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Biology

MICROBIAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY OF BACTERIAL PATHOGENS ISOLATED FROM PAPER CURRENCIES CIRCULATING IN SOME RESTAURANTS AND BUTCHERIES IN HAWASSA, SIDAMA, ETHIOPIA, 2020-21

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ABSTRACT Currency notes could play a significant role in transmitting pathogenic microorganisms amongst individuals in the society. This study was aimed to determine the microbial profile and Antibiotic susceptibility of bacterial pathogens isolated from Ethiopian paper notes in circulation. 64 currency paper notes of different denomination were tested for bacterial contamination using standard microbiological methods. Antibiotic susceptibility profiles of the isolates were determined with approved methods. Results were analyzed using descriptive statistics. Overall mean AMBC was 4.08 log units, with the highest 6.58 log units recorded from denomination 5 followed by 4.50, 3.03, 2.20 log units from denominations 10, 50 and 100 respectively. Total Coliforms (TC) displayed the same pattern with the highest mean counts of 6.52 log units, from denomination 5 and lowest counts of 2.19 log units from denomination 100. Out of 64 currency notes, 35 (54.7%) were contaminated with bacteria. The predominant bacteria isolates were E. coli (60.5%), Salmonella spp. (23.6%) and Shigella spp. (13.2%). Each isolate was resistant to four or more antibiotics tested. All isolates were resistant against Cefepime and Tetracycline and sensitive to Ceftriaxone. This study revealed that currency notes are contaminated with pathogenic bacteria and in most cases these bacterial isolates were resistant to commonly prescribed antibiotics. Therefore, contaminated notes are identified as potential public health threat, because pathogens can be spread by circulating the notes and become source of infection. Awareness creation is important among public in this regard.

KEYWORDS : Aerobic mesophilic bacteria, E. coli, Hawassa, microbial contamination, Salmonella,Shigella

INTRODUCTION

Background of the Study:

Paper currency is one of the items most frequently passed from hand to hand globally. During passage, money can get contaminated and may play a role in the transmission of microorganisms among people. Microorganisms spread from person to person via the surface of paper currency (Pope *et al.*, 2002; Umeh *et al.*, 2007). The contamination of currencies by pathogenic microorganisms is much of public health concern as they can be sources of transmission of microorganisms.

Currencies are widely used and each currency is exchanged many times. If some of these currency notes are contaminated with pathogenic microorganisms, there is a potential to spread these microbes (Ahmed *et al.*, 2010).

Paper currencies are often contaminated with pathogens through counting using saliva, coughing, sneezing on hands followed by exchanging currency, placement, storage on dirty surfaces, and poor hand washing after using toilet, thus serves as a vehicle to spread microorganisms to the next user (Barolia *et al.*, 2011; Ngwai *et al.*, 2011). Lamichane *et al.*, (2009) reported that microbial contamination rate of currency is variable; handled by butchers (78.0 %) and food sellers (62.1 %).

A study conducted in Nigeria Naira (Nigerian currency) by Umeh *et al.*, (2007) indicated the isolation of *E. coli* (80%), *Aerobacter* (59%), *Salmonella* (31.8%), *Enterococcus faecalis* (31.8%), *S. aureus* (27.3%), and coagulase negative *staphylococci* (18.2%). Saeed and Rasheed (2011) reported from Pakistan that Rupee currency notes harbored by *Bacillus* spp. (49.3%), *Micrococcus sadentarius* (19.7%), *E. coli* (14.1%), *S. aureus* (4.9%), *Klebsiella pneumoniae* (2.8%), and coagulase negative *staphylococci* (4.2%).

The problem of spread of pathogenic microorganisms through currency notes is compounded by the emergence of multidrug resistant pathogens. Antibiotics resistance is recently a global issue of disease and death as a result of treatment failures and increased health care costs (WHO, 2018). While antibiotics resistance has steadily been increasing, e.g., with Extended Spectrum of Beta Lactamases producing *Escherichia coli* and *Klebsiella* spp. contaminated banknotes and coins contribute to the transmission of these multidrug resistant microorganisms (Gedik *et al.*, 2010).

In Ethiopia, few published studies indicated the microbial colonization of currency notes. In one study done in Jimma, Ethiopia, Girma *et al.* (2014) reported that *Staphylococcus* spp. (34.06%), *Bacillus* spp. (31.88%), *Enterobacteriaceae* spp. (13.39%), *Micrococcus* spp. (9.55%) and *Streptococcus* spp. (9.03%) were isolated from Ethiopian paper currency. Solomon (2015) from Debre Marcos town, Ethiopia indicated seven different types of bacterial species such as *Staphylococcus aureus* (26%), *Escherichia coli* (24%), *Enterobacter* spp. (12%), *Salmonella* spp. (10%), *Klebsiella* species (8%), *Pseudomonas* spp. (6%) and *Shigella* spp. (5%). However, studies of the contamination of currency notes with microbial agents is lacking in southern Ethiopia, especially in Hawassa city. Awareness of the microbial diversity of currency notes in circulation can provide the idea of health awareness in people during currency handling and effective control of infection transmission. Hence, this study was initiated with the objective of identifying microbial contamination of currency notes circulating in some restaurants and butcheries and the antibiotics susceptibility profiles of the pathogenic isolates.

