Antibiogram of the predominant bacterial contaminants of Nigerian currency notes in circulation in Ogun State, Nigeria.

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Abstract
Currency notes can act as a vehicle for the transmission of pathogenic organisms. This study was carried out to determine the antibiotic susceptibility patterns of the predominant bacterial contaminants of Nigerian currency notes in circulation in parts of Ogun State. A total of 240 naira notes of 8 different denominations were collected from various persons into sterile polythene bags, transferred into universal bottles containing 10 mL of sterile buffered peptone water. The notes were removed; the resulting solution incubated overnight and the overnight solution inoculated onto Blood agar, Mannitol salt agar, Eosin Methylene Blue agar and MacConkey agar plates and incubated at 37^0C for 24 hours. The isolates were then identified by Gram reactions and Biochemical tests and their susceptibility profiles against 8 commonly used antibiotics were determined. A total of 95 out of the 240 samples showed bacterial contamination and the prevalent isolates include: *Escherichia coli* 31 (32.6 %), *Salmonella typhi* 26 (27.4 %), *Staphylococcus aureus* 20 (21.1 %) and *Bacillus subtilis* 18 (18.9 %) respectively. There was no bacteria growth on the control samples. 52.3 % of the isolates were resistant strains. EC 1 isolate showed the highest susceptibility with an inhibitory zone diameter (IZD) of 25 mm against ciprofloxacin while on the whole, 26.6 % of the isolates were susceptible to all the antibiotics evaluated with 20.9 % showing intermediate susceptibility. This study showed that most of the circulating currency notes harbored one or more bacteria, especially the resistant strains which could pose a severe public health challenge.

Key words: Antibiogram, Bacteria contaminants, Currency notes, Ogun State.

Introduction

Naira, the official legal tender in Nigeria, became operational in 1973, replacing the Nigerian pounds and shillings. Over the years it has gone through a number of improvements to enhance its aesthetics, security as well as durability. Apart from Nigeria, the paper currency is equally used in the rest of the world (Sadawarte *et al*, 2014). The currency notes currently available for business transactions are: ₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500 and ₦1000. The first four currency denominations are made with polymers and the others are made of papers. These Nigerian currency notes are passed from hand to hand by different categories of individuals whose personal hygiene may be poor, thereby contaminating the notes with pathogenic organisms (Borah *et al*, 2012; Umeh *et al*, 2007). Thus, currency notes serve as a vehicle for the further transmission of these disease-causing agents, thereby constituting serious public health concerns **(**Kawo *et al*, 2009; Charnock, 2007; Xu *et al*, 2005). Earlier studies report that the lower denominations of the naira notes tend to harbor more of the pathogens than the higher ones (Barua *et al*, 2019; Khalil *et al*, 2014). It has also been said that the paper currency is impregnated with disinfectants of high potency to inhibit the colonization of these currency notes by pathogenic organisms, notwithstanding some disease-causing agents have been isolated from these notes in a number of countries (Hanash *et al*, 2015; Ahmed *et al*, 2010; Taro, 2005; Goktas and Oktay, 1992). These pathogenic agents include *Staphylococcus aureus*, *Enterococci sp*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Shigella sp*, *Salmonella sp*, *Mycobacterium tuberculosis*, *Escherichia coli*, *Klebsiella sp* among others (Hanash *et al*, 2015; Moosavy *et al*, 2013). Fungi are also one of the important groups of microorganisms isolated from the Nigerian currency notes: *Penicillium sp*, *Aspergillus fumigatus*, *Rhizopus sp* (Shahram *et al*, 2009; Barro *et al*, 2006). These isolates from the currency notes have been shown to carry antibiotic resistant strains; further compounding public health concerns (Firoozeh *et al*, 2017).

There is insufficient data on the probable microbial contaminants of Nigerian currency notes in Ogun Central and Ogun East Senatorial districts. This research therefore seeks to investigate the prevalent bacterial contaminants in Nigerian currency notes in circulation in the study area as well as to determine the antibiogram of these bacterial contaminants.

