

ANTIBIOTIC SUSCEPTIBILITY PROFILES OF BIOFILM-FORMING BACTERIAL ISOLATES FROM CURRENCY NOTES IN NIGERIA

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ABSTRACT

The health hazards associated with the handling of contaminated money have been attributed to the ability of contaminated money to act as a vehicle by which pathogens can be transmitted among human beings as well as the capacity of the associated organisms to form biofilm. The consequences of biofilm formation are increased rate of mortality and morbidity, increased cost of treatment and prolonged hospital stay, among others. This study, therefore, aimed at evaluating the bacterial isolates from contaminated money for their ability to form biofilm and determining the susceptibility profiles to selected antibiotics.

A total of 100 pieces Nigerian currency notes were aseptically withdrawn from the Automated Teller Machine (ATM) of five different banks located within Obafemi Awolowo University campus. The bacteria associated with their surfaces were isolated and characterized using conventional biochemical tests. The ability of the isolated bacteria to form biofilm was determined using the Congo red agar (CRA) method. The susceptibilities of the CRA-positive bacterial isolates to selected antibiotics were determined using the Kirby-Bauer disc diffusion method.

Result revealed the presence of *Bacillus flexus* (89.7%), *Bacillus subtilis* (2.8%), *Staphylococcus aureus* (5.6%) and *Staphylococcus hyicus* (1.9%) of one hundred and seven (107) total isolated bacteria. All fifty (50) CRA-positive isolates were susceptible to gentamicin and resistant to penicillin G. However, 58 and 88 percent resistance were observed for fosfomycin and meropenem antibiotics, respectively.

The study concluded that currency notes can be a source of pathogenic bacteria capable of causing infections and resistant to many antibiotics aside their capacity to form biofilms.

Keywords: Biofilm, Currency; Antibiotics, Susceptibility, Isolates

INTRODUCTION

Money, any item or verifiable record that is generally accepted as payment for goods and services, can be used as a medium of exchange, a measure of value, store of value, unit of account and standard for deferred payment (Girma *et al.*, 2014). The ability of money to perform these functions has been attributed to some qualities as acceptability, uniformity, portability, divisibility, and durability, among others.

Microbiologically, money can serve as a source of infections as microorganisms majorly of bacterial and fungal origins have been isolated from their surfaces. For instance, bacteria as *Pseudomonas aeruginosa*, *Citrobacter* spp, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Klebsiella* spp, *Streptococcus* spp and *Mycobacterium* spp have been isolated from currency (Debajit *et al.*, 2012; Awe *et al.*, 2010). Moreover, several species of fungi such as *Candida* spp., *Aspergillus flavus*, *A. niger*, *A. parviticus*, *Penicillium* spp., *Rhizopus* spp., *Alternaria tenuis*, *Trichoderma* spp., *Fusarium* spp., and *Sporotrichum* spp have also been isolated (Debajit *et al.*, 2012; Awe *et al.*, 2010; Michael, 2002; Charnock, 2005; Lamichhane *et al.*, 2009; Alwakeel and Nasser, 2011). All these isolates have been reported to differ in their pathogenicity and virulence. One major virulence factor is biofilm formation. High resistance to antimicrobial agents often displayed by biofilms has resulted in increased level of mortality and morbidity, increased cost of treatment, as well as increased stay in hospital by patients.

However, that money is portable suggests the possibility of microbial transmission of the associated microbe either directly from hand to hand or indirectly through food and other inanimate objects. Poor money handling habits often leads to currency notes contamination (Girma *et al.*, 2014). For instance, some women are in the habit of squeezing money and keep under their bra; some market men and women do keep money in their dirty pockets while others such as the meat sellers in abattoirs handle money with hands stained with body fluids and animal droppings (Mensah *et al.*, 2002; Brady and Kelly, 2000). Furthermore, many people wet their fingers with saliva when counting money thereby contaminating the currency notes (Igumbor *et al.*, 2007). The process of circulating currency notes and their passing through hands in the course of daily transactions before it would reach bank aids microbial transmission (Awodi and Nock, 2001; Yakubu, Ehiowemwenguan and Inetianbor, 2014).

Nonetheless, despite the abundance of information about microbial contamination of currency note especially from Africa such as Nigeria in literature, information about the biofilm-forming ability of isolates associated with currency notes contamination is insufficient. This work therefore aimed at determining the biofilm-forming and the antibiotic susceptibility profiles of bacteria associated

with naira notes aseptically withdrawn from automated teller machines located on the campus of Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out on Nigerian currency notes from the Automated Teller Machines in Obafemi Awolowo University (O.A.U) campus, Ile-Ife, Osun state, Nigeria.

