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Microbial and parasitic contamination on circulating Pakistani Currency

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Abstract:

Background: Fomites are nonliving objects that are capable of imbibing, harboring and spreading infectious microorganisms. Currency notes and coins, as exchangeable fomite, are constantly subjected to contamination. The objective of this study was to determine microbial and parasitic contamination of Pakistani currency thus highlighting the potential of money for spreading pathogens in the Pakistani community.

Methods: In the present study, a total of 81 Pakistani currency notes and coins in circulation were randomly collected from different shopkeepers, vendors, canteen owners and restaurant cashiers in Lahore and analyzed for parasitological, fungal, aerobic and anaerobic microbial analyses by using various microbiological techniques.

Results: The study revealed 92.5% of Pakistani currency to be contaminated with pathological microorganisms. Potential pathogens such as *Staphylococcus* spp. (48.05%), *Streptococcus* spp. (3.89%), *Micrococcus* spp. (5.19%), *Bacillus subtilis* (11.68%), *Corynebacterium* spp. (7.79%), *Cronobacter sakazakii* (2.59%), *Burkholderia cepacia* (1.29%), *Klebsiella pneumoniae* (2.59%), *Serratia rubideae* (1.29%), *Bacteriodes* spp. (34.46%) and Yeast and Mold (3.89) % respectively were isolated. The parasitological analysis of the currency evinces 13.58% of the samples with parasitic ova and cysts. Predominant ova and cysts of *Entamoeba histolytica* & *Giardia lamblia* were identified.

Conclusion: This study indicates that currency notes and coins are excellent fomites that can harbor the microorganisms very well. The current analyses points out towards the unhygienic practices of the people spending money in the form of currency notes and coins. Launching effective and frequent awareness campaigns in the society can help to stop the spread of microorganisms to a greater extent.



Introduction

Money, in the form of currency notes and coins, is extensively traded for goods and services in countries all over the world [1]. Perhaps, it is the most widely handled article throughout the world; being exchanged by several hands each day. Money goes through clean and dirty hands and can get contaminated with pathogens [2]. Even though money can transmit these pathogens, the likelihood of currency notes and coins as vehicle of pathogenic transmission was first suggested in 1972 [3]. Contamination of currency notes and coins may occur during production, storage or use [4]. Pathogenic microbes, if present on the hands, can be transferred from cashiers and salesman to the general public via currency notes and coins [5]. Improper handling of money in hospitals also plays an important role in the contamination of currency notes and coins with pathogenic microbes [6]. Similarly, simultaneous handling of food and money by waiters or vendors can have serious consequences as the food they serve is ready to eat and does not require any further heating. Additionally, the people ordering that food usually do not wash their hands prior eating [7]. Currency counting machines and rooms are also found to be contaminated with different bacterial and fungal pathogens [8]. The habit of wetting of fingers with saliva or the use of contaminated water as lubricant for fingers during the money counting, can lead to the transfer of bacteria and parasitic ova or cysts to currency notes and coins [9]. Even the currencies used in public transport system were also reported to be contaminated with pathogens [10].

Therefore, we can say that paper currency offers a larger surface area as a propagation medium for pathogens. Microorganisms may endure on it for longer periods of time. A large number of microbes can be found accumulated on older paper notes [11]. At the same time, material of currency notes also plays an important role in bacterial attachment [12]. Moreover, the storage of currency note and coins in polythene, cotton and leather bags increases the microbial load. This is because of humid and dark conditions present in these bags, which ultimately favor the growth and propagation of the microorganisms [13].

Bacteria that are commonly reported in association with currency notes and coins are *Staphylococcus aureus*,

hemolytic and non-hemolytic *Streptococci*, *Micrococci*, *Escherichia coli*, *Proteus*, *Klebsiella*, *Salmonella*, *Pseudomonas aeruginosa*, and *Enterobacter* [14].

Yeast, *Aspergillus* species and *Penicillium* species have been frequently found on contaminated currency notes and coins [15].

Recently, larvae and ova of many parasites have been reported from currency notes and coins. The reported ova and larvae belong to different species of *Microsporidia*, *Cryptosporidium*, *Taenia*, *Trichuris*, *Enterobius*, and *Ascaris*. All of the reported parasites are known enteric pathogens [16].

