# Microbiological Safety Levels of South Sudanese Bank Notes in Circulation at University of Juba Food Restaurants

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# Abstract

Food borne infections arise from either a host of bacteria, viruses and parasites originating in food or pathogens introduced through cross contamination. This study assessed the potential microbiological cross contamination risk posed by South Sudanese Pounds in circulation at University of Juba food restaurants by examining the level of microorganisms on banknotes. Bacterial contamination on the South Sudanese Pounds in circulation at University of Juba were determined using currencies collected from five different food serving points coded A,B,C, D and E respectively. From each food serving points, five samples of banknotes 5, 10and 25 South Sudanese Pounds denominations were randomly selected and their surface bacterial content enumerated. High and varying proportions of Total Coli forms (TC), Escherichia coli (E. Coli) and Staphylococcus aureus (S. aureus) were detected. Findings revealed a significant correlation between microbial levels and the denominations of the bank notes, with the smallest having the highest levels of microorganisms per square centimeter. However, there was no specific pattern in contamination levels between banknotes obtained from the different food points. Another factor that influenced the level of contamination was period the banknotes took in circulation with the older notes having higher levels of microorganisms. High levels of microorganisms on banknotes coupled with unhygienic food handling practices predisposes consumers to health risks. Strategies to reduce the risk of transmission of pathogens from the South Sudanese Pounds with specific emphasis on awareness programs and improvement in food hygiene & handling practices through physical contact between food and money in restaurants at University of Juba were mentioned in order to reduce risk of food borne illness or otherwise potentially lethal outbreak of food borne diseases.

Keywords: South Sudanese pound note (ssp), contamination, bacteria, University of Juba

#### List of acronyms

*E. coli*: Escherichia coli
EPA: Environmental Protection Agency
MO: Microorganism
PC: Plate Count
S. aureus: Staphylococcus aureus
SS: South Sudan
SSP: South Sudanese Pounds
TC: Total Coliform
USA: United States of America

# 1. Introduction

World over the frequency in occurrence of food borne infections has become a public health issue of major concern. In the United States alone, the economy's cost associated with outbreaks of five major food borne pathogens was estimated in the region of US\$ 6.9 billion per year (Hall et al., 2008). In addition to acute

gastroenteritis, food borne infections may result into deaths or chronic disability, for example *E. coli O157:H7* infection was found to be the leading cause of hemolytic uremic syndrome which results into acute kidney failure in children (Boyce et al., 1995).

Several factors contribute to emergence of food borne diseases ranging from human demographics and behavior, technology in the food industry, microbial adaptation but most importantly ineffectiveness of public health measures (Institute of Medicine, 1992). Foodborne disease outbreaks are also more likely to originate from commercial food service premises with restaurants and hotels accounting for more than 75% incidence in many countries (Little & Gillespie, 2008; Altekruse, 1996). Accordingly, the main channels for pathogen entry were identified as cross contamination, unhygienic food storage facilities and infected food handlers (Little & Gillespie, 2008; Altekruse, 1996).

In general, the level of consumer awareness about food safety issues is dependent upon the number of food borne disease outbreaks reported worldwide (Jevšnik et al., 2008). Studies revealed that 79 percent of sampled consumers had been alerted about food safety issues including stories related to *E-Coli:015H7* bacteria, salmonella, food handling and preparation among others through media, and more than 50% of the consumers surveyed were likely to respond to negative stories concerning safe drinking water, bacteria in food and food preparation

Research has also shown that several attributes of paper currency make it an ideal breeding ground for microorganisms. First, the paper bills offer a large surface area for organisms and organic debris to collect. Secondly, folds and/or deliberate depressions or projections specifically engineered into the bills' as anti-counterfeit designs serve as settling sites for both organisms and debris, which allow the microorganisms to live longer in banknotes also (Creswell, Munsell, Fultz, & Zirbel, 1997) and also weave their way through the population for many years before they come to rest (Alemu, 2014; Girma et al., 2014).

