Advanced Journal of Microbiology Research Vol. 2013
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Enterohaemorrhagic *Escherichia coli* 0157:H7 Prevalence in meat and vegetables sold in Benin City, Nigeria

Stephen A. Enabulele[1]* and Nduka Uraih[2]

[1]Department of Biological Sciences, College of Natural and applied Sciences, Igbinedion University, Okada, Nigeria.
[2]Department of Microbiology, Faculty of Life Science, University of Benin, Benin City, Nigeria.

Accepted 23 January, 2013

Food samples made up of three meat types of 72 samples each of fresh meat from abattoir and open traditional market and "ready to eat" grilled meat (*suya*) and three vegetable types consisting of 72 each of cabbage, lettuce and tomatoes all totaling 432 samples, were screened to determine the presence of *Escherichia coli* 0157:H7 in these food items sold in Benin City, Nigeria. Of the total food samples analyzed *E. coli* was isolated from 365(84.45%) out of which 10(2.32%) had *E. coli* 0157:H7. 72(100%) each of the abattoir and open traditional market samples had *E. coli*, while 41(56.9%) *suya* samples had *E. coli* isolated from them. Of these, 5(6.94%) abattoir samples, 2(2.78) open traditional market and 3(4.17%) *suya* samples had *E. coli* 0157:H7 present on them. The cabbage, lettuce and tomatoes had 48(66.67%), 68(94.99%) and 64(88.8%) samples with *E. coli* respectively. *E. coli* 0157:H7 was not detected in any of the vegetable samples. Most significant of the result is the finding that *E. coli* 0157:H7 is present in meat sold in Benin City, Nigeria and especially in the "ready to eat" grilled meat (*suya)* which is consumed directly without further processing. There is therefore the need for health authorities to put in place measures to prevent possible *E. coli* 0157:H7 outbreak.

Key words: *Escherichia coli* 0157:H7, meat, vegetables, suya.

INTRODUCTION

Escherichia coli 0157, which is a strain of the entero-heamorrhagic *E. coli* group, is recognized as an organism whose presence in any food material can lead to serious disease outbreak (Anonymous, 1999). The growth of this strain in the human intestine is known to produce large quantity of toxins, which can cause severe damage to the lining of the intestine and other organs of the body (Nataro and Kaper, 1998). These toxins are very similar to the toxins produced by *Shigella dysenteriae* (Paton and Paton, 1998; Perna et al., 1998). The organism is particularly associated with the development of hemolytic uremic syndrome, known to result in a mortality rate of 2 - 10% (Law, 2000) . The potentially high mortality associated with *E. coli* 0157 infection, therefore make its presence in any food material worrisome and of serious public health concern as most of the outbreaks recorded has been traced to consumption of beef contaminated with the *E. coli* 0157:H7 strain (Hussein, 2007) and Vegetables such as lettuce (Hilborn et al., 1999).

In spite of the wide knowledge of the organism and its interaction, there seem to be no report on the prevalence of the organism in Africa and particularly Nigeria. In the light of these prevailing circumstances, and an increased reported cases of *E. coli* 0157 infection outbreak worldwide (Anonymous, 2002), it becomes apparent that a thorough study be conducted in this part of the world to ascertain its presence in our food products. This study is aimed at determining the prevalence of *E. coli* 0157 in meat and vegetables sold in Benin City, Nigeria, since consumption of food contaminated with the organism can result in serious disease outbreak and even death.

MATERIALS AND METHODS

Collection of Samples

Samples of three different types of beef meat namely, fresh meat from abattoir, fresh meat from open traditional market and "ready to eat" grilled meat (*suya*) and three different types of vegetables namely, cabbage, lettuce and tomatoes, were collected randomly

*Corresponding author. E-mail: amadinbulele@yahoo.com. Tel.: +2348033526243.

Table 1. Prevalence rates of *E. coli* and *E. coli* 0157:H7 in meat samples.

