Prevalence and antibiotic susceptibility pattern of bacteria isolated from Nigerian currency note in 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 bacterial 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*(35.3%), *E.coli* (15.0%), *Pseudomonas aeruginosa* (2.5%), *Bacillus*sp (13.6%), *Salmonella* sp (2.8%), *Klebsiella*sp(10.1%), *Streptococcus*sp(7.3%), *Acinetobacter*sp (1.6%), *Serratia marcescens* (1.7%), *Proteus mirabilis* (3.8%), *Enterococcus faecalis* (2.8%), *Citrobacter*sp(3.0%) and *Actinomycete* sp(0.3%). TheGram positive and Gram negative bacteria showed resistance to cloxacilline (87%) and amoxicillin (84%). Further, 93.4% of all the bacterial isolates had an index >2 while 6.5% had a multi-antibiotic resistance index < 2.

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

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

INTRODUCTION

It has been well documented that bacteria iscosmopolitan 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).Currency notes, constantly exchanged, can harbour pathogens from handling. This facilitates the spread of bacteria during transactions and can easily be transferred between locations through coughs, sneezes, and dirty surfaces. (Elshariefet *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 cooperate 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.)Alemu, (2014). *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.

Materials and Methods

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 sp, Klebsiella aerogenes, Streptococcus sp, Acinetobacter sp, Serratia marcescens, Proteus mirabilis, Enterococcus faecalis, Citrobacter spp*and *Actinomycetes sp*

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), cephalexin (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 was obtained to ensure that all the sources of from different locations in the study area was represented, of which ₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500, ₦1000 notes were randomly obtained by exchanging sample notes for new and fresher ones of the same value(Igumbor*et al*., 2007).Five (5) mint Naira notes were gotten from First bank of Nigeria and served as control to provide a base line for comparism. Samples were obtained from different artisan groups of meat sellers, food sellers, palm oil sellers, mechanics and non artisansin the study area. Samples were collected from the surface of the Naira currency note using a damped 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^{o}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^{o}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 all the testedisolates 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 inoculums 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^{o}C. The IZD (inhibition zone diameter) were measured using a graduated meter rule and resultswererecorded and interpreted following the guideline of CLSI (2023).

Determination of Multiple antibiotic resistance index (MARI):

The multiple antibiotic resistance index (MARI) for each isolate was determined using the formula as describe by Sandhu*et al.* (2016); MARI= $\frac{a}{b}$where a;is the number of antibiotics to which the test isolate was resistant to; b is the total number of antibiotics to which the isolates where subjected.

Result

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 that13 different bacteriaspecies swere isolated from the Naira note and the total percentage prevalence from each isolate in all the denomination and out of 632 bacteria were isolated there were 223(35.3%) *Staphylococcus aureus*, 95(15.0%) *Escherichia coli*, 86(13.6%) *Bacillus subtilis*, 64(10.1%) *Klebsiella aerogenes*, 46(7.3%) *Streptococcus sp*, (3.8%) *Proteus mirabilis*, other organisms that were isolated include 16(2.5%) *Pseudomonas aeruginosa*, 18(2.8%) *Salmonella sp*, 10(0.3%) *Acinetobacter sp*, 11(1.6%) *Serratia marcescens*, 18(2.8%) *Enterococcus faecalis*, 19(3.0%) *Citrobacter sp*, 2(0.3%) *Actinomycete sp*. Gram positive isolates made up 56.2% of the total isolate while the rest were gram negative (43.8%). The antibiotic resistance pattern(%) of the bacterial isolated from all denomination were shown in (Table 3 and Fig 2) all Gram positive bacteria isolated from naira currency notes showed high susceptibility to cefuroxime, ciprofloxacin, and ofloxacin. However, Clindamycin and Erythromycin were not effective against *Staphylococcus aureus* and *Streptococcus sp*. (Table 4 and 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 indices are shown in Table 5.