Statement of the Problem:

Microorganisms are ubiquitous on living and nonliving things including on currency notes that frequently circulating among

different people, for trade, occupation, commercialization, salary payment and investment purpose. Therefore, contaminated currency notes can result in spreading of disease caused by pathogenic microorganisms. Currencies circulate in People with different body condition like sweating, body secretions contributes to different types of diseases (skin, digestive and respiratory tract diseases) caused by bacterial and fungal activities which can be transferred by handling currency notes. Due to these, microbes are circulated with currencies and also distributing various type of diseases including food borne disease. Currency becomes contaminated by different types of microorganisms due to repeated handling by people during its turnover life time. Some of these microorganisms may be pathogenic, food borne or direct contact.

There is absence of published work on the problem specifically from Hawassa city administration of Sidama Regional State, one of the tourist hotspots. Hence this study was initiated and the results of this study will be useful to serve as early warning information to avert potential problems. .

General Objective:
To assess the microbial profile and Antibiotics susceptibility of bacterial pathogens and indicator organisms isolated from paper notes circulating in some restaurants and butcheries in Hawassa, Sidama Regional State of Ethiopia, 2020-21

Specific Objectives
- To estimate the aerobic mesophilic bacterial count (AMBC) and total coliform bacterial count (TCC) of paper currencies of different denomination (ETBs 5, 10, 50 and 100 notes) circulating in some selected restaurants and butcheries in Hawassa city, Sidama Regional State of Ethiopia.
- To isolate and identify some common bacterial pathogens (*Salmonella*, and *Shigella*) and indicator organisms (*E.coli*) from the surface rinse of Ethiopia paper currencies of different denomination (ETBs 5, 10, 50 and 100 notes)
- To determine the antibiotic susceptibility profiles of the potentially pathogenic bacteria isolates (if there are any) based on the standard set of commonly prescribed antibiotics.

Research Questions
1. What is the microbial load (AMBC and TCC) of the different denominations of the paper currencies? Is the observed difference in microbial load among the different denominations of paper currencies from restaurants and butcheries statistically significant?
2. What are the common bacterial pathogens and indicator organisms from the surface rinse of Ethiopia paper currencies of different denomination (ETBs 5, 10, 50 and 100 notes)?
3. What is the antibiotics susceptibility profile of the pathogenic bacterial isolates?

MATERIALS AND METHODS
Description of the Study Location:
This study was conducted in Hawassa, the administrative center of Sidama Regional state of Ethiopia which is located on the shores of Lake Hawassa in the Great Rift Valley; 273 Km south of Addis Ababa. Geographically, it is situated between 7°3′ N latitude to North and 38 ° 28′ E longitudes to East and it is elevated at 1697m above sea level. The city administration has an area of 157.2 KM^2 and is divided into eight sub-cities and 32 Kebeles(smallest administrative unit) with total population of 351,469 (CSA, 2015). Hawassa city is one of the hotspot tourisms in Ethiopia which serves over 200 thousand tourists each year. The major tourist destination includes Fish market, Millennium Park (Tikur Wuha Forest), Lake Hawassa, Gudumale Park (Amora Gedel), Mount Tabor and Alamura,

Teha Goda Island, and Sidama Cultural Hall, etc. The major economic activity of Hawassa city is business and investment. Commercial activities in the city include wholesale, retail, small business and different types of activities (Assefa et al., 2009).

Figure 1: Map of Hawassa City Administration (Source: Assefa et al., 2009 E.C)

Study Design:
A cross-sectional study method was conducted on four denominations of Ethiopian paper currencies(ETB-Ethiopian Birr) circulating in some restaurants and butcheries in Hawassa city. Two samples of each denomination ETB5, ETB10, ETB50 and ETB100 Ethiopian currency notes was obtained from randomly selected restaurants and butcheries in Hawassa city from September 2020 to January 2021 G.C

Sampling Points and Sample Size:
Convenient arbitrary sampling was employed by considering available resources and time. Accordingly, one restaurant and one butcher were randomly selected from each of four sub-cities of Hawassa (with significant hubs of business activities), including Atote, Menaharya, Bahil adarash and Arab sefer. Therefore, a total of 8 sampling points were included in the study. From each sampling point (restaurant or butchery) two each of the four denominations of Ethiopian paper currencies (ETB5, ETB10, ETB50 and ETB100) were collected. Therefore, a total of 64 (2x4x8) paper currency samples were included in the study.

