

## Determination of Bacterial Contamination of Nigerian Currency Notes Using a New Mathematical Model

Lugbe, Popgbara Beenen\*., Ogan, Abiye Caleb. and Lugbe, Queen

Department of Science Laboratory Technology, School of Applied Sciences,  
Kenule Beeson Saro-Wiwa Polytechnic, P.M.B. 20, Bori, Rivers State, Nigeria.

\*Corresponding Author: [lecturerlugbe.kenpoly@gmail.com](mailto:lecturerlugbe.kenpoly@gmail.com)

### ABSTRACT

Nigerian currency notes come in paper and synthetic polymer denominations. The present study investigated used paper notes in the Naira (₦) denominations of ₦100, ₦200, ₦500 and ₦1000 notes for bacterial load and quality using standard microbiological methods. This investigation was carried out to address inaccurate methodologies in works on microbial contamination of Nigerian currencies, which do not take into account the total surface area of study notes. Each note was immersed in 150 ml physiological saline for 20 minutes with shaking at intervals to dislodge cells into suspension. This was followed by 10-fold serial dilutions and plating on nutrient agar and MacConkey agar. Colonies that developed on the plates were counted and populations were accurately calculated in CFU/cm<sup>2</sup> using a mathematical formula developed in this study. Identification of isolates was carried out based on cultural characteristics, cell morphology, Gram reactions, and biochemical tests. Results showed that populations were highest on ₦100 notes ( $4.6 \pm 1.2 \times 10^5$  CFU/cm<sup>2</sup>) and least on ₦1000 notes ( $0.4 \pm 0.2 \times 10^5$  CFU/cm<sup>2</sup>). Isolates were identified as *Staphylococcus aureus*, *Bacillus* sp., *E. coli*, *Micrococcus* sp., and *Pseudomonas aeruginosa*. Distribution of these species showed that *Bacillus* sp. was the most prevalent whereas *Pseudomonas aeruginosa* was the least. It was concluded that fairly used Nigerian currency notes harbour a variety of bacterial species whose populations in CFU/cm<sup>2</sup> can be determined using a general mathematical formula. Since these organisms are known pathogens, particularly to immunocompromised individuals, it becomes important to treat currency notes with utmost care so that they do not constitute fomites for transmission of diseases.

**Keywords:** Currency Notes, Bacterial Contamination, *Staphylococcus aureus*, *E. coli*, Surface Area, Mathematical Formula.

### Introduction

Nigerian currency notes are called naira notes. They exist in eight denominations – 1,000, 500, 200, 100, 50, 20, 10 and 5 naira notes. The first four are paper notes whereas others are synthetic polymers. Being the medium for exchange for goods and services, the naira is handled from time to time by huge populations in Nigeria (Beg and Fisher, 1997). The manner of handling varies according to culture, gender, level of education, and so on. Even against government orders, naira notes are sprayed at wedding and thanksgiving ceremonies where the notes are trampled underfoot without regard to the conditions of the ground (Ogo *et al.*, 2004). Men keep the notes in cloth pockets and socks. Women may do so in their brassieres and waist bags. During counting of currency notes, some wet their fingers with saliva while some may do so with water from doubtful sources (Oyedemi *et al.*, 2013).

The many conditions to which currency notes are exposed have led to gross contamination by microorganisms (Anidiobu *et al.*, 2022).

Organisms isolated from naira notes include *Salmonella* spp; *Escherichia coli*, *Shigella* spp; *Citrobacter* spp; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella* spp; *Bacillus* spp and *Micrococcus* spp. (Moses *et al.*, 2018; Anidiobu *et al.*, 2022). Many of these bacteria are of public health concerns. Contamination of naira notes is of worry because many see the notes as clean. This explains why people touch their tongues to wet their fingers when counting money, and may handle notes even while eating. There is therefore need to provide information concerning the hygienic status of Nigerian currency notes from time to time. When working on surfaces of objects many do not understand the technicalities involved. This has led to methodologies that are inaccurate. For instance, measurements of surface areas from which organisms were collected are rarely reported, showing that these, most probably, are not done. Again, soaking of naira notes in 10 ml volume of diluents that can hardly cover the 'soaked' material have been documented (Allan *et al.*, 2018; Dike-Ndudim *et al.*, 2021; Anidiobu *et al.*, 2022).