Table 1: Number of currency note according to source

Denomination (₦)	hospital/ laboratories	Students	Meat vendors	Food vendors	Palm oil vendors	Bus conductors/ Driver	Mechanics	Total
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
Total	65	27	61	20	33	54	40	300

Table 2: 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	
% Prevalence	15.0	35.3	2.5	13.6	2.8	10.1	7.3	1.6	1.7	3.8	2.8	3.0	0.3	

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

$$\textbf{\%prevalence} = \frac{total\ occurence\ in\ a\ particular\ organsim}{total\ number\ of\ all\ isolate} \times 100$$

Table 3: Antibiotic resistance profile of Gram positive bacteria isolates from Nigerian currency notes

Antibiotics	**nc (%)** ***Sa***	**nc (%)** ***St***	**nc (%)** ***Bs***
Cefuroxime	10(41.6)	7(77.7)	6(75.0)
Ciprofloxacin	5(20.8)	3(33.3)	2(25.0)
Gentamycin	2(8.3)	0	1(12.5)
Ofloxacin	6(25.0)	4(44.4)	3(37.5)
Clindamycin	11(45.8)	6(66.6)	0
Erythromycin	17(70.8)	3(33.3)	2(25.0)
Ceftriaxone	8(33.3)	0	5(62.5)
Ampicillin	17(70.8)	4(44.4)	5(62.5)
Levofloxacin	7(29.1)	2(22.2)	2(25.0)
Cloxacillin	19(79.1)	9(100)	7(87.5)

Key*: Sa*; *Staphylococcus sp*,*St*; *Streptococcus sp*, *Bs*;*Bacillus subtilis*

