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Original Research Article

Assessment of Microbial Contamination of Indian Currency in Circulation in A Tertiary Care Hospital Setting

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Conflict of interest: Nil

Abstract:

Background: Currency represents a universal medium for transmission of microorganisms in the environment and serve as an unrecognized reservoir for pathogenic bacteria contaminated by droplets while coughing, sneezing, touching with contaminated hands. Paper currency notes are continuously contaminated by poor handling and poor storage practices. Contaminated currency can also cause nosocomial infections and care should be taken while handling these currencies. Simultaneous handling of currency notes along with food can cause contamination of food. The pathogenic or potentially pathogenic microorganisms could survive on paper currency for days and may cause healthcare associated infections. The aim of this study was to investigate the occurrence and identify the microorganisms contaminating Indian currency notes.

Methods: This is a prospective study based on laboratory investigations on various denominations of currency randomly collected from various sources of our hospital. Each currency note was collected from the respective people in a sterile plastic bag and transferred to the laboratory immediately and finally the swab samples are subjected to laboratory analysis. Similarly, a total of 12 new paper currencies (mint or uncirculated paper currency) and 12 new coins (mint coins) collected from the local bank, were used as negative control.

Results: A total of 90 (69%) organisms were isolated from 130 currency notes. Among the 90 isolates 14% were pathogenic and 86% were non-pathogenic environmental organisms. Among the different categories or the sources, the highest percentage of contamination was occurred in food handlers followed by hospital visitors and healthcare workers (HCWs) while the least level of contamination was occurred in non-healthcare professionals.

Conclusion: The study is to add to the limited body of literature on microbial contamination of Indian currency. The outcome of this study reflects that paper currency notes contaminated with microbes and can be a source for microbial transmission causing infectious diseases, foodborne illness, nosocomial infections and thus could be public health hazard. Public awareness should be created to avoid cross contamination of currency notes.

Keywords: HCWs, Currency, Hospital Visitors, Contamination, Food Handlers, India.

Introduction

The word "money" is accepted to originate from a temple of Juno Moneta, which is on Capitoline, one of the seven hills of Rome. Before currency was introduced, economic exchange was practiced by barter system. The barter economy, which involved exchange of one good for certain amount of a different good. Money either in the form of paper or coins, represent a universal medium for transmission of microorganisms in the environment and among humans, may serve as an unrecognized reservoir for pathogenic and non-pathogenic bacteria. [1-7]

Money is very important in human life since it involves economic and trade needs, currency notes

are vital for goods and services worldwide. Contaminated notes and coins are a public health risk when associated with the simultaneous handling of food and currency may spread and lead to contamination of food, because currency note has a large surface area thus providing breeding ground for microbial contaminants. Immunocompromised persons are at risk of acquiring opportunistic infection, through handling of contaminated currency. [1-4,8-15]

Currency is an exchangeable fomite continuously subjected to contamination by various substances, contamination may occur during production, during storage and use. Currency can be contaminated by droplets while coughing, sneezing, touching with contaminated hands, anal region, placement on dirty surface. The practice of licking or applying saliva to the fingers while counting paper money is an important potential route of exposure to bacteria and enteric pathogens. In India, poor-currency-handling culture is widespread and indiscriminate abuse of currency notes. Care should be taken by those handling these currencies. [1-5, 8-10,13,14,16,17,18]

The pathogenic microorganisms may spread from contaminated currency include Enterobacteriaceae, Mycobacterium tuberculosis, Vibrio cholerae, Bacillus species, Micrococcus species, Corynebacterium species, MRSA strains and ESBLs producers. Infected or colonized patients act as reservoirs and transient hand carriage by healthcare workers may be mode of transmission from one patient to another. [2,4,6,10-12,18-20]

Currency in hospital environment is contaminated with different pathogenic and potential pathogenic organisms and people handling currency are invariably exposed to these microorganisms. Contaminated currency may play a significant role in the transmission of potentially harmful microorganisms that are resistant to commonly used antibiotics and therefore represents risks and public health hazards to the community and individuals. [1,3,5,6,12,13,17,21,22]

Furthermore, many researches have also shown that paper currencies could also be contaminated by several fungal and parasitic species of different helminths. Parasites that have been observed to be contaminants of paper money are mainly of faecal origin, when hands used in cleaning up the anus after passing out faeces are not properly washed. [1,4,7,10,13,14,22]

A majority of the people does not carry money in wallets and squeezing of paper currency is common. For instance, women especially among the unenlightened, often place money underneath their brassieres, in the handkerchiefs, under the carpet or rugs. The spread of the bacteria among the handlers of their body flora on the notes. Bacteria are incredibly adaptable and may thrive in harsh conditions. [5-7,10,16]

Simultaneous handling of money and food by food handlers, waiters or vendors should be discouraged, otherwise, can have serious consequences as the food they serve is ready to eat and does not require any further heating. People who are working in the medical field, the food and catering business should wash their hands after handling cash. [2,4,6,8-10,12-14,16,20,23]

Thus, the objective of this study is to investigate the occurrence of microorganisms such as bacteria, parasites and fungal contamination that might play a significant role in order to explore the possibilities of transmission of infectious diseases through currency notes collected from different sources of the hospital, food handlers and others.

Objectives

1. To explore the presence of bacteria, parasites and fungal elements contaminating the Indian currency notes in the hospital settings.
2. To compare the microbial contamination of paper currency notes handled by different groups such as HCWs, hospital visitors, food handlers and non-healthcare professionals of currency contamination in the hospital settings.

Materials and Methods

Study Design: This was a prospective study based on laboratory investigations on various denominations of currency randomly collected from various sources of our hospital. The investigation was conducted from February to April 2025 at Sri Siddhartha Institute of Medical Sciences, a tertiary care centre, T. Begur, Nelamangala taluk, Bangalore Rural, India. Before the collection, the purpose and procedure of the study was explained to the subjects and written informed consent of each subject was obtained. Money was collected only from individuals who accepted to participate freely in the study. We approached the HCWs such as doctors, staff nurses, laboratory technicians, other department technicians, nursing teaching staff, nursing students, interns, pharmacy staff, reception staff, house-keeping staff, security personnel, medical students, office staff, attenders, food handlers of hostels and hospital cafeteria, bank employees, out-patients, in-patients and patients' attenders (who accompanied with patients). Each currency note was collected from the respective people in a sterile plastic bag and transferred to the laboratory immediately and finally, the swab samples are subjected to laboratory analysis. Similarly, 12 new currency (mint or uncirculated paper currency and coins) notes and 12 new coins of various denomination were procured from the local bank in the campus and used as negative control and processed as study samples. The term 'mint'

describes uncirculated currency notes that had been newly produced. Such currency had not gone into circulation as such was used as control in the present study (Table: 1 and Figure: 10).

