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Bacteriological Assessment of Naira Currency Notes From End Users In Anambra State, Nigeria

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Abstract
Naira is the legal tender used in Nigeria which appears in cotton, linen and polymer-based patterns with an average life span of 24 months. It is however observed that mutilated and dilapidated currency notes probably older than the stipulated life span are more in circulation than mint notes and thus draws a public health concern as repertoires of pathogens and agents of disease transmission. The aim of this research is to evaluate Nigerian currency denominations from end users in Anambra State as potential sources of agents of infectious diseases. A total of 120 mutilated currency denominations (₦ 5, ₦ 10, ₦ 20, ₦ 50, ₦100, ₦ 200, ₦ 500 and ₦ 1000) were collected from end users within the study area using random, stratified and cluster sampling techniques, while mint notes obtained from the Central Bank of Nigeria (CBN) served as control. Bacteria were isolated from the notes and identified using standard routine bacteriological procedures, and molecular typing of some choice isolates was carried out using Gene sequencing. Statistical analysis was carried out using analysis of Variance and mean partitioning was performed using Tukey test. The result showed that 74.16% of examined currency notes were dirty/mutilated, with ₦100 notes having the highest total viable counts of 9.86 x 10^4cfu/ml while #1000 notes showed the least viable count of 2.52 x 10^4cfu/ml. The bacteria isolates include Streptococcus faecalis, Micrococcus luteus, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli. These findings thus suggest that currency notes are indeed carriers of agents of disease.

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I. Introduction

Naira is the legal tender in Nigeria, issued and regulated by the Central Bank of Nigeria (CBN). According to the CBN Act of 1958. The expected life span of the naira notes in circulation is about 24 months but the mishandling practices resulting in abuse of the naira notes, reduces this to less than 6 months (Ofoedu *et al*., 2021).

Money is the most widely used and sought-after financial instrument on planet earth. Currency notes move from one user to the other, and concomitantly have the potential to serve as vehicles of disease transmission; consequently, could be said to pose a public health risk. Currency is handled by large number of people under a variety of personal and environmental conditions thus increasing its possibility of acting as environmental vehicle for transmission of potential pathogenic organisms. The naira notes are of two types: a mixture of 75% cotton and 25% linen and polymer-based paper currencies. Currently there are eight denominations of the naira. Four (N100, N200, N500 and N1000) are in paper form while the other four (N5, N10, N20, N50) notes are polymer based (Brady and Kelly, 2000; Ofoedu *et al*., 2021).

In Nigeria, the naira notes presently in circulation are abused by squeezing, stapling, cellotaping and writing on them. Damaged, soiled or cellotaped naira notes are referred to as mutilated naira notes. Daily transactions have made the naira to pass through many hands and pathogens become imposed on them. They get contaminated with normal flora and pathogens from the skin, respiratory secretions, gastro intestinal tract, water, soil and aerosols before they are finally deposited in the banks. Paper currency provides a large surface area as a breeding ground for pathogens. The contaminated notes go in circulation and contaminate the hands of others, transmitting microorganisms and parasites in the process (Awodi *et al*., 2000, Brady and Kelly, 2000, Thiruvengadam *et al.,* 2014, Yakubu *et al.,* 2014).

Age of the notes and materials used for the production of the notes influence(s) the degree of contamination. Paper notes have been shown to be more contaminated than polymer notes. Microbial contaminants may be transmitted either directly through hand to hand contacts or indirectly via food or other inanimate objects like formites. These routes of transmission are of great importance in the health of many populations in developing countries where the frequency of infection is a general indication of local hygiene and environmental sanitation levels. When hands used in cleaning up the anus after passing out faeces are not

properly washed and are used to touch the naira notes in anyway, the tendency is contamination by pathogenic zonotic fungi, bacteria and the trophozoites of developed parasites, eggs, cysts or even the Oocysts (Brady and Kelly, 2000, Oyero and Emikpe, 2007). The objectives of this research were to conduct preliminary examination of the physical conditions of the paper currency notes; isolate bacteria from mint and mutilated old naira notes; identify the bacteria using cultural and molecular methods; determine the prevalence of bacteria isolated from different currency denominations.

