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ORIGINAL ARTICLE

Microbial Profile of Egyptian Currency

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ABSTRACT

Background*: Currency notes and coins are widely exchanged for goods and services in countries worldwide. Currency is handled by a large number of people, under a variety of personal and environmental conditions thus increase the possibility of acting as environmental vehicle for transmission of potential pathogenic microorganisms.* ***Objectives:*** *This study aimed to isolate microorganisms that may be carried by Egyptian currency (paper & coins), determine the antibiotic sensitivity of the isolated bacteria and detect unusual modes of transmission of certain common diseases.* ***Methodology****: Money samples of different denominations were collected from different places (120 samples: 60 paper money and 60 metal coins).Twenty samples were collected from each place. Paper money and coins were collected in sterile plastic bags using sterile gloves and the bags were labeled.* ***Results****: Nine bacterial species were isolated; Coagulase negative staphylococci (48.3%), Staphylococcus aureus (46.7%), Bacillus spp. (30%), E.coli (10.0%), Klebsiella pneumonia (9.2%), Pseudomonas auroginosa (8.3%), Acinetobacter bumannii (7.5%), Enterococcus fecalis (3.3%), Acid fast bacilli (1.7%). And 5 fungal species were isolated; Candida albicans (38.3%), Aspergillus niger (25%), Aspergillus fumigatus (21.7%), Cryptococcus (10.8%)and Penicillium (5.8%).This study revealed the presence of antibiotic resistant strains of bacteria on Egyptian currency as(MRSA) (35.7%) and (VRSA) (25%) of the total isolated Staph.aureus, (MRSE) (34.5%) and (VRSE) (17.2%) of the total isolated Staph.epidermides and (VRE) (50%)of the total Enterococcus fecalis isolated .* ***Conclusion:*** *Egyptian paper currency and coins are highly contaminated and must be considered as an unusual mode of transmission of several bacteria and fungi.*

INTRODUCTION

Money has a wide circulation among the general public and hence has a role in transmitting disease-causing microorganisms. In poorer societies, money and especially low value denominations change hands frequently unlike rich communities using plastic money. Human hands are a major source of disease transmission and money also serves as important vehicles in transmission of disease or disease causing organisms [1, 2].

The possibility that currency notes and coins might act as environmental vehicles for the transmission of potential disease-causing microorganisms was suggested in the 1970s [3].

Paper notes and coins which are handled by a large number of people increase the possibility of acting as environmental vehicle for the transmission of potential pathogenic microorganisms [4].

Studies have shown that currencies serve as an ideal breeding ground for microorganisms for several reasons. First, the paper bills offer a large surface area for organisms and organic debris to collect [5]. Secondly, folds and/or depressions or projections serve as settling sites for both organisms and debris, which allow the microorganisms to live longer[6].

Lastly, banknotes weave their way through the population for many years before they come to rest. An individual living in unhygienic conditions and having unhygienic habits will contaminate the notes with bacteria e.g. improper hand washing after using the toilet, counting paper notes using saliva, coughing and sneezing on hands then exchanging money, and placement or storage of paper notes on dirty surfaces lead to the contamination and these notes which will act as a vehicle delivering bacteria to contaminate the hands of the next user. The money contributes in easy transfer of bacteria and thus cross contamination[7].

Microbial contaminants may be transmitted directly, through hand-to-hand contact, or indirectly, via food or other inanimate objects. As a result, hand hygiene is considered critical for preventing food outbreaks and healthcare-associated infections [8].

Studies reported that currency notes and coins were contaminated with bacteria as *(Klebsiella pneumoniae, Escherichia coli, Staphlococcus aureus, Pseudomonas species* and *Salmonella typhi*), fungi as (Aspergillus) and parasites[9]. Studies also found that viral transmission via money is possible as Influenza, Rhinovirus, HAV, Rotavirus and Astrovirus [10].

METHODOLOGY

In this study a total of 120 samples were collected (60 coins, 60 notes) in circulation within a period of seven months (October 2016: April 2017) according to:

A- Sites:

1. Hospitals.
2. Food handlers& Restaurants
3. Greengrocers.
4. Bus stops
5. Butchers
6. Fish markets.

20 samples were collected from each site.