nc: Number of isolate that were resistant to a particular antibiotics

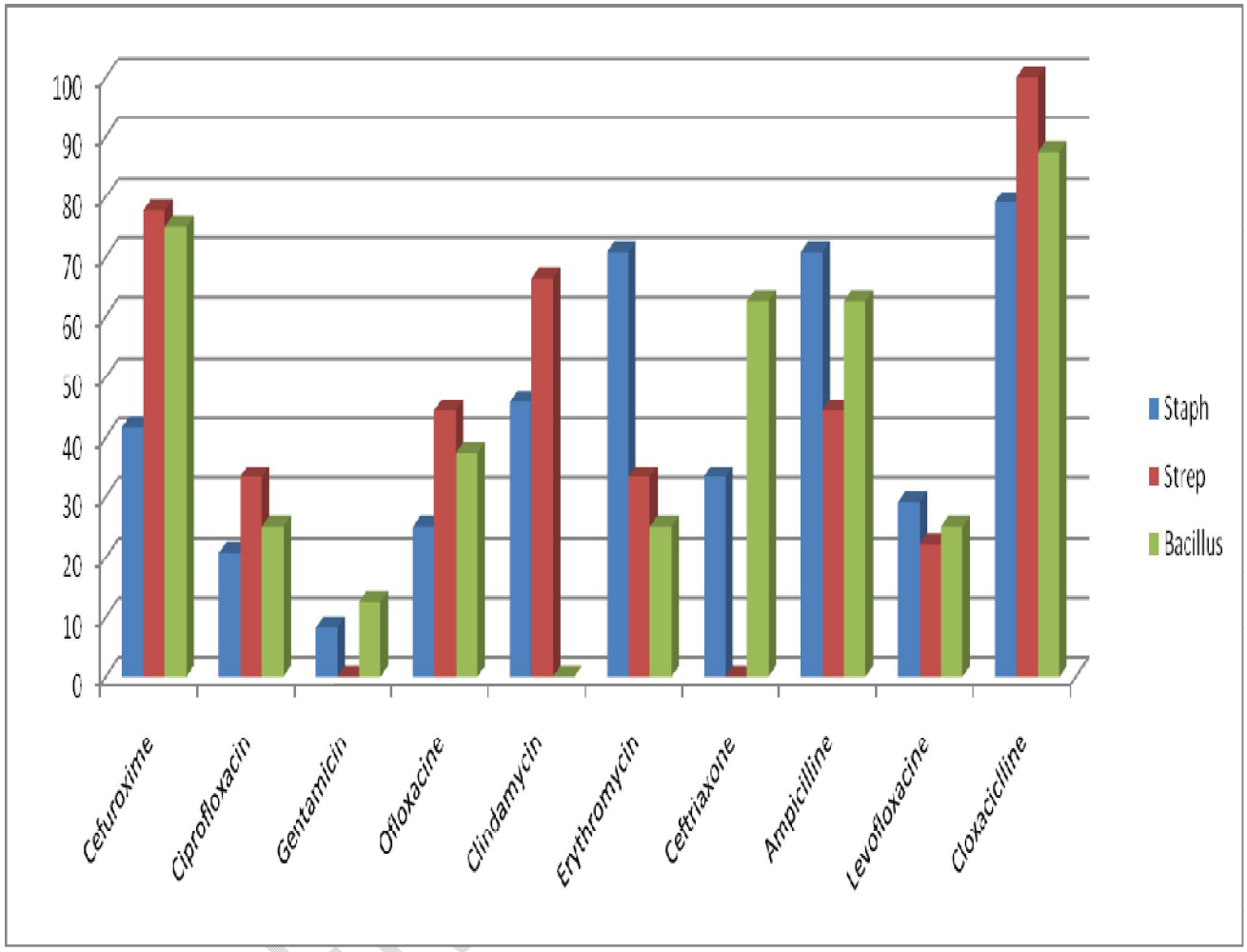

Fig 2: Percentage antibiotic resistance pattern ofGram positive bacteria.

Table 4: Antibiotic resistance profile of Gram negative bacterial isolates from Nigerian currency notes

Antibiotics	**nc (%)** ***Sm***	**nc (%)** ***Pm***	**nc (%)** ***Ec***	**nc (%)** ***Ka***	**nc (%)** ***Pa***	**nc (%)** ***Acto***	**nc (%)** ***Ct***	**nc (%)** ***Sal***
Meropenem	0	2(40.0)	10 (55.4)	3(37.5)	1(33.1)	1(50.0)	2(33.1)	2(40.0)
Nitrofurantoin	3(100)	3(60.0)	7(38.8)	2(25.0)	2(66.2)	2(100)	5(83.1)	3(60.0)
Ciprofloxacin	1(32.7)	1(20.9)	1(5.5)	1(5.4)	1(33.1)	0	1(16.3)	0
Gentamicin	1(32.7)	1(20.0)	2(11.1)	2(25.0)	1(22.1)	0	1(1.16.3)	3(60.0)
Ofloxacin	1(32.7)	1(20.0)	3(16.6)	1(5.4)	1(33.1)	0	1(16.3)	0
Ceftriaxone	3(100.0)	0	5(27.7)	4(50.0)	1(33.1)	1.(50.0)	2(33.1)	2(40.0)
Amoxicillin	3(100)	3(60.0)	14(77.7)	7(87.5)	3(100)	2(100)	5(83.1)	3(60.0)
Streptomycin	2(66.6)	2(40.0	5(27.7)	4(50.0)	2(55.2)	0	2(33.1)	3(60.0)
Perfloxacin	1(32.7)	0	3(16.6)	3(37.5)	1(33.1)	0	2(33.1)	0
Chloramphenicol	2(66.6)	0	6(33.3)	6(75.0)	2(66.2)	1(50.0)	2(33.1)	0

