INTRODUCTION:

Money is the widely accepted means of exchange for goods and services in the community and also serves as an economically stable gathering of wealth. It is circulated widely and handled by almost every member of the society like beggars, vendors, shopkeepers, food handlers, office employees, under various personal and environmental conditions and even by children who play with coins. Recently there is a growing concern about inanimate objects/fomites playing major role in indirect transmission of infectious diseases like gastroenteritis, respiratory infections, trachoma and diarrhoeal disorders (1). They can also serve as potential vehicle in the spread of drug-resistant strains of various pathogenic microbes in the community (2,3,4,5) posing public health hazard. Immuno compromised persons are at risk of acquiring opportunistic infections while handling contaminated currency (7). Contamination of currency can be either from environment (soil, dirty surfaces, contaminated materials) or by droplet nuclei during actions like coughing, sneezing and while handling with contaminated hands. High rates of microbial contamination of currency notes in circulation has been reported in studies conducted in different parts of the world (6,7,8,9,10,11). In our country, where practices like using saliva for counting money, keeping the currency in undergarments in contact with body surfaces and holding the currency in hands for long period of time- a common scenario, implies the prevailing meager awareness about contaminated currency notes and coins which pose a risk of microbial transmission in the community. Our study aims at identifying the bacterial and fungal profile of currency notes and coins in circulation in our region among various groups of people, to compare the degree of contamination based on their physical nature, to study the antimicrobial susceptibility pattern and specific resistance mechanisms like MRSA (Methicillin resistant Staphylococcus aureus), ESBL (Extended spectrum beta lactamase production) of the pathogenic bacterial isolates. The available knowledge can provide the basis to address the risk of microbial transmission associated with handling of money and to raise the health awareness among people while handling money- a potential fomite for microbial transmission and also for infection control measures.

MATERIALS AND METHODS:

SAMPLE COLLECTION:

We conducted an observational cross sectional study during the period of June 2011 – November 2012 during which a total of 300 samples were collected randomly from volunteers after obtaining informed consent and persons showing definite and visible signs of infections were excluded from the study. Samples of currency notes (Indian Rupee) of denominations 5, 10, 20, 50, 100 and coins-50p,1Rs,2Rs were collected by exchanging notes/coins from volunteers with new notes. Volunteers included Beggars(n-50), Shopkeepers(n-50), Bank employee(n-50), Fishermen(n-50), Laboratory personnel(n-50) and samples collected in sterile petri dishes and transported immediately to Microbiology laboratory for analysis.
PHYSICAL CONDITION OF CURRENCY:
Based on their physical condition, currency notes were categorized as clean and dirty. Rupee notes that had a clean appearance without any obvious damage were designated "clean". Rupee notes and coins which were damaged or soiled were designated "dirty".

ANALYSIS OF MICROBIAL PROFILE AND DETERMINATION OF BACTERIAL LOAD:
Under aseptic conditions, sterile cotton-tipped swab moistened with sterile physiological saline was used to swab both sides of every sample. These swabs were inoculated into BHI (Brain heart infusion) broth and incubated at 37°C for 24 hrs (4, 6). Bacterial load of each sample was determined by semi-quantitative swab culture technique described by Herruzo-Cabrera et al which is a classic semi-quantitative technique involving sequential streaking of the initial swab over four quadrants of Blood agar plate and determining the highest quadrant with bacterial growth after incubation under aerobic conditions at 37°C for 24 hrs (13, 14). Sub culturing of the BHI broth was also done on MacConkey agar, incubated aerobically at 37°C for 24hrs and Sabouraud's dextrose agar slopes in duplicate-incubated at 25°C for 3 weeks. Culture plates were observed for growth. In the semi-quantitative method, bacterial growth in quadrant 3 or quadrants 3 & 4 was taken as significant (13, 14, 15). Identification of bacterial isolates done by standard techniques – colony characteristics, Gram reaction and various biochemical reactions (16,17).