Sample Collections and Transportation:
The samples were collected aseptically by exchanging or replacing equivalent currency to the cashers in a sterile polyethene plastic bags and the cashers were told to put the currency/money into a sterile polyethene plastic bags and sealed as fast as to avoid additional contamination and labeled accordingly to identify the sample collected site and sources and taken immediately to the Hawassa University veterinary microbiology laboratory for microbial analysis. A newly minted Ethiopian currency notes that were not in use or in circulation were also obtained from Commercial bank of Ethiopia (CBE), Hawassa branch and were used as control sample group.

Preparation of Rinse for Microbial Isolation:
Each currency note was transferred and soaked within physiological saline (0.85% NaCl v/v) aqua solution for few minutes at room temperature with intermittent shaking to dislodge the cell suspension and then were transferred by forceps and immersed aseptically in to 100ml of sterile distilled water. The tube was capped and then shaken using vortex shaker for two to three minutes so that microbes adhered over the note surface come out to distilled water to serve as test sample. Serial dilutions were performed from the test sample $1:10^1$, $1:10^2$.........$1:10^8$ by transferring 1ml of test sample into 9ml of distilled water, then vortex and 1ml aliquot transfer into next tube using micropipette.

Preparation of Culture Media:
All the microbiological media were prepared following

recommended procedures according to the manufacturer (Hi media, India depending on the labels on the media packaging bottle).

Aerobic Mesophilic Bacteria Count (AMBC):
From appropriate serial dilutions prepared in 3.5 above, 0.1ml aliquots were aseptically transferred into plate count agar (PCA, Hi media, India) and spread plated with sterile bent glass rod. The inoculated plates were incubated at 37°C for 48 to 72 hrs. At the end of the incubation, plates having between 30 and 300 colonies were selected to calculate the average AMBC. A digital colony counter with magnifying glass was used to count the colonies.

Total Coliform Count:
Appropriate dilutions were inoculated by pour plating on to VRBA plates and incubated at 37 °C for 48 hr. At the end of the incubation plates with typical pink colonies from countable plates were used to calculate the colony forming unit (cfu) using the following formula:
To calculate the average load from multiple plates the following formulae was used:

N = Sum of all colonies from all plates with countable plates

$$N = \frac{\Sigma C}{(N1 + 0.1 N2) D}$$

$(N_1 + 0.1N_2) D$

Where: N1 is the number of plates with countable colonies in the first dilutions, **N2** is the number of plates with countable colonies in the second dilution; **D** is the dilution factor corresponding to the first dilution. Final values were transformed into log10 units for ease of manipulations.

Screening and Detection of *Escherichia coli*:
From the plates used for TCC in 3.7 above three to five well isolated colonies were purified by repeated sub-culturing. The pure isolates were then streaked onto plates of Eosin methylene blue agar and MacConkey and also subjected to the IMViC biochemical test. Isolates that showed black colonies with green metallic sheen on EMB agar and pink colonies on MacConkey agar plates, positive for indole and methyl red test but negative for Vouges Proskauer and citrate utilization test were identified as *E. coli*.

Detection of *Salmonella* Species:
To detect Salmonella species a loop full of the rinsate from each sample of currency was directly streaked on to XLD agar plates. In order to resuscitate metabolically injured cells, streaking on the same media was also done after overnight culture of 0.1 ml aliquots of the rinsate into a tube containing 5 ml of sterile nutrient broth. All plates were incubated at 37 °C for 48 h and at the end of the incubation typical colonies (black) were picked and purified by repeated sub-culturing. Purified presumptive *Salmonellae* isolates were subjected to selected biochemical tests for confirmation by inoculation into sulfide – indole – motility (SIM) agar, triple sugar iron (TSI), Urea agar. Salmonella species are gram negative small rods, hydrogen sulfide positive, indole negative, motile, and non-lactose fermenters (Andrews, 1992; Aslanzadeh, 2006).

Determination of Antibiotic susceptibility
Profiles of Potentially Pathogenic Isolates:Antibiotic susceptibility testing of putative pathogenic isolates was performed using Kirby-Bauer disk diffusion method on Muller Hinton agar plates as per the National Committee for Clinical Laboratory Standards Institute (CLSI, 2020).