The potency of currencies as means for transmission of potential pathogenic microorganisms was first suggested by Abrams and Waterman 1972. Particularly because money is frequently transferred from one person to another, if contaminated, it could play a significant role in spreading communicable diseases. However, there are limited studies on currency contamination by MOs, there is indeed limited knowledge especially among local populations in South Sudan about the risk of banknotes carrying potentially harmful pathogens.

#### 1.1 Statement of the Problem

The SSP is widely used in South Sudan for exchange for goods and services. These banknotes are handled using bare hands, stored under unhygienic conditions and frequently dropped in dirty places. It is also believed that due to cultural and financial market constraints, people keep money in clothes like socks, underwear and bras. Poor waste disposal further exposes the SSP to microorganisms including pathogenic ones when money drops in places contaminated with urinal and fecal matter which can easily be transferred on to foods.

The problem of improper handling of banknotes is compounded with unhygienic food environments. In the public restaurants for example, preparation and serving of food are done by the person receiving money and often without washing hands or use of knives and cutting boards. Similarly, consumers make payments as they dine or eat without washing their hands which could potentially lead to contamination and therefore cause food borne illness. Due to the rising number of students, there has been a demand led emergence of food eating points, which inherently increases the health risks associated with improper handling of banknotes in food restaurants if not given the due attention it deserves.

Although studies in Sub-Saharan Africa (SSA) have investigated the contamination on some currencies, these have been mainly been limited to the Nigerian Naira, South African Rand and Ghanaian Cedi (Kawo et al., 2009; Yazah et al., 2012; Ngwai et al., 2011; Ehwarieme, 2012; Tagoe & Adams, 2011; Igumbor et al., 2012). Assessing the potential risk of the SSP as a medium for contamination of food remains to be investigated. Findings of this study will increase awareness about the potential health risk associated with unhygienic food servings and currency handling in restaurants contributing to the effective control and prevention of foodborne diseases.

# 1.2 Study Objectives

This study examined microbiologically safety of the South Sudanese Pounds used in University restaurants by specifically;

- a. Determining level of Total Coliform on South Sudanese banknotes;
- b. Determining the level of Escherichia coli on South Sudanese banknotes; and
- c. Determining the level of Staphylococcus aureus on South Sudanese notes.

# 1.3 Hypothesis

The level of Total Coliforms, Escherichia coli and Staphylococcus aureus on the South Sudanese banknotes used at University of Juba restaurants are above acceptable limits

# 2. Materials and Methods

South Sudan currency, SSP was used for purposes of this study. After random selection of banknotes from identified points, horizontal methods were applied to obtain MOs from surfaces by use of contact plates and swabs. Subsequent enumeration of MOs were done following the ISO 18593 guidelines.

# 2.1 Sampling Procedures

Five food serving points A, B, C, D and E at University of Juba were randomly identified. From each point, five (5) random samples of 5SSP, 10SSP and 25SSP denominations were selected for enumeration of their bacterial content. The sampled banknotes of 5, 10, and 25SSP were swabbed with sterile swabs as follows, the tip of sterile swabs were initially moistened by immersing in a tube containing sterile quarter strength ringer's solution and pressed against container walls to remove excess liquid while rotating the swabs between the thumb and finger a demarcated area of  $100 \text{cm}^2$  was swabbed in two directions at right angles to each other. Either side of the banknotes were swabbed and treated separately and the swab sticks were aseptically broken into bottles of 100ml sterile quarter strength ringer's solution, diluted and mixed. Serial dilutions were made from the initial suspension to form the samples which were analyzed. MOs were enumerated using procedures **2.2** for Coliforms, *E. coli* and **2.3** for *S. aureus* as described below. The number of microorganisms per swab was calculated from the mean number of colonies grown on petri dishes.

# 2.2 Enumeration of Coliforms and Escherichia coli

Following ISO 4832:2006 guidelines for the enumeration of Coliforms and *E. coli*, the colony count techniques was applied as below;

# 2.2.1 Reagents

- a) Violet Red Bile Lactose Agar
- b) Brilliant Green Lactose Bile Broth
- c) Peptone water
- d) Kovac's reagent

# 2.2.2 Procedure

Media was prepared by weighing the required amount of media powder and mixing thoroughly with distilled water. The mixture was heated until boiling while occasionally stirring. It was allowed to boil for 2 minutes and then cooled immediately in a water bath at 44 °C – 47 °C.