Antibiotic susceptibility pattern of the pathogenic isolates were tested by Kirby-Bauer disc diffusion method on Mueller Hinton agar using the following antibiotic discs - Penicillin (10 units), Ampicillin (10µg), Erythromycin (15µg), Cephalexin (30µg), Cotrimoxazole (1.25+23.75µg), Gentamicin (10µg), HLG - 120µg), Ciprofloxacin (5µg), Amikacin (30µg), Vancomycin (30µg), Ceftazidime (30µg). Methicillin resistance among Staphylococcus aureus isolates was detected using cefoxitin disc (30µg) and screening and confirmatory test for ESBL production was carried out using ceftazidime (30µg), cefotaxime (30µg) discs alone and in combination with clavulanic acid (30µg/10µg) as per CLSI (Clinical and Laboratory Standards Institute) guidelines. The zones of inhibition around the discs were measured and interpreted as per CLSI guidelines (19). All the media and antibiotic discs used for the above said procedures were procured from Hi Media Laboratories Pvt Ltd, Mumbai, India.
The study has also been approved by our institutional Ethical committee.

**STATISTICAL ANALYSIS:** The results were analyzed statistically by Chi-square test.

**RESULTS:** Of the total 300 samples, 58 were coins and 242 currency notes. Out of these 300 samples 117 samples were clean samples and 183 were dirty samples. All samples showed growth of organisms.

Table 1. Group wise distribution of Bacterial and Fungal isolates among the clean and dirty samples.

<table>
<thead>
<tr>
<th>Source group</th>
<th>Nonpathogenic bacteria</th>
<th>Pathogenic bacteria</th>
<th>Fungal isolates</th>
<th>Total isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CL (n=150)</td>
<td>DR (n=150)</td>
<td>T (n=300)</td>
<td></td>
</tr>
<tr>
<td>Beggar s (n=60)</td>
<td>65 (63%)</td>
<td>54 (54%)</td>
<td>46 (48%)</td>
<td>165 (55%)</td>
</tr>
<tr>
<td>Shop keeper s (n=50)</td>
<td>54 (100%)</td>
<td>44 (88%)</td>
<td>34 (68%)</td>
<td>132 (84%)</td>
</tr>
<tr>
<td>Bank employee (n=50)</td>
<td>60 (100%)</td>
<td>45 (90%)</td>
<td>34 (68%)</td>
<td>139 (86%)</td>
</tr>
<tr>
<td>Fishermen (n=50)</td>
<td>60 (100%)</td>
<td>52 (87%)</td>
<td>34 (68%)</td>
<td>146 (91%)</td>
</tr>
<tr>
<td>Health care workers (n=50)</td>
<td>54 (100%)</td>
<td>45 (90%)</td>
<td>34 (68%)</td>
<td>133 (86%)</td>
</tr>
<tr>
<td>Laboratory personnel (n=50)</td>
<td>54 (100%)</td>
<td>45 (90%)</td>
<td>34 (68%)</td>
<td>133 (86%)</td>
</tr>
<tr>
<td>Total (n=300)</td>
<td>186 (62%)</td>
<td>156 (52%)</td>
<td>110 (37%)</td>
<td>452 (100%)</td>
</tr>
</tbody>
</table>

n- Total samples CL-Clean samples, DR-Dirty samples T-Total NP-non pathogen P-pathogen From the 300 samples, a total of 524 bacterial isolates and 110 fungal isolates were recovered. Of these total 524 bacteria, 435 (85.5%) were non pathogens and 89 (14.5%) were pathogens (Significant, p value <0.001**). Majority of samples showed polymicrobial growth with maximum of 4 bacteria and 2 fungi from a single sample. About 80% pathogenic isolates showed significant growth (3+,4+) and 20% showed minimal growth (1+, 2+). The dirty samples showed higher level of contamination (63.6%) (significant, p value <0.026*) compared to the clean samples (36.4%). Majority of the pathogens were isolated from fishermen (24%), laboratory personnel (19%) and healthcare workers (18%) followed by others. Hence the degree of contamination is highest in samples collected from fishermen, laboratory personnel, health care workers followed by shop keepers, beggars and bank employee.

![Figure 6. Group wise distribution of Non Pathogenic isolates.](image)

The most commonly isolated nonpathogens were *Bacillus Spp.* 232 (53.3%) (other than *B.anthracis*), Coryneforms other than *Corynebacterium diphtheriae* 148 (34%) followed by *Coagulase negative Staphylococi* 31 (7%) and *Micrococcus* spp. 24 (5.5%).