The antibiotic discs used were: Azithromycin (15μg), Amoxicillin/clavulanic acid (30μg), Cefazolin (30μg), Ampicillin (10μg), Meropenem (10μg), Tetracycline (30 μg), Ertapenem (10μg), Cefotaxime (30μg), Ceftriaxone (30μg), Nalidixic acid (30μg), Cefepime (30μg), Chloramphenicol (30μg), Erythromycin (15μg), Gentamycin (10μg), Cefuroxime (30μg), and Sulfamesthoxazole (5μg).

Each test isolate was refreshed by streaking on nutrient agar plates and incubated overnight at 37°C. Well-isolated single colony was selected and emulsified in 5ml sterile normal saline solution in a sterile test tube. The turbidity of the suspension was adjusted to the density of a barium chloride standard (0.5 McFarland) in order to standardize the size of inoculum. A sterile cotton swab was dipped into the standardized suspension of the bacterial culture, squeezed against the sides of the test tube to remove the excess fluid and inoculated onto Mueller-Hinton agar and allowed to dry the flood. Thereafter, antibiotic discs were placed on the Medium with forceps and gently pressed down to ensure firm contact. The plates were then allowed to stand for 30 minutes for diffusion of active substance of the agents.

Plates were inverted and incubated at 35-37°C for 24 hrs. At the end of the incubation the inhibition zone diameter of each antimicrobial was then measured using graduated ruler and interpreted as 'Resistant', 'Intermediate' and 'Sensitive' by comparing with standard chart (CLSI, 2020).

Data Analysis:
All data were recorded and analyzed in Microsoft excel 2010 and SPSS version 23. Data were summarized and presented in tables depicting frequencies and percentages. The average microbial load between currency denominations as well as among the restaurants and butcheries were compared using one-way ANOVA and p values less than 0.05 was used as cut of point to determine statistical significance.

RESULTS
The microbial load of the paper currency samples:
The average microbial load of the currency samples in general was highest for the lowest denomination both in terms of the average aerobic mesophilic bacterial count (AMBC) and total coliform count (TCC). The overall average microbial load of AMBC and TCC was 3.76 and 3.74 log unit respectively. The mean AMBC of the currency samples from the restaurants ranging from: 6.09 log units for ETB5, 4 log units for ETB10, 3.46 for ETB50 and 1.49 log units for ETB100 (Table 1). The mean TCC of the same samples was also in the order of ETB5 (6.04 log units) ⟩ETB10 (3.99 log units) ⟩ETB50 (3.44 log units) ⟩ETB100 (1.48 log units).

The overall mean AMBC and TCC of the currency samples from butcheries were 4.39 and 4.36 log units respectively (Table 1). These values were higher than that of the currency samples from the restaurants. The same pattern of mean microbial load (both for AMBC and TCC) was detected as before in that: 7.07 log units was recorded from Ethiopian birr five (ETB5), followed by 5.00 log units for ETB10, 2.60 for ETB50 and 2.9 log units for ETB100 respectively (Table 1). Similarly, the mean TCC of the same samples also declined in the directions ETB5 (7.00 log units) ETB10 (4.98 log units) ETB50 (2.58 log units) < ETB100 (2.90 log units).

Table 1: The average microbial load in $\log_{10}$ CFU ml^{-1} of rinsates of paper currencies by type of denominations and sampling sites in Hawassa city, Sidama region, Ethiopia.

Colony Group	Samples Sites	Denomination				Average Mean (log CFU/ml
		ETB5	ETB10	ETB50	ETB100	
AMBC	Restaurants	6.09	4.00	3.46	1.49	3.76
	Butcheries	7.07	5.00	2.60	2.90	4.39
Denomination averages		6.58	4.5	3.03	2.20	4.08
TCC	Restaurants	6.04	3.99	3.44	1.48	3.74
	Butcheries	7.00	4.98	2.58	2.90	4.36
Denomination averages		6.52	4.49	3.01	2.19	4.05

AMBC = aerobic mesophilic bacterial count, TCC= total coliform count, ETB = Ethiopian Birr, CFU = Colony forming unit

A one-way ANOVA was conducted to compare the average microbial load values of the currencies among the different denominations and between the sampling sources (See appendix Table). There was a significant difference in mean microbial loads of currencies samples of the four denominations with regards to mean AMBC [F (3, 60) = 4.853, p = 0.004 as well as mean TCC values [F (3, 6) = 4.490, p = 0.007]]. However, there was no statistically significant difference between the mean microbial loads of currencies samples of all denominations from Restaurants and that of from the Butcheries with regards to both AMBC [F (1, 62) = 0.440), p = 0.510 and TCC [F (1, 62) = 0.351), p = 0.556]].