Although a lot of studies on microbial status of currency are carried out in different countries, there are only a few studies conducted to detect microbial and parasitic contamination of Pakistani currency. The current study was designed to add to limited body of literature on contamination of Pakistani currency and to accentuate the risks related to handling of contaminated money. Hence the study was undertaken to investigate pathogenic contamination of currency circulating in Lahore.

Methods

Sampling

Total 81 samples of Pakistani currency were collected and investigated for microbial contamination. Currency notes that were included in the study were mostly of lower denominations (Rs 5, 10, 20), as there are more chances of their frequent exchange and thus an increased probability of carrying various types of microorganisms. Currency notes of high denominations such as Rs 100, Rs 500, and Rs 1000 were also included in the study. Coins that were included in the study comprised of all denominations that were in circulation including Rs 1, 2 and 5. Currency was randomly collected from different shopkeepers, vendors, canteen owners and restaurant cashiers. Additionally, the currency that was in routine circulation around was also included in the current microbiological study. Study was conducted in Lahore, Pakistan from November to May, to include different temperature variations in the environment. The sample currency notes and coins were collected in sterile plastic bags after wearing sterile gloves. The sampled currency notes and coins were kept in sterile conditions to avoid any further contamination

though air or any other contact which might have had compromised the actual contamination.

Physical condition of currency notes and coins

The currency notes and coins were in various physical conditions. The currency notes and coins were categorized as of good, moderate, and bad condition. The term “good” refers to currency notes and coins that were recently produced and were as new. The term “moderate” refers to currency notes and coins that only retained half of their original condition. The term “bad” refers to currency notes and coins that were either badly mutilated or in case of currency notes were held together with the help of adhesive tape.

Microbial analysis

Isolation and identification of aerobic bacteria

Isolation of different microbial pathogens from currency notes and coins was carried out by following a little modification in the protocol described by Lamichhane *et al* [10]. For inoculum preparation a sterile cotton swab moistened with sterile normal saline was used to swab both sides of currency notes and coins thoroughly. The swab was directly inoculated on Blood agar, MacConky agar, Cysteine Lactose Electrolyte Deficient (CLED) agar, and Lowenstein-Jensen medium tubes while Nutrient agar, Brain heart infusion broth and Mannitol salt agar were not used. CLED agar was used in replacement of nutrient agar specially to isolate urinary tract microbes. The plates were incubated at 37°C for 24 hours except for Lowenstein-Jensen medium tubes for which incubation was given up to 8 weeks. The microbes from selective media were further purified on Nutrient agar and Blood agar, where required. The identification was done using different staining techniques such as Gram’s staining, spore staining and acid fast staining, by performing various biochemical tests such as catalase test, coagulase test and oxidase test, by using different biochemical media such as TSI, motility, indole, urea, citrate and API-20E strips.

Isolation and identification of anaerobic bacteria

Isolation and identification of anaerobic bacteria was performed using Merck Anaerocult® C mini kit (Cat # 113682). A sterile cotton swab moistened with sterile normal saline was used to swab both sides of currency notes and coins thoroughly. The swab was directly inoculated on Blood agar plates. As for negative control *Pseudomonas aeruginosa* was also streaked on the blood

agar plates. The plates were then packed in polyethylene bags with Merck Anaerocult® C mini kit (Cat # 113682) and incubated at 37°C for 48 hours. Identification of microbes was done by Gram staining, by biochemical tests for indole production, glucose and mannitol fermentation and by antibiotic disc resistance tests as described by Duerden *et al* [17]. The bacteria were identified but not quantified.

Isolation and identification of fungi

The fungi was isolated and identified by few modifications in method described by earlier by Neel [18]. The growth of fungi was examined on Sabroaud dextrose media after 24 hrs of incubation instead of 1 week. The observed colony was Gram stained instead of mounting on Lacto phenol cotton blue and the fungal species were identified with help of compound microscope [19].

