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ISOLATION AND IDENTIFICATION OF BACTERIA PREVALENT AMONG NIGERIAN CURRENCY NOTES IN SELECTED AREAS IN AND AROUND AKPERAN ORSHI COLLEGE OF AGRICULTURE, YANDEV

Aorkwagh, M.T., Iko Wanen, Alye, C.T., Gberindyer, A.I., Asue, U
Department of Basic Sciences
Akperan Orshi College of Agriculture Yanev, Benue State, Nigeria

ABSTRACT

The unhygienic state of Nigerian currency notes in circulation in most areas in the country and particularly in Akperan Orshi College of Agriculture Yandev (AOCAY), metropolis of Gboko Local Government Area of Benue State necessitated this research. The research aims at isolating and identifying the bacteria prevalent on those currency notes and associated risk factors. A total of forty (40) different Naira notes of all denominations (₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500, ₦1000), were randomly collected from artisans, sellers (vendors) and students in selected areas around AOCAY. Each currency of both paper and polymer were rinsed with sterile distilled water and part of the solution was examined under the microscope for microorganisms. The other part was used for culture by pour plate method and biochemical tests. The mean average bacterial count on those notes was between 1.4 X 10^3 CFU/ml to 7.6 X 10^2 CFU/ml. The lowest and the highest bacterial counts were found in the ₦20.00 and ₦200.00 notes respectively. The bacterial species isolated were Staphylococcus aureus, Klebsiella pneumonae, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi. The bacteria species were isolated by simply performing culture tests, Gram's Stains, and biochemical tests. It was concluded that the naira notes in circulation in AOCAY may serve as a vehicle for the transmission of potentially pathogenic microorganisms and this might be as a result of poor money- handling culture. Hygienic handling of money is therefore stressed.

Keywords: Bacteria, Currency, culture, Colonies, Denominations, Diseases.

INTRODUCTION

Bacteria are members of tiny single celled micro- organisms which can either be parasitic or free living which are almost found everywhere on earth. There are members of recognized phyla- the dominant ones are: proteobacteria, firmicutes, Actinobacteria. The bacteria that cause health problems fall in the phylum proteobacteria. Examples of such bacteria are; E. coli, Salmonella spp, vibrio spp, Helicobacteria and numerous other genera (Alan, 2007). The possibility that bacteria can be found almost everywhere on earth also makes it possible in transmission on many contact surfaces. Many scientists have conducted research on transmission of microbes via (Money) currency.

Money is used as medium of exchange for goods and services, settlement of debts and for differed payments in economic activities. It might act as formites or environmental vehicles for the transmission of potential microbes. The use of paper and polymer currency for every type of commerce makes it susceptible for germs contamination, with the lower- denomination notes receiving the most handling and thus, the most contamination because they are exchanged frequently (Gadsby, 1998) and (Uneke, 2007).

In Nigeria, the currency is highly abused especially in the manner in which it is handled in transactions. Presently, it is commonly seen faded, torn, stapled, cello- taped, squeezed, and writings on them.

As the result of daily transactions, money is often contaminated with germs. The contamination of the Naira notes could as well be from the atmosphere, during production, storage, usage or handling (Matur *et al*., 2008). Daily transaction has made the naira notes to pass through many hands or is placed in a dirty environment and pathogenic organisms become imposed on them.

Awodi *et al*., (2002), reported that, the source of contamination could be as a result of poor or negative money handling or practices like spraying around during ceremonies; here the notes are sprayed on the celebrant(s) and in the process fall on the ground where a large number of people dancing, step on them with soiled shoes on bare ground.

Corresponding Author's: Iko Wanen; E- mail: waneniko@yahoo.co.uk

Also, keeping them in stockings, brassieres by individuals and tongue- wets of fingers with saliva or use of contaminated water to lubricate the hands when counting, failure to wash hands after visiting toilet and touching of naira notes with dirty hands could be responsible for the microbial contamination (Ameh and Balogun, 1997). When hands used in cleaning up one's self after going to the toilet (passing out faeces) are not properly washed and are used in touching money notes in any way, there is tendency that there will be contamination with trophozite of the developed parasite or bacteria (Orji *et al*., 2012).

STATEMENT OF THE PROBLEM: Transition of communicable diseases is generally on the increase in underdeveloped counties because of poor health management practices. People adopt many practices to prevent infectious diseases, while leaving the fact that, most infections are contacted through contact surfaces; for example, currency notes in circulation (Elumalai and David, 2012).

AIM: The aim of this work is to isolate and identify bacteria prevalent among Nigerian currency notes in selected areas in and around Akperan Orshi College of Agriculture Yandev, Benue State.