Key*: Sm;Serratia marcescens,Pm;Proteus mirabilis,Ec;Escherichia coli, Ka;Klebsiella aerogenes, Pa*; *Pseudomonas aeruginosa, Acto*; *Acinetobacter, Sal*; *Salmonella sp, Ct*; *Citrobacter sp.*

nc: Number of isolate that were resistant to a particular antibiotics

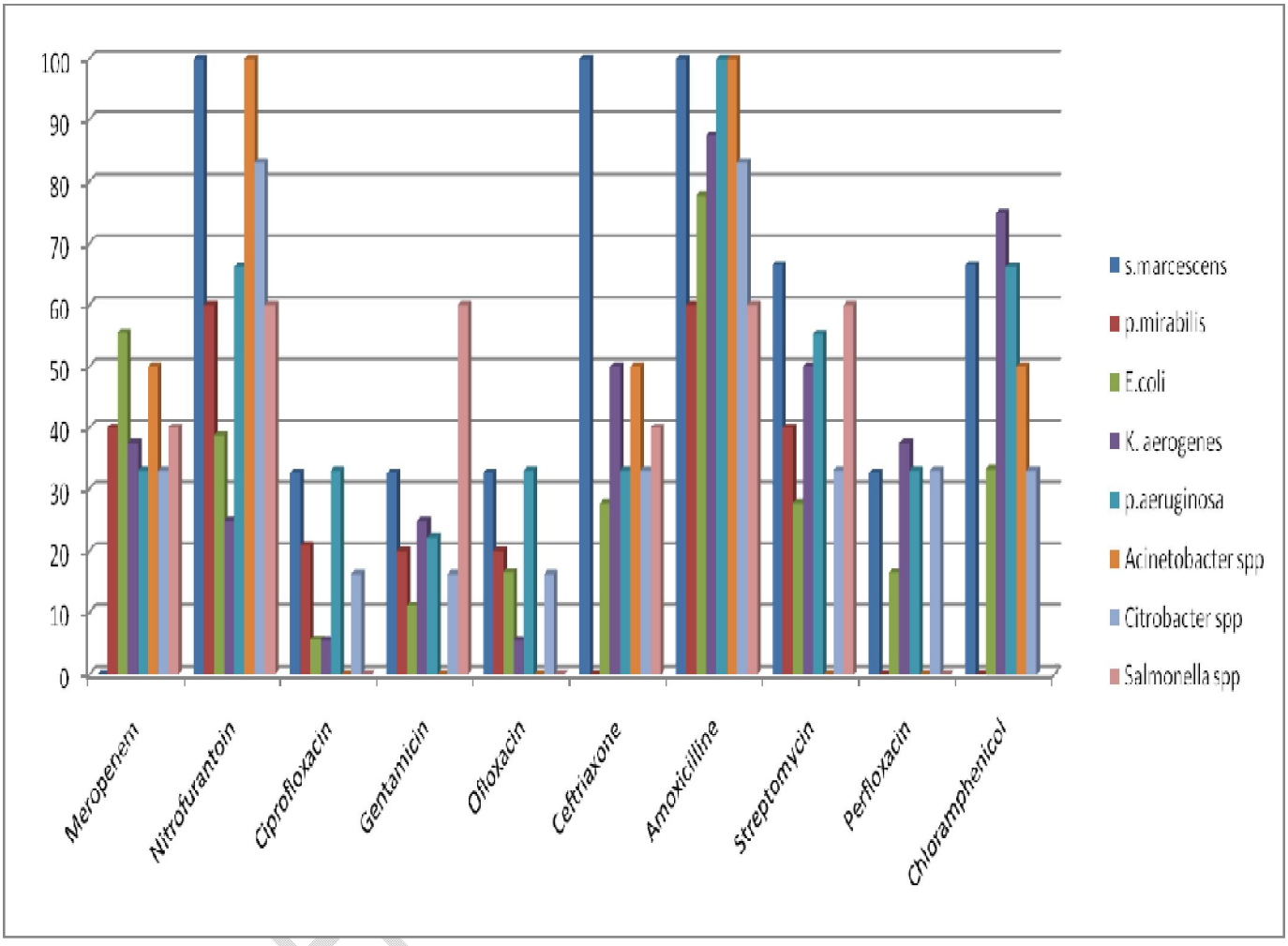

Fig 3: Percentage antibiotic resistance of Gram negative bacteria.

Table 5: Determination of Multiple Antibiotic Resistant Index (MARI)

MAR INDEX	***Sa*** **n(%)**	***St*** **n(%)**	***Bs*** **n(%)**	***Sm*** **n(%)**	***Pm*** **n(%)**	***Ec*** **n(%)**	***Ka*** **n(%)**	***Pa*** **n(%)**	***Acto*** **n(%)**	***Ct*** **n(%)**	***Sal*** **n(%)**
0.1	1(4.1)	1(11.1)	-	-	1(20)	2(11.1)	-	-	-	1(11.1)	-
0.2	3(12.5)	1(11.1)	-	-	2(40))	2(11.1)	-	1(33.3)	1(50)	-	1(20)
0.3	4(16.6)	1(11.1)	2(25)	-	1(20)	2(11.1)	4(50)	-	-	2(2.2)	3(60)
0.4	8(33.3)	2(22.2)	3(37.5)	2(66.7)	-	5(27.8)	1(12.5)	1(33.3)	-	-	-
0.5	3(12.5	1(11.1)	3(37.5)	-	1(20)	3(16.7)	1(12.5)	-	1(50)	3(33.3)	1(20)
0.6	2(8.3)	2(22.2)	-	-	-	2(11.1)	2(25)	-	-	1(11.1)	-
0.7	1(4.1)	1(11.1)	-	-	-	1(5.6)	-	-	-	-	-
0.8	2(8.3)	-	-	-	-	1(5.6)	-	-	-	-	-
0.9	-	-	-	1(33.3)	-	-	-	1(33.3)	-	-	-

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

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 fromhospital/laboratory, students, meat vendors (butchers), mechanics, bus conductors/drivers, palm oil vendors and food vendors. Some freshly printed naira (clean mint) noteswere 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, atotal of 632 microorganismswere isolated. *Staphylococcus aureus* was the prevalent bacterium (35.3%) followed by *Escherichia coli* (15.0%) and*Bacillus subtilis* (13.6%), 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; Elemem*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 account 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 andaddressthe need for public health interventions through to reduction of faecal contamination in the environment, hand washing (Greene*et al*., 2012; Navab-Daneshmand *et al*., 2018). The prevalence of *Klebsiella* sp was 10.1%, which is in agreement with the findings of researcher (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 understandablebecause 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).

The majority of thetest bacteria showed resistance to most of the antibiotics used. Comparing fig 2 and 3, 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).

In table 4, different sample sizes of isolates were randomly selected and the multiple antibiotic resistance indices (MARI) of different isolates had their ranges. The multiple antibiotics resistance indices of*staphylococcus aureus*and *E.coli*ranged from of 0.1 – 0.8 and 0.1-0.8 respectively with an average of 0.4, Five(5) samples of *proteus mirabilis* were selected and MARI ascertained with a range of 0.1- 0.5, while *Serratia marcescens*ranged from 0.4-0.9. This shows that greater percentage of all the isolates recorded a MARI ≥ 0.2. Hence, appropriate hygienic measures should be adopted while handling Naira notes within the study area. The high resistance pattern recorded against these antibiotics implies inappropriate use of antimicrobial agents in the study area. Most of the testisolateshad a MAR index $\geq$0.2 indicating their source to where antibiotics are commonly used or previous exposure of the organism to antimicrobial agents as reported by other researchers (Gunasekar, 2017,Mthembu *et al*. 2019,Afunwa*et al*. 2020). In order words, the test isolates were obtained from high risk sources of antibiotic resistance.These isolates from Naira notes gotten from artisans and non-artisan , points to the fact that antibiotic resistance and development of superbugs are not limited to hospital acquired pathogens only, but can be from community acquired pathogens too. It has been shown that MARI$\geq$ 0.4 or higher is associated with human faecal sources of contamination (Jospeh *et al*., 2017), this highlights a highly compromised hygienicenvironment forsale and processing of food in this study area.

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.

consent

No ethical clearance was needed because informed consent was sought from the all currency note users

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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