

Antibiotic Resistance and Public Health Perspective of Bacterial Contamination of Nigerian Currency

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Abstract

Nigerian bank notes like other currencies in the world pass through different hands and have been implicated in the carriage of medically important pathogens. This study was undertaken to determine the types of bacteria present on Nigerian currency in Ile-Ife, Osun State, with a view to determining the potential of naira notes as environmental vehicle for the transmission of potential pathogenic bacteria. Standard microbiological methods were used to isolate, characterize and identify the bacterial isolates from different banknotes. Disc diffusion method was used to determine the sensitivity of the isolates to ten antibiotics. Representative multiple antibiotic resistant isolates were profiled for plasmid deoxyribonucleic acid (DNA). A total of three hundred and five comprising 216 Gram positive and 89 Gram negative bacteria were isolated from two hundred and five bank notes sampled; naira in mint condition was used as control. Thirteen bacterial genera namely *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Lactobacillus*, *Micrococcus*, *Aeromonas*, *Citrobacter*, *Edwardsiella*, *Klebsiella*, *Moraxiella*, *Pseudomonas*, *Enterobacter* and *Proteus* were recovered. Resistance to antibiotics was generally high and varied among the isolates. Resistance ranged from 18.18% to 100% in Gram negative bacteria and 26.6% to 100% in Gram positive isolates. *Enterobacter* sp. and *Edwardsiella* sp. recovered were 100% resistant to all the antibiotics except septrin. All the isolates were resistant to more than one antibiotic. Multiple antibiotic resistant isolates harboured plasmids of various sizes, ranging from 1,356 bp to 17,367 bp in Gram- positive bacteria and 1815 to 23130 bp in Gram negative isolates. Nigerian currency harboured different types of multiple antibiotic resistant bacteria which can be implicated in human infections; hence constituting a potential public health hazard.

Keywords: bacteria, multiple antibiotic resistance, currency, plasmids, pathogens, mint

Introduction

Contaminated currency is a potential public health hazard due to its high circulation among man, hence facilitates dissemination of pathogens to susceptible hosts. Money, being a fomite can be contaminated by different microorganisms associated with unclean hands or dirty environment and therefore present high risk to public health (Shakir *et al.*, 2010; Ngwai *et al.*, 2011; Yazah *et al.*, 2012; Neel, 2012; Gedik *et al.*, 2013). Confirmation of contamination of money by drugs has been detected in the United States and United Kingdom (Ritter, 1997; Jenkins, 2001, Thompson, 2002). Contamination from the skin, anal region, wounds, nasal secretions and aerosols generated by sneezing and coughing are potential sources of transfer of microorganisms to currency notes during handling (Mackintosh and Hoffman, 1984). Unhygienic practices among money handlers such as keeping in bra (women), handling after attending to patients with unwashed hands (physicians and medical personnel), and money transactions with hands contaminated with body fluids (commercial sex workers) do not only deface but contaminate the currency.

Bacteria causing different infections like pneumonia, tonsillitis, peptic ulcers, urino-genital tract infection had been reportedly associated with money (Pope *et al.*, 2002; Pal *et al.*, 2013).

A study by Hosen *et al.* (2002) in Bangladesh revealed coliform contamination of 80% of thirty old two-taka notes, Pope *et al.* (2002), isolated pathogenic or potentially pathogenic organisms from 94% of one-dollar bills and Basavarajappa *et al.* (2005) found 96 out of 100 currencies contaminated with bacteria, fungal and protozoa.

Contamination of objects by pathogenic microorganisms is of much public health concern as contaminated materials can be source of transmitting pathogens (Gedik *et al.*, 2013). Contaminated bank notes therefore remain a potential risk to public health, since communicable diseases can spread through contact with fomites. The study evaluates the level of bacterial contaminants of Nigerian bank notes and determines their antibiograms and the presence of transmissible plasmid deoxyribonucleic acid (DNA) in multiple antibiotic-resistant (MAR) bacterial contaminants.

2.0 Materials and Methods

2.1 Survey and Collection of samples

Nigerian banknotes were collected from bus passengers, bus conductors, food vendors, Ile-Ife Markets (Oja Ife and Oja-Tuntun) and students and staff of Obafemi Awolowo University, Ile-Ife, Nigeria. The samples were

collected randomly and put in sterile plastic containers with lids. They were immediately transferred to the Department of Microbiology Laboratory at Obafemi Awolowo University, Ile-Ife, Nigeria, where all microbiological analysis was carried-out.

2.2 Isolation of bacterial isolates

The surface of the naira note was swabbed with saline moistened swab stick and spread on nutrient agar plates, incubated at 37°C for 24h. Pure culture of the isolates was identified based on morphological characteristics and standard biochemical tests (Cheesbrough, 2000).

