EVALUATION OF PATHOGENIC POTENTIALS OF MICROBIAL CONTAMINANTS FROM NAIRA NOTES IN NIGERIA

ABSTRACT

The study was done to determine burden of fiat currencies. A total of Six hundred and twenty four pieces of different denominations of naira notes obtained from banks in Enugu metropolis and samples of nose swabs aseptically collected from fifty two note counters from those banks were examined for similar bacterial and fungal contaminants. All sequences were identified using the Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology Information (NCBI) website. While the fungi amplicons yielded DNA bands of approximately 650 base pair, that of the bacterial isolates were approximately 850 base pair. *Proteus mirabilis*(NR11449.1) and *Escherichia coli* (LN831043.1) were identical and selected from the bacterial category while *Aspergillus fumigatus* (MK910068), *Aspergillus flavus* (JQ860302) and *Aspergillus niger* (MK461093) were identical and selected from the fungal category. Rats inoculated orally with *Escherichia coli* and *Proteus mirabilis* presented with watery stool and reduction of weight by $16^{\pm}0.4$g after two weeks of commencement of inoculation. They showed reduction in activity and reduced locomotion when compared with the control. There were no physically observable changes in the other test groups. In the hematological investigation, the mean PCV in % were $39^{\pm}1.0$ for the *E.coli*, $40^{\pm}0.4$ for *P.mirabilis*, $35^{\pm}0.2$ for *A.niger*, $40^{\pm}0.7$ for *A.fumigatus* and $37^{\pm}0.1$ for *A.flavus*. These varied significantly at $p<0.05$ with the control which has mean PCV of $45^{\pm}0.3$. The differential leucocyte count showed a marked increase in the % neutrophil (*E.coli* $73^{\pm}0.1$, *P. mirabilis* $70^{\pm}0.1$, *A.niger* $78^{\pm}1.1$, *A. fumigatus* $59^{\pm}0.3$ and *A. flavus* $62^{\pm}1.0$) when compared with the control rats with percentage neutrophil of $20^{\pm}0.2$. There was also an increase in the white blood cell count of the test groups when compared with the control. Histopathological study of the lungs of the rats inoculated nasally with *Aspergillus niger* showed necrosis of the alveolar epithelium. This study has shown that naira notes could be a

reservoir of microorganisms of medical importance which in turn could become vectors for the transmission of diseases in the society.

Keywords*: pathogenic potentials, microbial contaminants, naira notes*

INTRODUCTION

The earliest form of paper money originated in China between 1050-1450 .The early banknotes were exchange notes or private bills showing a definite amount of credit, but the use was normally the same.(Allan *et al*,2018) Paper currency is one of the most essential medium for exchange of goods and services all over the world (Sadawarte et al., 2014)

In Nigeria, Naira is the official currency and is minted by the Nigerian Security Printing and Minting Company under the regulation of the Central Bank of Nigeria (CBN). Presently there are eight denominations of the Naira notes from ₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500, ₦1000. According to CBN, the projected lifespan of the Naira notes is 24 months. (Popoola and Popoola, 2019)

There is an **i**ncrease in the level of abuse of naira notes. Though there are penalties specified for individuals that abuse these naira notes, they are scarcely enforced. Some naira notes stay for so long in circulation both in the hands of handlers and the banks without being mopped up for destruction.

A note is deemed to have been tampered with if the note has been impaired, diminished or lightened or has been defaced by stumping, engraving, mutilating, piercing, stapling, writing,

tearing, soiling, squeezing. Spraying, dancing or matching on the Naira notes also constitutes an abuse. (CBN Acts 2007).

The crude materials that are utilized for making these paper currency haves been reported to play significant roles in harboring of microorganisms. (Vriesekoop et al, 2010). Earlier studies have associated paper money with transfer of various microorganisms. (Oha et al, 2016). This is because paper currencies are contaminated in the course of business transactions and other routine activities. Some unhygienic activities such as sneezing, coughing, and placement in obscure places like pockets, socks, shoes and under floor covering also expose these notes to contamination.(Oyero and Emikpe, 2007)

On daily basis bank workers come in contact with these naira notes while discharging their official duties. These notes usually pass through humans from different walks of life and ultimately end up in the bank where they are counted and kept for further processing.

This study assessed the pathogenic potentials of similar bacterial and fungal contaminants isolated from naira notes and bank workers in Enugu metropolis.