Post hoc comparisons using Turkey test were carried out. There was a significant difference between ETB5 and ETB50 (p = 0.028) and there was also a significant difference between ETB5 and ETB100 (p = 0.004). Microbial loads of AMBC on ETB5 was on average 3.55 log units more than those on ETB50 and microbial loads of AMBC on ETB5 was on average 4.38 log units more than on ETB100 respectively. Similarly, there was a significant difference between ETB5 and ETB50 (p = 0.031) and there was also a significant difference between ETB5 and ETB100 (p = 0.007). Microbial loads of TCC on ETB5 was on average 3.51 log units more than those on ETB50 and microbial loads of TCC on ETB5 was on average 4.20 log units more than on ETB100 respectively. (see in appendix the SPSS results).

Common bacterial pathogens isolated from the surface of currency samples :
Out of the total 64 currency notes analyzed in the present study, 35 (54.7%) were contaminated with different species of bacteria. Of these, 38 bacterial isolates were recovered from currency samples which were putatively identified into 3 different types of bacterial genera or species (Table 3) consisting of 23 (60.5%) *E.coli*, 10 (26.3%) *Salmonella* spp. and 5 (13.2%) *Shigella* Spp. (Table 2). The majority of the isolates were recovered from currencies collected from the butcheries (20 of 38 or 52.6%) while 47.4% (18 of 38) were isolated that from Restaurants. The predominant bacteria recovered from the currencies in the restaurants were isolates related to *E.coli* (13 of 38 or 34.2%), followed by those related to *Salmonella* spp. (3/38 or7.8%) and Shigella spp (2/38 or 5.3%). Likewise, 10(26.3%) *E.coli*, 7(18.4%), *Salmonella* spp., and 3(7.8%) *Shigella* spp. were isolated from the currencies in the butcheries (Table 2).

Table 2: Frequency of bacterial Isolates from samples of four denomination currencies with respect to their sampling sources (Restaurants and Butcheries) in Hawassa City.

Source	Denomination	E. coli	Salmonella	Shigella	Total
Restaurants	ETB5	5	1	1	7
	ETB10	4	1	1	6
	ETB50	3	0	0	3
	ETB100	1	1	0	2
Total from restaurants		13(34.2%)	3(7.8%)	2(5.3%)	18(47.4%)
Butcheries					
	ETB5	3	3	1	7
	ETB10	2	2	0	4
	ETB50	3	1	1	5
	ETB100	2	1	1	4
Total from butcheries		10(26.3)	7(18.4%)	3(7.8%)	20(52.6%)
Grand Total		23(60.5%)	10(26.3%)	5(13.2%)	38(100%)

Antibiotic susceptibility profiles of potentially pathogenic isolates:
All of the 38 putatively identified bacterial pathogens were subjected to drug sensitivity testing to 16 different antibiotics. All of the isolates showed resistance to four or more of the antibiotics tested (Table 3). Among the 23 *E. coli* isolates tested, 11(47.83%) showed resistance to six of the antimicrobials, five (21.74%) showed to seven antibiotics and four (17.39%) showed to eight antibiotics and one (4.35) isolate to nine antibiotics (Table 3). All of the 23 E. coli isolates were resistant to Cefepime (FEP) and Tetracycline (TE) but were sensitive to Ceftriaxone (CRO), Cefotaxime (CTX), Ertapenem (ETP) and Nalidixic acid (Table 4).

With regards to *Salmonella* species, all the isolates were resistant to five or more of the 16 antibiotics tested (Table 3). Half of the ten isolates (50%) related to *Salmonella* species were resistant to five of the drugs, and four (40%) were resistant to seven or more of the drugs tested (Table 3). All of the isolates related to *Salmonella* species were resistant to Erythromycin (ERY), Cefepime (FEP), and Tetracycline (TE), but sensitive to Ceftriaxone (CRO) and Nalidixic acid (NA). More over 90% of the *Salmonella* isolates were also sensitive to Ampicillin (AMP), Augmentin (AUG), Gentamycin (CN), Ertapenem (ETP), Meropenem (MEM) and SXT -Sulfa mesthoxazole-Trimethoprim (Table 4).

Likewise, all of the five isolates related to *Shigella* species were resistant to seven or more of the antibiotics tested and two showed resistance to eight and 11 of the antibiotics tested (Table 3). All were resistant to AMP, CTX, Cefazolin (CZ), ERY, FEP and TE but sensitive to AUG, CRO, MEM and SXT.

