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Research Article

# MULTIDRUG RESISTANCE OF ISOLATES OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN PAPER CURRENCY NOTES FROM RESTAURANTS AND HOTELS IN LUSAKA IN ZAMBIA

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**ABSTRACT**

Objective: Isolation and determine antibiotic sensitivity test for coagulase positive *Staphylococcus aureus* isolates from restaurants and fast food serving hotels from Lusaka, Zambia.

Methods: A total of 205 Paper currency notes of Zambia Kwacha (50,100,500,1000,5000,10,000,20,000 and 50,000)were randomly collected from market place and swab samples were analyzed in microbiology lab for *S.aureus* bacterial contamination and antibiotic sensitivity test and by conducting catalase and coagulase tests; hemolysis, sugar fermentation, and other biochemical tests, including tests for indole production, citrate utilization (for glucose, sucrose, and lactose fermentation); gas and hydrogen sulfide production tests; and oxidase tests. Gram positive cocci bacteria were inoculated on blood agar and MacConkey agar. Coagulase test was conducted to know coagulase positive S.aureus. Antibiotic sensitivity test was conducted for *S.aureus* isolates.

Result: 53(25.85%) *S.aureus* isolated from 205 paper currency notes and 6(2.92%) of Vancomycin resistance was found. All *Staphylococcus aureus (S.aureus)* isolates showed multidrug resistance. *S.aureus* isolates resistant resistant to Penicillin. Isolates resistant to Vancomycin were also resistant to Methicillin. All isolates were sensitive to Lenozoid antibiotic. The incidence of *S.aureus* is more in lower denomination paper currency notes than higher denominations. Currency notes may be the reservoir of infection in society.

**Keywords:** Market places, *S.aureus* isolates, Antibacterial activity, Paper currency notes.

## INTRODUCTION

*Staphylococcus aureus Staphylococcus aureus* is a Gram-positive coccus. It is a non-motile, non-sporing and facultative anaerobic, is ubiquitous in nature and a known colonizer in humans. Community acquired soft tissue infections due to *S. aureus* is quite common. During the past four decades, Methicillin-resistant *Staphylococcus aureus* (MRSA) has evolved from a controllable nuisance into a serious public concern [1]. CA-MRSA infections typically occur as skin or soft tissue infections, but can develop into more invasive, life-threatening infections. CA-MRSA is occurring with increasing frequency in the United States and around the world and tends to occur in conditions where people are in close physical contact, such as athletes involved in football and wrestling, soldiers kept in close quarters, inmates, childcare workers, and residents of long-term care facilities [2].

Currency notes are used as a medium for exchange for goods and services, settlement of debts and for deferred payments in economic activities [3].The contamination of the naira notes could also be from several sources, it could be from the atmosphere, during storage, usage, handling or production [4]. The predominant bacterial isolate was Bacillus sps followed by coagulase negative Staphylococci and Micrococcus sps. Other bacteria that are either potential or confirmed pathogens included *K. pneumoniae*, *E. coli*, *S. aureus*, Pseudomonas sps and *S. typhi*. Only two notes were positive for Acid fast bacilli. 28 samples did not yield any fungal growth [5].

Paper currency offers a larger surface area as a breeding ground for pathogens. Microbes may persist on it for longer periods. The older the paper note the more accumulation of microbes occurs [6]. Eighty-nine percent of Nigerian Naira notes studied were contaminated with bacteria [7]. Other studies have shown the more paper currency stays in circulation the higher the risk of becoming contaminated. Egyptian paper notes minted in the year 2000 had more bacterial contamination than those minted in 2003 [6].

Lower denomination notes harbor the greatest bulk of infectious agents since they are exchanged more than higher denomination notes [8]. Antimicrobial resistance is a global phenomenon that has resulted in high morbidity and mortality as a result of treatment failures and increased health care costs [9].

The aim of this study was to isolation and determine antibacterial activity of *s.aureus* isolated from Zambian currency. This is the first kind of work in Zambia.

## MATERIALS AND METHODS

### Collection of Samples

The study was conducted from August 2012 to September 2012. 205 notes of different denominations paper currency notes of Zambian Kwacha (50,100,500,1000,5000,10,000, 20,000 and 50,000 were collected from restaurants and fast food serving hotels from Lusaka, Zambia. Simultaneously we collected new paper currency notes from Bank as reference for bacteriological analysis. We did not collect coin currency.

