BVG Trust

J. Sci. Trans. Environ. Technov. 2015, 8(4) : 185-188

Scientific Transactions in Environment and Technovation

Isolation and identification of microflora from Indian currency notes and evaluation of virulence of pathogens using PCR

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Abstract

Currency note contamination is of important to public health as it can provide a vehicle for easy transmission of pathogen between handlers. An attempt has been made to isolate bacterial and fungal pathogens from different categories of Indian currency notes and evaluation of potential pathogens and its virulencehave been determinedusing PCR. The present article deals with microbial load of the currency notes and their potentiality to cause diseases in human beings.

Keywords: Currency notes, Pathogens, Public Health, Virulence.

INTRODUCTION

Fomites are inanimate objects capable of absorbing, harbouring and transmitting infectious microorganisms. Dust and dirt that commonly accumulate on such objects contain spores of infectious agents (Bailey and Scott, 1974). A fomite currency which are contaminated through droplets during coughing, sneezing, touching with hands and placement on dirty surface are commonly handled by various categories of people during transactions. Paper currency is widely exchanged for goods and services, settlement of debts and for differed payments in the economic activities in different countries. Currency contamination is of important to public health as it can provide a vehicle for easy transmission of pathogen between handlers.

Environment plays a critical role in disease transmission to human, with many environmental materials serving as vehicles (Anderson and May, 1991). Microbial contaminants may be transmitted, either directly through hand to hand contactor indirectly via food or other inanimate objects. Other attitudes such as the wetting of hands or fingers with saliva or use of contaminated water to lubricate the hand in counting money could lead to possible transfer of parasite or bacteria from such a medium to the notes (Ameh and Balogun, 1997).

Studies from other parts of the world (Havas, 2000) have also shown that bank notes revealed the presence of high load of germs which could cause several illness. So the infected currency is identified as potential public health hazard as pathogen spread by circulation.Immuno-compromised persons are at the risk of acquiring opportunistic infection, through handling or contamination. Opportunistic infection can occur when immune system is not functioning properly. So bacteria and fungi that contaminate the currency notes are considered potential pathogens to cause diseases.

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The Regional Sophisticated Instrumentation Center (RSIC) at the North Eastern Hill University Shilong, India, examined Indian banknotes and found that the microbes contaminating the currency notes could cause tuberculosis, meningitis, tonsillitis, peptic ulcers, throat infections and genital tract infections.The microbes from the banknotes infect the body through scratches on the hands or when the hand touches the mouth or nose.

Over the last two decades, the observed data indicated that simultaneous handling indeed was a cause of sporadic food borne-illness and survival of pathogens on currency notes in Turkey (Pope *et al.*,1999) and in India (Singh *et al.,* 2002). Failure of food service workers to adequately sanitize hands or food – handling tools (tongs, spoons, utensils or bakery/serving papers) between handling of money and serving of food could put food service persons at risk (Michaels, 2002). However, reports on the degree at which the paper money is contaminated with bacteria are few (Abrams and Waterman,1972). Scientific information on the contamination of currency notes by microbes is lacking in most of the developing countries. The present article deals with the microbial load of the currency notes and their potential role as pathogens to cause diseases in human beings.

MATERIALS AND METHODS:

Isolation of bacteria and fungi

Isolation of bacteria by subjecting the currency notes (A total of 25 currency notes of five viz each Rs.5, Rs.10, Rs.20, Rs.50 and Rs.1000 in circulation were randomly collected from bank, vendors in open –air market, super markets, traders and conductors) to the routine laboratory methods using nutrient agar medium (Feglo and Nkansah, 2010). The plates for isolation, enumeration and counts were incubated at 37°C for 24 hours. Besides, selective and differential growth media viz *Pseudomonas* agar, TCBS Agar, SS agar and EMB agar were used for the identification and confirmationof different groups of microorganisms.

