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ASSESSMENT OF BACTERIA ASSOCIATED WITH NAIRA NOTES

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ABSTRACT

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Background: Microbial transmission on the surface of any currency note can either be through direct (hand to hand contact) or indirect (food or other inanimate objects) means.

Objectives: This study was designed to assess and compare microbial load between two currencies (N100 and N1000 naira note).

Methods: The identification of bacterial species was achieved through gram staining, microscopic analysis and biochemical test (catalase, coagulase, urease, citrate, indole and motility test).

Results: Out of 10 samples, 8 samples were found to be more contaminated by different species of bacteria. N100 notes harbor the highest bacteria load, while N1000 notes harbor the least bacterial load. This study also shows that, Streptococcus pyrogenes was the least encountered as compared to Staphylococcus aureus. Most of the naira notes were wrinkled and dirty especially the N100 naira notes. The bacterial counts were generally high: ranging from 2.0 to 6.2×10^5 cfu/cm². The N100 notes harbor the highest bacterial load (average of 6.2×10^5 cfu/cm²) while N1000 notes had the least $(2.0 \times 10^5 \text{ cfu/cm}^2)$. The occurrence of bacteria isolates on one hundred (N100) notes, four species: Escherichia coli, Staphylococcus aureus, pseudomonas aeruginosa, Streptococcus pyrogenes were isolated. The occurrence of bacterial isolated on one thousand (N1000) notes, three bacterial species: Escherichia coli, Staphylococcus aureus, Pseudomonas aeroginosa. were isolated. The occurrence of the bacterial isolates, i.e Streptococcus pyrogenes was the least encountered (20%) while Staphylococcus aureus was the most encountered (70%).

Conclusions: The study indicated a high concentration of bacteria on one hundred (N100) notes compared to one thousand (N1000) naira notes with 65% and 35% respectively.

Keywords: Bacterial contamination, Naira notes, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Strepto-coccus pyogenes*

INTRODUCTION

Worldwide, currency notes and money in general serve as means of economic exchange of goods and services, to defer payment (Ogunleye, 2005, Awe *et al.*, 2010). Between the late 1800s and early 1900s, Scientists postulated the association of handling money with disease transmission. Subsequently, by modern scientific techniques, these postulations confirmed that pathogenic organisms can be isolated from currency/money surfaces (Awe *et al.*, 2010; Alemu,Alemu, 2014). For example, *Citrobacter ssp.*, *Escheriachia coli*, *Mycobacterium spp.*, *Pseudomonas aerogenosa*, *Salmonella ssp.*, and *Staphylococcus aureus*, are among the examples of food borne pathogenic microorganism reported on currency notes (Awe *et al.*, 2010). In Nigeria, the naira notes presently in circulation are abused by the different ways they are handled and stored which may include but not limited to squeezing, spraying, stapling, cello-taping, keeping naira notes in brassiere, socks and pockets, under the carpet or rugs, writings on them etc (Ameh and Balogun, 2001). However, there is well documented evidence suggesting that currency notes could act as fomites with enormous potential to carry microbes. The contamination of the naira notes could be from several sources, it could be from the atmosphere, during storage, usage, handling or production (Ogba, 2007). The contaminated currency notes go in circulation and contaminate the hands of others and across borders transmitting microorganisms in the process since money is not screened for microbes (Pope et al., 2002). The survival of various microorganisms on money and other fomites, with their transmission via the hands of market men and women and other users is often overlooked as enteric disease reservoir (Michaels, 2002). Pathogenic microorganisms that may survive on currency notes may serve as a potential source of enteropathogens (Michaels, 2002; Cardoen et al., 2009; Lamichhane et al., 2009). Carrier micro-organisms apart from reducing the lifespan of the notes, have been documented to cause infections in the skin, eye, gastrointestinal tract, internal organs (Yildiran, et al., 2006), as well as the respiratory tract (Denning, 2006) in humans. Microorganisms such as Micrococcus spp., Corynebacterium spp., Vibrio cholerae, Mycobacterium tuberculosis and members of the Enterobacteriacea family top the list subsequently. An investigation that was reported in 2008 and that involved swabbing and culturing from various coins and paper money collected at random from doctors, laboratory staff, and other employees at a New YOR hospital resulted in the recovery of many pathogenic microorganisms (FSA, 2000). Many study shows that, the presence of coagulasepositive staphylococci on the money surface was confirmed. This suggested that without hygienic intervention, human occupational activities, especially those involving simultaneous money handling, could introduce the risk of cross-contamination to foods (FSA, 2000). Oddly, publications regarding the degree to which paper money is contaminated with