Sample size

A total number of 100 pieces of ₦500 denomination Nigerian currency notes were aseptically obtained from Automatic Teller Machine in five different banks namely: Guarantee Trust Bank, First Bank, Wema Bank, Access Bank and UBA into a sterile ziplock nylon bag and thereafter taken to the Pharmaceutical Microbiology Laboratory for microbiological analysis.



Figure 1 A picture of a typical Nigeria five hundred naira note

Sampling, isolation and identification methods

The two surfaces of each sampled currency note placed on pre-sterilized aluminum foil that was larger than the size of paper currencies were swabbed with sterile

swab stick moistened with sterile peptone water solution. The swabs were separately soaked into 10 mL sterile buffered peptone water solution and incubated at 37 °C for 24 hours. A loopful of the overnight grown culture was streaked on nutrient agar plate and incubated at 37 °C for 24 h. The grown colonies were morphologically examined and subjected to conventional biochemical tests such as the Gram's stain, catalase, indole, sugar fermentation, hydrogen sulphide production, citrate utilization etc for identification. The remaining colonies were put on a slope and stored at 4 °C in a refrigerator until they are needed.

Biofilm-forming ability of the isolates using Congo red Agar (CRA) method

This was done as described by Freeman *et al.* (1989). Congo red stain (0.8 g/L) prepared as concentrated aqueous solution and sterilized by autoclaving at 121 °C for 15 min. was added to sterile Brain Heart Infusion agar containing Brain Heart Infusion (BHI) broth (HiMedia, India) (37 g/L) and agar number 1 (HiMedia, India) (10 g/L) supplemented with sucrose (5 g/L) at 55 °C. The isolated bacteria were streaked on the CRA plates and incubated aerobically at 37 °C for 24 h. Formation of biofilm was indicated by distinct black colonies with a dry crystalline consistency.

Antimicrobial susceptibility testing

Susceptibility of bacterial isolates to seven selected antibiotics each was evaluated by the disc diffusion method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2021). (Oxoid, United Kingdom) The antibiotics (Oxoid, United Kingdom) used include: Gentamicin (10 µg), Ciprofloxacin (5 µg), Meropenem (10 µg), Fosfomycin (200 µg), Nitrofurantoin (300 µg), Penicillin G (10 units) and Piperacillin/Tazobactam (100/10 µg). McFarland standard equivalent of 0.5 ($A_{625\text{ nm}} = 0.09$) was prepared by inoculating 10 mL of sterile distilled water with five colonies of the test bacterium and rigorously mixed with a spin mixer. The turbidity of the resulting suspension was visually compared and adjusted to match the turbidity of 0.5 McFarland standard prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), with 9.95 mL of 1% sulfuric acid (H_2SO_4). The final suspension was evenly spread on the surface of Mueller–Hinton agar using a sterile swab stick. Selected antibiotic discs were then pressed onto the surface of the agar using a pair of sterile forceps following 20 minutes incubation at 37 °C for acclimatization and growth of the inocula. All plates were incubated at 37°C for 18 h after a 30-minute refrigeration at 4°C to ensure sufficient diffusion of antibiotics. *Escherichia coli* ATCC 25922 was used as control strain. The diameters of inhibition zones were measured in millimeters and interpreted according to the CLSI manual.

RESULTS

Table 1 Distribution of bacterial isolates from contaminated money by banks

Species/Genera	WB	FB	AB	UB	GT	Total
<i>Bacillus spp</i>	23	15	20	13	28	99
<i>Staphylococcus spp</i>	2	2	0	1	3	8
Total	25	17	20	14	31	107

WB: Wema Bank; FB: First Bank; AB: Access Bank; UB: United Bank for Africa; GT: Guaranty Trust Bank

Table 1 reveals the distribution of two bacteria genera isolated from contaminated money withdrawn from the automated teller machine of selected banks used in the study with GT Bank having the highest number of both the Bacilli and Staphylococci and UBA with the least. However, no staphylococcus was isolated from money withdrawn from the Access bank automated teller machine.

