Research Journal of Medical Science

Antibiotic Resistance Profiles of Enteric Pathogens Isolated from Nigerian Currency Notes

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Key words: Enteric pathogens, antibiotic resistance, Naira notes, Nigeria

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Page No.: 72-77
Volume: 15, Issue 4, 2021
ISSN: 1815-9346
Research Journal of Medical Sciences

Abstract: Currency notes are exchangeable fomites handled by many persons on a daily basis and have the potential for carrying enteric pathogens on their surfaces. Infections caused by these pathogens have a global impact on public health. This study was aimed at analysing the Nigerian currency notes Naira for the presence of enteric bacteria, particularly drug resistant strains. A total of 64 Nigerian currency notes were randomly collected from various sources. Samples were analysed using standard microbiological techniques. Antimicrobial profiles of bacterial isolates was determined using the Kirby-Bauer modified disc diffusion method. Preliminary analysis showed that 67.2% of the samples were contaminated. The 100 Naira notes had higher contamination rates with bacterial mean counts of 1.22×10^{10} cfu mL^{-1}. Three different species of enteric bacteria were isolated. The predominant pathogens were *Escherichia coli* (44.2%) and *Salmonella* sp. (44.2%). All isolates recovered were 100% susceptible to amikacin, gentamicin and ciprofloxacin while 97.7% were resistant to penicillin. *Escherichia coli* was resistant to ampicillin (100%) and trimethoprim-sulfamethoxazole (89.5%). This study showed that the Nigerian currency notes harbour enteric pathogens capable of causing serious infections to handlers. Therefore, improved hygiene practices, especially after handling currency notes should be encouraged to prevent the spread of these pathogens.

INTRODUCTION

Enteric infections and diarrheal diseases constitute pervasive health burdens globally with much of the disease burden attributed to infection with enteric pathogens including *Salmonella*, rotavirus and many other bacterial, viral and protozoan organisms[1]. These faecal pathogens are transmitted through different routes which may be direct (contaminated fingers and fomites) or indirect (food, flies, water). Currency notes which are exchangeable fomites handled daily by many persons, have the potential for carrying enteric[2] as well as drug-resistant pathogens[3] on their surfaces despite containing disinfectant that inhibits microorganisms[4].

Poor handling practices such as spraying of currency notes during ceremonies where such notes are trampled

on[5], concurrent handling of currency notes and food items, use of saliva to wet fingers during counting, placing or storing currency notes in or on dirty surfaces among others have been documented as contributing to the widespread contamination of currency notes with various pathogenic microorganisms[6].

Globally, the contamination of currency notes with pathogenic microorganisms have being reported. A multi-national study involving 1,280 currency notes obtained from ten different countries showed that bacterial contamination of currency notes is greatly influenced by age of the notes and the nature of material used to produce the notes[7]. In the United States, 94% contamination of circulating one dollar bill was reported in 2002[8]. A similar contamination rate was reported in Estonia[9], Croatia[10] and Pakistan[11]. Several other studies have also reported high levels of bacterial contamination in commonly circulated currency notes[12-17]. In sub-Saharan Africa, few studies have reported microbial contamination of currency notes such as the Nigerian Naira[18-21], the South African Rand[22] and the Ghanaian Cedi[23].

These microorganisms apart from reducing the lifespan of the currency notes, have been documented to cause infections in the skin, eye, gastrointestinal tract[13, 24], internal organs[25, 26] as well as the respiratory tract[27] in humans. A study by Elumalai *et al*.[28] reported eight different types of bacterial species (*Escherichia coli*, *Proteus mirabilis*, *Vibrio* sp., *Staphylococcus aureus*, *Pseuodomonas* sp., *Salmonella* sp., *Bacillus* sp. and *Klebsiella* sp.) isolated from 30 Indian currency notes consisting of five notes each of Indian Rupee 5 and 10 denominations. These bacteria are well known to cause a wide variety of diseases ranging from food poisoning, wound and skin infections, respiratory and gastrointestinal problems to life threatening diseases such as meningitis and septicaemia. Other studies have reported the isolation of bacteria causing foodborne diseases such as typhoid fever, gastroenteritis and shigellosis from currency notes[29, 30].

