Nat. Volatiles & Essent. Oils, 2021; 8(4): 12089-12099

NVEO
Natural Volatiles & Essential Oils

Isolation, Characterization And Assessment Of Microbial Contamination From Metallic Coins

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Abstract :

Every day, individuals in all societies exchange cash, so mixing bank coins could serve as a medium for bacterial infection transmission. Many countries have looked into the function of coins in the transmission of pathogenic microflora. Metallic alloys have recently attracted a lot of attention as a new antibacterial weapon for sectors where surface hygiene is crucial. In the environment and among humans, currency coins aid as a universal medium for the blowout of microbes. The goal of our research is to quantify and liken the overall microbial load of Pseudomonas sp., Staphylococcus aureus, E. coli, Bacillus sp., Klebsiella sp., Salmonella sp., and Proteus species exist on money coins mingling in the market arena and hospital counter. Separation of Gram positive and negative sp., from coins indicated that coins may play an essential function as a vector in the spread of pathogenic microbes in the communal in the current investigation. The pathogenic microbesidentified on money coins, such as E. coli, S. aureus, Bacillus sp., Klebsiella sp., Salmonella sp., and Pseudomonas sp., can cause a wide range of illnesses, including food intoxication, wound infections, skin contagions, respiratory issues, and gastrointestinal complications.

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

Introduction

Money is among the most commonly handed items from handed to hand around the world. Money can become polluted during its transit and hence have a part in the transfer of microbes to other individuals. During counting, for instance, money may become caused by bacteria from the respiratory and gastro-intestinal tract. Furthermore, a community's overall hygiene concentrations may influence the

proportion of germs present on coins and notes, and hence the risk of transmission through money handling [1]. Although the types of bacterial isolates studied differed according on the procedures utilized, the season, the location, and the kind of money utilized, Gram positive bacteria were most common.

Communicable diseases rates will continue to climb if the coins are infected with dangerous microorganisms. Money has been found to include a variety of microbes from all across the world, even wealthy countries. Bacillus sp. and Staphylococcus aureus have been recovered from paper currency and have been found as prevalent pollutants [2-5]. Other species have been recovered from currency, including Micrococcus sp., Corynebacterium sp., Vibrio cholerae, Mycobacterium tuberculosis, and representatives of the Enterobacteriacea family. Harmful bacteria such as S. aureus, E. coli, Klebseilla, and enterobacter have been recovered from US coins and paper bills [8]. The focus of this research was to isolate and assess the amount of microbiological bacterial contamination of..... currency coins, as well as to investigate any possible causes in the study location.

Methodology

Eight currency coins of each denomination (1, 2, and 5) developing it as a overall 30 coins were gathered, amongst that five coins of 1, 2, and 5 were obtained randomly from open markets (Group I)and also from hospital cash counters (Group II). The research specimens were gathered depends on the concentration of use and thus motion. Coins were consistentbased on year of printing, for instance, currency coins published in the year 2012 were taken. currency coinsare taken to the laboratory immediately for identification of microorganisms.

Microbiological study

Both surfaces of every coin were swabbed with a clean cotton swab wet with sterile physiological saline (0.85 percent NaCl). The swab has been used to streak microorganisms onto MacConkey agar media to extract microorganisms, as seen in figure 1. After that, the samples were incubated aerobic capacity for 24-48 hours at 35°C - 37°C to enable microorganisms to proliferate. By streaking a tiny amount of cells from such a cell suspension on the appropriate medium and exposing plates night at 35°C–37°C, pure cultures were created. Colonial structure was seen, and also hemolytic responses. The mobility of pure cultures was assessed using Gram staining. The total number of bacterial present in the unit surface area

of the coins was calculated using established biochemical procedures. The total amount of germs found in the money coins was measured.

