

Usman et al. Afr. J. Clin. Exper. Microbiol. 2021; 22 (2): 244 - 251

<https://www.afrcem.org>African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X
AJCEM/2061. <https://www.ajol.info/index.php/ajcem>

Apr 2021; Vol.22 No.2

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Microbial contamination of Naira notes circulating in Bauchi metropolis: prevalence, microbial load and detection of extended spectrum beta-lactamase producing Gram-negative bacteria

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Background: Globally, contamination of banknotes with various microbial species is increasingly being reported. This usually results from improper handling during exchange of goods and services. In the present study, we aimed to determine the microbial load, prevalence and the presence of Extended Spectrum Beta Lactamase (ESBL) among bacteria isolated from the Nigerian Naira notes circulating in Bauchi metropolis.

Methodology: A total of 400 Naira notes of various denominations were randomly collected aseptically, cultured and total viable counts determined. The isolated microbial species were identified using standard microbiological techniques. Antibiotic susceptibility of the isolates and detection of ESBL were determined by Kirby-Bauer's disc diffusion method and Double Disc Synergy Test (DDST), respectively.

Results: All the 400 samples collected were contaminated with various microbial species. The highest mean colony count was detected in 20 Naira notes (28.5%), while the least was observed in 1000 Naira note (3.3%). Fourteen different microbial species were isolated from the contaminated currency notes, predominantly *Escherichia coli* (25.0%), and *Staphylococcus aureus* (12.0%). Some fungal species mainly *Aspergillus flavus* and *Aspergillus niger* were also isolated. Majority of the bacteria isolates resistant to the third generation cephalosporins (72.1%) were ESBL positive.

Conclusion: The study shows that Naira notes circulating in Bauchi metropolis were heavily contaminated with various microbial species, and a high proportion of the isolated Gram-negative bacteria were ESBL producers. Efforts should thus be made to improve hygiene practices in the study area. Importantly, businesses should be encouraged to adopt the use of electronic transactions.

Keywords: Currency notes, Naira, Microbial contamination, ESBL

Received Jul 24, 2020; Revised Jan 2, 2021; Accepted Jan 3, 2021

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Contamination microbienne des notes de Naira circulant dans la métropole de Bauchi: prévalence, charge microbienne et détection de la production de bêta-lactamase à spectre étendu Bactéries Gram-négatives

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Abstrait:

Contexte: À l'échelle mondiale, la contamination des billets de banque par diverses espèces microbiennes est de plus en plus signalée. Cela résulte généralement d'une mauvaise manipulation lors de l'échange de biens et de services. Dans la présente étude, nous avons cherché à déterminer la charge microbienne, la prévalence et la présence de bêta lactamase à spectre étendu (BLSE) parmi les bactéries isolées des notes nigérianes naira circulant dans la métropole de Bauchi.

Méthodologie: Un total de 400 billets Naira de différentes dénominations ont été collectés au hasard de manière aseptique, cultivés et le nombre total viable déterminé. Les espèces microbiennes isolées ont été identifiées à l'aide de techniques microbiologiques standard. La sensibilité aux antibiotiques des isolats et la détection des BLSE ont été déterminées respectivement par la méthode de diffusion sur disque de Kirby-Bauer et le test de synergie à double disque (DDST).

Résultats: Tous les 400 échantillons prélevés étaient contaminés par diverses espèces microbiennes. Le nombre moyen de colonies le plus élevé a été détecté dans 20 billets nairas (28,5%), tandis que le moins a été observé dans les billets 1000 nairas (3,3%). Quatorze espèces microbiennes différentes ont été isolées des billets de banque contaminés, principalement *Escherichia coli* (25,0%) et *Staphylococcus aureus* (12,0%). Certaines espèces fongiques, principalement *Aspergillus flavus* et *Aspergillus niger*, ont également été isolées. La majorité des isolats bactériens résistants aux céphalosporines de troisième génération (72,1%) étaient BLSE positifs.

Conclusion: L'étude montre que les notes de Naira circulant dans la métropole de Bauchi étaient fortement contaminées par diverses espèces microbiennes et qu'une forte proportion des bactéries Gram-négatives isolées étaient des producteurs de BLSE. Des efforts devraient donc être faits pour améliorer les pratiques d'hygiène dans la zone d'étude. Surtout, les entreprises devraient être encouragées à adopter l'utilisation des transactions électroniques.

