Antibiogram of Bacteria Isolated from Nigerian Currency Notes obtained from Meat Vendors in Abakaliki, Ebonyi State, Nigeria

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Abstract: Currency is one of the most potential vehicles in the transmission of pathogens. This study was designed to isolate, identify, and determine the antibiotic susceptibility profiles of bacterial pathogens isolated from different denominations of Naira notes. A total of sixty Naira note samples ranging from ₦100.00 to ₦1000.00 were randomly collected from meat vendors at the International and Kpirikpiri market, Abakaliki, Nigeria. Collected samples were analyzed using standard microbiological procedures. Antibiotic susceptibility test was done using Kirby-Bauer disc diffusion technique. Results showed that bacterial counts ranged from 1.2 x 10^3cfu/mL to 2.3 x 10^4cfu/mL for ₦1000.00 and ₦100.00 Naira notes respectively. The bacteria isolated in this study were *Escherichia coli* (27.7%), *Pseudomonas aeruginosa* (16.7 %), *Klebsiella* species (16.7 %), *Salmonella* species (22.2 %), and *Staphylococcus aureus*(16.7 %). The Gram-negative isolates (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* species, and *Salmonella* species) were highly resistant (75 % -100 %) to cefotaxime, ceftazidime, and ertapenem but susceptible (25 % - 100 %) to fluoroquinolones. Interestingly, *S. aureus* isolates were completely susceptible (100 %) to gentamicin and amikacin but with a resistance percentage of 33.3 % to oxacillin. This study has demonstrated that Naira notes are potential vehicles in the transmission of bacterial pathogens from person to person. From the foregoing, citizens are therefore advised to wash their hands regularly with soap and water after handling Naira notes.

Keywords: Antibiotics, Naira notes, Resistance, Susceptibility

INTRODUCTION

Currency is the system of money in general use in a particular country. In Nigeria, paper currency, commonly known as Naira notes is the legal tender. World over, money is one of the most frequently exchanged material in human communities as it is used daily as a means of exchange for goods and services (Djouadi *et al*., 2020), thus, changing hands from one person to another. As it circulates, it is exposed to different unhygienic environmental conditions which subject it to microbial contamination (Krishan, 2017).

Money plays an important role in all aspects of life because every transaction is based on money usage. There is evidence that paper notes have the ability to act as fomites with the potentials of carrying pathogenic microorganisms (Awodi *et al*., 2000). This is true because most paper notes are rough and dirty, hence, can act as reservoir for microbes with the potential of transmitting such organisms and subsequently causing diseases to the handlers (El-Daris and Hassan, 2015). A person living in an unhygienic environment with unhygienic habits can contaminate Naira notes. Habits such as keeping money inside stockings, shoes, under the carpet or rugs, and squeezing with wet hands frequently introduce microorganisms to Naira notes(Basavarajappa *et al*., 2005).Most people usually wet their fingers with their saliva while counting money, thereby contaminating it with the normal flora of their bucalcavity; others use the toilet without washing their hands to count money, this practice can introduce faecal bacteria, while others use fingers in picking their nose to count money, these practices further contaminates currency notes (Awe *et al*., 2010). Other negative money handling practices such as placing money on the faces of individuals and throwing money on people during occasions where other individuals step on them are ways in which money can contaminated by the normal flora of people and by organisms from soil and dust (Ogo *et al* ., 2004). The Naira notes used in Nigeria are ₦ 5.00, ₦10.00, ₦20.00,₦ 50.00,₦100.00,₦ 200.00,₦ 500.00 and ₦1000.00.The ₦ 5.00,₦ 10.00, ₦ 20.00, and₦50.00 notes are made of polymer material, and based on the economic condition of the country, are not in regular use, thus, it is

envisaged that their microbial load will be minimal. Several organisms have been implicated in Naira note samples and include *E. coli*, *Salmonella*, *Citrobacter*, *S. aureus*, *Pseudomonas*, *Klebsiella*, *Shigella*, and a host of others (Oyejola and Adebayo, 2004). Some of these organisms are commonly known to inhabit the lower intestinal tracts of warm blooded animals and have been found to contaminate Naira notes as a result of improper handling (Barolia *et al.,* 2011). Most of these organisms are pathogenic and harbor resistant genes which has conferred antibiotic resistance on the organisms.

