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Antibiogram and Phenotypic Characterization of *E. Coli* Isolated from Nigeria's Paper Currencies obtained from Butchers in Ebonyi State

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Abstract

This study was designed to phenotypically characterize and determine the antibiotic resistance patterns of *E. coli* isolated from Nigeria's paper currencies obtained from butchers in Abakaliki meat market, Ebonyi State. A total of 98 samples of naira notes in different denominations (N5 to N1000) were randomly collected from butchers and were analyzed using standard microbiological procedures. The isolated bacterial pathogens were subjected to antibiotic susceptibility test on Mueller-Hinton agar (Oxoid, England) by Kirby Bauer disc diffusion technique. A total of 7 (7.1%) *Escherichia coli* isolates were obtained in which two exhibited multi-drug resistance trait. The *Escherichia coli* isolates were highly resistant to ceftazidime (85.7%). The highest susceptibility frequency was observed for ciprofloxacin (100%), imipenem (100%), meropenem (100%) and ofloxacin (100%). There was a statistically significant difference in the mean percentage resistance and mean percentage susceptibility ($P < 0.05$) of the *E. coli* isolates. The multiple antibiotic resistance indices (MARI) of the *Escherichia coli* isolates ranged from 0.14-0.43. These high MARI values might be an indication of abuse and misuse of antibiotics. *Escherichia coli* can cause serious community-acquired infections. Hence, appropriate hygienic measures should be adopted while handling naira notes.

Keywords: Antibiotic resistance; Butchers; Paper currencies; *Escherichia coli*; Multi-drug resistance

Introduction

Currency is handled by all categories of people and may be contaminated during coughing, sneezing, touching with hands and placement on dirty surfaces. Many people tongue-wet their finger when counting money thereby contaminating their

fingers as well as currency notes. So, it is obvious that anything that gets on hands may be transferred to money *vice versa*. Search for contaminated currency which started in early 1970s, reported potential pathogens of 13% on coins and 42% on paper currency. Contaminated banknote (paper currency) is any currency that has been exposed to some biological and chemical contaminants. Banknotes serve as an ideal breeding ground for microorganisms. Contaminated banknotes or coins could be a public health risk as it is handled by everyone [1,2]. Contamination may occur from the general public in community, hospitals and food outlets or during use, storage or production. During handling, contamination from the skin, anal region, wounds, nasal secretions and aerosols are main sources of transferring microorganisms to banknotes during handling [1]. Therefore, banknotes have an important role in the transmission of pathogenic microorganisms and present a risk to public health. The biological contamination could be virus, fungal spores, or bacteria. The microorganisms known to be transferred by circulating banknotes in community are *Escherichia coli*, *S. epidermidis*, *Streptococcus*, *Pseudomonas aeruginosa*, and *Klebsiella aerogenes* [3]. The main microorganisms that are known to be transferred by circulating banknote recovered from hospitals are *E. coli*, *S. aureus*, *Salmonella spp.* [4,5] and that recovered from food outlets is *S. aureus*, and sometimes *E. coli*. It is noted that there is an increasing multi-drug resistant (MDR) bacterial strains where the bacteria demonstrate resistance to more than one class of antibiotics [6]. Few studies had focused on antibiotic resistance among bacteria recovered from banknotes [7]. Contaminated banknotes and coins contribute to the transmission of multi-drug resistant microorganisms in the community such as MRSA and vancomycin-resistant enterococci (VRE) [8,9]. It is also noted that antimicrobial resistance such extended-spectrum beta lactamases (ESBL)-producing *E. coli* and *Klebsiella spp.* has steadily been increasing [10]. Today, disinfectants used for human hygiene purposes make an essential component of human health care approach as they aim

to remove and destroy harmful and undesirable microorganisms. This study is therefore designed to determine the antibiotic resistance patterns of *E. coli* isolated from Nigeria's paper currencies obtained from butchers in Abakaliki meat market, Ebonyi State.

Materials and Methods

Study area: Ebonyi State is popularly known as the 'Salt of the Nation,' apparently because of the large deposits of salt water in the state. The State capital is Abakaliki. It lies approximately within longitude 70 30' and 80 30' E and 50 40' and 60 45'N. According to data from the 2006 Population and Housing Census, Ebonyi State has an estimated population of 2.3 million and a land mass of 5,935 km².

