Screening of currency in circulation for bacterial contamination

Fomites or inanimate objects play a major role in indirect transmission of infections like diphtheria, trachoma, gastroenteritis, whooping cough and pathogenic agents causing diarrhoea[1]. Currency contaminated by microbes might also act as fomite, playing an important role in the transmission of microorganisms and also in the spread of drug-resistant strains in the community, as it is the most widely handled article by people from all walks of life[2,3]. Currency is bound to get contaminated either from the environment or from the persons handling it.

There have been numerous reports on the contamination of currency with illicit drugs, including cocaine, heroin and amphetamines[4]. However, data regarding the bacteriological contamination of currency is scanty and a recent medical literature search revealed only a few studies on this topic. Moreover, in India, few studies have reported the microbial colonization of currency. Hence, the present study was undertaken to screen the currency circulating in Mangalore for microbial contamination, to study the antibiotic susceptibility pattern of the pathogenic isolates and to type the pathogenic isolates by random amplification of polymorphic DNA (RAPD) technique.

Twenty-five samples each of currency notes and coins were collected from the general population by following simple random sampling technique. The study population included people from various occupations and walks of life, like beggars (n = 6), street food vendors (n = 11) and school children (n = 8). The samples were collected in a sterilized paper packet, numbered serially and transported to the laboratory for microbial screening.

All the media and antibiotic discs used for isolation, identification and antibiotic susceptibility testing were procured from HiMedia Laboratories Pvt Ltd, Mumbai, India. The collected coins were inoculated into brain–heart infusion (BHI) broth and incubated at 37°C for 1 h, after which they were removed with sterile tweezers. Sterile swabs moistened in sterile saline were used for sampling the currency notes. These swabs were also inoculated into the BHI broth[3]. Inoculated BHI broths were incubated at 37°C for 24 h, after which 50 µl of the BHI broth was subcultured onto solid media like blood agar, chocolate agar, MacConkey's agar and Sabouraud's dextrose agar plates and incubated at 37°C for 24 h.

Culture plates were observed for growth and a Gram smear was performed from different types of colonies. On the basis of Gram reaction and colony morphology, and standard biochemical reactions, the isolates were indentified to genus level[5].

Using Mueller Hinton broth and Mueller Hinton agar, antibiotic susceptibility testing was performed by the disk diffusion method[6,7]. Methicillin resistance was detected using cefoxitin disc (30 µg). The zones of clearing around the discs were measured and compared with standards of Clinical Laboratory Standards Institute[8], and interpreted as either sensitive (*S*), resistant (*R*) or intermediate (*I*).

DNA from the bacterial isolates was prepared by following the procedure described by Onasanya *et al.*[9]. Briefly, biochemically confirmed bacterial isolates were emulsified in 100 µl of TRIS EDTA (TE) buffer (pH 8.0), treated with proteinase K (250 µg/ml) and incubated at 37°C for 1 h, after which they were heated for 15 min in a dry bath (Bangalore Genei Pvt Ltd, India) at 100°C to inactivate proteinase K. Lysates were centrifuged and 1 µl of the supernatant was used for polymerase chain reaction (PCR).

Two custom-synthesized decamer random primers R_1 (5′GCGATCCCCA3′) and R_2 (5′CAGCACCCAC3′) procured from Bangalore Genei were used in the RAPD reaction[10]. DNA amplification and detection of amplified product was done according to Neilan[11], and Sambrook *et al.*[12]. Amplifications were performed in 25 µl reaction mixture consisting of genomic DNA, 1× reaction buffer, 100 µM each of dATP, dCTP, dGTP and dTTP, 0.2 µM single random primer, 2.5 µM $MgCl_2$ and 1U of *Taq* polymerase. PCR reaction was carried out up to 35 cycles in a thermocycler (Bio Rad Inc., USA). The reaction conditions were: initial delay at 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2 min and the final delay at 72°C for 10 min. The amplified product was resolved by agarose gel electrophoresis using 2% agarose in 1× TAE buffer containing 0.5 µg ethidium bromide/ml. Gels were visualized under UV trans-illuminator; gel pictures were photographed using Alpha Imager and analysed visually.