II. Materials And Methods

Study area

The study area was some selected towns. The towns were selected from 177 towns that fall into the three (3) Senatorial zones (Anambra North, Anambra Central and Anambra South) in Anambra State, Nigeria.

Study population and sample size determination

The target population was mutilated Nigerian currency denominations (₦5, ₦ 10, ₦ 20, ₦ 50, ₦ 100, ₦ 200, ₦ 500, and ₦ 1000) collected from end users (Bus conductor, meat sellers, food vendors, Petrol pump attendants, commercial motorcycle riders, Students and Civil servants) within the selected study area. The inclusion criteria were mutilated currency of all denominations (Soiled, cello taped, stapled, dirty and torn) while the exclusion criteria were currency denominations that were not mutilated. The sample size (n) was determined using Cochran's formula. The formula states as follows:

$$n=\frac{z^2pq}{e^2}$$

n= sample size

z= Standard deviation at 95% which corresponds to confidence interval of (1.96).

p= Proportion of the population having the desired characteristics.

q= (I-P), Proportion of the population without the desired characteristics

e= degree of precision ie the margin of error that is acceptable (0.05)

Since the proportion of the naira notes (P) with the characteristics was not known then 50% (0.5) was used (Susan *et al.,* 2015).

In this study, one hundred and twenty (120) mutilated currencies were used (Yakubu *et al*.,2014).

Sampling techniques

In this study, random, stratified and cluster sampling techniques were employed. Samples were collected from end users in selected towns in the three senatorial zones in Anambra State, Nigeria. Each of the selected towns was considered a stratum and the strata were clustered into groups.

Sample collection

A total of 120 samples of Nigerian currency notes consisting of fifteen(15) pieces each of the different denominations ₦ 5, ₦ 10, ₦ 20, ₦ 50(Polymer type), ₦ 100, ₦ 200, ₦ 500 and ₦ 1000 (Paper type) were collected randomly from different occupational groups (bus conductors, meat sellers, food vendors,Petrol pump attendants, Okada riders, Students and civil servants) in the three (3) senatorial zones, forty samples were collected from each of the zones. Five (5) pieces of minted notes of each of the currency denominations were also obtained from Central Bank of Nigeria (CBN), Awka to serve as control samples. Samples were collected in separate polythene bags and were conveyed to the Applied Microbiology and Brewing Laboratory, Nnamdi Azikiwe University Awka for analysis.

Grouping of samples and examination of their physical conditions

The currency notes were grouped according to their make and were categorized as paper and polymer; their physical conditions: Dirty/mutilated (damaged, soiled and squeezed with tapes). The production year of the different notes was also recorded.

Preparation of culture media

All culture media to be employed in this study were prepared according to the manufacturer's instructions. They were sterilized by autoclaving at 121°C, 15psi for 15 minutes. Salmonella-Shigella agar and Thiosulphate Citrate Bile Salt Sucrose agar were sterilized by boiling at 100°C and allowed to cool to 45°C before use.

Determination of bacterial load of currencies and their prevalences

The rinse method of Matur *et al*. (2010) was adopted for the isolation and determination of bacterial load. Each paper currency note was soaked separately in universal bottle containing 10 ml of sterile normal saline and shaken vigorously and allowed to stand for 30 minutes in order to dislodge the microorganisms. The resulting fluid served as the test sample. The currency note was removed aseptically using sterile forceps dried to recover the note. Tenfold serial dilution was prepared from the test sample (10^{-1}-10^{-3}) using one milliliter of the test sample in sterile buffered peptone water. One milliliter of the samples was inoculated into nutrient agar plates, in duplicates, using the pour plate method.