Collection of samples

A total of 98 samples of naira notes in different denominations (N5 - N1000) were randomly collected from butchers at meat market, within Abakaliki metropolis between the months of April and June, 2016. The naira notes were collected with hands covered with sterile gloves. The notes were then immediately placed inside sterile polythene bags and labeled accordingly. In some instances, the naira notes were obtained during exchange for bigger denominations; others were obtained after buying items with bigger denominations. The naira note samples were then immediately transported to the department of Applied Microbiology laboratory, Ebonyi State University, Abakaliki, Nigeria for bacteriological analysis. The currencies were observed to have been in circulation for about 2-4 years (2011-2014). Mint paper currencies were used as controls.

Culturing, isolation, phenotypic characterization and identification of the isolates

A sterile swab stick moistened with sterile physiological saline (0.85% NaCl) was used to swab both sides of each note. Each of the swabs was then aseptically transferred into test tubes containing 5 ml of peptone water and incubated overnight at 37°C. After incubation, the swabs were then streaked onto already prepared Eosin Methylene Blue (EMB) agar (Oxoid, UK). The inoculated plates were then incubated at 37°C for 24 hours. Colonies displaying typical *Escherichia coli* characteristics (green metallic sheen) were picked and sub-cultured on freshly prepared EMB agar plates so as to obtain pure colonies. Pure colonies (one pure colony per culture plate was selected) of *E. coli* isolates were picked from the EMB agar plates and inoculated on prepared nutrient agar slants and preserved in the refrigerator at 4°C for further tests. The suspected *E. coli* isolates were further characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining, catalase test, motility test, and other biochemical tests which include oxidase test, indole test, citrate utilization test, H₂S production test, Voges-Proskauer test, methyl red test, urease test, nitrate reduction test, and sugar fermentation test [11].

Antibiotic susceptibility test

Antibiotic susceptibility of the *E. coli* isolates was done using the Kirby Bauer disc diffusion method and interpreted according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2007) formerly known as National Committee for Clinical Laboratory Standards (NCCLS). Mueller-Hinton agar was prepared according to the manufacturer's instructions. The medium was cooled to 45-50°C and poured into plates. Plates were allowed to set on a level surface to a depth of approximately 4 mm. When the agar has solidified, plates were allowed to dry before use. An 18-24 hour old broth culture of the *E. coli* isolate was standardized by diluting to 0.5 McFarland's standard. A sterile swab stick was inserted into the standardized *E. coli* inoculum, drained to remove excess inoculum load and inoculated by spreading on the surface of prepared Mueller-Hinton agar plate. After this, the inoculated Mueller-Hinton agar plate was allowed to dry for a few minutes at room temperature with the lid closed. After the agar surface has dried for few minutes, antibiotic impregnated discs (Oxoid, UK) of known concentrations; gentamycin (30 µg), aztreonam (30 µg), ofloxacin (5 µg), ceftazidime (30 µg), cefepime (30 µg), meropenem (10 µg), ciprofloxacin (5 µg), and imipenem (10 µg) were carefully applied on the inoculated Mueller-Hinton agar plates using sterile forceps. The plates were then incubated at 37°C for 18-24 hours. After incubation, the diameters of the zones of inhibition were measured with a ruler to the nearest millimeter and recorded. The results were recorded as resistant, intermediate and susceptible according to the guidelines of Clinical Laboratory Standards Institute [12].

Determination of multiple antibiotics resistance index (MARI)

Multiple antibiotic resistance indices (MARI) of the *E. coli* isolates were calculated using the technique described by Christopher et al. [13] and Subramani et al. [14]. This was calculated as the number of antibiotics to which the tested isolate was resistant to (a), divided by the total number of antibiotics that was tested on the isolates (b).

Statistical Analysis

Categorical variables of the mean resistances of the isolates were compared their mean susceptibilities using T-test and Pearson correlation index at P<0.05 with statistical software, SPSS Data Editor Version 16.

