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Prevalence and antimicrobial resistance pattern of microorganisms isolated from Naira notes in Ogbomoso North, Nigeria

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ABSTRACT:

Nigerian naira notes (#5, #10, #20 and #50) obtained from different locations and occupational groups in Ogbomoso North Local Government Area in Oyo State, Nigeria were analyzed microbiologically for evidence of microbial contamination using MacConkey Agar, Nutrient Agar, Mannitol Salt Agar and Potato Dextrose Agar. About seven-nine different microorganisms were isolated from the naira notes, while the prevalent microorganisms include; *Staphylococcus aureus* (3.8 %), *Bacillus subtilis* (10.1%), *Enterobacter aerogenes* (8.9%), *Staphylococcus epidermidis* and *Aspergillus niger* (11.4%), *Bacillus megaterium* (12.7%), *Escherichia coli* (3.8%), *Pseudomonas putida* and *Aeromonas hydrophila* (6.3%), *Fusarium solani* and *Colletotrichum truncatum* (5.1%), and *Trichoderma reesei* and *Colletotrichum gloesporoides* (7.6%). About 75% of the isolates were resistant to the broad spectrum antibiotics and the Multi- Antibiotics resistance pattern among the bacterial isolates ranged from 7 to 11. The fungi isolates also showed resistance to different concentration of Fulcin and Mycoten used (500Mg/l, 250Mg/ml and 100Mg/ml). Zone of inhibition observed with the fungal isolate that is susceptible to those antifungal agents is <15mm.

Keywords:
Prevalence, Drug resistance, Susceptibility, Small Denominations, Enteropathogenic Microorganisms.

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INTRODUCTION

Paper money was introduced into Nigeria in 1945 and since then several changes have been made to different denominations we are using as legal tender. Paper currency can act as an environmental vehicle for the transmission of potential pathogenic microorganisms (Abrams and Waterman, 1972), since the currency is transferable from person to person and from one country to another.

Although paper money is impregnated with disinfectant to inhibit microorganisms, however pathogens have been isolated from currency notes and coins (Talaro, 2005). Research has shown that currency paper provides a large surface area as breeding ground for pathogenic microorganisms. Money on which pathogenic microorganisms can survive represents an often overlooked reservoir for enteric diseases (Michaels, 2002) but recent studies from different parts of the world have revealed that currency, either as coins or paper have high rate of microbial contamination (Goktas and Oktav, 1992).

Environmental organisms such as *Bacillus* sp., *Staphylococcus aureus* have been identified as common contaminants isolated from paper money (Xu *et al*., 2005). While other organisms like, *Micrococcus* sp., *Corynebacterium* sp., *Vibrio cholerae*, *Mycobacterium tuberculosis* and members of the family Enterobacteriacea have been isolated from money too. Different microorganisms have been isolated from money worldwide including developed countries, pathogenic microbes like *Staphylococcus aureus*, *E. coli*, *Klebseilla enterobacter* have been isolated from United State coins and paper bills currencies (Abrams and Waterman, 1972; Gadsby, 1998). Another survey also isolated a total of 93 different types of bacteria belonging to the genera; *Staphylococcus*, *Streptococcus*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Klebseilla pneumoniae* and *E. vuluneris* (Pope *et al*., 2002). Improper handling of money by food vendors in which food vendors serve food with the hands and at the same time handle currency notes as they sell (Michaels, 2002; Lamichhane *et al*., 2009) can transfer bacteria from currency notes to humans through food (Lamichhane *et al*., 2009). Emikpe and Oyero (2007) had reported that most of the bacteria isolated from these currencies notes were resistant to the first line antibiotics which are cheap and common.

This study then focused on the prevalent bacteria and fungal contaminants present in small denominations naira notes from Ogbomoso North in Nigeria and the antimicrobial resistant pattern of the isolates.

MATERIALS AND METHODS

Sources and Collection of Nigeria Currency Notes: -

Samples were collected from different four locations in Ogbomoso North Local Government Area namely; LAUTECH campus area, Kuye, Sabo and Takie areas. Four different denominations that include #5, #10, #20, and #50 were collected at random from different classes of people in those four areas.

Microbial Isolation: -

Four different media, namely; Nutrient Agar, Potato Dextrose Agar, MacConkey Agar and Mannitol Salt Agar were used for the isolation of bacteria, fungi, coliforms and *Staphylococcus* sp. respectively.

