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Enumeration, Isolation and Identification of Pathogenic Microbes from Indian Paper Currency and its Antibiotic resistance susceptibility Pattern

Nikhi Verma [1], Dushyant Singh [2], Rudrangshu Chatterjee [2], Manoj Kumar [3], Anupam Dhasmana [4], Sanjay Gupta [4], Vivek Kumar [4]*

[1] Amity Institute of Microbial Technology, Amity University, Noida, Uttar Pradesh, India
[2] Department of Microbiology, Shriram Institute for Industrial Research, New Delhi, India
[3] Centre for Life Sciences, Central University of Jharkhand, Ratu-Lohardaga Road, Brambe, Ranchi, Jharkhand, India
[4] Himalayan School of Biosciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttrakhand, India

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Abstract: In the present study, total 77 Indian paper currency samples of different denominations (Rs 10, 20, 50 and 100) were randomly collected from fifteen different sources of Delhi in a sterile polybags. All the samples were analyzed by swab method for enumeration of total bacterial, total yeast and mould and coliform population as well as isolation and identification of relevant pathogenic bacteria. The result showed that total bacterial load in all 77 samples ranges from 52 to 3.8 x 10^6 cfu $swab^{-1}$ whereas 22 samples were contaminated with total yeast and mould lie in the range from 24 to 1.3 x 10^6 cfu $swab^{-1}$ and 22 samples were contaminated with coliform, ranges from 34 to 8.4 x 10^2 cfu $swab^{-1}$. The most prevalent microorganisms found were *Staphylococcus aureus* (6.49 %), *Pseudomonas aeruginosa* (5.19 %), *Salmonella* sp. (7.79 %), *Escherichia coli* (9.09 %) and *Shigella* sp. (1.29 %). Antibiotic susceptibility and resistance pattern was also checked by Kirby Bauer well diffusion method. Various antibiotics were used against each isolated pathogens. These microbes could be one of the major sources of transmittance of diseases in Delhi.

Keywords: Indian paper currency, microbial contamination, pathogenic microorganisms, antibiotic resistance.

Introduction

Indian currency is widely handled by low to high society people circulating every day on to various locations. Currency surfaces which harbor microbes might act as fomite and thus plays a significant role in the transmission of disease causing microbes. This route of transmission of bacteria is of great importance for health in developing countries, like India where the frequency of infection is general indication of local hygiene and environmental sanitation levels. Survival of various microbes on paper money and coins indicate that currency use represents a potential cause of sporadic cases of food borne illness reservoir [10].

Environment plays a vital role in transmission of microbes from one person to another, with many environmental materials serving as vehicles and one such example of it is money. Microbes are ubiquitous and are of two types; transient and resident. Paper currency is one of the most extensively exchanged articles. Microbes can be transmitted from one to other either directly

*Corresponding author (Vivek Kumar)
E-mail: < vivekbps@gmail.com >

through hand to hand contact or indirectly via food, drinks or other inanimate objects [3]. An individual living in unhygienic environment having unhygienic habits will contaminate the currency notes more. For example, Activities such as using saliva to count the paper notes also leads to the contamination and these notes will act as a vehicle delivering bacteria to contaminate the hands of the next user. The money makes easy transfer of bacterial and thus cross contamination [14].

Research has shown that paper currency offers a larger surface area as a breeding ground for pathogens [1]. The older the paper note the more accumulation of microbes occurs [4]. In India, poor currency handling culture is widespread, and there is indiscriminate abuse of currency notes. A great majority of the population do not carry money in wallets and squeezing of currency notes is a common occurrence. Many people tongue wet their finger when counting money thereby, contaminating their fingers used to handle or eat food without washing of hands. Practices like spraying money during ceremonies and keeping currency in socks are also prevalent. These activities not only enhance currency contamination but may also increase the risk of infection from contaminated notes [11].

Pathogenic strains like *E. coli* 0157:H7 and *Salmonella enteridis* can survive up to eleven days and up to nine days respectively on the surfaces of currency, thus making it possible for currency to transfer bacteria to human hands [7]. Bhat *et al.* [2] reported that bacteria on currency surface can cause tuberculosis, meningitis, tonsilitis, peptic ulcers, throat infections, genital tract infections.

Therefore, keeping the above in view, the present study has been planned to investigate the different types of bacterial species isolated from Indian currency notes and their antibiotic resistivity pattern.

Materials and Methods

Sample collection

A total 77 samples of Indian paper currency of different denominations (Rs 10, 20, 50, 100) were collected randomly in duplicates from fifteen different sources of Delhi viz., bus conductor, chicken shop, street food vendor, tea stall, juice corner, pan corner, flower shop, vegetable seller, rickshaw puller, fish seller, fruit seller, egg shop, nursery, wallets (Men and women) and spices seller in a sterile poly bags, sealed and taken to the microbiology laboratory for analysis. Coins were not collected because their circulation is low.

