http://dx.doi.org/10.4314/bajopas.v11i1.30S

Bayero Journal of Pure and Applied Sciences, 11(1): 185 - 190
ISSN 2006 – 6996

ANTIBIOTIC SENSITIVITY PATTERN OF BACTERIA ISOLATED FROM NIGERIAN CURRENCIES (NAIRA) CIRCULATING IN SOME HOSPITALS OF KANO METROPOLIS, KANO STATE, NIGERIA

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ABSTRACT
Microbial contamination of fomites such as currency notes is of public health concern as contaminated materials might act as vehicle for the transmission of pathogenic and drug resistant organisms. The aim of this study was to evaluate antibiotic susceptibility profile of bacteria isolated from Nigerian currencies circulating in some hospitals of Kano metropolis. Four hundred paper currency notes of all denominations obtained from three hospitals (Hasiya Bayero pediatrics hospital, Murtala Mohammad specialist hospital and Aminu Kano Teaching Hospital) and school environment (Bayero university Kano) were investigated. The samples were all screened for bacterial pathogens and some common nosocomial pathogens using standard microbiological procedures. Antibiotic susceptibility testing was done using disc diffusion method to detect the presence of resistant isolates including multidrug resistant organisms (MDR) and methicillin resistant Staphylococcus aureus (MRSA). The results of the study revealed that 84.7% of currencies were contaminated with pathogenic organisms. Bacteria isolated from currencies circulating in hospital were more resistant to antibiotics than non hospital source isolates (p< 0.05). The prevalence of MDR was found to be (16% and 6% p< 0.005) and (6.8% and 5.9% p>0.05) for MRSA from hospital and non hospital currencies respectively. Staphylococcus aureus, S. epidermidis, Escherichia coli, Bacillus species, Streptococcus species, Proteus species, Klebsiella spp, Salmonella spp and Pseudomonas aeruginosa were the most frequently isolated bacteria. Ampicillin, cotrimoxaxole, and amoxicillin/clavulanate showed high levels of inactivity. Ciprofloxacin had the greatest activity (40% to 100%) against the isolates. The study revealed that Currency notes circulating in hospital are highly contaminated with potentially pathogenic bacteria including drug resistant nosocomial pathogens, MRSA and MDR organisms. Measures should therefore be employed to ensure the safety of currency handlers in the hospital.

Key words: Antibiotic, Sensitivity pattern, Bacteria, Nigerian Currencies, Hospitals.

INTRODUCTION

Currency note (money) is one of the items most frequently handled by various categories of people during transaction. Thus it may get contaminated with various types of microorganisms. Microbial contamination of currency notes could be from several sources including counting machine, atmosphere, the skin, anal region, wounds, nasal secretion and aerosol generated by sneezing and coughing during handling and storage (Badvi *et al.*, 2017). Attitudes such as applying saliva or unclean water for counting improper storage and exchange of currency notes from one hand to another expose it to various types of microorganisms and makes it a good vehicle for their transmission (Sucilathangam *et al.*, 2016).

Nosocomial infection (hospital acquired infection) caused by drug resistance organisms is a growing problem in many health care institutions (Kennedy *et al.*, 2003). It has resulted in high mortality and morbidity due to treatment failures and increased health care cost. The most common bacteria pathogens that cause nosocomial infections are *Staphylococcus aureus, Pseudomonas aeruginosa , Escherichia coli* and antibiotic resistant strains of Gram negative rods (Jamal, 1981). The infections may include urinary tract infections, respiratory pneumonia, surgical site and wound infections, bacteremia, gastrointestinal and skin infections (Jamal, 1981).

Many studies have shown that the spread of nosocomial infection may be linked to contaminated surface of formites handle by health care workers (Aminu *et al.*, 2017; Nwanko *et al.*, 2014; Eugene and Erdoo, 2011).

The regular handling and exchange of currency notes by the patients, health care workers cashiers and other personnel's in the hospitals makes it vulnerable to contamination by various pathogenic organisms.

