

## RESEARCH ARTICLE

# Evaluation of Bacterial colonization of Naira Notes in Circulation as a Potential Fomite

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## Abstract

Colonization of surfaces like Naira note by pathogens has the ability to transmit infection. Hence, this research work was conducted to investigate the bacterial contamination of Naira notes in circulation. 80 samples of Naira notes in circulation at different denominations were randomly collected aseptically at Niger State Polytechnic. Serial dilution was used in the quantification of the bacterial and was plated into Nutrient and MacConkey agar. The bacteria isolates were identified based on their Grams and biochemical reactions. From the result, 56 (70%) of the Naira note in circulation were contaminated with various pathogenic bacteria. The bacteria count ranged from  $20 \times 10^3$  to  $1.0 \times 10^4$  cfu/g. Seven (7) bacterial species which includes: *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus pyogenes*, *Enterobacter arogenes*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* were isolated. *Staphylococcus aureus* was most predominant followed by *Bacillus subtilis* and the least encountered was *Streptococcus pyogenes*. Thus the Naira note in circulation could be heavily colonized by pathogenic bacteria and could be a potential fomite. Therefore, cashless transaction which does not allow direct contact with Naira notes should be highly encouraged and washing of hands with soap and water should be practiced regularly to prevent the transmission of infectious agents.

**Keywords:** Naira Note; Fomite; Pathogens; Infectious Agent

## Introduction

Various factors play important role in the transmission of disease agents from one individual to another. These include food, water, air current, direct contact, contact through items of clothing, pen, etc. [1]. Furthermore, disease transferred through these agents may be restricted to very limited locales but may be in some cases result in epidemic outbreaks [2].

Naira notes which are used as a mean of exchange for goods and services is usually passed from one person to another just like other essential needs (air, water, food, clothing, shelter, etc.) that man is always in contact with in close proximity, when mishandled under unhygienic conditions could serve as a fomite for the transmission of disease [3]. Practical evidences have shown that money is one of the most frequently handled materials by people all over the world; this is due to its usage for the exchange of goods and services [4]. Its usage in the exchange of goods and services has made money to be very mobile, changing hands from one person to another, from one environment to another and as it goes round in circulation, it is exposed to different unhygienic environmental conditions and thereby subjected to microbial contaminations [5].

Naira note can be contaminated with bacteria in the respiratory or gastro-intestinal tract during counting through the saliva that is often used to moisten the hand when counting the notes [2]. However, Naira note does not contain the necessary nutrient needed for the growth and survivals of microorganism on its surface hence, only those that can resist harsh environmental conditions or spores formers can survive for a long period of time [6]. This study was aimed at determining the level of bacterial contamination of naira note in circulation.

## Methodology

### Samples collection

A total of 88 samples of Naira currency notes were used for this study. 80 samples of the Naira notes in circulation, comprising of ten (10) samples for each of the eight (8) denominations (that is, N1000, N500, N200, N100, N50, N20, N10 and N5) were

randomly collected from student, motor-cyclist, food vendors and school shops in Niger State Polytechnic, Zungeru, Niger state, Nigeria. While the remaining 8 samples of fresh Naira mints for each denomination were obtained from Unity Bank Plc, Zungeru as a control. Each Sample was collected in sterile polythene bags using hand gloves and was labeled and taken to Aminchi Laboratory in Zungeru for analysis.

### Media preparation

The media used for this research work includes: Nutrient agar and Macconkey agar. They were prepared according to the manufacturer's instructions and were allowed to cool to about 45 °C after sterilization before dispensing into different sterile Petri dishes according to the method described [7].

### Preparation of money for analysis

Each Naira note samples was soaked in 20ml of sterile water for 30 minutes at room temperature with regular vigorous shaking in order to dislodge the cells into the water aseptically, according to the method described [7].

