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Abundance and Antibiotic Resistance of Cultivable Bacteria and Fungi on Ethiopian Cash-Money in Nekemte Town

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Abstract

In Ethiopia, marketing system depends on cash exchange that could be a vehicle for cross contamination. To investigate the loads of cultivatable bacteria and fungi on Ethiopian currencies, a total of 120 samples of Ethiopian money-cash called birr (ETB) with different denominations (10 ETB, 5 ETB, 1 ETB note, 1 ETB coin and 50 cents) were collected from street food sellers, taxi drivers, gamblers, restaurant workers, beggars, bank workers and butchers in Nekemte Town. The isolates were tested for resistance to antibiotics (µg/ml), Ampicillin (10), Cefalexin (16), Cefixime (5), and Cefuroxime (8). The highest number of Aerobic Mesophilic Bacteria (AMB) was 255.8 CFU/ml on 1 ETB note notes collected from beggars. Similarly, the maximum number of Enterobacteriaceae and *Bacillus* was 138.1 CFU/ml and 33.8 CFU/ml counted on 1 ETB note collected from beggars. In addition, 44 CFU/ml of Coliforms and 103 CFU/ml of Staphylococcus bacteria were counted on 1 ETB note from street food sellers. The highest colony of yeast and molds was counted on 1 ETB note from street food sellers (125 CFU/ml) and from beggars (20.2 CFU/ml), respectively. The maximum number of pathogenic bacteria was Staphylococcus (91.7 CFU/ml) followed by *E. coli* (44 CFU/ml) on 1 ETB notes from street food sellers. *Bacillus* was not detected on 50 cents coin from almost all sources. *Bacillus* was resistant to the test dose of the antibiotics. Besides, Shigella was only sensitive to Cefixime and the antibiotics were effective against the pathogens except *Bacillus*. In general, Ethiopian cash-money collected from Nekemte town is loaded with microorganisms and Cefixime is effective against most of the pathogens.

Keywords: Birr; Antibiotics; Contamination; Pathogenic; Bacteria; Fungi

Introduction

In world, people exchange money by different mechanisms. Citizens of most developed countries use credit or debit card system, whereas the majority of developing countries are still depend on hand-to-hand cash turnover for marketing. From its nature, cash-money is fragile and easy to be lost in addition to its exposure to contamination by different microorganisms. As a result, cash-money exchanges are considered as one of the potential vehicle for the spread of pathogenic microorganisms. Communities have different habits of storing, counting, using and managing cash-money that could be conducive for the growth and transmission of pathogenic microorganisms. As a result, both bacterial and fungal pathogens of human are reported from different countries [1-3].

The abundance and type of pathogenic microorganisms existing on cash-money varies based of the livelihood of the bearer [3,4]. For instance, in India, the cash-money obtained from bank, hospitals and municipals are highly contaminated with various pathogenic bacteria as compared to those obtained from butchers and food sellers [5]. A study conducted at some parts of Ethiopia showed the high prevalence of *E. coli* that ranges from 67.2% at open market milk sellers to 87.2% at dairy station milk sellers [6]. Cash-money collected from different classes of Libyans also showed variation in load of pathogenic microorganisms in which the abundance of *Enterobacter agglomerans*> *Pseudomonas* spp.>*Staphylococcus aureus*>*Enterobacter cloacae*>*Klebsiella pneumonia*>*E. coli* [7]. Similarly, in Nepal abundance of heterotrophic aerobic bacteria>coliform bacteria>Staphylococci are found on cash-money [1].

Contamination level of cash-money varies based on its type and value of the money [1,6]. A study showed the negative correlation between value of the money and microbial load [2]. For instance, prevalence of *E. coli* on Ethiopian Birr (ETB) of hundred notes is less than on ETB of one note [6]. Pathogenic microorganisms from cash-money vary in their resistance to the minimum inhibitory concentration of antibiotics. *E. coli* isolated from Ethiopian cash-money circulating in the western part of the country were resistant to neomycin than other antibiotics tested [6].

Ethiopia is known by enormous culture, customs and classes that vary among regions and localities. Contamination level of Ethiopian cash-money could be the reflection of the community lifestyle. Prevalence and drug susceptibility study of *E. coli* on Ethiopian birr was studied at the Eastern part of the country where majority of the communities' livelihood depends on business but not studied in the western part of the country where the main livelihood is farming. Therefore, this study was to investigate abundance of pathogenic microorganisms on Ethiopian cash-money of different birr notes and coins collected from Nekemte town communities having different livelihood and to test their antibiotics susceptibility.

Materials and Methods

Description of the study sites

The study was conducted at Nekemte town, 331 km from Addis Ababa to the western part of the country. Nekemte is a market

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town; and according to 2012 national census, its total population is estimated to 110,688. Majority of the community lifestyles include businesspersons, smallholder farmer, and government employers. As the town is connecting many town of the country, money exchange rate is expected to be high.

