

Isolation and Identification of Bacterial Contaminants on Naira Notes Used in Parts of Ohaji/Egbema LGA, Imo State, Nigeria

Nwabueze E.U.¹; Ezekwe, C.O.²; and Nwabueze N.O.³

¹Department of Biological Sciences, University of Agriculture and Environmental Sciences Umuagwo.

²Department of Microbiology, Imo State Specialist Hospital Owerri. ³Department of Animal and Environmental Biology, Imo State University, Owerri.

Corresponding author: ekeuzo20@gmail.com

DOI: <https://doi.org/10.62154/nk05ke19>

Abstract

This study was designed to isolate and identify the various micro-organisms that contaminate naira notes used in parts of Ohaji/Egbema LGA. A total of one hundred (100) pieces of ₦10, ₦20, ₦50, ₦100, ₦200, ₦500 and ₦1,000 denominations were collected from different points which include traders, beggars, bus conductors, students, food vendors and banks. These notes were separated into four on the basis of their physical state: mint, clear, dirty and mutilated. Each of the notes were immersed in 10ml of sterile distilled water and allowed to stand for 30 minutes. The solution was later inoculated into nutrient agar, MacConkey agar, mannitol salt agar. The colonies were also identified using standard biochemical test. From the result of the study, the highest and lowest bacterial count were found in ₦20 and ₦100 notes, whereas the mean average bacterial count on those notes were between 1.6×10^3 CFU/ml to 2.6×10^3 CFU/ml. the bacterial isolates identified includes staphylococcus aureus, Escherichia coli, pseudomonas aeruginosa and streptococcus spp. The result of the study revealed that naira notes in circulation in parts of Ohaji/Egbema LGA could be a potential source of transmitting pathogenic micro-organisms.

Keywords: Naira Notes, Ohaji/Egbema, Micro-Organisms, Isolation, Identification.

Introduction

Naira notes are legal tender in Nigeria. This money is in general use and it enjoys the backing of the Federal Government of Nigeria Paper currency is passed from one individual to another in the Nigerian population. As such it undergoes handling by numerous hands. Both clean and dirty notes can be contaminated by droplets during sneezing, touching with hands and placement on dirty surface. Money is commonly handled by various categories of people during transaction. Paper currency is widely exchanged for goods and services in most countries worldwide. If some of these are contaminated there is the potential to spread the organisms from person to person and across borders since money is not screened for microbes (Graig, 1996).

Fomites which are inanimate objects are capable of absorbing, harbouring and transmitting infectious microorganisms. Dust and dirt that commonly accumulate on such objects

contains spores of infectious agents, (Oyero et al., 2007). Contamination of objects by pathogenic microorganisms is of public health concern as contaminated materials can be a source of transmitting pathogens. Paper money, therefore present a particular risk to health since communicable disease can be spread through contact with fomites (Michael, et al. 2002).

The possibility that currency notes might act as environmental vehicle for the transmission of potential pathogenic microorganisms was suggested in the 1970s (Abrams and Waterman, 1972). Paper currency is widely exchanged for goods and services in countries worldwide and it is used for every type of commerce. Although paper currency is constantly abused in most parts of the world, including Nigeria, and other developing nations. The average U.S dollars, for instance - like most currency notes worldwide last a mere 18 months (Gadsby, 1998). Paper currency also provides a large surface area as a breeding ground for pathogens (Podhajny, 2004).

Money on which pathogenic microorganisms might survive represents an often over looked reservoir for enteric disease (Michaels, 2002). In most parts of the developed world, there is a popular belief that the simultaneous handling of food and money contribute to the incidence of food – related public health problems. Over the last two decades, data indicating that simultaneous handling could indeed be a cause of sporadic food borne illness. Data have shown from studies that microbial infections and survival of pathogens on coins and currency notes could constitute public health problems in Turkey (Dow-Jones News, 1998).

In Nigeria, naira notes are highly abused especially in the way and manner they are handled during transactions. These activities could lead to mutilation, tearing, squeezing and writing on them (Gadsby, 1998). As a result of daily transactions, naira notes usually pass through many hands or are placed in dirty places that can predispose them to microbial contamination. Airodi et al (2002) reported that the source of contamination of naira notes could occur as a result of poor handling or practices like spraying during ceremonies.