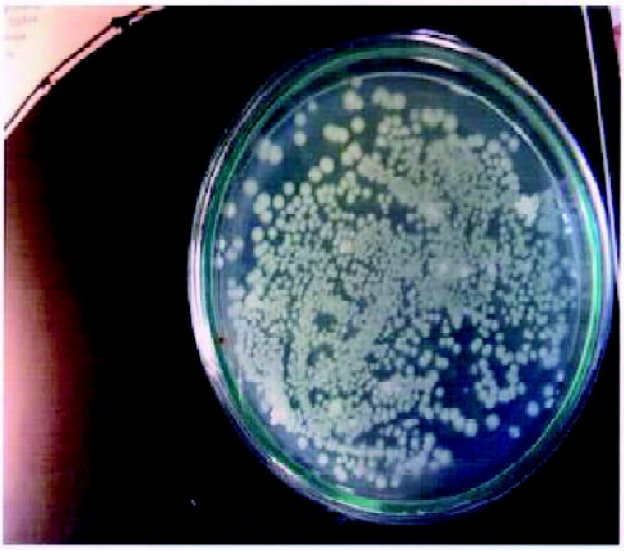


Figure 1.Growth of total heterotrophic bacteria on nutrient agar

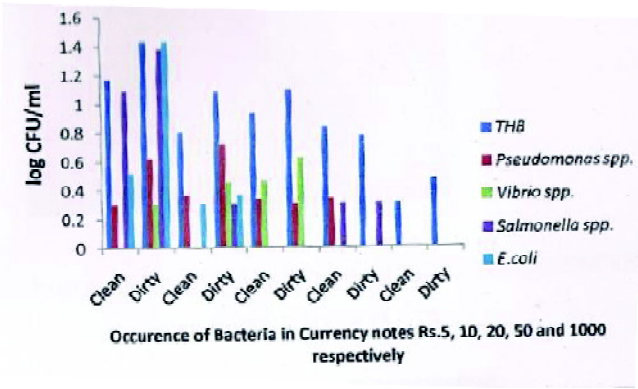
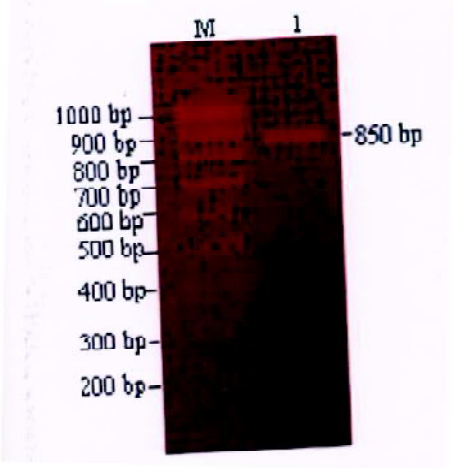
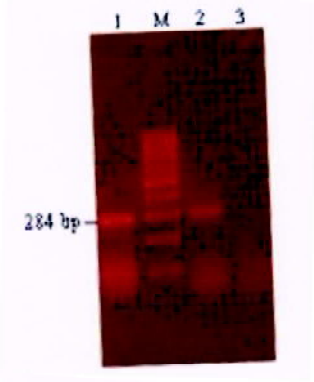


Figure 2.Occurrence Of Bacteria In Currency Notes



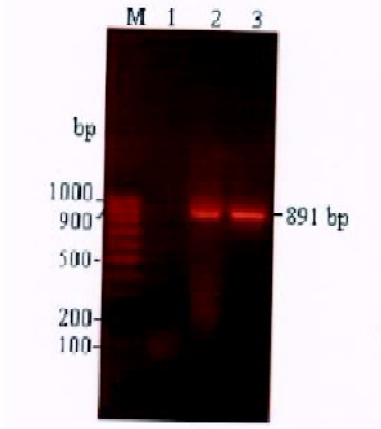
Lane M: 100 bp DNA Ladder
Lane 1 : 850 bp amplified *Pseudomonas spp.* specific gene product

Figure.3 P*seudomonas spp.* specific detection by using polymerase chain reaction (PCR)



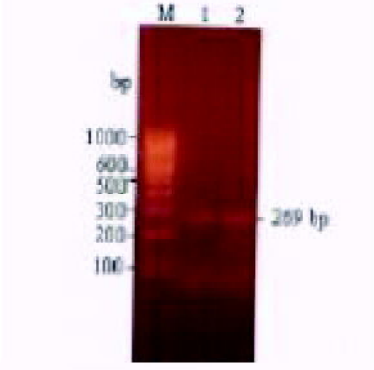
Lane M: 100 bp DNA Ladder
Lane 1: 284 bp amplified gene product of Positive Control DNA
Lane 2: 284 bp amplified gene product of Sample DNA
Lane 3: Negative Control

