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Evaluation of Banknote Contamination with Bacteria and Fungi among Falafel Vendors in Ibb City- Yemen

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Abstract:
Money, either in the form of paper notes or coins could be the most broadly handled article by people every day around the world. Banknotes can be contaminated in many ways like the droplets during sneezing, coughing, touching with previously contaminated hands or other articles, and placement on contaminated surfaces. The aim of this study is to isolate and identify microbial contaminating banknotes among falafel vendors in Ibb city, Yemen. A total of 100 samples of the Yemen banknotes (denomination - 1000, 500, 250, 200,100, and 50 Rials), were randomly collected from (Falafel vendors) in Ibb city. All collected currency were examined for total viable bacterial count and identification of pathogenic bacteria and fungi. The results showed that all collected samples (100%) were contaminated with one or more bacteria and fungi species. Our findings showed that the maximum total bacterial count was 240×10^3 CFU/ml and the minimum was 40×10^3 CFU/ml. The total coliform ranged from minimum value of 35×10^3 CFU/ml to maximum value of 171×10^3 CFU/ml. In this study, the isolated bacteria and fungi were *S.aureus* (23%), Coagulase Negative *Staphylococci* (16%), *S.pneumoniae* (7%), *S. pyogenes* (2%), *B. cereus* (18%), *L. monocytogenes* (3%), *E. faecalis* (6%), *H. influenzae* (13%), *Salmonella spp* (5%) and *E.coli* (3%), Yeast (10%), *Rhizopus* (31%), *Mucor* (24%) and *Aspergillus niger* (35%). All tested samples in our study were contaminated with many pathogenic and opportunistic bacteria and fungi. This contamination may play an important role in the transmission of infection especially during outbreaks of infectious diseases like COVID-19. Wrong behaviors in dealing with banknotes including licking the fingers with the tongue or wetting them with saliva while counting banknotes, could contaminate them with serious microbes. Due to the unlimited use of banknotes, many people may acquire serious diseases, especially with the reduction of sanitary measures such as washing hands after using currencies, which may reduce the risk of infection with pathogenic microbes. It is recommended that specific antimicrobial agents should be added to the composition of banknotes during its preparation.

Keywords: Banknotes Contamination, Infection Outbreak, Bacteria, Fungi, *S.aureus, E.coli, Salmonella Spp.*

INTRODUCTION

The transmission of human pathogens can occur between hosts though animals, food and environmental ways (Uneke and Ogbu, 2007; Saleem *et al.,* 2018 a,b). Direct contact (Person-to-person) and transfer via common contact surfaces are also additional routes that can lead to widespread infectious outbreaks of pathogens such as norovirus and methicillin resistant *Staphylococcus aureus* (Hassan *et al*., 2011). Besides that, Currency (Bank notes and coins) is a further common contact surface whereby pathogens can be transferred widely within a population although the significance remains unknown (Igumbor *et al*., 2007; Bhat *et al*., 2010). Owing to Currency is frequently handled and transferred over the world; there is a huge chance to disseminate contamination across the globe (Pope *et al*., 2002). Regarding to risk factors, the contamination carried on currency would be considered in food retail outlets whereby pathogens can be transferred to high risk products such as meat that can support growth of introduced microbes to hazardous levels (Shekarforoush *et al*., 2009; Feglo and Nkansah, 2010).

In almost all communities, People exchange currency on a daily activities, circulating banknotes which may play a role as an important vehicle for the transmission of bacterial infection and multidrug-resistant pathogens especially in immunocompromised people (El-Dars and Hassan, 2005; Ahmed *et al*., 2010). Banknotes may be contaminated during transaction, handling, storage, and upon contact with contaminated surfaces. A previous study reported that “Unhygienic practices such as wetting fingers with saliva prior to money counting can introduce an array of bacteria to the banknotes” (Enemuor *et al*., 2012); these routes of transmissions have a huge sanitary impact on public health in developing countries like Yemen, where the frequency of bacterial infection has been increasing (Saeed *et al*., 2015). Even though every geographical area has specific endemic bacteria, members of *Enterobacteriaceae*, *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., and *Corynebacterium* spp. have been identified as common contaminants isolated from banknotes in different countries (Noha *et al*., 2018). Furthermore, bacteria belonging to the genera *Vibrio* and *Pseudomonas* have also been isolated from banknotes in developing countries (Iruvengadam *et al*., 2014). Additionally, there is accumulated data highlighting the contamination of banknotes by antibiotic-resistant bacteria (Noha *et al*., 2018). This study aimed to investigate microbial contamination in Yemen banknotes in Ibb city, Yemen.

MATERIALS AND METHODS

Study samples and duration

One hundred samples of Yemen banknotes (denomination - 1000, 500, 250, 200,100, and 50 Rials), were randomly collected from (Falafel Vendors) in Ibb City. The study was conducted from November 2019 to January 2020. All laboratory works were carried out in the Microbiology Lab., faculty of Allied Medical Sciences, Al-Jazeera University.