Studies conducted across Nigeria have documented varying rates of microbial contamination of the Naira (Nigerian currency) notes[31-33]. However, data on the presence of enteric bacteria and their antimicrobial profile on Nigeria banknotes are scanty. This study therefore analysed Nigerian currency notes for the presence of enteric bacteria, particularly drug resistant strains.

MATERIALS AND METHODS

Study design and setting: This study employed a descriptive cross-sectional design which was carried out in eight months. The study was conducted in Anyigba, a community in Dekina Local Government of Kogi State, Nigeria. The community is located between latitudes 7°15'N-7°29'N and longitude 7°11'E-7°32'E and has an estimated population of 189,976 inhabitants[34]. The land use and economy of the area is predominantly agrarian[35].

Sample collection: A total of 64 Nigerian currency notes (Naira) were randomly collected from various sources namely: students/lecturers, commercial motorcyclists, clinic cashiers, shop attendants, eateries/snack bars and banks. The currency notes included 8 pieces each of the widely circulated denominations (N5, N10, N20, N50, N100, N200, N500 and N1,000). The different denominations sampled were either through the purchase of goods or by exchanging new currency notes for old ones. Currency samples were collected in sterile plastic bags and sealed to avoid further contamination. Samples were then labelled and immediately transported to the laboratory for bacteriological analyses.

Laboratory analyses: Each currency note was transferred aseptically into 10 mL sterile buffered peptone water and vortexed for 2 min to dislodge the microbial contaminants. Sevenfold serial dilutions of each suspension was made and an aliquot of 0.1 mL of 10^{-6} dilution was inoculated into Eosin Methylene Blue (EMB) agar and Hektoen Enteric Agar (HEA) plates. All plates were incubated aerobically at 35-37°C for 24 h and examined for growth. Discrete colonies were sub-cultured on corresponding medium to obtain pure cultures. Media used for isolation were products of Oxoid Limited, United Kingdom. Morphologic characteristics based on conventional microbiological methods were observed. Bacterial isolates were then Gram-stained, tested for motility and characterized using standard biochemical techniques.

Antibiotic susceptibility testing: Antimicrobial susceptibility testing of bacterial isolates was determined using the Kirby-Bauer modified disc diffusion method. Bacterial isolates were inoculated onto Mueller Hinton agar (Oxoid, UK) by streaking the surface of the agar plates. Plates were then allowed to dry before aseptically introducing antibiotic discs and incubated at 37°C for 18 h. Ten antibiotic discs were used. These included ampicillin (10 mg), gentamicin (10 mg), ciprofloxacin (10 mg), amikacin (30 mg), streptomycin (10 mg) doxycycline (30 mg), tetracycline (30 mg), chloramphenicol (30 mg), trimethoprim-sulfamethoxazole (25 mg) and penicillin (10 mg). Results were interpreted as resistant or susceptible based on the interpretative standard chart of the Clinical and Laboratory Standards Institute[36] manual.

Statistical analysis: Data analysed were presented as simple descriptive statistics which included proportions and percentages. The Statistical Packages for Social Sciences (SPSS) Version 16 software was used.

Ethical consideration: This study did not collect personal data from study participants. However, verbal consent and approval from participants was sought before the collection of sample.

RESULTS AND DISCUSSION

Out of the 64 Naira currency notes examined, 43 (67.2%) were contaminated. The mean bacterial counts from different denominations on HEA and EMB is presented in Table 1. The 100 Naira notes showed higher mean counts of 1.14×10^{10} and 1.22×10^{10} cfu mL^{-1} on HEA and EMB, respectively.