Sampling Technique: The different denominations of Indian currency notes (Rs. 10, 50, 100, 200 and 500) and coins (Rs. 10 and 20) were randomly collected from various sources of our hospital (Figure: 10). We approached the study participant in the hospital during working hours, explained them about the research and requested their participation. Those who volunteered to participate were included in the study. The participants who fulfilled the inclusion criteria were included in the study. The following demographic information such as name, age, gender, occupation, address and contact number was collected (Table: 1).

Sample Size: The study was conducted by Anitha et al,[4] reports that the prevalence of microbial contamination of currency notes, Staphylococcus aureus isolate is 22% (p=0.22).

Sample size is calculated using the formula (sample size estimation of prevalence).

$$n = \frac{Z^2_{(1-\alpha/2)}\, p(1-p)}{d^2} \quad n = \frac{(1.96)^2(0.22)\,(1-0.22)}{(0.1)^2} = 66$$

Where: n = sample size, z = Standard normal table value at a given level of significance (α), d= allowable error and p = estimated prevalence. Using the above formula, at 95% confidence interval and 10% allowable error, the estimated sample size is n=66. However, a sample size of 130 was processed for the above study.

Inclusion Criteria: Currency notes and coins of various denominations from various sources of the hospital.

Exclusion Criteria: Those participants mentioned in the study design who did not want to involve voluntary in the study.

Sample Collection and Transportation: A total of 130 currency notes of different denominations (Rs. 10, 20, 50, 100, 200 and 500) from 20 different categories mentioned in the study design, were collected and processed (Figure: 1). Of 130 currency samples, 4 coins of two denominations (Rs. 10 and 20) were collected and processed. Similarly, 12 new paper currency notes and 12 new coins of various denomination were collected from the local bank and used as negative control and processed as study samples. (Table: 4). Persons handling the notes and coins were asked to deposit them in a separate sterile polythene bag and immediately transported to the laboratory for microbial analysis. They were compensated with other currency of the same denomination (Figure: 1).

Figure 1: Collection of samples

Schematic overview of experimental programme

The protocol started with the collection of currency note samples, preparation of samples for microbial determination, inoculation, incubation, to identification of different bacterial isolates, is shown in Table: 1.

Laboratory Processing

Bacteriological Analysis: The samples from each currency notes were collected with two sterile cotton swabs dipped in normal saline. Swabbing was done from both sides of currency notes. Then the swab samples were subjected to laboratory analysis where they were inoculated on blood agar and McConkey's agar media. The plates were incubated aerobically at 37° C for 24 hours and observed for the growth of bacteria. Mixed growth was further subcultured to obtain a pure colony. The colony was identified phenotypically by colony characteristics, Gram staining and performed biochemical tests as per standard protocol.[24] In the present study, the cultural characteristic features and biochemical analysis of positive isolates were studied and represented in (Table: 2 and Figures: 2 to 6).

Table 1: Algorithm

<table>
<tr><td colspan="8">• STEP 1: SAMPLE COLLECTION</td></tr>
<tr><td>DAY 1</td><td colspan="2">Study participant: To go through the Patient Information Sheet (Kannada and English)</td><td colspan="2">Informed consent form: Accept and signature (Kannada and English)</td><td colspan="2">Patient data entry form: Name, age, gender, etc. (Kannada and English)</td><td>Currency collection: Sterile polythene bag</td><td>Replacement: currency on site</td><td>Transportation: To the Microbiology lab within 30 minutes for microbial analysis</td></tr>
<tr><td colspan="8">• STEP 2: SAMPLE PROCESSING PROTOCOL IN THE LABORATORY</td></tr>
<tr><td rowspan="3">DAY 1 (Continued)</td><td>Swabbing from currency: Sterile cotton swabs dipped in sterile normal saline</td><td>Sample: Placed in a screw capped sterile saline tube immediately</td><td>First: Inoculation on BA & MC agar & incubate at 37^0 C for 24 hours</td><td>Second: Saline & Iodine wet mount before centrifugation</td><td>Third: Remaining sample transfer to sterile tube and centrifuge for 5 minutes</td><td>Fourth: Deposit subject to Z-N stain</td><td>Fifth: Deposit subject to LPCB mount</td><td>Sixth: If LPCB shows fungal elements, culture on SDA & observe for 1 week</td></tr>
<tr><td colspan="8">Seventh: when coins are collected, the coins samples also should be processed similarly as currency notes.</td></tr>
<tr><td colspan="8">Eighth: Uncirculated or Mint currency notes and coins of different denominations procured from bank as control, swabbing, inoculation and incubation should be carried out as currency samples.</td></tr>
<tr><td>DAY 2</td><td colspan="7">• STEP 3: BACTERIAL CHARACTERIZATION AND IDENTIFICATION</td></tr>
</table>

Table 2: Cultural Characteristics and Biochemical Analysis of Bacterial Isolates

	Cultural characteristics				**Staining**	**Biochemical analysis**								
SL. No.	**Shape & arrangement**	**Elevation**	**Consistency**	**Colour**	**Gram**	**Catalase**	**Coagulase**	**Oxidase**	**Motility**	**Indole**	**Citrate**	**TSI**	**Urease**	**ISOLATES**
1	Cocci in clusters	Small, opaque & spherical	Moist, non-haemolytic	Grey white/ yellow	+	+	-	-	NT	NT	NT	NT	NT	CoNS
2	Cocci in tetrads	Small, circular & convex	Moist, non-haemolytic	Grey white	+	+	-	-	NT	NT	NT	NT	NT	Micrococci sp.
3	Rod shaped	Small, shiny & opaque	Small, dry colonies	white to cream	+	+	NT	NT	NT	NT	NT	NT	NT	Diphtheroids sp.
4	Long & bamboo stick	Large, flat	Ground glass appearance	Pink, yellow, or brown	+	NT	NT	NT	NT	NT	NT	NT	NT	Bacillus sp.
5	Medium size, rod	Flat colonies	dry, non-mucoid	Pink	-	+	NT	-	+	+	-	A/A	-	Escherichia coli
6	Small rod, plumpy	Medium, circular & convex	Mucoid	Pink	-	+	NT	-	-	–	+	A/A & gas	+	Klebsiella sp.
7	Medium size, rod	Flat with serrated edges	dry colonies	Green-blue	-	NT	NT	+	+	-	+	NC	NT	Pseudomonas sp.
8	Medium size, rod	Small convex	dry colonies	Pink to brown	-	NT	NT	+	+	+	NT	NT	NT	NFGNB

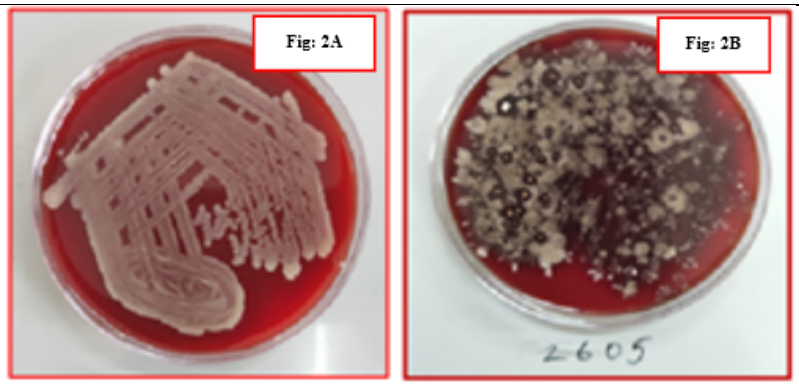

Figure 2: Aerobic bacterial spores' colony on Blood agar (Fig: 2A and 2B - white, dry and smooth colonies)