Samples contain lower and higher denominations. Each currency note was collected directly into a sterile plastic bag and transported to the Laboratory of the Department of Science, Sebastian Kolowa Memorial University, Lushoto, Tanzania soon after collection and examined for bacterial contamination. Swab samples were dipped in 1% peptone water. The swab samples were carried to lab for further examined for microbiological analysis.

### Bacteriological Analysis

Isolation of various bacterial contaminants from the currency notes was performed via standard techniques described previously [10,11]. Briefly, a sterile, cotton-tipped swab moistened with sterile physiological saline was used to swab both sides of the currency note. The swabs were directly inoculated on blood agar and MacConkey agar. The pairs of inoculated media were incubated aerobically at 35-370C for 24 hours and then examined for bacterial growth according to standard protocol described previously [12]. The isolated bacteria were further studied by colony characteristics and Gram reaction and by conducting catalase and coagulase tests; hemolysis, sugar fermentation, and other biochemical tests, including tests for indole production, citrate utilization tests (for glucose, sucrose, and lactose fermentation); gas and hydrogen sulfide production tests; and oxidase tests,coagulase test was conducted to know coagulase positive S.aureus. Antibiotic sensitivity test was conducted for *S.aureus* isolates according to protocols described previously [12].

### Antibiotic Susceptibility Test (AST)

Antibiotic susceptibility were determined by the agar diffusion technique on Mueller-Hinton agar (Kirby-Bauer NCCLS modified disc diffusion technique) using 8 antibiotic discs (Biotec Lab. UK) corresponding to the drugs most commonly used in the treatment of

human and animal infections caused by bacteria; Penicillin(PEN) (10 units), Streptomycin (STR)(30 µg), Methicillin(M)(30 µg), Ofloxacilin (OFL) (30 µg), Ciprofloxacin(CP)(30 µg), Vancomycin(V)(30 µg), Gentamycin (GEN) (30 µg), Linezolid(L)(30 µg), Amoxicillin(AM)(30 µg)and Ceftriaxone (CFX) (30 µg)(Hi Media,India) [13].

**RESULTS**

From the analysis of the *205* paper currency notes collected from restaurants and fast food serving hotels of Lusaka city of Zambia, See Table no.1.

We did not find a single *S.aureus* from Kwacha 10,000, 20,000 and 50,000. Bacteria were identified but were not quantified. In lower denominations *S. aureus* showed high incidence and exhibited resistance to multiple antibiotics.

All *S.aureus* isolates were sensitive to Linezolid antibiotic and 100% resistance to Penicillin. 76.9% resistance to vancomycin that was often considered as last line of defense was isolated.

Kwacha 50 lower denominations, showed high microbial load, 15 (50%) incidence was found. All 15 *S.aureus* isolates from 50 Kwacha were resistant to Penicillium and sensitive to Lenezoid, 80% were resistant to Methicillin, 3 (20%) were resistant to Vancomycin,5(33.33%) to Ciprofloxacin, 6 (40%)to Streptomycin, 7(46.66%) to Gentamycin, 9(60%) to Ofloxacin, 4(26.66% ) to Cefriaxome, and 9 (60%) to Amoxicillin.

Kwacha 100 lower denominations, showed high microbial load, 11(42.30%) incidence was found. All 11 *S.aureus* isolates from 100Kwacha were resistant to Penicillium and sensitive to Lenezoid, 9(81%) were resistant to Methicillin, 1 (9%) were resistant to Vancomycin, 3 (27.27%) to Ciprofloxacin, 6 (54.54%)to Streptomycin, 6 (54.54%) to Gentamycin, 8(72.72%) to Ofloxacin, 6(54.54%) to Cefriaxome, and 6(54.54%) to Amoxicillin.

500 Kwacha (27) lower denominations, showed high microbial load, 11(40.74%) incidence was found. All 11 *S.aureus* isolates from 500 Kwacha were resistant to Penicillium and sensitive to Lenezoid, 8(72%) were resistant to Methicillin, 1 (9%) were resistant to Vancomycin, 3 (27.27%) to Ciprofloxacin, 6 (54.54%)to Streptomycin, 4 (36.36%) to Gentamycin, 8(72.72%) to Ofloxacin, 5(45.45%) to Cefriaxome, and 3(27.27%) to Amoxicillin.