Parasitological analysis

For parasitological analysis, little modification in the protocol of Uneke and Ogbou [1] was made as 5 ml of 10% formal saline used instead of 10 ml. First the swab was made using a very light foam sheet with an approximate thickness of half inch. Foam was cut into pieces 2 cm x 2 cm, washed with detergent, and sterilized by dipping several times in a dilute solution of sodium hypochlorite. The pieces of foam were then rinsed in water instead of 70% ethanol and dried in air. The foam pieces were then tied with thin wooden sticks about 15 cm in length to make the final swabs. Finally the swabs were wrapped in paper, oven dried at 65°C for 24 hours and stored at room temperature till used. For each currency sample the swab was first moistened with a 10 % formal - saline solution and was swabbed on both sides of the currency sample. The swab was placed in a capped bottle containing 5 ml of 10% formal-saline solution, and was vigorously shaken. Thereafter, the swab was pressed against the inner sides of the bottle to squeeze the solution out of the swab and was removed. The solution was poured into a sterile 15 ml BD Falcon™ Tube and centrifuged at 14,000 rpm for 10 minutes instead of 2000 g for 5 minutes. The supernatant was discarded in 10% bleach solution, and a properly mixed drop of the sediment was placed on a glass slide. It was covered with a glass cover slip and examined microscopically for parasitic ova, cysts or the protozoan cells. Observations were made using 10X, 40X and 100 X magnifications.

Results

In the current study, total 81 samples of Pakistani currency notes and coins were screened for the presence of microorganisms. Seventy five (75) out of eighty one (81) Pakistani currency samples were found to be contaminated with different microorganisms.

Old and tattered currency notes were more likely to be contaminated.

From samples the samples we analyzed, 77 microbial strains were isolated. Many of the sample currency notes and coins were contaminated with more than one microbial species.

Strains	Currency denomination and frequency of isolated strains (percentages)									Total (%)
	Rs 1	Rs 2	Rs 5	Rs 10	Rs 20	Rs 50	Rs 100	Rs 500	Rs1000	
<i>Staphylococcus</i> spp.	13.51%	13.51%	16.21%	24.32%	8.10%	5.04%	13.51%	-	2.70%	48.05
<i>Streptococcus</i> spp.	-	33.33%	33.33%	33.33%	-	-	-	-	-	3.89
<i>Microcococus</i> spp.	25%	-	25%	50%	-	-	-	-	-	5.19
<i>Bacillus subtilis</i>	22.22%	33.33%	11.11%	1.11%	-	11.11%	11.11%	-	-	11.68
<i>Bacillus megaterium</i>	-	33.33%	-	33.33%	33.33%	-	-	-	-	3.89
<i>Bacillus cereus</i>	-	33.33%	33.33%	33.33%	-	-	-	-	-	3.89
<i>Corynebacterium</i> spp.	-	-	-	40%	20%	-	40%	-	-	7.79
<i>Chronobactor sakazakii</i>	-	-	50.00%	50.00%	-	-	-	-	-	2.59
<i>Klebsiella pneumonia</i>	50%	-	-	50%	-	-	-	-	-	2.59
<i>Serretia rubideae</i>	-	-	-	-	100%	-	-	-	-	1.29
<i>Burkholderia cepacia</i>	-	-	-	-	-	100%	-	-	-	1.29
<i>Bacteriodes</i> spp.	50%	100%	100%	40%	20%	40%	40%	20%	-	34.46
Mold spp.	33.33%	-	-	33.33%	-	-	-	33.33%	-	3.89
Yeast spp.	33.33%	-	33.33%	-	-	-	-	-	33.33%	3.89
Total	77									

Table 1: Pakistani currency and the microbial strains isolated

Denominations of Currency	Number of samples contaminated	Cyst Observed	Ova Observed
Rs 1	2	<i>Giardia lamblia</i>	<i>Entrobium vermicularis</i>
Rs 2	2	<i>Giardia lamblia</i>	<i>Diphylobothrium latum</i>
Rs 5	1	<i>Entamoeba histolytica</i>	<i>Schistosoma japonicum</i>
Rs 20	1	<i>Giardia lamblia</i>	<i>Ascaris</i> spp.
Rs 50	2	<i>Giardia lamblia</i>	<i>Entrobium vermicularis</i>
Rs 100	2	-	<i>Entrobium vermicularis</i>
Rs 500	1	-	<i>Entrobium vermicularis</i>
Rs 1000	-	-	-
Total	11(13.58%)		