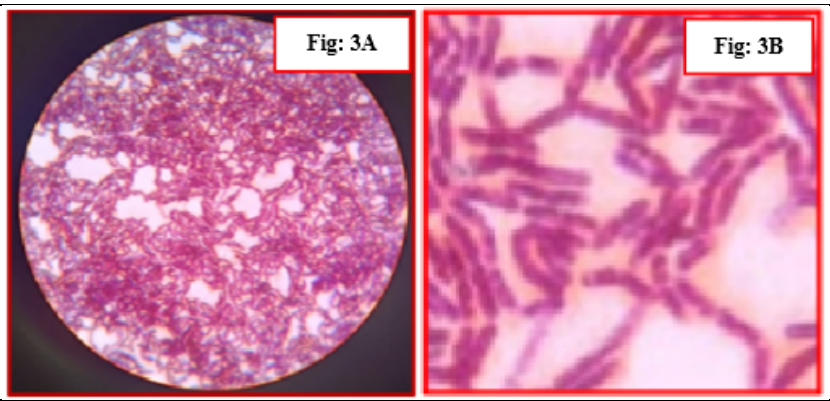

Figure 3: Aerobic bacterial spores by Gram stain (Fig: 3A) with spores enlarged picture (Fig: 3B)

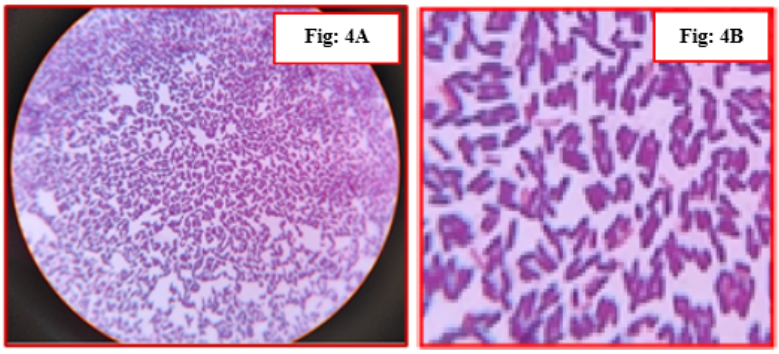

Figure 4: Diphtheroids (palisade arrangement) by Gram stain (Fig: 4A) with enlarged photo (Fig: 4B)

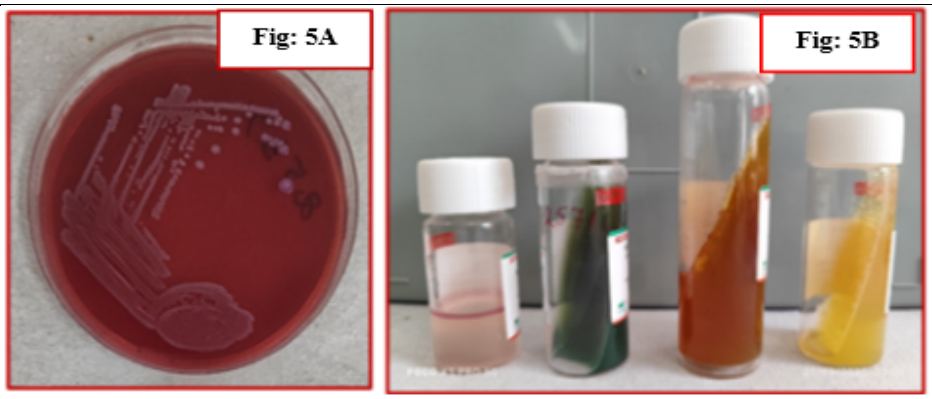

Figure 5: Escherichia coli: (Fig: 5A) LF colonies on MacConkey agar with biochemical tests. (Fig: 5B) (Indole: Positive, citrate: Negative, TSI: A/A and Urease: Negative).

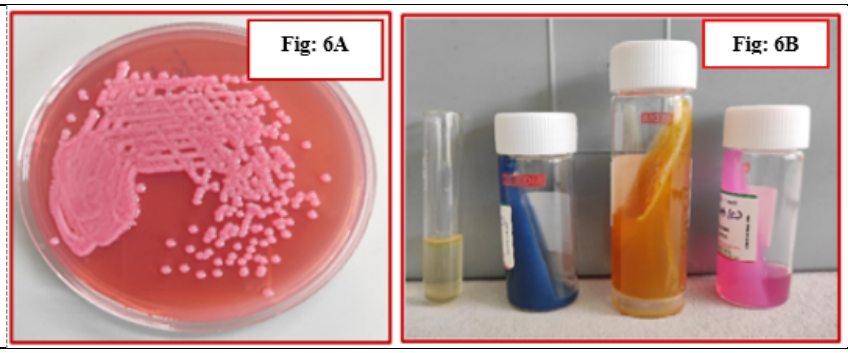

Figure 6: Klebsiella species: LF colonies on MacConkey agar (Fig: 6A) with biochemical tests. (Fig: 6B): (Indole: Negative, citrate: Positive, TSI: A/A with gas and Urease Positive.

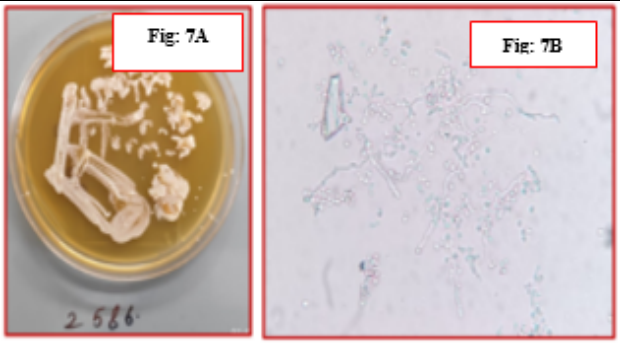

Figure 7: Candida species: White colonies on SDA medium (Fig: 7A) and (LPCB mount - Candida species and germ tube positive - (Fig: 7B)

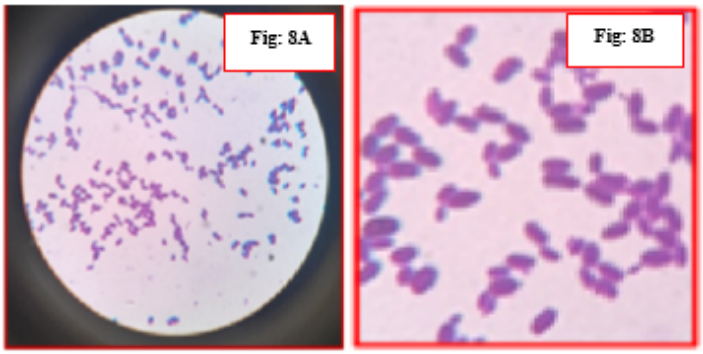

Figure 8: Candida species by Gram stain (Fig: 8A) with enlarged photo (Fig: 8B)