1000 Kwacha (27), 9(33.33%) incidence was found. All 9 *S.aureus* isolates from 1000 Kwacha 9(100%) were resistant to Penicillium and sensitive to Lenezoid, 9(100%) were resistant to Methicillin, 1 (33.33%) were resistant to Vancomycin, 8 (29.62%) to Ciprofloxacin,4(44.44%) to Streptomycin, 4(44.44%) to Gentamycin, 4(44.44%) to Ofloxacin, 3(33.33%) to Cefriaxome, and 4(44.44%) to Amoxicillin.

5000 Kwacha (26), 7(26.92%) incidence was found. All 7 *S.aureus* isolates from 5000 Kwacha 7(100%) were resistant to Penicillium and sensitive to Lenezoid, 7(100%) were resistant to Methicillin, all *S.aureus* were sensitive to Vancomycin, 3(42.8%) to Ciprofloxacin, 3(42.85%) to Streptomycin, 4(57.14%) to Gentamycin, 4(44.44%) to Ofloxacin, 2(28.57%) to Cefriaxome, and 3(42.85%) to Amoxicillin. We did not find single bacteria from unused paper currency notes from Bank.

**Table 1: Expression of antibiotic resistance patterns by *S. aureus* strains isolated from paper currency from restaurants and hotels of Lusaka in Zamia.**

| S. No. | Zambia currency notes with number. Total *205* | Number of *S.aureus* isolates and Percentage | % of *S.aureus* isolates resistance to penicillin (10 units) | % of *S.aureus* isolates resistance to methicillin(30 µg) | % *S.aureus* of isolates resistance to vancomycin (30 µg) | %of *S.aureus* isolates resistance to ciprofloxacin (30 µg) |
|---|---|---|---|---|---|---|
| 1 | 50 Kwacha(30) | 15(50%) | 100 | 12(80%) | 3(20%) | 5(33.33%) |
| 2 | 100Kwacha (26) | 11(42.30%) | 100 | 9(81%) | 1(9%) | 3(27.27%) |
| 3 | 500 Kwacha (27) | 11(40.74%) | 100 | 8(72%) | 1(9%) | 3(27.27%) |
| 4 | 1000 Kwacha (27) | 9(33.33%) | 100 | 100 | 1(3.70%) | 8(29.62%) |
| 5 | 5000 Kwacha (26) | 7(26.92%) | 100 | 100 | nil | 3(42.8%) |
| 6 | 10,000 Kwacha(24) | 00 | 00 | 00 | 00 | 00 |
| 7 | 20,000 Kwacha(23) | 00 | 00 | 00 | 00 | 00 |
| 8 | 50,000 Kwacha(22) | 00 | 00 | 00 | 00 | 00 |

**Table.2 Expression of antibiotic resistance patterns by *S. aureus* strains isolated from paper currency from restaurants and hotels of Lusaka in Zamia.**

| S. No. | Zambia currency notes with number. Total *205* | Number of *S.aureus* isolates and Percentage | % of *S.aureus* isolates resistance to Streptomycin (30 µg) | % of *S.aureus* isolates resistance to gentamycin (30 µg) | % of *S.aureus* isolates resistance to Linezolid(30 µg) | % of *S.aureus* isolates resistance to ofloxacilin(30 µg) |
|---|---|---|---|---|---|---|
| 1 | 50 Kwacha(30) | 15(50%) | 6(40%) | 7(46.66%) | nil | 9(60%) |
| 2 | 100Kwacha (26) | 11(42.30%) | 6(54.54%) | 6(54.54%) | nil | 8(72.72%) |
| 3 | 500 Kwacha (27) | 11(40.74%) | 6(54.54%) | 4(36.36%) | nil | 5(45.45%) |
| 4 | 1000 Kwacha (27) | 9(33.33%) | 4(44.44%) | 4(44.44%) | nil | 4(44.44%) |
| 5 | 5000 Kwacha (26) | 7(26.92%) | 3(42.85%) | 4(57.14%) | nil | 3(42.85%) |
| 6 | 10,000 Kwacha(24) | 00 | 00 | 00 | 00 | 00 |
| 7 | 20,000 Kwacha(23) | 00 | 00 | 00 | 00 | 00 |
| 8 | 50,000 Kwacha(22) | 00 | 00 | 00 | 00 | 00 |