Figure 4 Genus specific (inv A– g) detection of *Salmonella* spp. by using polymerase chain reaction (PCR)



Lane M: 100 bp DNA Ladder
Lane 1: Negative Control
Lane 2: 891 bp amplified gene product of Positive Control DNA
Lane 3: 891 bp amplified gene product of Sample DNA

Figure.5 Detection of enteropathogenic*Escherichia coli* (EPEC) –gene by using polymerase chain reaction (PCR)



Lane M. 100 bp DNA Ladder
Lane 1 & 2: Amplified 269 bp TDH-gene of Positive Control (1) and Sample DNA (2) of *V.parahaemolyticus*

Figure.6 Detection of TDH – gene *Vibrioparahaemolyticus* by using polymerase chain reaction (PCR)

Isolation of fungi was made by subjecting the currency notes to the routine laboratory methods using Czapek – Dox agar medium(Feglo and Nkansah, 2010)

Identification of bacteria and fungi

Identification of bacterial strains were done based on Bergey's manual of Determinative Bacteriology (Gram's staining and biochemical tests) and fungal isolates using standard manuals.

Evaluation of virulence gene of Pathogens

Identification and evaluation of virulence gene of bacterial pathogens were made by using Polymerase Chain reaction (PCR). The procedure for identification of bacterial strains included preparation of template DNA, DNA amplification (PCR), PCR analysis for the detection of bacterial strains, detection of amplified product by using Agarose gel electrophoresis and documentation of the results (gel documentation)

RESULTS

A total number of 25 currency notes of five different denominations viz.Rs.5, Rs.10, Rs.20, Rs.50 and Rs.1000 were analysed for bacterial load including THB, *Pseudomonas spp., Vibrio spp. and Salmonella spp.* According to the appearance and degree of dirtiness the notes were classified as mint, clean and dirty. Mint refers to the fresh notes just received from the bank. The notes which were relatively clean and the dirty referred to the notes that were handled and in circulation.The mint notes were sterile (i.e.) they didn't harbour any microbe.

The clean notes showed that the THB was in the range of $1.0x10^2$ to $1.3x10^8$ CFU/ml. Regarding pathogens,*E.coli* was in the range of $1.0x10^1$ to $1.1x10^3$ CFU/ml, *Pseudomonas spp*. was in the range of 0 to $1.2x10^2$ CFU/ml, whereas *Vibrio* and *Salmonella* were in the range of 0 to $1.5x10^1$ and $1.0x10^1$ to $2.3x10^3$ CFU/ml respectively. (Fig.I and II).

The dirty notes showedthat the THB was in the range of $1.0x10^3$ to $1.5x10^4$ CFU/ml. Regarding pathogens *E.coli* was in the range of 0 to $3.0x10^3$ CFU/ml, *Pseudomonas*was in the range of 0 to $1.3x10^3$CFU/ml., whereas *Vibrio* and *Salmonella* were in the range of 0 to $1.0x10^2$ and $1.0x10^1$ to$2.9x10^3$ CFU/ml respectively (Fig I and II).*Aspergillusniger* wasthe only fungus found in the paper currency.

Primers were designed to check the virulence of*E.coli* (891bp), *Salmonella spp*. (284bp) and *V.parahaemolyticus* (269bp). They possessed ETEC, inv A and TDH gene respectively. *Pseudomonas* was checked with genus specific primer which resulted in 850 bp product (Fig. 3, 4, 5 and 6).

DISCUSSION

The present study showed that bacteria were abundant in Indian currency notes especially in the lower denominations viz.5, 10, 20 and 50. It is similar to the study reported by Gadsby (1998).Surprisingly the fresh notes received from bank did not harbour any bacteria including the lower denominations.The study clearly indicated that in1000 rupee notes the bacterial load was too low and among pathogens except *Salmonella* no other pathogens were found.