2.3 Antibiotic sensitivity

Antibiotic susceptibility of the isolates was done by the antibiotic disc diffusion method. The disc containing the antibiotics (Fondisk, Lagos, Nigeria) namely amoxicillin (25 µg), ampiclox (10 µg), ceftriaxone (30 µg), cefuroxime (30 µg), ciprofloxacin (10 µg), erythromycin (5µg), gentamycin (10 µg), pefloxacin (5µg), septrin (30µg) and streptomycin (5 µg) were firmly placed on Mueller Hinton agar (HIMEDIA lab. Ltd Vadhani) plates previously seeded with standardized inoculums (10⁷ CFU/ml). The plates were incubated at 37°C for 24 h and the diameter of the zone of inhibition measured, and interpreted according to the guidelines of Clinical Laboratory Standards Institute (2006)

2.4 Plasmid analysis

Plasmid DNA extraction was carried out on the representative MAR isolates using TENS buffer (Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5% (Sigma products)) and separated by 0.8% (w/v) agarose gel in Tris-acetate-EDTA buffer containing ethidium bromide (20 ml of 50 X TAE and 6.0 µl of 10 µg/ml ethidium bromide per litre). Hind III DNA marker (Biomerx Lab, Hamburg, Germany) was used as control. Plasmid DNA fragments were visualized by UV light illuminator and photographed with a Leicaflex SL-camera.

3.0 RESULTS

A total of three hundred and five comprising 216 Gram positive and 89 Gram negative bacteria were isolated from two hundred and five banknotes sampled. Twenty of the bank notes and the naira samples in mint condition had no bacterial growth. Thirteen bacterial genera namely *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Lactobacillus*, *Micrococcus*, *Aeromonas*, *Citrobacter*, *Edwardsiella*, *Klebsiella*, *Moraxiella*, *Pseudomonas*, *Enterobacter* and *Proteus* were isolated with *Bacillus* (64.59%) appearing the most frequent (p>0.05) (Table 1).

Table 1. Frequency and percentage occurrence of isolates from naira notes

Isolates	Frequency	Percentage (%)
1. <i>Bacillus badius</i>	17	5.57
2. <i>Staphylococcus epidermidis</i>	2	0.66
3. <i>Bacillus polymyxa</i>	12	3.93
4. <i>Bacillus pumilus</i>	34	11.15
5. <i>Corynebacterium kutscheri</i>	6	1.97
6. <i>Corynebacterium hofmannii</i>	1	0.33
7. <i>Bacillus megaterium</i>	24	7.88
8. <i>Bacillus alvei</i>	15	4.91
9. <i>Bacillus subtilis</i>	46	15.08
10. <i>Bacillus coagulans</i>	47	15.41
11. <i>Staphylococcus aureus</i>	3	0.98
12. <i>Lactobacillus delbrueckii</i>	2	0.66
13. <i>Bacillus marinus</i>	1	0.33
14. <i>Bacillus circulans</i>	1	0.33
15. <i>Staphylococcus saprophyticus</i>	3	0.98
16. <i>Lactobacillus casei</i>	1	0.33
17. <i>Micrococcus luteus</i>	1	0.33
18. <i>Aeromonas hydrophilia</i>	2	0.66
19. <i>Pseudomonas</i> sp.	2	0.66
20. <i>Proteus mirabilis</i>	19	6.23
21. <i>Proteus penneri</i>	1	0.33
22. <i>Proteus vulgaris</i>	5	1.64
23. <i>Klebsiella pneumoniae</i>	11	3.61
24. <i>Aeromonas sobria</i>	11	3.61
25. <i>Citrobacter freundii</i>	14	4.59
26. <i>Enterobacter</i> sp.	1	0.33
27. <i>Citrobacter diversus</i>	4	1.31
28. <i>Edwardsiella tarda</i>	1	0.33
29. <i>Enterobacter aerogenes</i>	11	3.61
30. <i>Moraxella catarrhalis</i>	3	0.98
31. <i>Klebsiella oxytoca</i>	4	1.31
Total	305	100%

Tables 2a and b show the percentage antibiotic resistance of Gram-positive and Gram-negative isolates from naira notes, respectively. Resistance to antibiotics was generally high and varied among the isolates. Resistance ranged from 26.6 to 100% in Gram positive bacteria and was mostly against ceftriaxone as over 90% was resistant to this antibiotic. *Corynebacterium* sp. and *Bacillus circulans* were resistant to all the antibiotics except septrin and pefloxacin, respectively (Table 2a). Resistance to septrin by the isolates was low compared to other antibiotics ($P>0.05$).

Resistance to antibiotics ranged from 18.18 to 100% in Gram negative bacteria isolates (Table 2b). *Enterobacter* sp. and *Edwardsiella* sp. recovered were 100% resistant to all the antibiotics except septrin. Resistance ranged from 50 to 100% for chloramphenicol, 63.64 to 100% for sparfloxacin and 36.36 to 100% for ciprofloxacin. *Proteus penneri* was completely resistant to chloramphenicol, sparfloxacin and ciprofloxacin (Table 2b). Resistance to streptomycin, septrin and ofloxacin was low compared to other antibiotics ($p>0.05$).

Table 2. Percentage Antibiotic resistance of Gram-positive isolates from naira notes.