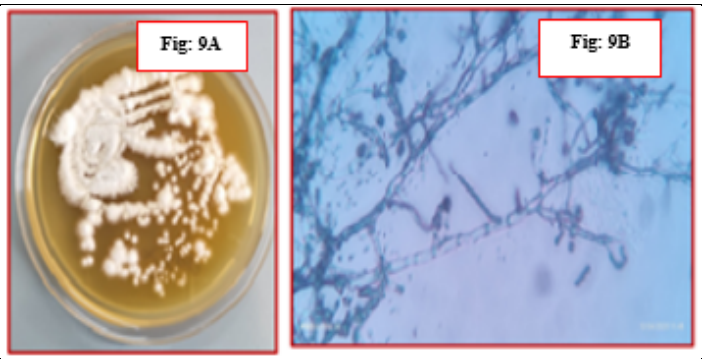

Figure 9: Fungal growth on SDA medium (Fig: 9A) and LPCB Mount- Septate hyphae with Branches and Conidia –?(Cladosporium species) (Fig: 9B)

Parasitological Analysis: The cotton swab moistened with sterile physiological saline is placed in a capped bottle containing 5 ml normal saline. Then, direct wet mount such as saline and iodine wet

mount preparation was carried. The saline and iodine wet mount preparation were screened for trophozoites, cysts and eggs. The preparations were then examined under low (10X) and high power (40X) objectives.[24]

Fungal Elements Detection: The cotton swab moistened with sterile physiological saline is used to swab both sides of the currency notes. The fungal contamination was identified with the presence or absence of fungal elements detection on currency notes by Lacto-Phenol Cotton Blue (LPCB) mount. If LPCB mount showed any fungal elements, then those samples were inoculated on Sabouraud chloramphenicol agar plate and incubated at room temperature. Finally, slide culture was done to study typical morphology for identification (Figures: 7, 8 and 9).[24]

Identification of Acid-Fast Bacilli: After inoculating the agar plates, the remaining test sample was transferred into sterile centrifuge tubes and centrifuged for 5 minutes. The supernatant was decanted; the smears were made on microscope slides from the deposits. The film was air-dried, heat fixed and stained with Ziehl–Neelsen method and finally examined under the microscope for acid-fast Bacilli.[24]

Statistical Analysis: The data obtained from this study was entered in Microsoft excel and analysed using Jamovi software. Microbial contamination is reported using frequency and percentage. The most prevalent organism was observed from statistical data. Chi-square test or Fisher's exact test was used to find the association among categorical variables. Comparison of data was carried out between various denominations of currency notes and statistical data analysis involved descriptive analysis of bacterial isolates, where by bar charts and frequency distribution tables were drawn. A p value < 0.05 was considered statistically significant.

Results

The currency notes collected from different sources of people and contamination percentage in the rural tertiary care hospital settings presented in Figure: 10. The Indian paper currency and coins of different denomination (paper currency: Rs. 10, 20, 50, 100, 200, 500 and coins: 10 and 20) were contaminated with both pathogenic and non-pathogenic organisms of bacteria, fungi and parasites and shown in the (Table: 3, 4 and 5).

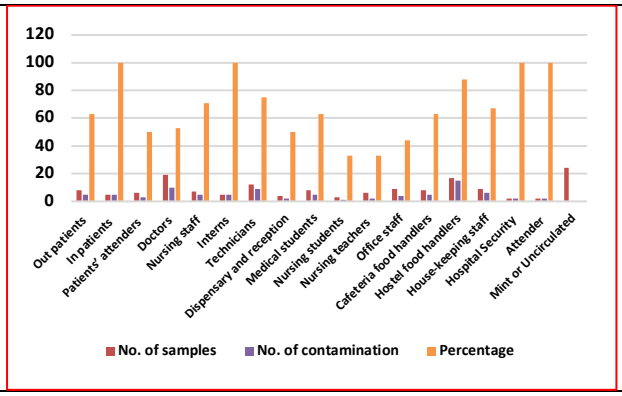

Figure 10: Sources of currency, Number of currencies and Contamination with Percentage

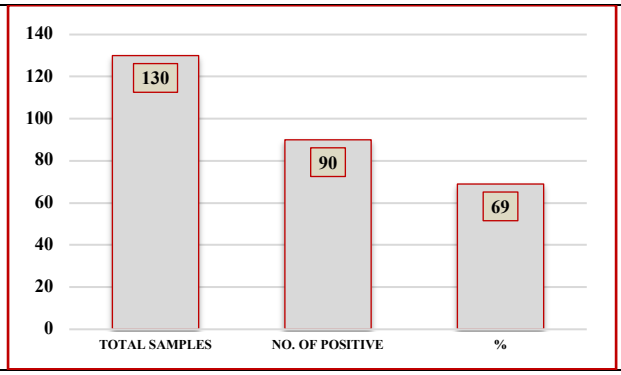

Figure 11: Prevalence of Microbial Contamination of Indian Currency Notes

A total of 90 (69%) organisms were isolated from 130 currency notes subjected to microbial analysis (Figure: 11). Out of the 130 samples, 126 were paper currency, of which 90 (69%) were contaminated,

but, of the total 4 coins collected, none of them were contaminated (Table: 3 and 4). A total of 8 different genera of bacteria, 2 genera of fungi and 1 genus of parasite were isolated from the Indian currency notes (Table: 4). In this study, the most common isolates were Bacillus species 38%, followed by Coagulase negative Staphylococcus (CoNS) 20%, Micrococcus species 16% and Diphtheroids 12%, from the currency notes. The prevalence of Gram-negative isolates were Escherichia coli 5%, Klebsiella species 3%, Pseudomonas species 1%, non-fermenting gram-negative bacilli (NFGNB) 1%. The fungal species (Candida species and (?) Cladosporium species) 2% (1 each) and Protozoan cysts (Entamoeba histolytica and Entamoeba coli (normal intestinal commensal protozoan parasite)) (1 each) 2% (Table: 4 and Figure: 12). Table: 4 shows the organisms isolated from the study according to locations sampled.

