157:H7 produce a toxin called Shiga toxin that can be deadly in humans and is similar to toxins produced by Shigella dysenteries. *E. coli* that produce Shiga toxins are also referred as (STEC).
E. coli O157:H7 grow with an optimum temperature of 37°C and can grow in temperatures of between 7°C to 50°C. It is a facultative anaerobe, which means it can grow with or without the presence of oxygen (Tarr *et al.*, 1990).
The aim is to screen for *Escherichia coli* O157:H7 in diarrheic patients in L.G.A Dutsin-ma general hospital Katsina state.

Methodology
Study Area: This study was carried out in the town of Dutsin-ma local government area Katsina state. Which lies between the coordinates of 12°27'17"N, 7°29'29"E (News Track India, 2015). The local Government has an area 527 Km2 (203sqkm) and a population of 169,671 as at 2006 census. The local Government is bounded by Kurfi and Charanchi local Government to the North, Kankia local Government to the East, Safana and Dan- musa local Government to the West, and Matazu local Government to the South. It also has the Zobe Dam lying to the South of the town (Wikipedia, 2014).

Fig 1

Sampling Collection
30 stool samples was collected from diarrheal patients at MallamMande General Hospital Dutsinma, and transported on ice packed to department of microbiology, Federal University, Dutsinma for microbiological analysis

Methods
Isolation of lactose and non-lactose fermenter
Lactose and non-lactose fermenter were isolated using Mac Conkey agar. Mac Conkey agar was prepared according to the manufacturer instruction. Thereafter, pink and colorless colonies appeared on the media. (Cheesbrough 2006) [11]
Isolation of Escherichia coli
Those with pink colonies were sub cultured on Eosin methylene blue (EMB) agar. EMB agar was prepared according to the manufacturer instruction. After 24hrs green metallic sheen colonies appeared on EMB which shows positive *E. coli.* (Cheesbrough 2006) [11]

Isolation of salmonella and shigella
Those with colorless colonies were sub cultured on salmonella shigella agar (SSA). SSA agar was prepared according to the manufacturer instruction. After 24hrs, creamy colonies with dark centered appeared on SSA which shows positive salmonella and Shigella. (Cheesbrough 2006) [11]

Gram staining
Smear was prepared on a clean and dry slide from overnight culture, the smear was allowed to dry and then fixation was done. The smear was then covered with crystal violent for 30 to 60 seconds and then washed. Iodine was added too for 30 to 60 seconds and washed. Decolorized with alcohol and washed immediately. Safranin was added for 2 minutes then washed. It was examined using 100xobjective lens with oil immersion.

Biochemical Test
Several biochemical tests were carried out in order to have a presumptive identification of the potential bacteria chosen before. Most of the methods were done according to the microbiology laboratory manual (Cappuccino and Sherman, 2005).

Indole test
Tryptophan broth was inoculated with colony of the test organism in tryptophan broth. Incubated at 37℃ for 24-48hrs in a incubator. Added 0.5ml of kovac's reagent to the both cultures. Positive results showed pink color in the reagent layer in 1 minute.

Methyl red test
MR-VP agar was aseptically prepared in agar slant test tubes according to manufacturer's instructions. Overnight culture were inoculated on the agar slant thereafter incubated for 24 hrs. Colour changes from yellow to red indicates positive results when a few drops of methyl-red reagent were added to the culture. S

Voges-Prokauer test
MR-VP agar was aseptically prepared in agar slant test tubes according to manufacturer's instructions. Overnight culture were inoculated on the agar slant thereafter incubated for 24 hrs. A few drops of Barri's reagent were added a copper-like change indicate positive result.

Citrate utilization test
Citrate utilization test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon by the enzyme citrate permease. Simmons citrate agar slants of 2ml in each vial were prepared by autoclaving at 15 psi 121℃. Using sterile technique, small amount of the experimental bacteria from 24hrs old pure culture was inoculated into the vials by means of a streak inoculation method with an inoculating needle and the vials were incubated for 48hrs at 37℃. Observe color change citrate positive change, color of media to blue. (Cappuccino and Sherman, 2005).

