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Bacteriological Assay for the Egyptian Currency Collected from Veterinary Field

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Abstract: Bacteriological examinations of Egyptian currency collected from veterinary markets included microbial count at 35°C and 25°C, besides that isolation of *Escherichia coli* (*E. coli*), Salmonellae, *Staphylococcus aureus* (*S.aureus*) and *Listeria monocytogenes* (*L. monocytogenes*). The public health significance of isolated organisms and suggestive measures were also discussed. Average bacterial content on the paper currency ranged from10^5 to10^8 while the contents on metal coins ranged from 10^3 to 10^5 and the more isolated bacteria were *S. aureus* and *E. coli* followed by *Salmonella* spp. while *L. monocytogenes* was not isolated from the metal coins. The bacterial isolates were sensitive to ofloxacine and ciprofloxacin and more resistant to penicillin, tetracycline, gentamicin, chloramphenicol and amoxicillin.

Key words: *Staphylococcus aureus* · *E. coli* · Salmonellae · Listeria monocytogenes · Egyptian currency

INTRODUCTION

Currency in the form of banknotes and metal coinage represents a universal medium for the transmission of bacteria in the environment and among humans [1, 2]. Microbial contaminants may be transmitted directly, through hand-to-hand contact, or indirectly, via food or other inanimate objects, these routes of transmission are of great importance in the health of many populations in developing countries, where the frequency of infection is a general indication of local hygiene and environmental sanitation levels [3].

Paper currency is widely exchanged for goods and servicesin countries worldwide. It is used for every type of commerce, all this trade is hard on currency and with lower-denomination notes receiving the most handling because they are exchanged many times [4]. Paper currency also provides a large surface area as a breeding ground for pathogens [5].

Money on which pathogenic microorganisms might survive represents an often overlooked reservoir for enteric disease [6]. In most parts of the developed world, there is a popular belief that the simultaneous handling of food and money contributes to the incidence of food-related public health incidents [7].

Oddly, publications regarding the degree to which paper money is contaminated with bacteria are few and far between [6, 8-10].

Accumulated data obtained over the last 20 years on the microbial status and survival of pathogens on coins and currency notes indicates that this could represent a potential cause of sporadic cases of food borne illness. Survival of various microorganisms of concern on money is such that it could serve as a vehicle for transmission of disease and represents an often overlooked enteric disease reservoir. Since communicable diseases can spread through contact with paper currency, data regarding the bacteriological contamination of paper currency are limited.

The objective of this study was to investigate the extent of contamination of some of the most used paper and coin denominations of the Egyptian currency to detect any potentially pathogenic bacteria that may pose a public health risk. Therefore, this work was carried out to assess the bacteriological contamination of the currency. This study was designated to perform bacteriological assay for the Egyptian money coins and currency papers collected from butcheries, poultry and fish markets, to identify the possible public health risk.

MATERIALS AND METHODS

Sampling

Coins: Money coins were collected from raw fish markets, raw poultry markets and raw meat markets (butchers), a total of five coins and five paper currency were

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randomly collected from vendors in each category of sampling and from different units. The coins were dropped into a 30 millilitres volume sterile plastic container. The container was promptly capped and the individual given a replacement coin. The containers were immediately transported to the laboratory.

Paper Currency: Samples were collected in sterile plastic bags.

Quantitative Bacterial Analysis: In the laboratory, 3 ml of sterile tryptone soy broth (TSB) were added into each container and bag. Five doubling dilutions of TSB from each (200 µl), were then prepared and 100 µl of each of the dilutions was plated, in duplicates, onto plate count agar plates, using the pour plate method. The plates were then incubated at 37°C for 48 hours after which colonies were enumerated [11] and the total bacterial (colony forming units) yield from each coin was calculated.