Naira notes were washed in 50ml of sterile distilled water, serial dilution was carried out and 0.2ml was plated out from appropriate diluents. Pour plate method was used for the isolation of the microorganisms. All plates were incubated at 37^{o}C for 24hrs with the exception of fungi plates that were incubated at 27^{0}C for 2 to 5 days depending on the visible growth on the plates. The isolated microorganisms were identified by using microscopic and biochemical tests as described by Holt *et al* (1994). Lactophenol cotton Blue was used for microscopic identification of fungi, using both microscopic and macroscopic observations; the probable identities of fungi were determined by using Alexopoulous and Mims, (1970) and Domsch *et al*., 1980.

Antimicrobial Sensitivity

Antimicrobial sensitivity test was carried out to determine the sensitivity profiles of the isolates to selected antibiotics discs. Disc diffusion method using Nutrient Agar was used for the sensitivity test. Antibiotics discs containing Tetracycline (30µg), Augmentin (30µg), Nitrofurantoin (200µg), Cotrimozazole (25µg), Amoxycillin (25µg), Streptomycin (30µg), Pefloxacin (5µg), Ceftriazone (30µg), Gentamycin (10µg), Ofloxacin (5µg), Ciprofloxacin (10µg), and Chloramphenicol (10µg) were used for the test. The plates were incubated at 37^{o}C for 48 hours. After incubation, zones of inhibition were examined and interpreted accordingly.

While mycelia plug was cut off from the previous grown plates of Potato Dextrose Agar

(PDA) and placed on already prepared plates of PDA. Different concentration of Mycoten and Nystatin were prepared (100, 250 and 500mg/ml) and sterile paper discs were dipped into each concentration and placed aseptically on the plates that contained the test fungi and incubated at 25°C for 2-3 days after which the zones of inhibition were measured.

RESULTS AND DISCUSSION

Table 1 showed the Total bacterial counts of different denominations, #10 notes had the count of 33.93 cfu/ml × 10^4, followed by #5 notes (20.68 cfu/ml × 10^4), while #50 notes showed the least bacterial load of 19.94 cfu/ml× 10^4. **Table 2** revealed that #10 notes had the highest fungal count of 22.60 cfu/ml × 10^4, while #5 notes had the least fungal load of 5.16 cfu/ml× 10^4. The Total coliform count was represented by **Table 3,** #10 also had the highest coliform count of 21.00 cfu/ml× 10^4, and followed by #20 notes with count of 18.12 cfu/ml × 104 and #5 notes had the least count of 9.58 cfu/ml × 10^4. Studies from other parts of the world have shown that bank notes contained high load of bacteria which could cause many diseases (Shukla, 1980; Oyler *et al*., 1996; Pachter *et al*., 1997; Havas, 2000). Kawo *et al*. (2009) also reported high load of bacterial and fungal count from abused naira notes in Kano metropolis, Nigeria. **Table 4** showed the occurrence of different microorganisms isolated from the different naira notes analysed in this study. *Bacillus megaterium* had the highest occurrence of 12.7% followed by *Staphylococcus epidermidis* and *Aspergillus niger* (11.4%), *Bacillus subtilis* (10.1), *Enterobacter aerogenes* (8.9%), *Trichoderma reesei* and *Colletotrichum gloesporoides* (7.6%), *Aeromonas hydrophila* (6.3%), *Fusarium solani* and *Colletotrichum truncatum* (5.1%), and *Escherichia coli* and *Staphylococcus aureus* (3.8%). Similar works by Awe *et al*. (2010), Feglo and Nkansah (2010), Matur *et al*. (2010), and Shakir *et al*. (2010) had also reported the occurrence of different microorganisms from the currency notes in their countries. The isolation of *Staphylococcus* on the currency notes could have been contamination from normal skin flora and soil (Igumbor *et al*., 2007; Larkin *et al*., 2009) and Staphylococci infection occur when *Staphylococcus* enter the body through breaks, cuts and abrasions in the skin (Shakir *et al*., 2010) and it is also associated with impetigo, carbuncles and food intoxication (Jensen *et al*., 1997). *Escherichia coli* which is due to feacal contamination is also responsible for many diseases when consumed in large doses, other bacterial isolates like *Enterobacter aerogenes*, *Bacillus subtilis*, *Staphylococcus epidermidis, Pseudomonas putida* and *Aeromonas hydrophila* have been associated with different types of diseases especially in immunocompromised and immunosuppressed patients (Kelly *et al*., 1993; Yang *et al*., 1996; Aurélie *et al*., 2005), *Fusarium solani* may also cause a range of invasive mycoses and a range of opportunistic infection in immunocompromised patients (Zhang *et al*., 2006), the fungal isolates could also produce mycotoxins in food, which is