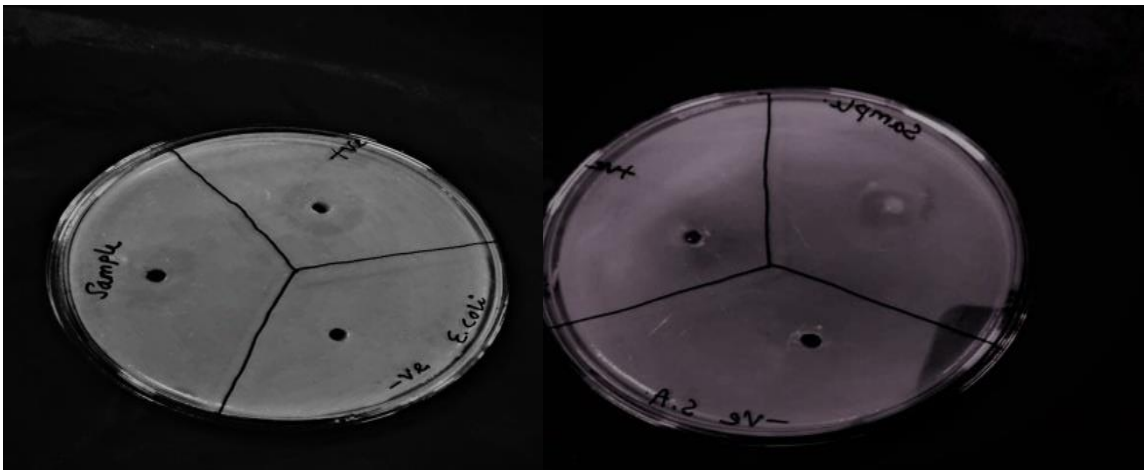

Figure 1. Microbial culture and colonies

Statisticalstudy

The Statistical Package for the Social Sciences, Version 21, was used to measure the results (IBM Statistics, 2019). The Chi-square tests were used to compare the groups, as well as the level of significance were fixed at 5%.

Results and Discussion

A microbiological testing was done on 30 currency coins, and all of the coins were found to be infected with germs. All cash coins acquired from the open market (Group I) and the hospital (Group II) were tainted (100%). Both microbes and fungi were found in the majority of the samples (mixed contamination). Species like E. coli, Klebsiella spp., S. aureus, and anerobic spore-bearing microbes were shown to be active participants. A total of >103 colony-forming units/plate were discovered. S. aureus (38%) and E. coli (21%), respectively, were discovered on all 15 currency coins of values 1, 2, and 5 (5 each), while Pseudomonas sp. (19%) and anaerobic spore-bearing microbes (9%) were identified in all denominations. S. aureus (54%) was isolated from all 15 monetary coins of denominations 1, 2, and 5 (5 each); E. coli (25%) and S. aureus (8%) were secluded from 1 and 2, and anaerobic spore-bearing microbes (9%) and non-hemolytic streptococci (4%) were secluded from all 15 currency coins of denominations 1, 2, and 5. Pathogenic bacterial isolates from the hospital and the outdoor specific

market. Table 1 shows the results of an intergroup comparative statistical data that use the Chi-square test, which is seen to be statistical significance (P 0.05).

Table 1: Potent pathogenic microbes on currency coins of denominations 1, 2, and 5 (5 each) from the marketplace and healthcare group

Potential pathogens	Open Market (Group l)				Hospital (Group ll)			
	Total microbial occurrence %	Denomination wise microbial occurrence %			Total microbial occurrence %	Denomination wise microbial occurrence %		
		1	2	5		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*							

Chi-square test, *Statistically significant

The separation of microbes from monetary coins, as proven by our research, suggests that money could play a major role in the spread of pathogenic microbes in the population, posing a public health risk. The coins were determined to be tainted in 100% of the cases. Lower-denomination currency coins (1 and 2) were most polluted, which is consistent with earlier research [8,9]. This is to be anticipated, given lesser

denomination coins are exchanged more frequently than larger denomination coins. Currency taint was shown to be greater in our research than in other poor countries' currencies[1-3]. In our research, currency coins from the public market area had higher E. coli infection (36%) than those from the hospital (25%) category, while S. aureus infection was higher in the healthcare group (54%) than in the marketplace group (38 percent). The proportion of E. coli and S. aureus was greater in the lesser denomination (1) than the larger denominations (2 and 5), which had never been discussed in the literature before. These discrepancies reflect regional disparities in hygienic procedures and currency coin management, as well as the fact that microbiological contamination of money coins is a worldwide issue.