Table 3: Types of bacteria based on Gram's reaction (N=86) (66%)

	Gram-positive bacteria (N= 77) (90%)				Gram-negative bacteria (N= 9) (10%)	
Sl. No	Gram-positive cocci N= 32 (37%)		Gram-positive bacilli N= 45 (52%)		E-coli	4 (5%)
1	CoNS	18 (21%)	Bacillus species	33 (38%)	Klebsiella species	3 (3%)
2	Micrococci	14 (16%)	Diphtheroids	12 (14%)	Pseudomonas species	1 (1%)
3	----	----	----		NFGNB	1 (1%)

Out of 86 bacterial isolates, 77 were Gram-positive and 9 were Gram-negative organisms in this study (Table: 3). A total five denominations of INR 10 (2), 20 (1), 50 (1) and 100 (1) were showed mixed contaminations. Out of two, INR 10 denominations, one, 10 INR, denomination showed two protozoan parasites, Entamoeba histolytica and Entamoeba coli, whereas other INR 10 showed filamentous fungus - (?) Cladosporium species with CoNS were contaminated. On 20, 50 and 100 notes, NFGNB and CoNS, Diphtheroids and Bacillus, and Klebsiella and Candida species were contaminated respectively.

Table 4: Relationship between source of currency and type of contamination (Microorganism isolations (N=90) (69%)

SL. NO	Source of Currency Notes/and or Coins	No. of samples (N=130)	Bacillus species (N=33) (37%)	Diphtheroids (N=12) (13%)	CoNS (N=18) (20%)	Micrococcus species (N-14) (16%)	E-coli (N=4) (5%)	Klebsiella species (N=3) (3%)	Pseudomonas species (N=1) (1%)	NFGNB (N=1) (1%)	Candida species & (?)Cladosporium species (N=2) (2%)	E. histolytica & Entamoeba coli (N=2) (2%)
1	Out patients	8	3	---	1	1	---	---	---	---	---	---
2	In patients	5	0	1	1	1	---	1	1	---	1	---
3	Patients' attenders	6	1	---	1	1	---	---	---	1	---	---
4	Doctors	19	5	2	2	---	---	---	---	---	1	---
5	Nursing staff	7	3	2	---	---	---	---	---	---	---	---
6	Interns	5	2	1	1	---	---	---	---	---	---	2
7	Technicians	12	4	---	3	2	---	---	---	---	---	---
8	Dispensary and reception	4	1	---	---	1	---	---	---	---	---	---
9	Medical students	8	3	---	2	1	---	---	---	---	---	---
10	Nursing students	3	1	---	---	---	---	---	---	---	---	---
11	Nursing teachers	6	---	2	---	---	---	---	---	---	---	---
12	Office staff	9	2	1	1	---	---	---	---	---	---	---
13	Cafeteria food handlers	8	1	---	1	2		1	---	---	---	---
14	Hostel food handlers	17	4	1	3	3	3	1	---	---	---	---
15	House-keeping staff	9	2	1	1	1	1	---	---	---	---	---
16	Hospital Security	2	1	---	1	---	---	---	---	---	---	---
17	Attender	2	---	1	---	1	---	---	---	---	---	---
18	Control currency: (Mint or Uncirculated)	24	00	00	00	00	00	00	00	00	00	00

Table 5: Relationship between currency denomination and type of contamination

Indian currency denomination (N=130)		Contamination isolated from different denominations (N=90) (69 %)				
		Bacteria	Fungus	Parasites	Total	%
Notes	10 (39)	24	1	2	27	69
	20 (32)	25	00	00	25	78
	50 (21)	14	00	00	14	67
	100 (23)	15	1	00	16	70
	200 (5)	3	00	00	3	60
	500 (6)	5	00	00	5	83
	Total: (126)	86	2	2	90	69
Coins	10 (3)	00	00	00	00	00
	20 (1)	00	00	00	00	00
	Total: 4	00	00	00	00	00

A total of 90 (69%) organisms were isolated from 130 currency notes subjected to microbial analysis. Out of 130 currency notes subjected to microbial analysis 40 (31%) were found free from microorganisms and found negative. Among the 90 isolates 13 (14%) were pathogenic and 77 (86%) were non-pathogenic environmental organisms.

Among the currency notes of different denominations, the trend of maximum percentage of contamination occurred on lower denominations i.e., with rupees 20 and 10 accounting at 25 (78%) and 27 (69%) respectively, followed by 50 rupees 14 (67%). However, higher denomination notes of 100 also showed higher contamination rate with 16 (70%). On the other hand, further higher denomination notes of 200 and 500 also showed higher contamination rate with 5 (60%) and 6 (83%) respectively. However, although contamination rates are high in the 200 and 500 denominations, this may be due to the fact that only a few currency samples were collected in higher value rupees, and thus may be the reason showed higher contamination rate when compared to the lower denomination currencies (Table: 5 and Figure: 13).

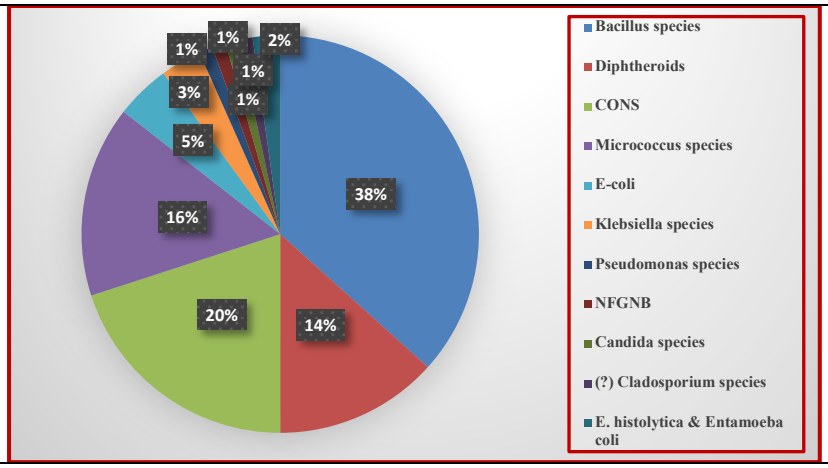

Figure 12: Graphical Representation of Prevalence of Microbial Isolates

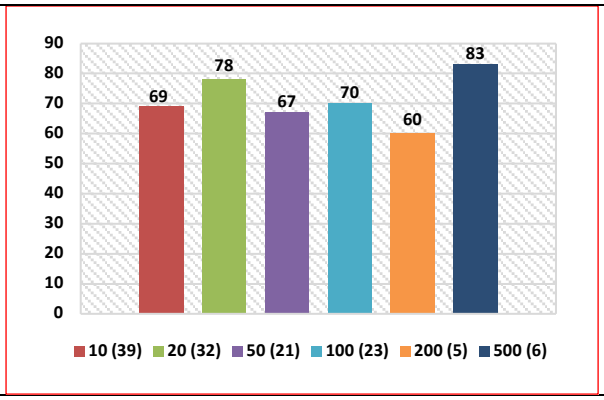

Figure 13: Total number of each currency denomination with contamination rate

Table 6: Association of contamination with various denomination of currency notes

Group	Currency contamination	
	Yes	No
R-1	66	26
R -2	24	10
R-1: INR: 10, 20 and 50 and R -2: INR: 100, 200 and 500		

A chi-square test was performed to find the association between currency notes of different denominations (R-1 Vs R-2) and contamination status (Table: 6). The test yielded a statistic value of 0.016 with 1 degree of freedom and p value 0.8990, indicating that there is no statistically significant association between the two groups.