This contamination may play a significant role in the transmission of different diseases. Knowledge on the type of pathogenic bacteria associated with currencies circulating in the hospital and their drug sensitivity pattern is important for controlling the nosocomial spread of these contaminants.

This study was therefore undertaken to identify contamination level, types of isolates and antibiotic sensitivity pattern of bacteria associated with Nigerian currencies circulating in some hospitals of Kano metropolis.

MATERIALS AND METHODS

Sample (Naira notes) for the study were obtained from Murtala Muhammad Specialist Hospital (MMSH), Hasiya Bayero Pediatric Hospital and Aminu Kano Teaching Hospital (AKTH) between January 2016 to January 2018.Ethical permission for the study were obtained from ethical committee of the respective hospitals.

A total of 400 currency notes consisting of all types of denominations were obtained from different locations in the hospitals and from the school environment (Bayero University Kano). One hundred fresh mints were also collected from first bank to serve as control. The notes were collected by exchanging with new notes in sterile polythene bags using aseptic techniques and transported immediately to the laboratory for analysis according to the procedure of Yukubu *et al*. (2014).

Sterile swab sticks were slightly moistened with physiological saline rubbed over both surfaces of currency notes, then inoculated on Blood agar, MacConkey agar and Mannitol Salt agar. The plates were incubated for 24 hours. Pure isolated colonies were observed morphologically, Gram stained and biochemically identified using Indole, Catalase, Oxidase, Citrate, Nitrate, Coagulase, Urease and other relevant test according to standard microbiological techniques (Cheesebrough, 2000).

The antibiotic susceptibility pattern of the isolates was determined using disc diffusion method (CLSI, 2012). The suspension of each isolates was prepared in peptone water to match concentration of 0.5 McFarland standards. Sterile plates containing Mueller Hinton agar were inoculated with the standardized suspension of the organisms and the antibiotic disc were placed and incubated at 37^0C overnight. The antibiotics tested include Ampicillin (10µg), Gentamycin (30µg), Amoxicillin/Clavulanate (30µg), Cotrimoxazole (30µg), Erythromycin (10µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Ofloxacin (5µg) and Ceftazidime (30µg).

Methicillin resistant *Staphylococcus aureus* (MRSA) strains were detected using Cefoxitin disc diffusion method according to CLSI (2012). A standardized suspension of *Staphylococcus aureus* (0.5 McFarland standards) was inoculated on Mueller-hinton agar plates. Cefoxitin (30µg) disc was placed on the plates aseptically and incubated at 37^0C for 24 hours. The inhibition zones were measured and compared with standard chart (CLSI, 2012). Multidrug resistant isolates were identified when any of the isolate resists at least 3 or more of the different classes of antibiotics used in the study (CLSI, 2012).

Statistical analysis

Data obtained were analyzed using SPSS version 2.1. The data was presented using descriptive statistics in form of percentages and compared using Chi-squared analysis and T-Test. The results were considered significant at $P<0.005$.

RESULTS AND DISCUSSION

The result of the present study revealed that 84.7% of naira notes were contaminated with potential pathogenic bacteria. The rate of bacterial contamination of naira notes circulating in hospitals (87.5%) was slightly higher than that of non hospital environment (82%) as presented in Table 1. The contamination level is favorably comparable with the reports of some researchers who observed 86.4 % contamination level of Indian currencies (Sucilathangam, *et al.*, 2016) and 89.8% of Nigerian currency notes (Umeh *et al.*, 2007).

Higher denominations of naira notes (₦500 and ₦1000) were found to be more contaminated(100% each) in naira notes circulating in hospitals while lower denominations(₦ 5 and ₦ 10) from non hospital source were more contaminated (88% and 92% respectively) Table 1. The high level of bacterial contamination of lower denominations of naira notes observed is in line with the work of (Sucilathangam *et al.*, 2016) and Umeh *et al.*, 2007). This could be due to the fact that lower denominations are the most frequently used or exchanged in daily monetary transactions.

In hospitals the high levels of contamination of higher denominations of currency notes disagree with the report of some researchers and could possibly be due to less movement or circulation of lower denominations relating to the cost of hospital services which commonly involve the use of higher denominations.