Mots clés: billets de banque, naira, contamination microbienne, BLSE

Introduction:

Bank currency notes are the commonest means of exchanging goods and services particularly in developing countries (1). They are one of the most frequently passed items from one hand to the other during transactions (2). Improper handling practices such as concurrent handling of banknotes and food items, use of saliva to wet fingers during counting, placing or storing paper notes in or on dirty surfaces among others has led to widespread contamination of banknotes with various microbial species (3).

Globally, contamination of banknotes with microbial species is being reported. A large multi-national study involving 1280 banknotes obtained from 10 different countries showed that bacterial contamination of banknotes is greatly influenced by age of the notes and the nature of material used to produce the notes (polymer-based versus cotton-based) (4). In the United States, 94% contamination of circulating one dollar bill was reported in 2002 (5). A similar high contamination rate has been reported in Estonia (6), Pakistan (7), Croatia (2) and Ghana (8).

The contaminated banknotes have been demonstrated to be a viable source of cross-contamination and a vehicle for transmission of infectious agents in the community (9). Bacteria causing foodborne diseases such as typhoid

fever, gastroenteritis, shigellosis, etc have been isolated from banknotes (10,11). Some other researchers have isolated parasites and viral particles from bank currency notes (12–14).

Studies across Nigeria have documented varying rate of microbial contamination of banknotes (15–17). In Bauchi, north-eastern Nigeria, the level of microbial bioburden and the prevalence of extended spectrum beta-lactamase (ESBL) among the circulating Naira notes is currently unknown. In the present study, we aimed to determine the microbial load, prevalence and the presence of ESBL among bacteria isolated from the Nigerian Naira notes circulating in Bauchi metropolis.

Materials and method:

Study setting

The study was carried out in the main market (Wunti market) in Bauchi metropolis, the capital of Bauchi State (geographic coordinates 10.7761° N, 9.9992° E). The city has an estimated population of 6,537,314, approximately 3.38% of the Nigerian population (18). The market is located in the heart of Bauchi metropolis, serving as point of exchange of goods and services for the inhabitants of the State. Field survey showed that the market has 2980 shops operating various businesses ranging from grocery stores, fashion houses, butcher houses, snack bars, canteens, and hardware shops.

Study design and sampling method

This was a descriptive cross-sectional study of Naira currency denominations among market shop owners, conducted between January and July 2019. Systematic random sampling method was used to select shops for participation in this study. Samples were collected from every 5th shop in the study area.

Ethical considerations

The approval to conduct this study was granted by the Bauchi State Ministry of Health (Ref No: NREC/12/05/2013/2017/38). Consent was also sought from the market men and women after carefully briefing them on the objective of the study.

Selection criteria

All the Naira currency denominations currently in use in Nigeria from the selected shop owners were included with the exception of the mutilated or grossly dirty notes. The samples were collected from only numbered shops. Constructed road side/temporary shops without numbering were excluded. Also, old notes that have been withdrawn from circulation were also excluded from the study.

Sample collection

Fifty (50) samples of each of the eight (₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500 and ₦1,000) Naira denominations in circulation totaling 400 samples were collected from different shop owners practicing different businesses of their choice in Wunti market in the metropolis of Bauchi State, Nigeria. The Naira note samples were collected aseptically by letting the owners drop them into sterile polythene bags, and the polythene sealed immediately. The sealed polythene bags were immediately taken to the Medical Microbiological Laboratory of the Abubakar Tafawa Balewa University Teaching Hospital in Bauchi.

Enumeration of microbial contaminants

The enumeration of microbial contaminants on the notes was carried out as previously described (17). Briefly, the collected Naira notes were placed aseptically into different sterile test tubes containing 10ml of sterile water and shaken for few minutes. After 3 minutes, the notes were removed from the test tubes and a 5 serial 10-fold dilution (10^{-1} to 10^{-5}) were made. An aliquot of 0.1 ml from the 10^{-4} dilution

was aseptically inoculated on Blood, MacConkey and Mannitol salt agar plates which had been prepared according to manufacturer's instruction. All plates were incubated at 37°C for 24 hrs, and examined for bacterial growth, and the number of colonies on each plate was counted using a digital colony counter.