OBJECTIVES: The objectives of this research work are to isolate and identify bacteria through culturing and gram staining, by use of microscope and identification through biochemical reactions. The research work is to also create awareness among the populace on the danger of transmission of diseases through contact surfaces like currency notes.

Study Area

The study was conducted in and around Akperan Orshi College of Agriculture Yandev of Gboko Local Government of Benue State, Nigeria. Yandev is located between latitude 7^0 22′0″N of the equator and longitude 9^0 3′0″E. Yandev is a populated place with a population of about 536, 068, located in Gboko Local Government of Benue state, Nigeria. It is located at an elevation of 315 meters above sea level. Its UTM position is NP01 and its Joint Operation Graphics is NB32- 03 (Benue State map, weather and photos, 2015). The community is host to Akperan Orshi College of Agriculture, the first state owned tertiary institution in Benue State. Although there are many small scale food processing outfits and artisan works around the community, the only industry of note is the Benue Cement Company, situated at about 7km from the community. The community has largely student population, an agricultural economy like most places in Benue state, which has the acronym, "The Food Basket of the Nation".

MATERIALS

The reagents used for this analysis were; sterile distilled water, nutrient agar, Macconkey agar, triple sugar iron agar (TSIA), crystal violet and Gram's iodine, acetone, 95% alcohol, dilute carbol fuchsin, safranin, 3% hydrogen peroxide, oxidase reagent, indole broth, Kovac's reagent, Simmons' citrate agar.

The apparatus used were; Petri dishes, beakers, spatula, wire loops/ sterile inoculating needle, test tubes and caps, racks, cotton wool and filter papers (Whatman No1), blotting papers, measuring cylinders, sterile sample bottles, wash bottles, Pasteur pipettes, conical flasks, sterile swab sticks, aluminum foil, glass rods, Bunsen burner, disposable hand gloves, and laboratory coat.

Equipments used were; autoclave, hot- air- oven, air laminar flow, microscope and slides, electronic weighing balance, heating mantle/ magnetic stirrer (hot plate), water distiller, incubator (digital), refrigerator, and colony counter.

METHOD

A total of forty (40) Nigeria currency notes which comprises of twenty (20) polymer notes and twenty (20) paper notes were randomly collected for this research. Collections were made from food vendors, artisan, farmers, a commercial bank, workers and students in and around AOCAY from April- June, 2014. The notes were collected into sterile plastic bags using disposable hand gloves and transported to the microbiology laboratory of University of Mkar, Mkar for analysis. Fresh new mint naira notes were collected from a commercial bank in Gboko and used as control. All the samples were collected as either change or balance after negotiation or purchase of an item or a transaction.

That is, the samples were randomly obtained by exchanging large-denominations notes to smaller denominations, as well as smaller- denominations to larger-denominations respectively.

Sterilization of Materials

Glass wares were sterilized by autoclaving at 121^0C for 15 minutes and pressure of 0.1 to 0.14mpa as well as the culture media. The inoculating wires were sterilized by red hot technique on the Bunsen burner, and the working surfaces were disinfected using cotton wool containing 90% alcohol rubbed at the surfaces.

Preparation of Culture Media

Each medium was prepared according to manufacturer's instructions and sterilized by autoclaving at 121^0C and 0.14mpa for 15 minutes.

i. 11.5g nutrient agar powder were dissolved into conical flask containing 500ml of distilled water, and stirred with a sterile glass rod.
   The mixture was heated constantly while stirring; it was allowed to boil for one (1) minute then removed from heat, covered and allowed to cool for few minutes. The melted agar was poured aseptically into the Petri dishes covering about $^1/_8$ to ¼ deep bottoms.
   The agar plates were placed on a counter top to cool and set like stiff gelatin at room temperature.
ii. 4g of modified Macconkey agar powder were suspended into a flask containing 80ml of distilled water and mixed thoroughly. The mixture was heated with frequent agitation on the heating mantle then allowed boil for one minute to completely dissolve the powder, then removed. The agar was sterilized by autoclaving at 121^0C for 15 minutes then removed, covered, and stored at room temperature in the refrigerator.

ISOLATION/ IDENTIFICATION OF BACTERIA

After putting on a sterile disposable hand gloves, each naira notes was swabbed using swab stick and thereafter folded and soaked into a sterile beaker containing 10ml sterile distilled water and was vigorously shaken for about 15 minutes (Orji *et al.*, 2012). The notes were then removed using a pair of sterilized forceps and transferred to sterile polymer bags. The mixture so obtained after soaking was mixed together in one sterile bottle for each denomination shaken thoroughly. A portion of each bottle was poured into a sterile centrifuge tube and centrifuged at 200rpm for three (3) minutes (Orji *et al.*, 2012). The supernatant was decanted while from the resultant sediments; a drop of it was placed on a clean slide, covered with a slip and examined microscopically at x10 and x40 for presence of bacteria and parasites. Earlier, a slide was prepared from the swab obtained above and examined microscopically as well.

