Original Article

Identification of Microbial Contamination: Isolation from Metallic Coins

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Abstract

People of various cultures exchange money on a daily basis, which means that contaminated bank coins might spread germs. Many countries have looked at whether or not coins might spread disease. Recently, metal alloys have attracted a lot of attention as a potential new antibacterial weapon for use in settings where surface cleanliness is paramount. Dollar coins serve as an environmental and interpersonal vector for the spread of bacteria and viruses. Our research aims to quantify and compare the microbiological load of Pseudomonas sp., Staphylococcus aureus, E. coli, Bacillus sp., Klebsiella sp., Salmonella sp., and Proteus species found on money coins found in circulation at the market and at the hospital counter. Separation of Gram-positive and -negative sp., from coins in this study revealed that coins may play a significant role as a vector in the dissemination of hazardous germs in the population. In addition to food poisoning, skin infections, wound infections, gastrointestinal difficulties, and respiratory disorders, the pathogenic bacteria including E. coli, S. aureus, Bacillus sp., Salmonella sp., Klebsiella sp., and Pseudomonas sp. discovered on money coins may cause.

Keywords: Bacteria, Gram, Coins, Wound, Isolation, Infections.

DOI: 10.47750/pnr.2022.13.S03.196

INTRODUCTION

Money is one object that is passed from person to person rather regularly. When money is carried from one location to another, it increases the likelihood that it may get contaminated. Bacteria from the mouth and nose could find their way onto a stack of bills while they are being counted. Depending on the level of sanitation across a society, there might be a wide range in the number of germs present on coins and bills, and hence the danger of transmission by touching money [1]. Although the kinds of bacterial isolates tested varied with respect to methodology, season, location, and source of funding, it was found that Gram-positive bacteria were the most common.

If the coins carry harmful microorganisms, the rates of communicable diseases will rise. It has been discovered that money contains a wide range of microbes from all over the world, including wealthy nations. Paper money has been recovered and has been found to contain Bacillus sp. and Staphylococcus aureus as common pollutants [2–5]. Currency has also been discovered to harbour other bacteria, including Micrococcus sp., Corynebacterium sp., Vibrio cholerae, Mycobacterium TB, and members of the Enterobacteriaceae family. S. aureus, E. coli, Klebsiella, and enterobacter are only a few of the harmful bacteria that have been isolated from US coins and paper cash [8]. The purpose of this research was to determine the extent of bacterial contamination in..... currency coins and to investigate the sources of this problem in the studied region.

METHODOLOGY

Group I consisted of open-market vendors and Group II consisted of hospital cash registers; each group collected 30 coins total, 10 of each denomination (1, 2, and 5). (Group II). The research samples were collected in different ways depending on the intensity of use and the resulting motion. Based on the printing year, coins were consistently produced; for example, currency coins from the year 2012 were used. Coins are immediately brought to the lab for microorganism identification.

Microbiological study

Each coin had a cotton swab dipped in sterile physiological saline and used to swab both sides (0.85 percent NaCl). The swab was used to streak bacteria onto MacConkey agar medium, as shown in Figure 1. After that, the samples were placed in an aerobic incubation chamber and heated to 35°C-37°C over the next 24 hours to allow the microbes to multiply. After exposing plates overnight at 35°C to 37°C, pure cultures were obtained by streaking a small number of cells from a cell suspension onto the appropriate medium. Hemolytic responses and the formation of colonies were detected. To determine how mobile pure cultures were, Gram

staining was used. The number of microorganisms per unit of coin surface area was calculated using established biochemical methods. The total number of microbes discovered in the coinage was calculated.