MATERIALS AND METHODS

Study Area

The study areas were Kpirikpiri and International markets both in Abakaliki metropolis, the capital of Ebonyi State. Ebonyi state is in Southeastern Nigeria. It is primarily inhabited by the Igbos, and it is predominantly an agricultural region with several solid mineral resources including lead, crude oil, and natural gas, though with few large scale commercial mining sites. Ebonyi State is located within longitude 7° 30¹ and 8° 30¹ E and 5° 40¹ and 6° 45¹ N with a land mass of 5,935 km^2 (www.ebonyistate.gov.ng). Kpirikpiri and International markets are the most popular in Abakaliki. Both wholesalers and retailers buy goods in these two markets. Slaughter houses are located within the markets from where meat vendors purchase meat for reselling. Individuals also purchase their meat here as well as other commodities. Buying and selling is at its peak, hence, predisposing the Naira notes to contamination as it is daily exchanged.

Sample Collection

A total of 60 Naira notes ranging from ₦ 100 – ₦ 1000, were obtained from meat vendors in Kpiripkiri market and the popular International markets, Abakaliki. The random sampling method was used to select the meat vendors. The ₦ 1000 notes were exchanged with the meat vendors (after briefly explaining to them that the notes are to be used for research), while the other denominations were collected as change after offering them ₦ 1000 Naira notes. The notes were collected using hand gloves. These were wrapped in sterile polythene bags (by market, and for each market, by denomination and) and transported to the laboratory for analyses.

Processing of Naira Note Samples

Using hand gloves, each Naira note sample was carefully rolled and soaked in 20ml of peptone water in test tubes and incubated at 37°C for 24 h.

Determination of Bacterial Load in the Naira Note Samples

Standard spread plate method was used to inoculate 1 mL of the 24 h old peptone water into the appropriate media and incubated for 24 h at 37 °C. After the incubation period, distinct colonies were counted using the colony counter and expressed in CFU/mL. Pure culture was obtained by sub – culturing onto the appropriate media and stored at 4°C for future use. The isolates were Gram stained and subjected to motility test. Biochemical tests were also carried out on the isolates to further identify the organisms (Cheesbrough, 2006).

Antibiotics Susceptibility Tests

The antibiotics susceptibility patterns of the isolates were tested using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar and interpreted according to the guidelines recommended by the Clinical Laboratory Standard Institute (CLSI, 2017). Susceptibility/ resistance of the organisms to each of the test antibiotics were determined on the basis of the size of the zone of growth inhibition. A 24 h old suspension of the identified test bacteria was standardized by diluting to 0.5 Mcfarland's turbidity standard. A sterile swab stick was dipped into the standardized test inoculum, and drained by rotating firmly against the sides of the tube to remove excess inoculum load and inoculated by spreading evenly across the surface of the already prepared Mueller-Hinton agar plates.

The inoculated plates were allowed to dry for few minutes at room temperature with the lid closed. After the agar plates had dried, sterile forceps were carefully used to place antibiotic impregnated discs of known concentrations on the already inoculated Mueller-Hinton agar plates. The plates were then incubated for 24 h at 37°C. After the incubation period, the diameters of zones of inhibition were measured in millimeter using a ruler and recorded.

The results were recorded as resistant (0-10mm), and susceptible (11mm and above) (CLSI, 2007).

The antibiotics used in this study were purchased from Oxoid Limited (Oxoid UK). Gentamicin (30µg),Oxacillin (5µg), ceftriaxone (5µg), Cloxacillin (10µg), Ofloxacin (10µg), Amikacin (10µg), cefotaxime (30µg), Ceftazidine (10µg), Ciprofloxacin (10µg) and Ertapenem (5µg).

RESULTS

Table 1 shows the total viable counts of bacteria obtained from different Naira note samples

Table 1: Total viable counts of bacteria from Naira note samples (CFU/mL)

Currency Type	CFU/mL
100	$2.3x10^4$
200	$1.4x10^4$
500	$1.0x10^4$
1000	$1.2x10^3$

Table 2 shows the biochemical test results, the total number, and the percentage occurrence of isolates obtained from the different Naira note samples

Table 2: Total number and percentage occurrence of bacteria from Naira note samples

Sample	GR	MT	Oxidase	Methyl red	Citrate	VP	Catalase	Indole	coagulase	Bacterial isolates	Total No. of isolates	Percentage occurrence (%)
1	-	+	+	-	+	-	+	-	-	*Pseudomonas aeruginosa*	3	16.7
2	-	+	-	+	-	-	+	-	-	*Salmonella* spp.	4	22.2
3	-	-	-	-	+	+	+	-	-	*Klebsiella* spp.	3	16.7
4	-	+	-	+	-	-	+	-	-	*E. coli*	5	27.7
5	+	-	-	+	+	+	+	-	+	*S. aureus*	3	16.7
Total											18	100