An aliquot of the remaining 1.0ml of each denomination was then inoculated by pour plate method unto sterile plates containing nutrient- agar. The plates were incubated at 37- 38^0C for 24 hours. Pure bacteria isolates were obtained by sub- culturing unto nutrient- agar and Macconkey agar under the same conditions. The procedure was the same for the new notes from the bank.

The bacteria isolates were subjected to Gram staining and biochemical tests using the methods described by Cheesbrough, (2000) as well as culture test unto Macconkey agar and nutrient agar as described by Benson, (1990) as earlier stated. The bacteria were identified by colony characteristics and color change of the medium after staining.

MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION OF BACTERIA ISOLATES

Culture and Gram stain

The bacteria isolates were subjected to culture test unto Macconkey agar and nutrient agar described by Benson (1990), as well as Gram's staining and biochemical test using the methods described by cheesbrough (2000). The bacteria were identified by colony characteristics color characteristics and their color in the medium after staining.

i. **Culture Method:** Pure bacterial isolates were inoculated unto the sterile pre-prepared macconkey agar and nutrient agar by streak plate method then incubated at temperature of 37-38^0C for 24hrs. Growth colony characteristics and color appearance were observed, identified and recorded.
ii. **Gram's Reaction:** Bacteria isolates were subjected to gram staining as follow:

Five (5) thinly smears of the cultures were prepared on a slide gently. The smears were air-dried and passed over a gentle flame on the Bunsen burner in a circular motion.
The smears were flooded with crystal violet and allowed to stand for 10 to 60 seconds; the excess stains were washed off gently with a stream of sterile water from plastic wash bottle.
Iodine solution was added on the smears enough to cover the applied culture growths (the smear) then allowed to stand for 10- 60 seconds, the iodine were poured off and the slides were rinsed with running water.
Smears were decolorized with few drops of acetone, and then rinsed with water after 5 seconds until color stopped flowing over the slide.
Smear were counter stained immediately with basic fuchsin solution (carbol fuchsin) for 40 to 60 seconds, then washed off with water and blotted with bibulous paper (blotting paper) to remove the excess water, then allowed to air-dried.
The slides were observed on the microscope with oil immersion on the objective lens. Results were recorded as positive for the blush to dark bacteria; and negative for the red or pink bacteria.

Biochemical Identification of the Bacteria Isolates

i. **Simmons's Citrate Test.**
Using a sterile needle, a single isolated colony from the pure culture were streaked lightly on the surface of the slant medium (Simmons's citrate agar) in the test tube and incubated gently at a temperature of 35^0C to 70^0C for 18 to 48 hours then observed.
The visible growth on the surface of a slant medium and intense Prussian blue appearance of the medium indicated that the bacteria utilized citrate as a carbon source. While trace, or no growth and no color change of the medium indicated the inability of a bacteria to utilize citrate as a carbon source.

ii. **Catalase Test: (Slide Test)**
A drop of 3% hydrogen peroxide (H_20_2) were mixed with the colony from the culture using a sterile needle and observed. Rapid and sustained appearance of effervescence indicated that the bacteria have catalase, while lack of gas, or bubbles formation on the slide indicated absence of catalase.

iii. **Tube Coagulase Test**
Into a test tube, several isolated colonies from the culture were emulsified in 0.5 ml of rabbit plasma to give a milky suspension.
The tubes were incubated at 35^0C in ambient air for four (4) hours. Then, it was observed for clot formation. Results were recorded as positive for stable clot formation while negative for absence of clot.

iv. **Oxidase Test**
Few (1-2) drops of oxidase reagent were applied to a filter paper, with a help of a sterile needle, a colony of a bacteria culture were picked and streaked on the reagent-soaked filter paper and observed for the result in 10 to 30 seconds. The dark blue-purple color change of the filter paper indicated the presence of cytochrome c oxidase. While no colour change or colour change after more than 30 seconds indicated absence of cytochome c oxidase.

v. **Indole Test**
Several colonies of pure bacteria isolates were added into a tube containing 1 ml of indole broth. Tubes were incubated at 37^0C for 2 hours, with the aid of a dropper, kovac's reagent was added to the culture in the tube, results were observed within 30 seconds.
Formation of cerise red ring at the surface of the broth in the tube indicated that the bacteria produced indole.