B-Control samples: Currency notes and coins that had been newly or recently produced were obtained from the bank. These notes and coins were included in the study as a control.

C- Denominations:

- **Paper:** 1&5pounds: 20 samples from each denomination.
  10pound: 9 samples.
  20pound: 6 samples.
  50pound: 3 samples.
  100pound: 2 samples.
- **Coins:** 25, 50 PT and one pound.
  20 samples from each denomination.

Materials:

a) Materials used for bacteriological study:

- **Control strains**:

- ***E.coli*** :(ATCC25922).
- ***Staphylococcus aureus*** :(ATCC25923).

- **Gram stain**.
- **Bacteriological culture media:**
  Ordinary nutrient agar, Blood agar, MacConkey′s agar, Bile eschulin,
  Lowenstein-Jensen medium tubes and Mannitol salt agar (Oxoid).
- **Identification tests:**
  Catalase test, Coagulase test, Oxidase test , Simmons citrate agar slope, Indole test, Sugar fermentation tests, Triple sugar iron agar slope (Oxoid). Motility Indole Ornithine (MIO) medium and Lysine iron agar from (HIMEDIA).

b) Materials used for fungal study:

- **Fungal culture medium:**
  Sabouraud's dextrose agar (Oxoid).
- **Identification tests:**
  Lacto phenol cotton blue stain (HiMedia) and Germ tube test were used in this study

Methods:

Paper money and coins were collected in sterile plastic bags (Clinilab) using sterile gloves and the bags were labeled. The coins and paper money were not touched by any other person using bare hands at any stage. The controls consisted of one bank coin and one paper note.

A) Bacteriological Examination:

Isolation of various bacterial contaminants from the currency notes and coins was performed according to standard techniques[11]. A sterile, cotton-tipped swab moistened with sterile physiological saline was used to swab both sides of the currency note and coin. The swabs were directly inoculated on nutrient agar, blood agar, MacConkey's agar and Lowenstein-Jensen media (Oxoid). The plates were incubated aerobically at 37°C for 24-48 hours except for Lowenstein-Jensen medium for which incubation was given up to 8 weeks.

B) Identification of fungi:

The growth of fungi on Sabouraud's dextrose agar was examined after 1-3 week using Lacto phenol cotton blue stain and identification of the fungal species was performed with aid of binocular compound microscope (10X&40X) (Needle mount technique)[12].

C) *Antibiotic sensitivity test*[13,14]*:*

Using the Kirby-Bauer disc diffusion method according to the clinical and laboratory standard institute (CLSI) guidelines (2016).

- Antibiotics used for gram positive bacteria:

- Ampicillin (10µg)
- Amoxicillin/Clavulinic acid (30µg)
- Erythromycin (15µg)
- Ciprofloxacin (5µg)
- Clindamycin (2µg)
- Gentamycin (10µg)
- Cefoxitin (30µg)
- Vancomycin (30µg)

RESULTS

Staphylococcus aureus was the most isolated bacteria and *Acid fast bacilli* were the least isolated bacteria from the surfaces of the paper money. One pound note was the most contaminated denomination as shown in table 1.

As regard metal coins; *Coagulase negative staphylococci* were the most isolated bacteria and *Enterococcus fecalis* were the least isolated bacteria while *acid fast bacilli* were not isolated from the surfaces of metal coins. One pound coin was the most contaminated while 25PT was the least contaminated denomination as shown in table 2. This study revealed that all *S.aureus* isolates were resistant to ampicillin and most of the isolates were resistant to amoxicillin\clavulinic acid (82.1%) and erythromycin (89.3%) but sensitive to gentamycin (89.3%), (46.4%) were sensitive to ciprofloxacin, (35.7%) of isolates were resistant to cefoxitin (MRSA) and (25%) of isolates were resistant to vancomycin (VRSA) as shown in table 3.

All of *S. epidermidis* isolates were resistant to ampicillin, most of the isolates were resistant to amoxicillin/clavulinic acid (79.3%) and arythromycin (86.2%) with intermediate sensitivity to clindamycin

(77.6%) and sensitive to gentamycin (82.8%), (34.5%) of isolates were resistant to cefoxitin (MRSE) and (17.2%) of isolates were resistant to vancomycin (VRSE) as shown in table 4.

