Isolation, Prevalence and Antimicrobial Sensitivity Pattern of Microbial Contaminants on Paper Notes; a Study of Naira Notes

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Abstract: Six hundred and twenty four pieces of different denominations of naira notes obtained from thirteen different banks in Enugu metropolis were examined for bacterial and fungal contamination using the swab-rinse technique. The isolates were first characterized using their morphological and biochemical properties. Identification of the Genomic DNA sequences successfully extracted were carried out using the Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology Information (NCBI) website. Statistical analysis was done using IBM SPSS statistical package. The bacteria amplicons yielded DNA bands of approximately 850 base pair while the fungi amplicons were approximately 650 base pair in length. Bacterial contaminants formed 65% of the total isolates while the fungal contaminants accounted for 35% of the isolates. Proteus mirabilis was the most isolated contaminant in the bacterial genera 64(9.6%) while Streptococcus pyogenes had the least frequency of isolation 10(1.5%). Other bacteria isolated included Escherichia coli 45(6.7%), Klebsiella pneumonia 14(2.1%), Bacillus subtilis 39(5.8%), Pseudomonas aeroginosa 45(6.7%). Alcaligenes feacalis 49(7.3%), Providentiastuartii 40(5.9%), Bacillus amyloliquefaciens 14(2.1%) and Staphylococcus epidermidis 59(8.8%). In the fungal genera, Aspergillus niger had the highest frequency of 11.7% while Sporothrixschenkii had the lowest frequency of occurrence of 1.3%. Other fungi isolated were Penicillinchrysogenum 13(1.9%), Candida albicans 50(7.5%), Fusarium solanii 14(2.1%), Aspergillus flavus 24(3.6%), Aspergillus fumigatus 25(3.7%) and Geotrichumcandidum 21(3.1%). The 100 naira notes showed the highest mean viable count of $54\pm0.1 \times 10^4$ CFU/M) and the 20 naira notes had the least $18\pm0.3 \times 10^4$ CFU/ML. Statistical significant difference was established between the means of the colony counts of isolates on the various naira notes at p<0.05.P. mirabilis showed high resistance to Cefatzidine 22(85%) and Cefriazone 20 (77%). E. coli was 24(92%) resistant to Cefriazone and 69% resistant to Ceftazidine. A.fumigatus was 56% resistant to Miconazole while A. flavus was 55% resistant to Miconazole. This study has shown that naira notes could be a reservoir of microorganisms of medical importance which in turn become vectors for the transmission of diseases in the society. Considering these findings, adequate measures should be put in place to reduce contamination of naira notes and ensure safety of handlers.

Keywords: Naira Notes, Paper Money, Microbial Contaminants, Colony Counts, Genomic DNA Isolation

I. INTRODUCTION

Paper currency is the most common medium for exchange of goods and services all over the world [19]. The crude materials that are utilized for making these paper currency have been reported to play significant roles in harboring of microorganisms[4],[23]. Earlier studies have associated paper money with transfer of various microorganisms. [15],[17]. This is because, paper currencies are contaminated in the course of business transactions and other routine activities as a result of some unhygienic activities, such as sneezing, coughing, contacting with tainted hands or materials and placement on grimy place like pockets, socks, shoes and under floor covering [16],[17]

Today, Currency notes, have been reported by researcher in very different countries [7] to be fomites and vehicles for the transfer of microflora from one user to another [10]. This spread of pathogen and Infection via contact with infected surfaces poses a greater dimension this time around more than any time in the past as a result of the ravaging COVID -19 all over the world. Coronavirus disease (COVID- 19) is major public health concern since December 2019. Since its detection in Wuhan, China. This virus called the severe acute respiratory syndromecoronavirus 2 (SARS-CoV-2) is highly infectious and has recorded more than 3 million cases within aspace of four months of outbreak.

Naira is the authorized currency in Nigeria and Enugu is the capital city of Enugu State located in South Eastern Nigeria. The Nigerian Security Printing and Minting is the firm that mints naira notes under the regulation of the Central Bank of Nigeria. The Central Bank of Nigeria (CBN) controls the volume of money supplied in the economy in order to ensure monetary and price stability. [18] Presently there are eight denominations of the Naira notes and these include N5, N10, N20, N500, N1000.