![Figure 7. Groupwise distribution of PATHOGENIC GRAM POSITIVE COCCI](image)

The most commonly isolated pathogens were *Staphylococcus aureus* 38 (42.6%), *Enterococcus spp.* 14 (15.7%). Out of the total 38 *S.aureus*, 4 were Methicillin resistant *S.aureus* (MRSA) 10.5% (significant, p value <0.001**) which were isolated from health care workers and bank employee.
The pathogenic Gram negative isolates included - *Klebsiella* spp. 23 (25.8%), *Escherichia coli* 6 (8.6%), *Enterobacter* spp 1 (1%) *Citrobacter* spp. 3 (3%), *Pseudomonas* spp. 2 (2%) and *Proteus* spp. 2(2%). Four isolates were ESBL producing Gram negative bacilli (significant, p value <0.001**) (*K.pneumoniae*-2, *Pseudomonas aeruginosa*-1, *Proteus vulgaris*-1), of which one *K.pneumoniae* showed 2+ and all others showed significant growth (3+) and these were isolated from health care workers, laboratory personnel, fishermen and bank employees.

Figure.9. Distribution of various Fungal isolates

A total of 110 fungal isolates were identified including *Candida* spp. 43 (39%), *Aspergillus* spp. 43 (39%), *Penicillium* spp. 13 (11.8%) and others 11 (1%) like *Absidia* spp. *Chetomium* spp. *Nigrospora* spp., *Cunninghamella* spp. *Syncephalurn* spp. Majority of the *Candida* isolates were non albicans group - 37 (86%) and only 6 (14%) were *C.albicans*. The isolates of *Aspergillus* included *A.nidulans*, *A.fumigatus*, *A.flavus*, *A.terreus*, *A.niger* and *A.glaucus* in the descending order of isolation. Fungal isolates of significant concern are *Fonseca pedrosai*, *Mucor* spp., *Rhizopus*, *Rhizomucor*, *A.terreus*, *A.fumigatus*.

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coins with various bacterial and fungal isolates which included similar results with 100% contamination of currency notes and spp. Klebsiella spp. and P. aeruginosa of contamination rate on Bangladesh (TAKA) currency notes and rate of 44.5% whereas in our study it was little higher, 63.6% in have isolated (9). (Table 1)

contamination of currency were from fish sellers and meat sellers to the report by Ahmed .S.U et al(2010), that higher level of laboratory personnel have shown higher contamination rates similar to the report by Ahmed. S.U et al(2010), that higher level of currency notes collected from fishermen and healthcare workers, dirty notes, a favorable environment for microbes. In our study, the currency notes are contaminated with gram positive and gram negative bacteria and fungi. We observed in our study,

susceptible to Amikacin, Piperacillin/tazobactum and Imipenem. We observed in our study, among the 23 Klebsiella isolates, 22(96%) were resistant to Ampicillin. 20 isolates were (87%) susceptible to Ciprofloxacin and all the 23 (100%) were susceptible to Amikacin. The 4 ESBL producing Gram negative bacilli (resistance to Ceftazidime) (significant, p value <0.001**) were K. pneumoniae-2, Pseudomonas aeruginosa-1, Proteus vulgaris-1. All the 4 ESBL isolates were susceptible to Amikacin, Piperacillin/tazobactum and Imipenem.

**Antibiotics tested only against ESBL producing strains. S - Susceptible. R - Resistant.

Among the 23 Klebsiella isolates, 22(96%) were resistant to Ampicillin. 20 isolates were (87%) susceptible to Ciprofloxacin and all the 23 (100%) were susceptible to Amikacin. The 4 ESBL producing Gram negative bacilli (resistance to Ceftazidime) (significant, p value <0.001**) were K. pneumoniae-2, Pseudomonas aeruginosa-1, Proteus vulgaris-1. All the 4 ESBL isolates were susceptible to Amikacin, Piperacillin/tazobactum and Imipenem.

DISCUSSION:

The Rupee notes and coins circulate through the hands of every member of the community while trading and utilization of services. So there are possibilities of contamination when the currency is handled by contaminated hands or from contaminated surfaces, dirty objects, from soil or from the microbial flora of skin and thus can be transmitted to other individuals. We observed in our study, the currency notes are contaminated with gram positive and gram negative bacteria and fungi.