Table 7: Association of contamination with various intra-category of Group-2 (HCWs)

Group	Currency contamination	
	Yes	No
S-1	26	18
S-2	14	6
S-1: Doctors, Nursing staff, Technicians and Nursing teachers. S-2: Interns, medical students, nursing students, dispensary and reception.		

A chi-square test was performed to find the association between groups (S-1 V/s S-2) and contamination status (Table: 7). The test yielded a statistic value of 0.698 with 1 degree of freedom and p value 0.403, indicating that there is no statistically significant association between the two groups.

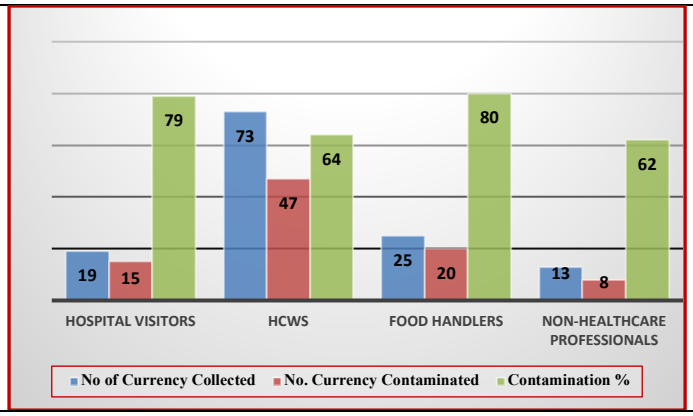

Figure 14: Total No of Currency Samples, Contamination and Percentage

Among the different categories and sources (Table: 8), the highest percentage of contamination rate was occurred in food handlers at 80%, followed by hospital visitors at 79%, and HCWs at 64%, while in non-healthcare professionals showed lowest level of contamination at 62% (Tables: 7 and Figure: 14). Fisher's exact test was applied to assess the association between participant group (HCWs V/s HV) and frequency of contamination. The test yielded a p value of 0.281, indicating that there is no statistical significance between the groups. Fisher's exact test was applied to assess the association between participant group (HCWs V/s NHP) and frequency of contamination. The test yielded a p value of 0.100, indicating that there is no statistical significance between the groups. Fisher's exact test was applied to assess the association between participant group (Group-2: Intra-category (Cafeteria food handlers and Hostel food handlers)) and frequency of contamination. The test yielded a p value of 0.283, indicating that there is no statistical significance between the groups.

Table 8: Percentage prevalence of microbial isolates from different categories

Group	Category	Source of Currency Notes/and or Coins	No. of Samples (N=130)	No. of Contamination and percentage (N=90) (69%)
Group-1	Hospital visitors (HV)	Out patients	19	15 (79%)
		In patients		
		Patients' attenders		
Group-2	Healthcare Workers (HCWs)[23]	Doctors	73	47 (64%)
		Nursing staff		
		Interns		
		Technicians		
		Dispensary and reception		
		Medical students		
		Nursing students		
		Nursing teachers		
		House-keeping staff		
Group-3	Food handlers (FH)	Cafeteria food handlers	25	20 (80%)
		Hostel food handlers		
Group-4	Non-healthcare professionals (NHP)[23]	Office staff	13	8 (62%)
		Hospital Security		
		Attender		
Group-5	Control notes (Bank notes)	Mint or Uncirculated	24	00 (%)

The table: 9 revealed that group-2: HCWs represented with the highest Gram-positive environmental isolates (48%) recovered. Next highest contamination was with non-pathogenic Gram-positive isolates occurred in group-3: FH (17%), followed by group-1: HV (12%), while least contamination with group-4: NHP (9%). Similarly, HCWs and FH groups also recorded the highest contamination with pathogenic isolates (6% and 6%), followed by hospital visitors also recorded with 4% pathogenic isolates. On the other hand, none of the NHP currency contaminated with pathogenic isolates.

Table 9: Isolation of various types of organisms from different groups (N=90) (69%)

	Environmental isolates N= 77 (86%)					Pathogenic isolates N= 13 (14%)								
	Gram-positive bacteria N= 77 (86%)					Gram-negative bacteria N=9 (10%)					Fungi N=2 (2%)		Parasites N=2 (2%)	
Group	Bacillus species (N=33) (38%)	Diphtheroids (N=12) (14%)	CoNS (N=18) (20%)	Micrococcus species (N-14) (16%)	Total Environmental isolates	E-coli (N=4) (5%)	Klebsiella species (N=3) (3)	Pseudo monas species (N=1) (1%)	NF GNB (N=1 (1%)	Total Gram-negative	Candida species (N=) (1%)	(?) Cladosporium species (N=1) (1%)	E. histolytica & Entamoeba coli (N=2) (2%)	Total Pathogenic isolates
Group -1 (HV)	4	1	3	3	11 (12%)	00	1	1	1	3 (3%)	1	00	00	4 (4%)
Group-2 (HCWs)	21	8	9	5	43 (48%)	1	00	00	00	1 (1%)	00	1	2	5 (6%)
Group-3 (FH)	5	1	4	5	15 (17%)	3	2	00	00	5 (6%)	00	00	00	5 (6%)
Group-4 (NHP)	3	2	2	1	8 (9%)	00	00	00	00	00 (0%)	00	00	00	0 (0%)

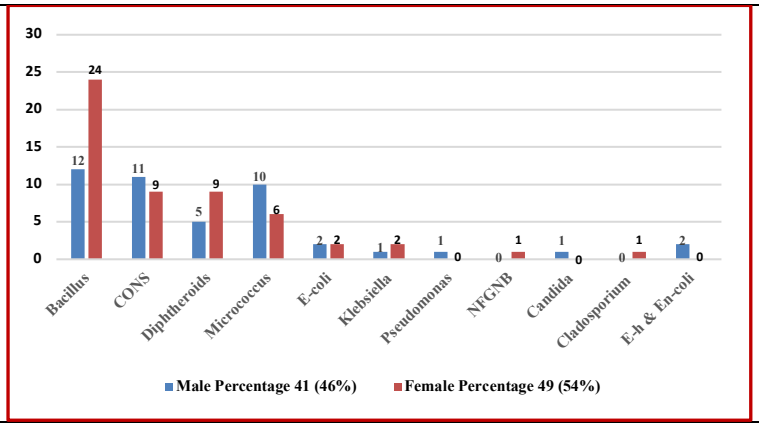

Figure 15: Percentage Prevalence of Microbial Isolates in Males and Females

In the sex ratio, a total of 61 (47%) male specimens were collected, out of this, 41 (46%) currency notes were contaminated (Figure: 15). In males, the highest parentage of isolates recovered were Bacillus species 11 (12%), followed by CoNS 10 (11%), Micrococcus species 9 (10%) and Diphtheroids 4 (5%). In addition, E-coli, 2 isolates and 1 isolate of Klebsiella species (Figures: 5 and 6) and 1 isolate of Pseudomonas species were grown from contaminated notes. Apart from this, males' specimens, 1 Candida species (germ-tube test positive (Figures: 7 and 8). And 2 protozoan parasites, (1 isolate of Entamoeba histolytica and 1 isolate of Entamoeba coli each) were identified by saline and wet mount preparation. Whereas, 69 (53%) female specimens were collected, out of this, 49 (54%) currency were contaminated. In females, the highest parentage of isolates recovered were Bacillus species 22 (24%), CoNS 8 (9%), Diphtheroids species 8 (9%) and Micrococcus species 5 (6%). In addition, E-coli, 2 isolates, Klebsiella 2 isolates (Figures: 5 and 6) and NFGNB I isolate were also isolated. Apart from this, females' specimens 1 filamentous (mould) species of (?) Cladosporium species also identified by LPCB mount and SDA slide culture method (Figure: 9 and 15).

