

**Prevalence and antibiotic susceptibility pattern of bacteria isolated from Nigerian currency note in Enugu, Enugu State, Nigeria.**

**ABSTRACT**

**Background:** Nigerian currency notes acts as fomites and thus can transmit pathogenic microorganisms from one person to another

**Aim:** This study was conducted to evaluate the prevalence and antibiotic susceptibility pattern of bacteria isolated from Nigerian currency note circulating in Enugu East local government area of Enugu.

**Method:** Isolation, characterization and antibiotic sensitivity tests were done using standard methods.

**Result:** A total of 632 bacteria strains comprising 13 different bacterial species were isolated and identified from the Nigerian currency notes. The respective isolation rates of the isolates were *Staphylococcus aureus*(74.3%), *E.coli* (31.6%), *Pseudomonas aeruginosa* (5.3%), *Bacillus spp* (28.6%), *Salmonella spp* (6.0%), *Klebsiella spp*(21.3%), *Streptococcus spp*(15.3%), *Acinetobacter spp* (3.3%), *Serratia marcescens* (3.6%), *Proteus mirabilis* (8.0%), *Enterococcus faecalis* (6.0%), *Citrobacter sp* (6.3%) and *Actinomycete sp* (0.6%). The Gram positive and Gram negative bacteria showed resistance to cloxacilline (87%) and amoxicillin (84%). Further, 79.5% of all the bacterial isolates had an index >2 while 20.5% had a multi-antibiotic resistance index < 2.

**Conclusion:** Nigerian currency notes harbour bacteria. Many of these bacteria are multidrug resistant organisms.

**Key words:** Prevalence, Bacteria, Antibiotic resistance, MARI.

## INTRODUCTION

It has been well documented that bacteria is cosmopolitan in distribution. Money, which is one of the most commonly used commodities in life, provides a niche for the microbial community. They are used for the purpose of goods and services worldwide. The word "money" has its origin in Rome (Snehalatha *et al.*, 2016). These papers, most frequently passed from hand to hand can be contaminated with pathogenic bacteria by different ways during handling, thus increases the possibility of the transmission of potential pathogenic bacteria, it is fact that we buy day to day commodities transferring microorganisms from one location to another location, bacteria adherence the notes during coughing or sneezing, and by placement on dirty surface (Elsharief *et al.*, 2018).

In Nigeria, the currencies exist as naira and kobo and various denominations of the naira notes have been minted by the Central Bank of Nigeria (CBN). They are released to the public, through the commercial banks. Currently, there are eight denominations of the naira notes: ₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500 and ₦1000 notes. The ₦5, ₦10, ₦20, ₦50, ₦100 and ₦200 naira notes are the most common and are more involved in daily cash transactions. They are common especially among the populace while the ₦500 are used in corporate transactions (Moses *et al.*, 2018). In day to day transactions, money is handled by persons of varying health and hygienic standards and also stored under varying environmental and personal hygienic conditions. Poor currency handling practices are common globally. People often exhibit unhygienic habits like store money in unsanitary places (socks, bras, shoes) or handle it carelessly, introducing germs. Unhygienic habits like licking fingers while counting money or handling bills with dirty hands significantly increase contamination (Mousa & Idress, 2023). Contaminated surfaces, dust, soil, and even the handler's own body can contribute to the spread of microbes on currency notes. These practices not only contaminate money but also increase the risk of infection for those who handle it (Awe *et al.*, 2010). This has been implicated in serious health hazards such as impairment of lung function. The contamination of the notes can be traced to dust, soil, water and microflora of the body of handlers (hand, skin, etc.). *Citrobacter* spp, *Salmonella* spp, *Shigella* spp, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been isolated from naira notes (Muhumuza *et al.*, 2018). Thus, the purpose of this study is to ascertain the degree of bacterial contamination and antibiotic resistance pattern of the bacteria isolated from Nigerian currency notes circulating in Enugu East Local Government Area of Enugu State.

**Comment [P H1]:** Revise, please and break down into shorter segments, capitalize the name of the currencies

**Comment [P H2]:** Citation needed

## Materials and Method

### Materials

**Study Area:** This research project work was carried out in Enugu east local government of Enugu state.

**Test sample:** Nigerian naira ( currency notes) (Figure 1)

**Test Organisms:** The test organisms were the bacteria isolated and they include: *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella spp*, *Klebsiella aerogenes*, *Streptococcus spp*, *Acinetobacter spp*, *Serratia marcescens*, *Proteus mirabilis*, *Enterococcus faecalis*, *Citrobacter spp* and *Actinomycetes spp*

**Culture media:** Media for isolation of bacteria were purchased from Titan Biotech LTD (India) and they include Nutrient agar, MacConkey agar, Eosin methylene blue (EMB) agar and Mannitol salt agar.

### Standard antibiotic disk

The selected antibiotics discs (Oxoid, UK) used for the study include meropenem (10 µg), nitrofurantoin (30 µg), cephalixin (30 µg), ciprofloxacin (10 µg), gentamicin (10µg), ofloxacin (5µg), clindamycin (10 µg), erythromycin (10µg), ceftriaxone (30µg), ampicillin (30µg), levofloxacin (5µg) amoxicillin (30µg), streptomycin (30µg), cloxacilline (10µg), perfloxacin (10µg), chloramphenicol (10µg).



**Fig 1:** Nigerian currency notes

## Methods

### Preparation and sterilization of media

The media used were prepared according to manufacturers' specification

**Sample Collection:** A total of 300 currency samples of different denominations of ₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500, ₦1000 notes were randomly collected using an adapted method from Igumbor *et al.* (2007). Samples were obtained from different artisan groups of meat sellers, food sellers, palm oil sellers, mechanics and non artisans in the study area. Samples were collected from the surface of the naira currency note using a dampened cotton wool swab stick and kept in a refrigerator for future use.

**Cultivation, isolation and characterization:** Each of the samples collected was inoculated into 5 ml of brain heart infusion (BHI) broth for enrichment and incubated for 12 h at 37°C. Following the enrichment, sub-culturing of the broth culture was done unto different selective media for presumptive isolation of different bacteria. Due incubation was done at optimum temperature of 37°C for 24 h. Then, organisms isolated were characterized based on morphology, biochemical, cultural and metabolic characteristics..

**Antibiotic susceptibility test:** Antibiotic susceptibility of all bacterial species were done using the Kirby Bauer disc agar diffusion method and interpreted according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2023). Sterile molten Mueller-Hinton agar plates were allowed to set and solidify on a level surface. An 18- 24 h old broth culture of the all the test isolates were standardized by adjusting its turbidity to 0.5 Mcfarland's standard. The sterile swab sticks were used for this cultivation by dipping it into a tube of standardized test isolates, drained to remove excess inoculum and inoculated by streaking 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 drying, antibiotic impregnated discs (Oxoid, UK) of known concentrations were placed on the inoculated plates and incubated overnight at 37°C. The IZD (inhibition zone diameter) were measured using a graduated meter rule and results recorded and interpreted following the guideline of CLSI (2023).

**Comment [P H3]:** Please also explain the process. Please mention why did you choose this sample size.

## Result

**Ethical approval:** The samples were collected with the consent of all the artisan and non artisan groups.

### Isolation of bacteria from the samples

Out of the 300 currency notes that were randomly sampled, a total of 632 bacteria were cultured. There was no bacterial growth detected on the control samples collected from First bank of Nigeria (N=5). The number of currency note according to source were shown in (Table 1) and the number in each denominations represented (65) hospital/laboratory, (27) students, (61) meat sellers, (20) food sellers, (33) palm oil sellers, (54) bus conductors/drivers and (40) mechanic. Table 2 shows the frequency of occurrence of bacteria isolate of naira note from whole sample collection. This shows that 13 different microorganisms were isolated from the naira note and 632 microorganisms in total were isolated from 300 samples. (Table 3) shows the total percentage prevalence from each isolate in all the denomination and out of 632 bacteria were isolated there were 223(74.3%) *Staphylococcus* sp, 95(31.6%) *E.coli*, 86(28.6%) *Bacillus subtilis*, 64(21.3%) *Klebsiella aerogenes*, 46(15.3%) *Streptococcus* sp, (8.0%) *Proteus mirabilis*, other organisms that were isolated include 16(5.3%) *Pseudomonas aeruginosa*, 18(6.0%) *Salmonella* sp, 10(3.3%) *Acinetobacter* sp, 11(3.6%) *Serratia marcescens*, 18(6.0%) *Enterococcus faecalis*, 19(6.3%) *Citrobacter* sp, 2(0.6%) Actinomycete. Gram positive isolates made up 53% of the total isolate while the rest were gram negative (47%). The antibiotic resistance pattern(%) of the bacterial isolated from all denomination are shown in (Fig 2) all Gram positive bacteria isolated from naira currency notes showed high susceptibility to cefuroxime, ciprofloxacin, and ofloxacin. However, Clindamycin and Erythromycin are not effective against *Staphylococcus* and *Streptococcus*. (Fig 3) Ciprofloxacin and Ofloxacin consistently demonstrate high resistance rates across multiple Gram-negative species and Meropenem and Ceftriaxone show relatively higher susceptibility rates compared to other antibiotics for several genera. The multiple antibiotic resistant indexes are shown in Table 4.

**Comment [P H4]:** What do you mean by this statement? Please explain. If any ethical body was involved, name it with proper reference number of the ethical permission issued. Otherwise you may mention no ethical clearance was needed, and if so, justify mentioning proper reasons.

**Comment [P H5]:** These control samples were not mentioned previously, declare it in methodology section, with description and reason for having a control sample.

**Table 1: Number of Currency note according to source**

| <b>Denomination (₹)</b> | <b>hospital/laboratories</b> | <b>Students</b> | <b>Meat vendors</b> | <b>Food vendors</b> | <b>Palm oil vendors</b> | <b>Bus conductor s/ Driver</b> | <b>Mechanics</b> | <b>Total</b> |
|-------------------------|------------------------------|-----------------|---------------------|---------------------|-------------------------|--------------------------------|------------------|--------------|
| 1000                    | 24                           | 20              | 40                  | 4                   | 0                       | 0                              | 8                | 96           |
| 500                     | 20                           | 6               | 2                   | 2                   | 14                      | 16                             | 15               | 75           |
| 200                     | 3                            | 4               | 2                   | 2                   | 2                       | 2                              | 3                | 18           |
| 100                     | 4                            | 5               | 1                   | 2                   | 3                       | 2                              | 1                | 18           |
| 50                      | 9                            | 0               | 6                   | 2                   | 4                       | 1                              | 7                | 29           |
| 20                      | 5                            | 11              | 7                   | 4                   | 7                       | 2                              | 4                | 39           |
| 10                      | 3                            | 8               | 2                   | 1                   | 2                       | 1                              | 2                | 19           |
| 5                       | 0                            | 0               | 1                   | 1                   | 1                       | 3                              | 0                | 6            |
| <b>Total</b>            | <b>65</b>                    | <b>27</b>       | <b>61</b>           | <b>20</b>           | <b>33</b>               | <b>54</b>                      | <b>40</b>        | <b>300</b>   |

**Table 2: Frequency of occurrence of bacterial isolates in the test Naira note.**

| Denomination<br>(₦) | EC | Sa  | Pa | Bs | Sal | Ka | St s | Acto | Sm | Pm | Ef | Ct | Ac sp, | Total |
|---------------------|----|-----|----|----|-----|----|------|------|----|----|----|----|--------|-------|
| 1000                | 35 | 76  | 5  | 16 | 1   | 20 | 17   | 1    | 1  | 12 | 6  | 5  | 1      | 196   |
| 500                 | 10 | 48  | 1  | 30 | 7   | 13 | 13   | 0    | 2  | 0  | 1  | 4  | 0      | 129   |
| 200                 | 8  | 13  | 0  | 9  | 2   | 3  | 1    | 0    | 0  | 0  | 1  | 1  | 1      | 39    |
| 100                 | 7  | 16  | 1  | 4  | 0   | 9  | 2    | 1    | 3  | 2  | 0  | 0  | 0      | 45    |
| 50                  | 12 | 25  | 6  | 8  | 6   | 5  | 4    | 1    | 2  | 6  | 1  | 2  | 0      | 78    |
| 20                  | 12 | 30  | 1  | 8  | 1   | 11 | 5    | 5    | 2  | 3  | 5  | 5  | 0      | 88    |
| 10                  | 9  | 10  | 2  | 7  | 1   | 2  | 3    | 0    | 1  | 1  | 3  | 1  | 0      | 40    |
| 5                   | 2  | 5   | 0  | 4  | 0   | 1  | 1    | 2    | 0  | 0  | 1  | 1  | 0      | 17    |
| Total               | 95 | 223 | 16 | 86 | 18  | 64 | 46   | 10   | 11 | 24 | 18 | 19 | 2      | 632   |

**Key:** *Ec*, *Escherichia coli*, *Sa*, *Staphylococcus sp*, *Pa*, *Pseudomonas aeruginosa*, *Bs*, *Bacillus subtilis*, *Sal*, *Salmonella sp*, *Ka*, *Klebsiella aerogenes*, *St s*, *Streptococcus sp*, *Acto*, *Acinetobacter sp*, *Sm*, *Serratia marcescens*, *Pm*, *Proteus mirabilis*, *EF*, *Enterococcus faecalis*, *Ct*, *Citrobacter sp*, *Ac sp*, *Acinomycetes sp*

**Table 3: Total percentage prevalence of each isolates from all the different denomination**

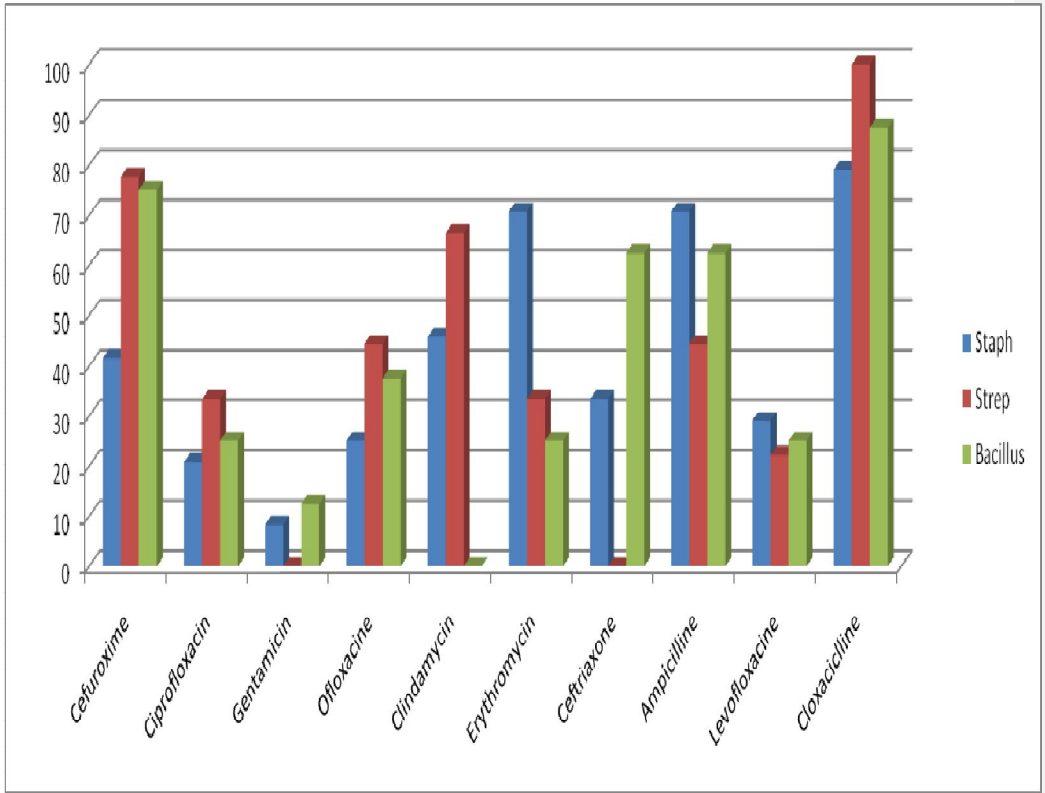
| Denomination (N) | EC   | Sa   | Pa  | Bs   | Sal | Ka   | St s | Acto | Sm  | Pm  | Ef  | Ct  | Ac sp | Total |
|------------------|------|------|-----|------|-----|------|------|------|-----|-----|-----|-----|-------|-------|
| 1000             | 35   | 76   | 5   | 16   | 1   | 20   | 17   | 1    | 1   | 12  | 6   | 5   | 1     | 196   |
| 500              | 10   | 48   | 1   | 30   | 7   | 13   | 13   | 0    | 2   | 0   | 1   | 4   | 0     | 129   |
| 200              | 8    | 13   | 0   | 9    | 2   | 3    | 1    | 0    | 0   | 0   | 1   | 1   | 1     | 39    |
| 100              | 7    | 16   | 1   | 4    | 0   | 9    | 2    | 1    | 3   | 2   | 0   | 0   | 0     | 45    |
| 50               | 12   | 25   | 6   | 8    | 6   | 5    | 4    | 1    | 2   | 6   | 1   | 2   | 0     | 78    |
| 20               | 12   | 30   | 1   | 8    | 1   | 11   | 5    | 5    | 2   | 3   | 5   | 5   | 0     | 88    |
| 10               | 9    | 10   | 2   | 7    | 1   | 2    | 3    | 0    | 1   | 1   | 3   | 1   | 0     | 40    |
| 5                | 2    | 5    | 0   | 4    | 0   | 1    | 1    | 2    | 0   | 0   | 1   | 1   | 0     | 17    |
| Total            | 95   | 223  | 16  | 86   | 18  | 64   | 46   | 10   | 11  | 24  | 18  | 19  | 2     | 632   |
| % Prevalence     | 31.6 | 74.3 | 5.3 | 28.6 | 6.0 | 21.3 | 15.2 | 3.3  | 3.6 | 8.0 | 6.0 | 6.3 | 0.6   |       |

**Comment [P H6]:** This total number is misleading, there were a total of 300 notes. Better omit this total. Mention clearly how you calculated those prevalences and percentages. Table 2 is probably unnecessary, as Table 3 is more complete and contains all the data from Table 2.

**Key:** *Ec*, *Escherichia coli*, *Sa*, *Staphylococcus sp*, *Pa*, *Pseudomonas aeruginosa*, *Bs*, *Bacillus subtilis*, *Sal*, *Salmonella sp*, *Ka*, *Klebsiella aerogenes*, *St s*, *Streptococcus sp*, *Acto*, *Acinetobacter sp*, *Sm*, *Serratia marcescens*, *Pm*, *Proteus mirabilis*, *EF*, *Enterococcus faecalis*, *Ct*, *Citrobacter sp*, *Ac sp*, *Acinomyces sp*