vi. **Test for Hydrogen Sulphide Production**
Inoculums from a pure culture were inoculated aseptically to a sterile triple sugar iron agar (TSIA) with a help of a sterile needle by streak and stab method and slant in the test tube. Inoculated tubes were incubated at 35^0C to 37^0C. Result were tested positive as the regions of agar turned black indicating the organism is producing H_2S. While no color appearance, or precipitate in the medium showed bacteria did not produce H_2S.

vii. **Test for Gas Production**
Using a straight inoculating needle, isolated colony were inoculated on the triple sugar iron agar (TSIA) and slant in the test tube by first stabbing and streaking the surface of the slant. Tube were loosely closed to permit access of air and incubated at 37^0C for 18 to 24 hours.

Positive result were indicated by formation of bubbles in the butt, and upward pushed of the agar by the amount of gas produced in the medium. While negative result where indicated by lack of formation of bubbles in the butt.

RESULTS

Results showed that different species of bacteria were present on the surfaces of different naira denominations used in transactions among artisans, vendors, farmers, and students in and around Akperan Orshi College of Agriculture Yandev. The results are shown on table 1 and 2 below.

TABLE 1: BACTERIAL COUNTS ON USED NAIRA NOTES.

Denominations (Naira)	Number of Samples	Number of Plates Used	Dilution Factor	Number of Colonies	Mean value of Bacterial Count (CFU/ML)
5	5	3	10^3	21	1.4×10^3
10	5	3	10^3	18	1.2×10^3
20	5	2	10^2	96	9.6×10^2
50	5	2	10^2	90	9.0×10^2
100	5	3	10^3	21	1.4×10^3
200	5	3	10^3	15	1.0×10^3
500	5	2	10^2	82	8.2×10^2
1000	5	2	10^2	76	7.6×10^2

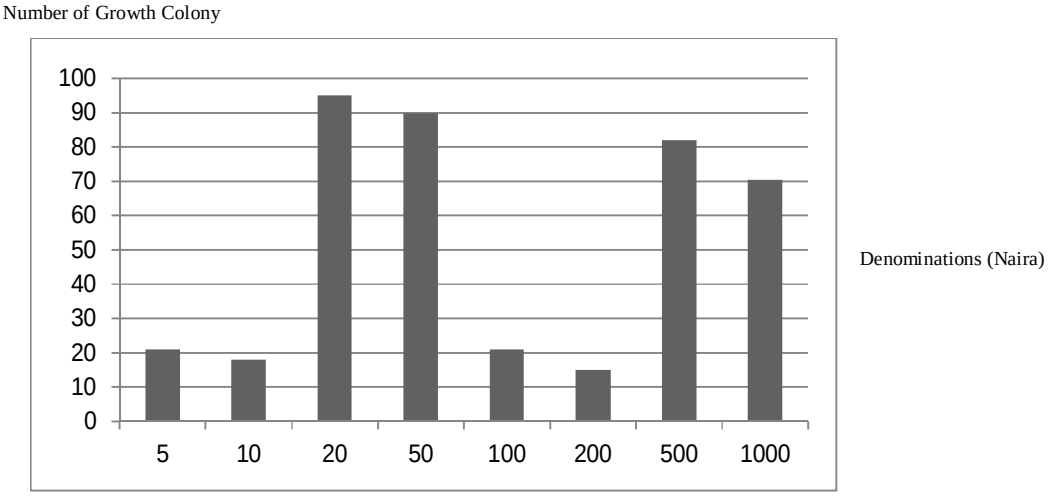
CFU/ML means colony forming units per milliliter.

TABLE 2: MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION OF THE BACTERIA.

APPEARANCE OF COLONIES ON PLATES	GRAM S STAIN	CIT-RATE TEST	CAT AL-ASE TEST	COA G-ULA SE TEST	OXI DA-SE TEST	IND OLE TEST	H_2S PRO D.	GAS PRODUCTION	BACTERIA IDENTIFIED
Smooth, circular, milky colonies on Nutrient agar	+ and cocci in shape	+	+	+	-	-	-	-	S. aureus
Pale colored colonies on macconkey agar	- and rod in shape	+	+	-	+	-	-	-	Pseudomonas aeruginosa
Smooth, round, yellow colonies on Nutrient agar.	+ and cocci in shape	+	+	-	-	-	-	-	S. Aureus S. aureus
Mucoid pink colonies on macconkey agar.	- and rod in shape	+	-	-	-	-	-	+	Klebsiella Pneumonae
Smooth, pink colonies on macconkey agar.	- and rod in shape	-	-	-	-	+	+	-	E. coli
Mucoid pink-brick red colonies on macconkey agar.	- and rod in shape	-	+	-	-	-	+	+	Salmonella typhi

+ means test carried out was positive, while - means the test carried out was negative.