Enumeration of total microbial count

The enumeration of total bacterial count, total yeast and mould count and coliform count was carried out (as per standard protocol) from currency surface by using a sterile cotton tipped swab dipped in the sterile diluent (0.1 % Peptone). The tip of the swab was then streaked on both sides of the currency note by rotating the swab between thumb and forefinger (as per ISO 18593:2004) and was incubated for at 37°C for 15 minutes.

Initially 1 ml of the sample from the swab was transferred to sterile petri plates and further dilutions were prepared by adding 1ml of sample to 9 ml diluent. For the enumeration of total bacterial count, the media used was plate count agar (PCA); for Total yeast and mould count media used was chloramphenicol yeast glucose agar (CYGA) and for the enumeration of Total Coliform media used was violet red bile agar (VRBA). The method used for enumeration was pour plate method. The plates of PCA and VRBA were kept at 37°C for 24 hours whereas the plates of CYGA were kept at 25°C for 3-5 days. Colonies were counted and reported in cfu/swab.

Isolation of microbes

For isolation of pathogenic microbes, 4 ml of swab sample was added to 50 ml of Nutrient broth (NB) and was incubated at 37°C for 24 hours. After overnight incubation a loop full inoculum from NB was further streaked onto different selective agar plates; Eosin methylene blue (EMB) and MacConkey agar (MCA) plates for *E. coli*, Baird Parker agar (BPA) plate for *S. aureus*, Deoxycholate citrate agar (DCA) plate for *Shigella*, Bismuth sulfite agar (BSA) and Brilliant green agar (BGA) plates for *Salmonella* and Cetrimide agar (CA) plate for *P. aeruginosa*. All the plates were incubated at 37°C for 24 hours (as per Indian standard protocol) and then observed for characteristic colonies and were further confirmed by Biochemical tests.

Identification of microbes

The bacteria were isolated by assessing colony characteristics and Gram staining and by performing various biochemical tests like Catalase tests; Oxidase tests; Coagulase tests; triple sugar iron (TSI) agar tests, Indole production, Nitrate broth test; Hugh Leifson test; Skim milk agar test; Gelatin Liquefaction test; Starch hydrolysis test. HiMedia Biochemical rapid detection kits were also used for confirmation of both *E. coli* and *Salmonella*.

Antibiotic susceptibility testing

Antibiotic susceptibility testing of the bacterial isolates was performed by Kirby-Bauer well diffusion method. Various antibiotics were used against each isolated bacterial strains.

Results

Microbiological profiling was carried out for 77 Indian paper currency samples of different denomination (Rs 10, 20, 50, 100) randomly collected from different sources of Delhi. In the present study, two types of controls with reference have used. Negative controls (mint) from Reserve Bank of India, Positive controls from the places having high probability of contamination. Results reveal that, all the currency notes were found to be contaminated by bacteria while 22 samples were contaminated by both Yeast and mould and coliform. The Total bacterial count in all 77 samples ranges from 52 to 3.8 x 10^6 cfu $swab^{-1}$ whereas 22 samples were contaminated with Total Yeast and mould lie in the range from 24 to 1.3 x 10^6 cfu $swab^{-1}$ and 22 samples were contaminated with coliform, ranges from 34 to 8.4 x 10^2 cfu $swab^{-1}$ (Figure 1). Bacterial, Yeast and mould concentration was found to be high in Egg shop samples while Coliform concentration found to be high in Chicken shop samples.

During the study, a total of 23 bacteria were isolated from Indian paper currency. The most prevalent microorganisms found were *Staphylococcus aureus* (6.49 %), *Pseudomonas aeruginosa* (5.19 %), *Salmonella* (7.79 %), *Escherichia coli* (9.09 %) and *Shigella* (1.29 %). These isolates were further confirmed by Biochemical tests. Isolated bacterial strains were then evaluated for their antimicrobial susceptibility pattern against eleven commonly prescribed clinically significant antibiotics (Table 1) by using agar well diffusion assay. Antibiotic resistance patterns were determined in terms of average zones of diameter considering 6 plates for each bacterial isolates against each of eleven antibiotics of 5 mg/ml concentration.

Data revealed that the most vulnerable antibiotic was found to be Vancomycin against which *Pseudomonas aeruginosa*, *Salmonella* and *Shigella* shows the 100 % resistance. Tazobactum was found in danger antibiotic as *Pseudomonas aeruginosa* and *Shigella* shows the 100 % resistance against it. Antibiotic Meropenem was found to be maximally susceptible for *S. aureus* with zone of 29 mm-36 mm, *P. aeruginosa* with zone of 29 mm-39 mm, *E. coli* with zone of 26

Table 1. Antibiotics used for checking susceptibility pattern

No.	Antibiotics	Stock concentrtatiom (10 mg/ml)
1	Tazobactum	980 µg/mg
2	Amikacin	900 µg/mg
3	Norfloxacin	990 µg/mg
4	Piperacillin	963 µg/mg
5	Doxycyclin Hcl	100 mg
6	Levofloxacin	500 mg
7	Ciprofloxacin	500 mg
8	Meropenem	500 mg
9	Gentamicin	590 µg/mg
10	Vancomycin	500 mg
11	Tobramycin	685 µg/mg

