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A Study of Bacterial Profiling on Coins and Currencies under Circulation and Identifying the Virulence Gene in Chennai (TN)

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Abstract: Coins and currency notes are widely exchanged between various communities for trade. These currency notes and coins may serve as a carrier of microbes, thus leading to the transmission of infectious diseases. In our research work, we have collected 31 currency notes and 31 coins from three different communities namely, bus conductors, public toilets agents and pharmacies to reveal the bacteria profile of circulating coins and currency notes. The samples were subjected to cultivation and isolation followed by staining and biochemical tests to identify the bacterial strain. In our analysis, we found that the currencies had *Staphylococcus aureus* and *Klebseiella pneumoniae* at higher frequencies; *Streptococcus pyogenes, Streptococcus pneumoniae, Enterococcus fecalis, Enterococcus faecium, Proteus mirabilis, Escherichia coli, Citrobacter freudii* and *Pseudomonas aeruginosa* at lower frequencies. Virulence of the strains, *S.aureus* and *K.pneumoniae* were determined by the presence of the conserved gene called HtrA in PCR. The study showed that the coins and currency notes can also act as vehicle for bacterial survival and transmission of diseases in humans while in continuous circulation.
Keywords: Currency, Coins, Virulence, PCR.

Introduction

The environment plays an important role in transmission of microorganisms to humans and many environmental materials serve as vehicles[1]. Currency and coins are important items most frequently passing from hand to hand; during its passing and counting currencies gets contaminated with normal flora and pathogens from the skin, respiratory secretions, gastro-intestinal tract, water, soil and aerosols. The chance of currency notes and coins may act as possible route for the transmission of potential pathogenic microorganisms was suggested in 1970s[2]. Most of the people do not care how dirty their hands are when handling and count the money. The contaminated notes and coins go in circulation and spread contaminated microbes to others hands and transmitting pathogenic organisms in the process[3]. These ways of transmission are significance in the health of many populations in all developed and developing countries. The type of infections is a general sign of local hygiene and environmental sanitation levels[1,4]. Evidence of carriage of pathogens on coins and currency notes have been reported in Turkey[5], United States[6], Australia[7], Egypt[8], China[9], Myanmar[10]. These studies implicate money as a vehicle for transmission of pathogens. Such information is necessary to facilitate

infection control strategies. The main objective of this study was to analyze the bacterial profiling in currencies under circulations in Chennai and identifying the virulence strain.

Materials and Methods

Collection of Samples

A total of 62 samples were collected (31 coins, 31 notes) among different communities i.e. from medical shops, bus conductors and public toilet agents. The samples collected were Rs.2 (same radius) coins and Rs.10 note, each community contributed 20 samples. The control consists of one bank coin and one currency note. Informed written consent was obtained from all the subjects after explaining the nature and purpose of the study. The samples were collected in a sterile petri plates and the plates were marked. The respective persons were asked to place the samples directly on the petri plates, notes were not touched by any other person using bare hands at any stage. The plates were secured with rubber band. The collected samples were divided into 2 groups. On the same day of the coin collection, the first set was used for further process and the second set was kept overnight in the petri plates for second or third day processing.

Culturing and Isolation

Culturing of diverse bacterial contaminants from the notes and coins was performed by standard techniques described by Gilchrist, 1993[11] and Singh *et al*., 2002[12]. Briefly, a sterile, cotton swab was moistened with sterile saline and used to swab both sides of the notes and coins. The swab was inoculated in the test tubes containing 2ml of Brain heart infusion broth. The test tubes were incubated for 24-48 hrs at 37°C with cotton plug on them. The second group of coins was kept in the petri plates for overnight and next day their swab was dipped in the broth following the same procedure as the first group. After checking the turbidity in broth, the organisms were streaked on petri plates poured with Nutrient agar, Mac Conkey agar and Blood agar for selective isolation of Gram positive and Gram negative organisms[13]. To test for sterility of medium, we placed uninoculted plates and broth along with streaked plates and inoculated tubes.

Staining and biochemical tests

Gram's staining and biochemical tests (Table-1 & 2) include Catalase test, Coagulase test, TSI test, IMViC tests, Nitrate reduction test, Urease test, Bile esculin test, Oxidase test, sugar fermentation tests were performed to identify organism in species level [9,12].