Materials and Methods

Study Area

This study was carried out in selected Local Government Areas (LGAs) in Ogun central senatorial district (Abeokuta North, Abeokuta South and Odeda) and some LGAs in Ogun East senatorial district (Ijebu Ode, Ikenne and Sagamu) of Ogun State in Nigeria. The climate in both districts is tropical with rainfall experienced in most months of the year and short dry season. Precipitation is about 77.6// per annum and average temperature of 26.1 ^{0}C (1970 mm) (Climate-Data.org. Retrieved10th June 2021). The inhabitants of these districts are predominantly farmers, traders, artisans and Civil Servants.

Sample collection

A total of 240 samples of Naira notes comprising 30 pieces each of the Naira denominations: ₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500 and ₦1000 were randomly collected from butchers, street beggars, filling station attendants, civil servants, water vendors and students into appropriately labeled sterile polythene bags between June and August, 2018 and transported to the Pharmaceutical Microbiology Laboratory of Olabisi Onabanjo University. Currency notes in mint condition, not yet in circulation, freshly obtained from the Central Bank of Nigeria (CBN) were used as control samples.

Bacterial Isolation

The naira notes were individually transferred from the polythene bags using sterile forceps under aseptic conditions into appropriately labeled individual universal bottles containing 10 mL of sterile buffered peptone water. The universal bottles were shaken vigorously for 1 minute so as to dislodge substantial microbes on the surface of the paper currency. The paper notes were then removed and the resulting solution was incubated at 37 ^{0}C for 24 hours. This incubated sample was then inoculated onto Blood agar (BA), MacConkey agar (MCA), Mannitol salt agar (MSA) and Eosin Methylene blue agar (EMB) plates, and incubated for 24 hours at 37 ^{0}C. The control samples were similarly treated.

Identification of Isolates

The discreet colonies obtained were grouped into Gram positive and Gram negative bacteria by Gram staining reactions following the method of Okore with a little modification (Okore, 2008). Briefly, a smear of the bacterial cells were prepared on clean glass slide and thereafter heat fixed. This smear was then flooded with a basic dye called Gentian violet (primary dye) and then allowed to stand for 60 seconds. The dye was washed off gently with water and an aqueous solution of iodine was applied for 60 seconds, which acts as a mordant to help bind the primary dye to the cells. The stained cells were then washed with 95 % ethanol for 30 seconds and rinsed with water for few seconds. The next stage was the application of Safranin, the counter stain, for about 60 seconds and rinsed with water for 5 seconds. Finally, it was blotted and examined under oil immersion.

Further authentication on the bacterial cells was done by carrying out Biochemical tests such as the catalase test, coagulase test, indole test, citrate utilization test, oxidase test according to Esimone *et al*, (2010); Willey *et al*, (2008).