bacteria are few and far between, as the authors found when they conducted a Medline search in December 2005 (Michaels, 2002; Pope *et al.*, 2002,; Singh *et al.*, 2002,; Xu *et* al., 2005; El-Dars and Hassan, 2005). Furthermore, the search found no documented study of the parasitological status of currency notes (as of December 2005). Scientific information on the contamination of money by microbial agents is also lacking in most sub-Saharan Africa, including Nigeria. This dearth of information may have contributed to the absence of public health policies or legislation on currency usage, handling, and circulation in many parts of Africa. Although the studies done in the United States and Australia have had no major impact on policies or legislation on currency handling and circulation in those countries, they have fostered a higher level of public awareness about the potential for currency contamination by microorganisms (FSA, 2000). In Nigeria, a whole division of the Department of Treasury deals with what is termed "mutilated currency," and the department Web site boasts many examples of beleaguered, burned, buried, water damaged money (Siddique, 2003). The bacteria associated with naira notes are highly contagious and relatively easy to transmit bacteria disease from one person to another by sharing the money from contaminated hands to uncontaminated ones. Also, bacteria can be transmitted by keeping the money in unsafe place like wallets, pockets or holding them with a hand that is contaminated with bacteria which may cause different kinds of bacterial diseases. This research aims at isolating, identifying and determining the levels of contamination of one hundred and one thousand naira notes with bacteria.

MATERIALS AND METHOD

Study area

The study area is Jega local government area, Gwandu emirate of kebbi state. It is located in the extreme north western corner of Nigeria, on latitude 10^0 to 15^0 N and longitude 33^0 and 602^0 East. The state shares a

border with Niger republic to the north, Benin republic to the west, sokoto state to the east and niger state to the south. The local government occupied a land mass of 891 kilometer square and is bound to the north east by Gwandu local government to the east by Aleiro local government area (Uzondu 2008). April is the warmest season with an average temperature of 40.3 ^oC at noon; December is the coolest season with average temperature of 16.2 ^oc at night.

Collection of samples:

Samples of 2 denominations of Nigeria currency notes: one hundred (100) and one thousand (1000) naira notes were collected, ten samples each within Jega metropolis, Kebbi State. And Collection was made from volunteers who included traders, motor conductors, students and food sellers. The samples were collected with gloves into separate sterile polythene bags labeled and were transported to the microbiology laboratory for microbial analysis.

Physical condition of the currency

The currency notes were in the various physical condition and were categorised as mint, clean, or dirty/mutilated. The term mint described currency notes that had been newly or recently produced. The term clean describes notes that had a clean appearance without any obvious damage. The term dirty/mutilated describes notes that either were not clearly more than one-half of the original notes or were in such condition that the value was questionable, or were damaged, soiled, or held together with bits of sticky tape.

Preparation of money for analysis:

Each of the ten abused naira note collected was soaked in 100ml aliquots of sterile buffered (0.1% w/v) peptone water for 20 minutes at ambient temperature with regular vigorous shaking to dislodge the cells into suspension.

Bacteriological analysis

To determine the total viable count, the washed water of the soaked notes was serially diluted $(10^{-1} \text{ to } 10^{-5})$ and the dilution (0.5ml) of each washing was inoculated (using pour-plate method) on sterile plates of nutrient agar

medium. The plates were incubated at 37°C for 24 hours. Representative colonies of bacterial isolates were selected and purified by sub-culturing on selective and enriched media. The pure culture was then characterized

and subsequently identified using Cowan and Steels Manual for the identification of medically important Bacteria. Data obtained were subjected to statistical analysis. Morphological characteristics, gram staining and biochemical tests were used to confirm the bacteria isolated.

Media preparation

The media was prepared by dissolving 28g of nutrient agar in one liter of distilled water. The mixture was dissolved on the hot plate to achieve the total dissolution of the nutrient agar. It was then corked with cotton wool and aluminum foil, and was sterilized in the autoclaved at 121°C for 15minutes. The media was allowed to cool to 45°C and was dispensed into a different sterile petri dish and allowed to solidify.

Gram staining

A smear of colonies isolated was made on a glass slide using a wire loop. It was dried and heat-fixed. Then, the fixed smear was flooded with crystal violet solution for 30 seconds and washed. This was later tipped off and covered with lugo's iodine for 60 seconds. This was then washed off and decolorized with ethanol 70%. The smear was then flooded with safranin solution for 60 seconds and then rinsed with water and air-dried.