Bacterial contamination of currency notes circulating in hospital and non hospital environment is presented in Table 2.

Staphylococcus aureus (56% and 50% for hospital and non hospital currency notes) was the most frequently isolated organism. *Acinetobacter spp* (5%) found only in hospital currency notes was the least isolated organism. A comparison of bacteria type and frequency of occurrence of isolates from hospital and non hospital currency notes showed a significant difference ($p<0.005$) with *Staphylococcus epidermidis* (38% Vs 32.5%), *Pseudomonas aeruginosa* (30% Vs 16%), *Bacillus spp.* (26.5% Vs 12.5%), *Escherichia coli* (25% Vs 15%), *Salmonella spp.* (14% Vs 24%) *and Acinetobacter spp.* (5% Vs 0%) respectively (Table 2).

This study has shown that most of the naira notes are contaminated with pathogenic bacteria. This is in support of the reports from other parts of the world that currency notes are usually contaminated by microorganisms that cause a wide range of diseases (Umeh *et al.,* 2007)

The preponderance of *Staphylococcus aureus* in currency notes is in agreement with the findings of Ahmed *et al.* (2010).This organism is part of normal skin flora and are responsible for a large number of hospital acquired infection (Nwanko *et al.,* 2014).Their presence in currency notes could be due to contact with hands of many people from various hygienic back ground. Some studies have documented the clinical significance of *S. aureus* as causative agent of urinary tract infection, toxic shock syndrome, skin infection and respiratory tract infection (Sucilathangam, *et al.*, 2016). The higher prevalence of *Staphylococcus epidermidis, Pseudomonas aeruginosa*, Bacillus *spp., Escherichia coli and Acinetobacter spp.* in currency notes circulating in hospital is in line with the report of Sepehri *et al.* (2009) and Nwanko *et al.* (2014) who associated these isolates with hospital acquired infection. Hospital environment play a significant role in the transmission of organisms associated with nosocomial infection (Jamal, 1981).This implies a serious concerned as to the possibility of currency notes to act as vectors in the spread of nosocomial pathogens.

The antibiotic sensitivity patterns of the isolates from hospital and non hospital currency notes are shown in Table 3 and Table 4 respectively. Table 5 summarized and compared the antibacterial activity of the antibiotics against the isolates. The isolates exhibited high level of resistance against ampicillin (83% and 82%), cotrimoxaxole (75% and 65%) and amoxicillin clavulanate (59 and 54%) for hospital and non hospital currency notes respectively. Ciprofloxacin had the greatest activity 80% for isolates from hospital currencies and 88% for non hospital isolates. The study also revealed that bacteria isolated from hospital currency notes were more resistant to antibiotics ($p<0.005$).

The *in vitro* antibiotic susceptibility pattern of the bacterial isolates varied greatly with ciprofloxacin showing highest activity (40 – 100%).This is comparable to 75 – 100% reported by Aminu *et al.* (2017) in isolates from hospital instruments and 83.5% in isolates from patients as reported by Iroha *et al* .(2013).Ciprofloxacin a quinolone compound which act by inhibiting nucleic acid synthesis can be considered as a drug of choice against bacteria associated with nosocomial infections. The high level of resistance exhibited by the isolates to ß lactam antibiotics conform with the work of Nwanko *et al.* (2017) which could be as a result of the production of ß lactamase enzyme that render the drugs ineffective (Mava *et al.*,2011).

Table 5 revealed that 38 out of 355 isolates were MDR (16% from hospital isolates while 6% from non hospital currency notes $P<0.005$). Table 5 further revealed that 22 out of 355 isolates were MRSA; 6.8% from hospital while 5.7% from non hospital currencies ($P>0.005$). The incidence of MRSA in relation to total *Staphylococcus aureus* is 21% and 18% for hospital and non hospital isolates respectively.