Table 2 shows the distribution of bacterial species isolated from the different Naira denominations. Three species of enteric bacteria were recovered. Of these 3, *Escherichia coli* (19/43, 44.2%) and *Salmonella* sp. (19/43, 44.2%) were the predominant isolates. *Shigella* sp. (05/43, 11.6%) accounted for the least isolated. The 100 Naira notes (8/43, 18.6%) showed the highest level of contamination followed by the 200 Naira notes (7/43, 16.2%). The least contaminated denomination was the 1000 Naira notes (2/43, 4.7%).

Table 3 shows the antimicrobial resistance profile of the enteric bacteria isolated. Generally, high resistance to penicillin was observed. The most effective antibiotics were amikacin, gentamicin and ciprofloxacin as all bacteria isolates (43/43, 100%) were susceptible to these agents. Isolates of *Shigella* sp. were also sensitive (4/5, 80%) to doxycycline, tetracycline and trimethoprim-sulfamethoxazole. *Escherichia coli*, one of the predominant isolates in this study was 100% resistant to ampicillin and 89.5% resistant to trimethoprim-sulfamethoxazole. *Salmonella* sp. also predominant, showed low susceptibility (04/19, 21.1%) to streptomycin and chloramphenicol.

Currency notes as a source of infection are one of the most common exchangeable medium for fomites[37,38] and could play an important role in the transmission of infectious agents in a community which could consequently constitute a public health threat[3]. In this study, the extent of contamination of the Nigerian currency notes was investigated. Forty-three (67.2%) of the currency notes examined had bacterial contaminants. This figure is higher than the 52.5% reported in a previous study in Nigeria[18] but comparable to those in Saudi Arabia[39] and Nepal[40]. This report was however lower than the 93.9% reported in Cameroon[3], 97.1% reported in Ghana[41] and 100% recently reported in Nigeria[42].

This study reported that the 100 and 200 Naira notes had higher contamination rates while the 1000 Naira notes were the least contaminated (4.7%). The finding in this study further revealed that the Naira notes of lower denominations (N5 and N10) were significantly contaminated with bacterial isolates. These findings supports reports from other studies that lower denominations show higher levels of contamination[42,41,43, 44, 45, 18, 46]. Lower denominations are very often handled for different daily transactions than higher denominations. This higher rate of exchange predisposes the lower denominations to higher contamination levels[3, 46].

Table 1: Mean bacterial load counts of each denomination of currency notes on different culture media

Media Naira denomination	HEA average TPC (cfu/mL)	EMB average TPC (cfu/mL)
N5	4.9×10^{9}	5.0×10^{9}
N10	3.8×10^{9}	4.3×10^{9}
N20	2.8×10^{9}	3.6×10^{9}
N50	2.7×10^{9}	2.7×10^{9}
N100	1.14×10^{10}	1.22×10^{10}
N200	7.8×10^{9}	7.5×10^{9}
N500	2.5×10^{9}	2.6×10^{9}
N1000	2.2×10^{9}	2.3×10^{9}

TPC = Total Plate Count; HEA = Hektoen Enteric Agar, EMB = Eosin Methylene Blue agar

The pathogenic bacteria species isolated from this study were *Escherichia coli*, *Salmonella* sp. and *Shigella* sp. This finding confirms the report that currency notes are particularly contaminated with entero-pathogens[14] and supports the idea that currency notes serve as a reservoir for enteric infection[46]. The finding further supports previous studies in Ghana and India where similar pathogens were recovered from currency notes[41, 47].

The enteric pathogens *Escherichia coli* and *Salmonella* sp. were widely distributed in the various denominations of the Naira notes (Table 2). This report corroborates with previous observation by Akoachere *et al*.[3]. The presence of these pathogens in the Naira notes suggests faecal contamination and poor personal hygiene habits among currency handlers[3], emphasizing the significance of hand washing after handling currency notes. *Shigella*, an important pathogen that requires a tiny inoculum to start infection was the least isolated in this study. This may be due to the organism's short life cycle when in dry condition[2].