Sampling and its preparation

Cash-money samples were purposively collected from butchers, beggars, gamblers, street food sellers, restaurant workers, bank workers and taxi drivers. Totally, 120 different ETB samples holding 50 cents, one birr (coin), one birr (note), five birr note and ten birr note were included in this experiment. Sampling was conducted in triplicates including a control of new ETB obtained from Commercial Bank of Ethiopia Nekemte district. Each collected cash-money was aseptically transferred into sterilized separate bottles containing 10 ml of sterile buffered peptone water solution. Then, the bottles were shaken vigorously for about 2 minutes to dislodge the microorganisms into the medium. The samples were removed aseptically from the bottle with a sterile forceps, rinsed with water and dried to recover.

Estimation of cultivatable bacterial load

From appropriate dilutions, 0.1 ml of the aliquot was spread plated on Plate Count Agar (PCA) and the plates were incubated at 35°C for 24 h. After incubation, plates with colonies between 30 and 300 were counted to estimate the abundances of aerobic mesophilic bacteria (AMB) [8]. Similarly, the diluents were spread-plate on Salmonella Shigella Agar (SSA), Xylose Lysine Deoxycholate (XLD) Agar, Eosin Methylene Blue (EMB) Agar, MacConkey (MAC) agar and incubated at 37°C for 24 h. After which, different types of colonies of bacteria with different colors like pink to red purple colonies on EMBA, Red to Black centered colonies on SSA and XLD Agar were counted as member of a family of Enterobacteriaceae [9]. A purplish colony surrounded by reddish zone on MAC agar and Metallic green sheen on EMB Agar were counted as Coliforms [8]. Spore forming bacteria (Bacillus) were differentiated by spore staining from PAC and the colonies were counted [10]. Typical golden yellow colour colonies and red colonies were counted as Staphylococcus and Streptococcus from Mannitol Salt Agar (MSA).

Estimation of fungal load

Colonies of yeast and mold were determined by direct plate count from Potato Dextrose Agar (PDA) supplemented with 0.06 g/L chloramphenicol. Smooth (non-hairy) colonies without extension at periphery were counted as yeasts whereas hairy colonies were counted as mold after 48 h of incubation at 29°C [9].

Antimicrobial susceptibility of cultivatable bacteria

The bacterial isolates were tested for sensitivity to different generations of antibiotics by disc diffusion method on Mueller-Hinton agar using the commercial antibiotics such Ampicillin (AMP), Cephalexin (LEX), Cefuroxime (CXM) and Cefixime (CFM). Concentration (µg/ml) of the antibiotics used during the experiment was 10, 16, 5, and 8 for AMP, LEX, CFM, and CXM, respectively [11]. The disc note was impregnated with appropriate amounts of antibiotics, and put on the surface of solid Mueller Hinton agar which was pre-seeded with a pure culture of the test organism and incubated for 24 h at 37°C. Sensitivity of the test organisms was verified with a clear zone and the level of sensitivity was estimated by measuring a clear zone using ruler and is expressed in mm. Based on the measurement of the inhibition zones (IZ in mm), effectiveness of the antibiotics against the inoculated bacteria was expressed as 'Sensitive', 'Moderately sensitive' and 'Resistance' for IZ>14, 7<IZ<14, and IZ<7, respectively [12].

Result

Abundance of cultivatable bacterial groups

Abundance of different bacterial groups such as aerobic mesophilic bacteria (AMB), Coliforms, Enterobacteriaceae, Staphylococcus, and *Bacillus* were significantly varied ($P<0.01$) based on sources of samples and type of money. The study showed that sample from "1 ETB note" and "50 cents" were the highest and the lowest in count of cultivable microorganisms, respectively. Amongst the sample sites, the maximum and minimum mean count of cultivatable microorganisms was from "Beggars" (255.8 ± 1.0 CFU/ml) and "Bank workers" (0.00 CFU/ml), respectively (Table 1).

The mean count of aerobic mesophilic bacteria (AMB) isolated from "beggars" was the highest on samples of "1 ETB notes" (255.8 ± 1.0 CFU/ml) and there was no AMB on "50 cents" from "butchers" and "restaurant workers". Similarly, the highest mean counts of Enterobacteriaceae were on "1 ETB note" sampled from "beggars" (138.1 ± 1.0 CFU/ml); whereas "50 cents" collected from "butchers" and "restaurant workers" did not contain Enterobacteriaceae (Table 1). The number of coliform bacteria and Staphylococcus bacterial groups from "street food sellers" were 44.0 ± 1.0 CFU/ml and 103.0 ± 1.0 CFU/ml, respectively on "1 ETB note".

Likewise, the mean counts of *Bacillus* bacteria counted from "1 ETB notes" were 33.8 ± 1.0 CFU/ml from "beggars" (Table 1). In general, AMB, Enterobacteriaceae, Staphylococcus, Coliform and *Bacillus* were observed on all samples isolated from the different bearer except from "restaurant worker" and "butcher". All types of money from taxi driver did not contain *Bacillus* except "1 ETB note" (Table 1).

Abundance of yeasts and molds

The mean counts of fungal load isolated from the sampling sources were higher for yeasts than molds. The highest load of yeasts was 125.75 ± 1.42 CFU/ml on samples of "1 ETB" from "street food sellers". Similarly, the maximum fungal molds count was 20.25 ± 0.52 CFU/ml on "1 ETB" isolated from "beggars". Yeasts and molds were not isolated from "50 cents" collected from "gamblers", "restaurant workers" and "butcher" (Table 2).