Isolation of Genomic DNA from Bacteria

Centrifugation was used to extract 5 mL of exponentially developing bacterial cells, which were then washed twice with wash buffer. The cleaned cells were resuspended in 20µl of solution I, then added 2 mg of lysozyme and incubated for 1 hour at 37 ˚C. SDS was introduced into a final concentration of 2% and thoroughly mixed using inversion. The contents were combined through inversion and stored at 20 ˚C for 10 minutes after adding 10µl of 5M NaCl. The mixture was centrifugated for 5 mins at 12,000 rpm, and an equivalent volume of phenol:Chloroform was introduced to the supernatant, which was then precipitated by 2.5 volumes of cooled 95 % ethanol. After drying the pellet, it was liquified in 25 µl of STE buffer. The tube was filled with RNAse (g) and heated to 37°C for 10 minutes. Up to 100 µl of sterile deionized water were used to make the solution. An equivalent volume of phenol:chloroform mixture (1:1,v/v) was introduced, assorted carefully, and centrifugated at 12,000 rpm for 5 mins. The upper aqueous stage was moved to a fresh microfuge tube, 2.5 µl of cold 95 % ethanol was added, and the mixture was left to precipitate overnight at -20°C. The DNA was pelleted through centrifugate at 12,000 rpm for 5 minutes, then dried and dissolved in 0.1X TE16Buffer after being washed with 70% ethanol. Electrophoresis on a 0.8 percent agarose gel in 0.5x TEBuffer was used to examine the DNA.

Polymerase Chain Reaction

The PCR reactions are executed in 0.2 ml tubes in Eppendorf Personal Mastercycler. Identification of the strain was done by magnification of partial 16SrRNA gene using 16S F 5' AGAGTTTGATCCCTGGCTCAG 3' and 16S R 5’GTACGGCTACCTTGTTACGAC 3'. The programme used for the magnification was 95ºC for 5 minutes for early denaturation and 34ºC cycles of 95ºC for 1 min, 56ºC for 2 mins and 72ºC for 1 min and 72ºC for 10 minutes for final extension.

Table 2: Components of Reaction Mixture

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

Agarose Gel Electrophoresis of DNA

The isolated pure DNA was run in agarose gel for confirmation of the presence.

a. Preparation of 1% Agarose Gel

1) Seal the gel casting tray with cellophane tape on both sides, check leakage and place the comb in a slot.
2) Prepare 1% Agarose in 0.5X TEB.
3) Weigh 250 mg of Agarose and dissolved in a small conical flask containing 25ml of 0.5XTEB.
4) Boil the Agarose either using microwave oven or water bath until Agarose appears as a clear solution.
5) Cool the agarose solution around 40°C.
6) Add 1µl of Ethidium Bromide (5mg/ml) when the agarose gel Taround 40°C.
7) Assemble the gel casting tray.
8) Pour Agarose gel on tray.
9) Do not disturb the tray until complete solidification.
10) Pour 0.5X TEB buffer in horizontal electrophoresis reservoir tank. (It should appear milky white).
11) Carefully remove the tape and placed in the electrophoresis tank.
12) Buffer level should be maintained just above the gel tray (5mm of buffer over the gel).
13) Carefully pull out the comb without damaging the wells.

b. Preparation of Samples, Loading and Running Electrophoresis

1) Place 3µl of loading dye onto square of parafilm.
2) Pipette 10µl of DNA sample to each onto this 3µl spot of loading dye slowly.
3) Pipette the mixture up and down to mix it.
4) Reset the micropipette to 10µl and carefully pipette the DNA sample/loadingdye mixture into wells of the gel.
5) Load 5µl of DNA marker in a separate well.
6) Connect the positive and negative electrodes into the appropriate connection on the power supply.
7) Turn the power supply "ON" a pre-set the voltage to 50 volts.

c. Gel Electrophoresis of Amplified DNA

The amplified DNA has been electrophoresed in the agarose gel and the fragments as the bands are eluted and purified by gel purification process

d. Elution And Purification Of Amplified DNA

After visualizing the amplified DNA fragments in a UV Trans illuminator, the bands has been eluted and purified by gel purification process. The bands of the DNA fragments have been cut out from the gel carefully with a sterile blade without disturbing the bands. Then the clean-up kit solution was added which will separate the DNA from the gel. Then the isopropyl alcohol was added to precipitate the DNA from other contaminates and centrifuged at 12,000 rpm for 2-3 mins. Then added the wash buffer to remove the alkali in the pellets and then again centrifuged. The pellet contains the purified amplified DNA fragments which are now ready for cloning.

