

Full Length Research Paper

Bacterial load on Ghanaian currency notes

Patrick Feglo^{1*} and Michael Nkansah²

¹Department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

²Department of Medical Laboratory Technology, Faculty of Allied Health Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Accepted 24 August, 2010

Ghanaian currency notes are handled by all manner of people including ready-to-eat food sellers who serve food and handle the currency notes as they sell making the notes dirty and cross-contaminated. Hence this study aims at determining bacterial species and level of contamination of the notes in circulation. Ghanaian currency notes were collected at random from ready-to-eat food sellers in Kumasi. Buffered peptone water (BPW) washings of the notes were inoculated onto plate count agar (PCA) for total viable count and then Blood and MacConkey agar for bacteria identification. The study reveals 98.6% of the currency notes were bacterially contaminated, 12 (17.14%) had acid-fast bacilli, and 1.43% *Taenia* sp. ovum. The bacterial mean viable count was $1.5 \times 10^4 \pm 1.1 \times 10^1$ CFU/Note, the GH¢1 had the highest mean viable count of 4.0×10^4 CFU/Note, the GH¢5 1.8×10^4 CFU/Note, and then the GH¢10 had 2.8×10^3 CFU/Note. The isolates were *Bacillus* species (41.07%), coagulase-negative staphylococcus (33.04%), *Staphylococcus aureus* (7.14%), *Enterococcus faecalis* (7.14%), *Citrobacter freundii* (4.46%), *Klebsiella pneumoniae* (2.68%), *Shigella dysenteriae* (2.68%) and *Escherichia coli* (1.79%). The Ghanaian currency notes in circulation were found to be contaminated with pathogenic microorganisms which can spread human diseases.

Key words: Bacterial contamination, currency notes, food poisoning.

INTRODUCTION

Paper currency is widely exchanged for goods and services in countries worldwide (Uneke and Ogbu, 2007). In Ghana, the currency notes are used for buying ready to eat food, uncooked meat from the market, charcoal, milk at a local store, drugs and are used in all sorts of trade. Many Ghanaians do not care how dirty their fingers are when handling money (Mensah et al., 2002). So, the butcher with the bloody fingers, the artisan with dirty-dusty and oily fingers, the teacher with the chalky and inky fingers, the street-food vendor with the wetly-oily fingers, etc., will just receive or pick the Ghanaian currency notes with the dirty fingers, leading to the contamination of the notes with microorganisms (Mensah et al., 2002). The contaminated currency notes go in circulation and contaminate the hands of others

transmitting pathogenic organisms in the process (Uneke and Ogbu, 2007; Mensah et al., 2002). The Ghanaian currency notes are often dirty, and even mutilated notes can be seen in circulation, although the notes were released into circulation in July, 2007 not long ago (Bank of Ghana, 2008). The survival of various microorganisms on money and their transmission via the hands of food vendors is often overlooked as enteric disease reservoir (Michaels, 2002). Pathogenic microorganisms that may survive on the Ghanaian currency notes may serve as a potential source of enteropathogens causing food poisoning because in Ghana food vendors serve food with the hands and at the same time handle currency notes as they sell (Michaels, 2002; Cardoen et al., 2009; Lamichhane et al., 2009). Such practices transfer bacteria from currency notes to humans through food (Lamichhane et al., 2009; Ministry of Health, 2007; Reither et al., 2007).

The aim of this study was therefore to determine the level of bacterial contamination of the Ghanaian currency

*Corresponding author. E-mail: pfglo.sms@knust.edu.gh, pfglo@yahoo.com.

notes in circulation, so as to determine whether or not the notes constitute a potential source of disease spread.

METHODS

Sample collection and transport

This study was conducted from November 2008 to February 2009 in which Ghanaian currency notes were collected at random from ready-to-eat food sellers on the campus of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. This was an observational cross sectional study involving 70 currency notes collected at random. The currency notes studied were 30 of the One Ghana Cedi Note (GH¢ 1), 30 of the Five Ghana Cedi Note (GH¢ 5) and 10 of the Ten Ghana Cedi Note (GH¢ 10). Each currency note was collected directly into a sterile plastic bag and transported to the Department of Microbiology Diagnostic laboratory, KNUST, soon after collection and examined for bacterial contamination.