Microscopy

The back of the glass slide was wiped clean and a drop of colorless thick oil (glycerin) was applied on the smear which was examined microscopically with x100 objectives lens for the observation of grams' reactions and morphological characteristics of the bacteria cell. Positive bacteria did not decolorize with ethanol and hence their cells appear purple, while gram negative cells retained the counter staining color of safranin and hence appear pink.

Biochemical reactions: Catalase test

This test was carried out mostly on gram-positive cocci to test their ability to produce the enzyme catalase. In this case, it differentiates between staphylococcus which is catalase-positive and streptococcus is catalasenegative. Catalase test is carried out also in both gram-positive and gram-negative bacilli and cocci. A colony of culture was emulsified in a drop of hydrogen peroxide on a clean glass slide. The presence of oxygen bubbies indicates positives result of a catalase test while; the absence of oxygen bubbles indicates a negative result of a catalase test.

Coagulase test

This test is used to differentiate between staphylococcus aureus from other staphylococcus species, due to their production of the enzyme coagulase by the S. aureus only. A looped of the isolated was emulsified in a drop of normal saline and a drop of citrated plasma was added and mixed. The slides were rocked gently for 2 minutes observing for coagulate reaction or dumping positive isolate gave agglutination reaction with the plasma.

Urease test

Urease test is applied for bacteria species that can decompose urea by an enzymatic reaction to produce ammonia. After solidification of the urea medium, the inoculums were inoculated into the slant bottles and incubated at 37°C for 24 hours. A positive test is indicated by purple-pink colour and for a negative test there is no change.

Citrate test

Koser's citrates medium was inoculated with the isolated and incubated at 37°C for 48hours. It was examined after two days. The presence of growth leads to an increase in pH resulting to change in colour table for a positive test and initial green colour for a negative test.

Indole test

Colonies were picked and inoculated into the test tube containing the indole medium and finally incubated at 37°C for 48 hours.

Sometimes 96 hours at 37°C may be required. 0.5ml of kovac's reagents was added dropwise to the test tubes and was shaken gently. This production of indole is confirmed by the formation of red ring colorations on the surface of the medium, which indicates a positive reaction while; in negative reaction red colorations is not produced.

Motility test

The motility test was aimed at identifying motile bacteria. A drop of normal saline was placed on a sterile slide and a colony of test organism was suspend and emulsified and then covered with a coverslip. The prepared slides were examined microscopically using 10x and 40x objective lens. Movement in different directions gave a positive test.

RESULTS

Most of the naira notes were wrinkled, toured and dirty; especially the N100 naira notes. The physical conditions of the various notes of both one thousand (N1000) and one hundred (N100) notes are shown in Table 1. The bacterial counts were generally high: ranging from 2.0 to 6.2×10^5 cfu/cm² (Table 2). The N100 notes harbour the highest bacterial load (average of 6.2×10^5 cfu/cm²) while N1000 notes had the least (2.0×10^5) cfu/cm²). For the occurrence of bacterial isolates on one thousand (N1000) notes (Table 3), three bacterial species: Escherichia coli, Staphylococcus aureus, P. aeroginosa. were isolated. For the occurrence of bacteria isolates on one hundred (N100) notes (Table 4), four species: Escherichia coli, Staphylococcus aureus, pseudomonas aeruginosa, Streptococcus pyrogenes were isolated. Similarly, the bacteria isolated from both naira notes using gram stain reaction were identified microscopically (Table 5). After biochemical test of bacteria on one thousand (1000) and one hundred (100) Naira notes, four species: Escherichia coli, Staphylococcus aureus, Streptococcus progenes, P. aeruginosa were identified on both dominations (Table 6 and 7). The occurrence of the bacterial isolates i,e Streptococcus progenes was the least encountered (20%) and Staphylococcus aureus was the most encountered (70%) (Table 8).

Considering the level of contamination based on different notes of one thousand (N1000) and one hundred (N100) naira, there is a high concentration of bacteria on one hundred (N100) notes compared with one thousand (N1000) naira notes (Table 9).