Table 2 Percentage Distribution (by banks) of CRA-positive bacterial isolates from contaminated money

	WB	FB	AB	UB	GT	Total
Number	11	6	10	4	19	50
Percentage	22	12	20	8	38	100

WB: Wema Bank; FB: First Bank; AB: Access Bank; UB: United Bank for Africa; GT: Guaranty Trust Bank

Table 2 shows the distribution of the fifty (50) CRA-positive bacterial isolates from the contaminated money used in the study. However, while GT bank has the highest number of isolates capable of forming biofilm as detected by Congo-red method, UBA has the least.

Table 3 Distribution of Resistance Profiles of CRA-Positive bacterial Isolates to selected Antibiotics

Antibiotics	% Resistance Pattern (n = 50)
Fosfomycin	58
Ciprofloxacin	4
Gentamycin	0
Nitrofurantoin	20
Meropenem	88
Penicillin G	100
Piperacillin/Tazobactam	4

Table 3 shows the distribution of the antibiotic resistance profiles of the fifty (50) CRA-positive bacterial isolates to seven (7) selected antibiotics. All the CRA-positive isolates in the study are resistant to penicillin G and sensitive to gentamycin. However, the isolates display equal resistance to ciprofloxacin and piperacillin/tazobactam antibiotics.

Table 4 Percentage distribution (by banks) of resistance profiles of isolated CRA-positive Bacilli genera

Antibiotics/Banks	WB (n = 11)		FB (n = 5)		AB (n = 10)		UB (n = 3)		GT (n = 17)	
	% R	% S	% R	% S	% R	% S	% R	% S	% R	% S
Fosfomycin	45	55	80	20	90	10	33	67	59	41
Ciprofloxacin	-	100	-	100	-	100	-	100	6	94
Gentamycin	-	100	-	100	-	100	-	100	-	100
Nitrofurantoin	9	91	-	100	-	100	-	100	24	76
Meropenem	100	-	100	-	80	20	33	67	88	12
Penicillin G	100	-	100	-	100	-	100	-	100	-
Piperacillin/Tazobactam	-	100	-	100	10	90	-	100	6	94

WB: Wema Bank; FB: First Bank; AB: Access Bank; UB: United Bank for Africa; GT: Guaranty Trust Bank. S = sensitive; R = Resistant

Table 5 Percentage distribution (by banks) of antibiotic resistance profiles of isolated CRA-positive Staphylococci genera

Antibiotics/Banks	FB (n = 1)		UB (n = 1)		GT (n = 2)	
	% R	% S	% R	% S	% R	% S
Fosfomycin	-	100	-	100	-	100
Ciprofloxacin	-	100	-	100	50	50
Gentamycin	-	100	-	100	-	100
Nitrofurantoin	-	100	-	100	-	100
Meropenem	100	-	100	-	100	-
Penicillin G	100	-	100	-	100	-
Piperacillin/Tazobactam	-	100	-	100	-	100

FB: First Bank; UB: United Bank for Africa; GT: Guaranty Trust Bank. S = sensitive; R = Resistant

The percentage distribution (by banks) of resistance profiles of CRA-positive Bacilli isolated in the study to selected antibiotics is shown in Table 4. Fifty nine percent (59%) of the isolates associated with money from GT bank are resistant to Fosfomycin while eighty-eight (88%) and six percent (6%) of the same isolates are resistant to meropenem and ciprofloxacin, respectively. All the CRA-positive bacilli (46) are resistant to penicillin G while all are sensitive to gentamycin.

All the CRA-positive staphylococci isolated in the study are sensitive to fosfomycin, gentamycin, nitrofurantoin and piperacillin/tazobactam antibiotics. On the other hand, all are resistant to meropenem and penicillin G, as shown in Table 5.

DISCUSSION

Contaminated money is a potential public hazard acting as a medium by which pathogens are disseminated to susceptible hosts (human or animals) thereby leading to the spread of communicable diseases. Microbial contamination of currency notes can be through many channels such as the automated teller machine (ATM), counting machine, atmosphere, storage environment, usage, handling or production (Awodi *et al.*, 2000; Prasai *et al.*, 2008). Moreover, water, soil, dust, and body of money dealers (such as skin, hand, and droplets) have also been implicated as contaminants of currency notes. As such, several microorganisms presenting high risk to public health have been isolated from contaminated money (Shakir *et al.*, 2010; Neel, 2012; Yazah *et al.*, 2012; Gedik *et al.*, 2013).

In this study, two genera of bacteria, namely bacillus and staphylococcus, were obtained from the one hundred samples of currency note (Table 1). This is in agreement with the report of Girma *et al.* (2015) who, among others, reported *Staphylococcus aureus* and *Bacillus* spp as the prevalent contaminants of currency note samples. From the data, it can be inferred that the currency notes are more contaminated with *Bacillus* spp because of their intrinsic capacity to form endospore and being able to withstand extreme environmental conditions. Routine transactions have predisposed the paper currency to colonization by pathogens by their passing through many hands before being finally deposited in banks.