Abundance of pathogenic bacteria

The result in Table 3 showed that the type of money collected from different sites had bacterial pathogens with different degree of contamination except "50 cents" from "butchers" and "restaurant workers" from which no cultivable type of pathogenic bacteria was isolated. Load of some specific pathogenic microorganisms on ETB from different type and different bearer showed significant variation ($p<0.01$) (Table 3). The bacterial contamination of note-money was exceeding the contamination of coins. Among note-money, 1 ETB was more contaminated with bacteria pathogens compared to the coins with six folds differences (Table 3). The highest load was of pathogenic bacteria was *Staphylococcus* (91.9 ± 1.0 CFU/ml) on "1 ETB note" from

Bacterial group	Sample	Sample site (CFU/ml)						
		Street food sellers	Taxi drivers	Gamblers	Restaurant workers	Beggars	Bank workers	Butchers
Aerobic Mesophilic Bacteria	10 ETB	159.1 ± 1.0^{f}	95.9 ± 1.0^{l}	143.9 ± 4.0^{h}	104.8 ± 1.0^{k}	219.1 ± 1.0^{d}	125.0 ± 1.0^{i}	237.9 ± 1.0^{b}
	5 ETB	79.9 ± 1.0^{n}	94.0 ± 1.0^{l}	117.8 ± 2.0^{j}	77.9 ± 1.0^{n}	152.0 ± 1.0^{g}	104.9 ± 1.0^{k}	125.9 ± 1.0^{i}
	1 ETB (note)	178.0 ± 1.0^{e}	123.9 ± 1.0^{i}	177.8 ± 1.0^{e}	143.8 ± 1.0^{h}	255.8 ± 1.0^{a}	153.9 ± 1.0^{g}	233.8 ± 1.0^{c}
	1 ETB (coin)	69.0 ± 1.0^{o}	47.8 ± 1.0^{q}	62.1 ± 2.0^{p}	48.0 ± 1.0^{q}	144.8 ± 1.0^{h}	62.9 ± 1.0^{p}	90.8 ± 1.0^{m}
	50 cents	9.8 ± 1.0^{t}	9.9 ± 1.0^{t}	17.0 ± 1.0^{s}	0.0 ± 0.0^{u}	87.9 ± 0.1^{m}	35.8 ± 1.0^{r}	0.0 ± 0.0^{u}
Coliform bacteria	10 ETB	35.8 ± 0.8^{b}	22.1 ± 1.0^{fg}	34.8 ± 1.0^{bc}	11.0 ± 1.0^{ij}	29.0 ± 1.0^{d}	8.0 ± 1.0^{kl}	25.1 ± 1.0^{e}
	5 ETB	28.9 ± 1.0^{d}	12.1 ± 1.0^{ij}	24.0 ± 1.0^{ef}	13.0 ± 1.0^{hi}	33.1 ± 1.0^{c}	5.0 ± 1.0^{mn}	20.1 ± 1.0^{g}
	1 ETB (note)	44.0 ± 1.0^{a}	25.9 ± 1.0^{e}	24.8 ± 1.0^{e}	14.8 ± 1.0^{h}	35.8 ± 1.0^{b}	11.1 ± 1.0^{ij}	22.1 ± 1.0^{fg}
	1 ETB (coin)	15.0 ± 1.0^{h}	5.0 ± 1.0^{mn}	4.9 ± 1.0^{mn}	$2.9 \pm 1.0^{n,o}$	9.9 ± 1.0^{jk}	2.8 ± 1.0^{no}	6.9 ± 1.0^{lm}
	50 cents	0.1 ± 0.1^{p}	0.8 ± 1.0^{op}	0.1 ± 0.1^{p}	0.0 ± 0.0^{p}	1.0 ± 1.0^{op}	0.1 ± 0.1^{p}	0.0 ± 0.0^{p}
Enterobacteriaceae	10 ETB	61.9 ± 1.0^{k}	44.0 ± 1.0^{n}	124.0 ± 1.0^{b}	90.0 ± 1.0^{e}	111.0 ± 1.0^{d}	43.9 ± 1.0^{n}	68.0 ± 1.0^{j}
	5 ETB	49.8 ± 1.0^{m}	29.0 ± 1.0^{o}	86.0 ± 1.0^{f}	80.1 ± 1.0^{g}	77.9 ± 1.0^{g}	42.9 ± 1.0^{n}	66.5 ± 1.5^{j}
	1 ETB (note)	70.9 ± 1.0^{i}	75.3 ± 1.1^{h}	113.9 ± 1.0^{c}	122.9 ± 1.0^{b}	138.1 ± 1.0^{a}	55.0 ± 0.9^{l}	90.0 ± 1.0^{e}
	1 ETB (coin)	15.8 ± 1.0^{r}	11.0 ± 1.0^{s}	24.0 ± 1.0^{p}	19.0 ± 1.0^{q}	25.1 ± 1.0^{p}	7.1 ± 1.0^{t}	25.0 ± 1.0^{p}
	50 cents	3.1 ± 1.0^{uv}	6.0 ± 1.0^{t}	5.1 ± 1.0^{tu}	0.0 ± 0.0^{w}	7.1 ± 1.0^{t}	1.1 ± 0.1^{vw}	0.0 ± 0.0^{w}
Staphylococcus bacteria	10 ETB	88.8 ± 1.0^{b}	30.9 ± 1.0^{jk}	49.1 ± 1.0^{e}	42.1 ± 1.0^{gh}	44.1 ± 1.0^{fg}	9.9 ± 1.0^{r}	27.9 ± 1.0^{lm}
	5 ETB	63.9 ± 1.0^{c}	33.0 ± 1.0^{ij}	28.9 ± 1.0^{kl}	44.8 ± 0.3^{f}	25.8 ± 1.0^{m}	10.0 ± 1.0^{r}	14.9 ± 1.0^{op}
	1 ETB (note)	103.0 ± 1.0^{a}	40.8 ± 1.0^{h}	51.9 ± 1.0^{d}	51.1 ± 1.0^{de}	50.9 ± 1.0^{de}	28.5 ± 1.5^{kl}	35.1 ± 1.0^{i}
	1 ETB (coin)	18.0 ± 1.0^{n}	11.0 ± 1.0^{qr}	13.1 ± 1.0^{pq}	12.8 ± 1.0^{pq}	10.8 ± 1.0^{qr}	5.8 ± 1.0^{s}	17.1 ± 1.0^{no}
	50 cents	15.0 ± 1.0^{op}	4.9 ± 1.0^{st}	1.9 ± 1.0^{uv}	0.0 ± 0.0^{v}	1.9 ± 1.0^{uv}	3.0 ± 1.0^{tu}	0.0 ± 0.0^{v}
Bacillus bacteria	10 ETB	3.0 ± 1.0^{ijk}	0.0 ± 0.0^{m}	6.8 ± 1.0^{gh}	24.8 ± 1.0^{c}	29.8 ± 1.0^{b}	13.1 ± 1.0^{f}	25.0 ± 1.0^{c}
	5 ETB	1.0 ± 1.0^{lm}	0.0 ± 0.0^{m}	5.0 ± 1.0^{hi}	20.8 ± 1.0^{d}	21.9 ± 1.0^{d}	9.1 ± 1.0^{g}	21.8 ± 1.0^{d}
	1 ETB (note)	8.9 ± 1.0^{g}	1.8 ± 1.0^{jkl}	8.0 ± 1.0^{g}	29.0 ± 1.0^{b}	33.8 ± 1.0^{a}	18.0 ± 1.0^{e}	21.8 ± 1.0^{d}
	1 ETB (coin)	0.1 ± 0.2^{m}	0.0 ± 0.0^{n}	3.9 ± 1.0^{ij}	3.0 ± 1.0^{ijk}	14.8 ± 1.0^{f}	1.0 ± 1.0^{lm}	1.8 ± 1.0^{jkl}
	50 cents	0.0 ± 0.0^{m}	0.0 ± 0.0^{n}	0.0 ± 0.0^{n}	0.0 ± 0.0^{n}	1.1 ± 1.0^{lm}	0.0 ± 0.0^{m}	0.0 ± 00^{m}