The nutrient agar plates were incubated at 37^{o}C for 24 hours. Colonies were counted and expressed as colony forming unit per milliliter (CFU/ml). The isolates were purified by sub-culturing and stored in slants of nutrient

agar (Cheesbrough, 2006). The sample was also inoculated into different selective and differential media (Mannitol salt agar, MacConkey agar, Cetrimide agar, Thiosulphate, Citrate Bile salt, sucrose agar, Salmonella-Shigella agar) and incubated at 35^{o}C for 24-48 hours (Kawo *et al*., 2009).

Identification and characterization of bacteria.

The bacterial isolates were identified based on their cultural, morphological and biochemical reactions as described by Cheesbrough (2006) and Erin, (2012). The tests include Gram Staining, spore staining, Oxidase, Catalase, Coagulase, citrate utilization, indole, urease, methyl red, Voges-Proskauer, gelatin hydrolysis, motility and sugar fermentation tests.

STATISTICAL ANALYSIS

The data obtained was subjected to one-way ANOVA using SPSS version 21. P-values < 0.05 were considered significant. Mean partitioning was performed using Tukey test.

III. Results

Physical Conditions and bacterial Load of Paper Currency used for the Study.

Currency notes from which bacteria were isolated were first examined to ascertain their physical conditions. Table 1 showed that 74.16% of the currency notes used were dirty/mutilated while 25.83% were dirty. Mint notes were used as control. The bacterial load on Table 2 showed that ₦100 notes had the highest total viable bacteria count of 9.86 x 10^4 cfu/ml while, ₦1000 notes had the least viable bacteria count of 2.52 x 10^4cfu/ml. No bacteria was recovered from the mint notes.

Isolation and Characterization of Bacteria from Currency Notes.

Bacteria isolates from the examined currency notes are *Streptococcus* faecalis, *Micrococcus* luteus, *Pseudomonas* aeruginosa, *Bacillus subtilis, Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* as shown in Table 3.

Prevalence of Bacteria from different Currency Denominations.

Prevalence studies from different currency denominations showed that ₦100 note had 22.4% bacterial load which accounts for the highest frequency, while ₦1000 note had the least bacterial load frequency of 5.52%. *Bacillus subtilis* had the highest frequency of occurrence of 15.6% while *Streptococcus faecalis* was the least occurring bacteria with a frequency of 1.3% as shown in Table 4.

Table 1: Physical condition of paper currency collected from different occupation groups.

Denominations (₦)	Paper	Polymer	Dirty/Mutilated	Production Year	Total
5	-	+	15	2013-2015	15
10	-	+	15	2014-2016	15
20	-	+	15	2012-2017	15
50	-	+	15	2013-2016	15
100	+	-	15	2012-2014	15
200	+	-	15	2015-2017	15
500	+	-	15	2007-2014	15
1000	+	-	15	2007-2016	15
Total			120		120

Table 2: Bacterial Counts from Mutilated Naira Notes.

Denomination(Naira)	Number Examined	Total Viable Bacteria Count (CFU/ml)	Total Coliform Count(CFU/ml)
5	15	$3.12x10^4$	0.0
10	15	$4.20x10^4$	$0.6x10^2$
20	15	$6.20x10^4$	$1.2x10^2$
50	15	$6.34x10^4$	$1.0x10^2$
100	15	$9.86x10^4$	$2.2x10^3$
200	15	$8.60x10^4$	$1.8x10^2$
500	15	$5.20x10^4$	$1.6x10^2$
1000	15	$2.52x10^4$	0.0