Similarly, we processed 12 new currency (mint or uncirculated paper currency and coins) notes and 12 new coins of various denomination procured from the local bank in the campus and used as negative control in the present study. None of the control samples showed growth and found free from microorganisms (Table: 4).

Discussion

We analysed a total 130 samples of currency comprising of 126 paper currency and 4 coins of various denominations and were contaminated with both pathogenic and non-pathogenic organisms of bacteria, fungi and parasites. In the present study, most of the currency notes were contaminated with non-pathogenic environmental organisms 77 (86%) such as Bacillus species, CoNS, Micrococcus and Diphtheroids. In addition, 13 (14%) pathogenic microbes also isolated with significant per cent in the present study such as Escherichia coli, Klebsiella species, Pseudomonas species and NFGNB representing with 10%. Apart from this, 2 fungi, 1 isolate of Candida species, and 1 isolate of (?) Cladosporium species and 1 pathogenic protozoan, Entamoeba histolytica and 1 normal intestinal commensal, Entamoeba coli also identified in the present study (Table: 4).

Studies in different parts of India and the World also showed that more or less common organisms isolated from contaminated currency were non-pathogenic environmental organisms such as Bacillus species, CoNS, Micrococcus species and Diphtheroids, Ahmed et al, (2017),[3] Anitha et al, (2020),[4] Kalpana et al, (2014),[7] Zarrin et al, (2018),[21] Singh et al, (2015),[22] Jane-Francis et al, (2014),[13] and Alemayehu et al, (2019).[23] Bacillus species, a vast group of hardy aerobic spore forming Gram-positive rods that inhabit the soil and thus ubiquitous in the environment, could also be transferred on money due to placement on dirty surfaces or with soil material. However, Bacillus species may cause a wide variety of diseases from food poisoning, wound and skin infections, respiratory and gastrointestinal problems (emetic exotoxin) to life threatening diseases such as meningitis and septicaemia, Elumalai et al, (2012),[11] Singh et al, (2015)[22] and Jane-Francis et al, (2014).[13] On the other hand, with respect to Gram-positive cocci isolates such as CoNS and Micrococcus are normally associated with the human skin as normal skin flora and can contaminate anything that comes in contact with hands. Similarly, Gram-positive rod, Diphtheroids also a normal skin flora and thus may be the reason of significant contaminant in the present study. These environmental organisms do not typically cause infections in healthy people rather they may cause significant infections in immunocompromised host.

In our study, 69% currency notes were contaminated with various kinds of pathogenic and non-pathogenic bacteria. Various studies conducted by many authors with the prevalence of contamination, such as Janardan et al, 75% (2009),[5] Muhumuza Allan et al, 100% (2018)[16] and Sucilathangam et al, 86% (2016).[20] The differences of contamination rates may be attributed to regional variations, hygienic practices and handling of currency in different areas and also show that microbial contamination of money is a global problem.

In the present study, of the 77 isolates, 90% were Gram-positive bacteria, whereas, 9 isolates, 10% were Gram-negative (Table: 9). Similarly, a study done by Ahmed et al, (2017),[3] 82% were Gram-positive bacteria and 18% were Gram-negative organisms. On the other hand, Anitha et al, (2020),[4] showed that 52% were Gram positive bacteria and 48% were Gram negative bacteria, these studies are not in agreement with our study.

Parasites that have been observed in our study was a protozoan parasite of 1 isolate of pathogenic fauna, Entamoeba histolytica and 1 isolate of intestinal normal fauna, Entamoeba coli, which represented 1% each. Neel et al, (2018) [14] also showed contamination in notes and coins with Entamoeba histolytica, 27% and 55% respectively but with very high prevalence rate and so, not comparable with our study. The intestinal parasitic infections mostly due to hands used in cleaning up the anus after passing out faeces are not properly washed and are used to touch paper moneys and tendency of contamination with the cysts or eggs.

A fungus that recovered from our study was Candida species with 1%. A number of studies have documented Candida species contamination in their studies, Kalpana et al, (2015) [7] Candida species 2%, comparable to our study, whereas, studies of Anitha et al, (2020),[4] Candida species prevalence was 19%. Another fungus, we isolated in our study was (?) Cladosporium species also with 1% prevalence. Singh et al, (2015),[22] also recovered, Cladosporium cladosporioides with 9% prevalence in their study. Candida, though a normal flora in human beings causes a secondary infection in HIV/AIDS affected people. Cladosporium, widespread environmental fungus, is a saprophytic and cosmopolitan in distribution. Some species of genus Cladosporium are associated with allergic rhinitis, respiratory arrest in asthmatics.

Contamination frequency of INR was found related to the denomination of currency. The observation in the present study showed that lower denominations had higher level of microbial contamination i.e., with rupees 20, 10 and 50 accounting at 78%, 69% and 67% respectively. However, higher denomination notes of 100 also showed higher contamination rate with 70%. Further, higher denomination notes of 200 and 500 also showed higher contamination rate with 60% and 83% respectively. Although contamination rates are high in the 200 and 500 denominations, this may be due to the fact that only a few currency samples were collected and thus may be the reason showed higher contamination rate when compared to the lower denomination currencies (Table: 5 and Figure: 13). The study observation is in agreement with the findings of other workers in India and abroad, Janardan et al, (2009) [5] Rupees 10 and 5 notes (75%) and Rupees 500 and 1000 notes (20%). Haile Alemayehu et al, (2019),[23] (ETB: Ethiopian Birr) ETB 1 (23.2%), 5 (19.7%) and 10 (23.5%). and Zarrin et al, (2018) [21] 10 and 20 INR was 23% followed by 50 INR: 22%. The fast circulation of lower denominations for daily transactions than higher denominations and the higher rate of exchange predisposes to higher levels of contamination. However, no denomination Indian rupees were protected from contamination as we detected microbial growth in all denominations of notes investigated.