Table 1: Total Bacterial Counts on Different Denominations

Locations	#5 Cfu/ml X 10^4	#10 Cfu/ml X 10^4	#20 Cfu/ml X 10^4	#50 Cfu/ml X 10^4
Sabo	8.30	18.00	3.30	13.30
LAUTECH	2.32	3.00	4.00	1.20
Kuye	3.96	9.60	3.72	4.04
Takie	6.10	3.33	9.33	1.40
Total	20.68	33.93	20.35	19.94

Table 2: Total Fungal Count on Different Denominations

Locations	#5 Cfu/ml X 10^4	#10 Cfu/ml X 10^4	#20 Cfu/ml X 10^4	#50 Cfu/ml X 10^4
Sabo	2.00	10.00	8.30	6.60
LAUTECH	NG	3.33	3.00	1.33
Kuye	1.00	NG	NG	3.00
Takie	2.16	9.33	6.70	6.00
Total	5.16	22.66	18.00	16.93

Table 3: Total Coliforms Count on Different Denominations

Locations	#5 Cfu/ml X 10^4	#10 Cfu/ml X 10^4	#20 Cfu/ml X 10^4	#50 Cfu/ml X 10^4
Sabo	7.23	11.0	8.45	7.80
LAUTECH	NG	2.50	3.42	2.16
Kuye	NG	1.30	2.50	1.76
Takie	2.35	6.20	3.75	5.20
Total	9.58	21.00	18.12	16.92

Key: NG- No Growth.

dangerous to human and other animals (Grundy and Grundy, 1974). **Table 5** shows the microbial rate of naira notes in relations to the locations and the denominations of the different naira notes used in this study. The results revealed that Sabo area which comprises of different artisans and beggars had the highest contamination rate followed by LAUTech campus area which also comprised of different food vendors, shop owners, different artisans and students, while Kuye area which is a residential area for Government workers and artisans had the least contamination. Similar work by Shakir et al. (2010) also revealed that Bangladesh paper currency notes collected from artisans groups had the highest contamination rate compared to money collected from educated people. #10 notes used in this work had the highest contamination rate followed by #20 notes, these two naira notes are commonly found among the poor people and the children, the least contamination rate observed with #5 notes may be due to the fact that it is very rare to found most commodities sold at #5 nowadays. In a similar work by Oyero and Emikpe (2007), they reported highest level of microbial contamination for #10 notes among Nigerian currencies notes in their work. Also, #10 notes are the most commonly used among the small denominations in Nigeria and it is exchanged many times especially amidst the artisans and lower economic class people. Shakir et al. (2010) had also reported highest contamination rate among the small denomination notes in Bangladesh currencies.

Table 6 showed the Antibiotics sensitivity

Table 4: The Occurrence of Microorganisms Isolated from Naira Notes

Organism	*A1*	*A2*	*A3*	*A4*	*B1*	*B2*	*B3*	*B4*	*C1*	*C2*	*C3*	*C4*	*D1*	*D2*	*D3*	*D4*	*TOTAL*	*CONTAMINATION RATE (%)*
Aeromonas hydrophila	+	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	5	6.3
Pseudomonas putida	-	+	-	-	-	+	-	-	-	+	-	+	-	+	-	-	5	6.3
Staphylococcus aureus	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	3	3.8
Bacillus megatarium	+	+	-	+	+	+	-	+	+	-	+	+	-	-	+	-	10	12.7
Enterobacter aerogenes	-	-	+	+	-	+	+	+	+	-	+	-	-	-	-	-	7	8.9
Staphylococcus epidermidis	+	-	+	+	-	-	+	-	-	+	-	+	+	+	-	+	9	11.4
Bacillus subtilis	-	+	-	+	-	+	-	-	+	+	+	-	-	-	+	+	8	10.1
E. coli	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	3	3.8
Aspergillus niger	+	-	-	+	-	+	+	+	-	-	-	+	+	+	-	+	9	11.4
Trichoderma reesei	+	-	-	-	-	+	-	+	+	-	+	-	-	-	+	-	6	7.6
Colletotrichum gloesporoides	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+	6	7.6
Fusarium solani	+	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	4	5.1
Colletotrichum truncatum	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	4	5.1
Total	8	3	4	6	1	8	4	7	4	3	6	6	4	5	5	5	79	100.0

KEYS:
Alphabets – Locations
A - Sabo (Market and Beggars' Area)
B - LAUTech (Students and Civil Servant's Area)
C - Kuye (Indigenes' Area but no market)
D - Takie Square (Motor Park and Commercial Area)