Sample collection

The bank notes were collected from the falafel Vendors directly by dropping it into a sterile polythene bag, and labelled accordingly; banknotes were not touched by the researcher using hands at any stage. The packet was sealed and immediately transported to the laboratory for analysis.

Isolation and identification of bacteria

All studied banknotes were weighed separately to prepare a 10^{-1} dilution solution using sterile normal slain (N.S) and the bottle shaken for 2 minutes. The banknote was removed. Serial dilutions were done until 10^{-3}, two methods were used to isolate, and counting Microorganisms from each specimen. First one is used for studying the microbial quality, by

counting Microorganism in all specimens, and then subculture from bacterial counted was done to (Mac Conckey Agar, Blood Agar and Nutrient Agar), the second one directly from each solution dilution, by using sterilized cotton swab the samples were inoculated on Blood Agar, Nutrient Agar, Eosin Methyl Blue (E.M.B), salmonella–Shigella agar (S.S.A) and mannitol salt agar (M.S.A), the plates were incubated aerobically overnight in an incubator at 37 °C. Pure isolated colonies were identified according to Bergey's manual of determinative bacteriology (Bergey and Holt, 2000) using their morphology, Gram reaction as well as biochemical tests (Iqbal *et al.,* 2015; Iqbal *et al.,* 2016; Yunus *et al.,* 2016a,b) such as the, Catalase, Coagulase, Oxidase, Urease, sugars fermentation and gas production on Triple sugar iron (T.S.I) medium.

Isolation and identification of fungi

The growth of fungi on sabouraud dextrose agar was examined after 2 to 5 days at 25°C aerobically. According to (CLSI, 2012) pure isolated colonies were identified, the prepared specimens were mounted on lacto phenol cotton blue and identification of the fungal species was performed with aid of binocular compound microscope (40X). Colony characters were considered for complete and confirmed identification of fungi.

Data analysis

The data collected from the results of microbiology tests, were analyzed by using Microsoft excel 2010.

RESULTS AND DISCUSSION

As mention before, Banknotes may be contaminated during transaction, handling, storage, and upon contact with contaminated surfaces. Microbiological study was done by analyse100 samples collected, and started as; total viable count on Nutrient agar medium, counting lactose fermenter bacteria on MacConkey (MA), coliform group counting on (EMB), using (SDA) for isolation of fungi, subculture from all above medium was done for isolation, and then Identification.

Table (1) illustrates the viable counts of bacterial colonies. Total viable count of bacteria in Nutrient agar medium ranged from minimum value of 40×10^3 CFU/ml to maximum value of 240×10^3 CFU/ml, while the coliform count on MacConkey (MA) ranged from minimum value of 35×10^3 CFU/ml to maximum value of 171×10^3 CFU/ml.

The percentage of contamination in our study was recorded up to 100%, which is in a good agreement with other studies (Zarei *et al.,* 2009; Tagoe *et al*., 2009). These studies reported 100% of contamination of the currency, while other studies have detected contamination levels of 80% (Hosen *et al.,* 2006), 94% by (Pope *et al.,* 2002) and 96% by (Igumbor *et al.,* 2007). The main source for the presence of these pathogens on the banknotes may be the hands of workers in falafel shops or the hands of customers. Where customers or workers deal in cash before or after preparing and eating food, which causes contamination of food with these pathogens consequently.

Table (2) shows that 100 banknotes were contaminated by 149 bacteria colonies some of them were repeated more than one. All the samples of banknotes are contaminated with many different bacteria. The classification of such bacteria based on gram stain that classified them to gram positive and gram negative bacteria. In our results, the majority was to gram positive bacteria 116 (77 %), while the frequency of gram negative bacteria was 33 (23%).

Table 1. Viable Bacterial count from the banknotes samples.

Bacterial count	Total viable count $\times 10^3$ (CFU/ml)	Coliform $\times 10^3$ (CFU/ml)
Maximum	240	171
Minimum	40	35

Table 2. Type of bacteria found in samples.

Type of bacteria	Number of bacteria	Percent %
G+ve bacteria	116	77
G-ve bacteria	33	23
Total	149	100

Our results showed that 10 different bacterial species isolated from banknotes were described in table (3) : (*S.aureus*, coagulase negative *Staphylococci, S.pneumoniae*, *S. pyogenes*, *B. cereus*, *L. monocytogenes*, *E. faecalis*, *H. influenzae*, *Salmonella* spp, *and E.coli*), the highest percent was in *S.aureus* 23%, and the lowest was *S. pyogenes* with 2%. Diagnostic characteristics are shown in the table (4) for gram positive and in table (5) for gram negative bacteria.

More precisely some of these isolated bacteria are considerable as coliform (faecal source) that can caused enteric infection and indicate to fecal contamination like (*E.coli, Enterococcus*. Non lactose fermenters were also isolated like *Salmonella spp*), others gram positive can cause food poisoning like (*S. aureus*, *B. cereus* and *L. monocytogenes*), these bacteria can be transmitted by oral fecal rout (ingestion of contaminated food).