1. **Catalase test:** This test was intended to differentiate Streptococcus (-) from Staphylococcus (+) as well as Bacillus (+) from Clostridium (-). A discreet colony was transferred into a clean glass slide and 1 drop of 3% H_2O_2 (Hydrogen peroxide) was added into it. The presence of bubbles due to the conversion of H_2O_2 to H_2O and O_2 indicates the presence of catalase.
2. **Coagulase test**: This test was important for this study in order to differentiate *Staphylococcus aureus* (+) from *Staphylococcus epidermidis* (-). Two drops of normal saline was dispensed onto a clean glass slide. The two drops of normal saline was then emulsified with the bacterial using inoculating loop. Next, a drop of plasma was added to the smear on the glass slide and rocked gently for about 15 seconds. A positive test would show clumping within 10-15 seconds.
3. **Indole test**: This test was intended to separate *Escherichia coli* (MR+, VP-, indole +) from *Enterobacter* (MR-, VP+, indole -) and *Klebsiella pneumonia* (MR-, VP+, indole-). Pure bacterial culture was inoculated into a tube containing peptone water and incubated for 24 hours. Thereafter, 5 drops of Kovac's reagent was added to the culture. A positive result is inferred by a red color in the surface layer of the culture and a negative result appears yellow.
4. **Oxidase test**: This test helps to distinguish *Neisseria* and *Moraxella spp.* (+) from Acinetobacter (-) and, enterics (all -) from pseudomonads (+). Briefly, 2 drops of 1 % oxidase reagents was added on a piece of filter paper on a clean glass slide. With the aid of inoculating loop, the bacterial cell was aseptically transferred onto the filter paper on the glass slide and emulsified with the reagent. A positive reaction turns the bacteria violet and negative reaction remains colorless within 30 seconds.
5. **Citrate utilization test**: This test was necessary in order to identify many of the enteric bacteria such as: Klebsiella (+), Enterobacter (+), Salmonella (+), Escherichia (-), Edwardsiella (-). Briefly, discreet bacterial colony of not more than 24 hours old was inoculated onto Simmons citrate agar and allowed to incubate at 37 ^{0}C for 7 days. Growth on the medium and sometimes with color change from green to blue is positive for citrate utilization and no growth signifies negative citrate utilization.

Antimicrobial Susceptibility Testing

This was carried out using the procedure according to Ngwai and coworkers with a little modification (Ngwai *et al*, 2010). Briefly, discreet bacterial colonies from nutrient agar slants were aseptically transferred into tubes containing Mueller Hinton Broth (MHB) and incubated at 37 ^{0}C for 24 hours. The bacterial suspensions were then standardized to 0.5 McFarland's standard and the entire surface of Mueller Hinton agar (MHA) plates were streaked with the suspension and then allowed to dry. The multiple discs (Rapid lab) containing ceftriaxone (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ampicillin (10 µg), ofloxacin (5 µg), augmentin (30 µg), nitrofurantoin (300 µg) and ciprofloxacin (5 µg) were placed aseptically onto the MHA plates, allowed 10 minutes for pre-diffusion and then incubated in inverted position at 37 ^{0}C for 24 hours. The inhibitory Zone diameters were measured in millimeter (mm) and then classified as Susceptible (S), Intermediate (I) and Resistant (R) based on the interpretative criteria provided

by the Clinical and Laboratory Standards Institute (CLSI, 2017).

Statistical Analysis

The generated data were analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0 by one way analysis of variance (ANOVA) and significance taken at $p<0.05$

Results

Table 1: Bacterial Isolates from the Different Currency Notes

Table 1: Shows the number of bacteria contaminants isolated from each of the denomination of the Naira notes evaluated.

Isolates	Denominations (₦)							
	5	**10**	**20**	**50**	**100**	**200**	**500**	**1000**
Escherichia coli	2	2	1	3	5	6	5	7
Staphylococcus aureus	1	0	0	3	4	5	3	4
Salmonella typhi	1	3	2	1	4	5	5	5
Bacillus sp.	0	2	0	1	4	3	4	4

Table 2: Isolation Rate for the Isolates Obtained

Notes Sampled (n)	Total Isolates (t)	Isolation Rate (t/n * 100)
240	95	39.6

Table 2 shows the isolation rate of the bacterial contaminants isolated from the currency note. We can observe from the table the relatively low isolation rate of 39.6 %.

Table 3: Biochemical Tests and Gram Staining Reaction Outcomes for the Isolates

Gram staining	Catalase test	Coagulase test	Indole test	Oxidase test	Citrate utilization test
			Escherichia coli		
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
-	-	-	+	-	-
			Staphylococcus aureus		
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-

+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
+	+	+	-	-	-
			Salmonella typhi		
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
-	-	-	-	-	+
			Bacillus sp.		
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+
+	+	-	-	-	+

Key: +: Positive, **-:** Negative

This table shows the staining reaction for the isolates, that is, whether Gram positive (*Staphylococcus aureus*, *Bacillus sp.*) or Gram negative (*Escherichia coli*, *Salmonella typhi*) and their biochemical reaction outcome to further narrow down on the genus and/ or species of the isolates.