There is an increase 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. Naira notes are meant to last for as long as24 months but the way the notes *are* handled reduces the life span[18]. Contamination of these naira notes could be from different sources, it could be from the atmosphere, during storage, usage, handling or production [16],[15]. In the process of handling, these notes come in contact with air, water, food, feaces, saliva (during counting) and soil among others. In a bid to avoid theft, some people hide these notes in their pants, brassiers, socks and other obscure places

Paper currency notes which are transferred from one person to another are known to carry bacteria on their surface [1]. Ghanaian paper notes in circulation were found to be contaminated with pathogenic microorganisms which can spread human diseases [7], [13].

Some researches carried out in Ghana, Kenya, Iran and Sudan have reported high rate of microbial contamination of their currency notes in circulation. [20], [3]. A research carried out in Kano, Nigeria also reported contamination of Naira notes in circulation [10]. Some of these researches showed that these contaminants include potential pathogens that may cause diseases in healthy individual[12].Naira notes in circulation in Nigeria poses risk to public health since infectious diseases can be transferred through this medium[22].

This study was designed to determine the nature and prevalence of bacterial and fungal contaminants on Naira notes from Banks in Enugu metropolis and their antimicrobial sensitivity pattern.

II. 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.

Collection of Samples:

Six hundred and twenty four pieces of various denominations of Naira notes were collected from thirteen commercial banks in Enugu metropolis. The respective persons were requested to place the samples directly into sterile bottles to avoid further contamination. These samples were immediately taken to the laboratory for analysis

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. Sterile forceps was used to remove the swabinto distilled water and was adequately shaken to dislodge the swabbed content into the solution. After removal of the swab, the solution was homogenized [8]. The solution was inoculated into Sabourad Dextrose Agar, Nutrient agar, MacConkey and Blood agar with sterile standard 10 μ l wire loop. The inoculated plates were incubated in an inverted position at 35 -37°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. For the colony count, the resulting solution was added to 90ml of sterile normal saline. 1ml of the resulting solution was serially diluted up to 10⁻⁴ magnitude. 0.1ml of sample homogenate from serial dilutions were inoculated onto freshly prepared nutrient agar and Sabouraud dextrose agar. The plates were labeled and incubated at 37°C for 24 hours to 72 hours to allow growth. The number of colonies was thereafter counted using a colony counter.

Antimicrobial Activity test

Antibacterial susceptibility test was carried out using the Kirby Bauer disc diffusion method as described by Wayne's Clinical Laboratory standard institute [24]. The isolates were tested against six conventional antibacterial agents which include Ceprofloxacilin 10 μ g, Cefriaxone 30 μ g, Augmentin 30 μ g, Ampicillin 10 μ g, Ceftazidin 30 μ g, Chloramphenicol 30 μ g. Sterilized Nutrientwere poured into sterile petri dishes and allowed to solidify. After solidification, the plates were spread with 0.1 ml of the bacterial inoculums (24 hours culture). Antibiotics discs (Fondisk, Lagos, Nigeria) were placed carefully on the plate using a sterile forceps and the plate incubated upside down at 37°C for 24hours andthe diameter of the zone of inhibition measured, and interpreted according to the guidelines of Clinical Laboratory Standards Institute [24].

In the fungal genera, the susceptibility of the isolates to Fluconazole, Miconazole, Ketoconazole and Griseofulvin were determined using the method described by Emenuga and Oyeka [5].The fungal isolates were subcultured and grown at 28^oC in 20mls quantity of sabouraud dextrose broth in bijou bottles for two days, after the second day, sterile glass beads were put into the suspension and homogenized by shaking gently with hand. Two tablets each of 200mg Fluconazole, Miconazole and Ketoconazole were dissolved in 1ml of distilled water to make a concentration of 400mg/ml. Two tablets of 500mg Grisiofulvin tab were dissolved in 2.5mls of distilled water to make a 400mg/ml concentration of all these drugs. Using 400mg/ml of the drug as the stock culture, serial dilution was carried out to obtain 200mg/ml, 100mg/ml and 50mg/ml concentration of the drug. [5].

The agar well diffusion procedure as described by Brooks et al.,[2] was used. Sabouraud dextrose agar was prepared according to manufacturer's instruction. On the surface of SDA in Petri dishes, 0.5mls of the fungal suspension was spread evenly. The plates were kept in that upright position. Using a 4mm diameter cork borer, holes were made in each Petri-dish. These holes were properly labeled. Different 0.5ml dilutions of the antifungal drugs viz 200mg/ml, 200mg/ml and 50mg/ml of Ketoconazole, Fluconazole, Miconazole and Griseofulvin were inoculated into the different holes by using sterile syringes while one hole each served as a control. All

experimental procedures were performed in triplicates. The plates were incubated at 28° c for 48-72hrs then the zone of inhibition was measured with a metre rule and the sensitivity patterns recorded and interpreted using a standard chart.