Table 1. Physical condition of a sample of each one thousand (1000) and one hundred (100) Naira notes

Samples (Naira)	N1000	N100
А	Fairly clean	Fairly dirty and wrinkle
В	Dirty, wrinkle	Dirty, torn and odorous
С	Dirty, torn	Dirty, torn wrinkle and odorous
D	Fairly dirty and torn	Dirty and wrinkle
Е	Dirty and wrinkle	Fairly dirty and wrinkle

Table 2: Average bacterial count of different one thousand(1000) and one hundred (100) Naira note

Sample	1000	100
А	2.0×10^5	2.6×10^5
В	5.4×10^5	4.4×10^5
С	5.8×10^5	6.2×10^5
D	2.0×10^5	5.8×10^5
Е	3.0×10^5	6.0×10^5

Table 3: Frequency of occurrence of bacteria isolate on 1000 naira note

Organisms	Frequency	Percentage of occurrence (%)
Staphylococcus aureus	4	50.0%
Escherichia coli	3	37.5%
Pseudomonas aeruginosa	1	12.5%
TOTAL	8	100%

Table 4: frequency occurrence	of the bacteria isolates on	n 100 naira note
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Organisms	Frequency	Percentage of occurrence (%)
Staphylococcus aureus	3	30.0%
Escherichia coli	3	30.0%
Pseudomonas aeruginosa	2	20.0%
Streptococcus pyogenes	2	20.0%
TOTAL	10	100%

Table 5: Bacterial load of one thousand (N1000) and one hundred(N100) Naira notes

Sample (naira)	Mean
N1000	$3.60 \mathrm{x} 10^5 \pm 0.581$
N100	$5.0 x 10^5 \pm 0.279$

1000 (Naira) sample of the isolate used	Microscopic arrangement and shape of the isolate	Gram reaction
A	Bacilli	-
B1	Cocci in cluster	+
B2	Cocci in cluster	-
C1	Bacilli	+
C2	Bacilli	+
D	Bacilli	-
E1	Bacilli	+
E2	Rod shape	-

Table 6: Microscopic Identification of the bacteria isolate by using gram stain Reaction

Table 7:	Microscopic	Identification	of the bac	teria isolate	by using gran	n stain Reaction

100 (naira) sample of the isolates used	Microscopic arrangement and shape of the isolates	Gram reaction
A1	Bacilli	-
A2	Cocci in cluster	+
B1	Rod shape	-
B2	Cocci in chain	+
C1	Cocci shape	+
C2	Bacilli	-
D1	Cocci in cluster	+
D2	Bacilli	-
E1	Cocci in chain	+
E2	Rod in pair	-

Table 8: Biochemical test for identification of bacteria of one thousand (1000)and one hundred (100) Naira notes

	Ca	Со	Ci	Mo	In	Ur	Glu	Lac	Organism
А	+	-	+	+	-	-	+	+	Escherichia coli
В	+	-	+	-	-	-	-	-	Streptococcus pyogenes
С	+	+	-	-	-	+	+	+	Staphylococcus aureus
D	+	+	+	+	-	-	+	+	Pseudomonads aeroginosa

Key: Ca = Catalase; Co = Coagulase; Ci = Citrate; Mo = Motility; In = Indole; Ur = Urease,Glu= Glucose, Lac = Lactose.+ = Positive; - = Negative

Table 9: The bacterial isolated from one hundred and one thousand Naira notes

Naira	No. sample	S. aureus	Strept. progenes	E. coli	P. aeruginosa	Total
1000	5	4(80.0)	0(0)	3(60.0)	1(20.0)	8(44.4)
100	5	3(60.0)	2(40.0)	3(60.0	2(40.0)	10 (55.6)
Total	10	7(70.0)	2(20.0)	6(60.0)	3(30.0	18(100)

DISCUSSION

Different species of bacteria isolated in this study are similar to those studied by Khin Nwe and co-workers (1989) in Rangoon, Myanmar; Goktas and Oktay (1992) in Turkey; and Pope and co-workers (2002) in Ohio. All of these researchers strongly suggest that money plays a role in the transmission of potentially harmful bacteria agents. Bacteria isolated in these studies, such as coagulase-negative staphvlococcus, alpha-hemolytic Streptococcus, nonaeruginosa species of pseudomonas, and Escherichia coli, do not typically cause infection in healthy people rather they have been known to cause significant infections in those with the depressed immune system, including those infected with HIV, undergoing cancer chemotherapy, or taking other medications that depress the immune system. Those bacteria may also cause infection in the hospitalized patient (Emori and Gaynes, 2007). The presence of the microorganisms on the notes suggests that the minimum conditions for their presence have been met (Brock et al., 2000). This brings to mind the question: how safe are our naira notes in circulation? Dirty notes are usually moist and thus provide a good surface for bacterial growth. They provide favorable conditions such as substrate acquired from the human body and due to handling as well as dust from the environment (Haque, 2003). Most of the bacteria encountered in this study are members of the human flora. This suggests that humans are the major source of bacteria on naira notes. The notes could have been colonized when placed in places where they make direct contact with the skin. The skin harbors a complex ecosystem of microorganisms, which could be transient or resident (Nester et al., 2004). The number of bacteria on the skin surface ranges from 10³ fu/ cm^2 in dry areas to more than 10^7 fu/cm^2 in moist areas (Willey et al., 2008). Colonization of the notes can also occur due to practices like moistening the fingers with saliva when counting money. It is common practice to keep notes in contact with surfaces such as the ground, soil, table surfaces and the likes. This is particularly common among traders and meat sellers. These bacteria could have been introduced via contaminated water used to moisten the fingers while counting or cross-contamination from