Aerobic Plate Count at 35°c (Mesophiles): The pouring technique recommended by AOAC [11] was applied. One ml of each dilution was separately pipetted in sterile Petri-dishes. Fifteen ml of melted standard plate count agar (SPCA;Oxiod;CM325) at 42-45°C were poured thoroughly mixed and then left to solidify. The inoculated plates were incubated at 35°C for 48 $\pm$ 2 hours. The average number of colonies was determined and the aerobic plate count per cm^2 was calculated as follows:

Mesophilic plate count/ cm^2/ organism = No. of colonies× dilution

Aerobic Plate Count at 25°c (Psychrotrophs): The same technique of the pouring method was done as previously mentioned in mesophiles but the inoculated plates were incubated at 25°C for 48 hours. The number of colonies/ cm^2 was calculated in countable plates as follows:

Psychrotrophs count/cm^2/ organism = No. of colonies× dilution

Isolation and Identification: The remaining TSB in the containers was incubated at 37°C for 12 hours. Thereafter the broth cultures were plated on selective and/or differential media, namely blood agar, MacConkey agar, Eosin methlene blue agar (EMB), Xylose lysine desoxycholate agar, *Salmonella Shigella* agar (S.S. agar) mannitol salt agar and PALCAM agar, The plates were incubated at 37°C overnight. Bacterial colonies in each medium were then characterized on the basis of colonial, cellular morphology and staining characteristics. On this basis, the colonies were categorized as Gram positive, catalase positive cocci; Gram positive short rods and Gram negative bacilli.

Biochemical Identification: Organisms in each category were then identified, when possible, on the basis of biochemical characteristics.

Sensitivity Test for the Isolates: It was carried out according to Finegold and Baron [12].

Preparation of Standard Suspension: Some of typical colonies of each isolate were suspended in Mueller-Hinton broth and incubated at 37°C for 8 hours till its turbidity exceeds the turbidity of standard Mcfarland tube No. 0.5.

Inoculation of the Test Plates: A sterile cotton swab was dipped into standardized bacterial suspension and the swab was then used to streak the dried surface of Mueller-Hinton agar plate in 3 different planes by rotating the plates to be sure for even distribution of the inoculums.

Placement of the Discs: The antimicrobial discs were placed on the inoculated place using gentle pressure by sterile pointed forceps on the agar to ensure complete contact with the surface. Then the plates were incubated at 37°C for 24 hours.

Reading the Results: The degree of sensitivity was estimated by measuring the visible clear zone of inhibition produced by diffusion of the used antimicrobial disc into the surrounding medium. Interpretation of the results was done according to National Committee for Clinical Laboratory Standards [13] and Koneman *et al.* [14].

RESULTS AND DISCUSSION

Money has mass circulation among the general public and has potentiality to transmit disease causing microorganisms so that paper currency is commonly contaminated with bacteria and this may play a role in the transmission of potentiality harmful microorganisms. A study in India found *S. aureus* contaminating paper currency collected from a wide variety of places, including shops, hospitals and restaurants and showed that the bacteria could survive for up to eight days on the money. Virulence genes and antibiotic sensitivity to

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

Microorganism	A.P.C.		*S. aureus*		*E. coli*		*Salmonella spp.*		*Listeria monocytogenes*		Total	
Paper currency samples	No	%	No	%	No	%	No	%	No	%	No	%
200.00	13	52	4	16	2	8	1	4	1	4	21/25	84
100.00	18	72	9	36	5	20	2	8	2	8		
50.00	23	92	19	76	4	16	1	4	1	4		
10.00	25	100	13	52	10	40	8	32	4	16		
5.00	22	88	11	44	8	32	6	24	2	8		
1.00	25	100	15	60	9	36	5	20	3	12		
0.50	22	88	16	64	6	24	2	8	1	4		
0.25	25	100	17	68	14	56	11	44	7	28		
Total	173	86.5										

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

Microorganism	A.P.C.		*S. aureus*		*E. coli*		*Salmonella spp.*		*Listeria monocytogenes*		Total
Metal coins	No	%	No	%	No	%	No	%	No	%	
1.00	10	40	6	24	3	12	2	8	-	-	
0.50	8	32	3	12	2	8	1	4	-	-	
0.25	7	28	2	8	1	4	-	-	-	-	
0.10	13	52	3	12	2	8	-	-	-	-	
0.05	9	36	1	4	1	4	-	-	-	-	
Total											