Table 3: The frequency distribution of resistant isolates to multiple antibiotics among three types of bacterial genera recovered from currencies circulating in Hawassa city

Multiplicity of resistance	E .coli (n = 23)		Salmonella sp (n = 10)		Shigella sp (n = 5)	
	Freq	%	Freq	%	Freq	%
4 antibiotics	1	4.35	0	0	0	0
5 antibiotics	1	4.35	5	50	0	0
6 antibiotics	11	47.83	1	10	0	0
7 antibiotics	5	21.74	2	20	3	60
8 antibiotics	4	17.39	1	10	1	20
9 antibiotics	1	4.35	1	10	0	0
> 9 antibiotics	0	0	0	0	1	20

Freq = Frequency, E. coli = Escherichia coli, sp = species, n = number of isolates

Table 4: The percentage distribution and sensitivity pattern of isolates to 16 drugs among three bacterial genera recovered from currencies circulating in Hawassa city

Type of Antibiotic (n =16)	E. coli (n = 23)			Salmonella sp (n = 10)			Shigella sp (n = 5)		
	R %	S %	I %	R %	S %	I %	R %	S %	I %
AMP	30	70	0	10	90	0	100	0	0
AZM	74	26	0	20	80	0	20	80	0
AUG	74	26	0	10	90	0	0	100	0
C	13	87	0	20	70	10	40	20	40
CN	74	26	0	10	90	0	40	60	0
CRO	0	100	0	0	100	0	0	100	0
CTX	0	100	0	90	10	0	100	0	0
CXM	22	48	30	20	40	40	80	20	0
CZ	78	2	0	90	10	0	100	0	0
ETP	0	100	0	10	90	0	0	80	20
ERY	83	17	0	100	0	0	100	0	0
FEP	100	0	0	100	0	0	100	0	0
MEM	0	96	4	10	90	0	0	100	0
NA	0	100	0	0	100	0	20	80	0

| SXT | 9 | 91 | 0 | 10 | 90 | 0 | 0 | 100 | 0 |
| TE | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |

AMP-ampicillin, AUG-Augmentin, AZM-Azithromycin, C-Chloramphenicol, CN-Gentamycin, CRO-Ceftriaxone, CTX-Cefotaxime, CXM-Cefuroxime, CZ-Cefazolin, ERY-Erythromycin, ETP-Ertapenem, FEP-Cefepime, MEM-Meropenem, NA-Nalidixic acid, SXT-Trimethoprim-Sulfamesthoxazole, TE-Tetracycline

DISCUSSION

Paper Currencies are often handled by various hands with different hygienic level, and could mediate as vectors of various pathogens for the transmission of potential pathogens among people. The isolation of bacteria from currency notes analyzed by this study showed that currency notes could play a potential role in the transmission of pathogenic microorganisms in the community.

Currency samples (notes) analyzed in the present study indicated different level of microbial loads with some denominations showing high counts and others lower counts. Thus, the lower denominations showed higher level of microbial contamination in line with the findings of different researchers (Bhat et al., 2010; Alemayehu and Ashenafi, 2019). This indirectly showed that the currency notes are more frequently passed from different hands among the society.

The overall mean microbial loads for aerobic mesophilic bacterial count of ETB5 was 6.58 log units which was higher than mean microbial loads of other denominations for AMBC. Likewise, mean microbial for AMBC of ETB10 was 4.50 log units. With regard to averages microbial loads for AMBC of ETB50 and ETB100 were 3.03 and 2.20 log units respectively. These mean microbial loads for AMBC of each denomination in this study was higher than the averages aerobic mesophilic bacteria count from similar study conducted in Addis Ababa, Ethiopia by Alemayehu and Ashenafi, (2019) which indicated 1.82 log units for ETB5, 2.35 log units for ETB10, 1.79 log units for ETB50, and 1.57 log units for ETB100 respectively.

This study showed that the average microbial loads for AMBC was much lower than the ones reported from Jimma town, Ethiopia which showed 6.83, 7.68, 4.72, and 4.66 log units for ETBs 5, 10, 50, and 100 respectively (Girma et al., 2014). The presence of high mean aerobic mesophilic bacterial count in this study could be linked to the frequency exchange of these currencies amongst the majority of the people i.e. lower denominations are more frequently passed through more hand during daily activities than the higher denominations.

The average microbial loads for total coliform count of ETB5 was 6.52 log units which was higher than 3.25 log units counted from Ethiopian birr five in Jimma town (Girma et al., (2014) and much higher than the 0.82 log units count for the same currency reported by Alemayehu and Ashenafi, (2019) from Addis Ababa.

On the other hand, mean microbial loads for TCC of Ethiopian birr ten, fifty and hundred were 4.49, 3.01, and 2.19 log units. These results were much higher than the 0.83, 0.60, and 0.71 log units for ETBs 10, 50, and 100, respectively recorded from a similar study in Addis Ababa. Girma et al., (2014) reported mean microbial loads of 4.09, 1.64, and 1.52 log units, for TCC of ETBs 10, 50, and 100, respectively. However, detection of total coliforms on currency notes indicated the lack of proper hand washing practice after using toilet among food handlers.

