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Microbial load of Ethiopian currency notes collected from various sources

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Abstract

Paper currency is widely exchanged for goods and services worldwide. The level of bacterial contamination of currency notes and the possible presence of foodborne pathogen on currency notes were studied on a total of 100 Ethiopian currency notes consisting of all denominations collected from students, receptionists butcher's shops, supermarkets and open markets. The highest mean counts of aerobic mesophilic bacteria and Staphylococci were log 2.35 cfu/cm^2 and log 1.71 cfu/cm^2, respectively. Average counts of Enterobacteriacae, yeasts and fungi were less than 100 cells/cm^2. Coliform counts were >100 cfu/cm^2 in samples obtained from butcher's shops. However counts within samples of the same currency notes or within samples from the same site of collection showed highly significant variations indicating inconsistency in the level of contamination. A total of 888 bacterial isolates were obtained in this study and the microflora was dominated by *Bacillus* spp. (41.9%), *micrococci* (26.8%) and *staphylococci* (19.5%). Pre-enrichment and selective enrichment did not yield *Salmonella* or *Shigella*. The study indicated that currency notes could be considered as sources of contamination and hands should be washed constantly after handling currency notes.

Keywords: currency notes, microbial load, dominant microflora

Introduction

Paper currency is widely exchanged for goods and services worldwide. Paper currencies circulate frequently among the population. Contamination of paper currency with a pathogen is a potential hazard if it reaches the hands of food handlers and, subsequently to food. Contact with contaminated currency notes with pathogens could also cause diarrhea and urinary tract infections (Kawo *et al.*, 2009). Paper currency, can be contaminated by droplets during coughing and sneezing as well as by placement on dirty surfaces (Ahmed *et al.*, 2010). Paper money, therefore, presents a particular risk to public health, since communicable pathogens can spread through dissemination to different people in a short time (Ahmed *et al.*, 2010). The risk of acquiring opportunistic infections through handling of contaminated currency notes might be more serious in immuno-compromised persons.

There are reports on the microbial load of different currency notes in various countries (Vriesekoop *et al.*, 2010) and from various sources (Bhat *et al.*, 2010). Microbial contamination of various national currency notes was reported from Bangladesh (Hosen *et al.*, 2006; Ahmed *et al.*, 2010), South Africa (Igumbor *et al.*, 2007), Nigeria (Oyero and Emikpe, 2007; Umeh, *et al.*, 2007; Kawo *et al.*, 2009; Awel *et al.*, 2010), Nepal (Prasai *et al.*, 2008; Lamichhane *et al.*,

2009), Saudi Arabia (Rashed, *et al.*, 2006; Ghamdi, *et al.*, 2010), Iran (Dehghani *et al.*, 2011), India (Rote *et al.*, 2010) and Sudan (Saadabi *et al.*,2010).

In Ethiopia, there is no documented data on microbial load and types of microbial contaminants on circulating currency notes. The aim of this study was, therefore, to evaluate the microbial load of Ethiopian currency notes circulating in Addis Ababa and assess the possible occurrence of some foodborne microorganisms on them.

Materials and Methods

A cross-sectional study was conducted to determine microbial load and isolate dominant food borne microorganisms from some parts Lideta Sub-city, Addis Ababa. A total of 100 currency notes (Ethiopian Birr, ETB) consisting of the various denominations were collected from students, receptionists working in food establishments, butchers, vendors in supermarkets and traditional open markets. The samples consisted of notes of ETB 100, 50, 10, 5 and 1 (Table 1).

Table 1: Collection sites and distribution of currency notes

	Number of the currency notes					
Collection site	100	50	10	5	1	Total
Students	2	2	5	2	1	12
Receptionists	3	5	1	6	0	15
Butchers	5	2	5	7	5	24
Supermarkets	5	2	6	5	1	19
Open markets	5	3	7	0	15	30
Total	20	14	24	20	22	100

Samples were directly collected into sterile plastic bags and brought to the laboratory for microbiological analysis upon arrival. Ten new currency notes of various denominations were obtained from a bank and were similarly processed as control. The collected samples were also subjectively categorized as dirty, moderate, and clean based on visible hygienic quality.