Using this small volume of diluent makes it obvious that note-contaminating organisms could not have been dislodged into suspension in these studies.

Population of organisms on any surface is given by number of organism per unit surface area – not per unit volume or per unit mass of substance. Hence, the standard unit for measurement of populations of microorganisms on surfaces of objects is colony forming unit per square centimetre (CFU/cm<sup>2</sup>) (Awe et al., 2010). Some working on surface of naira notes expressed their microbial counts in CFU/g (Aguoru et al., 2015). Others did so in CFU/ml (Dike Ndudim et al., 2021). Younos and Embaby (2024), working on sugarcane stem surface gave their population count in CFU/cm.

There are works where swabbing of just 1 cm<sup>2</sup> are reported to enable expression of microbial populations in CFU/cm<sup>2</sup>. Some, still, limit their work to qualitative studies to avoid quantitative expression (Anidiobu et al., 2022). Yet some leave their population counts in just CFU (Al-Easawi and Emran, 2017). Again, Oranusi and Braide (2012) reported bacterial counts on apple surfaces in CFU/sample. All these happen because of the rarity of available appropriate mathematical model for determination of microbial populations on surfaces of objects.

The present study shows the types of bacterial contamination of naira surfaces as well as general mathematical formula for determination of their populations. This formula applies not only to surfaces of the naira but to all study surfaces at large.

## Materials and Methods

### Study area

The present study was carried out in Kenule Beeson Saro-Wiwa Polytechnic, Bori, Rivers State, Nigeria. Bori is a city located 54km south of Port Harcourt, close the coast of Atlantic Ocean bathing southern Nigeria.

### Collection of samples

A total of 20 paper naira notes made of 5 each of ₦100, ₦200, ₦500 and ₦1,000 were randomly collected from students of the Kenule Beeson Saro-Wiwa Polytechnic, Bori. The notes which were fairly used but not dirty and torn were received from the students into sterile polythene bags and taken immediately to the Microbiology laboratory for analysis.

## Microbiological analysis

Each naira note was aseptically transferred by mean of sterile forceps into a 250-ml conical flask containing 150 ml sterile physiological saline. The notes were soaked for 20 minutes with repeated shaking to dislodge organisms into suspension. This was followed by 10-fold serial dilutions to 10<sup>-4</sup> dilution. From each of 10<sup>-2</sup> to 10<sup>-4</sup> dilutions, aliquots of 0.1 ml were transferred to separate nutrient agar and MacConkey agar plates in duplicates. Inocula were spread with sterile glass spreader and the plates were allowed to dry slanted against one another on surface-sterilized laboratory bench. The dried plates were incubated at 37°C for 24 hours. Each currency note was withdrawn from soak saline, allowed to dry, and the dimensions measured for determination of surface area. Pure cultures were obtained using the streak plate technique and identification of isolates was achieved using cultural characteristics, cell morphology, Gram reactions and biochemical tests.

## Determination of populations of isolates

Total surface area of a naira note was calculated using the formula-length x width x 2, since the note is two-sided. Also determined, was CFU/ml of diluent used to harvest organisms from each note. From this, the population of cells in the entire 150 ml employed to collect cells from each note was obtained. Since this population in the entire 150 ml diluent came from the two sides of a naira note, the population per square centimetre was calculated. On inspection of the procedure used in the calculations, a general formula was reached.

## Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA), using SPSS version 25 to determine differences in the capacity of test currency notes to hold bacterial species. All statistical analysis were carried out at  $\alpha = 0.05$ .