Genomic DNA Isolation

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

Polymerase chain reaction (PCR) for bacterial DNA viral DNA were performed in a total volume of 10µlAll amplification reaction were performed in a Gene Amp PCR system 9700

Table 3.1: Primer sequence for 16S RNA

Primer	Sequence
16S F	GTGCCAGCAGCCGCGCTAA
16S R	AGACCCGGGAACGTATTCAC

Table 3.2: Primer sequence for ITS

Primer	Sequence
ITS4	TCCTCCGCTTATTGATATGS
ITS5	GGAAGTAAAAGTCGTAACAAGG

DNA Sequencing

Sequencing was carried out with a Big Dye terminator cycle sequencing kit (Applied Bio Systems)[11].

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Sequence analysis

Sequences were identified using the Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology Information (NCBI) website.[11],[14].

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

III. RESULTS

The study revealed that bacteria contaminants were more predominant with a frequency of 65% and a total number of 434 isolates while the fungal contaminants formed 35% of the isolates with a total number of 234 isolates (Figure 4.1).

Proteus mirabilis was the most isolated contaminant in the bacterial genera (9.3%) followed by *Staphylococcus aureus* (8.2%) (see Table 4.3)

In the fungal genera, *Aspergillus niger* had the highest frequency of 11.7% while *Sporothrixschenkii* had the lowest frequency of occurrence of 1.3%. see (see Table 4.3).

The 100 naira notes showed the highest mean viable count of $54^{\pm}0.1 \times 10^{4}$ (CFU/ML) and the 20 naira notes had the least $18^{\pm}0.3 \times 10^{4}$ (CFU/ML).Figure 4.2

Using analysis of variance, statistically significant difference was established between the means of the colony counts of isolates on the various naira notes at p<0.05.

P. mirabilis showed high resistance to Ceftazidine (84%) and Cefriazone (74.6%). *E. coli* was 93% resistant to Cefriazone and 96% resistant to Ampicillin. *A.fumigatus* was 56% resistant to Miconazole while *A. flavus* was 48% resistant to Miconazole

N/S	Colony appearanc	Gram stai	Catalase	Coagulase	Oxidase	Citrate	Glucose	Sucrose	Lactose	Maltose	Xylose	Arrangem nt	Colony appearanc
1	Proteus mirabilis	Large swarmy creamy colonies on NA	-	+	-	+	+	+	+	+	-	I	Short single rods
2	Escherichia coli	Pink shiny smooth colonies on MacConkey	-	+	-	-	-	+	-	+	+	+	Short single rods
3	Bacillus Subtilis	White glossy round colonies on NA	+	+	-	+	+	+	+	+	+	+	Single and paired rods
4	Pseudomonas aeroginosa	Greenish glossy pigmented colonies on NA	+	+	-	+	+	+	+	-	+	+	Straight and slightly curved rods
5	Staphylococcu s aureus	Cream circular smooth colonies on NA	+	+	+	-	-	+	+	+	+	+	Single paired and irregular clusters
6	Alcaligenes feacalis	Non pigmented to grayish white on NA	+	-	-	-	-	+	+	+	+	+	Paired and short chains
7	Staphylococcu s Epidermidis	Smooth circular and creamy colonies on NA	+	+	-	-	-	+	+	+	+	+	Single paired and irregular clusters

Table 4.1 Identification of Bacterial Isolates

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8	Klebsiella pneumoniae	Creamy mucoid and round colonies on Blood agar	-	+	-	-	+	+	+	+	-	-	Short rods
9	Streptococcus pyogenes	Smooth white colonies	-	+	-	-	-	-	+	+	+	-	Short and long rods
10	Bacillus amyloliquefaci ens	Smooth white colonies on NA	+	+	_	-	-	-	+	+	+	_	Short and long rods
11	Providentiastu artii	Smooth white colonies on NA	-	+		-	+	-	+	-	-	+	Short and long rods