This can be explained by the unhygienic practices such as wetting fingers with saliva prior to money counting can introduce an array of bacteria to the banknotes (Enemuor *et al*., 2012) these routes of transmission have a great impact on public health in developing countries like Yemen, where the frequency of bacterial infection has been increasing (Saeed *et al*., 2015).

Other bacteria can cause upper Respiratory tract infection, some of them are normal flora in URT, and their transmission was done by droplets, like (*H. influenzae,* and *Corynebacteria)*, while the study conducted in Ghanaian (Tagoe *et al*., 2009) showed that total of 13 species of bacteria were diagnosed in which 100 samples including (Coagulase Negative *Staphylococci*, *S.aureus*, β- hemolytic *Streptococci*, α-hemolytic *Streptococci*, *E. coli*, *Yersinia* species, *Bacillus* species , *Klebsiella* species, *Shigella* species, *Enterobacter* specie, *Enterococci* species, *L. monocytogenes* and *Proteus* species and the highest percent was Coagulase Negative *Staphylococci* and *Bacillus* species with percentage equal to 23.4%, while the lowest percent was *Shigella* species and *L. monocytogenes* with percentage up to 0.9%.

This indicated that, banknote which is handled by large numbers of people, under a variety of environmental and personal conditions can be a source of infection (Tagoe *et al*., 2009). Even though, it may be almost impossible to trace the source of infection, coins and banknotes may carry potentially pathogenic organisms and serve as source in the transmission of infection (Singh *et al*., 2002).

Table 3. Frequency of different bacteria that isolates from banknotes.

Name of Bactria	No.	Percent%
S. aureus	35	23
Coagulase Negative *Staphylococci*	24	16
S.pneumoniae	11	7
S. pyogenes	3	2
B. cereus	28	18
L. monocytogenes	5	3
E. faecalis	10	6
H. influenzae	20	13
Salmonella spp.	8	5
E. coli	5	3

Table 4. Biochemical characteristics of isolated gram-positive bacteria.

G+ve Bacteria spp	Blood Agar	MSA	Shape	Catalase	Coagulase
S. aureus	β-hemolysis	Yellow colonies	G+ve cocci, grapes like	+	+
Coagulase Negative *Staphylococci*	γ-hemolysis	Pink colonies	G+ve cocci grapes like	+	-
S. pneumoniae	α-hemolysis	No growth	G+ve cocci / Chain	-	-
S. pyogenes	β-hemolysis	Pink colonies	G+ve cocci / Chain	-	-
B. cereus	β-hemolysis	Pink colonies	G+ve rods, spore forming	-	-
L. monocytogenes	β-hemolysis	Pink colonies	G+ve rods single /short chain	+	-
E. faecalis	γ-hemolysis	Yellow colonies	G+ve cocci a pairs shaped	-	-

Table 5. Biochemical characteristics of isolated of gram-negative bacteria.

G-ve bacteria spp.	Gram stain	EMB	Indole	Oxidase	T.S.I medium			
					Slop	Bottom	H_2S	Gas
H. influenzae	-v coccobacilli	No growth	+	+	R	Y	-	-
E. coli	-v rod	Green colonies	+	-	Y	Y	-	+
Salmonella spp.	-v rod	No growth	-	-	R	R	+	-

Fungi were also isolated on sabouraud dextrose agar from contaminated banknotes in our study. Table 6 shows the diagnostic characteristics of the fungi isolated. Our results indicated that 4 different fungi isolated from banknotes include (*Yeast, Rhizopus, Mucor,* and *Aspergillus niger*) with percentage up to 10%, 31%, 24%, 35% respectively.

Similar results obtained by the study conducted in Saudi Arabian (Suaad *et al*., 2011) that showed that total of 5 different species of fungi including (*Candida spp, Rhizopus spp, Aspergillus flavus,* and *Aspergillus niger*) were isolated.

Table 6. Diagnostic characteristics of the fungi isolated from banknotes.

Fungi	Macroscopic	Microscopic	Percent%
Yeast	Flat, smooth, large colonies	Ovoid shape with budding	10
Rhizopus	Cotton like white growth spotted with black color.	Sporangia contain spores, have rhizoids	31
Mucor	Cotton like white growth spotted with black color.	Sporangia contain spores, do not have rhizoids.	24
Aspergillus niger	Pin like black growth.	Non-Branched conidiophore with bulb end carries conidia like sun rays	35

CONCLUSION

All tested samples in our study were contaminated with many pathogenic and opportunistic bacteria and fungi. This contamination may play an important role in the transmission of infection especially during outbreak of infectious diseases like COVID-19. Wrong behaviours in dealing with banknote including licking the fingers with the tongue or wetting them with saliva while counting banknotes, could be contaminate them with serious microbes. Due to the unlimited use of banknote, many people may acquire serious diseases, especially with the reduction of sanitary measures such as washing hands after using currencies, which may reduce the risk of infection with pathogenic microbes. It is recommended that specific antimicrobial agents should be added to the composition of banknotes during its preparation

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not required.

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