The mean microbial loads of the currency samples in this study decreased with denominations from ETB5 to ETB100. Similar results were obtained from the study done in Ghana that indicated the decline in the mean microbial loads on Ghanaian Cedi from Cedi one to Cedi ten (Feglo and Nkanasah, 2010).

The mean microbial loads for AMBC of currency samples collected from both restaurants and butcheries were 3.76 and 4.39 log units respectively. Likewise, the average microbial loads for TCC were recorded as 3.74 and 4.36 log units of all currency samples collected from both restaurants and butcheries respectively. Thus, microbial loads were higher in butcheries than in restaurants of all currency samples analyzed. This study was in agreement with study conducted in Nekemte town, Ethiopia by Olijira and Kenasa, (2018) which indicated the higher microbial loads in Butchers than from restaurant workers.

Statistically significant difference in the microbial loads between currency notes of different denominations was observed between ETB5 and ETB50 as well as between ETB5 and ETB100. But there was no statistically significant difference observed between ETB5 and ETB10, ETB10 and ETB50, ETB10 and ETB100, ETB50 and ETB100 as well as between restaurants and butcheries.

Moreover, various reports showed that each denomination has direct association with degree of contamination as lower denomination notes had showed the most contaminations as reported by Moosavy et al., (2013) from Iran, Girma et al., (2014) from Jimma, Ethiopia, Alemayehu and Ashenafi, (2019) from Addis Ababa, Ethiopia and Sunil et al., (2020) from India. Hence, this study showed that lower denomination of Ethiopian currency notes (ETB5 and ETB10 notes) had the higher contamination than the higher denomination (ETB50 and ETB100 notes). This could be the fact that lower denominations are more frequently passed through more hand during daily activities than the higher denominations.

In the present study, bacterial pathogens such as E.coli, Salmonella and Shigella were isolated on currency samples (ETB) of different denominations (ETBs 5, 10, 50, and 100). Likewise, various types of pathogenic bacteria including Escherichia coli, Staphylococcus spp., Bacillus, Klebsiella, Streptococcus, Serratia, Salmonella, Pseudomonas, Citrobacter, Shigella, Listeria etc. were reported from Aksum, Northern Ethiopia (Saripalli et al., 2014). Similar pattern of pathogens were also reported from Nekemte town, Ethiopia by Olijira and Kenasa, (2018) which includes Escherichia coli, Staphylococcus spp., Bacillus, Klebsiella, Streptococcus, Salmonella, Pseudomonas, Shigella. Bacillus species, Staphylococcus aureus, Enterococcus faecalis, Citrobacter freundi, Klebsiella pneumoniae, Shigella dysenteriae and Escherichia coli were recovered from Ghanian Cedi in Ghana by Feglo and Nkanasah, 2010. Studies on the determination of pathogens on paper currency showed that currency notes are considered as a potential cause of food-borne diseases (Alemu, 2014).

There are evidences of isolation of food-borne pathogens including Salmonella spp. E. coli and S. aureus from the banknotes of different countries (Vriesekoop et al., 2010). The detection of salmonella, Shigella and E.coli on currency notes could suggest fecal contamination following poor hygienic practice.

The outcome of the present study revealed that 54.7% of the currency samples were contaminated. Based on cultural morphology and biochemical characteristics of the isolates, this revealed three different types of bacteria genera which account for 60.5% E.coli, 26.3% Salmonella sp., and 13.2% Shigella sp. The contamination rates of currency notes with these genera in this study was higher than the contamination rates from study conducted in Debre markos town, Ethiopia which showed 24% E.coli, 10% salmonella, 5% Shigella rates of contamination(Solomon, 2015).

This study was almost similar to study conducted in Dhaka, Bangladesh that showed *E.coli* (58%) and *Salmonella* Sp. (15%) (Ahmed *et al*., 2010). The contamination rate of *E.coli* (60.5%) in this study was lower than the report from Haramaya, Ethiopia which indicated 75% *E.coli* on currency notes (Hiko *et al*., 2016).

This study also showed that only 38 bacterial isolates were recovered from surface 64 currency samples analyzed. All the contaminated notes were found to be contaminated with each bacterial isolates. Among these isolates only three different types of bacteria genera or species and Gram-negative bacteria were obtained. Similar results were obtained from study conducted in Nigeria which yielded a total of three different species from Nigerian Naira (Barua *et al*., 2019 and Ofoedu *et al*., 2021). However, studies from other parts of Ethiopia had more species with five and nine different species respectively (Girma *et al*., 2014 and Olijira and Kenasa, 2018). Other studies conducted in different countries such as India (Sucilathangam *et al*, 2016), Bangladesh (Ahmed *et al*., 2010), and Zambia (Chauwa *et al*., 2020 showed more species with Six, seven, and eight respectively. The different in the results obtained from this study with other studies could be the factors such as sample size and methods used for isolation of bacteria.