A total of 50 samples were examined, which consisted of 25 coins and currency notes each. Among the currency screened, 10 coins and 15 notes showed growth of single bacterial species, 11 coins and 9 notes showed the growth of two types of bacterial species each, and three coins and one note had grown three different organisms. The common fungal isolate from two of the coins was *Aspergillus niger* (n = 2). The bacterial isolates include *Staphylococcus aureus* (n = 11), *Klebsiella* spp. (n = 6), *Escherichia coli* (n = 01), *Staphylococcus epidermidis* (n = 3), *Citrobacter* spp. (n = 7), *Pseudomonas* spp. (n = 4), *Acinetobacter* spp. (n = 4), *Enterobacter agglomerans* (n = 2), *Micrococcus* spp. (n = 1), α-haemolytic *Streptoscoccus* spp. (n = 1) and *Bacillus* spp. (n = 35).

We have observed that 96% of the coins and 100% of the currency notes were found to be contaminated with different bacterial species, of which *S. aureus*, *Klebsiella* spp. and *E. coli* can be considered as pathogens, as they have been implicated in various diseaseas. The other isolates being ubiquitous environmental organisms rarely cause disease in healthy hosts, but have been reported to cause serious nosocomial infections in immunocompromised individuals[13,14]. Even *Bacillus* spp., a common surface contaminant, is known to cause clinically significant infection in individuals with specific risk factors such as indwelling catheters[15].

Earlier studies reported the rate of contamination of currency to be between 78% and 96%, and they could detect the presence of different fungal species, acid-fast bacilli and even parasitic ova and cysts[16]. Our results show an average of 98% contamination of currency. The hot and humid climate of the place where this study was conducted could be a cause for the high rate of isolation of microorganisms from the currency, as microbial growth is favoured by such climatic factors.

The antibiogram of bacterial isolates suggests considerable resistance to commonly used antibiotics. The antibiotic susceptibility patterns of *Staphylococcus* spp. and Gram-negative bacterial

Table 1. Antibiotic sensitivity pattern of *Staphylococcus* spp. isolated from currency

	Staphylococcus aureus (*n* = 11)			*Staphylococcus epidermidis* (*n* = 3)		
Antibiotics tested	*S* (%)	*I* (%)	*R* (%)	*S* (%)	*I* (%)	*R* (%)
Cefoperazone	6 (54.5)	3 (27.2)	2 (18.1)	2 (66.7)	1 (33.3)	0
Erythromycin	3 (27.2)	2 (18.1)	6 (54.5)	2 (66.7)	0	1 (33.3)
Gentamicin	10 (90.9)	0	1 (9.1)	3 (100)	0	0
Netilmicin	11 (100)	0	0	3 (100)	0	0
Methicillin	5 (45.4)	2 (18.1)	4 (36.4)	1 (33.3)	1 (33.3)	1 (33.3)
Penicillin	0	0	11 (100)	1 (33.3)	0 (0)	2 (66.7)

S, Sensitive; *R*, Resistant; *I*, Intermediate.