**Table 3: Expression of antibiotic resistance patterns by *S. aureus* strains isolated from paper currency from restaurants and hotels of Lusaka in Zamia.**

| S. No. | Zambia currency notes with number. Total *205* | Number of *S.aureus* isolates and Percentage | % of *S.aureus* isolates resistance to Ceftriaxone (30µg) | % of *S.aureus* isolates resistance to amoxicillin(30 µg) |
|---|---|---|---|---|
| 1 | 50 Kwacha(30) | 15(50%) | 6(40%) | 10(66.66%) |
| 2 | 100Kwacha (26) | 11(42.30%) | 6(54.54%) | 6(54.54%) |
| 3 | 500 Kwacha (27) | 11(40.74%) | 3(27.27%) | 3(27.27%) |
| 4 | 1000 Kwacha (27) | 9(33.33%) | 3(33.33%) | 3(33.33%) |
| 5 | 5000 Kwacha (26) | 7(26.92%) | 2(28.57%) | 2(28.57%) |
| 6 | 10,000 Kwacha(24) | 00 | 00 | 00 |
| 7 | 20,000 Kwacha(23) | 00 | 00 | 00 |
| 8 | 50,000 Kwacha(22) | 00 | 00 | 00 |

## DISCUSSION

Communicable diseases spread through contact with fomites and transfer through paper currencies is a very possible route [14]. A review of the medical literature reveals few investigations involving the bacterial contamination of money in the United States. A study conducted in Australia in 2010 among currencies from 10 different countries showed that the lower the index values of the money, the higher the typical bacterial content of the currency. They further showed that the age of the notes and the material that was used to produce the notes influence the number of bacterial contamination [15]. Virulent genes of *S.aureus* were isolated from paper currency and these strains show resistance to the more common antibiotics [16].

*Staphylococcus aureus* can cause skin infection impetigo, Pneumonia, gastroenteritis localized collection of pus, known as an abscess, boil, food poisoning, vomiting with occasional abdominal cramping and urinary tract infections (UTIs), and bacteremia [17,18,19]. [20,21].

*S. aureus* which had highest occurrence has been recognized for cross implication in various types of infections. High level of antibiotic resistance was observed by the *S. aureus* isolates.

*S.aureus* in Kwacha 10,000, 20,000 and 50,000 was found to be nil in our reports. The percentage of *S.aureus* incidences were more in lower currency denominations and less in higher denominations.

53(25.85%) *S.aureus* isolated from 205 paper currency notes, showed multidrug resistant. Similar reports were found in Nigerian currency notes [22], with Bacterial contamination of *Staphylococcus aureus* (38%)in Saudi "one" Riyal paper notes [23]and Antibiotic Resistant Bacterial Contamination of the Ghanaian Currency Note [24].

All isolates were sensitive to Linezolid and resistant to Penicillin antibiotic, similar reports were found with (R Neel, 2012 and [5,25].

*S.aureus* isolates showed highest resistance to Ciprofloxacin, similar reports were found [26,27].

From this study, the bacterial isolates that were isolated, were associated with nasal, skin and hands. This is an indication that money contamination is associated to unhygienic practice of people. These practices include indiscriminate sneezing, coughing and defecation with indecent handling of currency notes [11,28].

All isolates were sensitive to Lenezoid antibiotic, similar reports found [26,29].

Vancomycin was choice of drug to treat MRSA. 6(2.92%) of Vancomycin resistance was found from 205 paper currency notes. In society the currency pass from patients, especially immunocomromised patients and are on chemotherapeutic agents get Vancomycin resistance [26,30].

In our reports isolates lower currency notes showed highest resistance Gentamycin, Streptomycin, Vancomycin, similar reports were found with Adegoke, Anthony A, 2011. Similar in case of Ceftriaxone [31].

Antibiotic abuse should be discouraged while currency notes should also be handled with care to prevent it from being the vehicle for infection transfer. Currency notes could be a source of contamination and cause variety of diseases. It is therefore suggested that the public should be further enlightened on the importance of hand washing at restaurants and homes, slaughter house, market place and after going to the toilet. Keeping notes under body surfaces and holding currency notes in hand for longtime should be avoided. Students should be educated at primary school level. Public should be educated through daily news papers.

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