Table 1: Abundance of bacterial groups isolated from Ethiopian cash-money of different types with respect to their sources of sampling.

Fungal groups	Samples	Sample site (CFU/ml)						
		Street food sellers	Taxi driver	Gamblers	Restaurant workers	Beggars	Bank workers	Butcher
Yeast	10 ETB	93.6 ± 0.5^{b}	79.5 ± 1.0^{d}	33.3 ± 1.2^{no}	68.8 ± 0.4^{f}	66.3 ± 1.3^{g}	55.5 ± 1.0^{ij}	51.8 ± 0.5^{k}
	5 ETB	84.6 ± 1.6^{c}	75.0 ± 1.0^{e}	42.0 ± 0.6^{m}	35.0 ± 0.5^{n}	59.8 ± 0.6^{h}	52.5 ± 1.0^{k}	51.5 ± 0.5^{k}
	1 ETB note	125.8 ± 1.4^{a}	68.0 ± 1.5^{f}	52.3 ± 1.1^{jk}	45.3 ± 2.6^{l}	75.5 ± 1.0^{e}	57.5 ± 1.7^{i}	72.3 ± 0.9^{e}
	1 ETB coin	30.9 ± 0.6^{o}	7.3 ± 0.5^{r}	2.8 ± 0.5^{s}	11.5 ± 0.5^{q}	31.8 ± 0.2^{o}	32.5 ± 2.0^{no}	10.8 ± 1.1^{q}
	50 cents	9.1 ± 1.0^{qr}	2.5 ± 0.5^{s}	0 ± 0.00^{s}	0 ± 0^{s}	27.0 ± 0.6^{p}	9.8 ± 0.5^{qr}	0 ± 0^{s}
Molds	10 ETB	4.1 ± 0.07^{ij}	6.5 ± 1.4^{gh}	1.8 ± 0.5^{lmn}	6.8 ± 0.5^{gh}	9.0 ± 0.6^{f}	8.5 ± 0.8^{fg}	13.0 ± 0.6^{d}
	5 ETB	0.4 ± 0.4^{mno}	3.0 ± 1.5^{jklm}	2.5 ± 0.5^{jklmn}	5.3 ± 0.5^{h}	14.3 ± 0.7^{d}	$6.3 \pm 0.5g^{h}$	15.8 ± 0.5^{c}
	1 ETB note	4.0 ± 1.0^{ij}	14.3 ± 2.0^{cd}	5.3 ± 0.5^{hi}	7.3 ± 0.6^{gh}	20.3 ± 0.5^{a}	10.0 ± 0.6^{e}	18.0 ± 0.6^{b}
	1 ETB coin	0 ± 0^{o}	1.3 ± 0.5^{lmn}	0.5 ± 0.5^{no}	0.5 ± 0.5^{no}	3.5 ± 0.5^{jk}	2.0 ± 1.0^{klmno}	1.3 ± 0.4^{mn}
	50 cents	0 ± 0^{o}	0.3 ± 0.4^{o}	0 ± 0^{o}	0 ± 0^{o}	3.3 ± 0.3^{jkl}	0.3 ± 0.4^{no}	0 ± 0^{o}