Half of *Enterococcus fecalis* isolates were sensitive to ampicillin and other antibiotics and the other half were resistant and (50%) of isolates were resistant to vancomycin (VRE) as shown in table 5.

Candida albicans was the most isolated fungus and Penicillium was the least isolated fungus from the surfaces of paper money. One pound note was the most contaminated and100 pound was not contaminated with any fungi as shown in table 6.

As regard to metal coins; *Candida albicans* was the most isolated fungus and Penicillium was the least isolated fungus from the surfaces of metal coins. One pound coin was the most contaminated and 25PT was the least contaminated denomination as shown in table 7.

Number of isolated bacteria from paper notes was (111) and from coins was (87) and P value was significant. While the number of isolated fungi from paper notes was (67) and from coins was (55) and P value was non-significant as shown in table 8.

Table 1: Incidence of isolated bacteria from the surfaces of different paper notes:

Denomination notes		*Staphylococcus aureus*	Coagulase negative staphylococci	Bacillus spp.	*E.coli*	*Klebsiella pneumoniae*	*Pseudomonas aeuroginosa*	*Acinetobacter bumannii*	*Enterococcus fecalis*	Acid fast bacilli	Total
1.00	**N:20**	7	15	5	4	0	5	2	1	1	40
5.00	**N:20**	14	5	7	0	3	0	3	0	1	33
10.00	**N:9**	4	3	3	3	1	2	1	1	0	18
20.00	**N:6**	2	2	3	1	2	0	1	0	0	11
50.00	**N:3**	3	1	1	0	0	0	0	1	0	6
100.00	**N:2**	1	2	0	0	0	0	0	0	0	3
Total	**N:60**	31	28	19	8	6	7	7	3	2	111
	%	51.7%	46.7%	31.7%	13.3%	10.0%	11.7%	11.7%	5.0%	3.3%	

Table 2: Incidence of isolated bacteria from the surfaces of different metal coins:

denomination coin		*Staphylococcus aureus*	Coagulase negative staphylococci	Bacillus spp.	*E.coli*	*Klebsiella pneumoniae*	*Pseudomonas aeuroginosa*	*Acinetobacter bumannii*	*Enterococcus fecalis*	Acid fast bacilli	Total
25.00	**N:20**	9	10	5	0	0	0	0	0	0	24
50.00	**N:20**	6	10	6	1	3	1	1	0	0	28
100.00	**N:20**	10	10	6	3	2	2	1	1	0	35
Total	**N:60**	25	30	17	4	5	3	2	1	0	87
	%	41.7%	50%	28.3%	6.7%	8.3%	5.0%	3.3%	1.7%	0%	

Table 3: Antibiotic sensitivity test of *S. aureus* isolates using disc diffusion (Kirby-Bauer) method:

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Ampicillin	56	100%	0	0%	0	0%
Amoxicillin/Clavulinic acid	46	82.1%	0	0%	10	17.9%
Erythromycin	50	89.3%	0	0%	6	10.7%
Ciprofloxacin	14	25%	16	28.6%	26	46.4%
Clindamycin	20	35.7%	26	46.4%	10	17.9%
Gentamycin	6	10.7%	0	0%	50	89.3%
Cefoxitin	20	35.7%	0	0%	36	64.3%
Vancomycin	14	25%	0	0%	42	75%

Table 4: Antibiotic sensitivity test of *S. epidermidis* isolates using disc diffusion (Kirby-Bauer) method:

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Ampicillin	58	100%	0	0%	0	0%
Amoxicillin/Clavulinic acid	46	79.3%	0	0%	12	20.7%
Erythromycin	50	86.2%	0	0%	8	13.8%
Ciprofloxacin	0	0%	30	51.7%	28	48.3%
Clindamycin	0	0%	45	77.6%	13	22.4%
Gentamycin	10	17.2%	0	0%	48	82.8%
Cefoxitin	20	34.5%	0	0%	38	65.5%
Vancomycin	10	17.2%	0	0%	48	82.8%

Table 5: Antibiotic sensitivity test of *Enterococcus fecalis* isolates using disc diffusion (Kirby-Bauer) method