Identification of bacterial isolates was done using a combination of morphological characteristics such as shape, colour, elevation, consistency etc. and biochemical characteristics including coagulase, novobiocin, triple sugar iron (TSI) agar, oxidase, catalase, oxidation-fermentation (OF), urease, motility, indole, citrate, methyl red (MR) and Voges-Proskauer (VP) tests as previously described (19).

For isolation of fungi, a loopful of the culture was inoculated in duplicate onto Sabouraud's Dextrose agar (SDA) supplemented with 0.01% chloramphenicol. The first plate was incubated aerobically at 37°C for 48 hours and the second plate was incubated for five days at room temperature (25°C). Identification was done on the basis of cultural (mould or yeast form, pigment production) and microscopic morphological characteristics following staining with lactophenol cotton blue as described (20).

Antibiotic susceptibility testing

Antimicrobial susceptibility pattern of bacterial isolates was determined using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (21). The tested antibiotics were as follow; ampicillin, ciprofloxacin, gentamicin, nalidixic acid, amoxicillin, aztreonam, cefoxitin, vancomycin, tetracycline and erythromycin (Mast company, UK). The standard strain of *E. coli* ATCC 25922 was used for quality control in susceptibility testing. Interpretation of results as susceptible, intermediate or resistant was done according to the criteria recommended by the CLSI guideline.

Screening test for ESBLs

Gram negative bacterial isolates resistant to third generation cephalosporins (3GC) were regarded as presumptive ESBL positive. This was confirmed by the double discs synergy test (DDST). Briefly, the confirmatory test was performed by placing a β -lactamase inhibitor (amoxicillin-clavulanic) disc between two third generation cephalosporins (3GCs) discs at a distance of 20 mm centre-to-centre on a plate

inoculated with a standardized inoculum of the test organism as previously described [22]. Formation of a characteristic keyhole effect or champagne-cork shaped zone of inhibition between the discs was considered as a phenotypic indication of ESBL production.

Statistical analysis

Data was analysed using IBM SPSS statistics software, version 24.0 (IBM Corporation, Armonk, NY) and presented as simple descriptive statistics or pictograms. Categorical variables were compared using Pearson's χ^2 test or Fisher's exact test. At 95% confidence interval, $p < 0.05$ was considered as statistically significant.

Results:

Mean microbial counts on each of the currency denominations

A total of 400 Naira notes samples of eight denominations (₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500 and ₦1,000) were collected from traders within the market. All the samples analysed were contaminated with various microbial species. The result of mean colony counts of the different currency denominations sampled from the markets is presented in Table 1.

Table 1: Mean colony counts of microbial pathogens across the currency notes

Currency denominations	Mean colony counts (CFU/ml x 10 ³)	
	Bacteria	Fungi
1000	7.0 ± 0.4	3.0 ± 0.9
500	9.0 ± 0.7	11.0 ± 0.3
200	15.0 ± 0.5	12.0 ± 0.4
100	24.0 ± 0.1	13.0 ± 0.5
50	21.0 ± 0.6	6.0 ± 0.1
20	48.0 ± 0.1	5.0 ± 0.7
10	39.0 ± 0.5	5.0 ± 0.2
5	34.0 ± 0.4	4.0 ± 0.1

CFU = Colony Forming Unit; ml = Millilitre

The result shows that 20 Naira notes had the highest mean count of $48.0 \pm 0.1 \times 10^3$ CFU/ml for bacteria, followed by 10 Naira note which had $39.0 \pm 0.5 \times 10^3$ CFU/ml while 1000 Naira notes had the least count of $7.0 \pm 0.4 \times 10^3$ CFU/ml. The fungal count of the Naira notes revealed that 100 Naira note has the highest count of $13.0 \pm 0.5 \times 10^3$ CFU/ml, followed by 200 Naira with $12.0 \pm 0.4 \times 10^3$ CFU/ml while the least was 5 Naira note which had $4.0 \pm 0.2 \times 10^3$ CFU/ml (Table 1).