Previous researches carried out in Ghana, Kenya, Iran and Sudan have reported high rate of microbial contamination of their currency notes. (Sharon and Sethu, 2017; Charnok, 2005). A research carried out in Kano, Nigeria also reported contamination of Naira notes in circulation. (Kawo et al., 2009) Some of these research showed that these contaminants include potential pathogens that may cause diseases in healthy individual. (Kuria et al., 2009). Naira notes in

circulation in Nigeria poses risk to public health since infectious diseases can be transferred through this medium (Umeh, 2007)

MATERIALS AND METHODS

The study was carried out at the Microbiology laboratory of Enugu State University of Science and Technology, Biotechnology Laboratory of Godffery Okoye University Enugu and Inqaba Biotec West Africa, Ibadan and Anatomy Department of University of Nigeria, Enugu campus.

Collection of Samples:

A total of six hundred and twenty four pieces of different denominations of naira notes were collected from banks in Enugu metropolis in sterile containers. The individuals were requested to place the samples directly into sterile bottles to avoid further contamination. Also samples of nose swabs were aseptically collected from fifty two Tellers from those banks using sterile swab sticks. These samples were immediately taken to the laboratory for processing.

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Processing of Samples

Sterilized wet swab moistened with physiological saline was placed and rolled on the surface of the notes. The swab of each sample was placed in a test tube containing 10 ml of distilled water. A Ssterile forcep was used to remove the swab into distilled water and was adequately shaken to dislodge the swabbed content into the solution. After removal of the swab, the solution was homogenized.(Gabriel et al, 2013) The solution was inoculated into Sabourad Dextrose Agar , Nutrient agar, MacConkey and Blood agar with sterile standard 10 µl wire loop. The same process was carried out with the nasal swabs. The inoculated plates were incubated in an

inverted position at 35 -37^{o}C for 24hrs for bacteria and 24 – 72 hours for the Sabouraud dextrose agar at room temperature. The plates were examined for bacterial and fungal growth after the incubation period. Mixed colonies on the Nutrient, MacConkey and Blood agar plates were subcultured into fresh media for further use.

Genomic DNA Isolation

Zymo Research Quick-DNA Fungal/Bacteria Miniprep kit (cat. D6005). 1000µl was used to extract the genomic DNA of the isolates from overnight bacterial isolate broth culture of the samples. DNA quality and concentration were checked by running 5µl of the DNA samples on 1% agarose gel.

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) for bacterial DNA fungal~~viral~~ DNA were performed in a total volume of 10µl. All amplification reactions were performed in a GeneAmp® PCR System 9700,

Table 1: Primer sequence for 16S RNA

Primer	Sequence
16S F	GTGCCAGCAGCCGCGCTAA
16S R	AGACCCGGGAACGTATTCAC

Table 2: Primer sequence for ITS

Primer	Sequence
ITS4	TCCTCCGCTTATTGATATGS
ITS5	GGAAGTAAAAGTCGTAACAAGG

DNA Sequencing

DNA sequencing was done using the Big Dye terminator cycle sequencing kit (Applied BioSystems),. (Kearse et al, 2012)

Sequence analysis

Sequences were identified using the Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology Information (NCBI) website.(Kearse et al 2012)

Pathogenicity Test

A total of 22 albino Wister rats from Animal house, College of Medicine, Enugu State University were used for the pathogenicity testing. The rats were 6-8 weeks old and weighed between 150-155 g before the commencement of the pathogenicity testing. They were randomly assigned to 6 groups with four animals per group except for group 1 which had 2 rats and served as the control group. Rat chow from Vital feeds Nigeria Ltd Bukuru Jos, Plateau State Nigeria and sterile water were provided for the rats ad libidum. *Eschericia coli* and *Proteus mirabilis* isolates were used for the pathogenicity testing in rats ~~the bacteria isolates~~. The organisms were asceptically inoculated into freshly prepared agar plates and incubated at 37^{o}C for 24 hours. 0.6 ml of the inoculum of the bacteria isolates, containing approximately 1×10^{7} cells/ml was administered to the rats orally using pyrogen-free sterile oral catether and intraperitoneally using pyrogen-free sterile disposable syringes. (Ali,*et al* 2018, Akinkumi *et al*, 2014) For the fungal isolates, *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus*

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were used for the pathogenicity testing and inoculated nasally and intraperitoneally. Spore suspension for inoculation was prepared by growing the different organisms in Sabouraud Dextrose agar medium for 5 to 10 days for profuse sporulation. For the harvesting of the spores, 5mls of sterile normal saline mixed with 0.1% Tween 80 was poured into the plate and gradually mixed with sterile cotton bud to harvest the spores. The mixture was gently poured into a sterile test tube with glass beads, shaken to disrupt the cells and allowed to sediment under gravity. The supernatant spore suspension was decanted. Cell count was carried out with a haemocytometer chamber, $5x10^6$ number of spores was used for the inoculation.(Chhabra and Dhakad, 2008) The animals in the test groups were repeatedly exposed but only animal feed and water were given to the rats in the control group. The animals were observed closely and any behavioral change was noted. After twenty one days of administration, capillary tube was used for retro-orbital blood sample collection. Collected blood samples were put in properly labelled sample bottles containing EDTA. The anti-coagulated blood was used for the determination of the Packed Cell Volume (PCV), White Blood Cell (WBC) and differential leucocyte analysis. PCV was determined by microhaematocrit technique using capillary tube.