Meat	No of samples analyzed	No. with *E. coli*	*%	No. with *E. coli* 0157:H7	*%	No. of *E. coli* Isolates	No. Identified as *E. coli* 0157:H7	%
Fresh Meat (Abattoir)	72	72	100	5	6.94	213	7	3.27
Fresh Meat (Open Market)	72	72	100	2	2.78	226	3	1.33
Suya	72	41	56.9	3	4.17	97	3	3.09
Total	216	185	85.65	10	4.63	536	13	2.43

*% = percentages based on No. of samples analyzed

from different locations in Benin City, Nigeria. A total of 72 of each food type were collected giving a total of 216 each for meat and vegetable respectively, and grand total of 432 samples were screened.

Sampling and screening was done between April, 2006 and February, 2007. Samples were collected in duplicates in sterile plastic containers, labeled and transported to the laboratory for immediate analysis. All the samples were screened initially for *E. coli* from which all positive isolates were further screened for *E. coli* 0157:H7

Isolation and identification of *E. coli* isolates

Each of the food samples was homogenized in a sterile mortar and 1 g of the homogenate food sample was suspended in 9 ml buffered peptone water. Serial dilutions of up to 10^{-7} were then made and 1 ml of each was plated on Eosin methylene blue (EMB) agar. They were then incubated at 37°C for 24 h. Pure cultures of all colonies exhibiting typical dark to purple red colonies with metallic sheen which is characteristic of *E. coli* on EMB were then made in readiness for biochemical tests. Biochemical tests to confirm *E. coli* was done using the API 20E test strips and in accordance with the method described by Holt et al. (1994).

Identification of *E. coli* 0157:H7

Pure cultures of all positive *E. coli* were cultured on cefixim tellurite sorbitol- MacConkey (CT-SMAC) agar using the recommended method of Vernozy-Rozand (1997) and incubated at 37°C for 18 - 24 h. Suspected colonies of *E coli* 0157:H7 were confirmed using slide agglutination test with *E. coli* 0157: H7 antiserum manufactured by DIFCO Laboratories, Michigan U.S.A.

RESULTS AND DISCUSSION

E. coli, which are normal flora of the human and animal intestine, have been identified as a leading cause of food borne illness all over the world. *E. coli* (Agbeyegbe and Uraih, 1982) and the *E. coli* 0157: H7 strain (Anonymous, 1993; Hussein, 2007) strain has previously been isolated from meat samples and has also been implicated in the contamination of vegetables such as lettuce (Hilborn et al., 1999). *E. coli* 0157: H7 strain was detected in some of the meat samples screened in this study but was not found in any of the vegetable samples examined.

Overall, *E. coli and E. coli* 0157: H7 prevalence rates (Table 1) were 85.65 and 4.63% respectively, with the fresh meat samples from abattoir and traditional open market each, recording 100% *E. coli* prevalence. Fresh meat from abattoir had the highest *E. coli* 0157: H7 prevalence (6.94%) while market samples had the lowest (2.78%). This result is consistent with the work of Agbeyegbe and Uraih (1982), whose study showed high prevalence rate of *E. coli* in raw meat samples. The result is also in agreement with the work of Hussein (2007), who recorded *E. coli* 0157: H7 prevalence rates in the range of 3 to 19.7% for beef cattle. These results are indication of the poor sanitary environment under which the animals are slaughtered and sold. These animals were slaughtered on the abattoir floor that is not properly disinfected after every kill, with butchers and retailers walking between carcasses as they transact their business, while those in the market are displayed on tables in the open for sale. Nkanga and Uraih (1981) reported that meat is frequently found to be contaminated due to poor sanitary environment during slaughter, transportation and usage and through handling.

The vegetable samples had an overall *E. coli* prevalence rate of 83.33% with lettuce recording the highest prevalence rate of 94.44% (Table 2) . *E. coli* is a normal inhabitant of the gut and has been isolated on several occasions from animal and human faeces (Agbonlahor and Odugbemi, 1982; Montenegro et al., 1990; Sayah et al., 2005). It has been observed that most of the vegetables in Nigeria are grown with irrigated water that are in contact with grazing cattle and this can be a source of contamination (Solomon et al., 2002). Another likely source of contamination with this organism is the vehicles on which the vegetables are transported to the various sales point. It has also been observed that vegetables are transported using the same vehicles used in trans-porting cattle from the north to the southern parts of the country with the cow dung as likely source of contamina-tion.