Distribution of the microbial isolates according to different denominations

As shown in Table 2, the highest contamination rate was in 20 Naira notes 114 (28.5%), followed by 50 Naira note 64 (17.5%), and the least rate was in 1000 Naira note 13 (3.3%).

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

Banknotes	Contamination
1000	13 (3.3)
500	23 (5.8)
200	40 (10.0)
100	53 (13.3)
50	64 (16.0)
20	114 (28.5)
10	56 (14.0)
5	37 (9.3)

Distribution of isolated microbial contaminants

The distribution of isolated organisms is presented in Table 3. Fourteen different microbial species were isolated from the contaminated currency notes. *Escherichia coli* (25.0%) and *Klebsiella pneumoniae* (24.5%), were the commonest Gram-negative bacteria isolated. This was followed by *Klebsiella oxytoca*, *Proteus* spp and *Pseudomonas aeruginosa*. Among the Gram-positive bacteria isolated, *Staphylococcus aureus* (12.0%) and *Bacillus* spp (3.5%) were the most frequently encountered. Some fungal species were also isolated, mainly *Aspergillus flavus*, *Rhizopus* spp and *Aspergillus niger*.

Table 3: Distribution of isolated microbial species

Isolates	Number (n)	Percentage (%)
Gram-negative bacteria (n=280)		
<i>Escherichia coli</i>	100	25
<i>Klebsiella pneumoniae</i>	98	24.5
<i>Klebsiella oxytoca</i>	40	10
<i>Proteus</i> spp	28	7
<i>Pseudomonas aeruginosa</i>	10	2.5
<i>Enterobacter</i> spp	2	0.5
<i>Salmonella typhimurium</i>	2	0.5
Gram-positive bacteria (n=140)		
<i>Staphylococcus aureus</i>	48	12
<i>Staphylococcus saprophyticus</i>	10	2.5
<i>Bacillus</i> spp	14	3.5
<i>Micrococcus</i> spp	2	0.5
Fungal isolates		
<i>Aspergillus flavus</i>	18	4.5
<i>Rhizopus</i> spp	18	4.5
<i>Aspergillus niger</i>	10	2.5
Total	400	100

Table 4: Distribution of ESBL producing among multidrug resistant *Enterobacteriaceae* isolates from Naira notes

Isolates	Number of 3GC resistant isolates	No of ESBL positive (%)
<i>Escherichia coli</i>	19	26 (30.2)
<i>Klebsiella oxytoca</i>	2	5 (5.8)
<i>Klebsiella pneumoniae</i>	44	22 (25.6)
<i>Proteus</i> spp	14	0 (0)
<i>Salmonella typhimurium</i>	1	0 (0)
<i>Pseudomonas aeruginosa</i>	5	8 (9.3)
<i>Enterobacter</i> spp.	1	1 (1.2)
Total	86	62 (72.1)

Antibiotic resistance pattern of the isolated bacteria

Out of the total of 280 Gram-negative bacterial isolated from various Naira notes, 86 (30.7%) were resistant to third generation cep-

halosporins (3GC). The 3GC resistant isolates comprise *E. coli* (n=19), *K. oxytoca* (n=2), *K. pneumoniae* (n=44), *Proteus* spp (n=14), *Salmonella typhimurium* (n=1), *P. aeruginosa*

(n=5), and *Enterobacter* spp (n=1). The result of phenotypic ESBL detection showed that 62 (72.1%) of the 3GC resistant bacteria were ESBL positive (Table 4). This was predominantly detected among *E. coli* 26 (30.2%) and *K. pneumoniae* 5 (5.8%) isolates.

Discussion:

The contamination of paper currency notes is almost inevitable due to poor handling practices. In this study, 100 % contamination rate was observed. This is consistent with high contamination rate reported by other researchers across the country (23). Similarly, in a neighbouring West Africa country, 100% contamination rate was reported (8). One hundred percent contamination rate of Pakistani and Euro currency notes have also been reported (24,25). The contamination may have arisen from simultaneous handling of the currency notes and various articles during exchange at selling points (26). This practice is particularly common among small businesses in the study area due to low level of penetration and acceptance of electronic transaction as a result of high internet cost and low-literacy level in the area (27,28). Additionally, activities of cyber-criminals popularly known as 'Yahoo-Yahoo' using deceptive banking tools to defraud unsuspecting clients makes the use of physical currency very popular, with attendant health risks (29). Though variation in mean colony counts among currency of the same denomination exist, the heavy contamination of lower denomination currencies (₦100 to ₦5) observed in this study concurs with the findings of other researchers (30,31). This may be attributed to high use and frequent exchange of lower denomination currencies than the higher denominations in daily cash transactions (31).

