IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 9, Issue 12 Ver. I (Dec. 2016), PP 34-39
www.iosrjournals.org

Evaluation of Occurrence of Escherichia Colio157: H7 FromNigerian Currency Notes (Naira) Collected from Butchers at The Meat Market in Karu Abattoir, Nigeria.

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Abstract: *Currency note is a medium of exchange for goods and services; buying and selling of various products including meat, at meat markets. This present study was designed to evaluate the occurrence of Escherichia coliO157:H7 from Nigerian currency notes (Naira) collected from butchers at the meat market in Karu Abattoir, Abuja. A total 189 currency notes sampled from the eight currency denominations were subjected to cultivation and isolation using Eosine methylene blue (EMB) and Sorbitol-MacConkay(SMAC) agar respectively, in which isolates were obtained. All the 189 (100%) naira notes sampled were contaminated with bacteria, andout of the 189 samples, 12 (19.7%) were contaminated with E. coli, of which5 (41.7%) were confirmed to beE. coli O157:H7. Currency notes can potentially serve as fomite in transmitting microorganisms such as E. coliO157:H7 which causes enteric diseases in humans. Also poor handling practices of currency notes poses a critical threat to public health.*

Keywords:*Naira, Karu, E. coli, Currency, Abattoir, Abuja*

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I. Introduction

Currency notes are widely exchanged for goods and services worldwide (Uneke and Ogbu, 2007). Naira note is the legal tender in Nigeria (Ogba, 2007), it is of two types Polymer and Paper. The paper naira note is a mixture of 75% cotton and 25% linen (Brady and Kelly, 2000), while the polymer is made from a polymer which is biaxially oriented polypropylene (BOPP) (https://en.wikipedia.org/wiki/Polymer_banknote). Currencies contaminated with pathogenic bacteria such as *E. coli*can potentially be a vehicle for the spread of the microorganisms when it is exchanged within individuals (Pinner *et al.*, 1996).

E. coli O157:H7 is commonly found in the intestines of cattle and cross contamination of any parts of the animal is possible when meat processing is not properly done. The bacterium is also found naturally in the intestines of other animals like pigs, sheep, goats and deer (Govindarajan, 1990; WHO, 2004; Yousuf*et al*., 2008).

According to Mir-Hassan *et al*., (2013), *E. coli* was frequently isolated from meat due to the transfer of faecal material during the slaughter process or via contact with other surfaces. Their study further suggested that it was not unexpected to encounter *E. coli* on currency notes derived from meat outlets where transfer to currency notes was via improper handling of meat in exchange for currency notes. Uneke and Ogbu, (2007) observed that this route accounted for the high prevalence of enteric bacteria recovered from banknotes sampled from poultry meat shops. The findings also showed that contamination could be introduced outside the retail environment. Butchers with the bloody and dirty fingers receive and give currency notes, leading to the contamination of the notes with microorganisms as reported by Mensah *et al*. (2002). Contaminated currency notes became a vehicle for the transmission of pathogenic organisms which were in circulation and contaminated the hands of other people in the process (Mensah *et al*., 2002; Uneke and Ogbu, 2007).

The survival of various microorganisms such as *E. coli* on money and other fomites, with their transmission via the hands of market men and women is often overlooked as enteric disease reservoir (Michaels, 2002). Pathogenic microorganisms that may survive on currency notes may serve as a potential source of enteropathogens (Michaels, 2002; Cardoen*et al*., 2009; Lamichhane*et al.*, 2009). Contamination of objects by pathogenic microorganisms is of much public health concern as contaminated meat, butcher's hands, utensils, tables and the exchange of currency can be sources of transmitting pathogens such as *E. coli* (Michaels, 2002; Pope *et al.*, 2002).

This study was carried out on isolation, serology andtotal viable count (TVC) of *E. coli* from Naira notes collected from butchers at the meat market in Karu Abattoir, Abuja. Nigeria.