Determination of bacterial load

With the aid of a pair of sterile forceps, each currency note was transferred aseptically into a sterile universal bottle containing 10 ml of sterile buffered peptone water. The bottle was capped and shaken vigorously by hand for about 2 min to dislodge the micro-organisms into the fluid. The resulting fluid (buffered peptone water) served as the test sample, whilst the currency note was removed aseptically from the universal bottle with a sterile forceps, rinsed with water and dried to recover the note.

Serial doubling dilutions were prepared from the test sample as shown thus - $1:10^1$, $1:10^2$, $1:10^3$... $1:10^{10}$. This was done by transferring dispensed 1 ml of test sample into 9 ml of sterile buffered peptone, vortexed, and then 1 ml aliquot transferred into the next tube using a micropipette. Starting with the highest dilution 0.1 ml of the test dilution (after agitation) was dispensed onto plate count agar also called PCA (Oxoid Ltd, Basingstoke, Hampshire, England) plates in duplicate. The inoculum was spread evenly over the entire surface of the PCA using a sterile bent spreader. All plates were incubated at 37°C, aerobically in an incubator overnight. After overnight incubation, all colonies on the plates containing 30 - 300 colonies were counted from the duplicate plates and the mean counts determined.

Bacteria isolation and identification

Using an automated pipette, 0.1 ml of the test dilution (after agitation) was dispensed onto Blood agar (Oxoid Ltd, Basingstoke, Hampshire, England) and MacConkey agar (Oxoid Ltd, Basingstoke, Hampshire, England) plates. Using a sterile microbiological loop the inoculums were streaked evenly. All the plates were incubated aerobically in an incubator at 37°C, overnight. After incubation both plates were examined for growth. Less than five identical colonies for a particular organism growing on a plate were ignored. Five isolated identical colonies growing on either the blood agar or MacConkey agar plates were each picked carefully and inoculated into buffered peptone water contained in sterile microtitre wells. Culture from each microtitre well was sub-cultured onto a Nutrient agar (Oxoid Ltd, Basingstoke, Hampshire, England) to obtain pure culture. Organisms which were identified to be the same from the microtitre wells were grouped as one isolate from the note sample analyzed. Bacterial identification was done using the pure culture on the nutrient agar. The first test was the gram staining and the results were followed by the appropriate

biochemical tests (catalase, coagulase, oxidase, sugar fermentation, indole, citrate utilization, urease production) and motility test. The catalase test was performed on the gram positive cocci. This was done by mixing a dense culture with two drops of H_2O_2 and looking for bubbles. Organisms positive (produced bubbles) in the test were considered to be *Staphylococci*, while those negative were *Streptococci*. The *Staphylococci* were further tested with the coagulase test. The coagulase test was performed by mixing a dense suspension of the culture with plasma contained in a small test tube. The set up was incubated overnight, and then observed for fibrin clot. Those positive in the coagulase test were identified to be *Staphylococcus aureus*, and those negative were coagulase negative staphylococci. The gram negative organisms were identified as follows: organisms which were non-fermenter of lactose on the MacConkey agar were subjected to the oxidase test using tetramethyl-*p*-phenylenediamine hydrochloride reagent sticks. The oxidase stick was used to touch the organism on the nutrient agar plate. The development of a black colour on the stick in less than 10 s was considered positive for oxidase production. *Pseudomonas aeruginosa* is positive in this test, so the oxidase test and other tests (growth characteristics smell and pigment production) were used to identify the organism. *E. coli* was used as negative control.

To test for citrate utilization, the Simon's citrate agar (Oxoid Ltd, Basingstoke, Hampshire, England) was used. Growth on the nutrient agar was inoculated onto the citrate agar and incubated overnight. Colour change from green to deep-blue is positive test. *Klebsiellae* are positive in this test and enabled it to be differentiated from *Escherichia coli*, which was also used as a negative control. Triple sugar iron (TSI) agar (Oxoid Ltd, Basingstoke, Hampshire, England), was used for the differentiation of the *Enterobacteriaceae*. Using a sterile straight wire, the TSI was stabbed deep to the bottom and the surface of the agar slant was streaked with the test organism. By the three different sugar fermentation and hydrogen sulphide production abilities of the enterobacteriaceae, they were identified. The indole test was performed by inoculating peptone water (Oxoid Ltd, Basingstoke, Hampshire, England), incubating it overnight and the detection of indole by the addition of Kovac's reagent. The formation of a red ring on the surface layer of the peptone water was positive test. *E. coli* is positive in this test so was used to separate it from *Klebsiella*. Motility of the organism was tested by using a sterile loop to pick culture from the microtitre wells, placed on a microscopic slide, covered with a cover slip and observed under the microscope. Care was taken to observe true darting locomotion and not brownian motion.