Statement of Problem

Bacterial contamination of naira notes has become a serious public health problem due to our activities such as spraying, selling, squeezing and defacing of naira notes. These acts usually make the notes to become vulnerable to pathogenic microbes which usually pose serious health problems whenever they infect man.

Objectives

To isolate and identify the different bacterial species that contaminate naira notes used in parts of Ohaji/Egbema L.G.A.

Research Questions

- Can micro-organisms survive on a dry paper and polymer.

- How can money survive as a vehicle for transmission of pathogenic micro-organisms.

Material and Method

Sample Collection

The naira notes were 10, 20, 50, 100, 200, 500, 1000 were obtained from five major sources. The samples were randomly obtained by purchasing an item or paying for a service using a large denomination note, this created the need for change (balance) to be given. The change was placed in a sterile aluminium foil, the foil was sealed with a masking tape and the sample was taken to the laboratory for analysis.

The currency notes were in their various physical conditions and were categorized as clean, or dirty/mutilated. The term clean describes notes that had a clean appearance without any obvious damage. The term dirty describes notes that had physical dirt on them.

Preparation of Samples

All the denominations collected were subjected to microbial analysis in a sterile atmosphere by swabbing the work benches with 95% ethanol. Samples of the naira notes were soaked into 10ml sterile distilled water contained in a beaker and allowed to stand for 30 minutes. After the time interval, the money was removed using a sterile forceps. The solution washed out from the money was then diluted serially into nine folds. Serial dilutions were carried out using a sterile pipette to pipette one ml from the mixed sample into the test-tube, labelled 10^1 and then shake thoroughly. One ml of the sample was pipetted from 10^1 into the next labelled test-tube 10^2 and then mixed. These same procedures were carried out in all the samples and were used for total viable count.

Preparation of Media

Media like MacConkey Agar, Nutrient agar and Sabouraud dextrose agar were used in this study. They were weighed and prepared according to the manufacturer's specification. The media were prepared in conical flask and was diluted with distilled water. The prepared media were carefully packed into the autoclave and sterilized at 121°C for 15 minutes prior to use. It was allowed to cool at 45°C before pouring them into different petri-dishes and allowed to solidify.

Inoculation of Test Samples

After solidification of the media on the plate, 1 ml of each sample solution was pipetted from the fifth tube and spread on the solidified agar plate. This was carried out in all the samples and was incubated at 37°C for 24 hours.

Identification of Test Organisms

Some of the test carried out for the identification of the bacterial isolates includes:

Gram Staining

A wire loop was sterilized in Bunsen burner and allowed to cool then a loopful of growth was collected from the agar plate and applied on a clean grease-free slide then a drop of normal saline was added, emulsified and heat fixed by passing over a flame three times. The smear was flooded with crystal violet for 30-60 seconds and then covered with iodine for 30-60 seconds and then washed off, it was decolorized with acetone until no colour runs off the slide and rinsed immediately. The slide was covered with safranin for 1 minute and then washed off with clean water. The slide was covered with safranin for 1 minute and then washed off with clean water. The slide was kept in a rack to air dry after wiping the back with cotton wool. The stained smear was then examined microscopically under oil immersion at 100x objective lens. Gram - positive bacteria appeared dark purple while gram-negative bacteria appeared pink.

Motility Test

Motility test was aimed at identifying motile bacteria.

Method: A drop of normal saline was placed on a sterile slide and colony of test organism was suspended and emulsified and then covered with a cover slip. The slide was examined microscopically using 10x and 40x objective lens. Movement in different directions gave a positive test.

Catalase Test

This was used to differentiate those bacteria that produce enzyme catalase such as *Staphylococcus aureus* and *Escherichia coli* used as positive and negative controls respectively.

Method: Three millilitres (3ml) of hydrogen peroxide solution was poured into a sterile test tube. Then a sterile glass rod was used to collect several colonies of the test organisms and inoculate into the hydrogen peroxide solution. It was observed for immediate active bubbling for positive test.

Coagulase Test

This was used to identify *Staphylococcus aureus* which produces the coagulase enzyme which causes plasma to clot converting fibrinogen to fibrin. The slide method was used.

