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ASSOCIATION OF MULTIDRUG RESISTANT *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* WITH SPECIFIC NAIRA DENOMINATIONS

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ABSTRACT

Currency serves as a global tool for commerce but is notorious for its association with potentially pathogenic bacteria. This has been further complicated by the growing evolution and emergence of drug resistance among bacteria, with various currencies associated with multidrug resistant (MDR) strains. This could be a silent factor in the current scourge of multidrug resistance. This study therefore set out to explore the presence of multidrug resistance Escherichia coli and Staphylococcus aureus in specific denominations of Naira, the Nigerian currency in Rivers State, Nigeria. A total of 50 naira notes comprised of two commonly used denominations (₦50 and ₦100) made up of two different materials (polymer and paper) were analyzed for microbial load, presence of key bacteria and drug resistance using standard tests. Results of the study revealed high bacterial loads with Log10 values ranging from 4.52 to 5.39 for the ₦100 notes and 4.52 to 5.31 for ₦50 notes. Samples had a higher occurrence of S. aureus (26) than E. coli (8). These isolates were associated with very high rates of resistance though the Gram-positive organisms were more resistant. Both S. aureus and E. coli had high rates of MDR (96.2% and 100% respectively). This study reveals an association of the Nigerian currency, Naira, with a variety of drug resistant bacteria and high levels of multidrug resistance in key pathogens and primarily underscores the need for continuous hand washing as the first key step in disease prevention

Keywords: Naira, fomite, drug-resistance, *Escherichia coli*, *Staphylococcus aureus*, Nigeria

INTRODUCTION

Currency, which serves globally as a tool for commerce, has long been noted for its association with bacteria belonging to classes of medical importance worldwide (Ofoedu et al., 2021; Yar, 2020; Sarwar et al., 2021; Hiko et al., 2016; Kalita et al., 2013; Al-Ghamdi et al., 2011). Currency contamination is as a result of the frequent person to person exchange of this item within the human population. Reports over the years have detected a wide variety of bacteria associated with various types of currencies worldwide. Some of these include, *Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas aeruginosa*, *Klebsiella* sp., *Escherichia coli* and *Proteus* sp. (Yazah et al., 2012; Mbata et al., 2016).

In more recent years with the rising global issues of antimicrobial drug resistance, studies have shifted from just the determination of microbial load and diversity of bacteria on currency, to focus on the potential role of currency as reservoirs of drug resistant bacterial strains. Unsurprisingly, considering the increasing rates of drug resistance, Nigerian currency notes have been found associated with drug resistant bacterial strains, which varied from study to study (Oluduro et al., 2014; Aminu and Yahaya, 2018, Mofolorunsho et al., 2021). While the more recent study by Aminu

and Yahaya (2018) carried out in the north of Nigeria reported moderate resistance levels with rates above 50% only reported for 3 of the 10 antibiotics tested, an older study in the west observed rates above 50% for 8 of the 10 antibiotics tested. Only the Aminu and Yahaya study clearly reported on the occurrence of multidrug resistant (MDR) isolates with a rate of 11%.

E. coli in addition to *S. aureus* have been described as the two most commonly detected isolates in clinical microbiology and make up key elements in the global story of multidrug resistance. The "S" in the ESKAPE acronym which represents the six main nosocomial pathogens of interest notorious for drug resistance and virulence, stands for *Staphylococcus aureus*. This organism has been linked to a wide variety of infections both in the hospital and the community, and has been known for its rapid development of drug resistance to antibiotics. Though not considered one of the ESKAPE organisms, *E. coli* is an important very ubiquitous, key indicator organism for monitoring the development and spread of drug resistance.

Few studies have specifically focused on exploring the presence of MDR strains of these two most commonly occurring organisms in currency. This study therefore set out to explore the presence of multidrug resistance *E. coli* and *S. aureus* in specific denominations of Naira, the Nigerian currency in Rivers State, Nigeria.