Table 1. Biochemical analysis result for Gram negative bacteria

Species	TSI	Indole	MR	VP	Citrate	Unrease	Nitrate
E.coli	(A/A),g	+	+	-	-	-	+
K. pneumoniae	(A/A)	-	-	+	+	+	+
P.mirabilis	(K/A), H_2s	-	+	-	+	+	+
K.oxytoca	(A/A),g	+	-	+	+	-	**v**
P.aeuringnosa	(K/NC)	-	+	+	+	-	+
C.freundii	K/A), H_2s	-	+	-	+	+	+

Note: A-acid, K-alkaline, g-gas, NC-no change, +-positive, -negative, v-variable, NA-not applicable

Table 2. Biochemical and cultural test results of Gram positive bacteria

Species	Catalase	Cogulase	Mannitol	Bile esculin
S.aureus	+	+	+	NA
S.epidermis	+	-	-	**NA**
S.pyogenes	-	**NA**	**NA**	-
S.pneumoniae	_	**NA**	**NA**	-
C.diptheriae	**Catalase**	**Blood Agar**	**Glucose**	**Maltose**
	+	+	+	+

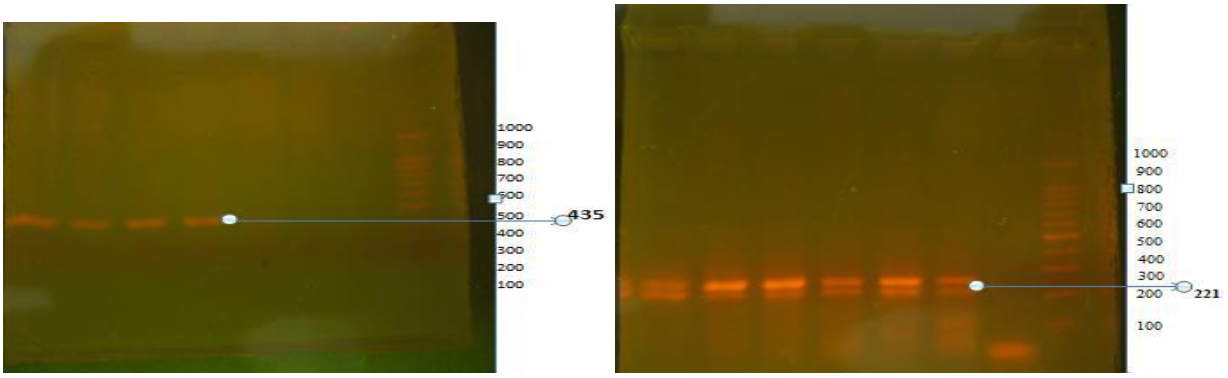
Note: +-positive, -negative, v-variable, NA-not applicable

Extraction of DNA and PCR

After the speciation process, the DNA from dominant species were isolated and subjected to PCR amplification of HtrA virulence gene[14,15]. The dominant species in Gram positive organism is *S.aureus* and *K.pneumoniae* in Gram negative are allowed to DNA extraction and polymerization with two different primers (Table- 3) according to the manufacturer's instructions (HELINI Biomolecules, Chennai). Electroporation were carried out by standard techniques in agarose gel along with maker (Figure- 1).

Table 3. Primer Used in PCR reaction

S.No	Organism	Gene	Primers	Product size
1	Staphylococcus aureus	HtrA gene	5'TGCTGCGACACAAAGTTGAAGGT3'5'TCGCCTTGGCCAGGTGATTGGAC3'	435bp
2	Klebsiella pneumoniae		5'AGAGTTCGCCGTTTTGCCAGGG-3' 5'ATCAGAGCGCGGATCTTTGCCG-3'	221bp



A B

Figure 1: (A)-PCR results for *S.aureus*, (B) -PCR results for *K.pneumoniae*

Statistical analysis

To find out the significance of the result obtained, statistical analysis was done by GraphPad Prism software. The input data of number of organisms against communities were fed into two way ANOVA table. Another input data of total number of isolates in currencies against communities were fed into one way ANOVA table.

Result and Discussion

A total of sixty two currencies (30 coins, 30 notes and 2 controls) were analyzed for bacterial contamination. Seventeen (17) different bacteria were isolated from sixty currencies, giving the percentage of contamination to be 28.33%. The bank notes and coins which were used as control showed that there were only *Micrococci* on the note and no organisms were present on the coins.

From Pharmacy

There were a total of 20 isolates in coins, out of which 4 isolates were of *Klebsiella pneumoniae* contributing 20 % to the total population on the coins. The next position is occupied by *Staphylococcus aureus, Diptheroids, Bacilli* and *Staphylococcus epidermidis* contributing 15 % of the total isolates. And only one isolates each of *Micrococcus, pseudomonas aeuroginosa,* and *Enterococcus faecalis* were identified. There are totally 21 isolates in the notes collected, of which *Bacillus* (29%) are present in large amounts. This is followed by *Diptheroids* (19%), *S. epidermidis* (14 %), *Micrococcus* (9%), and *S. aureus* (9%), while *Citrobacter freundii, K. pneumoniae, E. faecalis* and *Streptococcus pyogenes* contributed 5% each (Table-4).