KEY: + = Positive, - = Negative, GR = Gram reaction, MT = Motility test, VP = Voges Proskauer

Table 3 shows the susceptibility pattern of the Gram negative organisms isolated from the different denominations of Naira notes

Table 3: Susceptibility pattern of *Pseudomonas aeruginosa, Salmonella* spp., *Klebsiella* spp. and *Escherichia coli* isolated from Naira notes

Bacteria	Antibiotics	No. Resistant (%)	No. Susceptible (%)
P. aeruginosa			
	OX	3(100)	0(0.0)
	CRO	3(100)	0.(0.0)
	OB	3(100)	0(0.0)
	OFX	1(33.3)	2(66.7)
	AK	3(100)	0(0.0)
	CTX	3(100)	0(0.0)
	CAZ	3(100)	0(0.0)
	CIP	1(33.3)	2(66.7)
	ETP	3(100)	0(0.0)
***Salmonella* spp.**			
	OX	4(100)	00.0)
	CRO	3(75)	1(25)
	OB	1(25)	3(75)
	OFX	2(50)	2(50)
	AK	1(25)	3(75)
	CTX	4(100)	0(0.0)
	CAZ	4(100)	0(0.0)
	CIP	3(75)	1(25)
	ETP	4(100)	0(0.0)
***Klebsiella* spp.**			
	OX	3(100)	0(0.0)
	CRO	3(100)	0.(0.0)
	OB	2(66.7)	1(33.3)
	OFX	1(33.3)	2(66.7)
	AK	0(0.0)	3(100)
	CTX	1(33.3)	2(66.7)
	CAZ	3(100)	0(0.0)
	CIP	3(100)	0(0.0)
	ETP	3(100)	0(0.0)
Escherichia coli			
	OX	5(100)	0(0.0)
	CRO	4(80)	1(20)
	OB	3(60)	2(40)
	OFX	4(80)	1(20)
	AK	4(80)	1(20)
	CTX	5(100)	0(0.0)
	CAZ	5(100)	0(0.0)
	CIP	5(100)	0(0.0)
	ETP	5(100)	0(0.0)

Table 4 shows the result of the susceptibility pattern of the Gram positive bacteria(*S. aureus*) obtained from the different denominations of Naira notes

Table 4: Susceptibility pattern of *Staphylococcus aureus* isolated from Naira notes

Antibiotics	No. Resistant (%)	No. Susceptible (%)
CN	0(0.0)	3(100)
OX	1(33.3)	2(66.7)
CRO	3(100)	0(0.0)
OB	3(100)	0(0.0)
OFX	3(100)	0(0.0)
AK	0(0.0)	3(100)
CTX	2(66.7)	1(33.3)
CAZ	3(100)	0(0.0)
CIP	3(100)	0(0.0)
ETP	3(100)	0(0.0)

KEY:
CN = Gentamicin, OX = Oxacillin, CRO = Ceftriaxone, OB = Cloxacillin, OFX = Ofloxacin, AK = Amikacin. CTX = Cefotaxime. CAZ = Ceftazidime. CIP = Ciprofloxacin, ETP = Ertapenem, No. = Number

DISCUSSION

The results for the total viable counts (TVC) of different denominations showed that ₦100.00 notes had the highest colony counts (Table 2),this agrees with the report of Kawo *et al*. (2009) who attributed the high bacterial counts to higher frequency of usage in daily transactions; this was followed by ₦200.00 notes with TVC of $1.4x10^4$. The lowest TVC was observed in ₦1000.00 notes. This could be as a result of the fact that lower denominations are frequently exchanged among individuals of the lower class, hence, are prone to contamination; while the ₦1000.00 notes are handled by members of the social class with sophisticated lifestyle which reduces rate of contamination. This result agrees with the research of Usman *et al.* (2021) who obtained highest TVC of bacteria in smaller denominations and lowest TVC in bigger denominations of Naira notes. This result disagrees with the research of Ofoedu *et al.,* (2021) who obtained about 95% higher denominations that were more contaminated than lower denominations obtained from meat sellers and fish sellers in Owerri, Imo State, Nigeria, the reason is attributed to poor handling of Naira currency by the vendors.