MATERIALS AND METHODS

Collection and processing of Samples

A total of 50 naira notes comprised of two commonly used denominations (₦50 and ₦100) made up of two different materials (polymer and paper) were obtained randomly from the University of Port Harcourt population. These samples were aseptically obtained using sterile stomacher bags and transported to the Pathogenic Bacteriology laboratory for immediate processing. Samples were processed as previously described (Vriesekoop et al., 2010). In brief, 40 ml of extraction buffer [(g/L) NaCl (10); K_2HPO_4 (2)], was added into the stomacher bags and samples stomached twice for 5 mins using the Stomacher Lab-Blender 400 (BA 6021, Seward, UK) with a 30 min incubation in between. The resultant solution was then analyzed for total heterotrophic counts on Nutrient agar, Eosin Methylene blue (EMB) agar and Mannitol Salt agar (MSA), using standard bacteriological methods (Cheesbrough, 2000).

Identification and Susceptibility testing

Following culture, characteristic colonies on EMB and MSA were purified and identified using standard bacteriological methods (Cheesbrough, 2000). Next, susceptibility of the organisms to test isolates were determined using the standard Kirby Bauer method (Bauer et al., 1966). Following this, the resistance status of organisms was ascertained using the CLSI standard (CLSI, 2018).

RESULTS AND DISCUSSION

Results

Total Bacterial Counts

An assessment of the total heterotrophic counts (THC) associated with ₦50 and ₦100 naira notes showed similar Log10 values with a range of 4.52 to 5.39 for the ₦100 notes and 4.52 to 5.31 for ₦50 notes. The higher Log10 values had a higher representation of ₦100 rather than ₦50 (Figure 1).

An assessment of counts on MSA showed similar Log10 values for both ₦100 and ₦50 with values ranging from 4.51 to 5.36 and 4.53 to 5.37, respectively. Similar to the results obtained following THC, ₦100 had a greater representation among the higher Log_{10} values than ₦50 with 30% of ₦100 samples represented there as opposed to 16% of ₦50 samples. Results of counts on EMB differed from those on MSA. While similar ranges were observed, a lower representation of both currency denominations (12% each) were observed for the higher Log_{10} values (Figure 2).

Microbial Composition of Naira samples

A comparison of growth on both selective media showed that characteristic *Staphylococcus aureus* colonies occurred more commonly than characteristic *Escherichia coli* colonies in both

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naira denominations. Similar level of occurrence was however observed when comparing occurrence of each type of characteristic colonies between denominations.

A total of 32 isolates and 18 isolates were subsequently purified from the distinct characteristic colonies on MSA and EMB respectively. Biochemical characterization of these isolates revealed 7 distinct isolates (Figure 4) of which *S. aureus* and *E. coli* were the most commonly occurring organisms obtained from MSA and EMB respectively.

Antibiotic Susceptibility Profile

An analysis of antibiotics resistance showed high degrees of resistance (ranging from 75% to 100%) among the Gram-positive organism to most (6/8, 75%) of the antibiotics (Figure 5). Similar levels of resistance against most antibiotics were noted in isolates from both denominations. Organisms from the ₦100 notes however showed slightly higher resistance rates (with differences ranging from 12.5% to 25%) than those from ₦50 against 37.5% of isolates. The Gram-negative isolates had levels of resistance which were slightly lower ranging from 75 to 100% observed against 5 antibiotics for isolates from both denominations. Unlike results of the Gram-positive organisms, Gram negative organisms obtained from the ₦50 notes had higher levels of resistance with differences ranging from 30% to 52.9% against 2 of the antibiotics.

Further assessment of the susceptibility pattern shows that six antibiogram patterns were produced by the 32 Gram positive isolates (Table 1). One pattern was dominant, occurring in 75% of the isolates. A higher diversity was noted in the Gram negative isolates as observed by the nine antibiogram patterns produced by this group of 18 isolates. The most common antibiogram pattern occurred in 27.8% of isolates.

Most of the Gram positive isolates (87.5%) were resistant to less than 4 drug classes (Figure 6) unlike the Gram negative isolates for which most (88.8%) showed resistance to 4 or more drug classes. Focusing specifically on strains of *E. coli* and *S. aureus*, high levels of multidrug resistance was observed with values of 100% and 96.2% respectively (Figure 7)

Table 1: Antibiogram occurrence in the Gram positive **(A)** and Gram negative **(B)** isolates obtained from ₦100 and ₦50 notes