Table 2. Antibiotic sensitivity pattern of Gram-negative bacterial isolates

	Acinetobacter spp. (*n* = 4)			*Citrobacter* spp. (*n* = 7)		
Antibiotics tested	*S* (%)	*I* (%)	*R* (%)	*S* (%)	*I* (%)	*R* (%)
Amikacin	–	Not applicable	–	–	Not applicable	–
Ampicillin	1 (25.0)	0	3 (75.0)	1 (14.3)	1 (14.3)	5 (71.4)
Cefoperazone	0	1 (25.0)	3 (75.0)	5 (71.4)	2 (28.6)	0
Ceftazidime	1 (25.0)	1 (25.0)	2 (50)	3 (42.9)	3 (42.9)	1 (14.2)
Cephalexin	–	Not applicable	–	–	Not applicable	–
Chloramphenicol	2 (50.0)	2 (50.0)	0	7 (100)	0	0
Ciprofloxacin	–	Not applicable	–	7 (100)	0	0
Cotrimoxazole				7 (100)	0	0
Gentamicin	4 (100)	0	0	7 (100)	0	0
Piperacillin	0	0	4 (100)	5 (71.4)	2 (28.6)	0
Piperacillin tazobactum		–	Not applicable	–		
	Klebsiella spp. (*n* = 6)			*Pseudomonas* spp. (*n* = 4)		
Antibiotics tested	*S* (%)	*I* (%)	*R* (%)	*S* (%)	*I* (%)	*R* (%)
Amikacin	–	Not applicable	–	4 (100)	0.0	0.0
Ampicillin	2 (33.3)	0.0	4 (66.7)	–	Not applicable	–
Cefoperazone	4 (66.7)	2 (33.3)	0	2 (50.0)	2 (50.0)	0
Ceftazidime	4 (66.7)	1 (16.6)	1 (16.6)	2 (50.0)	1 (25.0)	1 (25.0)
Cephalexin	–	Not applicable	–	4 (100)	0	0
Chloramphenicol	6 (100)	0	0	–	Not applicable	–
Ciprofloxacin	6 (100)	0	0	4 (100)	0.0	0
Cotrimoxazole	6 (100)	0	0	–	Not applicable	–
Gentamicin	6 (100)	0	0	4 (100)	0.0	0
Piperacillin	3 (50.0)	0	3 (50.0)	3 (75.0)	1 (25.0)	0
Piperacillin tazobactum	–	Not applicable	–	4 (100)	0	0

S, Sensitive; *R*, Resistant; *I*, Intermediate.

isolates are shown in Tables 1 and 2 respectively. Among the Gram-positive bacterial isolates, *S. aureus* showed 100% resistance to penicillin. Methicillin-resistant *S. aureus* (MRSA) was found to be 36.4%, determined using cefoxitin disc.

Of the Gram-negative bacterial isolates, *Acinetobacter* spp., *Citrobacter* spp., *Klebsiella* spp. and *Pseudomonas aeruginosa* showed 100% sensitivity to gentamicin. *Acinetobacter* spp. showed 100% resistance to piperacillin and 75% of the isolates were resistant to ampicillin and cefoperazone. All the isolates of *Citrobacter* spp. and *Klebsiella* spp. were found to be sensitive to chloramphenicol, ciprofloxacin, cotrimoxazole and gentamicin. However, these isolates showed considerable resistance to ampicillin and ceftazidime. Fifty per cent of the *Klebsiella* spp. were resistant to piperacillin. Twenty-five per cent of the *Pseudomonas* isolates were resistant to ceftazidime, but they were 100% sensitive to piperacillin tazobactum combination antibiotics. *E. coli* was resistant to only ampicillin and piperacillin.

The samples were found to be significantly contaminated with *S. aureus* (36.4% MRSA), which is capable of surviving outside the host for prolonged periods. MRSA and *Klebsiella* spp. are capable of causing community-acquired and hospital-acquired infections, and are most commonly involved in respiratory tract infections[17]. Moreover, the antibiogram of these two organisms isolated showed resistance to some of the most commonly used antibiotics, which makes these organisms even more dangerous and capable of causing significant infections. Hence, contaminated currency might act as a fomite and play an important role in the transmission and spread