Serial dilutions were prepared by transferring 1ml of the sample or the appropriate dilutions to the center of each dish using a sterile pipette. About 10ml of medium was poured on each petri dishes, mixed with the inoculums and the mixture allowed to solidify. After complete solidification, about 5ml of the Violet Red Bile Lactose was poured onto the surface of the inoculated medium and allowed to solidify as before. A control plate with about 20ml of the medium was prepared concurrently to check for sterility. After complete solidification, the dishes were inverted and incubated at 37°C for  $24 \pm 2$  h for Coliform s and 41.5 °C for  $24 \pm 2$  h for *E. coli*.

# 2.2.3 Enumeration of Total Coliform and Escherichia coli

The purplish red colonies were considered as typical colonies of Coliforms and Escherichia coli. After counting using colony counting equipment, counts were taken from plates with colony numbers ranging from 30 to 300. In all cases spreading colonies and clusters were considered as single colonies.

# 2.2.4 Confirmation for coliforms

Brilliant Green Lactose bile broth was prepared by dissolving the medium powder in distilled water and boiling over a Bunsen flame. The medium was dispensed in quantities of 10ml in test tubes containing Durham tubes which were initially sterilized in an autoclave at 121 °C for 15 min. Care was taken to ensure that the Durham tubes did not contain air bubbles after sterilization. Five presumptive Coliform colonies were inoculated into tubes of the Brilliant Green Lactose bile broth and incubated at 37 °C for 24  $\pm$  2 h. Tubes that showed positive results were counted and number of coliform colonies calculated.

# 2.2.5 Confirmation for Escherichia coli

Peptone water was prepared and filled in tubes autoclaved at 121°C. Five presumptive E.coli colonies were

inoculated into tubes of the peptone water and incubated at 37 °C for  $24 \pm 2$  h. 3 drops of Kovac's reagent were dropped into the tubes and after a few minutes, red rings were observed which confirmed presence of *E.coli*. Tubes that showed positive results were counted and number of *E.coli* colonies calculated.

#### 2.3Enumeration of Staphylococcus Aureus

Following the ISO 6888-2:2003 method for the enumeration of *S. aureus*, the surface spread technique was applied as follows;

#### 2.3.1 Media and Reagents

- a) Baird Parker Agar (BPA)
- b) Tellurite egg yolk emulsion
- c) Brain-heart infusion broth
- d) Rabbit Plasma

#### 2.3.2Procedure

Required amount of media powder was weighed, mixed thoroughly with distilled water and sterilized by autoclaving at 121°C for 15 min. The mixture was immediately cooled in the water bath at 44 - 47° C. 50ml of egg yolk emulsion with tellurite was added to every 950ml of the molten BPA. About 20ml of the molten agar was aseptically poured on the petri dishes and left to set at room temperature. After complete solidification the plates inverted to avoid condensed water from dripping back onto the solidified agar

0.1ml of chosen dilutions was aseptically transferred onto the center of the solidified agar plate. The inoculum was evenly spread using a sterile spreader as quickly as possible on the agar surface, the plates inverted and incubated for  $24 \pm 2 h$  then re- incubated for a further  $24 \pm 2 h$  at 37 °C. We observed characteristic black /grey colonies surrounded by opaque clear zones and made counts from the plates that contained less than 300 colonies.

#### 2.3.3Enumeration of Staphylococcus aureuscolonies

Counts were taken from plates with colony numbers ranging from 30 to 300 using colony counting equipment. As in the first case, all spreading colonies and clusters were considered as single colonies.

# 2.3.4 Confirmation of Staphylococcus aureus

Selected colonies were transferred to a tube of brain-heart infusion broth, incubated at  $37^{\circ}$ C for 24h.Aseptically 0.1ml of culture was added to 0.3 mL of rabbit plasma and incubated at  $37^{\circ}$ C.The clotting was examined after 4h, and negative results were reexamined after an additional 24h.The test was considered positive if the clot exceeded half of the volume and from the tubes with positive results, number of *S. ureus* colonies were calculated.

#### 3. Results

#### 3.1 Occurrence of Microorganisms on Banknotes

On average, all the banknotes that were obtained from the various food serving points were contaminated with microorganisms. Three different microorganism species were isolated, with the most common being TC (81.4%) and *S. aureus* (18.6%) (Table 1). The 5SSP currency notes had the highest levels of MO contamination (50.7%) as compared to the 10SSP (16.5%) and 25SSP (32.8%).

Microorganism	Currency denominations (SSP)Variation of Microorganisms					
	5	10	25	Total (%)		
Total Coliform	398,494	113,430	183,768	695,692 (81.4)		
Escherichia coli	52	142	72	266 (0.0)		
Staphylococcus aureus	34,591	27,644	96,584	158,818 (18.6)		
Total	433,137(50.7)	141,215(16.5)	280,423 (32.8)	854,776 (100)		

Table 1. Relative occurrence of MOs on banknotes selected from 5 food serving points in Juba University café

# 3.2 Occurrence of MOs at Sampling Points

Microorganism	Sampling points		Variation of MOs			
	А	В	С	D	Е	Total (%)
Total Coliform	804,667	4,263	128,200	200,073	22,283	1,159,487(81.4)
Escherichia coli	37	67	110	174	57	444 (0.0)
Staphylococcus aureus	238	49,967	136,690	15,686	62,117	264,697 (18.6)
Total	804,941 (56.5)	54,297 (3.8)	265,000 (18.6)	215,933 (15.2)	84,457 (5.9)	1,424,628 (100)

Table 2. Relative occurrence of MOs in relation to sampling points

Serving point A recorded the highest levels of aggregated microorganism contamination (56.5%) amongst all the serving points from which the denominations were sampled. This was followed by serving point C (18.6%), serving point D (15.2%), serving point E (5.9%) and serving point B (3.8%) as shown in Table 2. A close analysis showed no specific pattern of MO contamination differences among the identified serving points.

Although no specific currency contamination level trends are observed at the different sampling points, relative distribution of MOs indicated higher concentration of TC, *E. coli* and *S. aureus* on the 5SSP, 10SSP and 25SSp respectively (Figures 1, 2, 3 & 4)

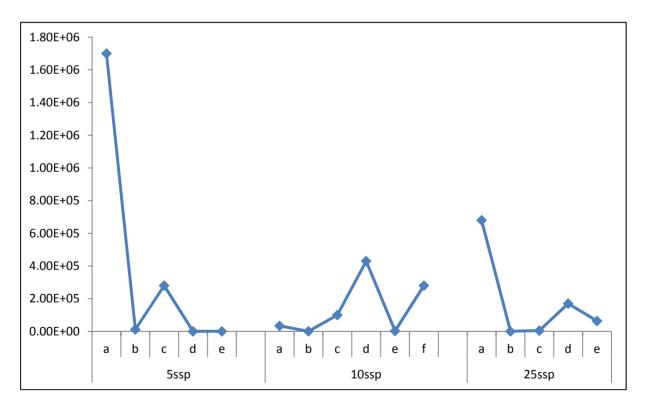


Figure 1. Variation of Total Coliform (TC) in different denominations from different serving points

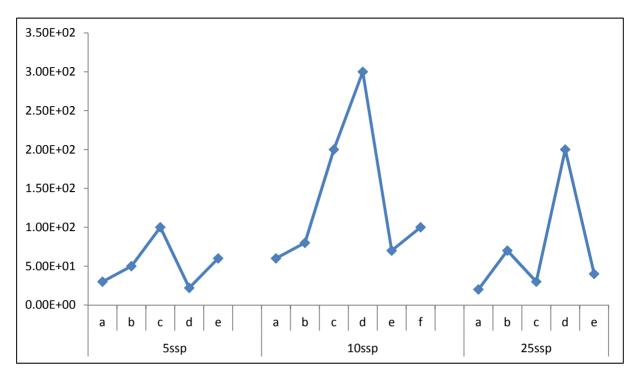


Figure 2. Variation of Escherichia coliin different denominations from different serving points