Antibiogram	Number of isolates		
	₦50	₦100	MDR
Gram Positive			
AUG-CAZ-CRX-CXC	1	-	No
AUG-CAZ-CRX-CTR-CXC	3	-	No
AUG-CAZ-CRX-CTR-CXC-ERY	12	12	Yes
AUG-CRX-CTR-ERY-GEN-OFL	-	1	Yes
AUG-CAZ-CRX-CTR-CXC-ERY-GEN	-	2	Yes
AUG-CRX-CTR-CXC-ERY-GEN-OFL	-	1	Yes
Gram Negative			
AUG-CAZ-CRX-CXM	-	1	No
AUG-CAZ-CXM-NIT	1	-	Yes
AUG-CAZ-CPR-CRX-CXM	1	1	Yes

AUG-CAZ-CRX-CXM-GEN	-	3	Yes
AUG-CAZ-CPR-CXM-NIT	1	-	Yes
AUG-CAZ-CRX-CXM-NIT	2	-	Yes
AUG-CAZ-CPR-CRX-CXM-NIT	1	-	Yes
AUG-CAZ-CRX-CXM-GEN-NIT	3	2	Yes
AUG-CAZ-CPR-CRX-CXM-GEN-NIT	2	-	Yes

KEY: Augmentin (AUG), Ceftazidime (CAZ), Ceftriaxone (CTR), Cefuroxime (CRX), Cloxacillin (CXC), Erythromycin (ERY), Gentamicin (GEN), Ofloxacin (OFL)

KEY: Augmentin (AUG), Ceftazidime (CAZ), Ciprofloxacin (CPR), Co-trimoxazole (CRX), Cefuroxime (CXM), Gentamicin (GEN), Ofloxacin (OFL), Nitrofurantoin (NIT)

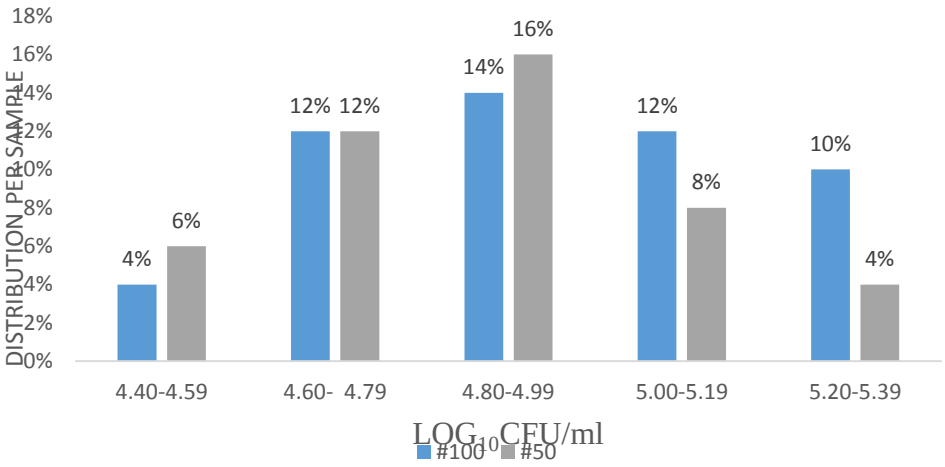

Figure 1: Total bacterial counts (Log_{10}CFU/ml) distribution occurrence of naira samples