Organism (n)	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
1. <i>Bacillus alvei</i> , n = 15	40.00	66.67	93.33	93.33	86.67	100.0	93.33	26.67	80.00	100.0
2. <i>Bacillus badius</i> , n = 17	58.82	94.12	100	100	88.24	100.0	70.59	95.29	76.47	100.0
3. <i>Bacillus circulans</i> n = 1	0.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
4. <i>Bacillus congulans</i> , n = 47	40.43	78.72	93.62	80.85	74.47	100.0	57.45	25.53	48.94	91.49
5. <i>Bacillus marinus</i> n = 1	0.00	0.00	100.0	0.00	0.00	100.0	0.00	0.00	0.00	100.0
6. <i>Bacillus megaterium</i> , n = 24	66.67	83.33	87.50	83.33	83.33	91.67	50.00	41.67	75.00	87.50
7. <i>Bacillus polymyxa</i> n= 12	41.67	83.33	83.33	83.33	75.00	91.67	58.33	33.33	66.67	91.6
8. <i>Bacillus pumilus</i> , n = 34	52.74	85.29	91.18	100.0	88.24	97.06	67.65	41.18	73.53	94.12
9. <i>Bacillus subtilis</i> , n = 46	28.26	65.22	95.65	82.61	84.78	97.83	60.87	30.43	60.87	84.78
10. <i>Corynebacterium</i> spp. n = 6	33.33	83.33	100.0	83.33	100.0	100.0	83.33	50.00	66.67	100.0
11. <i>Corynebacterium hofmanni</i> n=1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.00	100.0	100.0
12. <i>Lactobacillus casei</i> n = 1	0.00	0.00	100.0	100.0	100.0	100.0	100.0	0.00	0.00	100.0
13. <i>Lactobacillus delbruekii</i> n= 2	50.00	50.00	100.0	100.0	100.0	100.0	50.00	50.00	100.0	100.0
14. <i>Micrococcus luteus</i> n = 1	0.00	100.0	100.0	100.0	0.00	100.0	0.00	0.00	0.00	100.0
15. <i>Staphylococcus aureus</i> n = 3	66.67	66.67	66.67	100.0	100.0	100.0	66.67	66.67	100.0	100.0
16. <i>Staphylococcus epidermidis</i> n=2	0.00	100.0	100.0	50.00	50.00	0.00	50.00	50.00	50.00	0.00
17. <i>Staphylococcus saprophyticus</i> , n=3	33.33	66.67	100.0	66.67	33.33	100.0	100.0	33.33	33.33	66.67
Overall N = 216	43.52	77.31	93.06	87.04	81.94	97.69	63.89	37.50	64.81	90.74

KEY

PEF-Pefloxacin (5µg) CN-Gentamycin (10µg) APX-Ampiclox (10µg) Z-Cefuroxime (30µg) AM-Amoxacillin (25µg) R-Ceftriaxone (30µg) CPX-Ciprofloxacin (10µg)
 S- Streptomycin (5µg) SXT-Septrin (30µg) E-Erythromycin (5µg) n-number of Isolations R- Resistance
 S- Susceptible

Table 2b. Percentage Antibiotic resistance of Gram-negative isolates from naira notes.

	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
1. <i>Aeromonas</i> sp. n = 2	100.00	100.00	100.00	100.0	100.00	100.0	100.00	50.00	50.00	100.0
2. <i>Aeromonas sobria</i> n = 11	45.45	90.91	100.00	72.73	72.73	54.55	81.82	27.27	36.36	63.64
3. <i>Citrobacter freundii</i> n = 14	64.29	92.86	92.86	57.14	78.57	85.71	90.86	35.7	50.00	78.57
4. <i>Citrobacter diversus</i> n = 4	75.00	100.00	100.00	75.00	100.00	75.00	100.00	50.00	50.00	75.00
5. <i>Edwardsiella tarda</i> n = 1	0.00	100.00	100.00	100.0	100.00	100.0	100.00	0.00	100.0	100.0
6. <i>Enterobacter</i> sp. n=1	0.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
7. <i>Enterobacter aerogenes</i> n= 11	36.36	90.91	100.00	72.73	72.73	63.64	100.00	18.18	27.27	63.64
8. <i>Klebsiella oxytoca</i> n = 4	25.00	50.00	100.00	50.00	50.00	100.0	100.00	25.00	25.00	50.00
9. <i>Klebsiella pneumoniae</i> n=11	36.36	63.64	63.64	36.36	63.64	72.73	81.82	18.18	27.27	63.64
10. <i>Moraxella catarrhalis</i> n = 3	100.0	100.0	66.67	100.0	100.00	100.0	100.00	100.0	66.67	66.67
11. <i>Proteus mirabilis</i> n= 19	52.63	89.47	89.47	57.89	78.95	89.47	94.74	21.05	21.05	57.89
12. <i>Proteus penneri</i> n = 1	0	100.00	100.00	100.0	0.00	0.00	0.00	0.00	0.00	0.00
13. <i>Proteus vulgaris</i> n =5	80.00	100.00	80.00	80.00	80.00	80.00	100.00	60.00	60.00	80.00
14. <i>Pseudomonas</i> sp. n=2	0.00	100.0	100.0	50.00	100.0	100.0	100.0	100.0	50.00	50.00
OVERALL N = 216	50.56	65.17	89.89	64.04	76.40	78.65	92.13	32.58	37.08	66.29

KEY:

SXT- Septrin (30 μ g) CH-Chloramphenicol (30 μ g) SP- Sparfloxacin (5 μ g)
 CPX- Ciprofloxacin (10 μ g) AM- Amoxicillin (25 μ g) AU- Augmentin (30 μ g)
 CN-Gentamycin (10 μ g) PEF- Pefloxacin (5 μ g) OFX- Ofloxacin (5 μ g)
 S- Streptomycin (10 μ g) n- number of Isolations

Multiple antibiotic resistance patterns of the Gram-positive and Gram-negative bacteria isolates from naira notes are shown in Tables 3a and b, respectively. All the Gram positive and Gram negative isolates from the naira notes were resistant to more than one antibiotic with various multiple antibiotic resistance patterns (MAR) patterns. Gram positive bacteria isolates displayed various MAR patterns with the highest number of patterns observed in *B. subtilis* (Table 3a). The MAR patterns exhibited by the Gram negative isolates ranged from 2 to 8 with *Proteus mirabilis* displaying the highest number of patterns (Table 3b).