Triple sugar iron
Triple sugar iron was done to test microorganism's ability to ferment sugars and produce hydrogen sulfide. It is often used in selective identification of enteric bacteria including salmonella and shigella.

- With a straight inoculation needle, the top of the isolated colony was touched.
- TSI was inoculated by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant.
- The cap was left on loosely and incubate the tubes at 37℃ in ambient air for 18 to 24hrs.
- The reactions of the medium were examined

- **Isolation of *E. coli* O157:** H7 Ramnose Sorbitol MacConkey was prepared according to manufacturer instruction and was also diluted with cefrixime. The confirmed *E. coli* isolates were cultured onRamnose Sorbitol MacConkey Agar plates (CR-SMAC), the colonies that appeared colour less on CR- SMAC were tagged as presumptive *E. coli* O157.

Results
Table 1 shows results for Culture, Microscopy, and Gram reaction for the 30 stool samples collected.
The media used for the isolation are Eosine Methylene Blue Agar, *Salmonella Shigella* Agar Cefixime Rhamnose Sorbitol MacConkey Agar for the isolation of *Escherichia coli*, *Salmonella* and *Shigella spp* and *Escherichia coli* O157:H7 colonies respectively.
Microscopy and Gram staining was performed as all shapes for colonies revealed bacilli and Gram reaction, Gram negative.

Table 1: shows results for Culture, Microscopy, and Gram reaction for the 30 stool samples collected

Sample	Culture SSA	EMB	CR-SMAC	Microscopy	Gram Reaction	Organism
B1S1	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B1S2	BCC	GMSC	BC	Bacilli	Negative	*Salmonella, E.coli*
B1S3	NG	CC	-	Bacilli	Negative	-
B1S4	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B1S5	BCC, CC	GMSC	BC	Bacilli	Negative	*Salmonella, Shigella, E.coli*
B2S1	NG	CC	-	Bacilli	Negative	-
B2S2	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B2S3	BCC	GMSC	BC	Bacilli	Negative	*Salmonella, E.coli*
B2S4	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B2S5	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B3S1	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B3S2	BCC	GMSC	BC	Bacilli	Negative	*Salmonella, E.coli*
B3S3	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B3S4	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B3S5	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B4S1	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B4S2	BCC	CC	-	Bacilli	Negative	*Salmonella*
B4S3	NG	GMSC	CC	Bacilli	Negative	*E.coli E.coli* O157:H7
B4S4	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B4S5	BCC	GMSC	BC	Bacilli	Negative	*Salmonella, E.coli*
B5S1	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B5S2	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B5S3	BCC	CC	-	Bacilli	Negative	*Salmonella*
B5S4	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B5S5	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B6S1	NG	GMSC	BC	Bacilli	Negative	*E.coli*

B6S2	NG	GMSC	BC	Bacilli	Negative	*E.coli*
B6S3	BCC	GMSC	CC	Bacilli	Negative	*Salmonella*, *E.coli*, *E.coli* O157:H7
B6S4	NG	GMSC	BC	Bacilli	Negative	*E.coli*

Keys: B = Batch, S = Sample, SSA = *Salmonella-Shigella* agar, EMB = Eosine Methylene Blue, CR-SMAC = Cefixime Rhamnose Sorbitol Mac Conkey Agar, BCC = Black centered colonies, CC = Colorless colonies, NG = No growth, GMSC = Green metallic sheen colonies, BC = Blue colonies.

Table 2: Shows result for biochemical test for positive colonies on Eosine Methylene Blue and CR-SMAC agars to confirm *Escherichia coli* and *Escherichia coli* colonies respectively.