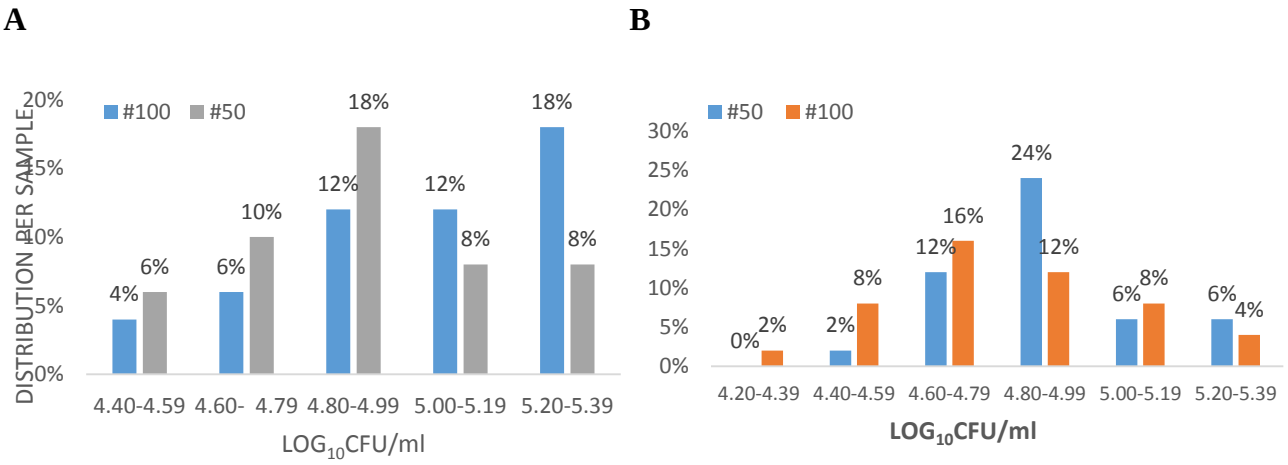

Figure 2: Bacteria counts distribution of ₦100 naira and ₦50 naira notes in (A) Mannitol salt agar and (B) Eosin Methylene Blue Agar

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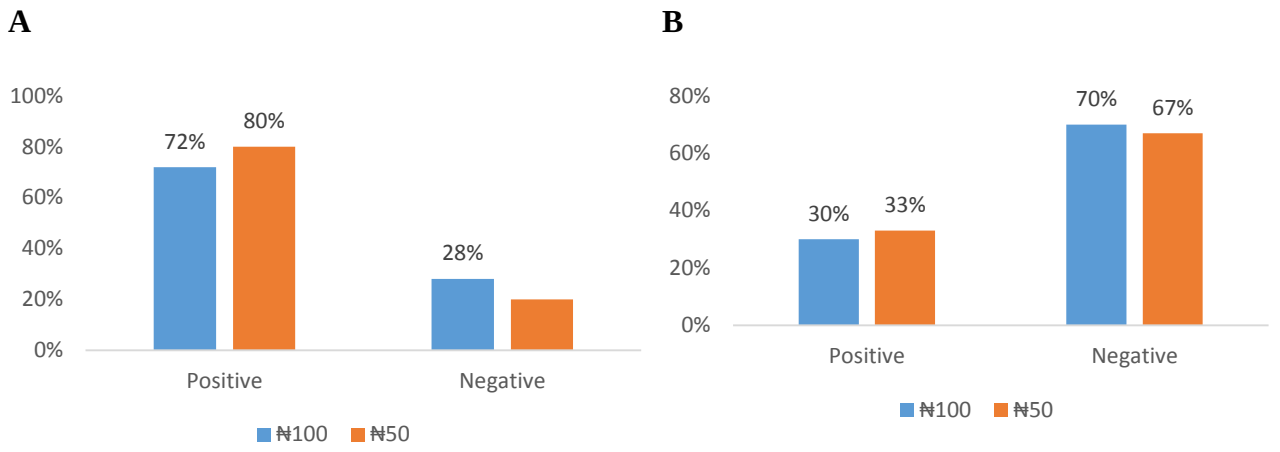


Figure 3: Percentage of occurrence of **(A)** *Staphylococcus aureus* characteristic colonies on MSA agar and **(B)** *Escherichia coli* on EMB for both ₦50 and ₦100.

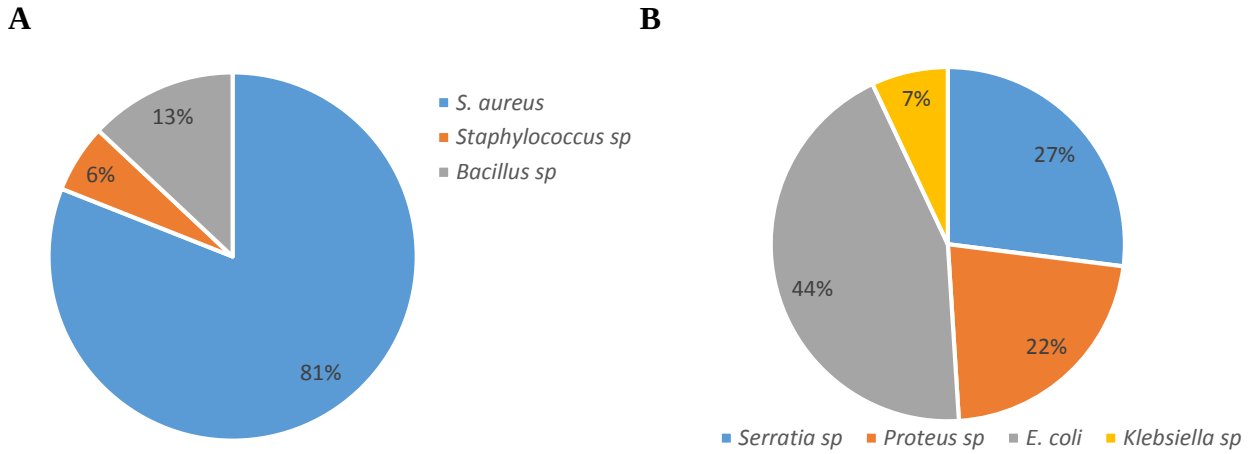
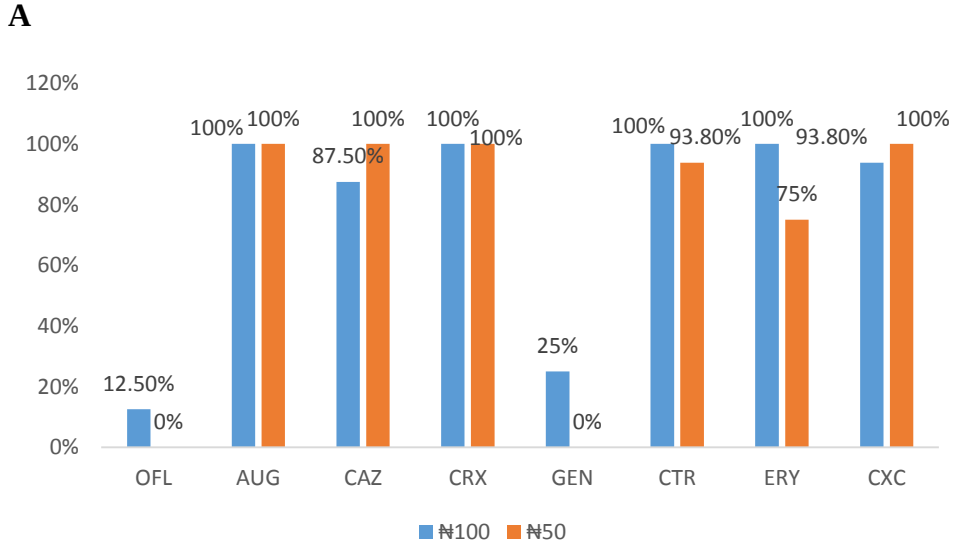
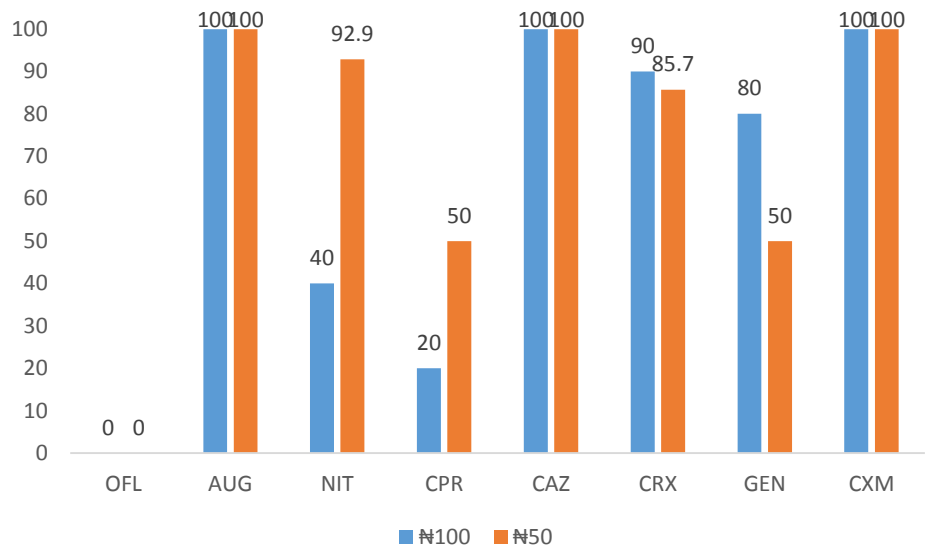


Figure 4: Distribution of organisms isolated on **(A)** MSA and **(B)** EMB from naira notes



KEY: Augmentin (AUG), Ceftazidime (CAZ), Ceftriaxone (CTR), Cefuroxime (CRX), Cloxacillin (CXC), Erythromycin (ERY), Gentamicin (GEN), Ofloxacin (OFL)

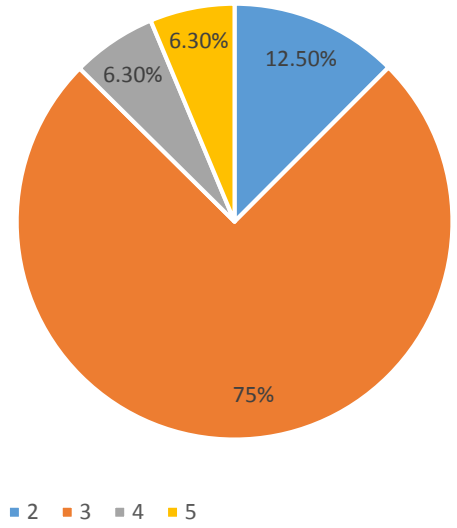
B



KEY: Augmentin (AUG), Ceftazidime (CAZ), Ciprofloxacin (CPR), Co-trimoxazole (CRX), Cefuroxime (CXM), Gentamicin (GEN), Ofloxacin (OFL), Nitrofurantoin (NIT)

Figure 5: Antibiotic Susceptibility Pattern of **(A)** Gram positive and **(B)** Gram negative isolates from ₦100 and ₦50 notes

A



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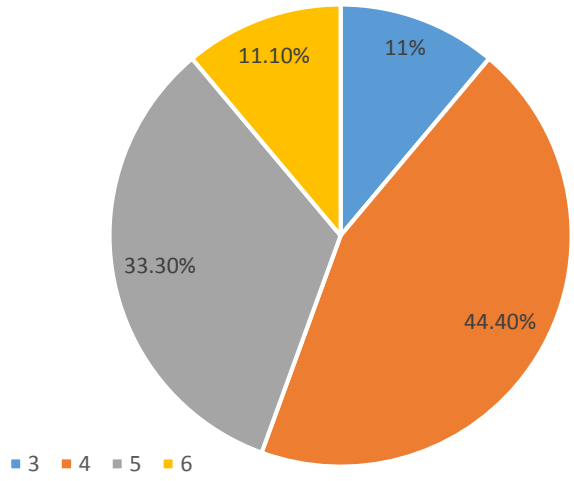

Figure 6: Percentage of **(A)** Gram Positive and **(B)** Gram Negative isolates with resistance to varying numbers of classes of antibiotics

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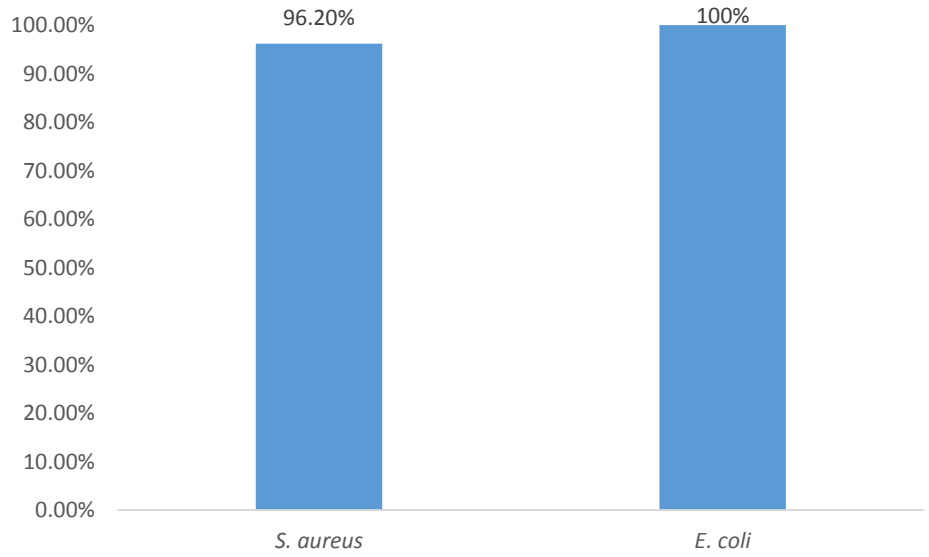

Figure 7: Occurrence of multidrug resistance in *Staphylococcus aureus* and *Escherichia coli*.

DISCUSSION

Currency has the potential to be a very successful fomite. With the rising rates of multidrug resistance, it is expected that currency also could serve as a potential source of MDR organisms. Sample processing protocol is a crucial element in assessing for this. This could be demonstrated by the total bacterial counts observed in this study (ranging from 10^4 to 10^5 CFU/ml) which were much higher than previously reported counts. The recent study by Ofoedu and colleagues reporting total bacterial counts in the 10^2 to 10^3 CFU/ml range, used the pre-incubation and a gentle shaking approach to dislodge bacteria into the test solution (Ofoedu et al., 2021). Similar results were also obtained by a 2017 study analyzing Naira, using a similar technique (Uko et al., 2017). A paper published on Ghanaian currency involved swabbing both sides of the currency note with a premoistened swab and extraction of the bacteria into 1 ml of sterile peptone water. This method expectedly reported bacterial counts in the 10^5 CFU/ml range. The hundred naira denomination had higher numbers represented in the higher counts. This differs from a number of other studies where higher bacterial load was associated with lower denominations (Yar, 2020; Badvi et al., 2017; Djouadi et al., 2020). These findings are probably a reflection of the variation in composition of the currency notes. The hundred naira is made from paper, while the fifty naira is polymer (Uko et al., 2017). Polymer has been found to be more resistant to bacterial contamination than paper (Vriesekoop et al., 2010).

Despite being the two most commonly isolated bacteria in diagnostic microbiology laboratories, reports on the prevalence of *E. coli* and *S. aureus* on different currencies around the world have observed variations in their occurrence. Some of these gave reports unlike this study with *E. coli* more commonly occurring with rates ranging from 28.23% to 100% (Yar, 2020; Hiko et al., 2016); Uko et al., 2017). Studies from Uganda and Algeria with similar results to this study reported low level of occurrences of *E. coli* among sampled currencies (Djouadi et al., 2020; Allan et al., 2018). Considering that *Staphylococcus aureus* as well as the coagulase negative Staphylococci are normal flora of both the skin and nasal cavity, they are expected to have higher occurrences. *E. coli* is more commonly identified as a normal flora of the gastrointestinal tract as well as soil. Therefore, its occurrence on currency denominations might point at faecal contamination or manhandling of the currency.

High levels of drug resistance have been reported in bacteria from various currencies but few have reported levels as high as observed in this study.

The exceptions to high level of resistance rates noted for most antibiotics was to gentamicin and ofloxacin. Isolates in this study were associated with low level resistance rates against these two antibiotics. This observation, with low levels of less than 30%, was also noted by many studies (Aminu and Yahaya, 2018; Akoachrere et al., 2014; Akond et al., 2015). The Iranian study by Firoozeh and colleagues in 2017 reported resistance rates of less than 50% for only gentamicin and ciprofloxacin out of the 10 antibiotics tested apart from vancomycin (Firoozeh et al., 2017). The potential toxicity associated with gentamicin and its controlled use might explain why these number of studies reported higher levels of sensitivity to gentamicin. Similarly, the fluoroquinolones which contain ofloxacin and ciprofloxacin are usually reserved for very severe bacterial infections. Hence, the levels of resistance here might be a reflection on the rate of use.

The extremely high MDR levels associated with currency notes in this study has not been widely described (Demirci et al., 2020). This is worrisome considering the important role both *S. aureus* and *E. coli* play as infectious agents. Additionally, this high level of multidrug resistance in commensal isolates would also be problematic as it points at a probable high level misuse of antibiotics in non-clinical settings.

CONCLUSION

The findings of this study, which associates the Nigerian currency, Naira, with a variety of drug resistant bacteria and high levels of multidrug resistance in key pathogens, underscores the need for continuous hand washing as the first key step in disease prevention. Since the use of currency cannot be eradicated and few avenues exist to sterilize currency already in circulation, mitigating potential role of these as fomites could focus on reducing contamination of the notes and preventing possible transmission.

Significance Statement

Results of this study showing high levels of multi drug resistant highlights the necessity for excellent hand-hygiene considering the frequent handling of currency generally, and suggest a potential negative impact on the health of the population.

Statements and Declarations

Competing Interests

All authors declare that they have no conflict of interest.

Ethical Statement

This article does not involve human participants or animals.

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