Method: A drop of sterile distilled water was placed on each end of a sterile slide. Then a colony of the test organism was emulsified on each spot to make two thick suspensions. A loopful of plasma was added to one of the suspensions and mixed gently. The slide was examined for clumping or clotting of the organisms within 10 seconds. Plasma was not added to the second suspension which serves as control.

Vogues-Proskauer Test

This test was used to identify members of the Enterobacteriaceae that produce acetylmethylcarbinol (acetone) a natural product formed from pyruvic acid in the course of glucose fermentation.

Method: Buffered glucose broth was inoculated with the test organism and incubated at 37°C for 3 days. Three millilitres (3ml) of naphthol was then added followed by 3ml of sodium hydroxide solution, mixed well and allowed to stand for hour at room temperature. The formation of a pink colour in the medium within 1 hour indicates a positive result. *Klebsiella pneumonia* and *Escherichia coli* were used as positive and negative controls respectively.

Indole Test

This test was carried out for indole production by test organism which is important in identifying enterobacteria.

Method: A sterile wire loop was used to incubate a colony of test organism into 2ml of peptone water containing tryptophan. The tube was stoppered and incubated at 37°C for 24 hours. Kovac's reagent was added to the medium. Observation of red coloration on the surface layer within 10 minutes showed a positive result.

Methyl Red Test

This was carried out to identify *Enterobacteria* based on the ability to produce and maintain stable acid end product from glucose fermentation. *Escherichia coli* were used as positive control.

Method: Glucose phosphate peptone water was used for inoculation of test organisms and incubated for 48 hours at 37°C after which few drops of methyl red solution was added to the culture and read immediately. Formation of red colour immediately showed a positive test.

Glucose Test

Trypticase sodium chloride and phenol red were weighed and dissolved in 100ml distilled water and transferred into conical flasks. 0.5% to 1% of desired carbohydrate was added into all flasks. An inverted Durham tube was sterilized at 1150°C for 15 minutes. Aseptically each labelled carbohydrate broth was inoculated with bacterial culture and was incubated at 18-2 hours at 37°C. Acid production changes the medium into yellow colour-organism. Acid production changes the medium into yellow colour-organism. It can be detected by the presence of small bubbles in the inverted Durham tube.

Results

Tables 1, 2, 3 below shows the bacterial isolates in different nutrient agar and their biochemical characteristics. The bacterial isolates were *Staphylococcus aureus*, *Escherichia*

coli, *Klebsiella spp.* and *Pseudomonas aeruginosa*. The most occurred organisms were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*

Table 1: Total Bacteria Count on Nutrient Agar

Sample	Dilution Factor	No. of Colonies	THBC (CFU/G)
A	10^{-3}	24	2.4×10^5
B	10^{-3}	27	2.7×10^5
C	10^{-3}	28	2.8×10^5
D	10^{-3}	32	3.2×10^5
E	10^{-3}	24	2.4×10^5
F	10^{-3}	30	3.0×10^5
G	10^{-3}	20	2.0×10^5
H	10^{-3}	22	1.2×10^5

Table 2: Total Coliform Count on MacConkey Agar

Sample	Dilution Factor	No. of Colonies	THBC (CFU/G)
A	10^{-3}	20	2.0×10^5
B	10^{-3}	22	2.2×10^5
C	10^{-3}	18	1.8×10^5
D	10^{-3}	10	1.0×10^5
E	10^{-3}	16	1.6×10^5
F	10^{-3}	20	2.0×10^5
G	10^{-3}	22	2.2×10^5
H	10^{-3}	24	2.4×10^5

Table 3: Percentage Occurrence of the Different Microorganism (Isolate)

Isolate	Frequency	Percentage (%)
<i>Staphylococcus aureus</i>	2	20
<i>Escherichia coli</i>	3	30
<i>Pseudomonas</i>	3	30
<i>Klebsiella spp.</i>	2	20
Total	10	100

Table 4: On Naira Identification/Biochemical Test Result of Bacterial and Fungi Isolates Found in Nigeria Currency Notes.