Table 4: Antibiogram of the Isolates

Inhibitory Zone diameters (mm)																								
CAZ			CRX			GEN			AMP			OFL			AUG			NIT			CPR			
S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	
Escherichia coli																								
		0			0	20				15		23					10			10	25			
		8			0	15					0		15				0			0		20		
		0			0	20					0	23					0			0	24			
		10			8	19				14		20			18			20				19		
		0			0	15				15				0			10	19				20		
		13		17		20			17			19			18			22			21			
		7			0			0			0	17			20			15				17		
		10	19					10	20			19				16		20				20		
		15			0	18				15		20			18			20			21			
		15			10	20				16		19					10	21				20		
		12			12	19				15		20				16		19				19		
	18				12	22			18			21				15		19			22			
		16		15		19					0	19					13	19				19		
		7		16		19					0	18				14		20				20		
		10		15		21					0	19				14		18			21			
		10			13	20					12	20			19			21				19		
		15	20			20				14		21					9	21				20		
		10	22			21					12			0			5	20			21			
		17	19			20			20					6			0	18				19		
		0		17		19			18					9			5	19				20		
		0			9	19			19					12			0	17						0
		9			10	15					5	19					0		16					5
	19				11	19					8	21					10		15					14
		15		15		21					13	20				14				0				15
		12		15		19					9			5		14				9		19		
		14	18			19				15				0			13			3		20		
		5			0			0			13			9	19					9				5
		9			7	15				15				6	20					6				0
		16			0			0			6	19			21			19						12
	19				0			0			6	19			22			21						0
	19		15			21					8	20			6			18						5
Staphylococcus aureus																								
	19		20					10			0	20					10			0		19		
	20		18					9			0	19					10			10		19		
	18		19					4			5	19					8			12	21			
		17	19					12			13	20					3			13	21			
	19		20				13				10	20					12	19				20		
	20		18				13				9	18					0		15			20		
	18		18			15					13	17					9	20				19		
	19			17				0			0		13				0			6	21			
	20			15				0			3			12			0			3		20		
		17		16				0			13		13				0			0		20		
		17			9			7			12	19					5			6		19		
	19				6	16					0			0			8			0				15
	20		21					9			11			3			9			2				14
	20				6			6			7			5			12			0				14
		17	18					9			7	19				15				10		18		
	19		19			17					5	22					12	18				19		
	19		20				14				0	20					10	19				20		
		7	19					0			2	20				15		19				19		
		15	18					3		14				12			13	21				19		
		16		15				5			12			0		15			15			17		
Salmonella typhi																								
	19				0			10			10	19					4			10		20		
	18				0	15					0	20					6			10		19		
	19				0	17					0	17					10			6	21			
		15			13	17					0		15				8			12		19		
		0		16		19					0			0			2			12		18		
		0	19			19					6			0			0			9		19		
		17			3	19					5			5			0			6		20		
		17			9			12			3	20					0			5		17		
	19		19					12			8	19					12			0		19		
		16	18					3			4	19					13			0				12
		17	21					3			0	21					11			7		19		

	15		15				5	5	17				9		13		20	
18		18					4	0			5		7	15				0
18			17				0	6	18				10	15				5
	9		17				0	10	19				3		12		19	
	9	18					0	0	19				0		9	21		
	8		17				0	8	20				0		3		19	
	5	19					0	10	21				4		2		19	
19		19					0	8	19				5		6		20	
18			16				9	9		15			5		9			15
	4		17		16			9		14			6		9			15
20			17		19			13	20				9		5		20	
	12			0			12	0			12	14			14		18	
19				4	18			11			0	14			14	21		
	8		15				2	10			0		6		12		19	
	16		16			14		0	21				3		8		20	
								Bacillus sp.										
	10			10	20			0	20				10		12		19	
	15			12	21			0	18				12		10		20	
	12			12	19			0	17			15			9		20	
	15			11	21			5	17			14			6	21		
	12			12	20			12	21				13		0		18	
18				0	19			13	19				12		0		18	
	16			0	19			6	19				11		13	21		
	15			0	17			9			0		13		5			15
	0			6	16			3			6	16			0			15
	0			7	18			2	19			16			0			13
	0			0	19			0		15			0		13	21		
	6			12	20			0		15			0		12		19	
	13			12	19			9		15			12	15			17	
	14			0	21			10		13			0		13		20	
	13			8	21			6	16				0		13		20	
	14		13		20			8		15			5		0	21		
	6	11				13		12	19				13		9			12
	9	10			15			3	18				12		8		19	