Table 3a. Multiple antibiotic resistance (MAR) profile of Gram- positive bacteria from naira notes

Isolate (n)	Resistance pattern	Frequency	No of MAR pattern		
<i>Bacillus alvei</i> (15)	APX, Z, R, E	1	7		
	Z, AM, R, CPX, E	1			
	APX, Z, R, CPX, SXT, E	1			
	APX, Z, AM, R, CPX, SXT, E	3			
	CN, APX, Z, AM, R, CPX, SXT, E	2			
	PEF, CN, APX, Z, AM, R, CPX, SXT, E	5			
	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	2			
<i>Bacillus badius</i> (17)	APX,Z,R,E	1	7		
	CN,APX,Z,R,CPX,E	1			
	CN,APX,Z,AM, R ,CPX,E	1			
	CN,AMP,Z,AM,R,CPX,SXT,E	4			
	PEF,CN,APX,Z,AM ,R,SXT,E	4			
	PEF, CN, AMP, Z, AM, R, CPX, E	1			
	PEF,CN,AMP,Z,AM,R,CPX,S,SXT,E	5			
	CN, APX, Z, AM, R, CPX, S, SXT, E	1			
<i>Bacillus circulans</i> (1)	CN, APX, R	2	1		
	APX, Z, R, E	5			
<i>Bacillus coagulans</i> (47)	PEF, APX, Z, R, E	7	8		
	CN, APX, Z, R, CPX, E	6			
	PEF, CN, Z, AM, R, CPX, E	6			
	CN, APX, Z, AM, R, CPX, SXT, E	6			
	PEF, CN, APX, Z, AM, R, CPX, SXT, E	5			
	PEF, CN, APX, Z, AM, R, CPX, S,SXT, E	10			
	APX, Z, AM, R, CPX, SXT, E	1			
	<i>Bacillus marinus</i> (1)	APX, R, E		1	1
		PEF, R, E		2	
	<i>Bacillus megaterium</i> (24)	APX, AM, R, SXT		1	8
		Z, AM, R, SXT, E		4	
CN, APX, Z, AM, R, S		1			
PEF, CN, APX, AM, R, SXT, E		4			
CN, APX, Z, AM, R, CPX, SXT, E		1			
PEF, CN, APX, Z, AM, R, CPZ, SXT, E		5			
PEF, CN, APX, Z, AM, R, CPX, S, SXT, E		6			
R, E		1			
PEF, CN, Z, R, CPX, E		2			
CN, APX, Z, AM, R, SXT, E		2			
<i>Bacillus polymyxa</i> (11)	CN, APX, Z, AM, R, CPX, SXT, E	1	6		
	PEF, CN, APX, Z, AM, R, CPX, SXT,E	3			
	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	2			
	PEF, Z	1			
	AM, Z, R, E	2			
	CN, APX, Z, R, E	1			
	CN, Z, AM, R, SXT, E	2			
	APX, Z, AM, R, CPX, S, SXT	7			
	PEF, CN, APX, Z, AM, R, CPX, E	6			
	PEF, CN, APX, Z, AM, R, CPX, SXT, E	6			
<i>Bacillus pumilus</i> (34)	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	9	8		
	APX, E	1			
	APX,AM,R	3			
	APX, R, CPX,Z	5			
	APX, AM, R, CPX	4			
	CN, APX, Z, R, E	4			
	APX,Z,AM,R,CPX,SXT,E	2			
	APX, CN, AM, Z, CPX, R,S	7			
	CN,APX, Z, AM, R, CPX, SXT, E	8			
	PEF CN, APX, Z, AM, R, CPX, SXT, E	5			
<i>Bacillus subtilis</i> (46)	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	7	10		
	APX, E	1			
	APX,AM,R	3			
	APX, R, CPX,Z	5			
	APX, AM, R, CPX	4			
	CN, APX, Z, R, E	4			
	APX,Z,AM,R,CPX,SXT,E	2			
	APX, CN, AM, Z, CPX, R,S	7			
	CN,APX, Z, AM, R, CPX, SXT, E	8			
	PEF CN, APX, Z, AM, R, CPX, SXT, E	5			
<i>Corynebacterium sp.</i> (6)	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	2	4		
	APX, AM, R, CPX, E	1			
	CN, APX, Z, AM, R, SXT, E	2			
	CN, APX, Z, AM, R, CPX, S, SXT, E	1			
<i>Corynebacterium hofmannii</i> (1)	PEF, CN, APX, Z, AM, R, CPX, SXT, E	1	1		
	CN, APX, Z, AM, R, SXT, E	1			
<i>Lactobacillus delbrueckii</i> (2)	PEF, APX, Z, AM, R, CPX, S, SXT, E	1	2		
	CN, APX, Z, R, E	1			
<i>Micrococcus luteus</i> (1)	CN, APX, Z, R, E	1	1		
<i>Staphylococcus aureus</i> (2)	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	1	2		
	PEF, CN, APX, Z, AM, R, S, SXT, E	1			
<i>Staphylococcus epidermidis</i> (2)	CN, APX, AM, R	1	2		
	CN, APX, Z, R, CPX, S, SXT	1			
<i>Staphylococcus saprophyticus</i> (3)	APX, R, CPX, C	1	3		
	CN, APX, Z, R, CPX	1			
	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	1			