Microbial enumeration

Both surfaces of a note were thoroughly swabbed with a sterile cotton swab dipped into sterile buffered peptone water (BPW) and directly inoculated in to a tube containing 10 ml of 0.1% BPW. This was serially diluted 10^1 to 10^6 and a volume of 0.1 ml of appropriate dilution was spread plated in duplicates on pre-dried surfaces of the appropriate medium.

Total aerobic mesophilic bacteria were counted on Plate Count Agar (PCA), (Oxoid, Basingstoke, Hampshire England). The plates were incubated at 37° C for 24 hrs for colony counting. Total staphylococci were counted on Mannitol Salt Agar (MSA), (Oxoid, Basingstoke, Hampshire England). The plates were incubated at 37°C for 24 h and typical yellow colored colonies were counted.

Total coliforms were enumerated on Violet Red Bile Agar (VRBA),Oxoid Basingstoke, Hampshire England). The plates were incubated at 37°C for 24 h and pink colonies surrounded by precipitated bile were counted.Total Enterobacteriaceae were counted on Violet Red Bile Glucose Agar (VRBGA). (Oxoid Basingstoke, Hampshire England) The plates were incubated at 37° C for 24 hrs and pink colonies were counted.

Yeasts and fungi were counted on Dichloran Rose-Bengal Chloramphenicol Agar (DRCA) (Oxoid Basingstoke, Hampshire England) after incubation at 25°C for 3 days. To determine the microbial load per cm^2 of the currency note surfaces, the surface area of each denomination was determined by measuring length and width of the paper using ruler and calculating the surface area of each accordingly. Counts were reported as colony forming unit per square centimeter (cfu)/cm^2 or log cfu/cm^2.

Isolation of *Salmonella* and *Shigella*

A volume of 1 ml of the swab suspension was separately inoculated into enrichment media consisting of 10 ml each of Selenite Cystine Broth and Tetrathionate Broth. A volume of 0.1 ml of the swab suspension was also inoculated in 10 ml of Rappaport Vassiliadis Enrichment Broth and all were incubated at 35°C for 18-24 hours. A loopful of the suspension was then streaked on to Xylose Lysine Deoxycholate (XLD) (Oxoid Basingstoke, Hampshire England) agar in order to check for the presence of *Salmonella* species.

Microbial flora analysis

After colony counting, 10 to 15 colonies were randomly picked from countable Plate count agar plates(Oxoid Basingstoke, Hampshire England) for further characterization. After repeated purification on Nutrient agar (Oxoid), isolates were microscopically characterized by cell shape, cell grouping, motility, and presence or absence of endospores. Gram reaction of isolates was tested by the KOH test (Gregersen, 1978). Production of the enzyme oxidase was tested according to Kovacs 1956 and formation of catalase was determined by flooding young colonies with 3% solution of H_2O_2. Oxidative or fermentative utilization of glucose by each isolate was assessed by the O/F test (Hugh and Leifson, 1953).

Data analysis

Descriptive statistics was used to compute percentage, mean, standard deviation and coefficient of variation by using SPSS version 16 statistical software. The Coefficient of Variation (CV) was used to examine presence of variation in microbial count within samples of the same denomination and variation >10% indicated significant variation in counts.

Results

The various currency notes were graded based on their physical condition as level of contamination could also be related to the physical condition of the currency. Dirtiness was most common among the 1 Birr notes (95.4%) and least common among 100 Birr notes (15%). Open markets were sources for 70% of the dirty notes. The least amount of dirty notes were collected from receptionists (6.7%) (Table2)

Table 2: Physical condition of currency by sampling sources and currency type.