Table 4: Shows the number of isolates in coins and currencies different communities

Sample No.	Bacterial Isolates					
	Pharmacy		Bus Conductor		Public Toilets Agents	
	Coins	Currency	Coins	Currency	Coins	Currency
1	S.epidermis, E.faecalis,	S.aureus, Bacillus.	Micrococcus, Diptheroids, S.aureus.	C.diptheriae, Micrococcus, S.epidermis.	S.aureus, S.pyogenes.	S.aureus, S.pneumoniae.
2	Bacillus, P.aeuroginosa.	Bacillus, S.epidermis, S.pyogenes.	E.coli	S.aureus, C.diptheriae.	S.aureus, Bacillus.	Micrococcus, E.faecium.
3	K.pneumoniae, S.epidermis.	Bacillus, Diptheroids.	S.aureus	S.aureus, Micrococcus.	Bacillus.	S.aureus, Bacillus, Micrococcus, S.pneumoniae.
4	Bacillus, K.pneumoniae.	Bacillus, Diptheroids.	S.aureus, C.diptheriae, Micrococcus.	S.pyogenes, S.epidermis.	Bacillus, P.aeuringnosa.	S.aureus, Bacillus.
5	Bacillus, S.aureus.	S.aureus, Bacillus.	E.coli .	Micrococcus, S.pyogenes.	Bacillus.	S.aureus, Micrococcus.
6	Diptheroids, K.pneumoniae.	S.epidermis, Micrococcus.	C.diptheriae, S.pyogenes .	K.oxytoca, S.epidermis.	S.epidermis, Diptheroids.	S.aureus, Bacillus.
7	K.pneumoniae, Micrococcus.	E.faecalis, Micrococcus.	K.pneumoniae, S.epidermidis .	S.epidermis, C.freundii.	S.pyogenes, Micrococcus.	S.aureus, Bacillus.
8	S.aureus, Diptheroids.	S.epidermis, C.freundii.	K.pneumoniae ,Diptheroids	C.freundii, P.mirabilis.	S.aureus, Micrococcus.	S.epidermis, Diptheroids.
9	S.epidermis. A.baumannii.	K.pneumoniae, Diptheroids.	S.aureus, Micrococcus .	K.oxytoca.	C.diptheriae.	S.aureus, Micrococcus.
10	S.aureus, Diptheroids.	Bacillus, Diptheroids.	Micrococcus .	S.epidermis.	S.aureus, Diptheroids.	Diptheroids.

From the above data it can be concluded that, all the samples had more than one strain including the normal flora. In the case of coins collected from pharmacies there were 9 different species of microbes present, of which 70 % were gram positive bacteria and 30% were of gram negative species. In case of notes out of 9 different species there were 90 % of gram positives and 10 % of gram negatives. On the whole there were 80 % gram positives and 20 % gram negatives were identified from the samples collected from pharmacies On the whole, there were totally 41 isolates. Though the nonpathogenic bacteria occur in higher frequencies, highly pathogenic species such as *S. aureus and P .aeuroginosa* are present in appreciable amount. Thus the chance of transmitting infections caused by the pathogenic organisms which were found in lower frequencies cannot be ruled out.

From bus conductors

In case of coins from bus conductors there were in all 18 isolates (Table- 4). Out of which 22 % were contributed by *S. aureus* and *Micrococcus*, followed by *Diptheroids*, *K. pneumoniae*, *Escherichia coli* and *Corynebacterium diptheriae* each forming 11 % of the total bacterial populations on coins. The remaining 12 % was equally shared by *S. epidemidis* (6%) and *S. pyogenes* (6%). From notes there were in all19 isolates of which *S. epidemidis* (29 %) was found to be present in maximum amount. It was followed by *Micrococcus* (16%), *S. pyogenes*, *C. diptheriae* and *C. freundii* each contributes 11% to the total isolates present on these notes. Isolates *Klebsiella. oxytoca* and *S. aureus* were found to be 10 % of total population, whereas *P. mirabilis* was just 5% of the total organisms. A total of 37 isolates of coins and currency contributes, 76 % were

gram positives and the remaining were gram negatives. From the gram positive, pathogenic *S. aureus* were found in significant number.