Table 3b: Multiple antibiotic resistances (MAR) profile of Gram-negative organisms

Isolate (n)	Resistance pattern	Frequency	No of MAR pattern
<i>Aeromonas</i> sp. (2)	PEF, S, SXT, CH, CP, CPX, AM, AU, CN	1	2
	OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	
<i>Pseudomonas</i> sp. (2)	PEF, CH, SP, AM, AU, CN	1	2
	PEF, OFX, S, CH, SP, CPX, AM, AU, CN	1	
<i>Proteus mirabilis</i> (19)	SXT, CH, CN	1	8
	SP, AM, AU, CN	3	
	SXT, CH, SP, CPX, CN	2	
	S, SXT, CH, SP, AM, AU	4	
	PEF, CH, SP, CPX, AM, AU, CN	3	
	S, SXT, CH, SP, CPX, AM, AU, CN	2	
	OFX, S, SXT, CH, SP, CPX, AM, AU, CN	2	
	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	2	
	CH, SP, CPX	1	
<i>Proteus penneri</i> (1)	CH, CN	1	1
	CH, CN	1	
<i>Proteus vulgaris</i> (5)	S, SXT, CH, SP, CPX, AM, AU, CN	1	3
	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	3	
	PEF, SXT, AM	1	
	S, SP, AU, CN	3	
	CH, SP, CPX, AU, CN	2	
<i>Klebsiella pneumoniae</i> (10)	OFX, S, CH, SP, AM, AU, CN	2	6
	OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	
	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	
	CH, SP, CPX, AM	1	
	S, CH, SP, AM, CN	2	
	SXT, CH, SP, CPX, AU, CN	5	
	PEF, S, CH, SP, CPX, AM, AU, CN	1	
<i>Aeromonas sobria</i> (11)	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU	1	6
	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	
	SXT, CH, SP	1	
	PEF, CPX, AM, AU, CN	2	
	S, CH, SP, AM, AU, CN	1	
	OFX, CH, SP, CPXZ, AM, AU, CN	2	
<i>Citrobacter freundii</i> (14)	S, SXT, CH, SP, CPX, AM, AU, CN	5	7
	PEF, OFX, S, SXT, CH, SP, AM, AU, CN	1	
	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	2	
	PEF, OFX, S, CH, SP, CPX, AM, AU, CN	1	
	S, SXT, CH, SP, AM, AU, CN	2	
<i>Enterobacter</i> sp. (1)	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	1
	PEF, OFX, S, CH, SP, CPX, AM, AU, CN	1	
	S, SXT, CH, SP, AM, AU, CN	2	
	PEF, OFX, CH, SP, CPX, AM, AU, CN	1	
<i>Citrobacter diversus</i> (4)	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	3
	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	
	PEF, OFX, S, CH, SP, CPX, AM, AU, CN	1	
<i>Edwardsiella tarda</i> (1)	OFX, S, CH, SP, CPX, AM, AU, CN	1	1
	CH, SP, CPX, CN	2	
<i>Enterobacte aerogenes</i> (11)	CH, SP, CPX, CN	2	5
	PEF, CH, SP, CPX, AU, CN	2	
	S, SXT, CH, SP, CPX, AM, CN	1	
	PEF, OFX, S, CH, SP, AM, AU, CN	2	
	OFX, S, SXT, CH, SP, CPX, AM, AU, CN	2	
	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	
	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	
<i>Moraxella catarrhalis</i> (3)	PEF, OFX, S, SXT, CH, CPX, AM, AU, CN	1	3
	PEF, OFX, S, SXT, CH, CPX, AM, AU, CN	1	
	PEF, OFX, S, SXT, CH, SP, CPX, AM, AU, CN	1	
<i>Klebsiella oxytoca</i> (4)	SP, CPX, AM, AU, CN	1	4
	S, CH, SP, AM, AU, CN	1	
	S, SXT, CH, SP, AU, CN	1	
	PEF, OFX, SP, CPX, AU, CN	1	

The molecular weights (bp) of the plasmid DNA recovered from the representative MAR Gram positive (Figure 1) and Gram negative (Figure 2) bacterial isolates are shown in tables 4a and b, respectively. Gram positive bacterial isolates contained large size plasmid DNA of molecular weights ranging from 1,356 to 17,367 bp. *Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus alvei* and *Bacillus polymyxa* harboured multiple plasmid DNA (Table 4a).