Currently, antimicrobial resistance has become a serious issue throughout the world. Indiscriminate uses of antibiotics leads to treatment failure and augment health cost (Sharma and Dhanashree, 2011). Since currency notes are commonly contaminated with pathogenic microorganisms in circulation, of which most of them are resistant to commonly used antibiotics reported somewhere else (Ayandele and Adeniyi, 2011 and Barau *et al*., 2019). Transmission of these antibiotic resistance microorganisms from one individual to another through currency notes could leads to public health threats.

Of the 16 different antibiotic agents tested for susceptibility pattern in this study, a higher level of resistance was recorded to Tetracycline, Cefazolin, Cefepime and Erythromycin Noting that antibiotic likes Cefepime and Tetracycline were not effective against all isolates (*E. col isolates*, *Salmonella* spp., isolates and *Shigella* spp., isolates) whereas sensitivity were seen in Ceftriaxone.

Most *E. coli* isolates were 74% resistant to Azithromycin, Augmentin, and Gentamycin, 78% to Cefazolin, 83% to Erythromycin, 100% to Cefepime and Tetracycline but 100% susceptible to Ceftriaxone, Cefotaxime, Ertapenem, and Nalidixic acid, 70% to ampicillin, 87% to chloramphenicol, 96% to Meropenem and 91% to Trimethoprim-Sulfamethoxazole. With regard to resistant of *Salmonella* sp., the highest resistant of 90% was observed in Cefazolin and Cefotaxime, and 100% in Erythromycin, Cefepime and Tetracycline respectively but 90% susceptible to ampicillin, Augmentin, Gentamycin, Ertapenem, Meropenem and Trimethoprim-Sulfamethoxazole, and 100% to Ceftriaxone and Nalidixic acid. Likewise, *Shigella* sp. isolates were 100% resistant to Ampicillin, Cefotaxime, Erythromycin, Tetracycline and 80% resistant to Cefuroxime. However, these isolates were 100% sensitive to Meropenem, Trimethoprim-Sulfamethoxazole, Ceftriaxone and Augmentin, 80% to Azithromycin, and Ertapenem. Similar pattern of antibiotics resistance was observed in Ghana where all isolates showed 100% resistant to Tetracycline (Dekugmen, 2020).

Multidrug resistant pattern in the present study showed that each isolates have a resistant pattern with at least four or more drugs of antibiotics tested. This study was in agreement with the study conducted in Sudan which indicated the resistant pattern of each isolates with at least four or more drugs (Alfadil *et al*., 2018). This was almost in line with another study done in Nigeria that showed each isolates have a resistant

pattern with at least six or more drugs (Unko *et al*., 2017). Therefore, currency notes could play an important role in spreading of multidrug resistant pathogens. Multi drug resistant bacteria represent a major risk to human health in linking to bacterial contamination and disease. The results from this study showed that currency notes in circulation are contaminated with potential pathogenic bacteria, as results one or more isolates were resistant to commonly prescribed antibiotics which may signify threats to individuals handling currency notes.

CONCLUSION

It is generally noted that currency samples are loaded with bacterial groups like aerobic mesophilic bacterial count and total coliform counts. However, the microbial loads were varied based on sample sources and denomination types. Therefore, the overall mean AMBC and TCC were 4.08 log and 4.05 log units, respectively. Moreover, three different genera; *E. coli* species (60.5%), *Salmonella* species (23.6%), and *Shigella* species (13.2%) were isolated from the different denominations, the majority of them from lower denominations which are frequently exchanged from hand to hand. Based on antibiotic susceptibility pattern, most isolates showed resistance to higher level of Tetracycline, Cefazolin, Cefepime and Erythromycin, and were susceptible to Ceftriaxone. In general, the present study demonstrated that currency notes circulating in Hawassa are contaminated with potentially pathogenic bacteria that could transmit different diseases. This contamination could play a vital role in the transmission of disease in the community and may also cause spreading of antibiotic resistance pathogens.

Recommendations
- Personal hygiene to reduce the risk of contamination is recommended especially for those who simultaneously handle food and money.
- There should be public awareness of the fact that currency notes might be source of infection and could be hazardous for health.
- Replacement of the traditional methods of trading with electronic money transactions would be good for the ease of problem.
- Further investigation of bacteria on the surface of currency notes is needed by employing necessary methods to isolates all types of bacteria that are able to survive on the surface of currency notes, especially Gram-positive bacteria which are not isolated in this study

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