Table 4.2 Identification of Fungal Isolates

S/N	Appearance of colony	Microscopy	Organism
1	Filamentous flat gray colonies at the centre and whitish at the periphery.	Septate hyphae with conidiophores that are branched	Penicillumchrysogenum
2	Wooly flat spreading colonies that appeared white	Septate hyphae with Macroconidia that are fusiform in shape.	Fusarium solanii
3	Rapid growing white wooly colonies that turned black over time	Septate hyphae with unbranched conidia enlarged at the tip	Aspergillus niger
4	White colonies that turned yellowish green over time	Septate hyphae with unbranched conidia of different lengths	Aspergillus flavus
5	Shiny white mucoid round colonies	Septate hyphae with characteristic dichotomous branching	Candida albicans
6	Moist and glabrous wrinkled and folded surface	Septate hyphae with ova shaped conidia resembling flower	SporothrixSchenkii
7	White colonies that turned greenish over time	Septate hyphae with unbranched conidia	Aspergillus fumigates
8	Fast growing whitish flat membranous surface	Wide septate hyphae segmented into rectangular arthroconidia with variable size	Geotrichumcandidum



FIG 4.1: Percentage Occurrence of Bacterial and Fungal Isolates

Table 4.3 Distribution of Microbial Isolates According To Denomination of Naira Notes

ISOLATES	₩ 5(%)		₩ 10(%)		₩ 20(%)		▶ 50(%)		₩ 100(%)		₩ 200(%)		₦ 500(%)		₦ 1000(%)		TOTAL
Proteus mirabilis	7	(10.9)	8	(12)	3	(4.7)	7	(10.9)	9	(14)	12	(18.7)	10	(15.6)	8	(12.5)	64(9.6)
Escherichia coli	4	(8.9)	3	(6.7)	2	(4.4)	5	(11.1)	10	(22.2)	8	(17.8)	8	(17.8)	5	(11.1)	45(6.7)
Klebsiella pneumonia	3	(21.4)	0	(0)	0	(0)	0	(0)	5	(35.7)	3	(21.4)	2	(14.3)	1)7.1)	14(2.1)
Bacillus subtillis	2	(5.1)	5	(13)	3	(7.7)	4	(10.2)	9	(23)	7	(17.9)	5	(12.8)	4	(10.2)	39(5.8)
Pseudomonas aeroginosa	8	(17.8)	4	(8.9)	6	(13.3)	7	(15.5)	5	(11.1)	0	(0)	7	(15.5)	8	(17.8)	45(6.7)
Staphylococcus aureus	8	(14.6)	5	(9.1)	3	(5.5)	7	(12.7)	11	(20)	8	(14.6)	4	(7.3)	9	(16.4)	55(8.2)

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Alcaligenes faecalis	3	(6.1)	7	(14)	4	(8.2)	3	(6.1)	8	(16.3)	10	(20.4)	11	(22.4)	3	(6.1)	49(7.3)
Staphylococcus epidermidis	7	(11.8)	4	(6.8)	5	(8.5)	8	(13.5)	10	(16.9)	9	(15.2)	7	(11.8)	9	(15.2)	59(8.8)
Streptococcus pyogenes	0	(0)	0	(0)	3	(30)	1	(10)	2	(20)	4	(40)	0	(0)	0	(0)	10(1.5)
Providentiastuartii	5	(12.5)	2	(5)	3	(7.5)	8	(20)	7	(17.5)	4	(10)	6	(15)	5	(12.5)	40(5.9)
Bacillus amyloliquefaciens	3	(21.4)	2	(14)	4	(28.6)	1	(7.1)	0	(0)	0	(0)	1	(7.1)	3	(21.4)	14(2.1)
Penicillumchrysogenum	0	(0)	1	(7.7)	0	(0)	4	(30.8)	1	(7.69)	2	(15.4)	3	(23.1)	2	(15.4)	13(1.9)
Aspergillus niger	11	(14.1)	8	(10)	9	(11.5)	7	(9)	15	(19.2)	13	(16.6)	8	(10.2)	7	(9)	78(11.7)
Aspergillus fumigates	2	(8)	3	(12)	0	(0)	5	(20)	8	(32)	0	(0)	4	(16)	3	(12)	25(3.7)
Aspergillus flavus	2	(8.3)	1	(4.2)	0	(0)	5	(20.9)	8	(33.4)	0	(0)	3	(12.5)	5	(20.9)	24(3.6)
Sporothrixschenkii	0	(0)	0	(0)	0	(0)	3	(0.1)	5	(0.45)	0	(0)	0	(0)	1	(0.1)	9(1.3)
Candida albicans	7	(14)	5	(10)	3	(6)	7	(14)	10	(20)	11	(22)	5	(10)	2	(4)	50(7.5)
Fusarium solanii	0	(0)	0	(0)	3	(21.4)	4	(28.6)	5	(35.7)	1	(7.1)	0	(0)	1	(7.1)	14(2.1)
Geotrichumcandidum	3	(14.3)	0	(0)	0	(0)	4	(19)	2	(9.5)	5	(23.8)	5	(23.8)	2	(9.5)	21(3.1)
TOTAL	75	(11.3)	58	(8.7)	51	(7.7)	90	(13.5)	130	(19.5)	97	(14.6)	89	(13.4)	78	(11.7)	668(100)
Chi square analysis showed statisticlly significant relationship between the occurrence of isolates and the various naira notes. (P<0.05)																	