Isolate	Gram Reaction	Cellular Arrangement	IND	C-T	V-P	MR	OX	MOT	CAT	GLU	Probable organism
S ₁	+ve	Cocci in clusters	NR	NR	NR	-ve	-ve	-ve	+ve	-ve	<i>Staphylococcus</i>
S ₂	+ve	Cocci in chains	NR	NR	NR	-ve	-ve	-ve	-ve	+ve	<i>Streptococcus</i>
S ₃	+ve	Cocci	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	<i>E.coli</i>
S ₄	+ve	Rods	+ve	+ve	NR	+ve	+ve	-ve	+ve	+ve	<i>Pseudomonas</i>
S ₅	+ve	Clustered spherical shape	NR	NR	NR	NR	NR	-ve	+ve	-ve	<i>Staphylococcus</i>
S ₆	+ve	Cocci in clusters	NR	NR	NR	-ve	NR	-ve	+ve	-ve	<i>Staphylococcus</i>
S ₇	-ve	Rods	+ve	+ve	NR	NR	+ve	-ve	+ve	+ve	<i>Pseudomonas</i>
S ₈	-ve	Cocci	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	<i>E.coli</i>

KEYS:

+: Positive

-: Negative

CT: Citrate test

VP: Voges-Proskauer

OX: Oxidase

CAT: Catalase

MOT: Motility

MR: Methyl red

IND: Indole

THBC: Total heterotrophic bacteria count

Discussion

The result of the study has shown that majority of the naira notes used in parts of Ohaji/Egbema L.G.A. were contaminated by micro-organisms. The micro-organisms isolated and identified from these currency notes include *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp* and *Pseudomonas aeruginosa*.

This finding is supported by the reports of Graig (2020) in Western Nigeria. They reported that *E. coli* appeared to be the commonest organism isolated for currency notes used in parts of Western Nigeria. The findings also justified the fact that currency notes represent a reservoir of enteric pathogens. The result of the study also revealed that lower denominations like N20, N50 and N100 were most infected by these pathogens. The high viable count of *staphylococcus aureus*, *Escherichia coli*, *pseudomonas aeruginosa* and *Klebsiella spp*s on these lower denominations could be as a result of constant handling of these denominations. Since these lower denominations are always used for change

(balance) by bus drivers, market women and food vendors. It could be possible that this constant exchange from one hand to another may have predisposed them to more contamination by these microbes.

S. aureus is usually able to cause infection whenever there is portal of entry into damaged tissues. They are also associated with exfoliations, impetigo and food poisoning (Grag, 2020). It can be transmitted from person to person to initiate infection. Also, *P. aeruginosa* being one of the species involved in human diseases can cause eye and skin infections as well as otitis media.

Colonization of the notes can also occur due to practices like tongue moistening of finger with saliva when counting money, since it has become common practice to keep money in places like socks, brassieres, underground and dirty surfaces increase the risk of contamination of currency. Therefore, proper use of naira notes will help to reduce contamination by these organisms and improve the public health conditions of the people who live in the study area.

Conclusion

The result of the study clearly shows that significant number of the naira notes used in parts of Ohaji/Egbema L.G.A. were contaminated with different species of pathogenic micro-organisms. The organisms if not properly managed and controlled could become a potential source of infection to the inhabitants of the area.

Recommendations

In order to reduce the concentration and spread of pathogenic microbes through paper currency. The following are recommended:

- The public are advised to observed good personally hygiene especially when they are handling money.
- Regular disinfection of currency notes deposited in bank through ultra—violet rays and formalin vapours.
- The ATM machine should be remodelled to be able to sanitize money through exposure to antibacterials.
- Tongue wetting of fingers used in counting naira notes should be discouraged.
- Cashless policy should be properly end effectively adopted.

References

- Abrams, H. and Waterman, N.G. (1972), Dirty Money. *Journal of the Medical Association*, 219:1202 - 1203.
- Airodi, M.K., Jolks, C.B., Treece, B.L., Julks, M.L.C. (2002). Pathogens associated with abuse of currency in Nigeria. *International Journal of Public Health*, 64(14): 1164 – 1170.
- Anderson, C.D. and May, R.T. (1997), Microbiology. *Journal on Dirty and Mutilated Money*, 20 (4): 520 - 522.
- Brady, D.H, and, Kelly, M.O. (2000), *Pathogens Associated with currencies*. Retrospective Study of Private Diagnostic Laboratories, University's Press, Australia 19; 122- 123.