Table 2: Parasitic ova/cysts identified from various currency denominations

Lower currency denomination Rs 10 was found to be highly contaminated with various microbes. Most of microbes isolated were potential pathogens such as *Staphylococcus* spp. (48.05%), *Streptococcus* spp. (3.89%), *Micrococcus* spp. (5.19%) *Bacillus subtilis* (11.68%), *Corynebacterium* spp. (7.79%), *Cronobacter sakazakii* (2.59%), *Burkholderia cepacia* (1.29%), *Klebsiella pneumoniae* (2.59%), *Serratia rubideae* (1.29%), *Bacteriodes* spp. (34.46%) and Yeast and Mold (3.89) % respectively (Table 1). However, no growth was observed of any *Mycobacterium* species on Lowenstein-Jensen medium after 8 weeks of incubation.

Parasitological analysis

A total of 11 out of 81 samples currency notes and coins were found to be contaminated with ova and cysts of enteric parasites. Ova and cysts were identified not numerated. Most of ova identified were of *Entamoeba histolytica* while most of cysts identified were of *Giardia lamblia* (Table 2). Cysts of *Entamoeba histolytica* as well as ova of *Diphyllobothrium latum*, *Schistosoma japonicum* and *Ascaris* spp. were also isolated (figure 1).

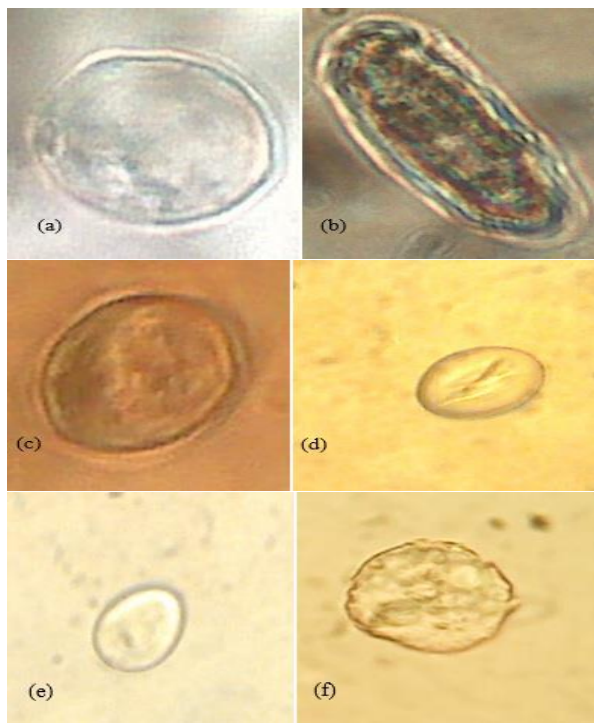


Figure 1: Parasitic ova and cysts isolated from currency notes and coins a) ova of *Diphyllobothrium latum* (b) ova of *Ascaris* spp. (c) ova of *Schistosoma japonicum* (d) cyst of *Giardia lamblia*, (e) ova of *Entrobisus vermicularis* (f) cyst of *Entamoeba histolytics*

Discussion

Abrams and Waterman were first to propose that currency notes and coins might act as source of microbial contamination [3]. Now several studies show that contamination of currency in circulation is a common phenomenon [20-22]. Unhygienic practices involving simultaneous handling of money could introduce risk of contamination. This contaminated money is identified as potential public health hazard as pathogens can spread by its circulation [5].

In the present study, 92.4% of Pakistani currency notes and coins were found to be contaminated with different microbes some of which were potential pathogens. Similar results were observed in previous studies in different countries. A study in America showed that 94% of US one dollar bills had various microbial contaminations [21]. While in Nigeria and Saudi Arabia 89% of their respective currency notes i.e. Nigerian (Nair) and Saudi Riyal were found to be contaminated with bacteria [22,23]. In another study, it was found that 80% of Bangladeshi currency (old two Taka notes) had coliform contamination [24], where as in Ghana 100% of currency notes were found to be contaminated with one or more bacterial species [14].