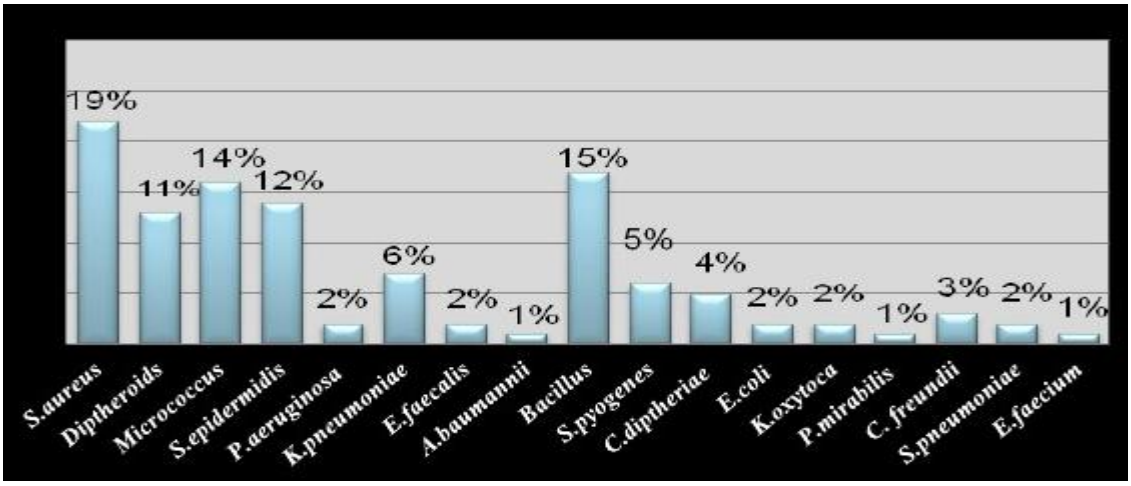


Figure 2: Complete profile of coins and currencies.

From public toilets agents

Out of the total 17 isolates (Figure- 2 & Table -4) from coins, *S .aureus* and *Bacillus* were the maximum contributing about 23 % of the total species. That is followed by *Diptheroids, Micrococcus* and *S .pyogenes* were the second highest each forming 12 % of the total isolates. The least contributors were *S. epidermidis* (6%), *P. aeuroginosa* (6%), and *Corynebacterium* (6%). Out of the 21isolates from currency, *S. aureus* contributed the maximum of 33 % to the bacterial flora on the coins. It was followed by *Micrococcus* and *Bacillius* each contributing 19%, *S. pneumoniae* and *Diptheroids* contributed 10 % and 9% respectively. While *S. epidermidis* and *E. faecium* contributed 5 % each. Public toilets, being the most prone community have the maximum isolates of the pathogenic *S. aureus*. Thus we can say from the sample collection that people infected with *S. aureus* have visited the public toilets more often.

Among 12 different species present on the coins, 82 %were gram positives and remaining 18 % were gram negatives (Figure- 3). In case of notes there were on all 14 different species of which 84% were gram positive and remaining 16% were gram negative. So it can be concluded that in the overall population 87 % were gram positive species and remaining 13% were gram negative.

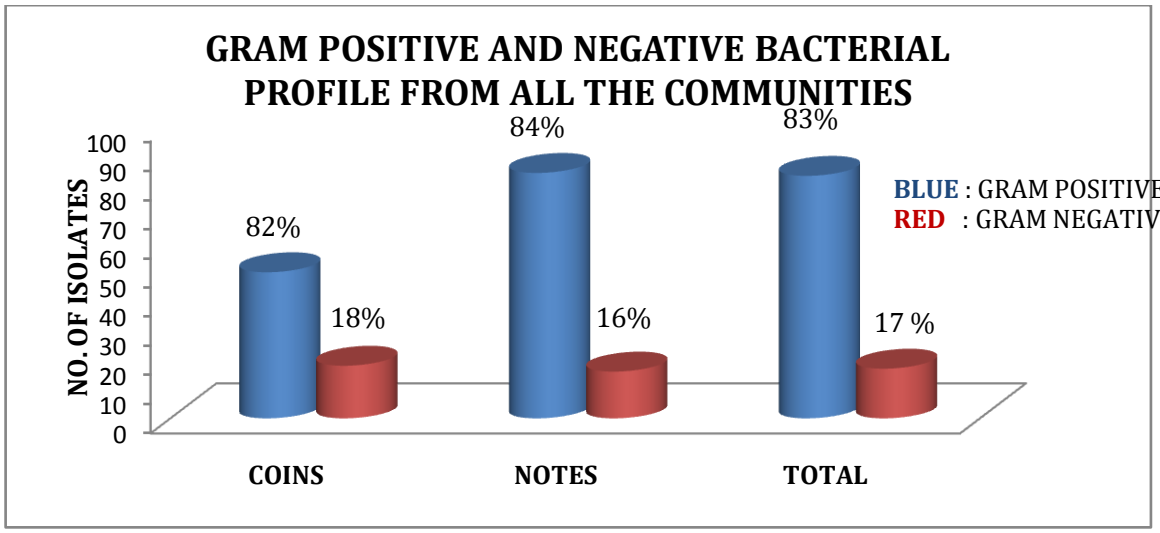


Figure 3: Gram positive and Gram negative bacterial profile of all the cmmunities.