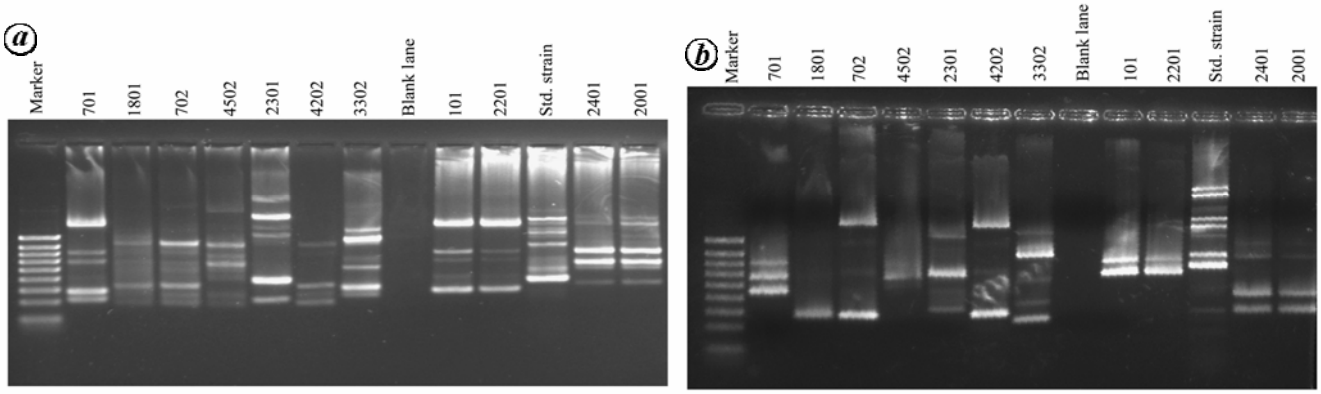

Figure 1 ***a, b.*** Gel picture showing RAPD fingerprint patterns of *Staphylococcus aureus* strains obtained with R1 and R2 primers respectively. Std. strain refers to standard strain of *S. aureus* ATCC 25923, and marker refers to 100 bp ladder.

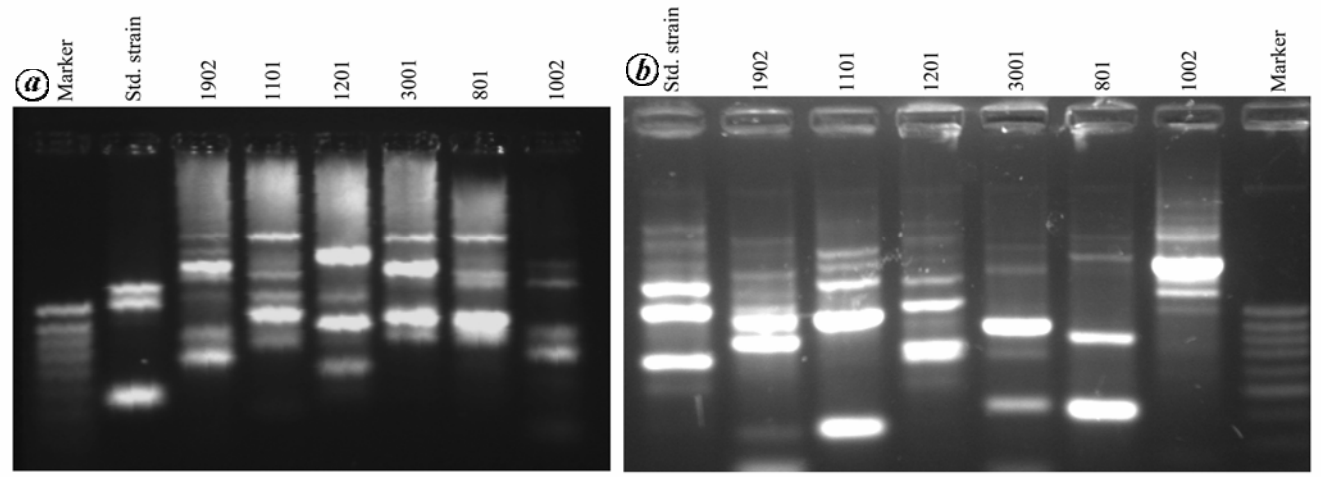

Figure 2 ***a, b.*** Gel picture showing RAPD fingerprint patterns of *Klebsiella* spp. obtained with R1 and R2 primers respectively. Std. strain refers to standard strain of *Klebsiella pneumoniae* ATCC 13883, and marker refers to 100 bp ladder.

of drug-resistant microorganisms in the community.

The predominant isolates from notes and coins were typed by RAPD. Bacterial strains that were isolated from a single source (either coin or note), isolates which were few in number ($n \leq 2$) and *Bacillus* spp. were excluded from molecular typing. *S. aureus* ($n = 11$), *Citrobacter* spp. ($n = 6$), *Klebsiella* spp. ($n = 6$) and *P. aeruginosa* ($n = 4$) were typed by RAPD. One of the *Citrobacter* spp. was not typed as it had lost viability in storage.