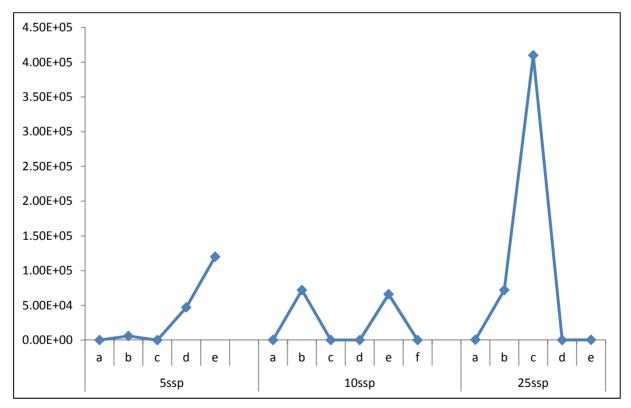


Figure 3. Variation of Staphylococcus aureusin different denominations from different serving points

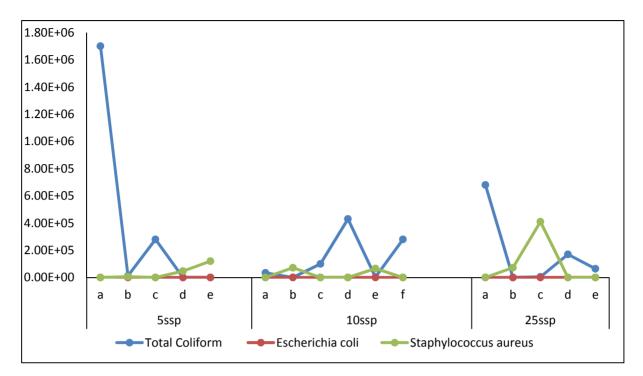


Figure 4. Variation of TC, E. coli and S. aureus in different denominations from different serving points

#### 4. Discussions

In this study five random samples of 5, 10 and 25 SSP notes respectively obtained from five serving points coded A, B, , D and E in Juba University restaurants were analyzed for microbial load. All the sampled banknotes were found to be contaminated with microorganisms in varying proportions. Similar (100%) currency contamination rates were previously reported in Ghana and Pakistan (Tagoe et al., 2011; Sabahat & Humaira, 2011). The presence of MOs on all banknotes demonstrates the critical role played by money in transmitting pathogens, hence poses a dimensional public health threat. This finding supports reports from other parts of the world that currency notes are usually contaminated by microorganisms that can cause a wide range of diseases (El-Dars & Hassan, 2005; Khinet et al., 1989; Siddique, 2003; Pope et al., 2002) including tuberculosis (Basavarajappa et al., 2005). Several factors contribute to currency contamination namely; storage of money in clothes, body surfaces or dirty surfaces, improper washing of hands, use of saliva to wet fingers when counting currency, coughing and sneezing (Akoachere et al., 2014)

In general the extent of contamination corresponded with currency denominations. The lowest currency in circulation (5SSP) notes exhibited a highest levels of contamination (56.5%). Such findings were also reported from studies in Bangladesh, Ghana and Pakistan (Tagoe et al., 2011; Sabahat & Humaira, 2011; Ahmed et al., 2010). The relatively high level of contamination of the 5SSP denominations could be attributed to the fact that it passes through many hands as the most frequently used banknote which predisposes it to continued contamination. This was consistent with studies in Cameroon which suggested that the tendency to mishandle money is also higher in lower denominations (Akoachere et al., 2014). Apart from denomination, the physical condition of the currencies also influenced the level of contamination. Old, tattered and dirty notes were more contaminated in line with previous studies in which soiled notes especially those held together with bits of sticky tape were particularly dangerous (Siddique, 2003).