Dhanasree et al. (2010) have reported 100% contamination of currency notes and 96% of coins, the most common isolates being staphylococcus spp and gram negative bacteria like Citrobacter spp. Klebsiella spp. and P. aeruginosa (6). Our study also showed similar results with 100% contamination of currency notes and coins with various bacterial and fungal isolates which included known pathogens like S. aureus(42.6%), Enterococcus spp(15.7%), Klebsiella spp (25.6%), Pseudomonas spp (2%) and Proteus spp. (2%) and E. coli (1%). Lamicichane. J et al. (2009) has reported 75% of contamination rate on Bangladesh (TAKA) currency notes and have isolated S. aureus, S. epidermidis, S. pyogenes, K. pneumoniae, E. coli and Enterobacter spp as the common pathogenic isolates (10). In their study, the dirty notes had shown microbial isolation rate of 44.5% whereas in our study it was lower, 63.6% in dirty notes. It could be attributed to the moist, soiled nature of the dirty notes, a favorable environment for microbes. In our study, the currency notes collected from fishermen and healthcare workers, laboratory personnel have shown higher contamination rates similar to the report by Ahmed. S.U et Al(2010), that higher level of contamination of currency were from fish sellers and meat sellers (9). (Table 1)

Feglo, P.et al, (2010) have reported 98.7% contamination rate in Ghanaian currency notes and have reported Bacillus spp (41.7%), Coagulase negative staphylococci (33.04%) as the common nonpathogenic isolates and S. aureus (7.14%) E. faecalis (7.14%) followed by Citrobacter spp (4.46%), E. coli (1.7%) (11). In our study, we have isolated Bacillus spp (53.3%), coenergy other than C. diphtheriae (34%), Coagulase negative staphylococci (7%) and Microccoci (5.5%) as non pathogens which shows slightly varying results with the above study. There are reports of Bacillus spp. and Coagulase negative staphylococci causing opportunistic infections especially in immune suppressed individuals (6).

In our study, most of the pathogenic isolates like S. aureus, Klebsiella spp. have shown significant growth as detected by the semi quantitative method followed by others. Majority of the S. aureus isolates were resistant to Penicillin(90%), Macrolides (92%) but were 100% sensitive to Aminoglycosides, Cephalosporins, Fluoroquinolones and Vancomycin and 4 S. aureus were identified as MRSA (10.5%). Enterococcal isolates have shown 50% resistance to Ampicillin, Gentamicin but 100% sensitive to Vancomycin.

Sharma, A, Dhanashree, et al 2010(6) have reported 36.4% of MRSA which is higher than our findings and they have also reported 100% sensitivity of gram negative bacteria such as Citrobacter spp. Klebsiella spp. and P. aeruginosa, to Gentamycin which is similar to our study results. The four ESBL producing Gram negative bacilli (10.8%)- K. pneumoniae, P. vulgaris were 100% sensitive to Amikacin, Piperacillin/Tazobactum and Imipenem. The isolation of ESBL producing organisms as in our study is not yet reported by others.

In our study, the fungal isolates identified were Candida spp 43(39%), Aspergilus spp. 43 (39%), Penicilium spp 13 (11.8%) and others 11(1%) like Absidia spp. Chetomium spp. Nigrospora spp., Cunninghamella spp. Syncephalastrum spp which is higher than the report given by Singh.DV, et al(3) from Shimla showing 14% Candida spp. and 11% Aspergilus spp.

CONCLUSION:

In conclusion, the MRSA & ESBL producing Gram negative bacilli along with other pathogens showing resistance to the commonly used antibiotics are capable of causing many community acquired and hospital acquired infections of serious life threatening nature especially respiratory infections resulting in high morbidity and mortality. Thus these heavily contaminated currencies can act as potential vehicle of microbial transmission within the community and hospitals placing both immunocompetent and immunocompromised individuals at risk. Hence, there is a need for strict hand hygiene practices like washing hands before eating, before and after handling the food and handling money. Most importantly, health care providers and laboratory personnel should practice strict hand washing practices before and after touching every patient/potentially infected materials and should not handle any inanimate objects during their work without washing their hands.

It also emphasizes the need for health education to the community on strict adherence to good hygienic practices and proper handling of currency and avoiding practices like using saliva for counting money and keeping coins and currency notes in mouth. In some countries, use of plastic currency and intermittent decontamination of currencies are recommended (6,9,10). There is also an urgent need to
create and spread awareness about this to all community members that the currency could be a potential fomite in microbial transmission and to stress the importance of hand washing after handling money to prevent microbial transmission.

REFERENCES: