# MICROBIAL LOAD AND ANTIBIOTICS RESISTANCE PATTERNS OF BACTERIAL ISOLATES FROM NIGERIA NAIRA NOTES

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**ABSTRACT**

In our society today, exchange of goods and services usually involves exchange of currency notes by hand. This can serve as a means of transferring microorganisms and antibiotics resistant bacterial isolates. This study was aimed at assessing the contamination of Naira notes in Ungwan Rimi area of Kaduna State and to determine the antibiotics resistance patterns of the isolated bacteria. A total of 160 samples of naira notes, were collected randomly from staff and students, marketers, hospital and pharmacies, food vendors and transporters at strategic spots in Ungwan Rimi. Bacteria isolation and identification of samples were carried out using spread plate technique on medium and standard biochemical characterization, respectively. Antibiotics susceptibility testing were carried out using agar disc diffusion method. Isolates that were non-susceptible to at least one antimicrobial agent in three or more antimicrobial classes were classified as multidrug resistant (MDR), those that remain susceptible to agent(s) in only one or two classes as extended drug resistant (XDR) while pandrug resistant (PDR) isolates were classified as those isolates that were resistant to all antibiotics used. Highest bacterial load was observed with N100 notes with marketers ($5.33 \times 10^5$) while the least bacterial load was $1.2 \times 10^4$ in N50 notes from hospitals and pharmacies. The following bacteria were isolated *Staphylococcus aureus* 70 (26.8%), *Bacillus* spp 60 (23.0%), *Clostridium* spp 31 (11.9%), *Escherichia coli* 30 (11.5%), *Streptococcus* species 25 (9.6%), *Klebsiella* spp 24 (9.2%), *Salmonella* 15 (5.8%), *Shigella* species 4 (1.5%) and *Pseudomonas* species 2 (0.8%). The most active antibiotics were gentamicin (81%) and ciprofloxacin (73.4%), while 60.8%, 17.7% and 1.3% of the isolates were multidrug resistant (MDR), extended drug resistant (XDR) and pan-drug resistant (PDR), respectively. The naira notes sampled were highly contaminated with multidrug resistant bacterial isolates. Proper hygienic handling of naira notes and cashless money transaction is highly recommended.

**Keywords**: Antibiotic resistance, bacteria, naira notes

## INTRODUCTION

Any object or method of exchange that is accepted by people as payment for goods and services is referred to as money. In Nigeria, naira notes, (N5, N10, N50, N100, N200, N500, N1000) is the currency commonly exchanged for goods and services, debts settlement and for deferred payments in economic activities, and is handled by a large number of people including bankers, traders, artisans, butchers, transporters, roadside vendors, fishmongers etc, under a variety of environmental conditions. The naira notes presently in circulation are abused by the different ways they are handled and stored which may include squeezing, spraying and stepping on sprayed money, keeping naira notes in brassiere, socks and pockets, under the carpet or rugs, writings on them etc. (Alemu, 2014). Use of saliva and contaminated water on naira notes to facilitate counting is another way of introducing microorganisms to naira notes (Girma et al., 2016). In fact use of saliva to facilitate money count can be a source of health hazard through repeated hand to mouth movement in contact with naira note. There have been reports of microbial organisms contaminating currency all across the world. An extensive international study comprising 1280 banknotes from 10 different nations revealed that the age of the notes and the type of material used to make them (polymer versus cotton-based) have a significant impact on the amount of bacteria that can be found on banknotes (Okon et al., 2003, Vriesekoop, 2010).

Although paper money is drenched with disinfectant to inhibit microorganisms (Talaro, 2005), its use for every type of commercial activity is hard on the currency, with the lower denomination notes being handled the most because of its frequent exchange (Ogbu and Uneke, 2007); hence the rapid spread of the microorganisms in the currency notes in circulation. A number of pathogenic microorganisms, including *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella* spp*, Pseudomonas* spp*, Aerobacter* spp, *Mycobacterium tuberculosis*, *Vibrio cholerae*, and *Streptococcus faecalis*, can be transferred through money, according to studies (Kawo et al., 2009; Mbata et al., 2016; Ademokoya, 2018). Some of these bacterial infections are known to be significant multidrug resistance gene repositories, which can be disseminated to other pathogens through mobile genetic elements such as plasmid, transposon, and insertion sequence. This is a potential source of public health hazard. This study was aimed at assessing the contamination of Naira notes in Ungwan Rimi area of Kaduna State and to determine the antibiotics resistance patterns of the isolated bacteria. The findings from this study will facilitate rational planning, proper and safe handling of Naira notes to prevent the spread of microorganisms in Ungwan Rimi and Kaduna State at large.

## MATERIALS AND METHODS

### Description of Study area

Kaduna is the state capital of Kaduna State in north-western Nigeria, occupying an area of approximately 45,711.2km$^2$ and had a population of approximately 6,113,503 people with an annual growth rate of 3% during the 2006 census (FRN, 2010). This research is restricted to the Ungwan Rimi area which is a large city that is a significant economic, political, and cultural center for Kaduna State, and is also an important hub for regional or international connections, commerce, and communications.

### Physical conditions of the samples

The currency notes were in different physical conditions, as the presence of microorganisms on a naira note is independent on its physical condition but on its frequency of usage. The currency notes were categorized based on physical observation as of good, moderate, and bad condition. The note in good condition referred to those recently produced and looked new. Notes in moderate condition referred to the ones that retained half of their original condition while those in bad condition referred to currency notes which were either badly handled or in case of currency notes; were held together with the aid of a cellotape.

### Sample collection

A total of one hundred and sixty (160) naira notes were randomly collected from persons representing each of these occupations of interest which includes: marketers (including provision sellers), hospital/pharmacies, staff/students, food vendors and transporters. A total of 32 samples (4 samples of each denomination x 8 different denominations) were collected per each occupational category.  A sterile swab stick was used to streak the surface of the note while being held by the individual handling it, the swab stick was then covered and labelled appropriately. The samples were immediately conveyed to the Pharmaceutical Microbiology Laboratory of Kaduna State University for analysis.

### Determination of total bacteria count

Each swab stick used for sample collection was soaked in a sterile test tube containing 10ml of sterile distilled water. A tenfold serial dilution was carried out from the resulting liquid and 0.1ml of the third and fourth dilution were spread on sterile nutrient agar in petri dishes; which were further incubated at 37˚C for 24 hours in an incubator. The pure cultures were Gram stained in accordance with standard Gram staining procedure and microscopic examination was done (Cheesbrough, 2006).

### Isolation and identification of microorganisms

Bacterial growth from the nutrient agar plates was sub cultured on to selective media: mannitol salt agar, cetrimide agar and MacConkey agar. The plates were incubated at 37˚C for 24 hours and examined for growth. Thereafter, catalase test, coagulase test, indole, oxidase, urease test, triple test sugar, methyl red - voger proskeur test and sugar fermentation (lactose, fructose and glucose) were carried out on the isolates for biochemical characterization and identification. (Cheesebrough, 2006).

### Determination of Antibiotics Susceptibility Tests on the isolates

Antibiotics susceptibility patterns of bacteria isolates was determined using agar disc diffusion method on Mueller Hinton agar plates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2016). The tested antibiotics were tetracycline (15µg), gentamicin (10µcg), erythromycin (15µg), ceftriaxone (30µg), ciprofloxacin (5µg),trimethoprim/sulfamethoxazole (25µg), amoxicillin/clavulanic acid (30µg), vancomycin (30µg), chloramphenicol (30µg) and cefoxitin (30µg). (Liofilchem Company, Italy).

A turbid suspension of the isolates was made in sterile normal saline and standardized according to 0.5 McFarland turbid standard. A sterile swab was dipped into the bacteria suspension, pressed on the side of the bottles to allow excess drip-off, and then used to evenly streak the entire surface of the Mueller-Hinton agar. The inoculated plates were allowed to dry for few minutes, after which sterile forceps were used to place the antibiotic discs in a circular pattern on the media. The process was carried out for all the identified isolates, and the plates were incubated at 37˚C for 24 hours.  Interpretation of results as either susceptible, intermediate or resistance was done according to the criteria recommendation by the CLSI guideline (2016).

### Determination of antibiotics resistance pattern of the isolates

In determining the antibiotics resistance pattern of the isolates, those isolates that were non-susceptible to at least one antimicrobial agent in three or more antimicrobial classes were classified as Multidrug resistant (MDR) bacteria, the isolates that showed non-susceptibility to at least one antimicrobial agent in all but two or fewer antimicrobial classes (that is, bacterial isolates remain susceptible to agent(s) in only one or two classes) as extended drug resistant (XDR); while pandrug resistant bacteria (PDR) were the isolates with non-susceptibility to all agents in all antimicrobial classes (Magiorakos et al., 2012).

## RESULTS AND DISCUSSION

The mean total bacteria count of naira denominations' notes from the various occupational group where samples were taken are presented in Table 1. Highest bacterial load was observed with N100 notes with marketers (5.33 x 10$^5$ cfu/ml) while the least bacterial load was 1.2 x 10$^4$ cfu/ml in N50 notes from hospitals and pharmacies.

**Table 1: The mean total bacteria counts (CFU/ml) of various denominations of naira notes**

| S/N | NAIRA CURRENCY NOTES (₦) | UNIVERSITY STAFF AND STUDENTS | MARKETS | TRANSPORTERS | PHARMACIES AND HOSPITALS | FOOD VENDORS |
|---|---|---|---|---|---|---|
| 1 | 5 | $2\times10^4$ | $1.42\times10^5$ | $1.05\times10^5$ | $2.5\times10^4$ | $6.25\times10^4$ |
| 2 | 10 | $1.75\times10^5$ | $2.3\times10^4$ | $1.10\times10^5$ | $7.1\times10^4$ | $2.5\times10^4$ |
| 3 | 20 | $1.23\times10^5$ | $1.04\times10^5$ | $7.35\times10^4$ | $1.20\times10^5$ | $1.35\times10^5$ |
| 4 | 50 | $9.35\times10^4$ | $4.37\times10^5$ | $3.57\times10^5$ | $1.2\times10^4$ | $4.8\times10^4$ |
| 5 | 100 | $6.65\times10^4$ | $5.33\times10^5$ | $4.74\times10^5$ | $2.2\times10^4$ | $1.65\times10^5$ |
| 6 | 200 | $1.24\times10^5$ | $1.66\times10^5$ | $2.46\times10^5$ | $1.43\times10^4$ | $1.19\times10^5$ |
| 7 | 500 | $1.25\times10^5$ | $1.21\times10^5$ | $1.62\times10^5$ | $4.73\times10^4$ | $2.71\times10^5$ |
| 8 | 1000 | $1.22\times10^5$ | $2.28\times10^5$ | $5.05\times10^4$ | $1.71\times10^5$ | $1.5\times10^5$ |

A total of 261 bacterial isolates were detected. *S. aureus* had the highest occurrence of 26.8% (Figure 1), while *Pseudomonas* spp was the least (0.8%). Other bacterial isolates were *Bacillus* spp (23%), *Clostridium* spp (11.9%), *Escherichia coli* (11.5%), *Salmonella* spp (5.8%) and *Shigella* spp (1.5%). Gram staining results showed 69.4% (181/261) Gram positive and 30.7% (80/261) Gram negative bacterial isolates.
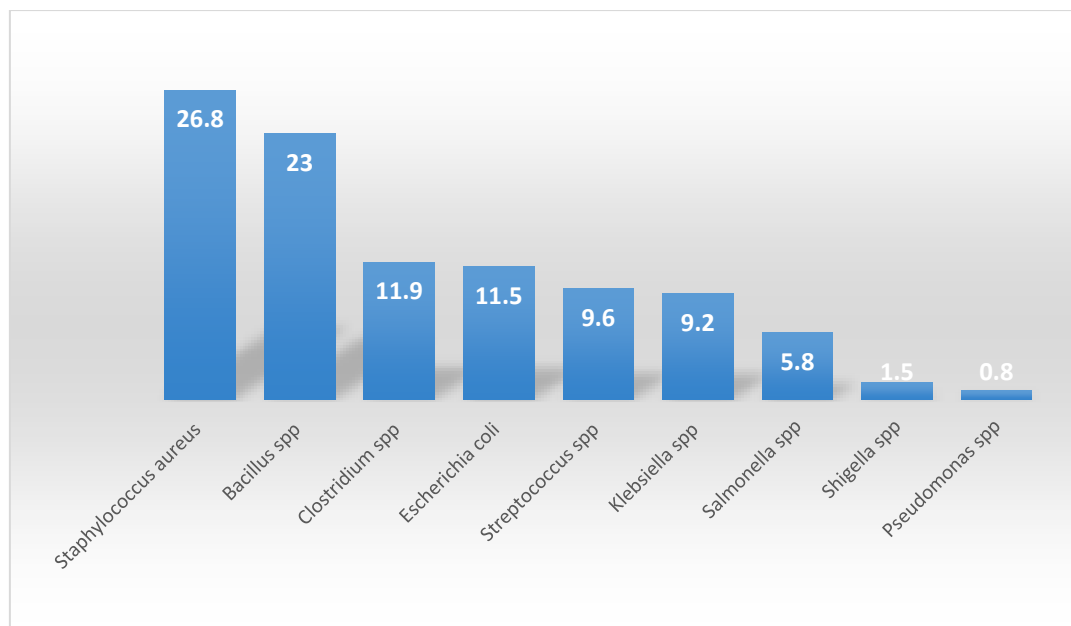


Figure 1: Percentage occurrence of microorganisms isolated

The results of the antibiotics susceptibility testing showed that the most active antibiotics were gentamicin (81%) and ciprofloxacin (73.4%), while amoxicillin-clavulanate (12.7%) and cefoxitin (22.8%) which are beta lactam antibiotics were the least active against the bacterial isolates, followed by erythromycin (0 %), a macrolide antibiotic (Figure 2).
Classifying the resistance pattern of the bacterial isolates into multidrug resistant, extended drug resistant and pan-drug resistant: 76% isolates were multidrug resistant, meaning they are resistant to three or more antibiotics from three or more classes of antibiotics used; 22% showed extended drug resistance (that is, bacterial isolates remain susceptible to agent(s) in only one or two classes) and 2% of the isolates were classified as pan-drug resistant because they were resistant all the classes of antibiotics tested (Figure 3).
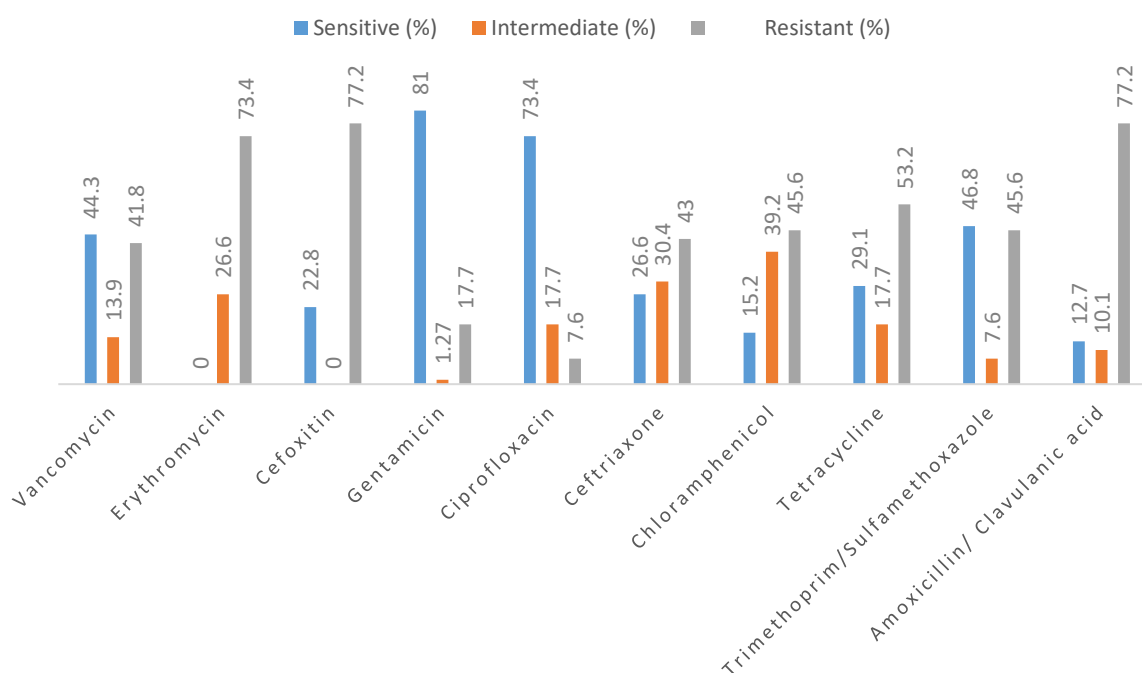


Figure 2: Percentage antibiotics susceptibility of the isolated bacteria
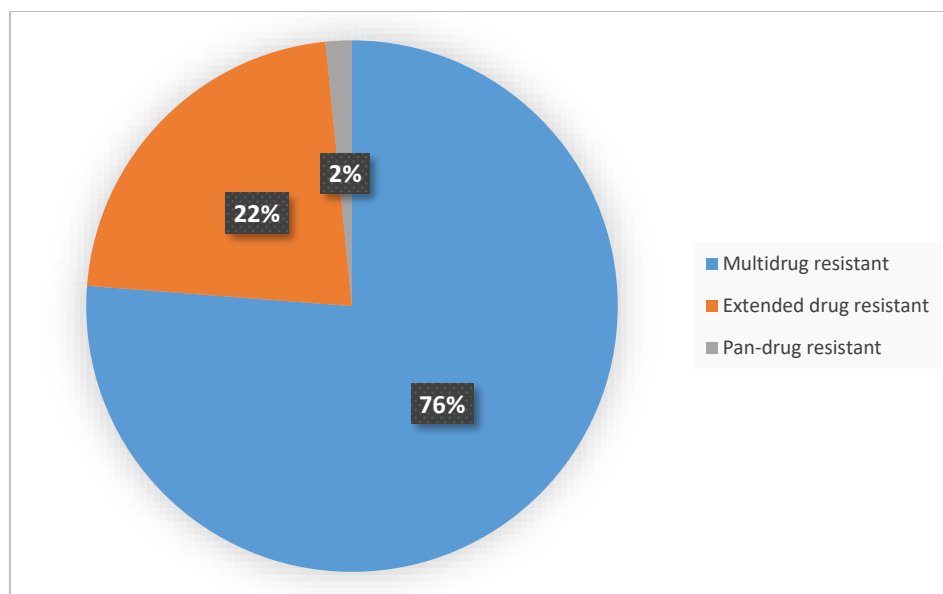
Figure 3: Antibiotic resistance pattern of bacteria isolates

## Discussion

The naira notes sampled from the various categories of people (marketers, transporters, food vendors, university staff and students, pharmacy and hospitals) were found to be contaminated with bacteria despite. Highest bacterial load was observed with ₦100 notes with marketers ($5.33 \times 10^5$) while the least bacterial load was $1.2 \times 10^4$ in ₦50 notes from hospitals and pharmacies. This can imply that ₦100 note is more in circulation among marketers that use it for buying and selling while higher denominations of naira notes are used more in the hospital and pharmacies as reflected in the highest total bacterial count observed on ₦1000 notes, and the lowest bacterial noticed on ₦50 notes in hospitals and pharmacies. The extent of bacteria load on the various naira denomination noted may suggest notes predominantly used among various categories of people of different occupation that were sampled in this study. However, from a similar study by Nwachi *et al.,* (2021) on Nigerian currency notes obtained from meat vendors in Abaliki, Ebonyi State, Nigeria, a lower bacterial count was reported ranging from $1.2 \times 10^3$ cfu/ml to $2.3 \times 10^4$ cfu/ml for ₦1000 and ₦100 notes, respectively.

Among the bacterial isolates *S. aureus* was the most prevalent, this might be because *S. aureus* is a normal human skin flora, it is estimated that 30% of the population is colonized with *S. aureus* and physical money (naira) transactions are conducted using hands. Other bacterial isolates from this study: *Bacillus* spp (23%), *Clostridium* spp (11.9%), *Escherichia coli* (11.5%), *Salmonella* spp (5.8%) and *Shigella* spp (1.5%) *Pseudomonas* spp (0.8%) were also reported by Nwachi *et al.,* (2021) and Morka, (2021) except *Clostridium* spp and *Shigella* spp. These bacterial isolates were pathogenic and their continuous presence on naira notes in circulation can constitute public health hazard. *S. aureus* had been reported to be a leading cause of skin and soft tissue infections such as abscesses, furuncles and cellulitis. *E. coli* can cause urinary tract infection, abdominal and pelvic infection, pneumonia and bacteremia, some can cause serious food poisoning (Makvana and Krilov, (2015). Among the infections caused by *Bacillus* spp are endocarditis, meningitis, and infections of wounds, the ears, eyes, respiratory tracts, urinary tract and gastrointestinal tracts (Baron, 1996). *Salmonella* infections include gastroenteritis, bacteremia, and enteric fever (Majowicz et al., 2010).

Gentamicin (aminoglycoside) and ciprofloxacin (quinolone) were the most active among antibiotics used in this study, while amoxicillin-clavulanic acid and cefoxitin, both of which are beta lactams were the least active. Similar activities of gentamicin and ciprofloxacin was reported by Morka. (2021) and Nwachi et al., (2021). The difference in the mechanisms of action of these antibiotics may be responsible for the observed difference in their activities. Ciprofloxacin's main mode of action is to stop DNA replication by blocking the A subunit of DNA gyrase (Shariati, 2022). Gentamicin inhibits protein synthesis by binding to bacterial ribosomal 30S subunit (Sahasranaman and Woolford, 2013)

The high level of multidrug resistant bacteria observed in this study is another concern especially to public health. Multidrug resistant bacteria are associated with nosocomial infections and are possible cause of community acquired infections and can lead to increased antibiotic use (van Duin and Paterson, 2016). Isolation of MDR bacterial isolates from naira notes in circulation can also imply that antibiotics are being abused or misused among the population using the naira notes sampled.
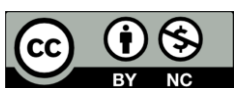
## CONCLUSION

Ciprofloxacin and gentamicin were the most active antibiotics observed in this study. The naira notes sampled were highly contaminated with multidrug resistant bacterial isolates. This is of public health risk because money is being used in exchange for goods and services every day. Proper hygienic handling of naira notes, e-commerce and cashless society is recommended including advocacy against abuse and misuse of antibiotics.

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