## Results

From 10<sup>-3</sup> dilution of a ₦100 note 67 colonies were counted. This gave colony forming unit per millilitre of suspension as follows:

$$\text{CFU/ml} = \frac{\text{Colony count} \times \text{dilution factor}}{\text{volume of inoculum}}$$

i.e.  $67 \times 10^3 / 0.1$  or  $6.7 \times 10^5$

This means that 1 ml contained  $6.7 \times 10^5$  CFU. Therefore, 150 ml contained  $150 \times 6.7 \times 10^5$  or  $1.005 \times 10^8$  CFU.

Length of each note was 15 cm whereas width was 7.5 cm. Hence total surface area of each note was 2 x 15 x 7.5 or 225 cm<sup>2</sup>. This means that 225 cm<sup>2</sup> had 1.005 x 10<sup>8</sup>CFU (the population in 150 ml suspension). Hence 1 cm<sup>2</sup> had 1.005 x 10<sup>8</sup>/225 or 4.5 x 10<sup>5</sup> CFU. That is, for 67 colonies from 10<sup>-3</sup> dilution with 0.1 ml inoculum, bacterial population of test ₦100 surface was 4.5 x 10<sup>5</sup> CFU/cm<sup>2</sup>. Inspection of the procedure used in these calculations produced the following formula:

$$\text{Population in CFU / cm}^2 = \frac{a \times b \times c}{d \times e}$$

Where a = colony count

b = dilution factor

c = volume of diluent for collection of organism from test surfaces

d = volume of inoculums for plating

e = Total surface area sampled in cm<sup>2</sup>

Using the formula, for 67 colonies from 10<sup>-3</sup> dilution with 0.1 ml inoculum, population in CFU/cm<sup>2</sup> was

$$\text{Population} = \frac{67 \times 10^3 \times 150}{0.1 \times 225} = 4.5 \times 10^5 \text{ CFU/cm}^2$$

Averages of populations obtained with this formula gave population values of test naira notes (Table 1).

Based on cultural characteristics, cell morphology, Gram reactions and biochemical tests, isolates were identified as *Staphylococcus aureus*, *Bacillus* sp; *Escherichia coli*, *Micrococcus* sp, and *Pseudomonas aeruginosa* (Table 2).

The prevalence of bacterial contamination of the currency notes (Table 3) showed that all test Naira notes harboured bacterial species. *Bacillus* sp was found on all the notes tested. This was followed by *Staphylococcus aureus* which occurred on 70% of the notes. *Escherichia coli* and *Micrococcus* existed on 25% as *Pseudomonas aeruginosa* occupied 20% of test naira notes.

Results also revealed that lower denominations (₦100 and ₦200) harboured higher varieties of bacteria than higher notes. The difference in contamination by a variety of bacterial species between lower and higher denominations was statistically significant (p < 0.05). Also significantly different at 5% level of F-test (ANOVA) was bacterial load on low and high currency notes.

**Table 1: Population of bacterial contamination of Nigerian currency notes**

Denomination	Number Sampled	Average Populations (x10 <sup>5</sup> cfu/cm <sup>2</sup> )
100	5	4.6 ± 1.2 <sup>a</sup>
200	5	3.9 ± 1.1 <sup>a</sup>
500	5	0.6 ± 0.1 <sup>b</sup>
1000	5	0.4 ± 0.2 <sup>b</sup>

Figures carrying different letters are significantly different

Table 2: Cultural, morphological and biochemical characteristics of bacteria isolated from test Nigerian currency notes

Cultural characteristics	Cell morphology													Identity of Isolate
		Gram reaction	Indole	Methyl Red	Voges Proskauer	Oxidase	Catalase	Citrate	Coagulase	Glucose	Maltose	Sucrose	Lactose	
Convex entire, golden yellow, small, moist on nutrient agar	Cocci mainly in clusters	+	-	+	+	-	+	+	+	A	A	A	-	<i>Staph. aureus</i>
Flat, irregular milky, large, dry on nutrient agar	Large rods mainly in short chains	+	-	-	+	-	+	+	NA	A	A	-	-	<i>Bacillus</i> sp.
Convex, entire, pink, small, moist on MacConkey agar	Rods in singles	-	+	+	-	-	+	-	NA	AG	AG	A	AG	<i>E. coli</i>
Convex, entire yellow, tiny, moist on nutrient agar	Cocci mainly in fours	+	-	NA	NA	+	+	+	-	A	-	A	-	<i>Micrococcus</i> sp
Flat, entire large, moist bluish green pigmentation on nutrient agar	Rods in singles	-	-	-	-	+	+	+	-	A	-	-	-	<i>Psuedomonas aeruginosa</i>

Key: AG = Acid and Gas; A = Acid; NA = Not applicable.