The incidence of multidrug resistant observed in this study is lower than the study of Aloma *et al.* (2016) who reported 41.3% of MDR bacteria from formites. The emergence of MDR organisms has become a public health problem creating a new burden on medical care in hospitals. The isolation of this organism from currency notes circulating in hospital further emphasized the public health significance of currency notes.

The rate of MRSA isolated in this study is less compared to the 44% and 44.3% from hospital instrument as reported by Aminu *et al.* (2017) and Eugene and Erdoo (2011) respectively. The isolation of antibiotic resistant strains of *Staphylococcus aureus* from currency notes circulating in hospital is of major health concern since the organism is the most frequently isolated in this research and have been reported to be one of the common causes of nosocomial infections (Jamal, 1981).

Table 1: Bacterial contamination level of different denomination of Nigerian currency notes

Denominations (₦)	Currency from Hospital Source		Currency from Non - Hospital Source	
	Number screened	Number contaminated (%)	Number screened	Number contaminated (%)
5	25	20 (80)	25	22 (88)
10	25	21 (84)	25	23 (92)
20	25	20 (80)	25	20 (80)
50	25	22 (88)	25	21 (84)
100	25	20(80)	25	21 (84)
200	25	22 (88)	25	22 (88)
500	25	25 (100)	25	17 (68)
1000	25	25 (100)	25	18 (72)
Total	200	175 (87.5%)	200	164 (82%)
				Mean % 84.7%

Table 2: Comparison of bacteria isolated from currency notes collected from hospital and non hospital environments

Type of bacteria	Hospital source N= 200	Non-hospital source N=200	P- Value
Staphylococcus aureus	112 (56%)	100 (50%)	0.56
Staphylococcus epidermidis	75 (38%)	65 (32.5%)	0.035
Klebsiella spp.	20 (10%)	23 (11.5%)	0.681
Pseudomonas aeruginosa	30 (15%)	16 (8%)	0.015
Proteus spp.	20 (10%)	25 (12.5%)	0.721
Bacillus spp.	53 (26.5%)	25 (12.5%)	0.023
Streptococcus spp.	29 (14.5%)	30 (15%)	0.0724
Escherichia coli	50 (25%)	30 (15%)	0.023
Salmonella spp.	28 (14%)	48 (24%)	0.041
Acinetobacter spp.	10 (5%)	0 (0%)	0.001

Table 3: Antibiotic sensitivity pattern of isolates from hospital currency notes.

Isolates	Number Tested	Number (%) of bacteria sensitive to antibiotic tested								
Gram positive		AMP	AMC	COT	CIP	CTZ	OFL	CRO	GN	E
S. aureus	56	10(18)	26(46)	12(21)	50(89)	42(75)	41(73)	43(77)	48(86)	48(86)
S. epidermidis	36	15(43)	15(43)	10(29)	28(80)	27(77)	28(80)	25(71)	20(57)	27(77)
Streptococcus spp.	10	5 (50)	6 (60)	03(30)	10(100)	07(70)	07(70)	07(70)	6(60)	07(70)
Gram Negative										
E. coli	25	0 (0)	9(36)	06(24)	20(80)	16(64)	18(72)	21(84)	20(80)	18(72)
Klebsiella spp.	25	0 (0)	3(30)	04(40)	07(70)	06(60)	07(70)	08(80)	06(60)	06(60)
Proteus spp.	10	0 (0)	3 (30)	02(20)	06(60)	09(90)	06(60)	07(70)	07(70)	03(30)
Pseudomonas aeruginosa	15	0 (0)	2 (13)	03(20)	06(40)	11(73)	06(40)	10(67)	10(67)	03(30)
Acinetobacter spp	05	0 (0)	0 (0)	0 (0)	05(100)	4 (80)	04(80)	04(80)	03(60)	01(21)
Salmonella spp.	14	0(0)	09(64)	02(14)	12(86)	11(79)	10(71)	10(71)	10(71)	06(43)

AMP-Ampicillin, AMC-Amoxicilin clavulanate, COT- Cotrimoxaxole, CIP- Ciprofloxacin, CTZ- Ceptaxidime, OFL- Ofloxacin, CRO- Ceftriaxone, GN- Gentamicin, E-Erythromycin.