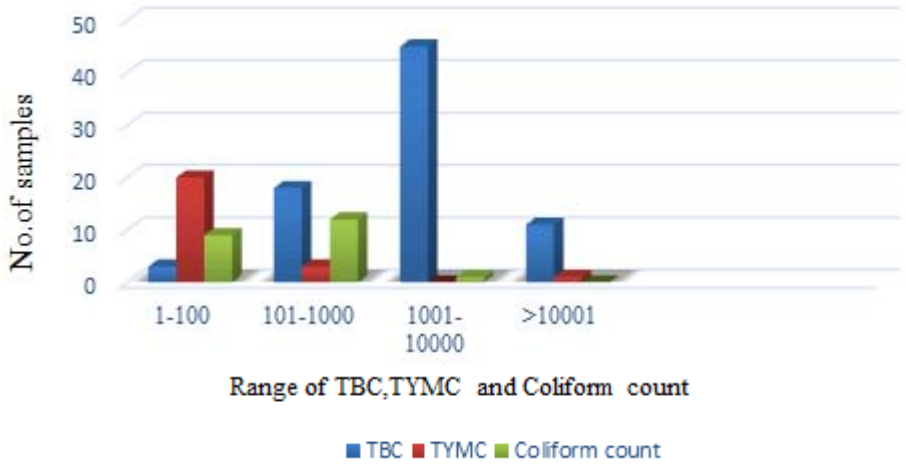

Figure 1. Graphical representation of total bacterial, yeast and mould and coliform count

mm-32 mm and *Salmonella* with zone of 30 mm-39 mm. Ciprofloxacin was also found to be most promising antibiotic against *P. aeruginosa* with zone of 29 mm-47 mm and *Shigella* with zone of 36 mm. Whereas Amikacin, Norfloxacin, Piperacillin, Doxycyclin Hcl, Levofloxacin, Gentamicin and Tobramycin were found to be intermediate antibiotics against each isolated bacteria.

Discussion

Indian currency provides surface area for microbial establishment and the microflora also changes depending on where the money has been passed. The large population, high density and weather conditions in India create ideal conditions for bacteria to proliferate. Also the prevalence of unhealthy money handling culture like counting money with salvia, keeping money under clothes and not washing hands after money usage increases the risk of transmission of diseases by currency [9]. Contamination was also related to the physical conditions of the currency; the high aging paper currency had the highest, moderate aging paper currency had lesser and new paper currency having least prevalence of contamination [13].

Lower the denomination of currency is directly proportional to microbial load. Since lower denimination notes are most widely exchangeable article. The presence of *S. aureus* and *P. aeruginosa* in the Indian paper currency indicates the practice of unhygienic habits. On the other hand presence of *E. coli* and *Shigella* isolates indicates the presence of poor sanitary conditions. Similarly, the presence of *Salmonella* which is responsible for the disease Salmonellosis (food poisoning) indicates to the usage of poor handling of food raw materials and water.

In Indian scenario, the women, particularly among the uninformed, generally place money beneath their brassieres, while on the other hand, men place it in their vests and socks. These doings not only enhance currency contamination but also may also upsurge the infection risk from dirty and filthy notes. In this study, isolation of Gram's negative as well as Gram's positive bacteria from currency notes showed that currency notes might play a significant role as transmission vector for pathogenic bacteria in the public. For eg. There are some strains of *Bacillus* which have been reported in food poisoning [8]. Likewise, *Micrococcus* sp. have been well documented as unscrupulous pathogens particularly in the patients which are immunocompromised [16]. Nevertheless, the *Staphylococcus aureus* are the usual flora of skin and mucous membrane their high occurrence has clinical implications and are deliberated as well-known pathogen. In several studies it has been documented that the clinical implication of *S. aureus* as a main causative agent of urinary tract infections [15]. The *S. aureus* has also been reported to associated with, skin infections, toxic shock syndrome e.g. frunculosis and respiratory tract infections. In this study, among Gram's negative bacteria isolated *Escherichia coli* is a contagious organism that may cause urinary tract infections, bacteremia, community acquired pneumonia, re-

current meningitis, sepsis etc. [6]. The *Klebciella pneumoniae* is also the significant cause of nosocomial and community acquired infections. The *Klebciella pneumoniae* has also been observed as one of the chief source of Gram's negative sepsis as well as bacteremia. It may lead to diseases like pneumonia, fatal acute bacterial, meningitis, myocarditis and wound infections [12].

Conclusion

From this study, it can be concluded that more general awareness is needed so that people can know that money can also pose a threat. Basic hygiene practices should be cultivated among people like washing hands properly with soap before and after handling money. Avoid practices like soling to count money, keeping money under clothes, spraying money in weddings and keeping money in mouth. Introduction of currency made from material having antimicrobial properties or plastic currency can serve as an alternate.

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