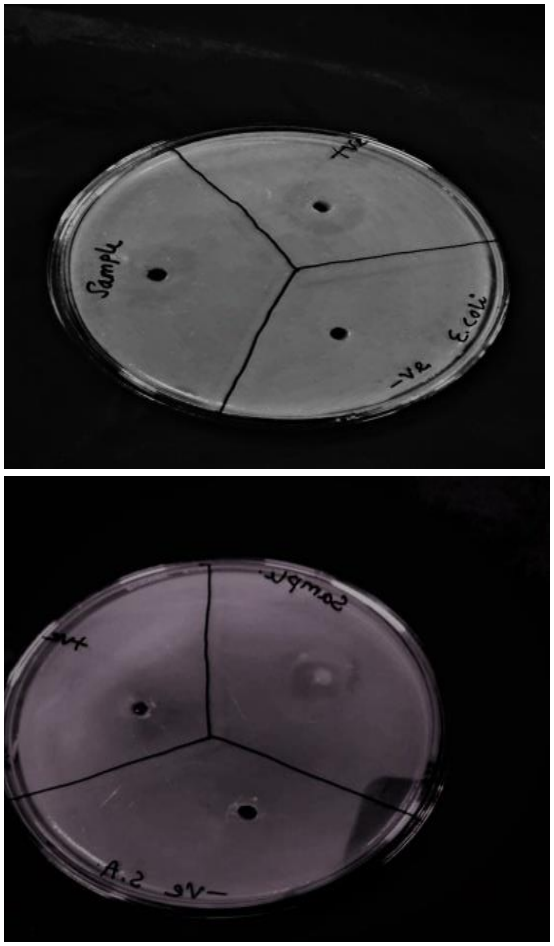

Figure 1. Microbial colonies and culture

Statistical Study

To calculate the outcomes, the Statistical Package for the Social Sciences, Version 21, was used (IBM Statistics, 2019). The level of significance was set at 5% and the groups were compared using Chi-square tests.

Results and Discussion

Thirty coins were examined using microbiological testing, and it was discovered that every single one of them was contaminated with bacteria. Group I market cash and Group II hospital cash coins were both compromised at 100%. Most of the samples contained both microbes and fungi (mixed contamination). Anaerobic spore-bearing microbes and gram-negative bacteria like Escherichia coli and Klebsiella spp. were also shown to play a role. Found at a rate of over 103 CFU/plate. All 15 coins were found to contain either S. aureus (38%) or E. coli (21%), with Pseudomonas sp. (19%) and anaerobic spore-bearing microbes (9%) also being present across all denominations. All 15 currency coins (1, 2, and 5) were tested, with S. aureus (54%) being isolated from all 15 of them; E. coli (25%) and S. aureus (8%) being isolated from 1 and 2, and anaerobic spore-bearing microbes (9%) and non-hemolytic streptococci (4%) being isolated from both 1 and 2. Isolates of harmful bacteria from healthcare facilities and the open market. Chi-square test results for group comparisons are shown in Table 1; these results show statistical significance (P 0.05).

Table 1: Market and healthcare group currency coins with highly harmful bacteria on them, in values of 1, 2, and 5.

Potential pathogens	(Group l)				(Group ll)			
	microbial occurrence %	wise microbial occurrence % 1 2 5			microbial occurrence %	wise microbial occurrence % 1 2 5		
S. aureus	38	24	6	8	54	27	13	14
E. coli	36	23	8	5	25	5	9	11
Pseudomonas sp	19	11	4	4	22	8	7	7
Salmonella sp.	15	8	5	2	8	2	2	4
Bacillus sp	32	9	12	11	30	12	14	4
Klebsiella sp	29	13	6	10	27	7	2	18
Anerobic spore-bearing bacteria	9	2	3	4	9	2	4	3
P	0.034*							

*Statistically significant, Chi-square test

Our study's findings that germs can be removed from coins show that currency may play a significant role in the dissemination of dangerous microorganisms in the community, endangering public health. In each and every instance, it was established that the coins were contaminated. Consistent with previous studies [8,9], the lowest value coins (1 and 2) were the most contaminated. That's to be expected, as smaller coins are traded for more often than their bigger counterparts. We found that the taint on our currency was far higher than on currencies from other developing nations. Our study found that E. coli infection rates were 36% higher in coins from the public market than in coins from hospitals, and S. aureus infection rates were 54% higher in hospitals than in the public market (38 percent). This finding—that E. coli and S. aureus were more prevalent in the smaller denomination (1) than in the bigger denominations (2 and 5)—was unprecedented in the literature. Microbiological contamination of money coins is a global problem, and these variations reflect geographical differences in sanitary

standards and currency coin management.