Table 4: Antibiotic sensitivity pattern of isolates from non- hospital currency notes

Isolates	Number Tested	Number (%) of bacteria sensitive to antibiotic tested								
Gram positive		AMF	AMC	COT	CIP	CTZ	OFL	CRO	GH	E
S. aureus	56	12(21)	29(52)	22(39)	54(96)	48(86)	40(71)	52(93)	50(89)	46(82)
S. epidermidis	35	14(40)	17(49)	15(43)	32(91)	30(86)	32(91)	30(86)	21(60)	27(77)
Streptococcus spp.	10	06(60)	08(80)	02(20)	10(100)	07(70)	09(90)	10(10)	06(60)	08(80)
Gram Negative										
E. coli	25	0 (0)	13(52)	09(36)	21(84)	19(76)	20(80)	20(80)	18(72)	20(80)
Klebsiella spp.	10	0 (0)	04(40)	03(30)	07(70)	08(80)	07(70)	08(80)	09(90)	06(60)
Proteus spp.	10	0 (0)	05(50)	02(20)	08(80)	09(90)	06(60)	06(60)	07(70)	04(40)
Pseudomonas aeruginosa	15	0 (0)	04(27)	05(33)	10(67)	12(80)	10(67)	12(80)	11(73)	05(33)
Salmonella spp.	14	0(0)	11(79)	04(29)	12(86)	11(79)	12(86)	10(71)	09(64)	06(43)

AMP-Ampicillin, AMC-Amoxicilin clavulanate, COT- Cotrimoxaxole, CIP- Ciprofloxacin, CTZ- Ceptaxidime, OFL- Ofloxacin, CRO- Ceftriaxone, GN- Gentamicin, E-Erythromycin.

Table 5: Antibacterial activity of different antibiotics against bacteria isolated from Nigerian currency notes.

Antibiotics	Hospital isolates (N= 180)		Non hospital isolates (N=175)	
	Number susceptible (%)	Number resistant (%)	Number susceptible (%)	Number resistant (%)
Ampicillin	30 (17)	150 (83)	32 (18)	143 (82)
Amoxicilin clavulanate	73 (41)	107 (59)	80 (46)	95 (54)
Contrimoxaxole	42 (23)	138 (77)	62 (35)	113 (65)
Ciprofloxacin	144 (80)	36 (20)	154 (88)	21 (12)
Ceftriaxone	135 (75)	45 (25)	148 (85)	27 (15)
Ceftaxidime	133 (74)	47 (26)	144 (82)	31 (18)
Erythromycin	119 (66)	19 (19)	122 (70)	53 (30)
Gentamicin	130 (72)	50 (28)	131 (75)	44 (25)
Ofloxacin	127 (71)	53 (29)	124 (71)	49 (29)

P<0.005

Table 6: Frequency of occurrence of MRSA and MDR isolate from currency notes

Currency source	Number screened	Number of MDR (%)	Number of MRSA (%)	No. of MRSA out of Total *S. aureus*
Hospital	180	28 (16)	12 (6.8)	12/56(21%)
Non- hospital	175	10 (6)	10 (5.7)	10/56(18%)
TOTAL	355	38 (11)	22 (6.2)	
P-Value		0.0042	0.0068	

MDR -Multidrug resistance, MRSA-Methicillin resistant *Staphylococcus aureus*

CONCLUSION AND RECOMMENDATION

The study revealed that Nigerian currency notes (naira) circulating in hospital are heavily contaminated with various bacterial agents including potential nosocomial pathogens of which most are resistant to commonly used antibiotics. The isolation of MRSA and MDR bacteria in this study confirms that currency notes may play an important role in the transmission of drug resistant bacteria. Intervention measures should therefore be employed to avoid the risk of transmitting nosocomial pathogens to patients and other money handlers in the hospital. This should include personal hygiene, adoption of electronic transactions in the hospitals and public enlightenment on the risk associated with handling currency notes without taking aseptic measures.

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