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Ampicillin	2	50%	0	0%	2	50%
Amoxicillin/Clavulinic acid	2	50%	0	0%	2	50%
Erythromycin	2	50%	0	0%	2	50%
Ciprofloxacin	2	50%	0	0%	2	50%
Vancomycin	2	50%	0	0%	2	50%
Gentamycin	2	50%	0	0%	2	50%
Tetracyclin	2	50%	0	0%	2	50%

Table 6: Incidence of isolated fungi from the surfaces of different paper notes:

denomination notes		*Aspergillus niger*	*Aspergillus fumigatus*	*Candida albicans*	Cryptococcus	Penicillium	Total
1.00	**N:20**	6	6	10	0	2	24
5.00	**N:20**	2	2	15	3	0	22
10.00	**N:9**	2	0	4	4	1	11
20.00	**N:6**	4	3	0	0	0	7
50.00	**N:3**	1	1	0	0	1	3
100.00	**N:2**	0	0	0	0	0	0
Total	**N:60**	15	12	29	7	4	67
	%	25%	20%	48.3%	11.7%	6.7%	

Table 7: Incidence of isolated fungi from the surfaces of different metal coins:

Denomination coin		*Aspergillus niger*	*Aspergillus fumigatus*	*Candida albicans*	Cryptococcus	Penicillium	Total
25.00	**N:20**	2	6	3	3	0	14
50.00	**N:20**	9	2	4	3	0	18
100.00	**N:20**	4	6	10	0	3	23
Total	**N:60**	15	14	17	6	3	55
	%	25%	23.3%	28.3%	10.8%	5%	

Table 8: Incidence of bacterial and fungal contamination in paper notes and coins:

		Bacteria	Fungi
notes	**N**	111	67
Coin	**N**	87	55
X^2		14.993	2.402
P value		0.001*	0.121

DISCUSSION

This study revealed that paper notes were more contaminated than as shown in table8. This finding is in agreement with previous studies found that among 186 samples subjected to laboratory investigation, 71 were coins, and 115 were paper notes. The number of bacteria was always significantly higher on notes than on coins and the difference was also higher in the case of fungi[15]. Another study reported that two hundred (93.9%) samples of two hundred and thirteen currency samples were contaminated and also notes (96.6%) showing higher contamination than coins (88.2%)[16].

This can be explained by that paper notes provide a large surface area suitable for survival of pathogens. Another study suggested that the coins might be toxic to bacteria and bacterial survival on coins was very poor[17]. The bacterial numbers on the coins decreased steadily to near zero over one week[17]. Bacteria such as *E. coli* and *Salmonella enteritidis* rapidly died-off when exposed to coins[18].

Also our study found that one pound note was the most contaminated denomination among paper notes. This is in agreement with another studies found that lower denominations had the highest level of contamination followed by other denominations[4,6,19,20,21].

This may be due to the possibility that the low values are more wide spread and exchangeable among population**.** On the other hand, a study was made in Nigeria reported that the highest bacterial and fungal contamination were found on higher denominations[22].

This may be due to that higher denominations are more exchangeable than the lower denominations in Dutse environment (Dutse is capital city of Jigawa), also most Nigerians are less interested in using lower denominations. This study showed that one pound note and five pounds were the only denominations that were contaminated with *acid fast bacilli* (1.7%). This is in agreement with another study found that only 2 lower denomination currency notes showed presence of *Acid fast bacilli* (3.7%)[23].

This may be attributed to the wide spread and usage of lower denomination currency. In our study 9 bacterial species were isolated; *Coagulase negative staphylococci* having the highest bacterial incidence (48.3%) and *acid fast bacilli* are the lowest bacteria isolated *(*1.7%*)* as shown in table1 and 2. This is in an agreement with another study reported that *Staphylococcus spp*. having the highest percentage (30.2%) and *Bacillus spp*. (26.5%)[22].

Another study also found that the predominantly isolated microbial groups were *Staphylococcus* spp. (34.06%) followed by *Bacillus* spp. (31.88%), *Enterobacteraceae* (13.39%)[19].

Another study also found nine bacterial species: among the bacteria isolated, *coagulase negative Staphylococcus* (54.9%) and *Staphylococcus aureus* (20.1%) were the predominant isolates[15]. On the other hand, a study was done in Croatia reported that the most common bacteria isolated was *S. epidermidis* but with higher percentage (86.33%), *Bacillus spp*. (13%), *Neisseria spp*. (2%) and *Klebsiella spp* (1%)[24].