Table 2: Abundance of fungal groups isolated from Ethiopian cash-money of different types with respect to their sources of sampling.

"street food sellers" followed by *Salmonella* (35.1 ± 1.0 CFU/ml) on "1 ETB note" and *Klebsiella* (34.8 ± 1.0 CFU/ml) on "10 ETB" from "gamblers" and "beggars", respectively (Table 3).

Samples from "street food sellers" and "taxi drivers" showed the dominancy of *Staphylococcus* with 91.9 CFU/ml but *Bacillus* was the least from the sources (Table 3). Similarly, samples collected from the "gamblers" were majorly loaded with *Salmonella* but lowest mean count was *Bacillus* from the source. Samples from "restaurant workers" were heavily loaded with *Klebsiella* as compared to the other

Pathogens	Samples	Sample site (CFU/ml)						
		Street food sellers	Taxi drivers	Gamblers	Restaurants workers	Beggars	Bank workers	Butchers
Salmonella	10 ETB	13.0 ± 1.0^{hi}	16.1 ± 1.0^{efgh}	33.7 ± 1.5^{a}	19.8 ± 1.0^{d}	18.0 ± 1.0^{def}	14.1 ± 1.0^{ghi}	25.0 ± 1.0^{c}
	5 ETB	18.9 ± 1.0^{de}	9.1 ± 1.0^{ijk}	24.1 ± 1.0^{c}	13.1 ± 1.0^{hi}	16.0 ± 1.0^{efgh}	9.1 ± 1.0^{ijk}	18.8 ± 1.0^{de}
	1 ETB (note)	12.0 ± 1.0^{ij}	23.8 ± 4.5^{c}	35.1 ± 1.0^{a}	26.9 ± 1.0^{bc}	29.8 ± 1.0^{b}	14.9 ± 1.0^{fghi}	17.1 ± 1.0^{defg}
	1 ETB (coin)	2.8 ± 1.0^{lm}	3.0 ± 1.0^{lm}	7.0 ± 1.0^{k}	3.0 ± 1.0^{lm}	8.8 ± 1.0^{jk}	1.0 ± 1.0^{m}	6.1 ± 1.0^{kl}
	50 cents	0.9 ± 1.0^{m}	0.0 ± 0.0^{m}	1.0 ± 1.0^{m}	0.0 ± 0.0^{m}	1.8 ± 1.0^{m}	0.8 ± 1.0^{m}	0.0 ± 0.0^{m}
Shigella	10 ETB	12.1 ± 1.0^{fg}	5.9 ± 1.0^{ijk}	24.8 ± 1.0^{a}	13.9 ± 1.0^{ef}	18.8 ± 1.0^{bc}	8.8 ± 1.0^{hi}	4.9 ± 1.0^{jkl}
	5 ETB	9.0 ± 1.0^{hi}	3.9 ± 1.0^{klm}	17.9 ± 1.0^{cd}	10.0 ± 1.0^{gh}	11.1 ± 1.0^{gh}	10.0 ± 1.0^{gh}	9.8 ± 1.0^{gh}
	1 ETB (note)	11.0 ± 0.9^{gh}	12.1 ± 1.0^{fg}	20.8 ± 1.0^{b}	19.1 ± 1.0^{bc}	24.1 ± 1.0^{a}	7.1 ± 1.0^{ij}	15.9 ± 1.0^{de}
	1 ETB (coin)	2.0 ± 1.0^{mnop}	1.9 ± 1.0^{mnop}	5.1 ± 1.0^{jkl}	2.1 ± 1.0^{mnop}	2.9 ± 1.0l^{mno}	1.9 ± 1.0^{mnop}	3.1 ± 1.0^{lmn}
	50 cents	0.1 ± 0.2o^{p}	1.0 ± 1.0^{nop}	1.0 ± 1.0^{nop}	0.0 ± 0.0^{p}	1.0 ± 1.0^{nop}	0.2 ± 0.1^{op}	0.0 ± 0.0^{p}