Similarly, large size plasmid DNA of molecular weight 23130 bp was each harboured by the representative MAR Gram negative isolates namely, *Pseudomonas* sp, *Aeromonas sobria*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Enterobacter aerogenes*, *Citrobacter diversus*, *Citrobacter diversus* (Table 4b).

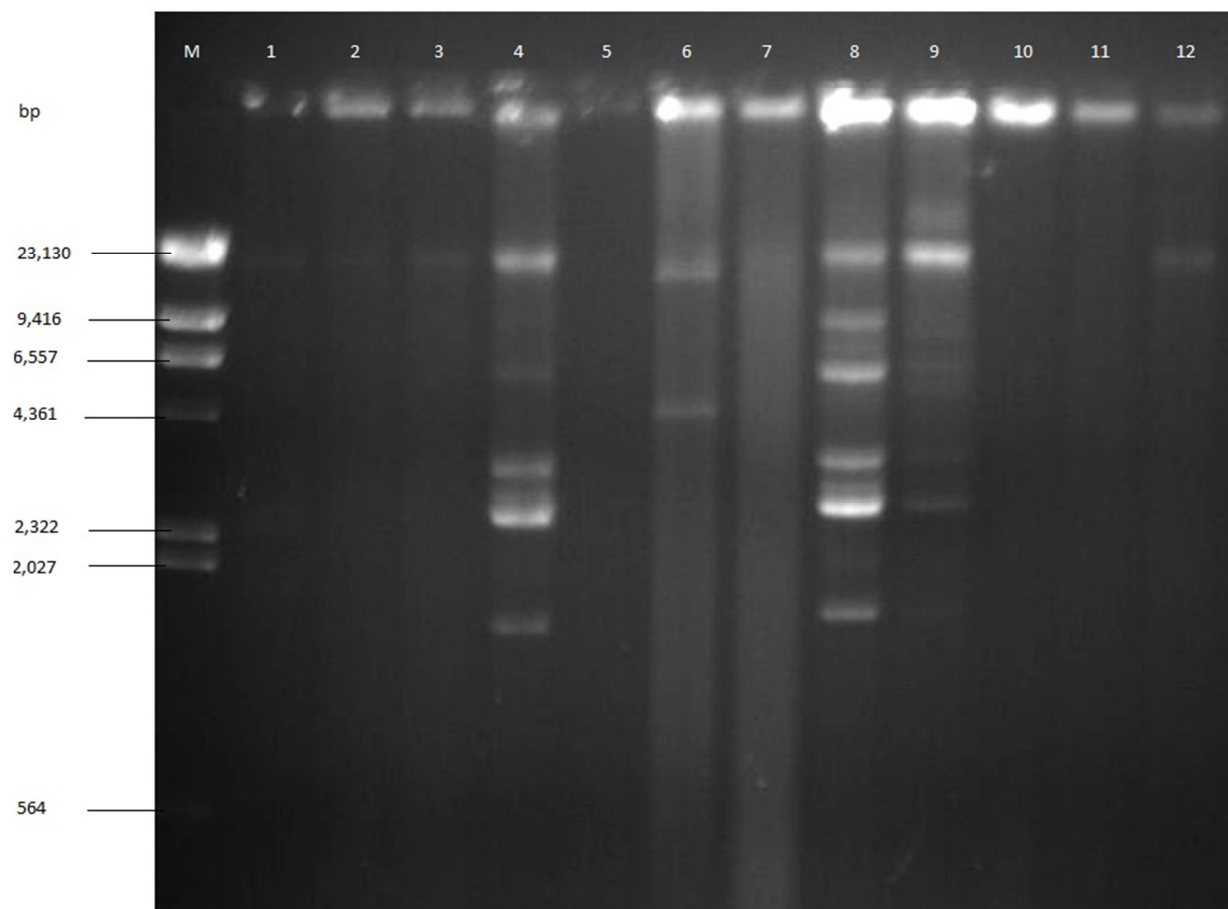


Figure 1. Gel electrophoresis plasmids results of Gram positive isolates

Key: M = HIND III digest of λ -DNA (DNA molecular weight marker).

Lane 1 = Sample 6 *Bacillus pumilus*, Lane 2 = Sample 35 *Bacillus badius*, Lane 3 = Sample 41 *Bacillus subtilis*, Lane 4 = Sample 44 *Bacillus megaterium*, Lane 5 = Sample 59 *Bacillus coagulans*, Lane 6 = Sample 73 *Bacillus polymyxa*, Lane 7 = Sample 87 *Corynebacterium kutscheri*, Lane 8 = Sample 203 *Staphylococcus aureus*, Lane 9 = Sample 220 *Bacillus alvei*, Lane 10 = Sample 264 *Staphylococcus saprophyticus*, Lane 11 = Sample 279 *Bacillus polymyxa*, Lane 12 = Sample 7 *Corynebacterium hofmannii*.