Key: CAZ: Ceftaxidine, **CRX**: Cefuroxime, **GEN:** Gentamicin, **AMP**: Ampicillin, **OFL:** Ofloxacin, **AUG**: Augmentin, **NIT:** Nitrofurantoin, **CPR**: Ciprofloxacin

Discussion

This study showed the presence of various bacterial contaminants on the naira notes in circulation in our study area. The specific microorganisms identified after subjecting the isolates to Gram staining reaction and further authentication by various biochemical tests (Table 3) include *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus sp.;* the first two being Gram negative bacteria and the last two Gram positive and the Gram negative bacteria isolates seem to be more than the Gram positive (Gram negative 57[60 %] and Gram positive 38 [40 %]) (Table 3). This agrees with earlier studies that microbes are usually isolated from currency notes (Kawo *et al*, 2009; Xu *et al*, 2005). The reasons for this microbial colonization of the currency notes had been giving to be due to the poor handling by various users with different levels of hygienic practices, which over time overwhelms the strong disinfectants impregnated in the currency notes (Hanash *et al*, 2015; Goktas and Oktay, 1992). It was further observed that *E. coli* constitutes the greatest bioburden among the bacterial isolated (Table 1). This once again is in agreement with earlier researchers (Moosavy *et al*, 2013; Kawo *et al*, 2009; Tagoe *et al*, 2009). The reason for the predominant *E. coli* isolates could be that there was faecal-currency notes communication among a sizeable number of the people whose currency notes were sampled. The study showed a relatively low isolation rate of 39.6 % (Table 2). This is at variance with earlier studies (Kawo *et al*, 2009; Tagoe *et al*, 2009). We also observed in this study that the lower denomination currency notes (that is; ₦5, ₦10, ₦20, ₦50) were the least colonized by bacterial contaminants compared with the higher denominations (that is; ₦100, ₦200, ₦500 and ₦1000) (Table 1). This again is at variance with earlier researches (Ayandele and Adeniyi, 2011; Umeh *et al*, 2007). The reason for this could be the nature of the notes made with polymers, which does not allow a firm grip by the bacterial contaminants and the population of the contaminants can easily be reduced by mere accidental water washing of the notes. Its poor moisture retention capacity, which does not encourage the multiplication of the bacterial

contaminants, could also be given for this observation. The isolates showed varying degree of responses against the eight commonly used antibiotics (Table 4). The EC 1 isolates showed the highest susceptibility with an IZD of 25 mm against ciprofloxacin (Table 4). 52.3 % of the isolates were resistant strains and this may pose a severe public health concerns.

Conclusion

This study showed that most of the circulating currency notes in Ogun-East and Ogun-Central Senatorial districts of Ogun State, Nigeria harbor one or more bacteria species, especially the resistant strains which could pose severe public health challenge if measures are not taken to mitigate this trend.

Recommendations

The populace in these districts sampled and even beyond should be encouraged to maintain proper hygiene and to handle the currency notes with clean hands and in wallets rather than in back pockets or braziers to prevent the colonization of the currency notes by pathogenic organisms. The currency notes made of polymer showed very low bacterial colonization and so the Central Banks are therefore encouraged to use polymers only in the minting of currency notes. The outcome of this research could serve as an interesting template for further studies.

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