offal's. High bacterial loads were found with the N100 notes, which are commonly used in daily cash transactions. The highest load was associated with N100, possibly because it dominates most daily cash transactions. The high bacterial loads also suggest danger especially with unhygienic practices such as intermittent moistening of the finger by touching the tip of the tongue while counting money. S. aureus is usually harmless but are often able to cause infections (pyogenic infections) once they gain entry into damaged skin or deeper body tissues. It is also associated with peeling of superficial skin layer (exfoliation), impetigo, carbuncles and food intoxication (Jensen et al., 2000). It can be easily transferred from the notes to person and initiate infection. P. aeruginosa is one of the three Pseudomonas species involved in human diseases. It can cause eye and skin infections as well as external otitis. E. coli are clinically important members of Enterobacteriaceace. Some strains of E. coli are associated with the production of heat-stable enterotoxins (Jensen et al., 2000; WHO, 2004). The naira notes pass from person to person without any sanitization or disinfection. They can therefore act as vehicles of transmission of infectious agents (that is, acts as formites). The likelihood of contacting infections due to contact with dirty naira notes is thus high. The multi-drug resistance observed among the bacteria further emphasizes the public health significance of the notes. Handlers of notes especially those who put them in their brassiere or other areas where there is intimate contact with the skin should exercise caution; as there is the risk of infection by bacteria resident on the notes. Also, the habit of wetting the finger with saliva while counting naira notes should be avoided; organisms on the notes could be transferred to the mouth by this action. Dirty and mutilated notes should be withdrawn from circulation from time to time. The central bank of Nigeria (CBN) should put in place a retrieval system, which ensures that notes do not remain in circulation for too long. Money handlers should generally improve on their habit and ensure that the notes are not abused or mishandled. These could

go a long way in checking the spread of infections through naira notes as formites. The climatic and environment conditions of the tropics favor the thriving of many pathogenic microorganisms, and in the face of underdevelopment, inadequate water and sanitation, crowded living conditions, lack of access on health care, and low levels of education, a greater proportion of the populace, particularly the poor, become highly susceptible to infection and infection and disease (Gwatkin 2000). Risk of infection is increased several fold when objects that change hands at a high frequency, such as currency notes, are contaminated with microbes. The risk is by no means restricted to residents of the country in question; it might even be greater for expatriates, tourists, and visitors from other countries, who may not be immune to the pathogens. In Nigeria, poor-currency-handling culture is widespread, and there is indiscriminate abuse of the currency notes. A great majority of the populace does not carry money in wallets, and squeezing of currency notes is a common occurrence. Women, especially among the unenlightened, often place money underneath their brassieres, while men place theirs in socks. The activities not only enhance currency contamination but may also increase the risk of infection from contaminated notes. The situation is further compound by the inability of the Nigeria government to consistently withdraw old, worn-out, and mutilated notes from circulation. The presence of damaged currency notes and the failure to consistently withdraw them from circulation are common phenomena in many parts of Africa and Asia (Podhajny 2004).

CONCLUSION

From the result of the study, it revealed that currency notes can be contaminated with pathogenic microorganisms that are capable of causing diseases and infections. The likelihood of contacting infections due to contact with mutilated naira notes is high considering the microbial count gotten in the study. The potential health danger of mutilated naira notes is obvious and the chances of infection is on the increase. Handlers of notes especially those who put them in their brassiere or other sensitive areas where there is intimate contact with the skin should exercise caution and also the habit of wetting finger with saliva while counting

naira notes should be avoided; organisms on the notes could be transferred to the mouth by this action. Therefore, Regular withdrawal of damaged notes and improvement of personal hygiene policies should be implemented by the federal authorities.

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