Table 3: Mean values of bacterial count of paper currency surfaces

Microorganism					
Paper currency samples	A.P.C.	*S. aureus*	*E. coli*	*Salmonella spp.*	*Listeria monocytogenes*
200.00	$3x10^5$	$6x10^3$	$5x10^2$	10^2	$<10^2$
100.00	$4x10^6$	$4x10^4$	$2x10^3$	$2x10^2$	10^2
50.00	$2x10^7$	$7x10^4$	$2x10^3$	10^2	10^2
10.00	$6x10^7$	$2x10^4$	$2x10^2$	10^2	10^2
5.00	$3x10^8$	$9x10^4$	$3x10^2$	$2x10^2$	$<10^2$
1.00	$5x10^8$	$3x10^5$	10^3	10^2	95
0.50	$4x10^8$	10^4	$2x10^2$	10^2	10^2
0.25	$2x10^7$	$3x10^3$	10^2	10^2	95

Table 4: Mean values of bacterial count of metal coins surfaces

Microorganism					
Metal coins	A.P.C.	*S. aureus*	*E. coli*	*Salmonella spp.*	*Listeria monocytogenes*
1	$4x10^4$	$2x10^3$	$4x10^2$	10^2	-
0.5	$3x10^5$	$2x10^4$	$3x10^2$	30	-
0.25	$2x10^5$	$3x10^3$	$5x10^2$	-	-
0.10	$6x10^4$	$2x10^3$	95	-	-
0.05	$5x10^3$	10^2	60	-	-

Table 5: Antibiotic sensitivity test of *S. aureus* isolates

	Resistant		Intermediate		Sensitive	
Antibacterial agent	No	%	No	%	No	%
1-Amoxicillin	40	33	8	7	71	60
2-Penicillin	119	100	0	0	0	0
3-Chloramphenicol	55	46	6	5	58	49
4-Ciprofloxacin	0	0	12	10	107	90
5-Ofloxacine	0	0	0	0	119	100
6-Gentamicin	71	60	6	5	42	35
7-Tetracycline	98	82 18	0	0	21	

The percent was calculated according to the total number of staph. isolates (119)

Table 6: Antibiogram sensitivity test of E. coli isolates

	Resistant		Intermediate		Sensitive	
Antibacterial agent	No	%	No	%	No	%
1-Amoxicillin	47	70%	0	0%	20	30%
2-Penicillin	67	100	0	0%	0	0%
3-Chloramphenicol	17	25%	0	0%	50	75%
4-Ciprofloxacin	12	18%	2	3%	53	79%
5-Ofloxacine	0	0%	0	0%	67	100%
6-Gentamicin	30	45%	3	4%	34	51%
7-Tetracycline	38	57%	3	4%	26	39%

The percent was calculated according to the total number of E coli isolates (67)

nine antibiotics were both investigated and those samples with all tested virulence genes had higher levels of antibiotic resistance [15].

From the results presented in tables (1 and 2); the bacterial isolation increases in the lower currency and less in the higher one due to their good paper quality. Rocha-Gámez *et al.* [16] found that Out of the 70 peso banknotes, 48 (69%) to be contaminated in Mexico while Pope *et al.* [17] revealed that pathogenic or potentially pathogenic organisms were isolated from 94% of One-dollar bills survey.

The results revealed that the more isolated bacteria were *S. aureus* and *E. coli* followed by *Salmonella* spp. while *L. monocytogenesis* was not isolated from the metal coins.

S. aureus isolates from money values (50, 0.25, 0.5, 1, 10, 5, 100 and 200) were (76, 68, 64, 60, 52, 44, 36 and 16%) respectively while *E. coli* isolates from money values (0.25,10.0,1.0,5.0,0.5,100,50 and 200) were (56,40,32,24,20,16 and 8%) respectively and these results slightly agree with that of Al-Ghamdi *et al.* [18] who detected *S. aureus* (13-38%) and *E. coli* (2-9%).

Salmonella isolates from money values (0.25,10.0, 5.0,1.0,0.5,100, 50 and 200) were (44, 32, 24, 20, 8, 8% and 4 and 4%) and these results coincide that of Khin *et al.* [19] who isolated Salmonella spp. frompaper-money samples obtained from butchers and fish mongers in Rangoon.