II. Methodology

Study Area

Karu is one of the satellite towns in Abuja Municipal Area Council (AMAC) of the Federal Capital Territory (FCT), Nigeria. It lies between latitudes 8° 59'38.6"N and 9° 01'39.6"N and longitudes 7° 33' 17.19"E and 7°34'49.61"E. Karu has an area of about 275 square kilometers. Karu abattoir, which is the study area, is located close to a residential area (Balogun, 2001). It is one of the major slaughter locations in the FCT which contains a meat market close to it, with high economic activities.

Materials and Methods

A total of 189 Naira notes (from all the 8 denominations) were collected from butchers at the meat market in Karu Abattoir. Convenient sampling technique was adopted for this study and on each day of sampling, based on informed consent, availability of currency and acceptance of the butchers to participate in the study. The participants were given an equivalent of the currency collected in exchange.This study was conducted from September to December 2014 (three months) in which naira notes of all the 8 denominations were collected from the butchers. The currency notes studied were 25 Five Naira Notes (N5), 24 Ten Naira Notes (N10), 24 Twenty Naira Notes (N20), 28 Fifty Naira Notes (N50), 28 One hundred Naira Notes (N100), 20 Two hundred Naira Notes (N200), 21 Five hundred Naira Notes (N500) and 19 One thousand Naira Notes (N1,000).

With the aid of a pair of sterile forceps, each currency notes were collected and transferred aseptically unto a sterile surface, swab stick dipped in peptone water was used to swab both surfaces of the currency notes. The swab stick was then placed in the sample bottle containing 20 ml of peptone water and the butcher was given a currency of equal value as a replacement. The sample bottle was capped, placed in a cooler with ice packs and then transported after collection to the Bacterial Zoonosis Laboratory in the Department of Veterinary Public Health and Preventive Medicine, AhmaduBello University, Zaria for processing (isolation, TVC and serotyping of *E.coli).*

Isolation and Identification of E.coli

The isolation of *E. coli* was conducted according to the procedure of ISO, (2001). The sample from the 1% buffered peptone water was incubated at 37^0C for 24 hours, a loop full was inoculated directly on EMB agar, streaked using a sterile loop and incubated at 37^0C for 24 hours. Greenish metallic sheen colony growth suggestive of *E. coli* which was stored on nutrient agar slant and refrigerated for further biochemical and serological analysis.

Total viable count

Serial dilutions were made from 1ml of the sample and 9ml of the normal saline solution, 2 drops were surface plated on plate count agar (PCA) for TVC. Plates were incubated at 37^0C for 24 hours. The number of distinct colonies on each plate were enumerated using a colony counter, colony forming units (CFU) per ml or cm^2 of sample were calculated, using the dilution factor of each and converted to log10, CFU/cm or ml values. Mean values of TVC were determined and reported.

Biochemical Tests Using MICROBACT® 12E Identification System.

A 24-hour pure culture of the organism growth suspected to be *E. coli* was obtained from nutrient agar slant. 1 to 3 isolated colonies were selected from the pure culture of the organism and emulsified in saline. A Test Strip or Microplate was placed in a holding tray and the seal was peeled back. Four drops of the bacterial suspension were added to each well. 2 drops of Mineral Oil (MB1093A) was added to the black wells. The seal was replaced and incubated at 35°C ± 2ºC for 24 hours. It was then removed from the incubator and appropriate reagents were added. The results were recorded on the report forms and were interpreted using the Microbact® Identification Package.

Identification ofE. coli O157:H7.

Latex agglutinations kit for *E. coli* O157:H7 (Wellcolex) having *E. coli* O157 and H7 antisera was used to further confirm *E. coli* O157:H7. A drop of the reagent was mixed on a card with the suspension of *E. coli* isolate.

Test Procedure for O157 (somatic antigen)

E. coli isolates obtained from samples on nutrient agar slants were sub-cultured on CT-Smac and incubated at 37^0C for 24 hours. Positive results showed agglutination with clear clumping of the latex particles indicating rapid agglutination occurred through the interaction of specific IgG and O157 lipopolysaccharide antigen.