### Bacteriological analysis

To determine total viable bacteria count, each of the bacteria cells suspension in the water were serially diluted into four folds ( $10^{-1}$  to  $10^{-4}$ ) and 0.5 ml of the dilution was inoculated into different sterile plates of Nutrient agar and MacConkey agar plates using pour plating techniques [1]. The inoculated plates were incubated at 37 °C for 24 hours. After 24 hours, the colony forming (CFU/g) for each samples was then determined with colony counter.

### Identification of isolated organisms

The representative colonies of the isolated bacteria were purified by sub culturing. The pure cultures were then characterized using Gram staining, colony morphology on selective and enriched media and biochemical test according to the techniques described [7].

#### Gram Staining

Smear of each of the isolate was made on a glass slide, air-dried and heat fixed. The fixed smear were Gram stained according to the method described [7].

### Biochemical Tests

#### Citrate test

About 25g of sodium citrate, 1.5g of sodium ammonium phosphate, 0.2g of mangnessim sulphate, 1g of potassim dehydrogen and 0.06g of bromothymol blue were dissolved in one litre of distilled water and heated on the hot plate for complete dissolution. It was dispensed in the test tube plugged with cotton wool and aluminum foil and sterilized at 121 °C for 15minutes. The Koser's citrates media was inoculated with the isolates and incubated at 37 °C for 48hours. It was examined after two days. The presence of growth leads to increase in pH, resulting in the change in colour for positive test and initial green colour for negative test.

#### Catalase test

This test was carried out mostly on gram-positive cocci to test their ability to produce the enzyme catalases. In this case, it differentiates between *Staphylococcus* catalase positive and *Streptococcus* catalases negative. Catalase test is carried out also in both Gram-positive and gram negative bacilli and cocci. A colony of culture was emulsified in a drop of hydrogen peroxide on a clean glass slide. The presence of oxygen bubbles indicates a positive result of a catalase test while; the absence of oxygen bubbles indicates a negative result of a catalase test.

#### Coagulase test

This test was used to identify *Staphylococcus aureus* that produce coagulase. A loopful of the isolate was emulsified in a drop of normal saline in a slide, a drop of plasma was added and rocked. The slide was rocked gently for 2 minutes observing for coagulation reaction with the plasma [8].

#### Sugar fermentation test

Triple sugar ion (TSI) Agar slant was prepared according to the manufacturers' specification and old culture of the isolate was stabbed into the slant in a test tube and incubated at 37 °C for 24 hours. Glucose fermentation was identified by redness at the bottom of the test tube and yellow indicate lactose fermentation while motility was identified by cloudiness in the media.

#### Urease test

Urease test was applied to identify bacteria species that can decompose urea to produce ammonia. About 25.2g of the urea base was weighted and dissolved in one liter of water and heated to achieve total dissolution before it was dispensed in universal bottle and was autoclave at 121 °C for 15minutes; 5ml of 40% urea solution was aseptically introduced into the media. This was allowed

to solidly in a slanting position. After solidification of the urea medium, the inoculums of each isolate was inoculated into the slant bottles and incubated at 37 °C for 24 hours. Purple pink colour indicates positive test result and for negative test result, there was no colour change [9].

**Indole test**

Colonies were picked and inoculated into the test tube containing the indole medium and finally incubated at 37 °C for 48hours. 0.5ml of kovac’s reagent was added to the test tubes and was shake gently. The production of indole was confirmed by the formation of red ring colorations on the surface of the medium.

**Hydrogen sulphate test (H<sub>2</sub>S)**

The hydrogen sulphate test media was prepared according to the method described [7] and was used to determine the production of H<sub>2</sub>S. Each of the isolate was inoculated by stabbing the media in the test tubes and was incubated for 24hours at 37 °C. Positive test result was indicated by black colouration along the line of stab.