Sample	Biochemical Tests				Organism confirmed
	Indole Test	Methyl Red	V.P	Citrate Utilization Test	
BIS1	+	+	-	-	***E. coli***
B1S2	+	+	-	-	***E. coli***
B1S3	-	-	+	+	
B1S4	+	+	-	-	***E. coli***
B1S5	+	+	-	-	***E. coli***
B2S1	-	-	+	+	
B2S2	+	+	-	-	***E. coli***
B2S3	+	+	-	-	***E. coli***
B2S4	+	+	-	-	***E. coli***
B2S5	+	+	-	-	***E. coli***
B3S1	+	+	-	-	***E. coli***
B3S2	+	+	-	-	***E. coli***
B3S3	+	+	-	-	***E. coli***
B3S4	+	+	-	-	***E. coli***
B3S5	+	+	-	-	***E. coli***
B4S1	+	+	-	-	***E. coli***
B4S2	-	-	+	+	
B4S3	+	+	-	-	***E. coli***
B4S4	+	+	-	-	***E. coli***
B4S5	+	+	-	-	***E. coli***
B5S1	+	+	-	-	***E. coli***
B5S2	+	+	-	-	***E. coli***
B5S3	-	-	+	+	
B5S4	+	+	-	-	***E. coli***
B5S5	+	+	-	-	***E. coli***
B6S1	+	+	-	-	***E. coli***
B6S2	+	+	-	-	***E. coli***
B6S3	+	+	-	-	***E. coli***
B6S4	+	+	-	-	***E. coli***
B6S5	+	+	-	-	***E. coli***

Keys: B = Batch, S = Sample, + = Positive, - = Negative, VP =Vorge's Proskauer

Table 3: Shows Biochemical Reaction for Colonies Presumptive For *Shigella* And *Salmonella* From Ssa.

Sample	Biochemical Test Triple Sugar Iron Agar Gas Slant Butt H_2S				Organisms Confirmed
BIS1	-	-	-	-	-
B1S2	+	R	Y	+	*Salmonella*
B1S3	-	-	-	-	-
B1S4	-	-	-	-	-
B1S5	+	Y	R	+	*Shigella, Salmonella*
B2S1	-	-	-	-	-
B2S2	-	-	-	-	-
B2S3	+	R	Y	+	*Salmonella*
B2S4	-	-	-	-	-
B2S5	-	-	-	-	-
B3S1	-	-	-	-	-
B3S2	+	R	Y	+	*Salmonella*
B3S3	-	-	-	-	-
B3S4	-	-	-	-	-
B3S5	-	-	-	-	-
B4S1	-	-	-	-	-
B4S2	+	R	Y	+	*Salmonella*
B4S3	-	Y	R		*Shigella*

B4S4	-	-	-	-	-
B4S5	+	R	Y	+	*Salmonella*
B5S1	-	-	-	-	-
B5S2	-	-	-	-	-
B5S3	+	R	Y	+	*Salmonella*
B5S4	-	-	-	-	-
B5S5	-	-	--	-	-
B6S1	-	-	-	-	-
B6S2	-	-	-	-	-
B6S3	+	R	Y	+	*Salmonella*
B6S4	-	-	-	-	-
B6S5	-	-	-	-	-

Keys: B = Batch, S = Sample, + = positive, R = Alkaline, Y = Acid - = Not applicable

Table 4: Occurrence of *Escherichia coli* of stool samples from Dutsin-ma General Hospital

Batches	No. of samples analyzed	No. of positive E. coli	Prevalence of *E.coli* (%)
B1	5	4	15.4
B2	5	4	15.4
B3	5	5	19.2
B4	5	4	15.4
B5	5	4	15.4
B6	5	5	19.2
Total	30	26	100

Keys: B1= Batch 1, B2=Batch 2, B3= Batch 3, B4=Batch 4, B5=Batch 5, B6=Batch

Table 4 shows the occurrence of *E.coli* from stool samples from six different batches where B3 and B6 have the highest Occurrence of *E.coli* (19.2%) and the lowest occurrence of *E.coli* which is (15.4%) in respect to other batches.

Table 5: Demography of stool samples collected with respect to age

Age	No. of samples analyzed	No. of positive E.coli	Percentage (%)
0-5	12	10	38.5
6-10	5	4	15.4
11-15	4	4	15.4
16-20	4	4	15.4
21-25	3	3	11.5
26-30	2	1	3.9

Table 5 shows result of stool samples with respect to age group. Stool samples from patients with the age group of 0-5 Years have the highest number of *E.coli* 10 (38.5%), and 26-30 have the lowest number of *E.coli* 1 (3.9%).