Table 3: Prevalence of bacterial contamination of Nigerian currency notes

Organism	Currency notes (₦)				
	100 n(%)	200 n(%)	500 n(%)	1000 n(%)	Total N(%)
<i>Staph. aureus</i>	5(100)	4(80)	3(60)	2(40)	14(70)
<i>Bacillus</i> sp.	5(100)	5(100)	5(100)	5(100)	20(100)
<i>E. coli</i>	2(40)	2(40)	1(20)	0(0)	5(25)
<i>Micrococcus</i> sp.	3(60)	2(40)	0(0)	0(0)	5(25)
<i>Pseudomonas</i> sp.	2(40)	1(20)	1(20)	0(0)	4(20)

n = number of positive notes out of 5; N = number of positive notes out of 20

## Discussion

Currency notes are materials constantly handled by many individuals everyday in Nigeria. This is because digital monetary transactions are still at infancy. Payments for goods and services are frequently done with naira notes. Since these notes move from hand to hand and from one environment to another, they become exposed to contamination by microorganisms. This explains why all the notes tested in the present study showed microbial contamination. This finding agrees with reports of numerous workers on currency notes (Allan *et al.*, 2018; Usman *et al.*, 2021).

The notes studied revealed that lower currencies, such as ₦100 and ₦200, had higher bacterial load than higher ones (₦1000). This is also in consonance with the findings of Allan *et al.* (2018) who reported that lower Ugandan currency notes harboured more bacteria than higher ones. In Nigeria, Dike-Ndudim *et al.* (2021) and Anidiobu *et al.* (2022), also found, in separate studies, that lower Nigerian naira notes have more bacteria than higher denominations. This finding is attributed to the fact that lower currency denominations exchange hands more frequently than the higher denomination notes. Also, the lower notes suffer more abuse than higher notes. For instance, it is the low currency notes that are frequently sprayed and trampled underfoot during ceremonies.

Besides bacterial load, it was found that bacterial types were more on lower currencies than higher ones. This position is corroborated by studies of Oyedemi *et al.* (2023) and Pradeep *et al.* (2012). Understandably, notes that are frequently abused and commonly passed from hand to hand would contain many microbial forms (Ahmed *et al.*, 2010). Those that are usually saved away or kept with some respect as with high currency notes will experience reduced contamination with a variety of microorganisms.

*Staphylococcus aureus*, *Bacillus* sp., *E. coli*, *Micrococcus* sp. and *Pseudomonas aeruginosa* are among bacteria isolated from contaminated currency notes in previous studies (Mbajiuka *et al.*, 2014; Dike-Ndudim *et al.*, 2021, Anidiobu *et al.*, 2022). *Staphylococcus aureus* may be found in commensal relationship on the skin of humans. This explains its presence on naira notes frequently in contact with the human body. Though commensal, this organism could be highly pathogenic if debilitating conditions exist in the body (Adamu *et al.*, 2012).

*Bacillus* species are common inhabitants of the soil. Their endospores easily get air-borne. Hence these organisms are ubiquitous in distribution. This explains the presence of *Bacillus* sp. on all the currency notes tested. Handling food and money together may introduce *Bacillus* sp. from money to food. Where this occurs, there is possibility that enterotoxin produced by some species of *Bacillus* may develop, leading to food poisoning (Ejaz *et al.*, 2018).

*Escherichia coli* is a bacterium commonly associated with the intestine of warm-blooded animals. It does not survive long in the external environment. For these reasons, its presence in any environment is attributed to faecal contamination of recent origin (Goзде and Emek, 2019). Just like the other species discussed, *E. coli* could be pathogenic under certain conditions.