Different types of bacteria were also isolated which included *Enterobacter cloacae*, *Klebsiella ozaenae*,

Cedecea davisae, *Yersinia pseudotuberculosis*, *Acinetobacter iwoffii*, *Staphylococcus warneri* and *Enterobacter agglomerans* [4].

The differences in types and percentages of isolated bacteria may be due to differences in sites from which samples were collected and differences in geographical locations. As regard antibiotic sensitivity, in this study we found that all *S.aureus* isolates were resistant to ampicillin (100%) and most of the isolates were resistant to amoxicillin\ clavulinic acid (82.1%) and erythromycin (89.3%), (35.7%) of isolates were resistant to cefoxitin (MRSA), (25%) of isolates were resistant to vancomycin (VRSA), (89.3%) were sensitive to gentamicin and (46.4%) were sensitive to ciprofloxacin. This is in an agreement with a study showed that *S. aureus* had 100% resistance to ampicillin and Methecillin-resistant *S. aureus* (MRSA) was found to be (36.4%)[25].

Another study found that (100%) of the isolates were sensitive to gentamicin and (81.4%) were resistant to erythromycin[15]. On the other hand, a study in India revealed that only one isolate of *Staphylococcus aureus* was MRSA[26].

In Cameroon, another study showed that *Staphylococcus aureus* isolates were completely sensitive (100%) to ampicillin and resistant to vancomycin [16].

Our study revealed that; all of *S. epidermidis* isolates were resistant to ampicillin, most of the isolates were resistant to amoxicillin/Clavulinic acid (79.3%) and erythromycin (86.2%), (34.5%) of isolates were resistant to cefoxitin (MRSE), (17.2%) of isolates were resistant to vancomycin (VRSE), (82.8%) were sensitive to gentamicin and (48.3%) were sensitive to ciprofloxacin nearly similar to *S.areus* isolates. On the other hand, a study In Cameroon showed that all *Coagulase-negative Staphylococcus* isolates were resistant (100%) to vancomycin and sensitive to erythromycin and gentamicin [16].

For fungal contamination; 5 fungal species were isolated: *Candida albicans* having the highest fungal incidence with total percentage of (38.3%) followed by *Aspergillus niger* (25%), *Aspergillus fumigatus* (21.7%), Cryptococcus (10.8%) and Penicillium (5.8%). This is in an agreement with a study reported the following fungi: ***Aspergillus niger,*** *Candida spp* and *Penicillium* spp [4]. Another study also revealed that the *Penicillium spp.* had the lowest occurrence of (7.6%)[21].

In Nanded city (India) a report revealed heavy contamination of currency notes and coins by *Aspergillus niger* (60.37%), *A. flavus* (3.98%) in addition to another fungal species like *A. nidulans* (0.2%) and *Penicillium citrinum* (17.80%)[27].

Another study also revealed *Aspergillus niger* had the highest fungal percentage occurrence of (30.2%) followed by *Penicillium spp.* (29.5%), *Rhizopus spp.* (26.5%) and *Trichoderma spp.* (13.8%)[28].

Aspergillus was represented by maximum number of species which accounted for (29.7%) of the total recovered fungi, followed in decreasing order by *Penicillium* (18.9%) and *Fusarium* (10.8%)[29]. The differences in types and percentage of isolated fungi may be due to differences in sites from which samples were collected, sanitation levels and differences in geographical locations. Also the present study reported that one pound note was the most contaminated and 100 pound was not contaminated with any fungi. This is in agreement with a study found that the 500 Saudi reyal notes had no fungal contamination[4]. This is due to higher denominations are less used and spread than lower denominations.

CONCLUSION

Egyptian paper notes and coins are highly contaminated and must be considered as an unusual mode of transmission of several bacteria and fungi. *Coagulase negative staphylococci* are the most isolated bacteria and *acid fast bacilli* are the least isolated bacteria. MRSA and VRSA represent a considerable percentage of isolates. *Candida albicans* is the most isolated fungus and Penicillium is the least isolated fungus. Paper notes are more contaminated than coins.

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