Table 4a: Molecular size (bp) of plasmid DNA in Gram positive bacterial isolates from naira notes

Isolate	Number of plasmid	Molecular size (bp)
<i>Bacillus pumilus</i>	0	–
<i>Bacillus badius</i>	0	–
<i>Bacillus subtilis</i>	1	12682
<i>Bacillus megaterium</i>	5	12682, 6307, 3607, 2925, 1356,
<i>Bacillus coagulans</i>	0	–
<i>Bacillus polymyxa</i>	2	11421, 5881
<i>Corynebacterium kutscheri</i>	0	–
<i>Staphylococcus aureus</i>	6	13600, 9261, 6763, 2925, 1506, 1356
<i>Bacillus alvei</i>	5	17367, 13600, 7004, 3868, 2925
<i>Staphylococcus saprophyticus</i>	0	–
<i>Bacillus polymyxa</i>	0	–
<i>Corynebacterium hofmannii</i>	1	1360

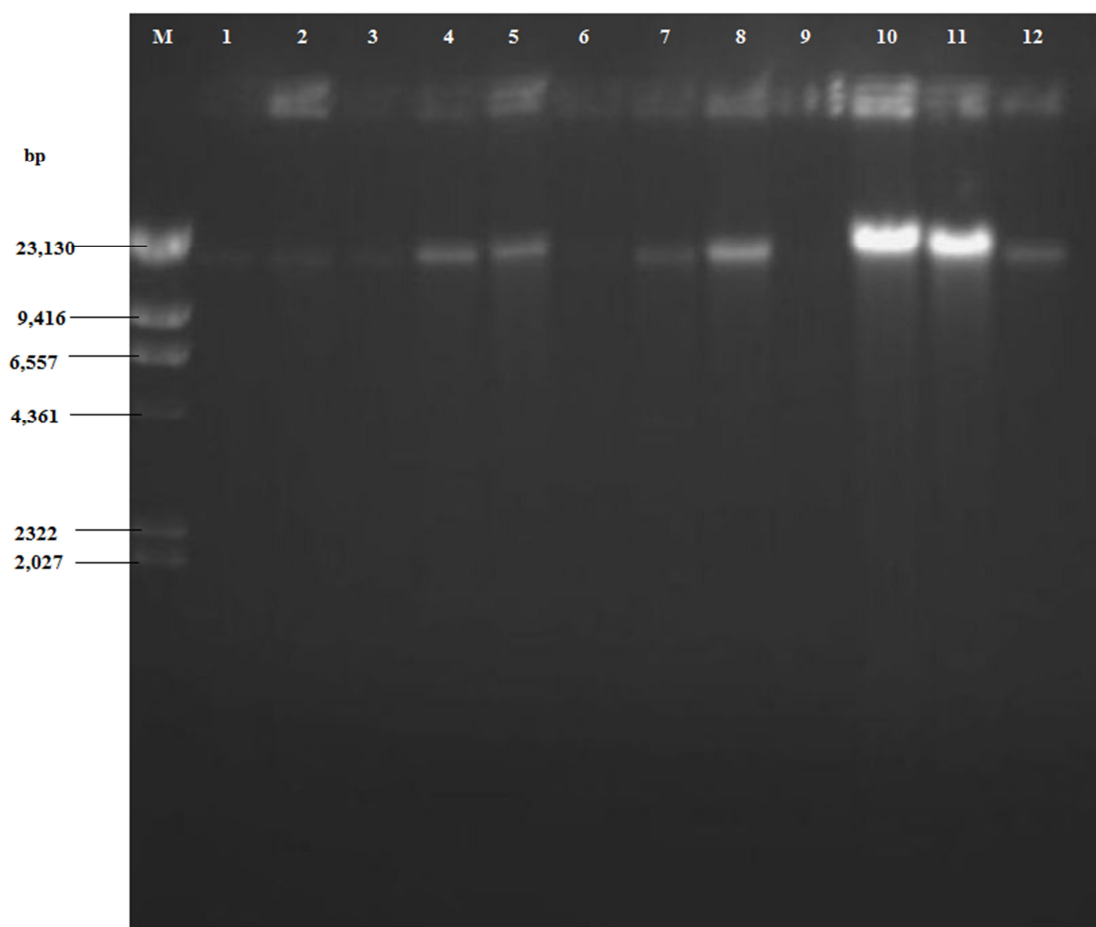


Figure 2. Gel electrophoresis plasmids result of Gram negative isolates

Key: M = HIND III digest of λ -DNA (DNA molecular weight marker).

Lane 1 = Sample 14 *Aeromonas hydrophilia*, Lane 2 = Sample 69 *Proteus vulgaris*, Lane 3 = Sample 78 *Citrobacter freundii*, Lane 4 = Sample 90 *Aeromonas sobria*, Lane 5 = Sample 162 *Klebsiella pneumonia*, Lane 6 = Sample 215 *Proteus vulgaris*, Lane 7 = Sample 246 *Moxarella catarrhalis*, Lane 8 = Sample 254 *Pseudomonas spp*, Lane 9 = Sample 259 *Proteus vulgaris*, Lane 10 = Sample 297 *Enterobacter, aerogenes*, Lane 11 = Sample 92 *Citrobacter diversus*, Lane 12 = Sample 92 *Citrobacter diversus*

Table 4b. Molecular size (bp) of plasmid DNA fragments in Gram-negative bacterial isolates from naira notes