Packed cell volume (PCV)

10 mls of retro orbital blood sample was collected into and EDTA bottle. A microhaematocrit tube was filled through capillary action with the blood sample and centrifuged at 10,000rpm for five minutes. This seperates the blood into layers. The PCV was determined by measuring the length of the layers against a standard chart.

Leucocyte differential count

A thin smear of the blood sample was prepared with blood from the EDTA bottle. Leishman stain was carried out- the smear was completely covered with Leishman's stain and left to stand for 1-2 minutes. The slide was then flooded with distilled water and allowed to stand for 10 minutes ~~es~~. The slide was washed off with distilled water, drained and allowed to dry by keeping it in a slanting position. The slide was then viewed under the microscope and counted with a leucocyte counter.

White blood cell count

Retro orbital blood sample was collected into an EDTA bottle and a 1in 20 dilution of the blood was prepared with Tuck's solution, the Tuck's solution lysis the red blood cell. With the aid of a pipette, a counting chamber was loaded with the mixture and counted under the 10X objective of the microscope.

The animals were sacrificed after 30 days using chloroform soaked in cotton wool in a desiccator.

Extraction of organs

For extraction of organs, the rats were placed on a dissecting board in the anatomical position with their anterior (ventral side) facing upwards and their legs and fingers stretched and pinned for easy dissection. A pair of blunt scissors was used to make a midline incision along thorax

and abdominal region in each rat to avoid damage to the visceral organs. The lungs, kidney, stomach, liver and intestines were harvested and preserved in 10% buffered formalin for histopathological examination.

Statistical analysis: Statistical analysis was carried out using Window SPSS. One way analysis of variance (ANOVA), T test and Chisquare were adopted for comparison. The data were expressed as Mean±Standard Error and values of $p<0.05$ were considered significant.

Results

While the fungi amplicons yielded DNA bands of approximately 650 base pair, that of the bacterial isolates were approximately 850 base pair. Identification of the DNA sequence using the Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology Information (NCBI) website revealed that *Proteus* mirabilis(NR11449.1) and *Escherichia coli* (LN831043.1) Table 3 were identical among the naira notes and the nasal swab isolates for the bacteria while *Aspergillus fumigatus* (MK910068), *Aspergillus flavus* (JQ860302) and *Aspergillus niger* (MK461093) were identical and selected from the fungal category(Table 4.2)

BLAST Analysis of the sequences generated from the bacterial and fungal isolates

The output of the BLAST query of the sequences produced significant hits. The percentage identity ranged from 89% - 99%, total bit score obtained in all ranged from 937–1563 for the bacteria isolates sequence while the percentage identity for the fungi isolates sequence was

99%-100%, total score obtained in all the sequence ranged from 1112 - 1779. The query coverage spanned between 88% and 99% while the e-value for all sequence was zero for both bacteria and fungi isolates.

Sample IDs	Sequence length	Hit in NCBI database	Total score	Query coverage	E-value	% Identity	Accession No
Isolate 1	899	*Bacillus subtillis*	1110	96	0	89.93	MF983544
Isolate 2	897	*Alcaligenes faecalis*	1522	95	0	98.61	AJ277669
Isolate 3	909	*Providencia stuartii*	1531	95	0	98.73	CP031508
Isolate 4	920	*Alcaligenes faecalis*	937	88	0	87.95	MH801132
Isolate 5	889	*Bacillus amyloliquefaciens*	1563	96	0	99.42	KR045286
Isolate 6	880	*E.coli*	1509	99	0	99	LN831043
Isolate 7	840	*Proteus mirabilis*	1530	99	0	99	NR114419

Table 3: BLAST outputs of total score, query coverage, e-value, percentage identity and accession number obtained from the bacterial isolates sequence.