Table 6: Demography of stool samples collected with respect to gender

Gender	No. of samples analyzed	No. of positive *E.coli*	Percentage (%)
Male	12	8	30.8
Female	18	18	69.2

Table 6 also shows result of stool samples with respect to gender. Stool from male patients have the lowest number of *E.coli* 8 (30.8%) in this table and female have the highest number of *E. coli* 18 (69.2).

Table 7: Prevalence of the occurrence of *E.coli* O157:H7

Isolate	No. of *E.coli*	No. of *E.coli* O157:H7	Prevalence of *E.coli* O157:H7 (%)
B1	4	0	0
B2	4	0	0
B3	5	0	0
B4	4	1	3.33
B5	4	0	0
B6	5	1	3.33

Keys: B1=Batch1, B2=Batch 2, B3=Batch3, B4=Batch4, B5=Batch5 and B6=Batch 6

Table 7 Shows the occurrence of *E.coli*O157:H7 from stool samples, from different batches where B4and B5 shows the Present of *E.coli* O157:H7 and absent of *E.coli* O157:H7 in respect to other batches.

Table 8: Prevalence of the organisms

Organisms	Occurrence	Prevalence (%)
Escherichia coli	26	72.22
Salmonella	8	22.22
Shigella	2	5.56
E.coli O157:H7	2	7.7
Total	36	100

Table 8 shows the prevalence number of organism present in stool samples where *E.coli* have the highest occurrence (72.22%) and *Shigella* have the lowest occurrence (5.56%).

Discussion

The finding in this study indicates that age remains a major risk factor in diarrhea disease, children between the Age of 0-5 are highly vulnerable to diarrhea. The prevalence of 38.5% diarrhea in respondent 0-5 in this work is higher than the 9.8% obtained by (H.O Abdulazi*et al.,* 2016) [19], lower than 43.1% obtained by Ifeanyi*et al.,* in Abuja, and higher than 2.6% obtained by Yilgwan andOkolo in Jos plateau. These differences might be due to breaches in sanitation and hygiene infrastructure of the respondents from these cities. The high occurrence rate of diarrhea among children 0-5 years in this study may be due to the fact that children within this age group on their own cannot differentiate between what to eat and what not to eat; they have not learnt the rudiment of adherence to aseptic or hygienic practices. Another reason for their high vulnerability to diarrhea may be due to weaker immunity as a result of them having lost their inborn immunity after being weaned from breast milk. Young children use more water over the course of a day given their higher metabolic rates, also their kidneys are less able to conserve water compared to older children and adults as such diarrhea is usually prevalent and often life threatening too. In this study, it was observed that the number of diarrheic stool gotten from adults was quite small compared to that obtained from children and this might not be unrelated to the fact that, adults in the locality rarely visit health institutions when they have diarrhea unless they perceive the diarrhea as being serious, usually if blood is present as reported by Okeke*et al.,*

The 7.7% prevalence rate of *E.coli* O157:H7 in this study is higher than the 1.39% prevalence by Abdulaziz *et al* in Zaria and the 6% prevalence by (Olorushola *et al.,*2006) in Lagos, the 3.1% prevalence obtained by (Ngbede*et al.,* 2006) in jos and lower than the 20% prevalence recorded by Esumeh*et al.,* in Benin state. Although there are differences in prevalence rate of Escherichia coli O157 in the stool samples in different parts of Nigeria, this result however shows that *Escherichia coli* O157 remains an Aetiological agent for diarrhea in Nigeria. The presence of *Escherichia coli* O157 in stool samples might not be unconnected to the fact that patients have been exposed to unsanitary conditions such as consumption of contaminated water, food, fruits and vegetables. Thus the 22.2% and 5.56% prevalence of *Salmonella spp* and*Shigellaspp* is lower than the 47.4% and 10.2% prevalence obtained by Meltzer *et al* 2007 and the 50% prevalence obtained by (wool *et al.,* 2006). Therefore, the prevalence of *E.coli* (72.22%) is higher than *Salmonellaspp* (22.2%) while the prevalence of *Shigellaspp* (5.56%) is lower than *Salmonella spp* and also lower than *E.coli*O157:H7 (7.7%).