After inoculating the agar plates, the remaining test sample was transferred into sterile centrifuge tubes and centrifuged at 3000 rpm for 5 min. The supernatant was decanted. Two smears were made on microscope slides from the deposit. To one smear, a drop of Dobell's iodine was added, covered with a cover slip and examined under the microscope for parasites. The other film was air-dried, heat fixed and stained with Ziehl-Neelsen method and then examined under the microscope for acid-fast bacilli.

Statistical analysis

Continuous data were expressed as mean±SEM and categorical data expressed as proportion. In all cases a p-value <0.05 was considered significant. The data was analyzed using Stata/IC 10.0 for windows (StataCorp LP, USA, <http://www.stata.com>).

RESULTS

A total of seventy (70) Ghanaian currency notes were

Table 1. Bacterial Isolates and their percent distribution amongst currency notes.

Isolates	Currency notes			Total
	GH¢1	GH¢5	GH¢10	
<i>Bacillus species</i>	22 (19.64)	17 (15.18)	7 (6.25)	46 (41.07)
<i>Coagulase-negative staph</i>	22 (19.64)	14 (12.50)	1 (0.90)	37 (33.04)
<i>Staphylococcus aureus</i>	3 (2.68)	4 (3.57)	1 (0.89)	8 (7.14)
<i>Enterococcus faecalis</i>	3 (2.68)	5 (4.46)	0.00%	8 (7.14)
<i>Citrobacter freundii</i>	2 (1.79)	2 (1.79)	1 (0.89)	5 (4.47)
<i>Klebsiella pneumonia</i>	2 (1.79)	1 (0.89)	0.00%	3 (2.68)
<i>Shigella dysenteriae</i>	2 (1.79)	1 (0.89)	0.00%	3 (2.68)
<i>Escherichia coli</i>	1 (0.89)	1 (0.89)	0.00%	2 (1.78%)
Total	57 (50.90)	45 (40.17)	10 (8.93)	112 (100)

Table 2. Mean viable count of bacteria on the currency notes (Standard deviation = 1.1×10).

Denomination	Mean viable count CFU/Note	Log ₁₀ (CFU/Note)
GH¢1	4.0×10^4	4.60
GH¢5	1.8×10^4	4.26
GH¢10	2.8×10^3	3.45
Overall mean count	1.5×10^4	4.18

analyzed for bacterial contamination. One hundred and twelve (112) different bacteria were isolated from sixty nine (69) currency notes, giving percentage of contamination to be 98.57%. One of the currency notes which appeared new and 'seemingly clean' did not grow any bacterium. Types of bacteria isolated from the notes were *Bacillus species* (41.07%), coagulase-negative *staphylococci* (33.04%), *S. aureus* (7.14%), *Enterococcus faecalis* (7.14%), *Citrobacter freundii* (4.46%), *Klebsiella pneumoniae* (2.68%), *Shigella dysenteriae* (2.68%), and *E. coli* (1.79%), as shown in Table 1.

For the thirty (30) of the One Ghana Cedi (GH¢1) notes analyzed for bacteria, a total of fifty-seven (57) bacteria was isolated from 29 of them out of which 45 different bacteria isolates were obtained. The bacteria species isolated from the GH¢1 notes were as follows; *Bacillus species* (38.59%), coagulase-negative *Staphylococci* (38.59%), *S. aureus* (5.26%), *E. faecalis* (5.26%), *K. pneumoniae* (3.51%), *S. dysenteriae* (3.51%), *C. freundii* (3.51%), and *E. coli* (1.75%). All five Ghana Cedi (GH¢5) notes grow bacteria and the isolates obtained were: *Bacillus species* (37.78%), coagulase-negative *Staphylococci* (31.11%), *E. faecalis* (11.11%), *S. aureus* (8.89%), *C. freundii* (4.44%), *K. pneumoniae* (2.22%), *S. dysenteriae* (2, 22%), and *E. coli* (2.22%). The GH¢10 notes analyzed for bacteria yielded ten different bacteria (Table 1), and the isolates obtained were *Bacillus species* (70%), *S. aureus* (10%), coagulase negative *staphylococcus* (10%), and *C. freundii* (10%).