Results

The *Escherichia coli* isolates were resistant to ceftazidime (85.7%). This was closely followed by aztreonam (42.9%) and cefepime (28.6%). The highest susceptibility frequency was observed for ciprofloxacin (100%), imipenem (100%), meropenem (100%) and ofloxacin (100%). This was closely followed by gentamicin (85.7%). The results of the one sample t-test conducted showed that there was no statistically significant difference in the mean percentage resistance of the *E. coli* isolates (mean=21.4375, SD=30.54462), t=1.985, P=0.088 (at

$P < 0.05$). One sample t-test showed that there was a statistically significant difference in the mean percentage susceptibilities of the *E. coli* isolates (mean=78.5625, SD=30.54462), $t=7.275$, $P=0.000$ ($P < 0.05$). Results of the independent samples t-test conducted to compare the percentage resistances and percentage susceptibilities of the *E. coli* isolates to different classes of antibiotics showed that there was a statistically significant difference in the mean percentage resistance (mean=21.4375, SD=30.54462) and mean percentage susceptibility (mean=78.5625, SD=30.54462); $t=-3.740$, $P=0.002$ ($P < 0.05$).

Discussion

Paper currencies have been accepted and used as a means of exchange and legal tender for centuries. However, these currencies are often times predisposed to microbial contamination due to the way they are handled. Bacteria, such as *Escherichia coli*, have frequently been implicated in the contamination of paper currencies used for transactions. This study was designed to determine the antibiotic resistance patterns of *E. coli* isolated from Nigeria's paper currency obtained from butchers in Abakaliki meat market, Ebonyi State.

Table 1 Currency type positive for *E. coli*.

S/N	Currency Type(N)	Smooth and Neat	Rough and Dirty	<i>E. coli</i> growth after 24 hours
1.	10	-	+	+
2.	50	-	+	+
3.	100	-	+	+
4.	1000	-	+	+
5.	200	-	+	+
6.	20	-	+	+
7.	500	-	+	+
8.	Control (mint)	+	-	-

Key: + indicates Positive; - indicates Negative

All the naira notes (rough and dirty) were positive for *E. coli* except the control (mint).

A total of 98 naira notes ranging from N 5 to N1000 obtained from butchers at Abakaliki meat market were used for this study while a Naira note in mint condition was used as a control. All the 98 naira notes obtained from butchers at meat market, Abakaliki showed bacterial growth after 24 hours. All the naira notes that had bacterial growths were rough and dirty in appearance. The control (mint) obtained from a commercial bank in the same study area did not show any bacterial growth. Out of the 95 naira notes obtained for this study, 7 were positive for *E. coli* growth after 24 hours of incubation at 37°C (Table 1).

This report is in agreement with the work of Ayandele and Adeniyi [15] who also isolated *Escherichia coli* from Naira notes obtained from traders in Ogbomoso, South-Western Nigeria. We recorded 7.14% *E. coli* frequency in our study. This *E. coli* frequency of 7.14% in our study is similar to a report from Pakistan by Abiodoun et al. (2011), who recorded 13% *E. coli* frequency among isolates recovered from paper currencies. The *Escherichia coli* isolates were resistant (85.7%) to ceftazidime. This was closely followed by aztreonam (42.9%) (Table 2).

Table 2 Percentage occurrence of responses of the *Escherichia coli* isolates to different antibiotics.

Antibiotics Used	Resistance (%)	Susceptible (%)
Ciprofloxacin	0 (0.00)	7 (100)
Cefepime	2 (28.6)	5 (71.4)
Gentamicin	1 (14.3)	6 (85.7)
Aztreonem	3 (42.9)	4 (57.1)
Meropenem	0 (0.00)	7 (100)
Imipenem	0 (0.00)	7 (100)
Ofloxacin	0 (0.00)	7 (100)
Ceftazidime	6 (85.7)	1 (14.3)

This observation is similar to the study carried out by Anyadele and Adeniyi [15] in Ogbomoso, South-Western Nigeria where they reported that *Escherichia coli* recovered from paper currencies were resistant to ceftriaxone, gentamicin, trimethoprim-sulfamethoxazole, ofloxacin and amoxicillin. In our study, the highest susceptibility frequency was observed for ciprofloxacin, imipenem, meropenem, and ofloxacin with susceptibility frequency value of 100% each. This was closely followed by gentamicin and cefepime with susceptibility frequency value of 85.7% and 71.4% respectively (Table 2). The results of the one sample t-test conducted showed that there was no statistically significant difference in the mean percentage resistance of the *E. coli* isolates ($P=0.088$ at $P < 0.05$). One sample t-test showed that there was a statistically significant difference in the mean percentage susceptibilities of the *E. coli* isolates ($P=0.000$ ($P < 0.05$). Results of the independent samples t-test conducted to compare the percentage resistances and percentage susceptibilities of the *E. coli* isolates to different classes of antibiotics showed that there was a statistically significant difference in the mean percentage resistance and mean percentage susceptibility ($P=0.002$ ($P < 0.05$). The result of our work shows that ciprofloxacin, gentamicin, meropenem, imipenem and ofloxacin are still active against bacterial infections caused by *Escherichia coli* in Abakaliki, Ebonyi state. Interestingly, our study also showed that ceftazidime, a third generation cephalosporin, is no longer effective in treating bacterial infections caused by *Escherichia coli* as 6 (85.7%) out of the seven isolates obtained in this study were resistant to this particular antibiotic. Two (28.6%) out of the seven (7) *Escherichia coli* isolates exhibited multi-drug resistance traits as