	Source/ currency	Total tested	Quality statues of the currency		
			Dirty No. (%)	Moderate No. (%)	Clean No. (%)
Sampling source	Butchers	24	10 (41.6)	13 (54.2)	1 (4.2)
	Supermarkets	19	10 (52.6)	9 (47.4)	0 (0.0)
	Open markets	30	21 (70)	9 (30.0)	0 (0.0)
	Students	12	1 (8.3)	10 (83)	1 (8.3)
	Receptionists	15	1 (6.7)	13 (86.7)	1 (6.6)
Denomination	ETB 1	22	21 (95.4)	1 (4.5)	0 (0.0)
	ETB 5	20	8 (40)	10 (50)	2 (10.0)
	ETB 10	24	9 (37.5).	15 (62.5)	0 (0.0)
	ETB 50	14	2 (14.3)	12 (85.7)	0 (0.0)
	ETB 100	20	3 (15)	16 (80)	1 (5.0)
	Total	100	43 (43)	54 (54)	3 (3.0)

The currency notes showed various levels of microbial load (Table 3). The average count of aerobic mesophilic bacteria ranged from log 1.57 cfu/cm^2 to log 2.35 cfu/cm^2. The highest count was observed in ETB 10 notes and the lowest in ETB 100 notes. Variation in counts within each currency note was significant (CV>10%). Notes of ETB 1 and 5 had the highest variation and those of ETB 10, 50 and 100 had the lowest. The count of staphylococci ranged between log 0.94 cfu/cm^2 and log 1.71 cfu/cm^2. Variation in counts within samples of the same currency notes was significantly very high in all denominations (CV>10%).

The counts of Enterobacteriace, coliforms and yeasts and fungi also showed a pattern similar to staphylococci (Table 3). Bacterial counts were highest in notes of ETB 1 and lowest in those of ETB 100. The counts, however, were less than log 2 cfu/cm^2 in all cases. The coefficient of variation for these microbial groups was very high and ranged between 49% to 62% for Enterobacteriaceae, over 95% for coliforms and over 80% for yeasts and molds. The level of contamination was significantly different within samples of the same denomination in all currency notes.

When the source of the currency notes were compared with one another, currency notes collected from students, open market vendors and butchers had a mean count greater than log 2 cfu/cm^2 for aerobic mesophilic bacteria and those collected from receptionists and supermarket vendors had counts less than log 2 cfu/cm^2 (Table 4). Variations in counts within samples of the same source were significant (CV>10%). Enterobacteriaceae mean counts were >log 2 cfu/cm^2 in samples obtained from butchers, although the variation in counts was over 50%. Samples from all the other sources yielded mean coliform counts of <log 2 cfu/cm^2. Similarly, very low counts of coliforms, and yeast and molds were seen in currency notes collected from all sources (<10^2 cfu/cm^2). Coefficient of variation for each source was very high for the microbial count (around or over 100%).

A total of 888 dominant bacterial isolates were obtained from the various currency notes in this study (Table 5). Most of the isolates were obtained from notes of ETB 1 (23.2%), ETB 5 (19.7%) and ETB 10 (23.5%). All isolates were grouped to the genus level. *Bacillus* spp. (41.9%), micrococci (26.8%) and staphylococci (19.5%) dominated the aerobic mesophilic flora. Members of Enterobacteriaceae, *Alcaligenes* spp., *Pseudomonas* spp., and lactic acid bacteria (LAB) together made only 11.8% of the total isolates. ETB 1 notes yielded the highest proportion of staphylococci (44.6%).

Of the total isolates, 31.4% were obtained from currency notes collected from open markets followed by those from butcher's shops (23.5%) (Table6). *Bacillus* isolates dominated the microflora obtained from currency notes collected from Supermarkets. *Micrococci* dominated those from butcher's shops (27.3%) and staphylococci from open markets (50.3%). Enterobacteriaceae and *Alcaligenes* isolates appeared more frequently in currency notes obtained from open markets (55.6% and 40.5%, respectively).

Discussion

The various currency notes used in this study showed different levels of contamination with some denominations having high counts and others lower counts. However, it should be noted that counts within samples of the same currency notes or the same collection site had very high variation. Although, in this study, coefficient of variation greater than 10% was considered as a significant variation, variation up to 40% were observed for aerobic mesophilic counts within currency notes of the same denomination and within samples coming from the same collection sites. Coefficient of variations up to or greater than 100% were even observed for other groups of microorganisms. Although analysis was based on means of counts, the high coefficient of variation indicated that counts were not consistent within samples of same denomination.

Most of the notes were dirty and moderately dirty, particularly notes of ETB 1 and ETB 10. The bacterial counts were generally related to the physical condition of the notes as seen in the high bacterial load in ETB 10 and ETB 1. These are among the currency notes which are commonly used in daily cash transactions in markets with small business. In contrast, the currency which is not frequently used for daily activities like , ETB 100, had least bacterial load. This, of course, also depends on how long a currency stayed in circulation.

Aerobic mesophilic bacteria were encountered in all currency notes considered in this study. The source of contamination of the circulating notes would be due to contamination during circulation and handling as no microorganisms were isolated from new bank notes used as controls. Moreover, the observation in the present study that showed lower denominations had higher level of microbial contamination is in agreement with the findings of other workers in other countries (Bhat *et al.,* 2010; Kawo *et al.,* 2009). The fast circulation of these notes among different social groups of the society could explain the observation.

Table 3. Mean counts (log cfu/cm^2) of the various microbial groups from currency notes

Currency Notes (ETB)	No.	AMB*			Staphylococci			Coliforms			Enterobacteriaceae			Yeast and molds		
		Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
1	22	2.19	0.87	40	1.71	0.56	32.8	0.90	0.66	72	0.57	0.55	95	0.59	0.47	79
5	20	1.82	0.61	34	1.33	0.45	34.2	0.82	0.52	63	0.49	0.46	93	0.29	0.47	158
10	24	2.35	0.64	27	1.66	0.69	41.7	0.83	0.40	48.5	0.53	0.46	86	0.32	0.48	149
50	14	1.79	0.36	20.5	1.24	0.36	29.6	0.60	0.35	59.5	0.35	0.43	125	0.36	0.46	127
100	20	1.57	0.42	27	0.94	0.48	517	0.71	0.36	51.8	0.14	0.36	252	000	0.001	447

AMB, aerobic mesophilic bacteria; SD, standard deviation; CV, coefficient of variation.

Table 4. Coun (log cfu/cm^2) of various microbial groups isolated from currency notes collected from variou sources.

Collection sites	No.	AMB*			Staphylococci			Enterobacteriaceae			Coliforms			Yeast and molds		
		Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Butchers	24	2.14	0.62	28.8	1.53	0.72	47.3	1.07	0.60	56.3	0.51	0.60	117	0.56	0.51	92
Supermarkets	19	1.70	0.58	34	1.32	0.48	36	0.53	0.45	86	0.04	0.47	100	0.22	0.30	134
Open markets	30	2.05	0.81	39.6	1.55	0.65	42	0.73	0.39	53	0.40	0.50	126	0.34	0.52	151
Students	12	2.26	0.68	30.3	1.35	0.51	37.8	0.86	0.34	40.7	0.66	0.45	69	0.17	0.33	198
Receptionists	15	1.67	0.44	26.7	1.06	0.45	33	0.72	0.36	50.6	0.28	0.36	128	0.11	0.31	283

AMB, aerobic mesophilic bacteria; SD, standard deviation; CV, coefficient of variation;

Table 5. Frequency distribution of dominant microflora on various currency notes

Currency notes (ETB)	No. of isolates (%)	*Bacillus*	Micro-cocci	Staphylo-cocci	Entero-bacteriaceae	*Alcali-genes*	*Pseudo-monas*	LAB
1 (N=22)	206 (23.2)	36 (17.5)	56 (27.18)	92 (44.6)	2 (0.97)	15 (7.28)	0	5 (2.42)
5 (N=20)	175 (19.7)	102 (58.2)	45 (26)	16 (9.1)	6 (3.4)	6 (3.4)	0	0
10 (N=24)	209 (23.5)	73 (34.8)	73 (34.8)	52 (25)	8 (4)	3 (1.4)	0	0
50 (N=14)	133 (15.0)	77 (58)	32 (24)	4 (3)	6 (4.5)	6 (4.5)	7 (5.3)	1 (0.75)
100 (N=20)	165 (18.6)	84 (51)	32 (19.4)	9 (5.5)	32 (19.4)	7 (4.2)	0	1 (0.6)
Total (N=100)	888	372	238	173	54	37	7	7

LAB, lactic acid bacteria

Table 6. Frequency Distribution of dominant flora among collection sites

Collection sites	No. of isolates (%)	*Bacillus*	Micro-cocci	Staphylo-cocci	Entero-bacteriaceae	*Alcali-genes*	*Pseudo-monas*	LAB
Butchers (N=24)	209 (23.5)	82 (22.0)	65 (27.3)	53 (30.6)	2 (3.7)	5 (13.5)	0	2 (28.6)
Supermarkets (N=19)	169 (19.1)	92 (24.7)	37 (15.5)	15 (8.7)	18 (33.3)	7 (18.9)	0	0
Open market (N=30)	279 (31.4)	81 (21.8)	61 (25.6)	87 (50.3)	30 (55.6)	15 (40.5)	0	5(71.4)
Students (N=12)	99 (11.1)	44 (11.8)	38 (16.0)	14 (8.1)	0	3 (8.1)	0	0
Receptionists (N=15)	132 (14.9)	73 (19.6)	37 (15.6)	4 (2.3)	4 (7.4)	7 (18.9)	7 (100)	0
Total (N=100)	888	372 (41.9)	238 (26.8)	173 (19.5)	54 (6.1)	37 (4.2)	7 (0.8)	7 (0.8)

Contamination comes from common unhygienic practices where currency notes of small denominations circulate among different people frequently. A similar observation was also made by Lamichhane *et al.,* (2009) in Nepal and Awel *et al.,* (2010) in Nigeria, where currency notes of lower denomination were found to be the most contaminated due to passing through more hands than larger denominations. As observed in our students counts, Birr 50 and Birr 100 notes were less contaminated than those of the lower denominations. Thus Most of the smaller unit currency notes were visually dirty and could be sources of microorganisms to hands and subsequently can contaminate food.

The currency notes considered in this study were contaminated by both Gram positive and Gram negative bacteria. *Bacillus* species were the most dominant isolates because they produce resistant spore, and are widely distributed in nature. The other dominant Gram positive isolates, staphylococci and *micrococci*, are normally associated with the human skin and can contaminate anything that comes in contact with hands. The dominance of Gram positive bacteria was also observed by Ahmed *et al.,* (2010) in currency notes from Bangladesh.

The presence of coliforms and Enterobacteriaceae is a point of concern as some of the potent enteric pathogens belong to these groups. Similar studies in other countries showed isolation of bacterial groups with potential pathogens (Feglo and Nkansha, 2010; Gamdi *et al*., 2010). Multiplication of microorganisms on currency notes might not be probable because of absence of required nutrients. However, their survival would make currency notes a source of contamination to foods where proliferation is possible. The absence of pathogenic enteric bacteria *Salmonella* and *Shigiella* in our study might be due to the fact that such pathogens are generally not competitive in the presence of high background flora which might limit their survival on contaminated currency notes.

In conclusion, a particular currency note or source of the notes could not necessarily be considered as having a higher or lower level of contamination as the difference in counts of same type of currency or currency from same source was highly variable. The duration of circulation could be a major factor in determining the microbial load. However mean values indicated that lower denomination currency notes are more contaminated due to frequent circulation.

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Authors' contributions

HA, designed and conducted all laboratory experiments; analyzed and interpreted experimental results and manuscript preparations. MA, supervised the laboratory work, analyzed the result and involved in manuscript preparation. All authors read and approved the final manuscript.

Conflict of interest

There is no Conflict of interest among the authors.

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