Sample IDs	Hit in NCBI database	Strain	Total score	Query coverage	E-value	% Identity	Accession No
Isolate 1	*Aspergillus sp.*	SS3	1115	99	0	100	KX928746
Isolate 2	PS	-	PS	PS	PS	PS	PS
Isolate 3	*Aspergillus niger*	IR3	1112	96	0	99.51	MK461093
Isolate 4	*Aspergillus fumigatus*	-	1133	99	0	99.36	MK910068
Isolate 5	*Aspergillus niger*	01NT.1.5.4	1134	99	0	99.52	MH095994
Isolate 6	*Aspergillus Flavus*	-	1779	99	0	99	JQ860302
Isolate 7	*Candida albicans*	WM 2	1761	100	0	99	JN941105

Table 4: BLAST outputs of total score, query coverage, e-value, percentage identity and accession number obtained from the fungal isolates sequence.

In the pathogenicity test, the rats inoculated orally with *Escherichia coli* and *Proteus mirabilis* presented with watery stool and reduction of weight by $16^{\pm}0.4$g after two weeks of commencement of inoculation. They showed reduction in activity and reduced locomotion when compared with the control. There was no observable changes in the other test groups.

The haematological study revealed that the mean PCV in % are $39^{\pm}1.0$ for the *E.coli*, $40^{\pm}0.4$ for *P.mirabilis*, $35^{\pm}0.2$ for *A.niger*, $40^{\pm}0.7$ for *A.fumigatus* and $37^{\pm}0.1$ for *A.flavus*. These varied significantly at $p<0.05$ with the control which has mean PCV of $45^{\pm}0.3$. The differential leucocyte count showed a marked increase in the % neutrophil (*E.coli* $73^{\pm}0.1$, *P. mirabilis* $70^{\pm}0.1$, *A.niger* $78^{\pm}1.1$, *A. fumigatus* $59^{\pm}0.3$ and *A. flavus* $62^{\pm}1.0$) when compared with control with percentage neutrophil of $20^{\pm}0.2$.(Table 4.1) There was also an increase in the white blood cell count of the test groups when compared with the control.(Table4.2)

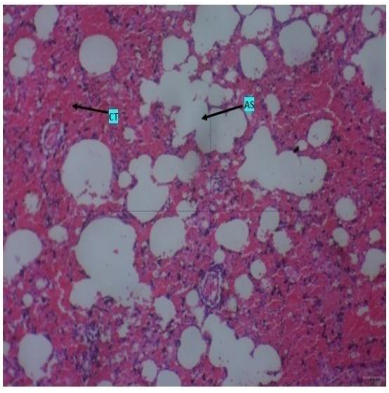
TABLE 5: FULL BLOOD COUNT ANALYSIS

		LYMPHO CYTES	NEUTRO PHIL	EOSINO PHILS	BASO PHILS	MONO CYTES	PCV	WBC COUNT
CONTROL		74±0.7	20±0.2	3±0.1	1±0.1	2±0.6	45±0.3	12300±1.5
E.coli	ORAL	30±0.1 [ab]	65±0.4 [ab]	3±0.4	0	2±0.3	39±1.0	13500±2.2 [b]
	INTRA.	20±0.3 [ab]	73±0.1 [ab]	3±0.1	0	0	36±1.2	14200±0.9 [b]
P.mirabilis	ORAL	30±0.2 [ab]	59±0.7 [ab]	5±0.2	2±0.1	4±0.5 [b]	40±.4 [b]	14000±1.2
	INTRA.	25±1.0 [ab]	70±1.0 [ab]	4±0.3	1±0.2	1±0.1 [b]	34±.5 [b]	13400±1.5
A.niger	INTRA.	22±0.6 [ab]	78±1.1 [ab]	1±0.4	0	0	35±0.2	13000±1.9
	NASAL	34±0.5 [ab]	62±0.3 [ab]	3±0.2	0	0	32±0.4	11400±2.5
A.fumigatus	INTRA.	31±0.6 [a]	59±0.3 [ab]	4±0.3 [b]	2±0.3	2±0.1	40±.7 [b]	13500±1.1
	NASAL	30±1.2 [a]	66±0.1 [ab]	2±0.1 [b]	1±0.1	1±0.3	33±.3 [b]	12800±1.3
A.flavus	INTRA.	24±1.0 [ab]	68±0.8 [ab]	3±0.4	1±0.2	2±0.1	43±0.2	12900±0.8 [b]
	NASAL	34±0.6 [ab]	62±1.0 [ab]	2±0.5	0	1±0.2	37±1.0	14100±2.1 [b]

INTRA - Intraperitonal. T test analysis between each test group and control and between the two routes of inoculation. Superscript ‘a’ show significant difference between test and control, while ‘b’ shows difference between the two routes of inoculation ($P<0.05$)