The results for the morphological and biochemical tests revealed that the bacteria isolated were *Pseudomonas aeruginosa* (16.7%), *Staphylococcus aureus* (16.7%), *Salmonella* spp (22.2%), *Klebsiella* spp (16.7 %), and *Escherichia coli*(27.7 %).

The isolation of *Escherichia coli* (27.7%) is in agreement with the research of Uneke and Ogbu (2007) who obtained 13.2 % *E. coli* from paper currency in Nigeria. Also, the presence of *E. coli*, with *Klebsiella* spp and *Staphylococcus aureus* corroborates with the work of Ofoedu *et al.* (2021) who isolated the same bacteria in varying degrees from Naira notes collected from local food vendors, while the isolation of *Salmonella* species is in tandem with the research of Moses *et al.* (2018) who isolated *Salmonella* from Naira note samples obtained from butchers. Generally, the presence of enteric organisms in Naira note samples obtained from meat vendors could be as a result of poor hygiene of the meat vendors who obviously did not wash their hands after handling the meat before touching money as these organisms are found

in the intestinal tracts of animals. This correlates with the findings of previous study of Yazah *et al.* (2012) who assessed the bacterial contamination of Nigerian currency notes and associated risk factors in Northern Nigeria.

Also, the presence of *Pseudomonas aeruginosa* on Naira note samples as observed in this study corresponds with the research of Imarenzo *et al.* (2018) who isolated *Pseudomonas aeruginosa*, *Escherichia coli,* and other organisms from Naira notes used in Wukari metropolis, Taraba state. *Staphylococcus aureus* is the only gram positive bacteria that were isolated from Naira notes in this study, the bacteria may have been shed from the skin of individuals as the organism is a normal flora of the human skin (Chiller and Murakawa, 2001).

Antibiotics susceptibility tests carried out on the isolated bacteria revealed that, all the isolates exhibited multi drug resistance traits as all the bacteria were highly resistant (100%) to Oxacillin, Ceftriaxone, Ceftazidime and Ertaperem. This finding is in agreement with the research of Imarenzor *et al.* (2018) where all the organisms were resistant to all the test antibiotics.

All the gram negative bacteria (*Pseudomonas aeruginosa, Salmonella* spp*., Klebsiella*spp. and *Escherichia coli*) isolated from this study were resistant (75-100%) to Cefotaxime, Ceftazidime and Ertaperem, but susceptible (25-100%) to the fluoroquinolones. This result is in agreement with the research of Moses *et al.* (2018) who obtained similar resistance result from *Salmonella* spp. obtained from butchers in Abakaliki meat market, Ebonyi State. Strains of *S. aureus* isolated from this study were completely susceptible (100%) to gentamicin and amikacin but resistant (33.3%) to oxacillin. This implies that gentamicin can be effectively used in treating infections caused by *S. aureus* in Abakaliki. This contrasts the work of Imarenzor *et al.* (2018) where *S. aureus* and *P. aeruginosa* strains were completely resistant to gentamicin in Wukari metropolis, Taraba state, such resistance could be possible if citizens of Wukari have abused the antibiotic overtime. *Salmonella* spps. were susceptible (75%) to cloxacillin and amikacin but 50% susceptible to ofloxacin. This corroborates the research of Moses *et al.* (2018) where *Salmonella* species were 88.9% susceptible to ofloxacin. Hence, these antibiotics can be effectively used in the treatment of infections caused by *Salmonella* spp.

The resistance patterns of these organisms could be due to misuse of the test antibiotics in the study area.

CONCLUSION

This study has revealed the presence of *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella* spp*., Klebsiella* spp. and *Escherichia coli* in Nigerian currency circulating in Abakaliki metropolis, Ebonyi State. The presence of these pathogenic organisms in Naira notes is of public health concern as they can serve as a vehicle for the transmission of infectious organisms which may cause diseases that may be difficult to contain, as most of these organisms are multi-drug resistant to some of the commercially available antibiotics.

RECOMMENDATIONS

It is therefore recommended that members of the public should be sensitized on the effect of improper handling of currency notes.Poor hygienic practices should be discouraged as this can contribute to the increase in microbial load of currency notes**.** Awareness should be created on the potentials of Naira notes to transfer pathogenic organisms. Cash payment systems should be discouraged to minimize cash handling, that is, cashless policy should be encouraged. Although this can only be achieved in developed countries and among high class citizens as the level of poverty in Nigeria may not permit such.

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Footnotes

₦ 5 - ₦ 50 notes were excluded from the study because they are made of polymer, and based on the economic situation in the country, these notes cannot buy any meaningful thing.