Conclusion

The result on this studies indicate that diarrhea is higher among younger children between the age of 0-5 (38.5%) than adult between the age of 26 -30 (3.9%). This is due to the fact that children within this age group on their own cannot differentiate between what to eat and what not to eat. Also the prevalence of*E.coli* (72.22%) in this study is higher than the *E.coli* O157:H7 (7.7%) and the prevalence of *salmonella* (22.22%) is higher than the prevalence of *Shigella* (5.6%).

Therefore *E. coli* O157:H7 is an important Aetiology for diarrhea. It is also important to note that an exceptionally low dose of this organism is able to cause infection and once introduced into a closed group or family, it can spread by person to person transmission especially by children.

Recommendations

- Health inspector in the local government area of Dutsin-ma should be vigilant and ensures that campaigns are mounted to educate our citizens on ways of improving on the unsanitary environment.
- Potential source of infection as well as food and meat inspection must be followed up.

In addition, our physicians should make inquiry on *E.coli* O157:H7 infection whenever patients present at their clinic with bloody or non-bloody diarrhea so as to subdue possible outbreaks early enough.

References

1. Alisky J, Iczkowski K, Rappaport A, Troitsky N. 1998. Bacteriophages show promise as antimicrobial agents. J. Infect,1998:36:5-15.
2. Anderson RC, Buckley SA, Kubena LF, Stanker LH, Harvey RB, Nisbet DJ. Bactericidal effect of sodium chlorate on Escherichia coli O157:H7 and Salmonella typhimurium DT104 in rumen contents *in vitro*. J. Food Prot,2000:63:1038-1042.
3. Armstrong GL, Hollingsworth J, Morris JGJ. Emerging foodborne pathogens: Escherichia coli O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiol. Rev,1996:18: 29-51.
4. Barker J, Humphrey TJ, Brown MWR. Survival of Escherichia coli O157 in a soil protozoan: implicationsfor disease. FEMS Microbiol. Lett,1999:173:291-295.
5. Bell BP, Goldoft M, Griffin PM, Davis MA, Gordon DC, Tarr PI *et al*. A multistate outbreak of Escherichia coli O157:H7–associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. J. Am. Med. Assoc,1994:272:1349-1353.
6. Besser TE, Hancock DD, Pritchett LC, McRae EM, Rice DH, Tarr PII *et al*. Duration of detection of fecalexcretion of Escherichia coli O157:H7 in cattle. J. Infect. Dis,1997:175:726-729.
7. Buchko SJ, Holley RA, Olson WO, Gannon VPJ, Veira DM. The effect of different grain diets on fecal shedding of Escherichia coli O157:H7 by steers. J. Food Prot,2000:63:1467-1474.
8. Buchanan RL, Doyle MP. Foodborne disease significance of Escherichia coli O157:H7 and other enterohemorrhagic E. coli. Food Technol,1997:51:69-76.
9. Chapman PA, Wright DJ, Siddons CA. A comparison of immune magnetic separation and direct culture for theisolation of verocy to toxin-producing Escherichia Coli O157 frombovinefaeces. J. Med. Microbiol,1994:40:4424-427.
10. Chapman PA, Siddons CA, Cerdan-Malo AT, Harkin MA. A 1-year study of Escherichia coli O157 in cattle, sheep, pigs