Bacteria isolated were generally susceptible to the following antibiotics; amikacin, gentamicin and ciprofloxacin. These drugs were the most active and they were effective against all isolates (100%). Previous studies have reported similar observations for amikacin[41, 23] and gentamicin[3]. Other antibiotics were effective against *Shigella* sp. These included doxycycline, tetracycline and trimethoprim-sulfamethoxazole (Table 3). However, penicillin was inactive against almost all bacterial isolates while resistant strains were found mostly among *Escherichia coli* and *Salmonella* sp. Trimethoprim-sulfamethoxazole and streptomycin showed low susceptibilities of 10.5% and 21.1% in these pathogens. A previous study in Nigeria reported strains of antibiotic resistant bacteria been

Table 2: Distribution of bacteria isolates according to the different currency denominations

Naira denomination	*Escherichia coli*	*Salmonella* sp.	*Shigella* sp.	Total (%)
N5	2	3	1	6 (14.0)
N10	3	3	0	6 (14.0)
N20	4	1	0	5 (11.6)
N50	1	4	0	5 (11.6)
N100	3	3	2	8 (18.6)
N200	2	4	1	7 (16.2)
N500	2	1	1	4 (9.3)
N1000	2	0	0	2 (4.7)
Total (%)	19 (44.2)	19 (44.2)	5 (11.6)	43 (100)

Table 3: Antibiotic susceptibility profile of bacteria isolates from Nigerian currency notes

	Escherichia coli No. of isolates (%)		*Salmonella* sp. No. of isolates (%)		*Shigella* sp. No. of isolates (%)	
Antibiotic tested	R	S	R	S	R	S
STR10	13(68.4)	6(31.6)	15(78.9)	4(21.1)	3(60)	2(40)
AMK30	0(0.0)	19(100)	0(0.0)	19(100)	0(0.0)	5(100)
AMP10	19(100)	0(0.0)	15(78.9)	4(21.1)	4(80)	1(20)
GEN10	0(0.0)	19(100)	0(0.0)	19(100)	0(0.0)	5(100)
DOX30	4(21.1)	15(78.9)	14(73.7)	5(26.3)	1(20)	4(80)
CIP10	0(0.0)	19(100)	0(0.0)	19(100)	0(0.0)	5(100)
TET30	5(26.3)	14(73.7)	14(73.7)	5(26.3)	1(20)	4(80)
SXT25	17(89.5)	2(10.5)	19(100)	0(0.0)	1(20)	4(80)
PEN10	18(94.7)	1(5.3)	19(100)	0(0.0)	5(100)	0(0.0)
CHL30	1(5.3)	18(94.7)	15(78.9)	4(21.1)	2(40)	3(60)

R = Resistance, S = Sensitivity; STR10 = Streptomycin 10 mg; AMK30 = Amikacin 30 mg; AMP10 = Ampicillin 10 mg; GEN10 = Gentamicin 10 mg, DOX30 = Doxycycline 30 mg; CIP10 = Ciprofloxacin 10 mg; TET30 = Tetracycline 30 mg; SXT25 = Trimethoprim-sulfamethoxazole 25 mg; PEN10 = Penicillin 10 mg, CHL30 = Chloramphenicol 30 mg

isolated from Naira notes[48]. Our findings have public health implications as these currency notes could serve as a possible route for the transmission of drug resistant pathogens and could result in treatment failure upon exposure to such pathogens[49].

CONCLUSION

Finding from this study has shown that the Naira notes are contaminated with resistant enteropathogenic bacteria capable of causing serious infections. This study further underscores the importance of hand hygiene after handling of currency.

Author contributions: Kehinde C. Mofolorunsho conceptualized and designed the study; Victor O. Obaje and Hannah O. Ocheni collected samples; Victor O. Obaje, Ruth F. Aminu and Olabisi O. Olowonibi ran the Laboratory analyses; Kehinde C. Mofolorunsho and Hannah O. Ocheni wrote the draft manuscript. All authors read and approved the final manuscript.

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