The mean viable bacterial count on the currency notes examined was expressed as log₁₀ was $1.5 \times 10^4 \pm 1.1 \times 10^1$ CFU/Note (as shown in Table 2). The mean viable count on the GH¢1 notes was 4.0×10^4 CFU/Note, with the range being 1.0×10^3 CFU/Note to 8.0×10^{10} CFU/Note. The GH¢5 notes had a mean viable count of 1.8×10^4 CFU/Note and the viable count ranged from 1.0×10^3 CFU/Note to 9.5×10^5 CFU/Note. The GH¢10 notes had the least mean viable count of 2.8×10^3 CFU/Note with the range being 3.0×10^2 CFU/Note to 1.0×10^4 CFU/Note.

Twelve (12) of the currency notes (17.14%) yielded acid-fast bacilli, whilst the wet-film analyses yielded the ovum of *Taenia species* on one (1.43%) of the currency notes.

DISCUSSION

This study determined the level of contamination of the Ghanaian currency notes. It demonstrated that, Ghanaian Currency (Cedi) notes in circulation are contaminated with both gram positive and gram negative bacteria, acid bacilli and the ovum of a parasite. The overall mean viable count on the currency notes was 1.27×10^4 CFU/Note. The One Ghana Cedi notes showed the highest average mean viable count of 4.0×10^4 CFU/Note, followed by the five Ghana Cedi notes and Ten Ghana Cedi notes respectively. The One Ghana Cedi notes were most contaminated, but there were no significant difference in the levels of contamination between the

notes ($p < 0.05$). Currency notes are therefore possible vehicles through which infectious agents can be transmitted to humans (Lamichhane et al., 2009; Umeh et al., 2007). Some bacteria isolates, such as *S. aureus* and *S. dysenteriae* are pathogenic microorganisms which can cross contaminate food, supporting reports from other parts of the world that paper currency are usually contaminated by microorganism that can cause a wide range of diseases (Umeh et al., 2007; Pope et al., 2002; El-Sakka et al., 2005; Kuria et al., 2009; Abrams and Waterman, 1972; Sapsford et al., 2004) including acid fast bacilli Basavarajappa et al., 2005) which can cause either tuberculosis, leprosy or buruli ulcer, depending on the *Mycobacterium* species. These diseases are common in Ghana and the mode of transmission of buruli ulcer is not clear and sufferers of leprosy are unable to tell when they got infected.

Culture of the currency washings led to the isolation of various bacteria. The bacteria isolated were *Bacillus* species (41.07%), which was the dominant isolate, followed by coagulase-negative *staphylococci* (33.04%), *S. aureus* (7.14%), *E. faecalis* (7.14%), *C. species* (4.46%), *K. species* (2.68%), *S. species* (2.68%) and *E. coli* (1.79%), just as *Bacillus* species was the predominant bacterial isolate in studies in India (Basavarajappa et al., 2005; Singh et al., 2002). This study revealed higher prevalence of bacterial contamination of the currency notes than of parasites (98.57 vs. 1.43% respectively) similar to a report from Nigeria (Uneke and Ogbu, 2007). The ovum of *Taenia* species was found on one of the currency notes and it is a reflection of poor local environmental sanitation and personal hygiene (Hotez et al., 2003).

This situation is not limited to the underdeveloped countries alone, for a study in the United States of America indicated that, handling money and ready-to-eat food with the same gloved hands or without hygienic intervention between these activities can introduce the risk of cross-contamination to foods provided in food service establishments (Michaels, 2002). That study also determined the survival of pathogens on coins and currency notes and found that various microorganisms may survive and multiply on currency notes (Michaels, 2002; Lamichhane et al., 2009). This is of concern, because the currency notes could serve as a vehicle for transmission of diseases and represents an often overlooked enteric disease reservoir (Michaels, 2002; Emikpe and Oyero, 2007); meanwhile in Ghana gloves are not worn at all. Food vendors use their bare hands which they occasionally wash in a bowl of water. This is not a flowing tap water but a stagnant bowl of water which is kept for as long as the food remains, until it is sold out to another overlooked reservoir.