- Brady, D.H., and Keny, Z.B. (2002). Review of microbial contaminants of currency used in Jos, Plateau State. *International Journal of Advance Research in Science and Technology*, 18: 123-129.
- Cappuccino, J.G. and Sherman, N. (1999). *Microbiology a Laboratory Manual*. The Benjamin/ Cummings publishing Company, Redwood City, California 15; 12- 13.
- Cheeshbroght, M. (1984), *Medical Laboratory Manual for Tropical Countries*. Volume 11, The Thetford press Ltd.
- Christopher, W.K. (1995). Microbiological Survey into the Presence of Clinical Significant, Bacteria on Surfaces, University of Newcastle Upontyne, 34; 12-16.
- Cooper, A.D. (1999). District Laboratory Practices in Tropical Countries 11. Microbiology (ELBS) Butterworths Kent, U.K. 8; 9-20.
- Craig, W.K. (1997). Growth Patterns of some Bacteria Isolated from Nigerian Currencies. *Diagnostic Microbiology and Infectious Disease*, 27(1-2): 49-53.
- Dow, Jones News, (1998). *Dirty and Contaminated Money*. A new short book if Medical Parasitic Infection. ELBS Publishers: 576 - 582.
- El-Din El - Cars Farida, M.S. and M.H. Hassan Wael (2005). A Preliminary Bacterial Study of Egyptian Paper. *International Journal on Environmental Health Research*, 235 - 240.
- Gadsby, A.D. (1996). *Medical Microbiology*. Third Edition, Mosby Publishers, 6:186-190.
- Gadsby, A.D. (1998). Implication of isolating staph aureus in paper money. *International Journal of Advances Research in Science and Technology*, 28: 116 -119.
- Gilchrist, F.A. (1993), Basic Laboratory Procedure in Clinical Laboratory. *General World Health Organization*, 5; 52 – 193.
- Goktas, R.T. and Oktary, A.D. (1992). The Challenges of Emerging Infectious Disease Development and Spread of Multiple Bacteria Pathogens. *Journal of the American Medical Association*, 20: 275:300 - 304.
- Gray, M.B. (1996). Microbial contamination of currency in parts of western Nigeria. *International Journal of Current Research*, 16: 121-126.
- Lavins, E.S., Lavins, B.D. and Jenkins, A.J. (2004). Cannabis (marijuana) Contamination of United States and Foreign Currency. *Journal the Analysis Toxicology*, 28:439-442.
- Michael, M.T., Marlinko, J.M. and Parker, J. (2002). *Brock Biology of Microorganisms*. gin Edition, Prentice Hall, Englewood's Cliffs, New Jerseys10: 957 - 974.
- Moore, C.U. and Miller, G.T. (2005). Publication of Rate of Paper Contamination. *Anatomical and International Medical Journal*, 106: 467-468.
- Olutiola, P.O. and Famorewa, O. and H.G. (2000). An introduction to General Microbiology. *Bola Bay Publication Nigeria* 23: 112-113.
- Oyero, O.G., and Emenike, BO, (2006). Preliminary Investigation on the microbial Contamination of Nigerian Currency. *International Journal Tropical Medicine*, 34:46-50.
- Padhajny, Z.N. (2004). Bacterial contaminants found in paper money in parts of Pakistan. *International Journal of Infectious Diseases*, 33; 1123 – 1128.
- Padhajny, Z.N. (2004). Paper Currency as a Breeding Ground for Pathogens. *Southern Medical Journal*, 94 (4): 365- 369.
- Pelzar, M.J., Chan, E.C.S., and Krieng, N.R. (1998). *Microbiology*. 5th Edition, McGraw - hill College, 5261 Highland Road, PMB 339 Baton Rouge, La 70808,4: 596-599.
- Pinner, R.W., Teutsch, S.T. and Berkelman R.L. (1996). Trend of infectious Disease Mortality in the United States. *The Journal of the Medical Association*, 273 (3): 189-193.
- Siddique, A.C. (2003). Currency Susceptible to being Burnt and damage with Water. Basic Laboratory Procedures in Clinical Laboratory Geneva: *World Health Organization*, 7: 52-193.

- Singh, A.Y., Thakur, B.A. and Kalpana, R.E. (2002). *Isolation of Various Contaminations in Currency*. Medical Microbiology, third Edition, Mosby Publishers. 3:1 70-186.
- Tortora, G.J., and Funks C.L. (1995). *Microbiology An Introduction*. 5th Edition. 6: 753-780.