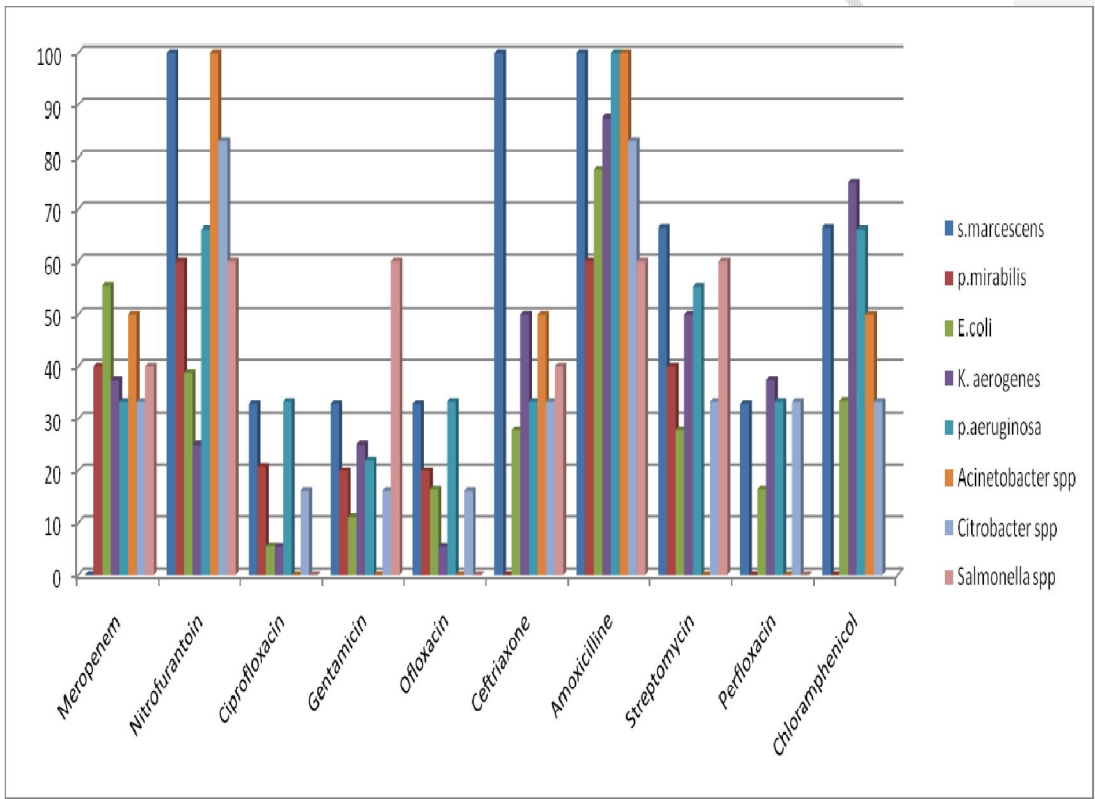


**Fig 2: Percentage antibiotic resistance pattern of Gram positive Bacteria.**



**Fig 3: Percentage antibiotic resistance of Gram negative Bacteria.**

**Comment [P H7]:** Where is explanation for the Figure 2 and Figure 3? And exact values at the end of the columns would be useful for the readers. You can also put a separate table.



**Table 4: Determination of Multiple Antibiotic Resistant Index (MARI)**

**Comment [P H8]:** Please provide explanation for Table 4

| Bacteria                      | Range of MARI |
|-------------------------------|---------------|
| <i>Staphylococcus</i> sp      | 0.1 – 0.8     |
| <i>Proteus mirabilis</i>      | 0.1 – 0.5     |
| <i>Streptococcus</i> sp       | 0.1 – 0.7     |
| <i>Bacillus subtilis</i>      | 0.3 – 0.5     |
| <i>Serratia marcescens</i>    | 0.4 – 0.9     |
| <i>Klesiella aerogenes</i>    | 0.3 – 0.6     |
| <i>Pseudomonas aeruginosa</i> | 0.2 – 0.9     |
| <i>Acinetobacter</i> sp       | 0.1 – 0.5     |
| <i>Citrobacter</i> sp         | 0.1 – 0.6     |
| <i>Salmonella</i> sp          | 0.2 – 0.5     |