Listeria monocytogenes indicates bad hygienic measures and may cause particular risk to public health.

From the results presented in table (3), it is evidence that the aerobic plate count of paper currency ranged from 10^5 to 10^8 which agrees with that of El-Dars andHassan [20] who detected that over 65% of paper currency in Egypt had abacterialcount above 10^5 cm^2.

From the results presented in table (4); it is evidence that the aerobic plate count of metal coins ranged from 10^3 to 10^5.

As shown in Table (5) 100, 82, 60, 46 and 33% of the isolates were resistant to penicillin, tetracycline, gentamicin, chloramphenicol and amoxicillin respectively. While 100 and 90% were sensitive to ofloxacine and ciprofloxacin respectively. These results agree with Espinosa *et al.* [21] who mentioned that the rate of ciprofloxacin, gentamicin and amoxicillin sensitivity for *S. aureus* is 100, 40 and 60% respectively. The isolates showed 100% resistance to penicillin while Pourakbari *et al.* [22] stated that the rates of Amoxicillin and penicillin resistance was 79 and 66%, respectively.

Table 7: Antibiogram sensitivity test of Salmonella isolates

	Resistant		Intermediate		Sensitive	
Antibacterial agent	No	%	No	%	No	%
1-Amoxicillin	35	90	0	0	4	10
2-Penicillin	39	100%	0	0%	0	0%
3-Chloramphenicol	0	0%	0	0%	39	100%
4-Ciprofloxacin	4	10%	0	0%	35	90%
5-Ofloxacine	0	0%	0	0%	39	100%
6-Gentamicin	16	41%	4	10%	19	49%
7-Tetracycline	27	69	0	0	12	31

The percent was calculated according to the total number of Salmonella isolates (39)

As shown in Table (6) 100, 70 and 57% of the *E coli* isolates were resistance to Penicillin, Amoxicillin and Tetracycline, respectively, While 100, 79, 75 and 51% were sensitive to Ofloxacine, Ciprofloxacin, chloramphenicol and Gentamicin, respectively. These results greatly agree with that of Espinosa *et al.*[21] who mentioned that *E. coli* isolates are resistant to Amoxicillin 70% *E. coli* isolates were sensitive to Ciprofloxacin 100% [23], 99% [24] and 91.4% [25].

As shown in Table (7) 100, 90, 69 and 41% of the Salmonella isolates were resistance to penicillin, amoxicillin, tetracycline and gentamicin, respectively While 100, 100 and 90%, were sensitive to chloramphenicol, ofloxacine and ciprofloxacin, respectively. These results nearly agree with that of Espinosa *et al.* [21] who mentioned that Salmonella isolates were100% resistant to amoxicillin and 100% sensitive to ciprofloxacin. Also, Dechen *et al.* [23] and Sang *et al.* [26] mentioned that Salmonella isolates were 100% sensitive to ciprofloxacin.

CONCLUSION

Data regarding the bacteriological contamination of metal coins were low in number regarding APC, *S. aureus*, *E. coli*, Salmonella spp. and *L. monocytogenes* isolation.

The contact surface and the material ability to absorb potentially pathogenic bacteria in paper currency is more than that of metal coins so ratio of their contamination is very high and represent public health risk.

Paper currency also was difficult to wash while the metal coins are safer due to their easy wash and remove the contamination.

In poorer socialites money and especially low value denomination coins charge hands frequently unlike in more rich communities, moreover due to the habits of Egyptian people who prefer to use the paper currency due to their light weight.

Recommendations:

- Using of metal coins which have narrow hands area surface resulting in little contamination and shelf-life very long but the drawback that they not preferred to use by Egyptian people because of their heavy weight.
- Using plastic money is the best which have light weight, easily sterilized and long shelf-life.
- Adding a bacteriostatic agent to paper currency to reduce their harbored microorganisms and improve their quality to increase their shelf-life.
- Hand hygiene is strongly recommended, especially for those who simultaneously handle food and money.

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