Test Procedure for H7 (flagella antigen)

Cultures that were positive with O157 test latex antigen were grown overnight at 37^0C in tryptonesoya broth(TSB). Positive results showed agglutination with clear clumping of the latex particles indicating rapid agglutination occurred through the interaction of specific IgG and O157 lipopolysaccharide antigen.

III. Data Analysis

Data was analyzed using the statistical package for social sciences (SPSS) version 20. Frequency of occurrence for *E. coli* on currency notes was determined and is presented in tables, charts and graphs. Chi square and independent T-test were used, where necessary to test for relationship between the occurrence of *E. coli* and the currency denominations. Percentage of polymer to paper currency notes.

IV. Results

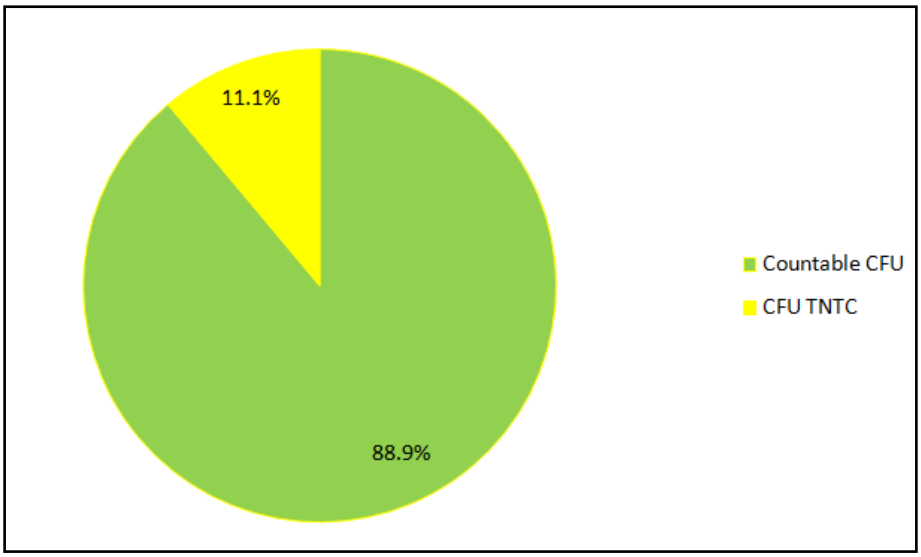

Figure 1 Level of microbial contamination among currencies sampled from butchers at the Karuabattoir,meat market Abuja.

All the 189 (100%) Naira notes sampled frombutchers at the Karu abattoir, in Abuja, were contaminated; 168 (88.9%) had colonies that could be counted (≤ 300cfu/cm^2),while 21 (11.1%) of the samples collected produced colonies that were too numerous to count (TNTC) (>300cfu/cm^2) (Figure 1). The mean microbial load on the currency notes with countable colonies, obtained from the butchers at the Karu abattoir meat market, was 190.08±83.92 cfu/cm^2. Distribution of the microbial load from the currency notes showed that the currency denominations on which the TVC with the highest load of microbial contamination, was the N5 five Naira notes (219.67±67.01 cfu/cm^2), this was followed by microbial contamination on N100 one hundred Naira notes (212.64±77.66 cfu/cm^2). The notes that the least microbial contamination among all the currency denominations that were sampled was N50 Fifty Naira notes (150.68±87.81 cfu/cm^2) (Table 1).

Table 1 Mean microbial load from currency notes of the eight denominations sampled from butchers at Karu abattoir, meat market. Abuja.

Currency Denominations	No of samples collected	No. of samples with TVC	Mean (±SD)
N5	25	15	219.67±67.01
N10	25	25	189.28±79.44
N20	25	18	210.39±89.88
N50	26	22	150.68±87.81
N100	28	28	212.64±77.66
N200	21	21	187.48±85.73
N500	20	20	174.45±85.73
N1,000	19	19	180.26±91.19
Total	189	168	190.08±83.92

The result shows that paper currency had higher microbial load (190.97±83.58) than polymer currency notes (189.11±84.81). The relationship was however not statistically significant (t=-0.143, p=0.887) (Table 2).

Table 2 Mean microbial load among currency types sampled from butchers at the Karu abattoir meat market. Abuja.