Currency notes, usually made of 75% cotton and 25% linen, offer large surface area to microbes for attachment, as a result currency note are more frequently contaminated with pathogens like *S. aures*, *E. coli*, *Klebsiella* and other coliforms [11]. A substantial relationship was also found between the physical condition of the paper notes and microbial contamination in our study. The dirty and mutilated currency notes which were present in circulation from a very long time had higher levels of contamination on them. This fact is supported by another study where a hundred percent of dirty and tattered older version notes were found to be contaminated with pathogens while seventy six percent of new version notes had bacterial contamination [24]. We also found that lower denomination currency note (Rs 10) had high levels of microbial contamination. It can be due to the fact that lower currency notes receive most handling and are exchanged many times. Similar results were obtained by Igumbor *et al* [5] who investigated South African currency and by Vrieskoop *et al* [7] who investigated different currencies of the world. However, coins made

up of metals like copper, aluminum, nickel and brass seems to be limiting factor for bacterial survival and they are found to carry opportunistic pathogens like Gram positive *Bacillus*, *Corynebacterium* and few fungal species [25].

In current study, we found currency notes and coins contaminated with normal skin flora microbes and potential pathogens. Microbes like Staphylococci, Streptococci and Micrococci might be resident of skin and nasal passage [26]. Their presence indicates contamination of currency by touching after nose rubbing, cough and sneezing droplets [27]. As different species of *Bacillus* and *Corynebacterium* are widely spread in nature, their presence on currency notes and coins signify soil contamination. These isolated microorganisms do not usually cause infections in healthy people, but have been known to cause diseases in immune-compromised patients, including those infected with HIV, undergoing cancer chemotherapy or those taking other immune suppressants [1].

Enteric bacteria, *Klebsiella pneumonia*, *Burkholderia cepacia*, *Enterobacter aerogenes* and *Chronobacter sakazakii* have been reported as common isolates from paper currency in several studies [1, 2, 11, 20-22, 27]. These enteric bacteria commonly called as coliforms are present among intestinal flora of humans and other warm-blooded animals so their presence indicate fecal contamination [28].

Anaerobic Gram negative *Bacteriodes* spp. was also isolated after anaerobic analysis of currency notes and coins. *Bacteriodes* species are considered as opportunist pathogens that can cause gastrointestinal infections only in the immuno-compromised patients [17].

Unlike Basavarajappa *et al* [2], in the present study, no *Mycobacterium* spp. were isolated from currency notes and coins, the possible reason explaining the failure of its recovery from LJ medium might be its insufficient revival of organism after staying on currency note or coin for a long period of time.

In our study, we also found currency notes and coins contaminated with yeast and Molds species. Similar results were obtained in many other studies [2, 15, 18]. Isolation of different fungal species is an indication of environmental contamination of currency.

In the present study, it was found that currency notes and coins serve as potential vehicles for protozoan cysts and helminthes as ova and cysts of different parasites were found to be attached with surface of currency notes and coins. *Giardia lamblia* and *Entrobilus vermicularis* were the most prevailing species on contaminated Pakistani currency notes and coins. Other parasitological studies on currency notes and coins in different countries reported similar results [1, 2, 16, 29, 30]. Parasitic contamination on currency notes and coins shows poor local sanitation conditions and personal hygiene.

The city of Lahore is cosmopolitan with people from different socioeconomic and ethnic backgrounds. These people may have various hygienic habits; large number people interact with each other daily and transmit pathogens without knowing. This explains high level of microbial and parasitic contamination on the currency notes and coins. The trend of using credit cards is increasing, which could reduce the frequency of getting infected from currency. However, due to unavailability of credit card facility in many areas, the use of low denomination currency will continue in day to day purchasing and hence the problem is expected to continue.

As a conclusion, we recommend following strategies to reduce the contamination

1. The introduction of washable plastic currency, as was done in Australia (the first country to do so) in 1988 [31].
2. Efficient washing of hands by food handlers both in restaurants and home.
3. Regular disinfection of circulating currency notes and coins by ultraviolet light or formalin vapors in banks [32].
4. Regular retraction of circulating currency by federal authorities and the improvement of personal hygiene. Scientists have noted the possibility of bioterrorism, terrorists can contaminate the circulating currency notes and coins with pathogens so regular microbial testing of currency notes and establishment of a method for large-scale replacement of contaminated notes is needed.
5. Finally, we recommend launching of awareness programs on microbial and parasitic contamination

of currency as the issue is becoming a major public health concern worldwide.

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