At the level of food service points there was no specific pattern of MO distribution between different sampling points. This could be because the banknotes don't necessarily get contaminated from the restaurants but other points of exchange like neighboring small vendors, bars and butcheries. Even then currencies could have moved from one part of the country to another during processes of trade, travel and recirculation.

Upon isolation of colonies, presence of TC, *E. coli* and *S. aureus* were confirmed on all bank notes albeit varied proportions. The highest levels of MOs were TC, *S. aureus* and *E. coli* in descending order.

TC presented significant prevalence in all the currency notes with the highest contamination levels registered with

the 5SSP currency notes. The coliform group consist of a collection of different types of bacteria usually present in the environment and feces of warm blooded animals. Although a small amount of coliforms may not cause illnesses, high presence of coliforms indicates possible presence of additional harmful pathogens which requires further scrutiny (Connecticut Department of Public Health, June 2010). Coliformsmay be an indication of fecal contamination from poor hygiene and inappropriate waste disposal. According to the US Environmental Protection Agency (EPA) presence of TC is a health concern and for water systems, TC levels exceeding 5% were regarded as acute risk (Connecticut Department of Public Health, June 2010).

Investigation also confirmed presence of *E. coli* on banknotes. This finding concurs with reports that currency notes act as reservoirs for enteric pathogens (Goktas & Oktay, 1992; Xu et al., 2005). Presence of *E. coli* indicates recent fecal contamination, dirty environments and inadequate personal hygiene of money handlers. *E. coli* is opportunistic and has a shelf life of up to 11days on currencies during which it can be easily transferred from one medium to another. If ingested, *E. coli* may result into bloody diarrhea or acute kidney failure in children moreover person to person transmission is well documented (Boyce et al., 1995).

#### 5. Recommendations and Conclusions

#### 5.1 Recommendations

This study has shown that SSPs are contaminated with potentially pathogenic microorganisms and all the people handling this currency are invariably exposed to infections. Based on the results and outcomes of our laboratory diagnosis, the following suggestions are proposed to improve food safety and quality assurance:

Its strongly recommend provision of health education. The importance of basic personal hygiene though practices like frequent and thorough hand washing, avoiding use of saliva during counting money, compulsory separation of cashier from other restaurant jobs and cleanliness of power houses like kitchens, bars and restaurants and shops that serve as food outlets are strongly recommended to reduce the risk of infection.

Furthermore the study also recommend formation of market and community health education committees to oversee the day today market and business outputs/ inputs like market cleanliness, awareness campaigns, and act as advocates for behavior change and communication. This would also improve local initiative and ownership of public health improvement efforts.

Adoption and integration of a food safety framework and strategy into National Health Policy to aid food safety assurance and quality control. This should involve critical scrutiny and health certification for restaurants to operate. Impromptu inspection of restaurants and reprimands for food outlets that do not comply with food safety standards or have high frequency of public health problems be initiated. Periodic disinfection of the South Sudanese currency for 24 hours before being re-circulated to minimize cross contamination from use of dirty banknotes is also encouraged.

#### 5.2 Conclusions

This study has shown that the SSPs circulating in University of Juba restaurants have high levels of contamination which could potentially facilitate spread of pathogens. Although contamination could have originated from other currency users like butcheries and street vendors, restaurants present a higher health risk since MOs can easily get into food at any point before or during consumption. This hazard is aggravated by poor food handling practices. Given that the most affected banknotes were the lower denominations (5SSP), we postulate that people belonging to weaker social and economic status may be at a higher risk of infections arising from cross contamination. Infants, children, the elderly or sick may be particularly vulnerable.

Due to comparative cost advantage and relative ease of implementation, we recommend emphasis on improvement of hygiene. Knowledge, appropriate behavior & attitude towards hygiene during and after handling money could be fostered by increasing awareness through health education, campaigns and advocacy. Prevention rather than treatment is targeted and improvement in hygiene presents the best means to reduce food contamination (Todd et al., 2010). At the central bank level, removal of mutilated and worn-out banknotes and periodic sanitization of the SSP before recirculation is advised.

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