## Discussion

Bacteria colonize both living and non living materials including food, stuff, meat, different fishes, wines, inert surfaces, currency notes and can be transferred from person to person through unethical practices (Adonu *et al.*, 2024). Whenever there is a presence of microorganisms on fomites, it suggests that the minimum conditions for their presence have been met. In the case of Nigerian naira notes, due to high frequency of use, one may question: 'How microbiologically safe are our naira notes in circulation'? In the present study, we isolated different bacteria that contaminate Naira currency notes. More so, we determined the antibiotic susceptibility pattern of the bacteria isolated from the test currency notes. This study made use of a total of 300 samples of naira notes (209 paper notes and 93 polymer notes) ranging from ₦5 to ₦1000 collected from hospital/laboratory, students, meat vendors (butchers), mechanics, bus conductors/drivers, palm oil vendors and food vendors. Some freshly printed naira (clean mint) notes were used as a control. All the naira notes that had bacterial growths were rough and dirty in appearance. The control group obtained from a commercial bank in the same study area did not show any bacterial growth. Of all the 300 samples used, the total of 632 isolates was isolated. *Staphylococcus aureus* was the prevalent bacterium (74.3%) followed by *Escherichia coli*

**Comment [P H9]:** Mention in methodology

(31.6%) and *Bacillus subtilis* (28.6%), which is in agreement with results of other researchers (Umeh *et al.* 2007; Shekarforoush *et al.* 2009; Abdumoniem *et al.* 2010; Alwakeel and Nasser. 2011; Elememet *et al.* 2016; Firoozeh *et al.* 2018; Nanda *et al.* 2019)). *Staphylococcus aureus* forms part of the normal flora of the skin of human hands and this accounts for high prevalence of this organism in the test currency notes. Similarly, *Escherichia coli*, which form part of the normal flora of the gut, frequently contaminate hands in individuals that do not maintain proper hygiene. The prevalence of *Klebsiella spp* was 21.3 %, which is in agreement with the findings of researchers (Chigozie *et al.*, 2021). Other bacteria which we isolated contaminated the currency notes in a manner that poses threats to public health. The results of this research shows that lower denomination was the most contaminated, this is consistent with a previous report (Tswana *et al.* 2000; Igumbor *et al.* 2007) and this is understandable because a good number of individuals currently have higher denominations in exchange for lower denomination when trading. It is important to note that bacterial growth was not detected in 5 samples of mint banknotes and this might be attributed to the fact that they had not been in circulation, which normally exposes them to usage and contamination. However, some researchers believed that some currency notes that are not yet in circulation could still be contaminated with fastidious organisms (Ahmed *et al.*, 2018).

**Comment [P H10]:** Any similar findings by other researchers? Mention importance of such findings

The majority of the test bacteria showed resistance to most of the antibiotics used. The highest resistance was seen against Gram positive bacteria. Many of the bacterial isolates showed resistance to amoxicillin (78.4%), cloxacillin (68.6%), ampicillin (63.4%), cefuroxime (56.0%) and erythromycin (53.6%), which was in agreement with the result of work done elsewhere (Obajuluwa *et al.*, 2023). The high resistance pattern recorded against these antibiotics implies inappropriate use of antimicrobial agents in the study area. Most of the test bacteria had a MAR index > 0.2 indicating their source to where antibiotics are commonly used or previous exposure of the organism to antimicrobial agents as reported by other researchers (Gunasekar, 2017, Mthembu *et al.* 2019, Afunwa *et al.* 2020). In other words, the test isolates were obtained from high risk sources of antibiotic resistance. These isolates were from artisans and non-artisan groups, pointing to the fact that antibiotic resistance and development of superbugs are not limited to hospital acquired pathogens only, but can be from community acquired pathogens too. It has been shown that  $MARI \geq 0.4$  or higher is associated with human faecal sources of contamination (Joseph *et al.*, 2017). This demonstrates a highly compromised hygienic environment for selling and processing of food.

**Comment [P H11]:** Explain MAR index in methodology section.

**Comment [P H12]:** Could you make your point clearer?

## Conclusion

This study showed that Nigerian currency notes harbour bacteria. Many of these bacteria are multidrug resistant and this poses a serious threat to public health. Inappropriate use and antibiotic misuse might have contributed to the presence of superbugs in the study area.

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