Although studies have shown that *E. coli* 0157:H7 could contaminate vegetables, especially through irrigation water and other sources such as soil (Solomon et al., 2002), this study did not detect the serotype out of all the

Table 2. Prevalence rates of *E. coli* and *E. coli* 0157:H7 in vegetable samples.

Vegetable	No of samples analyzed	No with *E. coli*	*%	No with *E. coli* 0157:H7	*%
Cabbage	72	48	66.67	0	0
Lettuce	72	68	94.44	0	0
Tomatoes	72	64	88.89	0	0
Total	216	180	83.33	0	0

*% = percentages based on No. of samples analyzed

all the *E. coli* isolated from all the vegetable samples screened (Table 2). This is in spite of the very high *E. coli* prevalence (83.33%) recorded among the vegetable samples. This findings lend credence to the findings of Johnson et al. (1996), who reported that humans are exposed more to non-0157 shiga-toxin *E. coli* (STEC) from food and environmental sources considerably more often than *E. coli* 0157, yet the incidence of non -0157 STEC infection is far lower than that of *E. coli* 0157.

Most significant of this study is the fact that *E. coli* 0157:H7 was isolated from "ready to eat" grilled meat *(suya*). *E. coli* 0157:H7 prevalence rate on the basis of number of sample analyzed showed that *suya* recorded 4.17 and 3.09% on the basis of the number of *E. coli* isolated (Table 1). These results are even higher than that recorded for open market fresh meat which will still be processed before consumption. This is quite significant, considering the low infective dose of the organism which is put at between 20 to 700 organisms in a study by Turtle et al. (1999) and 10 to 100 cells by Petridis et al. (2002). It was observed that the *suya* samples from which the organism was isolated were those obtained from hawkers who carry the product around and sell in trays especially to travelers in motor parks (bus station), without re-heating them in open fire as it is the practice with sale of *suya* in *suya* stands. Storage of food at room temperature and preparing food too long before consumption allow organism such as *E. coli* and *salmonella* to multiply and reach critical levels and have been identified as the most common cause of food poisoning (Adam and Mass, 1999) . Contamination by these organisms therefore could be as a result of improper handling of the *suya* after preparation and most especially from vectors like flies and hands of the sellers themselves. Enabulele el al. (1989) isolated *E. coli* and *Staphylococcus aureus* from the hands of 57.5 and 47.5% respectively of food handlers in Benin City.

Conclusively it is to be noted that with the low level of sanitary practices observed and lack of adequate data on infections outbreak in Nigeria the organism could spread easily without early detection and as such it is important to take seriously the isolation of the organism in food materials from this study. Consequent upon this, it is recommended that consumers cook their meat properly before they are consumed and also avoid indiscriminate eating of meat sold in the open that is not properly re-heated. Fruits and vegetables should be washed properly using appropriate food grade anti-microbial preparations and should not be carried in the same containers used for fresh meat to avoid cross contamination with the organism.

REFERENCES

Adam MR, Mass MO (1999). Food Microbiology. 2nd Edition. Royal Society of Chemistry, Cambridge p. 390.

Agbeyegbe JM, Uraih N (1982). The prevalence of *E. coli* in meat samples in Benin City, Nigeria. Microbios Lett. 20: 21–28.

Agbonlahor E, Odugbemi T (1982). Enteropathogenic, enterotoxigenic and enteroinvassive *E. coli* isolates from acute gastroenteritis from Patients in Lagos, Nigeria. Trans. R. Soc. Trop. Med. 76: 265-267.

Anonymous (Centers for Disease Control) (1993). Multi-state outbreak of *Escherichia coli* 0157:H7 infections from hamburgers - Western United States. 1992 – 1993 *MMWR*. 42(14): 258-263.