Klebsiella	10 ETB	20.1 ± 1.0^{ef}	8.0 ± 1.0^{j}	17.0 ± 1.0^{gh}	25.0 ± 1.0^{d}	34.8 ± 1.0^{a}	6.8 ± 1.0^{jk}	20.1 ± 1.0^{ef}
	5 ETB	11.1 ± 1.0^{j}	6.9 ± 1.0^{jk}	11.0 ± 1.0^{j}	22.1 ± 1.0^{e}	27.8 ± 1.0^{bc}	8.1 ± 1.0^{j}	22.1 ± 1.0^{e}
	1 ETB (note)	27.1 ± 1.0^{cd}	18.9 ± 1.0^{fg}	15.8 ± 1.0^{h}	29.8 ± 1.0^{b}	33.0 ± 1.0^{a}	12.0 ± 1.0^{j}	26.1 ± 1.0^{cd}
	1 ETB (coin)	8.1 ± 1.0^{j}	3.1 ± 1.0^{lm}	3.1 ± 1.0^{lm}	5.1 ± 1.0^{kl}	7.1 ± 1.0^{jk}	1.9 ± 1.0^{mn}	6.9 ± 1.0^{jk}
	50 cents	0.3 ± 0.0^{n}	1.1 ± 1.0^{mn}	1.0 ± 1.0^{mn}	0.0 ± 0.0^{n}	1.1 ± 1.0^{mn}	0.1 ± 0.1^{n}	0.0 ± 0.0^{n}
Bacillus	10 ETB	3.0 ± 1.0^{ijk}	0.0 ± 0.0^{m}	6.8 ± 1.0^{gh}	24.8 ± 1.0^{c}	29.8 ± 1.0^{b}	13.1 ± 1.0^{f}	25.0 ± 1.0^{c}
	5 ETB	1.0 ± 1.0^{lm}	0.0 ± 0.0^{m}	5.0 ± 1.0^{hi}	20.8 ± 1.0^{d}	21.9 ± 1.0^{d}	9.1 ± 1.0^{g}	21.8 ± 1.0^{d}
	1 ETB (note)	8.9 ± 1.0^{g}	1.8 ± 1.0^{jkl}	8.0 ± 1.0^{g}	29.0 ± 1.0^{b}	33.8 ± 1.0^{a}	18.0 ± 1.0^{e}	21.8 ± 1.0^{d}
	1 ETB (coin)	0.1 ± 0.2^{m}	0.0 ± 0.0^{n}	3.9 ± 1.0^{ij}	3.0 ± 1.0^{ijk}	14.8 ± 1.0^{f}	1.0 ± 1.0^{lm}	1.8 ± 1.0^{jkl}
	50 cents	0.0 ± 0.0^{m}	0.0 ± 0.0^{n}	0.0 ± 0.0^{n}	0.0 ± 0.0^{n}	1.1 ± 1.0^{lm}	0.0 ± 0.0^{m}	0.0 ± 0.0^{m}
Pseudomonas	10 ETB	11.9 ± 1.0^{fg}	5.1 ± 1.0^{kl}	24.0 ± 1.0^{a}	13.1 ± 1.0^{ef}	18.0 ± 1.0^{bc}	8.0 ± 1.0^{ij}	4.1 ± 1.0^{lm}
	5 ETB	8.2 ± 1.0^{ij}	3.1 ± 1.0^{lmn}	17.1 ± 1.0^{cd}	9.2 ± 1.0^{hij}	10.9 ± 1.0^{fgh}	9.2 ± 1.0^{hij}	9.0 ± 1.0^{hij}
	1 ETB (note)	9.9 ± 1.0^{ghi}	11.9 ± 1.0^{fg}	20.0 ± 1.0^{b}	18.9 ± 1.0^{bc}	23.9 ± 1.0^{a}	6.9 ± 1.0^{jk}	15.1 ± 1.0^{de}
	1 ETB (coin)	1.2 ± 1.0^{nop}	1.1 ± 1.0^{nop}	4.9 ± 1.0^{kl}	1.9 ± 1.0^{mnop}	2.1 ± 1.0^{mnop}	1.1 ± 1.0^{nop}	2.9 ± 1.0^{lmno}
	50 cents	0.0 ± 0.0^{p}	1.1 ± 1.0^{nop}	1.0 ± 1.0^{nop}	0.0 ± 0.0^{p}	1.8 ± 1.0^{mnop}	0.2 ± 0.10^{p}	0.0 ± 0.0^{p}
Enterobacter	10 ETB	4.1 ± 1.0^{jk}	8.9 ± 1.0^{h}	24.1 ± 1.0^{bc}	18.9 ± 1.0^{d}	21.9 ± 1.0^{c}	6.1 ± 1.0^{ij}	13.9 ± 1.0^{efg}
	5 ETB	2.0 ± 1.0^{kl}	6.1 ± 1.0^{ij}	15.9 ± 1.0^{e}	25.1 ± 1.0^{b}	12.1 ± 1.0^{g}	5.9 ± 1.0^{ij}	7.1 ± 1.0^{hi}
	1 ETB (note)	11.9 ± 1.0^{g}	12.9 ± 1.0^{fg}	22.1 ± 1.0^{c}	28.1 ± 1.0^{a}	28.0 ± 1.0^{a}	15.1 ± 1.0^{ef}	15.1 ± 1.0^{ef}