and poultry. Epidemiol. Infect,1997:119:245-250.
11. Cheesbrough M. District Laboratory Practice in Tropical Countries Part 2, 2006.
12. Cray WCJ, Moon HW. Experimental infection of calves and adult cattle with Escherichia coli O157:H7. Appl. Environ. Microbiol,1995:61:1586-1590.
13. Doyle MP, Schoeni JL. Survival and Growth Characteristics of Escherichia coli O157:H7 Associated with Haemorrhagic Colitis. Applied and Environmental Microbiology,1984:48(4):855-856.
14. Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmarie M, Laegreid WW. Correlation of enterohemorrhagic Escherichia coli O157 prevalence in feces, hides, and carcasses of beef cattle during processing Proc Natl Acad Sci,2000:97:29993003.
15. Esumeh FI, Isibor JO, Egbagbe IDS. Screening For Escherichia Coli O157:H7 In Diarrheic Patients InBenin City, Nigeria Journal of Microbiology and Biotechnology Research,2011:1(4):1-4.
16. Fenlon DR, Wilson J. Growth of Escherichia coli O157 in poorly fermented laboratory silage: a possible environmental dimension in the epidemiology of E. coli O157. Lett. Appl. Microbiol,2000:30:118-121.
17. Griffin PM, Tauxe RV. The epidemiology of infections caused by Escherichia coli O157:H7, other enterohemorrhagic E. coli, and the associated hemolytic uremic syndrome Epidemiol Rev,1991:13:6096.
18. Hancock DD, Besser TE, Kinsel ML, Tarr PI, Rice DH Paros MG. The prevalence of Escherichia coli O157:H7 in dairy and beef cattle in Washington State Epidemiol Infect,1994:113:199-207.
19. AbdulAziz HO, Maryam Aminu, Machido DA, "Isolation and Characterisation Esherichia coli O157 in Human Stool Samples from Parts of Kaduna Metropolis Nigeria. American Journal of Food Science and Technology,2016:4:5.
20. Isobol SB, Ratnam S. Sorbitol-MacConkey medium for detection of Escherichia coli O157:H7 associated with hemorrhagic colitis J ClinMicrobiol,1896:23:869-87.
21. Janisiewicz WJ, Conway WS, Brown MW, Sapers GM, Fratamico P, Buchanan RL *et al*. Fate of Escherichia coli O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies Appl Environ. Microbiol,1999:65:1-5.
22. Johnson RP, Wilson JB, Michel P, Rahn K, Renwick SA, Gyles CL *et al*. Human infection with verocytotoxigenic Escherichia coli associated with exposure to farms and rural environments in CS Stewart and HJ Flint, eds Escherichia coli O157 in farm animals. CABI Publications, New York, NY,1999:147-168.
23. Kudva IT, Hatfield PG, Hovde CJ. Escherichia coli O157:H7 in microbial flora of sheep J Clin Microbiol,1996:34:431-433.
24. MacDonald IA, Gould IM, Curnow J. Epidemiology of infection due toEscherichia coli O157: a 3-year prospective study Epidemiol Infect.1996:116:279-284.
25. Nataro JP, Kaper JB Diarrheagenic Escherichia coli. Clin. Microbiol Rev,1998:11:142-201.
26. Schmidt H, Beutin L, Karch H. Molecular analysis of plasmid-encoded hemolytic of Escherichia coli O157:H7 strain EDL 933 Infect Immun,1995:63:1055-1061.
27. Shere JA, Bartlett KJ, Kaspar CW. Longitudinal study of Escherichia coli O157:H7 dissemination on four dairy farms in Wisconsin Appl Environ Microbiol,1998:64:1390-1399.
28. Swerdlow DL, Woodruff BA, Brady RC, Griffin PM, Tippen S, Donnell HDJ *et al*. A waterborne outbreak in Missouri of Escherichia coli O157:H7 associated with bloody diarrhea and death Ann Intern Med,1992:117:812-819.
29. Rice DH, Hancock DD, Besser TE.Verotoxigenic E coli O157 colonization of wild deer and range cattle Vet Rec,1995:137:524.
30. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR *et al*. Hemorrhagic colitis associated with a rare Escherichia coli serotype N Engl J Med,1983:308:681-685.
31. Van Donkersgoed, Berg J, Potter A, Hancock,D, Besser T, Rice D *et al*. Environmental sources and transmission of Esherichia coli O157 in feedlot cattle Can Vet J,2001:42:714-720.
32. World Health Organization. DA,2011.5/1/2014 "Enterohaemorrhagic Escherichia Coli (EHEC) Fact Sheet" http://www.who.int/mediacentre/factsheets/fs125/en/