Currency type	No. of samples with TVC	Mean ±SD	Statistic	
Polymer	80	189.11±84.81	t=-0.143	p=0.887
Paper	88	190.97±83.58		
Total	168	190.08±83.92		

A statistically significant difference was observed between the nature of microbial contamination and type of currency notes. All (100.0%) of the paper currency notes had bacterial growth that was countable and none of them had colonies TNTC while 79.2% of the polymer notes had bacterial colonies that were countable and 20.8% of the notes had colonies that were TNTC (Table 3).

Table 3 Relationship between level of microbial load and currency type obtained from butchers at Karu abattoir meat market. Abuja.

Currency type	Level of microbial load		Total
	TVC	TNTC	
Polymer currency	80 (79.2)	21 (20.8)	101 (53)
Paper currency	88 (100)	0 (0.0)	88 (47)
Total	168 (88.9)	21 (11.1)	189 (100)

χ^2=20.584 df=1 p=0.000

Statistical test to determine the relationship between the type of currency and occurrence of *E. coli* shows that, of the 12 *E. coli* isolates, more of the polymer currency notes 8 (40.0%) were found to be contaminated with *E. coli* than the paper currency notes 4 (36.4%). The findings were however, not statistically significant as shown in Table 4.

Table 4 Relationship between the occurrence of *E. coli* and currency types collected from butchers at Karu Abattoir meat market, Abuja.

Sample category	Bacterial Isolate Category		Total
	E.coli	Other bacterial organisms	
Polymer currency	8 (40.0)	12 (60.0)	20 (64.5)
Paper currency	4 (36.4)	7 (63.6)	11 (35.5)
Total	12 (38.7)	19 (61.3)	31 (100.0)

χ^2=0.040 df=1 p=0.842

Among the 61 isolates that were suspected from Sorbitol-MacConkay agar, 12 (19.7%) were confirmed as *E. coli* using the Microbact® 12E identification system.

Using the Serological test; from the 12 *E. coli* isolates that were confirmed using the Microbact® 12E identification system; Serological test using Rapid Latex Agglutination Test for *E. coli* **(**Wellcolex*E. coli* O157:H7 kit) indicated that 5 of the *E. coli* isolates were confirmed to be Positive for *E. coli* O157:H7 (ten naira, five hundred, five hundred, five hundred and one thousand naira notes), 3 were positive for *E. coli* O157 (twenty, two hundred and one hundred naira notes) while 4 were Negative for *E. coli* O157:H7 (ten, ten, fifty and one hundred naira notes). According to currency denomination (Table 5).

Table 5 Serology using Rapid Latex Agglutination Test for the 12 *E. coli* isolates

S/NO	Currency Denomination	Isolates of *E.coli*	
		O157:	**H7**
1	**N10**	-	-
2	**N10**	+	+
3	**N10**	-	-
4	**N20**	+	-
5	**N50**	-	-
6	**N100**	+	-
7	**N100**	-	-
8	**N200**	+	-
9	**N500**	+	+
10	**N500**	+	+
11	**N500**	+	+
12	**N1,000**	+	+
	Total	**8**	**5**

Using the Serological test; from the 12 *E. coli* isolates that were confirmed using the Microbact® 12E identification system; Percentage forSerological test using Rapid Latex Agglutination Test for *E. coli* (Wellcolex*E. coli* O157:H7 kit) indicated that 41.7% of the *E. coli* isolates were confirmed to be positive for *E. coli* O157:H7, 25% were positive for *E. coli*, while 33.3% were negative for *E. coli* O157:H7 (Table 6).