The contamination of the notes with several known human pathogens concurs with several other reports (30,32). The predominance of *E. coli* and other enteric Gram-negative bacteria among the isolated bacteria may be due to poor hygiene (both personal and environmental) in the study area. This is in contrast with a result of a study in Uganda where *E. coli* isolates were not detected among the bacteria isolated from their paper currency notes (30). Similar to the finding of this study, currency notes in both Nigeria and elsewhere have been shown to be contaminated with various fungal isolates (11,33,34). Among the Gram-positive bacteria, *Staphylococcus* spp predominates. This could have been shed from the skin of the hands during handling and exchange (34).

The high ESBL detection rate (72.1%) in

the isolated bacteria is not surprising. This is in line with 7.5 93.3% rate reported in a systematic review on the prevalence of ESBL producing Gram-negative bacteria in Nigeria (35). Another study has reported contamination of automated teller machine (ATM) touch surfaces by ESBL producing bacteria (36). Bacteria harbouring other antibiotic resistance mechanisms have also been isolated from Nigerian currency notes (23). The high ESBL rate among the isolated bacteria may be due to overuse of beta-lactam antibiotics in the study area (37). In Nigeria generally, beta-lactam antibiotics are the most widely used in both hospital and community settings (38). Sometimes, these antibiotics are sourced over-the-counter for self-medication (39). This practice has over the years led to the emergence of beta-lactamase producing bacteria threatening the therapeutic efficacy of most the beta-lactam antibiotics.

The finding in this study has significant health implications. Majorly, the contaminated currency notes may serve as vehicle for transmission of infectious agents, playing significant role in the community transmission of infectious agents (3). This is worrisome particularly in this era of global pandemic due to SARS-CoV-2. Moreover, handlers of these notes especially women may put the currency notes in their brassieres or other areas where there is intimate contact with the skin and thus predisposing them to infections. Also, some individuals usually wet their fingers with saliva to ease the counting of the currencies, consequently resulting in cross-contamination. Food vendors too simultaneously handling monies and ready to eat food could also facilitate the transmission of potential pathogenic organisms from currencies to their clients.

Preventive measures aimed at breaking the chain of transmission of infections and contamination of the currency notes should therefore be instituted. This should include public enlightenment on personal and environmental hygiene and proper handling of Naira notes. Old and mutilated notes should be frequently withdrawn and replaced. Lastly and most importantly, electronic transactions such as the use of digital money, point of service (POS), electronic transfer of fund and others should urgently be promoted as a safer alternative.

This study was characterised by some limitations. First, the study was restricted to bacterial and fungal contaminants. Potential contamination by viruses and some pathogenic parasites could not be inferred. Also, the ESBL detection test was restricted to phenotypic method. As such, the predominant genotype of

the detected ESBL producing strains remains unknown. Recently, genotypic methods including meta-genomics is being explored for investigation of microbial pathogens and antibiotic resistance genes on currency notes (14). However, until now, no previous report on the microbial contamination of Naira notes in the State. This study thus provides an important baseline data on contamination of Naira notes in Bauchi State, North-eastern Nigeria.

Conclusion:

The study shows that Naira notes circulating in Bauchi metropolis were heavily contaminated with microbial species, predominantly *E. coli*, *K. pneumoniae* and *S. aureus*. A high proportion (72.1%) of the isolated Gram-negative bacteria were ESBL producers. Because of the potential role of banknotes in transmission of pathogenic organisms, advocacy to improve hygiene practices in the study area should be urgently undertaken. Most importantly, businesses should be encouraged to adopt safer alternatives such as the use of electronic transactions.

Authors' contributions:

The study was designed and conducted by MU and AI. AO analysed and interpreted the data and produced the first manuscript draft, which was revised by all authors. All authors read and approved the final manuscript.

Conflict of interest:

Authors declare no conflict of interest

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