Histopathological study did not reveal any distortion of the normal architechture of the stomach, liver, spleen and heart both for the normal and the test samples. However the lungs of the rats inoculated nasally with *A.niger* showed necrosis of the alveolar epithelium (Fig 1)

A B



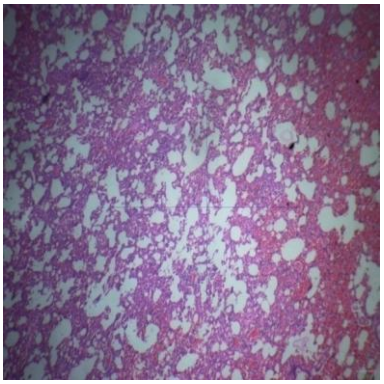


Fig 1

A. Photomicrograph of the lungs showing necrosis of alveolar epithelium(**AS)** and acute inflammation. Connective tissue **(CT)**show eosinophillic inclusions.

B. Photomicrograph showing normal histoarchitechture of the lungs.

DISCUSSION

From the present study, microorganisms of both bacterial and fungal origin were found on ~~contaminate~~ naira notes from banks within Enugu metropolis. This may not be unconnected with unhygienic handling of these notes by individuals from different walks of life. Since naira notes are a major means of exchange of goods and services, there is little or no restriction on the handling. Though penalties exist for individuals that abuse these naira notes according to CBN Acts 2007, they are hardly enforced hence the upward trend in the level of abuse. The present study has further buttressed the findings of Kumar et al, 2009, which identified paper currency as another mode by which community acquired Staphylococcus aureus may be transmitted since paper currency is frequently transferred from one person to another. Likewise Allan *et al* (2018) in a research carried out in Uganda revealed that the Ugandian Paper money in circulation was heavily contaminated with microorganisms of public health concern.

The constant exposure of unprotected bank workers to aerosols from the notes during counting with counting machines puts them at risk of inhalation of spores from these notes. According to Enemour *et al.* (2012) , about 70% of all the currency counting machines from banks studied were contaminated with microorganism of both bacterial and fungal origin. These machines could also be another reservoir for these contaminants and pass them on to the naira notes during counting. Preliminary and molecular identification of the

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isolates revealed that some of these contaminants belong to the following bacterial and fungal genera – Enterobacter, Staphylococcus, Streptococcus, Pseudomonas Aspergillus and Penicillium etc. Possible sources of these organisms include unwashed hands, sc clothing, ~~feacal~~fecal materials.~~.~~ Some of the isolated organisms are potential disease-causing agents. The pathogenicity result of the present study revealed that rats inoculated orally with *Escherichia coli* and *Proteus mirabilis* presented with watery stool and reduction of weight after two weeks of commencement of inoculation. This calls for serious caution during handling of these naira notes as similar effects may be obtained in humans who may ingest some of these organisms through unwashed hands after handling these coliforms that are commonly used as an indicator of sanitary quality of foods and water. According to Sushil *et al.* (2011) who demonstrated that paper currency in Ajmer, India were contaminated with a number of coliforms, the high prevalence of *E. coli* points to the fact that these notes may be reservoir of enteric pathogens. The significant increase in the mean Packed cell volume and differential leucocyte count of test rat blood sample compared with the control suggests that these organisms are potential disease causing organisms . Histopathological study of the lungs of the rats inoculated nasally w *Aspergillus niger* showed necrosis of the alveolar epithelium which is suggestive aspergillosis. Aspergillosis is an infection caused by *Aspergillus*, a common mold (a ty of fungus) the people with weakened immune systems or lung diseases are at a higher r of developing health problems due to *Aspergillus*. The types of health problems caused by

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Aspergillus include allergic reactions, lung infections, and infections in other organs.(Danner et al, 2008)

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Recommendation and Conclusion

Potentially pathogenic microorganisms have been isolated both from naira notes and nasal swabs of tellers from selected banks in Enugu metropolis and this should be an issue of public health concern. Safety measures should be put in place both by individuals and Government to ensure safety of handlers. I strongly recommend washing of hands with detergents or use of hand sanitizers before and after handling naira notes. This will help to reduce person to person transmission of these microbial contaminants particularly those who may have been involved in activities that exposed these notes to pathogenic organisms. Protective covering eg face mask should be made available to all bank note counters whose duties involve processing of these naira notes. Regular servicing and cleaning of the counting machines with disinfectant is recommended to keep the microbial load low. This is because these machines could serve as reservoir for these potentially pathogenic organisms. Enlightenment programmes should be embarked on to educate the masses. Various E-payment options should be promoted to reduce the volume of naira notes in circulation and ultimately the risk of handling.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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