Table 6 Percentage for Serology using Rapid Latex Agglutination Test for the 12 *E. coli* isolates

S/NO	*E. coli* Isolates	Confirmation	Percentage (%)	
1	*E. coli* O157:H7	Positive	41.7	
2	*E. coli* O157	Negative	25	
3	*E. coli* O157:H7	Negative	33.3	

V. Discussion

From this study, the data obtained showed that cultivable*Escherichia coli* O157:H7 was isolated from currency notes obtained from butchers at the Karu Abattoir meat market. All the 189 (100%) naira notes sampled from butchers at the meat market, were contaminated; 168 (88.9%) had colonies that could be counted ($\leq$ 300 cfu/cm^2) while 21 (11.1%) of the samples collected produced colonies that were too numerous to count (TNTC) (>300cfu/cm^2) (Fig. 1). This could be attributed to the fact that these currency notes got into the hands of several people including butchers who may have contaminated the currency notes with blood that serves as a good medium for bacteria growth, other handlers of the naira notes may have been traders, beggars and people who carry out other jobs that could contaminate hands and without cleaning or washing such hands, could result to cross contamination. The currency notes were also observed to be kept in clothes, pockets, wallets, purses and bags which are hardly washed, as a result, these pockets, wallets, purses and bags could also be the source for contamination of the currency notes.The contamination of currency notes by coliforms shows that humans are the major source of currency contamination similar to a study by Ngwai*et al*., (2011) which showed that all newly printed currency notes obtained from commercial banks prior to their introduction into circulation and subsequent handling by the public revealed no bacterial growth when compared to currency notes collected from different groups of people. This is similar to a study by Abrams and Waterman (1972), who stated that there is a possibility that currency notes might act as environmental vehicles (fomites) for the transmission of potential pathogenic microorganisms.

The isolation of the pathogen *E. coli* O157:H7 in Karu urban area, indicated that contaminated currency notes exchanged hands between butchers and buyers of meat which could also be due to improper processing and transporting from the abattoir which agrees with a similar study by Nkaga and Uriah, (1981) or cross contamination from the buyers with poor hygiene practice themselves as observed by Oyero (2007) or outside the retail environment (Mir-Hassan *et al*., 2013). This also agrees with various studies which identified cattle to be a major reservoir of *E. coli* O157:H7 (Faith *et al.*, 1996; Chapman *et al.*, 1997; Hancock *et al*., 1997; Rice *et al*., 1997). It could also be due to improper washing the utensils and tables as observed by Gill *et al*., 1998; Jones *et al*., 2008; Endale and Hailay, 2013. Human diseases caused by *E. coli* O157:H7 can be linked directly or indirectly to cattle (Bhat *et al*., 2007). This shows further that meat hygiene and currency notes handling practices remain a great challenge.

The contamination was found more in lower currency denomination (5-200 Naira notes) than in higher currency denomination (500-1000 Naira notes) as shown by the total viable counts. This can be attributed to the fact that the lower denomination currency notes are frequently more used in daily, petty transactions than the higher currency (500-1000 Naira notes). This agrees with the findings in in the studies conducted in Idaharea of Kogi State, Federal Capital Territory and Benin City all in Nigeria (Mailafia*et al*., 2011; Ukwuru and Gabriel, 2012; and Yakubu*et al*., 2014).

From the data analysis, T-test showed that there was no statistically significant association between the nature of the currency and microbial contamination as paper currency notes had higher contamination rate when compared to the polymer currency notes as seen in the amount of total viable count which is 190.97±83.58 and 189.11±84.81 for paper and polymer currency notes respectively, however, this was contrary to a study conducted by Gedik*et al*., (2013) who observed that the structure of polymer notes allows growth and transmission of pathogens compared with that of paper currency notes.

More microorganisms were obtained on polymer note (53%) than their counterpart, paper notes (47%) currency. Polymer notes form the smaller denomination of Nigerian currency and are more in circulation than their paper counterpart which has higher denominations handled by business people in the higher class who are probably cleaner, this was in agreement to a study by Mailafia*et al*., (2011).

VI. Conclusion

The results of this study showed that Nigerian currency notes collected from butchers at the meat market, in Karu abattoir, were contaminated by a potentially pathogenic enteroheamorrhagic*Escherichia coli* O157:H7, with a prevalence of 2.6%, have public health implications, because *E. coli* O157:H7 has low infective dose of <100 cells and the public to be mindful that microorganisms such as *E. coli* O157:H7 could be found on currency notes acting as fomites.

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