Numbers – Denomination
1 - #5 2 - #10 3 - #15 4 - #20

Table 5: Microbial Contamination Rate of Naira Notes in Relation to Different Locations and Denominations

LOCATION	#5 (cfu/ml × 10^4)	#10 (cfu/ml × 10^4)	#20 (cfu/ml × 10^4)	#50 (cfu/ml × 10^4)	TOTAL (cfu/ml × 10^4)	CONTAMINATION RATE (%)
SABO	10.30	28.00	11.60	19.90	69.80	44.30
LAUTECH	2.33	12.33	16.03	2.53	33.22	21.08
KUYE	4.96	9.60	3.72	7.04	25.32	16.07
TAKIE	8.16	6.66	7.00	7.40	29.22	18.55
TOTAL	25.75	56.99	38.35	36.87	157.56	
CONTAMINATION RATE (%)	15.30	35.08	24.40	23.34		

pattern of the isolated bacteria against selected antibiotics. All the bacteria isolates showed 100% resistant to Augmentin, Nitrofurantoin and amoxicillin, they had 87.5% resistant to tetracycline, chloramphenicol and streptomycin, the isolates showed 50% resistant to Ceftriazone, Cotrimoxazole and Gentamycin, 37.3% and 25.0% resistant to Ofloxacin and Pefloxacin respectively. While all the bacterial isolates were susceptible to Ciprofloxacin with zones of inhibition ranging from 16 to 5mm. The fungal isolates also showed varied activities against the two antifungal (Mycoten and Fulcin) used for the sensitivity test at varying concentrations (100, 250 and 500mg/ml). At 100, 250 and 500mg/ml concentrations for Fulcin, the resistant percentage was 80, 40 and 40% respectively, and for Mycoten, the resistance percentage were 80, 60 and 40% for 100, 250 and 500mg/ml respectively (**Table 7**). Similar work by Emikpe and Oyero (2007) revealed that organisms isolated from Nigerian Naira notes were resistant to first line antibiotics and this was also observed in this work. The resistance observed in this work might also be due to the indiscriminate use of antibiotics by people. The resistance to the antifungal agents might be as a result of the carriers of some diseases who might have been exposed to different antifungal agents especially mycoten which is used by many women in the treatment of *Candida albicans* infection.

The findings from this work showed that infections that may occur from the microorganisms isolated from this work might be difficult to treat and also the treatment might be very expensive because of the resistance of these isolates to the common and cheaper antibiotics drugs.

In conclusion, this study showed that naira notes collected from different areas in Ogbomoso North Local Government of Nigeria is contaminated with different pathogenic organisms

Table 6: Antimicrobial Sensitivity Pattern of Some Bacterial Isolates Against Selected Antibiotics

ORGANISM	TET	PFX	AUG	CRO	NIT	GEN	COT	OFL	AMX	CPX	CHL	STR
Aeromonas hydrophila	R	12	R	14	R	R	R	11	R	9	R	7
Pseudomonas putida	7	R	R	R	R	R	R	R	R	10	R	R
Staphylococcus aureus	R	9	R	6	R	R	8	R	R	10	10	R
Bacillus megatarium	R	14	R	R	R	8	10	17	R	14	R	R
Enterobacter aerogenes	R	R	R	R	R	R	R	R	R	8	R	R
Staphylococcus epidermidis	R	6	R	R	R	8	R	9	R	5	R	R
Bacillus subtilis	R	16	R	15	R	8	10	15	R	16	R	R
Escherichia coli	R	8	R	9	R	11	8	8	R	7	R	R
Percentage Resistance (%)	87.5	25.0	100	50.0	100	50.0	50.0	37.5	100	0.00	87.5	87.5

Table 7: Antimicrobial Sensitivity Test of Fungal Isolates Against Antifungal Agents

ORGANISM	FULCIN			MYCOTIN		
	100 mg/ml	250 mg/ml	500 mg/ml	100 mg/ml	250 mg/ml	500 mg/ml
Aspergillus niger	R	R	R	8	R	R
Trichoderma reesei	9	6	9	R	8	8
Colletotrichum gloesporoides	R	8	14	R	R	12
Fusarium solani	R	R	R	R	R	R
Colletotrichum truncatum	R	10	8	R	9	6
Resistance Rate (%)	80	40	40	80	60	40

and the microbial load is also very high. The isolated organisms showed high resistance to available antibiotics and antifungal agents. Therefore, there is need to educate the populace on the effect of improper handling of naira notes and women most especially because of their children who always put money in their mouth.

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