	1 ETB (coin)	1.1 ± 1.0^{l}	2.0 ± 1.0^{kl}	4.0 ± 1.0^{jk}	7.0 ± 1.0^{hi}	4.9 ± 1.0^{ij}	1.9 ± 1.0^{kl}	6.1 ± 1.0^{ij}
	50 cents	2.0 ± 1.0^{kl}	2.1 ± 1.0^{kl}	1.1 ± 1.0^{l}	0.0 ± 0.0^{l}	2.1 ± 1.0^{kl}	0.1 ± 0.2^{l}	0.0 ± 0.0^{l}
Escherichia coli	10 ETB	35.8 ± 0.8^{b}	22.1 ± 1.0$^{f g}$	34.8 ± 1.0^{bc}	11.0 ± 1.0^{ij}	29.0 ± 1.0^{d}	8.0 ± 1.0^{kl}	25.1 ± 1.0^{e}
	5 ETB	28.9 ± 1.0^{d}	12.1 ± 1.0^{ij}	24.0 ± 1.0^{ef}	13.0 ± 1.0^{hi}	33.1 ± 1.0^{c}	5.0 ± 1.0^{mn}	20.1 ± 1.0^{g}
	1 ETB (note)	44.0 ± 1.0^{a}	25.9 ± 1.0^{e}	24.8 ± 1.0^{e}	14.8 ± 1.0^{h}	35.8 ± 1.0^{b}	11.1 ± 1.0^{ij}	22.1 ± 1.0$^{f g}$
	1 ETB (coin)	15.0 ± 1.0^{h}	5.0 ± 1.0^{mn}	4.9 ± 1.0^{mn}	2.9 ± 1.0^{n,o}	9.9 ± 1.0^{jk}	2.8 ± 1.0^{no}	6.9 ± 1.0^{lm}
	50 cents	0.1 ± 0.1^{p}	0.8 ± 1.0^{op}	0.1 ± 0.1^{p}	0.0 ± 0.0^{p}	1.0 ± 1.0^{op}	0.1 ± 0.1^{p}	0.0 ± 0.0^{p}
Staphylococcus	10 ETB	84.9 ± 1.0^{b}	22.8 ± 1.0^{g}	25.1 ± 1.0^{fg}	24.0 ± 1.0^{g}	23.0 ± 1.0^{g}	3.9 ± 1.0^{op}	14.8 ± 1.0^{ij}
	5 ETB	62.0 ± 1.0^{c}	27.1 ± 1.0^{ef}	13.8 ± 1.0^{jk}	19.8 ± 1.0^{h}	13.9 ± 1.0^{jk}	4.9 ± 1.0^{op}	7.9 ± 1.0^{mn}
	1 ETB (note)	91.9 ± 1.0^{a}	28.1 ± 1.0^{de}	29.9 ± 1.0^{d}	23.1 ± 1.0^{g}	23.1 ± 1.0^{g}	13.9 ± 1.0^{jk}	20.1 ± 1.0^{h}
	1 ETB (coin)	17.0 ± 1.0^{i}	9.8 ± 1.0^{lm}	9.9 ± 1.0^{lm}	6.0 ± 1.0^{no}	6.1 ± 1.0^{no}	4.1 ± 1.0^{op}	11.8 ± 1.0^{kl}
	50 cents	13.9 ± 1.0^{jk}	2.9 ± 1.0^{pq}	1.0 ± 1.0^{qr}	0.0 ± 0.0^{r}	0.2 ± 0.1^{r}	3.0 ± 1.0^{pq}	0.0 ± 0.0^{r}
Streptococcus	10 ETB	3.9 ± 1.0^{klm}	8.1 ± 1.0^{h}	24.0 ± 1.0^{bc}	18.1 ± 1.0^{e}	21.1 ± 1.0^{d}	6.0 ± 1.0^{hijk}	13.1 ± 1.0^{fg}
	5 ETB	1.9 ± 1.0^{lmn}	5.9 ± 1.0^{hijk}	15.1 ± 1.0^{f}	25.0 ± 1.0^{b}	11.9 ± 1.0^{g}	5.1 ± 1.0^{ijk}	7.0 ± 1.0^{hi}
	1 ETB (note)	11.1 ± 1.0^{g}	12.1 ± 1.0^{g}	22.0 ± 1.0^{cd}	28.0 ± 1.0^{a}	27.8 ± 1.0^{a}	14.9 ± 1.0^{f}	15.0 ± 1.0^{f}
	1 ETB (coin)	1.0 ± 1.0^{n}	1.8 ± 1.0^{lmn}	3.8 ± 1.0^{klm}	6.8 ± 1.0^{hij}	4.1 ± 1.0^{jkl}	1.1 ± 1.0^{mn}	5.9 ± 1.0^{hijk}
	50 cents	1.8 ± 1.0^{lmn}	2.0 ± 1.0^{lmn}	0.9 ± 1.0^{n}	0.0 ± 0.0^{n}	1.9 ± 1.0^{lmn}	0.2 ± 0.1^{n}	0.0 ± 0.0^{n}