Some of the bacteria isolated in this study, such as, *Bacillus* species, *E. freundii*, coagulase-negative *staphylococci* and *E. faecalis*, do not typically cause enteric infections in healthy people (Uneke and Ogbu,

2007; Jalgaonkar et al., 2007). These bacteria have, however, been known to cause significant infections in those with depressed immune systems, including those infected with HIV (Human Immunodeficiency Virus), those undergoing cancer chemotherapy, or those taking medications that depress the immune system including hospitalized patients (Asikong et al., 2007). *E. faecalis* is known to cause important diseases such as abdominal abscess, urinary tract infection and endocarditis, which are well documented human pathogens (Kuria et al., 2009). The isolation of coagulase-negative *Staphylococci* on the currency notes could have been contamination from the normal skin flora (Uneke and Ogbu, 2007; Larkin et al., 2009) and from the soil (Igumbor et al., 2007). The coagulase-negative *staphylococci* are normal human flora and sometimes cause infections such as food poisoning (Udo et al., 1999) and other diseases often associated with implanted appliances and devices (Igumbor et al., 2007), especially in very young, old, and immunocompromised patients.

Citrobacter species, *Klebsiella* species, and *E. coli* are enteric microorganisms that are potential pathogens especially when they change their habitat (Basavarajappa et al., 2005; Igumbor et al., 2007) and may cause significant infections in those with depressed immune systems (Asikong et al., 2007). Though *E. coli* appeared to have the least percentage of bacteria, isolating it goes to confirm other reports that currency notes can be commonly contaminated with enteropathogens (Umeh et al., 2007; Larkin et al., 2009) and, the notion that, currency notes represent a reservoir of enteric diseases (Udo et al., 1999).

Only twelve (12) notes were positive for acid fast bacilli, giving prevalence of contamination to be 17.14%. This finding is higher than the two (2) notes which were positive for acid fast bacilli out of one hundred (100) notes examined in India (Basavarajappa et al., 2005) where it was reported that, currency notes may be a vehicle for the spread of tuberculosis, but in our study the acid fast bacilli found could not be assigned to *Mycobacterium tuberculosis* alone because tuberculosis, leprosy and buruli ulcer are endemic in Ghana.

From this study, the bacterial isolates that were isolated were associated with oral, nasal, skin and faecal contamination. 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 (Singh et al., 2002; Emikpe and Oyero, 2007).

Shigella species are common causes of food-borne and water-borne illnesses worldwide (Sapsford et al., 2004). The isolation of *S. dysenteriae*, the most virulent strain gives cause for concern, as it is often linked to large outbreaks of food/water associated dysentery and other forms of gastroenteritis (Guerin et al., 2003). *S. aureus* is a major pathogen for humans. *S. aureus*

causes diseases ranging from food poisoning or minor skin infections to severe life-threatening infections (Udo et al., 1999). The isolation of *Shigella* species and *S. aureus* from the currency notes and the fact that some food vendors serve food with their hands and also handle currency notes as they sell to their patrons, currency notes contaminated with pathogenic microorganisms such as *Shigella* species and *S. aureus* may cross-contaminate the food and may cause food-borne illness (Lamichhane et al., 2009). The Ghanaian currency notes are therefore possible vehicles through which pathogenic microorganisms can be transmitted to humans.

It is therefore suggested that individuals should improve upon their personal health consciousness by washing hands after handling of currency notes (Jalgaonkar et al., 2007; Guerin et al., 2003). Babies must be prevented from handling currency notes and adults should avoid using saliva during counting of paper currency notes (Hosen et al., 2006). The Bank of Ghana should educate the public and enforce rules on proper way to handle money. Hands should not be taken into mouth without washing and should be washed before and after handling currency notes (Emikpe and Oyero, 2007), just as we are advised to do after visiting the toilet, before and after handling food and finally before and after visiting hospitals. Ready-to-eat food sellers should be educated to avoid possible cross contamination between currency notes and food by avoiding handling currency notes as they sell (Reither et al., 2007; Jalgaonkar et al., 2007). There should be public awareness of the fact that currency notes could be a source of infection and could be dangerous to health (Emikpe and Oyero, 2007; Hosen et al., 2006). Although the number of currency notes studied was small compared to notes in circulation in Ghana, this study may draw a representative indication of the danger in handling of currency notes. All efforts should be made to reduce contamination of currency notes by investing on hygienic/sanitary education and proper handling of the currency notes.

Conclusion

The Ghanaian currency notes in circulation were found to be contaminated with various types of microorganisms. Ready-to-eat food sellers should be educated to avoid possible cross contamination between currency notes and food by avoiding handling currency notes as they sell. There should be public awareness of the fact that currency notes could be a source of infection and could be dangerous to health.

ACKNOWLEDGEMENT

We are very grateful to the Department of Clinical Microbiology, School of Medical Sciences, KNUST, for allowing us to use their laboratory facility for the study.

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