Studies in different parts of the world reported that in community based and hospital settings showed variation in contamination rates and compared with the present study contamination frequencies. The present study was conducted in the hospital setting with the most common prevalent isolates were non-pathogenic environmental organisms representing 86% occurrence. The prevalence of pathogenic bacteria at 10%, fungi 2% and protozoan parasite 2% (Table: 9 and Figure: 12). Similarly, studies conducted in community-based works also showed that most contamination rate more or less similar with our study with respect to environmental organisms, however, there is differences in percentage frequency, Ahmed et al, (2017) [3], where currencies were maximally contaminated with Bacillus species (57%). Haile Alemayehu et al, (2019),[23] where maximally contaminated with Bacillus species (51%) and Micrococci species (19%). Similarly, the pathogenic bacteria present in the currency are also isolated by many authors, Agarwal et al, (2015) [2] 79%, Anitha et al, (2020) [4] 48% and Zarrin et al, (2018) [21] 49.53%. However, in the present study, we isolated pathogenic organisms were 14% and not in agreement with authors mentioned above. The discrepancy in the bacterial pattern may be attributed to the regional variation of bacterial profile and habits of the local people.

In our study, we categorised the currency samples into 5 groups such as group 1 (HV), 2 (HCWs), 3 (FH), 4 (NHP) and 5 (Control) (Tables: 8 and 9). Notably, the highest prevalence of currency contamination occurred in group-2-HCWs with Gram-positive non-pathogenic environmental

organisms and Gram-negative pathogenic isolates at 48% and 5% respectively. Similarly, the second highest notes contamination happened with 17% and 6% in group-3-food handlers. Apart from this, group-1- hospital visitors' currency also contaminated significantly with both Gram-positive as well as Garm-negative organisms 12% and 4% respectively. On the other hand, group-4-NHP paper notes, least contaminated only with Gram-positive organisms, however none of the paper notes contaminated with pathogenic Gram-negative organisms. Pathogenic organisms can cause disease in healthy host while non-pathogenic organisms can cause disease in immunocompromised host. Group-5- Control notes include samples, where mint or uncirculated paper currency and coins were collected from the local bank, used as negative control. None of the control samples showed growth and found free from microorganisms (Table: 9 and Figure: 14). Coliforms detection in currency is indicative of faecal contamination and poor sanitary conditions and personal hygiene practices of currency handlers. The absence of other pathogenic enteric organisms such as Salmonella and Shigella in the present study might be due to the fact that such pathogens are generally not competitive in the presence of high commensal flora may affect their survival on the contaminated currency notes. Pseudomonas species and NFGNB were other potentially pathogenic organisms recovered in currency. Pseudomonas and NFGNB species are important opportunistic pathogens causing a wide range of acute and chronic infections in immunocompromised individuals. The bacteria isolated belong to Enterobacteriaceae family found in the air and also in in faeces as normal enteric flora, which are known to cause watery diarrohea, gastrointestinal diseases, food poisoning and nosocomial infections. In addition, Gram-negative sepsis may also be caused by E. coli, Klebsiella and Pseudomonas species. According to several studies, pathogenic bacteria isolates contaminate food items. E. coli O157:H7 contaminated with paper currency could serve as a potential vehicle for transmitting the infection, Anitha et al, (2020)[4] Jaswinder Singh et al, (2022)[6] Girma et al, (2015) [10] and Sunil et al, (2020).[15]

In the present study, Group-2 is representing with HCWs (Classifying Health Workers) [25] where non-pathogenic environmental organisms and pathogenic organisms were found in 48% and 5% respectively (Table: 9). In the study carried out by Sucilathangam et al, (2016) [20] where HCWs showed that currency contamination with 6% in their study and comparable to our study. Zarrin et al, (2018) [21] study revealed that HCWs currency contaminated with 49.53% pathogenic and 50.47% were non-pathogenic organisms. Sunil et al, (2020) [15] found in their study of the hospital group, isolated pathogenic organisms S. aureus 54% with MRSA 8% and E. coli 25%.

Group - 3 comprises of food handlers and we found that 17% of currency notes contaminated with environmental bacteria whereas, 6% were with pathogenic bacteria. None of the notes were contaminated with fungus or parasites. In the study carried out by Ofoedu et al, (2021)12 found in 13% of Naira currency contaminants recovered from different food vendors and Jane-Francis et al, (2014) [13] reported that 15% of food vendors currency were contaminated with bacteria and fungi in agreement with the present study.

Conclusion

Currency contamination is associated with diseases because of significant percentage of transaction for routine lifestyle is by paper currency, mostly in lower denominations. Currency notes are often touched during everyday life. Paper currency made of cotton/linen composition and offers a large surface as breeding ground for microbes which can persist on it for longer periods. Women, especially among the unenlightened, often place money underneath their brassieres, handkerchief, under the pillow, rug or bed, while men place theirs in their pockets, socks etc. Several behavioural practices in our study site may contribute to currency contamination, keeping money under body surfaces, improper washing of hands after using the toilet, wetting fingers with saliva when counting currency, coughing and sneezing on hands and handling currency.

The significant per cent of currency contamination prevalence of both Gram-positive and Gram-negatives isolates documented in HCWs from the present study indicated that the need of more precaution in the hospital premises to control the spread of the infections. Microorganisms may survive on paper currency may serve as a potential source of enteropathogens causing sporadic cases of foodborne illness because food vendors may serve food with the hands and at the same time handle paper currency as they sell. Improved personal hygiene standards are highly solicited to reduce risk of infection from currency. Washing hands thoroughly by food handlers, whether at a restaurant or at home after handling currency and before handling food. Thus, simultaneous handling of money and food should be discouraged unless proper hygiene is observed. Public awareness should be created to avoid cross contamination of currency notes. Shortening the duration of circulation, use of credit cards and introduction of plastic currency notes as in Australia, which can be washed easily. Finally, we recommend that similar studies on the microbial contamination of currency be undertaken with larger sample size across the World to enrich the global information on the subject.

Abbreviations

CoNS: Coagulase negative Staphylococcus species, ESBLs: Extended Spectrum Beta-Lactamase Producers, FH: Food handlers, HCWs: Healthcare workers, HV: Hospital visitors, IPD: Inpatient department, LF: Lactose fermenter, LPCB: Lactophenol cotton blue, MRSA: Methicillin resistant staphylococcus aureus, NFGNG: Non-fermenter Gram-negative bacilli, NHP: Non-healthcare professionals, OPD: Outpatient department, SDA: Sabouraud's dextrose agar.

Ethical Considerations

The study protocol was approved by the Institutional Scientific and Ethics Committees.

Authors' Contribution

All authors have made substantial, direct and intellectual contribution to the work and approved by all authors for publication.

Limitations of the Study

This study had several limitations could be that small sample size may not demonstrate the clear picture, so the results cannot be generalized. Methods used to isolate microorganisms detected only culturable organisms. The samples were processed by conventional method; we did not use any automated or molecular methods for microbial analysis. Inability to quantify the cell numbers of the bacterial agents. It did not record the presence of another category of potential pathogens such as viruses that might contaminate currency.

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