Of the 11 *S. aureus* typed by RAPD, two isolates from coins (strain nos. 701 and 101) and one isolate from notes (strain no. 2201) showed similarity in their banding patterns with R1 primers. Strains 101 and 2201 showed smilarity with R2 primer (Figure 1). RAPD profiles of *Citrobacter* spp. and *Pseudomonas* spp. did not reveal any similarity by both the primers (data not shown). However, of the *Klebsiella* spp., one isolate each from coin (strain no. 801) and note (strain no. 3001) showed similarity in banding pattern with both R1 and R2 primers (Figure 2). The results reveal that *S. aureus* isolates (MRSA) and *Klebsiella* isolates showing similarity in banding pattern might have some clonal relationships among themselves. Further studies with more number of isolates using highly discriminatory typing methods may help trace the transmission of pathogens from the currency in circulation.

The presence of a heavy contagion of microflora with significant antibiotic resistance is a cause of concern and warrant the use of standard precautions. Medical professionals and food handlers should be more careful, especially while handling currency. If adequate hand sanitation is not practiced by medical professionals in handling money and examining patients, or by food handlers in preparing food or drink, patients and food service patrons could be placed at risk. Food-handling tools (such as tongs, spoons, utensils or bakery/serving papers) would help prevent cross-contamination occurring between money and food via hands. In the case of medical professionals too, hands should always be washed after handling currency.

Although documentation of actual transmission of pathogens from one individual to another through currency was beyond the purview of this study, such a high rate of contamination with drug-resistant microbes suggests the possibility

of transmission of infection in the community. To the best of our knowledge, molecular typing has not been used to trace the spread of pathogens in the currency. However, an attempt has been made in the present study to know the clonal relationship between the commonly isolated bacterial species using RAPD technique, though 50 samples tested represent a small fraction of the large number of coins and currency notes circulating in the community.

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Oily fraction of nuts of *Semecarpus anacardium* Linn. enhances iNOS expression in macrophages

Recently, we have reported that non-polar oily fraction (NPF) and polar fraction of nuts of *Semecarpus anacardium* Linn. (Anacardiaceae; SA nuts) exhibit opposite property towards the pattern of release of nitric oxide (NO) by macrophages. NPF shows induction of NO production, similar to lipopolysaccharides (LPS), but the polar fraction has inhibitory effect against LPS-induced NO production[1]. Since no information is available about the role of NPF on the activity of iNOS enzyme or its gene expression, this aspect has been studied on rat peritoneal macrophage culture. The kernel of SA nuts is widely used in the Ayurveda and Siddha system of Indian medicine as anti-helminthic, anti-fungal, anti-carcinogenic, anti-nervous disorders and antiarthritis[2–7]. We have previously reported its antioxidant property[8] and anti-cancer property in DU-145 cells[4]. It also prevented localized bone loss and showed enhancement in calcification in osteoblast-like cells[9]. We have also developed a novel polyherbal formulation (BHUx patented in USA, EU, China and India), consisting of purified SA extract along with four other medicinal plants to manage hyperlipidemia and atherosclerosis, through its antioxidant and anti-inflammatory properties[10–11]. These properties were later associated with its active compound, tetrahydroamentoflavone, a biflavonoid. In Ayurveda, emphasis has been given towards its purification before clinical use[12].

LPS, phenylmethylsulphonyl fluoride, aprotinin, leupeptin, sodium dodecylsulphate and quercetin were purchased from Sigma, USA. Methelyne blue, ethylenediaminetetraacetic acid, NaCl, RPMI-1640, hydrogen peroxide and fetal calf serum (FCS) were purchased from Hi Media, Mumbai, India. Rabbit polyclonal anti-iNOS primary and secondary antibodies were purchased from Santa Cruz Biotechnology and other chemicals were of analytical grade.

Next, 250 g of dried SA nuts was carefully crushed and extracted in a soxhlet extractor with hexane for 30 h. The solvent-free dried hexane extract was standardized in terms of its percentage of yield, and TLC fingerprint was developed as described elsewhere[13]. Its stock solutions were made in dimethyl sulfoxide (DMSO) as 300 mg/ml and serially diluted with phosphate buffered saline (PBS) in three different working solutions (100, 10 and 1 mg/ml), which were used in culture wells in such a way that the final concentrations of NPF were arrived as given in the respective table, but the final concentration of DMSO was lower than 1%.

Animal experiments were approved by the Ethical Committee for Animal Welfare at the R&D Centre, Prof. S.N. Tripathi Memorial Foundation, Varanasi. Inbreed albino rats of Charles Faster strain were