The total number of isolates from all the coins put together was 55, of which *S. aureus* occupies the highest position contributing 20 %. The second position was occupied by *Diptheroids, Micrococcus* and *Bacillus* each forming 13 % of the total population. This is followed by the pathogenic *K. pneumoniae* which

forms 11 % of the populations. The next highest is *S. epidemidis* having 9 % of the total isolates. Other organisms contribute a small fraction to the overall microorganisms. There were in all 61 isolates on the notes of which *S.aureus* were found to be contributing 18 %. This is followed by the non-pathogenic species, namely *Bacillus, micrococcus, S. epidermidis* and *Diptheroids* contributing 16%, 15%, 15 % and 10% each. Other pathogenic species contributes some minor levels to the total populations.

At the final stage it was found that there were in all 116 isolates of which *S. aureus* occupied the highest fraction of 19 %. This is followed by the normal flora of *Bacillus*, *Micrococcus*, and *S. epidermidis* and *Diptheroids* contributing 15 %, 14 %, 12 % and 11 % respectively. The next highest pathogen found in the sample collected was *K. pneumoniae* contributing 6 % of the population. Other pathogenic species such as *P. aeuroginosa* (2%), *E. faecalis* (2%), *S. pyogenes* (5%), *C. diptheriae* (4%), *E. coli* (2%), *K. oxytoca* (2%), *P. mirabilis* (1%) *C. freundii* (3%) *S. pneumoniae* (2%) and *E. faecium* (1%) were also present.

The isolation of bacterial pathogens from currency and coins in this study and earlier report of Janardan *et al* (2009)[16] in Nepal confirmed that currency and coins acting as vector for the transmission of pathogenic and non pathogenic bacteria in the community. In between this currencies, more isolates where done in note in respect to number and different pathogens especially in lower denomination notes due to high circulation rate. The lower isolation rate in coins may be antimicrobial activity of metals used to make. Isolation of pathogens not merely mean that it produce infection, it depend upon the individual immunity, infective dose and route of entry.

Among the different bacterial isolates, *K. pneumoniae* is a virulent organism that can cause lung infection along with urinary tract and wound infections, especially in immuno- compromised individual. *E. coli* and *S. epidermidis* are usually non-pathogenic but some strains can cause serious food poisoning and UTI in human[16]. Though *Bacillus* species, *S. epidermidis*, coagulase-negative *staphylococci* and *E. faecalis* is usually non-pathogenic it causes infection in patients who possess compromised immune system. *Citrobacter* species, *Klebsiella* species, and *E. coli* are common enteric microorganisms that are possible pathogens especially when they change their environment. Pathogenic *Staphylococci* excreted either by an asymptomatic carriers or a person with a disease, can be spread by the hands or respiratory secretions. The *Staphylococci* are common inhabitants of the animal skin and hair, which is the source of those found elsewhere. As saprophytes, *Staphylococci* are found on normal skin, nose, mouth and intestine as well as in the air, water, milk and sewage and on fomites and it act as opportunistic pathogen. Pyogenic infections occur when *Staphylococci* enter the body through skin abrasions, wounds, burns and cuts.

Conclusion

Present study reveals that currency and coins are circulated among different classes of people contaminated and act as vehicle for the bacterial survival and spreading of pathogenic and non –pathogenic organisms. In the genomic analysis of more prone isolates, have shown the presence of HtrA gene virulence gene for pathogenicity and it shows that bacteria present have potential to cause infection under a variety of personal and environmental conditions. Thus suitable strategies must be adopted to reduce the contamination and spread of infection. To practice good personal hygiene, general awareness must be created among the public in handling currency notes and coins to reduce the contamination. In Reserve bank UV treatment or fumigation of money can be done once in a while as sterilization process to reduce the risk. Finally, we recommend that similar studies on the microbial contamination of currency note and coins must be undertaken in other part of Tamil Nadu and India to enrich the national information bank on the subject; the issue is becoming a major public health concern.

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