The *Bacillus* spp (*B. subtilis* and *B. flexus*) isolated in this study may be attributed to bad habit of keeping money under carpet or rugs by individuals thereby encouraging settling of dust particles on money. On the other hand, the *Staphylococcus aureus* isolated in this study may be from individuals who keep money in their bra, pants, armpit and other sensitive area of the body, as *S. aureus* is a normal body flora. However, some studies have reported clinical association between *S. aureus* and urinary tract infection, toxic shock syndrome, skin infection and respiratory tract infection (Sucilathangam, *et al.*, 2016).

Money is not usually suitable for the survival of microorganisms. This may suggest that those that are able to survive may possess special attributes that promote their virulence and/or pathogenicity or may be intrinsically resistant to external conditions. One of such virulence factors is the ability to form biofilms. Biofilm formation is said to occur when microbial cells attach to each other on biotic or abiotic surfaces within an autogenic matrix of extracellular polymeric substances. It has been reported that sessile bacteria are 10-1000 times more tolerant to antibiotics than planktonic ones (Roy *et al.*, 2018). The consequences of high antimicrobial resistance by biofilms are increased rate of morbidity and mortality, increased cost of treatment and length of hospital stay, among others. In this study, the ability to form biofilm, using Congo red agar (CRA) method, was detected in fifty isolates (Table 2). Apart from the fact that CRA method is sensitive, rapid and reproducible, it also has the advantage that colonies remain viable on the medium. Furthermore, the method lacks the challenge of inter-batch variation of media (Freeman *et al.*, 1989).

Despite the high resistance by biofilm to antibiotics, antibiotics still remain the mainstay of bacterial treatment (Fair and Tor, 2014). Classification of antibiotics can be done based on their chemical structure, mechanism of action, or spectrum of activity which can be broad or narrow and targets bacterial functions or growth processes (Calderon and Sabundayo, 2007).

In this study, the resistance profiles of the fifty CRA positive isolates to seven (7) selected antibiotics namely Gentamicin, Ciprofloxacin, Meropenem, Fosfomycin, Nitrofurantoin, Penicillin G and Piperacillin/Tazobactam were evaluated (Tables 3-5). All of the 50 isolates showed resistance to Penicillin G (100%), Meropenem (88%), Fosfomycin (58%), Nitrofurantoin (20%), Ciprofloxacin (4%), Piperacillin/Tazobactam (4%). However, all the CRA positive isolates were susceptible to Gentamicin (Table 3). While all the CRA-positive bacilli were sensitive to gentamycin and resistant to Penicillin G (Table 4), all CRA-positive staphylococci were sensitive to fosfomycin, gentamycin, nitrofurantoin and piperacillin/tazobactam and resistant to meropenem and penicillin G (Table 5).

Resistance to antibiotics, depending on its class, can be acquired through several mechanisms as (i) enzymatic drug modification and inactivation e. g. gentamicin, meropenem, penicillin G, (ii) enzymatic modification of the drug binding site e. g. ciprofloxacin, (iii) bypass mechanisms involving acquisition of a novel drug-resistant target (iv) drug efflux e. g. meropenem, ciprofloxacin, (v) displacement of the drug to protect the target e.g. ciprofloxacin (Foster, 2017). It is to be noted that one bacterial cell may develop resistance to a class of antibiotic using several mechanisms. For instance, resistance to ciprofloxacin, a fluoroquinolone antibiotic, can occur through any of (i) protection of the target site (ii) over-expression of efflux pumps that prevent the drug from being accumulated in the cell, and (iii) changes in genes encoding the target site (DNA gyrase and topoisomerase IV). However, all these mechanisms may exist together in the same bacteria thereby increasing the levels of resistance. Conversely, bacterial species appear to have evolved a preference for some mechanisms of resistance over others. For example, the predominant mechanism of resistance to β -lactams in gram-positive organisms is mostly achieved by modifications of their target site, the penicillin-binding proteins (PBPs) (Munita and Arias, 2016).

CONCLUSION

The study concluded that currency notes can be a source of pathogenic bacteria capable of causing infections and resistant to many antibiotics aside their capacity to form biofilms.

Conflict of interest: The authors declare there is no conflict of interest in the course of this work.

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