Table 3: Abundance of bacterial pathogens isolated from Ethiopian cash-money of different denomination from different sites.

pathogens. Samples from "beggars", "butchers" and "bank workers" were dominantly contaminated by *E. coli, Klebsiella* and *Bacillus* with load of 35.8, 26.1 and 18.0 CFU/ml, respectively (Table 3).

Drug susceptibility of pathogenic bacteria

As shown in Table 4, *Bacillus* was resistant to the antibiotics tested in this study. Besides, *Shigella* was resistant to Ampicillin and Cefalexin, and *E. coli* was resistant to Cefuroxime (Table 4). *Staphylococcus, Enterobacter* and *Pseudomonas* were "highly sensitive" to "moderately sensitive" to the antibiotics tested in this experiment [12].

Cefuroxime was about four times effective of Ampicillin and Cefalexin in inhibiting growth of *Salmonella.* Besides, *Shigella*, *Klebsiella* and *E. coli* were "highly sensitive" to Cefixime. In general, Cefixime and Cefuroxime were highly effective and broad in inhibition of the pathogens except *Bacillus* and *E. coli* (Table 4).

No	Antibiotics	Test dose (µg/ml)	Pathogenic bacterial groups								
			Salmonella	*Shigella*	*Klebsiella*	*Bacillus*	*Pseudomonas*	*Enterobacter*	*E. coli*	*Staphyllococcus*	*Streptococcus*
			Inhibition zone (mm)								
1	Ampicillin	10	4.50	0.00	3.00	0.00	20.00	9.00	14.00	12.00	14.00
2	Cefalexin	16	4.00	0.00	3.00	0.00	7.00	8.00	0.00	22.00	14.00
3	Cefixime	5	11.00	17.00	15.00	0.00	12.00	10.00	15.00	11.00	2.50
4	Cefuroxime	8	15.00	6.00	8.00	0.00	18.00	12.00	0.00	14.00	9.00

Table 4: Intrinsic antibiotics resistance of bacterial pathogens isolated from Ethiopian cash-moneys

Discussion

Money acts as the reservoir of different groups of microorganisms for the transmission among community. In this study of bacterial pathogens such as *Staphylococcus*, *Streptococcus*, *Escherichia coli*, *Salmonella*, *Shigella*, *Enterobacter*, *Klebsiella*, *Bacillus* and *Pseudomonas* were detected on ETB samples of "50 cents", 1 ETB coin", "1 ETB note", "5 ETB" and "10 ETB". In addition, yeasts and molds were also detected on the Ethiopian money samples. Similarly, different kinds of pathogenic bacteria and fungi were reported from currencies of Ghana [13], Saudi Arabia [14], and Bangladesh [15] although the load and survival rate of microorganisms on the currencies of different countries varies [16]. Ethiopian cash-money collected from different bearer at Easter and Southwestern part of the country were heavily loaded with microbes [6,17]. However, the mean microbial load in the present sample was up to 10-200 folds higher as compared to the one reported from the Southwestern part of the country [17]. The big difference in microbial load on the cash-money collected from different areas could depend of lifestyle of the community, and duration of the samples in market and its transfer rate in the area. Particularly, poor handling practices and personal hygiene of the bearers could contribute to the observed differences on microbial counts. For instance, cash-money collected from community working in hospitals, municipals and banks are more contaminated than those sampled from butchers and food sellers in India [5].

In this study, money notes were more contaminated than the coins. Particularly, "1 ETB note notes" were more contaminated by bacteria and fungi followed by 10 ETB. This could be also associated with the long duration of "1 ETB note" in market as it was on the process of replacing with 1 ETB coin". Besides, "1 ETB note" could be in high rate of transfer because it is small in value for change. Similarly, the inverse correlation between money value and microbial load on Sudanese currencies [2,18], Libyan note currencies [7] and Ethiopian currencies [19] was previously reported. The less contamination of "ETB coins" as compared to "ETB notes" could be associated with the nature of coins as it is not comfortable for the existence and reproduction of microbial pathogens. However, report from Nepal showed that coins are more contaminated with bacteria than note or polymer notes that are in contrary to the current result [1].

The load of bacterial pathogens and fungus on the samples collected from "beggars" and "street food sellers" were high as compared to samples from other sources. Similarly, in Nepal the cash-money sampled from public transport were found to be extremely contaminated with various pathogenic bacteria followed by the cash-money obtained from "butchers" and "food sellers" [20]. The lifestyle of the bearers could give chance for the reproduction of the pathogens, which make them potentially vehicles for the transmission of these pathogens.

The current global health problem is resistance of pathogens to multiple antibiotics. In this study *Bacillus* from all samples were found resistant to Ampicillin, Cefalexin, Cefixime, and Cefuroxime. *E. coli* is also resistant to Cefalexin and Cefuroxime although it was highly sensitive to Ampicillin and Cefixime. However, previous study showed the high susceptibility of *E. coli* from samples of Ethiopian cash-money to different antibiotics [6]. Therefore, variation could depend on target action of the antibiotics.

Conclusion

Ethiopian cash-moneys are loaded with bacteria and fungi. The bacterial group like Aerobic mesophilic bacteria, Coliform, Enterobacteriaceae, *Staphylococcus* and *Bacillus* were isolated from the cash-money. The specific pathogens like *Salmonella, Shigella, Klebsiella, Bacillus* and *Pseudomonas* were isolated from the cash-money. However, the abundance of these bacteria varied based on bearers and types of money. "1 ETB note" was more contaminated by the bacterial and fungal group as compared to other money types, and microbial load on coins were significantly low. The genus *Bacillus* was very resistant to Ampicillin, Cefalexin, Cefixime and Cefuroxime. *Shigella* responded only for Cefixime and the antibiotics tested in this experiment were effective against *Staphylococcus*.

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