Table 3: Identification of bacteria

Isolated code	Gram reactoin	Cellular morphology	Probable identity
1	+	cocci	Micrococcus species
2	-	Rods	Flavobactreriumspeices
3	+	Rods	Bacillus species
4	+	Rods	Bacillus species

5	-	Rods	Enterobacter species
6	+	Rods	pneumoniae species
7	+	Rods	Pseudomonas species
8	+	Rods	Enterobacter species
9	-	Rods	Acinetobacterspecies
10	+	Rods	Bacillus species
11	-	Rods	Acinetobacter species
12	+	Rods	pneumoniae species
13	+	Rods	Streptococcus species
14	+	Rods	Bacillus species
15	-	Rods	Choromobacter species
16	+	Cocci	Micrococcus species
17	-	Rods	Bacillus species
18	-	Rods	Pseudommonas species
19	+	Rods	Salmonella species
20	+	Rods	Streptococcus species

Maintaining money underbody surfaces, compliance with hand washing after using the restroom, persistent cough on hands and ability to handle currency coins, and placing or storing money on dirty areas of skin during exchanges are all examples of behaviours that may make a contribution to currency coin contamination in our research location. Currency coins have a huge surface area that can serve as a breeding habitat for bacteria that can survive for extended periods of time. Many distinct types of microorganisms can adhere to the surface of coins. Nasal colonisation with community-acquired S. aureus is becoming more common in places where schoolchildren come into direct contact with unsanitary environments [4-7]. There are no faecal infections. Bacteria of faeces, nose, throat, and skin origins are also most prone to be affected through the hands, emphasising the importance of good hand cleanliness and other pathogen-prevention measures such avoiding bare-hand touch with ready-to-eat food. Foodborne infections as well as the global development of resistant or multidrug-resistant bacterial isolates are linked to E. coli and Salmonella species [8,9].

Table 1 demonstrates that staphylococci (coagulase negative and S. aureus) were the most common samples in currency from across all sources, demonstrating their pervasiveness. Long thought to be non-pathogenic, coagulase-negative staphylococci have recently been recognised and examined for their crucial function as infections and growing occurrence [6-10]. S. aureus is a well-known pathogen. The pathogen's ability to survive on money coins for a long time allows it to spread. These microorganisms were discovered in the studied normal flora samples. E. coli was more broadly disseminated than the other pathogens in various values of cash coins gathered both from hospital and open market groups. The presence of these bacteria in money coins indicates faecal contamination and poor hygiene among cash handlers. Therefore, touching money and food at the same time must be avoided unless basic sanitation is practised or handling food instruments are utilised to separate the two activities.

More research is necessary to confirm whether multidrug-resistant bacteria can be transmitted through touch with circulating money coins. Because the study focused on the presence of bacteria in lower-denomination currency coins in circulation, future research will include all denominations, such as coins and notes, with such a larger sample, locations where large-scale money transactions take place, and a greater health centres.

Conclusion

The monetary coins gathered from health facilities and open marketplaces were found to be infected with dangerous bacteria, according to this study. In comparison to the open market category, the cash coins obtained from clinics were much more infected with S. aureus. Although there is no clear indication that the existence of germs on coins causes disease, initiatives to limit currency coins contamination were implementedthroughpresenting sanitization in regular intervals. Disinfection of currency coins deposited in the banks is to be done by means of ultraviolet light or even throughdisinfection. Concerned officials advise that old coins be removed because they may be contaminated. Hands should also be washed among holding currency coins and meals. To remove contaminants, education system on safe handling and preservation of money coins is recommended. Handling money during washing hands and food handling necessitates a repetition of the handwashing procedure. Most countries lack research on the infection of banknotes with harmful bacteria. Because the present study looked at the prevalence of microbes in lesser-denomination money in circulation, more study with a bigger specimen size is needed.

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