they were resistant to at least two different classes of antibiotics (Table 3).

Table 3 Antibiotic resistance patterns of the *Escherichia coli* isolates.

S/N	Isolate Code	Resistance Patterns
1.	E 7	CAZR
2.	E 8	FEPR CNR ATMR CAZR
3.	E 9	ATMR
4.	E 10	CAZR
5.	E 11	CAZR
6.	E 12	ATMR CAZR
7.	E 13	CAZR

Key: R= Resistant, FEP=Cefepime, CN=Gentamicin, ATM=Aztreonam, CAZ=Ceftazidime

Only two (E8 and E12) out of the seven *Escherichia coli* isolates exhibited multi-drug resistance traits as they were resistant to at least two different classes of antibiotics.

The average multiple antibiotic resistance index (MAR1) value of the *Escherichia coli* isolates was 0.335 and this signifies their high antibiotic resistance frequency values to commonly used antibiotic for treating bacterial infections (Table 4).

Table 4 Multiple Antibiotic Resistance Index (MARI) of *Escherichia coli* isolates.

Isolates	E 14	E 16	E 18	E 19	E 20	E 37	E 38	E 17	E 46	Total	Average
MARI value	0.143	0.57	0.63	0.143	0.143	0.143	0.143	0.43	0.143	2.345	0.335

The multiple antibiotic resistance indices (MARI) of the *E. coli* isolates ranged from 0.143 to 0.335.

The presence of microorganisms on paper currencies suggests that it can be a favourable environment for their growth. Presence of *Escherichia coli* on paper currencies could possibly suggest faecal contamination. It is a common practice among traders (butchers/meat sellers inclusive) to keep paper currencies on tables or squeeze them in their pockets or apron pockets. These unhygienic practices predisposes these traders to *Escherichia coli* infections especially *Escherichia coli* food-poisoning. Consequently, proper handling of banknotes is of public health importance considering the fact that some communicable disease can spread by means of fomites including money.

Conclusion

This report has shown that naira notes collected from butchers in Abakaliki, Ebonyi State is contaminated with *Escherichia coli*. This may be as a result of faecal contamination through the faeces of the animals. This study also revealed that the *E. coli* isolates recovered were susceptible to most of the antibiotics used for this study, possibly due to lack of abuse of such antibiotics within the study area. This research work showed that few of the *Escherichia coli* isolates were multi-drug resistant. Our study has shown that ceftazidime is no longer effective in treating bacterial infections caused by *E. coli* as virtually all the *E. coli* isolates were resistant to this antibiotic, thus indicating a possible abuse of ceftazidime in our study area. The result of this study also revealed that ciprofloxacin,

gentamicin, meropenem, imipenem and ofloxacin are still effective in treating bacterial infections caused by *Escherichia coli*. *Escherichia coli* contamination of paper currencies could pose a serious public health problem. Thus, proper sanitary handling of paper currencies and education of the populace on the negative effect of unhygienic handling of currencies should be done in possible cases to reduce community acquired infection in our society.

Recommendation

Currency is handled by all categories of people and may be contaminated during coughing, sneezing, touching with hands and placement on dirty surfaces. Many people tongue-wet their finger when counting money thereby contaminating their fingers as well as currency notes. So, it is obvious that anything that gets on hands may be transferred to money vice versa. Owing to the role of *E. coli* in infectious disease, it is therefore of public health importance to educate the populace on the effect of improper handling of paper currencies, especially the nursing mothers in our study area with the habit of allowing their children to play with paper currencies. These children, most times, end up putting these paper currencies in their mouths, thus predisposing them to bacterial infections. Further studies on the public health implication of bacterial contamination of paper currencies is therefore recommended to unveil more mysteries associated with paper currency contamination.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest

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