**Results**

The physical conditions of the various notes are shown in Table 1 as most of the Naira notes in circulation were old and dirty. Out of the 80 notes that were bacteriological analyzed, 56 (70%) were found to contain different kinds of pathogenic bacteria though, the bacterial load were more on Naira notes which were physically dirty and old compared to others that were clean. No bacterium was isolated from the 8 mint Naira notes that were used as control. The bacterial counts ranged from 2.0×10<sup>3</sup> to 1.0×10<sup>4</sup> cfu/g. The N100 notes had the highest bacterial load while N5 notes had the least with an average of 1.0×10<sup>4</sup> cfu/g) and (2.0×10<sup>3</sup> cfu/g) respectively. The average bacterial counts for each denomination of Naira notes are shown on Table 2. The microscopic identification of the bacterial isolates by Gram staining reaction and the Biochemical test of the bacteria characterization are shown in Table 3.

The seven (7) species of bacteria which include: *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterobacter arogenes*, and *Pseudomonas aeruginosa* that were isolated and their frequency of occurrence is showed on Figure 1. *Streptococcus pyogenes* was the least among the isolates (4.2%) while the most encountered was *Staphylococcus aureus* with 25% numbers of occurrence.

Denominations (Naira)	No. of samples collected	Condition of the Naira note
Mint (control)	8	Clean and neat
5	10	Dirty, toured, wrinkle and odorous
10	10	Dirty, wrinkle and odorous
20	10	Fairly clean and wrinkle
50	10	Dirty and wrinkle
100	10	Dirty, wrinkle and old
200	10	Fairly dirty, wrinkle and old
500	10	Fairly dirty and wrinkle
1000	10	Fairly dusty and wrinkle
<b>Total</b>	<b>88</b>	

Table 1: Physical conditions of a sample of each Naira denominations

Denominations (Naira)	Average colony count ( cfu/g )
5	2.0×10 <sup>3</sup>
10	4.0×10 <sup>3</sup>
20	1.0×10 <sup>3</sup>
50	4.0×10 <sup>3</sup>
100	1.0×10 <sup>4</sup>
200	4.0×10 <sup>3</sup>
500	6.0×10 <sup>3</sup>
1000	4.0×10 <sup>3</sup>

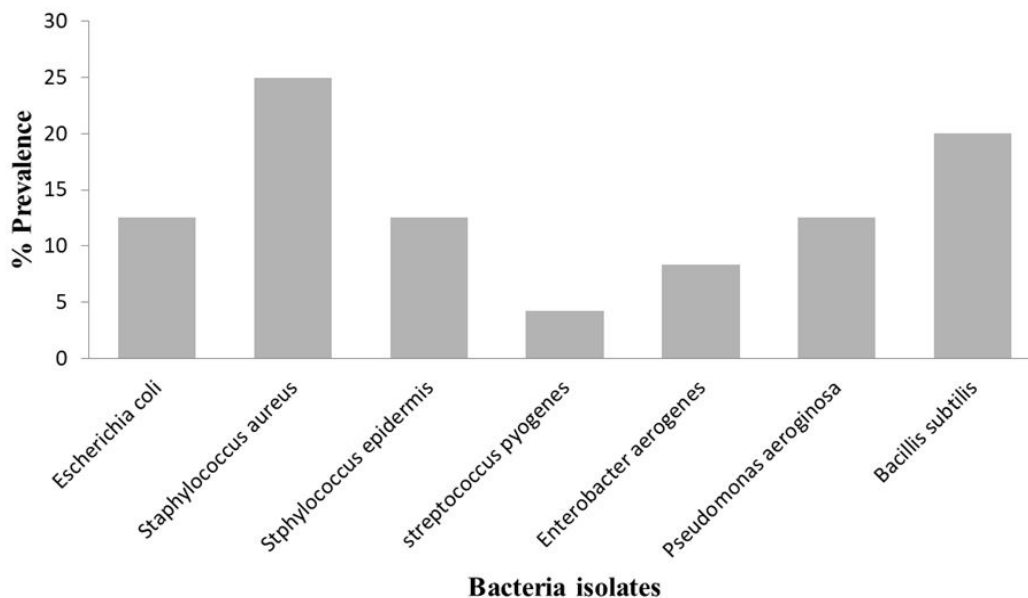
Table 2: Average bacterial count based on Naira denominations

Table 2 Shows the average bacterial count of different denominations ranging from 2.0 × 10<sup>3</sup> to 1.0 × 10<sup>4</sup> cfu/g. The N100 notes had the highest bacterial load while N5 notes had the least with an average of 1.0 × 10<sup>4</sup> cfu/g) and (2.0 × 10<sup>3</sup> cfu/g) respectively.