Denominations

Fig 4.2: Colony counts on paper notes (CFU/ML X 10⁴)

Using ANOVA test, statistical significant difference was established between the means of the colony counts of isolates on the various naira notes at p<0.05.

IV. DISCUSSION

The present study revealed that naira notes from banks within Enugu metropolis South Eastern Nigeria, are contaminated with microorganisms of both bacterial and fungal origin. It was also observed that the $\aleph100$ and $\aleph200$ had the highest microbial load and coincidently were the most mutilated in that category. The high prevalence of *E. coli*points to the fact that these notes may be reservoir of enteric pathogens. Sushil et al., [21] also demonstrated that paper currency in Ajmer, India were contaminated with a number of coliforms. These coliforms are commonly used as an indicator of sanitary quality of foods and water. These notes come in contact with different parts of the human body which may be responsible for the high percentageof *Staphylococcus aureus* and other normal flora of the human body in the recovered organisms. According to Kumar et al, [12], paper currency has recently been identified as another mode of spread by which community acquired *Staphylococcus aureus* may be transmitted since paper currency is frequently transferred from one person to another. Similarly, Allan*et al.*,[1] in a research carried out in Uganda revealed that the Ugandian Paper

money in circulation were heavily contaminated with *Staphylococcus aureus*.

Alcaligenes feacalis also recovered from this present study is an organism predominantly found in human feaces. It is an opportunistic pathogen that may trigger infection including peritonitis, meningitis, otitis media and blood stream infections [9]. Streptococcus pyogenes which accounted for 1.5% of the isolated organisms is also a gram positive organism of medical importance. It is an infrequent, but usually pathogenic part of the skin microbiota. They could colonize the throat and skin of individuals and are responsible for a number of supportive infections. [25]. Candida albicans isolated from these naira notes is an opportunistic pathogenicyeast that is a common member of the human gut flora [6]. The practice of counting naira notes with saliva will likely expose these notes to contamination with this organism. Though commensals can become pathogenic in immuno compromised individuals under a variety of conditions causing infection ranging from superficial infection of the skin to life threatening diseases. The multidrug resistance of some of the isolates egProteus mirabilis which had high resistance to Cefatzidine (84%) and Cefriazone (74.6%) and which Escherichia coli had high resistance to Cefriazone(93%) and Ampicillin (96%) and Ampicillin(96%) is indicative of an upward trend in antimicrobial resistance. In cases where human beings get infected with these organisms, some of these antimicrobial agents are meant to be used for therapeutic purposes. This trend therefore has a serious public health implication.

V. RECOMMENDATION AND CONCLUSION

Many potentially pathogenic and multidrug resistant microorganisms have been isolated from currency notes both in this study and previous ones within and outside Nigeria making it an issue of global concern. Hence control measures should be put in place both by individuals and Government to ensure safety of handlers. Hand washing cannot be overemphasized both before and after handling naira notes. Adequate provisions should be made for all bank note counters whose duties involve counting these naira notes on a daily basis. In the process of counting, aerosols from the notes and the counting machines may be inhaled so as a matter of policy, nose masks should be worn by these individuals while discharging their duties. Hand gloves are also recommended for these set of bank workers. Fume extractors should be installed and maintained in the counting rooms of banks as it was observed during the course of the study that most counting rooms had unpleasant odour as a result the abused and poorly handled naira notes. There is a serious need for Nigerian government to start enforcing the penalties associated with the violation of Naira handling policies. Banks should be encouraged to retrieve bad and mutilated bills from individuals and move to Central Bank of Nigeria for destruction. Knowledge is power so there should be awareness campaigns from time to time on the implication of unhygienic handling of these naira notes. In the present study, isolation of bacteria as well as fungi of medical importance from Naira notes confirmed that these notes might be playing a key role as a reservoir and vector in the transmission of diseases in the society.

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