Pseudomonas was an ubiquitous and non-pathogenic bacteria, whereas other representative haemolytic strains such as *E.coli, Vibrio* and *Salmonella* were selected to check their virulence.*E.coli* showed the presence of EPEC gene, *V.haemolyticus* possessed TDH gene and*Salmonella* strain showed Invasive A *gene* (*inv* A). Ginocchio and Gyles, 1992. The species of *Pseudomonas* was also established using genus specific gene of 850 bp as *Pseudomonas aeruginosa*. Though non-pathogenic, the concern here is that the immune compromised persons who handle these currency notes may get infected. This gene also can indicate the presence of other *Pseudomonas*strains. *Aspergillus niger* was the only fungus found in the paper currency. It is because of the fact that the species of the fungi as could be present as dormant propagules and also as casual residents.

The study proved that frequency of handling might be the deciding factor for the density of microbial load.Among the pathogens tested using specific media, all of them were present in higher densities than expected levels.Especially *Salmonella* population was found to be surprisingly high. This showed that high risk of *Salmonella* infection is possible while handling these notes. Some of these notes, especially lower denominations were collected from beggars, milk vendors, fish vendors and gobblers. Some of the notes collected from these people, were literally wet and sticky with dirt. As beggars, gobblers and small vendors are in and around bus stands and public places, the notes transferred by them may reach passengers or tourists who may carry these notes to far off places. The currency notes used by public transport in Nepal (Mini-Buses) were found to be extremely contaminated by various pathogens (2009). In public health point of view, the presence and emergence of *Salmonella* in large numbers in currency is of great concern. In the present, study, a specific PCR product of 284bp DNA fragment was obtained from the *Salmonella* strain isolated from dirty currency notes (Rs.5 denomination). This gene is not only useful in detecting *Salmonella* but also helpful in the confirmation of their invasive property,virulence. among 630 strains belonged to over 100 serovars it was found that this gene was able to detect 99.4% of *Salmonella* strains, and they suggested that this gene is a suitable PCR target with potential diagnostic application. The present study also confirms the same.

Regarding Vibrios only *V. parahaemolyticus* was found. Reason for this is unknown and deserves further research using increased number of samples. These strains were truly haemolytic in Wagatsuma agar which proved that their virulence was of at its highest degree.

The haemolytic strain was found to possess Thermolysin Direct Haemolysin (TDH) gene, which is capable of producing harmolysin toxin. Presence of virulent *V.parahaemolyticus* strains indicated the high risk involved in handling these notes. V.*parahaemolyticus* is an unusual gut pathogen. It is also an aquaculture pathogen. The abundance of shrimp farming activities in this area may be the reason for the presence of this strain in the circulating rupee notes. The study proved that the lower denomination notes might reflect more local hygienic conditions. However this may not be true always. Kamruzzaman *et al.* (2008) reported that the TDH gene encoding the thermostable direct haemolysin was considered as an important virulence gene, as most clinical strains carry this gene. It is interesting to note that TDH gene in V.*paraharmolyticus* is carried by plasmids and hence horizontal transfer is possible. In the present study also, the presence of TDH gene referred the potential health hazard related to handling contaminated currency notes.

According to Oyero and Emikpe (2007) the bacteria isolated were also found associated with skin and facial contamination. In the present study also, such bacterial isolates were also recorded viz. *Pseudomonas* and *E.coli*. This indicates the unhygienic practice of people as reflected by the presence of representative microorganisms. The practices include indiscriminate sneezing, coughing and defecation without minding the hygienic practices to be adopted.

E.coli was also recorded in very high density from the dirty notes.It is an adaptive species that is both a commensal resident of the intestine and a versatile pathogen of human and animals. ETEC is a major cause of diarrhoea in children in developing countries particularly under the age of five. ETEC infections are comparable to that *V.cholerae* (Sommer *et al.*, 2010). So the present study also emphasizes the health hazard due to the presence of virulent *E.coli* in the currency notes.

The present study highlights the abundance of microbes harboured in the Indian paper currencies and their disease producing potentiality. The study also proved that PCR could be used as a tool to rapidly identify the potential pathogens present in the currency notes. The study also emphasizes the importance of personal hygiene as well as the need for the use of other modes of paper currency less transactions.

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