Genomic DNA Isolation of from Bacteria

After centrifuging 5 ml of the rapidly multiplying bacteria, the pellet was washed twice with wash buffer. The cells were washed, then resuspended in 20 l of solution I containing 2 mg of lysozyme for an hour in a 37 °C incubator. After inverting the mixture, SDS was added to a final concentration of 2% and well incorporated. After 10 litres (5 US gallons) of 5M NaCl were added, the container was turned upside down and left at 20 degrees Celsius for 10 minutes. Five minutes of centrifugation at 12,000 rpm separated the mixture, which was then treated with an equal volume of phenol and chloroform in the supernatant before being precipitated using 2.5 volumes of cooled 95% ethanol. After being dried out, the pellet was mixed with 25 litres of STE buffer. Ten minutes of heating the tube to 37 degrees Celsius was followed by the addition of RNAse (g). Up to 100 litres of sterile deionized water was used to make the solution. The mixture was then subjected to centrifugation at 12,000 rpm for 5 minutes after being stirred by hand with a phenol: chloroform combination (1:1,v/v). An further 2.5 l of cold 95% ethanol was added to the upper aqueous stage, and the whole thing was refrigerated to -20°C for overnight precipitation. Following a wash in 70% ethanol, the DNA was pelleted in a centrifugate at 12,000 rpm for 5 minutes before being dried and dissolved in 0.1X TE16Buffer. The DNA was analysed by running it on an agarose gel at 0.8 percent in 0.5x TEBuffer and electrophoretically tracing its migration.

Polymerase Chain Reaction

The PCR reactions are performed in Eppendorf Personal Mastercycler using 0.2 ml tubes. 16S F 5' AGAGTTTGATCCCTGGCTCAG 3' and 16S R 5' GTACGGCTACCTTGTTACGAC were used to magnify the 16SrRNA gene and identify the strain. The temperatures used for the magnification were 95 degrees Celsius for 5 minutes to begin the denaturation process, 56 degrees Celsius for 2 minutes, 72 degrees Celsius for 1 minute, and 72 degrees Celsius for 10 minutes to complete the extension process.

Table 2: Reaction Mixture Components

S.No	Reagents	Concentration	Volume (µL)
1	Sterile water	-	34.5µL
2	10X Taq buffer	10X	5µL
3	2mm Dntp MIX	0.2mM	5µL
4	Primer I(M13 forward)	4µM	2µL
5	Primer II(MI3 reverse)	4µM	2µL
6	Template DNA	˜50ng	1µl
7	Taq DNA Polymerase	5U/µL	0.5µL
TOTAL			50µL

Gel Electrophoresis of DNA

To verify the presence of DNA, an agarose gel was used to run the isolated sample.

a. 1% Agarose Gel Preparation

1) Using cellophane tape, seal both sides of the gel casting tray, ensure there are no leaks, and insert the comb into a slit.
2) Mix 1% Agarose with 0.5X TEB.
3) Dissolve 250 mg of Agarose in 25 ml of 0.5XTEB in a tiny conical flask.
4) Bring Agarose to a boil in a microwave oven or a water bath until a clear solution forms.
5) Bring the agarose solution down to around 40 degrees Celsius.
6) When the agarose gel temperature is about 40 degrees Celsius, add 1 l of Ethidium Bromide (5mg/ml).Assemble the gel casting tray.
7) Spread Agarose gel on the tray.
8) Don't touch the tray until it's completely solidified.
9) Fill the tank used for horizontal electrophoresis with 0.5X TEB buffer. (It has to look like milk.).
10) Remove the tape and insert the sample in the electrophoresis tank (10) with care.
11) The gel tray should be kept at a level slightly above the buffer (5mm of buffer over the gel).
12) Avoid injuring the wells by gently removing the comb.

b. Samples Preparation, Running and Loading Electrophoresis

1) On a parafilm square, load 3 l of loading dye.
2) Carefully load a 3 l spot of loading dye onto each microplate using 10 l of DNA sample in a pipette.
3) Mix the Contents by Pipetting Up and Down.
4) Reduce the volume of the micropipette to 10 l, and then gently transfer the DNA sample and loading dye mixture to the gel wells.
5) Add 5 l of DNA marker to a designated well.
6) Plug in the positive and negative electrodes to the power source.
7) Activate the power source and adjust the voltage to 50 volts.

c. Amplified DNA

d. After electrophoresis on an agarose gel, fragments of the amplified DNA are eluted and purified by means of the gel purification procedure.

e. Amplified DNA Elution And Purification

The bands were then eluted and purified by means of a gel purification method after being seen as amplified DNA fragments in a UV Trans illuminator. A sterile blade was used to delicately remove the bands of DNA fragments from the gel without damaging the bands. A clean-up kit solution was then added to the mixture to extract the DNA from the gel. After adding isopropyl alcohol to separate the DNA, we

centrifuged at 12,000 rpm for two to three minutes to remove any additional contaminants. The pellets' alkalinity was eliminated by the addition of the wash buffer and subsequent centrifugation. The pellet stores the cloning-ready DNA fragments that have been purified and amplified.