Anonymous (Centers for Disease Control), (2002). Multi-state outbreak of *Escherichia coli* 0157:H7 infections associated with eating ground beef - United States. *MMWR*. 51(29): 637-639.

Enabulele OI, Gardner ET, Ukah I (1989). Aerobic bacteria flora on the hands of food handlers in the University community. Microbios. Lett. 41:17-21.

Hilborn ED, Mermin JM, Mshar PA, Hadler JL, Voetsch A, Wojtkunski C, Swartz M, Mshar R, Lambert-Fair MA, Farrar JA, Glynn MK, Slutsker LL (1999). A Multi-state outbreak of *Escherichia coli* 0157:H7 infections associated with consumption of mesclun lettuce. Arch. Intern. Med. 159(15): 1758-1764. PMID: 10448779.

Holt JG, Krevy HS, Sneathe RHA, Williams ST (1994). Bergey's Manual of Determinative Bacteriology 9th Edition. Williams and Wilkens Company, Baltimore, USA.

Hussein HS (2007). Prevalence and Pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. J. Anim. Sci. 85: E63-E72. Doi: 10.2527/jas.2006-421

Johnson RP, Clarke RC, Wilson JB, Read SC, Rahn K, Renwick SA (1996). Growing concerns and recent outbreak involving non-0157: H7 serotypes of verotoxigenic *E. coli.* J. food prot. 39: 1112 – 1122.

Law D (2000). Virulence factors of *E. coli* 0157 and other Shiga – toxin – producing *E. coli* - A review. J. Appl. Microbiol. 88: 729 – 745. Doi:10.1046/j.1365-2672.2000.01031.x

Montenegro MA, Bulte M, Trumpf T, Aleksic S, Reuter G, Bulling E, Helmuth R (1990). Detection and characterization of fecal verotoxin-producing *E. coli* from healthy cattle. J. Clin. Microbiol. 28: 1417 – 1421. PMID: 2199502

Nataro JP, Kaper JB (1998). Diarrheagenic *E. coli.* Clin. Microbiol. Rev. 11: 142 – 201. PMID:9457432

Nkanga EJ, Uraih N (1981). Prevalence of *Staphylococcus aureus* in meat samples from traditional market in Benin City Nigerian and possible control by use of condiments J. food protect. 44: 4 – 8.

Paton JC, Paton AW (1998). Pathogenesis and diagnosis of Shiga toxin producing *E. coli* infections. Clin. Microbiol. Rev. 11: 450 – 479.

Perna NT, Mayhew GF, Posfai G, Elliot S, Donnenberg MS, Kaper JB (1998). Molecular evolution of a pathogenicity Island from enterohemorrhagic *E. coli* 0157:H7 Infect. and Immun. 66: 3810 – 3817. PMID: 9673266.

Petridis H, Kidder G, Ogram A (2002). *Escherichia coli* 0157:H7 A potential Health Concern. Institute of Food and Agricultural Sciences,

University of Florida. http://edis.ifas.ufl.edu/ss197.

Sayah RS, Kaneene JB, Johnson Y, Miller R (2005). Patterns of antimicrobial resistance observed in *Escherichia coli* isolates from domestic and wild animal fecal samples, human septage and surface water. Appl. Environ. Microbiol., 71: 1394-1404. Doi: 10.1128/AEM.71.3.1394-1404

Solomon EB, Yaron S, Mathews KR (2002). Transmission of *Escherichia coli* 0157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Appl. Environ. Microbiol., 68(1): 397-400. Doi: 10.1128/AEM.68.1.397-400.2002

Turtle J, Gomes T, Doyle MP, Wells JG, Zhao T, Tauxe RV (1999). Lessons from a large outbrake of *E. coli* 0157:H7 infection, insight into the infectious dose and widespread contamination of hamburger parties. Epidemiol. Infect. 122; 185-192.

Vernozy–Rozand C (1997). Detection of *Escherichia coli* 0157:H7 and other verocytotoxin – producing *E. coli* (VTEC) in food. J. Appl. Microbiol., 82; 537 – 551. PMID: 9172396. DOI: 10.1111*ft*.1365-2672 1997.tb03584.x