Sample	Ca	Co	La	GI	Su	H <sub>2</sub> S	Gas	Mo	In	Ur	Ci	Gram rxt	Organism
A	+	-	+	+	+	-	+	+	-	-	+	+	<i>Bacillus subtilis</i>
B	+	-	+	+	+	-	+	-	-	-	+	+	<i>Streptococcus pyogenes</i>
C	+	+	+	+	+	-	-	-	-	+	-	+	<i>Staphylococcus aureus</i>
D	+	-	-	+	+	-	+	-	+	+	-	+	<i>Staphylococcus epidermidis</i>
E	+	+	+	+	+	+	-	+	+	+	+	-	<i>Pseudomonas aeruginosa</i>
F	-	-	+	+	+	-	-	+	-	-	+	-	<i>Enterobacter aerogenes</i>
G	+	-	-	+	-	-	-	+	-	-	-	-	<i>Escherichia coli</i>

**Table 3:** Biochemical test for identification of bacterial isolated from Naira notes

**Key:** Ca = Catalase; Co = Coagulase; La = Lactose fermentation; GI = Glucose; Su = Sucrose; Mo=Motility; In = Indole; Ur = Urease; Test; + = positive; - = Negative.



**Figure 1:** Shows the occurrences of each bacterial isolated from naira notes in circulation collected from Niger state polytechnic Zungeru, Niger State

## Discussion

The study shows that there is high prevalence of bacteria on Naira notes in circulation which agrees with the findings of Hobner, *et al.* [10] that demonstrated that bacteria are capable of surviving on currency notes when contaminated with pathogen or normal flora of the skin during handling. Both pathogenic and non-pathogenic bacteria can be transferred from one place to another through air and can as well contaminate objects or surfaces such as Naira note, which can serve as a fomite for its transmission from one person to another [11]. From the study, it was observed that there is a relationship between bacterial contamination and the physical condition of the Naira note, the dirty and old notes had a higher bacterial load. This finding also agrees with that of Ahmed *et al.*, [12] that old currency notes favour and harbour more bacteria than new notes.

The bacteria isolated in the course of this study, such as *coagulase-negative Staphylococcus*, *Streptococcus pyogenes*, *Enterobacter aeruginosa*, *Pseudomonas aeruginosa* and *Bacillus subtilis* have been known to cause infections especially in immunocompromised individuals or people with depressed immune systems, like those suffering from HIV [13]. The high prevalence of *staphylococcus aureus* and *Bacillus subtilis* among the bacterial isolates, confirmed the ubiquitous nature of the organisms which give them the ability to colonize surfaces or objects and the ability of *Bacillus* spores to resist and survive under harsh environmental conditions for a moderate period of time [14]. The study reported here shows that there is a prevalence of bacteria among different denominations of Naira notes in circulation; this could be as a result of handling under unhygienic conditions, which makes it easy for the transfer of bacteria from one person to another [3,15-19].

## Conclusion

The results of the study show that most of the sampled Naira notes in circulation were highly contaminated with various types of bacteria, including pathogens; hence, Naira notes could be a possible vehicle for bacterial or viral infection transmission. Therefore, cashless transactions which do not allow direct contact with currency notes should be highly encouraged, and the practice of licking or applying saliva to the fingers while counting paper money should be discouraged. The washing of hands thoroughly after handling currency and before handling food, as well as personal hygiene, should be practiced regularly to prevent the transmission of infectious agents.

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