Isolate	Number of plasmid	Molecular weight (bp)
<i>Aeromonas hydrophilia</i>	0	–
<i>Proteus vulgaris</i>	0	–
<i>Citrobacter freundii</i>	0	–
<i>Aeromonas sobria</i>	1	23130
<i>Klebsiella pneumoniae</i>	1	23130
<i>Proteus vulgaris</i>	0	-
<i>Moraxella catarrhalis</i>	1	23130
<i>Pseudomonas sp.</i>	1	23130
<i>Proteus vulgaris</i>	0	-
<i>Enterobacter aerogenes</i>	1	23130
<i>Citrobacter diversus</i>	1	23130
<i>Citrobacter diversus</i>	1	23130

4.0 Discussion

Money being one of the most circulated of inanimate objects which cut across all ages and all works of life; there is cause for alarm owing to the varieties and quality of bacteria recovered from the study. The study has shown

that naira notes are potential vector for the transmission of pathogenic and non pathogenic microorganisms. Various species of Genus *Bacillus* were frequently recovered. This supports the findings of Hadwen *et al.* (2003) which reported high occurrence of *Bacillus* sp. from currencies in their studies on the assessment of the public health risk associated with the simultaneous handling of food and money in the food industry-Central Goldfields Shire Council. These organisms have ability to produce spores and hence persist in the environments over a long period of time.

Other microorganisms with high incidences in the present study (*Proteus mirabilis*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Aeromonas sobria* and *Corynebacterium kutscheri*) may be implicated in nosocomial infections with possible cross transfer from hospital environment to the community, and vice versa. In this study, organisms namely *Lactobacillus casei* and *Lactobacillus delbrueckii* isolated are probiotics which are found in fermented milk products like cheese, would have contaminated the naira notes by unhygienic eating practices among some people in Ile-Ife, Nigeria. Though these organisms are not pathogenic, but *Lactobacillus casei* have been implicated in some cases of sepsis, meningitis and infections in some organs which also make it a potential threat to man. A few number of these organisms have been reported in earlier studies on currencies (Emikpe and Oyero, 2007; Umeh *et al.*, 2007; Abdulmoneim *et al.*, 2009; Rote *et al.*, 2010), and the climatic and environmental conditions of the tropics favor the thriving of many of these potential pathogenic microorganisms on surfaces, coupled with poor sanitation.

The high level of contamination of Nigerian currency notes is a potential threat to man because of the fact that money is continuously circulated hence the possibility of bacteria been transmitted as demonstrated by Hardy *et al.* (2006). In addition, the relative abundance of the resident or normal skin flora, as well as transient bacteria that may be found on the skin could enhance an easy transfer to inanimate objects like currency (Goktas and Oktay, 1992). The climatic and environmental conditions of the Tropics favour the thriving of many pathogenic microorganisms, and in the face of under-development, inadequate water and sanitation crowded living conditions, lack of access to good health care services, low populace, particularly the poor, become highly susceptible to infections and diseases (Anderson and May, 1991; Gwartkin, 2000). Risk of infection is increased several fold when objects that change hands at a high frequency, such as currency notes, are contaminated with microbes. The risk is by no means restricted to residents of the country in question; it might even be greater for expatriates, tourists, and visitors from other countries who may not be immune to the pathogens in their new environment (Janardan *et al.* 2009).

Resistance to antibiotics was generally high particularly with Gram positive bacteria. This may probably result from abuse of antibiotics, accessibility, cost, emergence and survival of resistant strains of microorganism

Bank notes have been reported to harbour antibiotic resistant pathogens (Umeh *et al.*, 2007; Awe *et al.*, 2010; Adegoke and Okoh, 2011; Ngwai *et al.*, 2011; Yazah *et al.*, 2012).

All the isolates on naira notes in the present study were multidrug resistant which pose a big challenge to human survival and continued existence in relation to bacterial infection and diseases. It has also being noted in the reports of Emikpe and Oyero, (2007) that treatment of infections caused by bacterial contaminants of currency may be very expensive and a death trap. The presence of antibiotic resistant human pathogens on paper money is highly consequential when contracted by the debilitated individuals. Most of the isolates are related to humans and the health risks associated with majority of these bacteria are well documented. Some of the enteric bacteria encountered in this study are opportunistic pathogens of man and have been associated with nosocomial infections (Ducel *et al.*, 2002; Prescott *et al.*, 2002), meanwhile very few are non-pathogenic to human being.

The reason for multidrug resistance in some of the bacteria could be attributed to the presence of plasmid DNA as observed in the present study. This gives an insight that these bacteria even if primarily not pathogenic could transfer the resistance factor to pathogenic ones (Scientific Correspondence, 2011; Saeed *et al.*, 2009; Aleshin and Levy, 2007; Walsh and Fanning, 2008).

The report of the presence of plasmid DNA in some of the multiple antibiotic resistant bacteria isolates on naira notes is unique in the study. The absence of plasmid DNA in some MAR bacteria may probably be that multi-drug resistance factor was chromosomally-linked.

The implication of the presence of similar plasmids of large sizes in some of the multiple antibiotic resistant isolates in the study is that resistance to these antibiotics may continue to persist through transmissibility within bacteria of same species and across other species. This could invariably enhance antibiotic resistant pathogens diversity on money with high tendencies of transmission within and outside the community.

Conclusion

Nigerian currency notes sampled were highly contaminated with a number of medically important bacteria, hence could be a potential source of transmission of potential pathogenic organisms. Public enlightenment by proper education on the health risk of contaminated currency and proper handling is highly canvassed.

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