*Micrococcus* species are common inhabitants of water, soil and dust. They are also found as commensals on the human skin where they break down compounds in sweat to generate unpleasant smell. The occurrence of *Micrococcus* in these environments explain its presence on naira notes which contact human skin, soil, dust and water of doubtful source from time to time. *Micrococcus* is another opportunistic pathogen of man (Smith *et al.*, 1999). *Pseudomonas aeruginosa* is naturally associated with water and soil.

These are therefore possible sources of pseudomonad contamination of currency notes investigated in the present study. This organism is yet another established human opportunistic pathogen (Duguid *et al.*, 1983). From the foregoing discussion, it is obvious that bacteria isolated from naira notes in this study are known to be involved in causing human health challenges.

The standard unit for population of organisms on surfaces of objects is colony forming unit per square centimetre (CFU/cm<sup>2</sup>) (Awe *et al.*, 2010). Many studies erroneously use units such as CFU/g (Aguoru *et al.*, 2015), CFU/ml (Allan *et al.*, 2018; Oranusi and Braide, 2012, Dike-Ndudim *et al.*, 2021), CFU/cm (Younos and Embaby, 2024) and simply CFU (Al-Easawi and Emran, 2017). These occur due to inaccurate microbiological techniques involved in the determination of populations of microorganisms on surfaces of objects. In these methods, the surface areas from which organisms were harvested were not determined. In some cases, volumes of diluents used to 'soak' and collect organisms into suspension for plating were impossibly low (Anidiobu *et al.*, 2022; Dike-Ndudim *et al.*, 2021; Allan *et al.*, 2018). The present study therefore solves a huge problem in microbiology by developing an appropriate technique and a general mathematical model that can be used to accurately determine microbial populations on object surfaces.

The formula developed here recommends, among others, that the volume of diluent for collection of organisms from test surface must be known and adequate to wash organisms into suspension. Also, importantly, the total surface area involved in the organism-collection process must be calculated. Where the sample surface is irregular, graph sheet may be used to map the area from which organisms were collected and, thereafter, the number of squares counted in the mapping graph used to obtain the required surface area (Lugbe, 2020). Methods for obtaining microbial cells in suspension may be by submerging and shaking of sample surface in appropriate known volume of diluent by repeated cleansing of test surface into known volume of diluent using a sterile moist swab stick (Lugbe, 2020). From this initial suspension, further 10-fold dilutions may be made to reduce populations that may grow on inoculated plates to countable levels.

Use of spread plate or pour plate technique provides other parameters in the formula for calculation of microbial populations on object surfaces in colony forming unit per square centimetre (CFU/cm<sup>2</sup>).

## Conclusion

All currency notes investigated in this study showed bacterial contamination with populations ranging from  $0.4 \pm 0.2 \times 10^5$  to  $4.6 \pm 1.2 \times 10^5$  CFU/cm<sup>2</sup>. The highest bacteria load was found on the lowest denomination tested (₦100) whereas the lowest population occurred on the highest note (₦1000). Bacterial varieties were also highest on the lowest currency note and least on the highest note. Species isolated were *Staphylococcus aureus*, *Bacillus* sp., *E. coli*, *Micrococcus* sp., and *Pseudomonas aeruginosa*. These organisms have been implicated in human diseases, particularly, of immunocompromised individuals. Importantly, a mathematical model for determination of bacterial populations on test currency notes was developed. The formula gives population of microorganisms in CFU/cm<sup>2</sup> as  $abc(de)^{-1}$ , where, 'a' is colony count, 'b' is dilution factor, 'c' is volume of diluent used to collect organisms from test surface, 'd' is volume of inoculum on agar plate, and 'e' is total surface area sampled. Parameters provided by this formula elucidate appropriate microbiological technique for calculating microbial loads on surfaces of objects. It is believed that its use will solve, for all time, problems encountered by microbiologists seeking to determine microbial populations on object surfaces.

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