No organism was recovered from the mint currencies

Table 3: Cultural and Biochemical Characteristics of Bacterial Isolates

Cultural Characteristics	Gram's reaction	Cat	Ox	Cit	Coag	Urease	Mot	Indole	MR	VP	Spore	Gelatin hydrolises	Glu	Lac	mal	Suc	Fruc	Probable Organism
Colonies 1-2mm in diameter, mocoid and produced Beta hemolysis on blood sugar.	Gram-positive cocci in chains.	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	*Streptococcus faecalis*
Deep-yellow colonies reused and entire edges, 2mm in diameter and hemolysis in blood agar.	Gram-positive cocci	+	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-	*Micrococcus roseus*
Large flat and irregular edged circular colonies in NA, produces Alpha hemolysis on blood agar.	Gram-negative Rods.	+	+	+	-	-	+	-	-	-	-	+	+	-	-	-	-	*Pseudomonas aeruginosa*
Large grey-white colonies 2-5mm in diameter on NA.	Gram-positive Rods in chains.	+	-	+	-	-	+	-	-	+	+	+	+	-	+	+	+	*Bacillus subtilis*
Deep golden-yellow colonies, smooth, raised and glistening on NA, 1-3mm in diameter.	Gram-positive cocci	+	-	+	+	+	-	-	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*
Grape-like clusters, white, raised on Nutrient Agar, non hemolytic on blood agar.	Gram-positive cocci	+	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	*Staphylococcus sepidermidis*
Large grayish white moistened colonies on NA.	Gram-negative Rods.	+	-	-	-	+	+	+	+	-	-	-	+	+	-	-	-	*E coli*

Table 4: Prevalence of Bacteria Isolates from Different Currency Denominations

	Currency Denomination Frequency (%)							
Bacteria	₦5	₦10	₦20	₦50	₦100	₦200	₦500	₦1000	Total
S. faecalis	-	-	1(0.83)	-	3(2.5)	-	-	-	4(1.3)
Micrococcus luteus	2(1.67)	2(1.67)	5(4.2)	4(3.33)	2(1.67)	3(2.5)	2(1.67)	1(0.83)	21(6.8)
P.aeruginosa	-	1(0.83)	3(2.5)	1(0.83)	4(3.33)	8(6.67)	1(0.83)	2(1.67)	20(6.5)
B. subtilis	5(4.2)	6(5.0)	6(5.0)	5(4.2)	10(8.33)	10(8.33)	4(3.33)	2(1.67)	48(15.6)
S. aureus	2(1.67)	2(1.67)	4(3.33)	5(4.2)	8(6.67)	6(5.0)	2(1.67)	2(1.67)	31(10.1)
S.epidermidis	2(1.67)	4(3.33)	4(3.33)	6(5.0)	8(6.67)	5(4.2)	3(2.5)	1(0.83)	34(11.04)
E.coli	-	1(0.83)	2(1.67)	2(1.67)	6(5.0)	6(5.0)	4(3.33)	-	21(6.8)
Total	11(9.21)	16(13.33)	25(20.86)	23(19.23)	41(34.17)	38(31.7)	16(13.33)	8(6.67)	178(58.14)

(Figures in parentheses are percentages) n=120.

IV. Discussion

The role of fomites in disease transmission has been established over the years. An excellent example of such fomite is currency notes and coins. Disease transmission through paper currency notes have been studied in different countries (Saadabi *et al*., 2011; Uraku *et al*., 2012; Jawed *et al*., 2017; Ademokoya, 2018). Owing to the importance and daily use of currency notes, they have been potent and constant agents of disease transmission. Microbial load examined in this study showed that mutilated and dirty currency notes were good agents of disease transmission while mint notes were relatively safe. Exchange of these mint notes from one end user to the other, over time, results in their aging, mutilation and accumulation of vegetative and spore stages of different microbes (Jawed *et al*., 2017). Bacteria found in the examined notes were *Streptococcus faecalis, Micrococcus luteus, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia coli*. These results correspond with the reports of Saadabi *et al*., (2011) and Ademokoya (2018). Prevalence studies showed that ₦100 notes had the highest microbial frequency of 22.4%, with *Bacillus subtilis* showing the highest prevalence. One hundred naira currency notes were most microbial burdened possibly because it is a frequently used currency note in Nigeria. Saadabi *et al*., (2011) also suggested that *Bacillus* spore forming ability helps in its ease of propagation and colonization of surfaces like currency

notes. The study has revealed that naira currency notes are very potent agents or vectors of disease transmission, thus the need to always exercise public health cautions while handling the notes.

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