BAR CHART OF THE BACTERIAL GROWTH COLONY IN THE RESPECTIVE DENOMINATIONS



DISCUSSION

The results in table 1 showed that used naira notes among artisans, vendors, farmers and students in areas around AOCAY are reservoir of bacteria. However, the control sample of naira currency notes did not have any growth observed from them.

This is closely similar to the earlier report by (Kawo *et al., 2009*). The lower denominations ₦20 and ₦50 had highest bacterial loads which ranged from 9.6 X 10^2 CFU/ML and 9.0 X 10^2 CFU/ML. The ranges obtained were lower as compared to the report by Kawo et al. (2009). However, a higher load of 8.2 x 10^2cfu/ml and 7.6x10^2cfu /ml were also obtained on the highest denominations in ₦500 and ₦1000 notes, but higher denominations of ₦100 and ₦200 notes were observed to have lower bacterial load as well as the lowest denominations ₦5, and ₦10 that ranged from 1.0 X10^3 CFU/ML to1.4x10^3CFU/ML. This has also been observed earlier by kawo *et al. (2009)* and Uraku *et al., (2012),* as well as Adeyemo *et al., (2014).*

The reason for the highest bacterial load observed in lower denominations could be attributed to the frequency at which they have been used in circulation among individuals than the higher denominations (Igumbor, 2007). This was also reported by Awe *et al.,* (2010).

And the result of the higher bacterial loads on the highest denominations could be attributed to the long time storage of dirty and overused cash in a process of savings. The lower bacterial load on the lowest denominations could also be attributed to the discrimination of the amount and depreciation in value among individuals (Kawo *et al*., 2009).

The cultural, morphological and biochemical properties of the isolates showed that, the following bacterial isolated were: Staphylococcus aureaus. Pseudomonas aeruginosa, Klebsiella pneumonae, and Eshelicia, coli, salmonella typhi.

The bacterial species such as Eschelicia coli and Staphylococcus aureus have been reported as some of the microbial contaminants isolated from Naira notes. The genera Klebsiella and pseudomonas have been isolated and reported (Adamu *et al., 2012*), (Oyero and Emikpe, 2007).

The study showed that, the poor handling of currency notes such as keeping them in stockings, brassieres by individuals and bad habit of putting saliva at the finger tips when counting, failure to wash hands after visiting toilet and touching of Naira notes with dirty hands could be responsible for the microbial contamination observed with the naira notes.

SUMMARY

A research was carried out to isolates and to identify bacteria prevalent on naira notes in circulation among individuals in some selected areas in and around Akperan Orshi College of Agriculture Yandev, AOCAY.

Samples were collected randomly from artisans, food vendors, farmers and students in those areas. A total of forty (40) pieces of different available denominations of naira notes were analysed for bacteria load in accordance to the standard protocol described previously by (Cheesbrough, 2000).
The bacteria isolates were identified by assessing colony characteristics and gram's reactions as well as conducting biochemical tests.
The bacteria isolated and identified were staphylococcus aureus, Pseudomonas aeruginosa, klebsiella, pneumonae, Eschericia coli, and salmonella typhi.
The results showed that, bacteria are prevalent on naira notes in circulations among people in areas around AOCAY.

CONCLUSION

These findings has left us with no doubt that, money does not only serves as a medium of exchange of goods and services, settlement of debts, and payments in economic activities, but also serves as the reservoir or vehicle through which microbes can transmitted from one person to another which can cause infectious diseases to animals and even humans.

RECOMMENDATIONS

To minimize the hazards that may arise from used contaminated notes. It is recommended that, a pilot scheme should be set up and currency Naira notes should be disinfected frequently by the pilot scheme set up by the government. Also, basic hygiene in terms of frequent and thorough hand washing with soap and water especially after use of toilet should be emphasized before handling of paper or polymer money. Furthermore, proper washing of hands after handling of money should be sacrosanct before handling of food (Richardson and George, 2000; Hadwen et al., 2003, Lalonde, 2007).
There is need for sensitization of the public on proper handling and storage of naira currency notes to reduce transmission of microbes and diseases.
Central bank of Nigeria should endeavor to step up its effort to withdraw from circulation naira notes and polymers notes that have been torn and or been over used. Co-operative societies and loans schemes should be enlightened to purchase and use hot-air ovens to sterilize the paper notes and use disinfectants to minimize bacteria load on polymer notes before disseminating to their members.

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