Table 3: bacteria Identification

Isolated code	Gram reaction	Cellular morphology	identity
1	+	cocci	Micrococcus species
2	-	…	Flavobactreriumspeices
3	+	…	Bacillus species
4	+	…	Bacillus species
5	-	…	Enterobacter species
6	+	…	*pneumoniae* species
7	+	…	*Pseudomonas* species
8	+	…	*Enterobacter* species
9	-	…	Acinetobacter species
10	+	…	Bacillus species
11	-	…	Acinetobacter species
12	+	…	*pneumoniae* species
13	+	…	*Streptococcus* species
14	+	…	Bacillus species
15	-	…	Choromobacter species
16	+	Cocci	Micrococcus species
17	-	…	Bacillus species
18	-	…	Pseudommonas species
19	+	…	S*almonella* species
20	+	…	*Streptococcus* species

Coins in our research region may be contaminated due to a number of factors, including the following: not washing hands after using the toilet, constantly coughing into hands and the capacity to handle currency coins, and putting or storing money on filthy parts of skin during exchanges. The large surface area of coins provides ideal conditions for the growth of bacteria capable of withstanding harsh conditions for lengthy periods of time. The surfaces of coins may attract a wide variety of bacteria and other microbes. Community-acquired S. aureus is increasingly seen in the nasal passages of students in areas where they are exposed to filthy conditions [4-7]. No diarrheal diseases have been reported. The need of basic hand hygiene and other pathogen-prevention measures, such avoiding bare-hand contact with ready-to-eat food, cannot be overstated. The hands are a major vector for bacteria that originate in the faeces, the nose, the throat, and the skin. E. coli and Salmonella species have been connected to both the rise of drug-resistant bacteria worldwide and the prevalence of food-borne illness in developed countries [8,9].

Staphylococci (coagulase negative and S. aureus) were found in the most samples from all different types of cash, as shown in Table 1. Coagulase-negative staphylococci were formerly assumed to be harmless, but their importance as infections and rising prevalence have just lately been recognised and studied [6-10]. Infectious S. aureus is a common name. Spreading quickly is the pathogen's capacity to cling to coins for a long period. Normal flora samples were found to include these bacteria. E. coli was found in greater numbers in coins of varying values from both hospital and open market samples than any of the other infections. This bacteria's presence in coins points to faecal contamination and a lack of cleanliness among those who work with money. In order to prevent the spread of disease, it is best to avoid touching money and food at the same time, and instead use different utensils for each.

More investigation is needed to determine whether MDR bacteria may be spread by touching coins in circulation. Due to the study's limited scope, future research will examine the presence of bacteria in all denominations of cash in circulation, a bigger sample (containing both coins and notes), sites where high volumes of money are exchanged, and a greater number of health centres.

CONCLUSION

This research indicated that coins collected from public markets and healthcare institutions were contaminated with harmful microorganisms. Coins purchased from clinics had much higher levels of S. aureus contamination than those purchased on the free market. Initiatives to reduce currency coin contamination were done by offering sanitization at regular intervals, notwithstanding the lack of evidence that bacteria on coins cause sickness. Bank-deposited money coins will be disinfected using ultraviolet light or other ways. Officials are worried that old coins may be tainted, therefore they are recommending that they be thrown away. Between handling money and eating, it's important to wash your hands. It is advised that a system of education be implemented on the proper handling and storage of money coins in order to eliminate impurities. Both the handling of money and the handling of food need a second round of handwashing. There is a dearth of study of the prevalence of germs on banknotes in most nations. More research with a larger specimen size is required since this study examined the frequency of microorganisms in lower-denomination money in circulation.

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