



Quantitative assessment and antibacterial activity of *Azadirachta indica* (neem) leaves against pathogens isolated from currency notes in circulation

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Abstract

Paper currency is commonly contaminated with microbes and this may play a role in the transmission of potentially harmful organisms. So cash should not be handled by children and should be kept away from food and cosmetics. Currency notes represent a universal medium for transmission of bacteria in the environment and among humans. Care should be taken by those handling these currencies to. Hence the present study was planned quantitatively assess of total bacterial load present on the currency notes circulating in the open market area and hospitals in Pattukkottai. A total of 24 frequently circulated currency notes were collected from open Markets and hospitals(12 from each groups).The bacterial isolates were characterized on the basis of their morphology, staining and biochemical tests. Currency notes were contaminated with *S. aureus*, *E.coli*, *P. aeruginosa*. In the hospital group, isolated organisms were *S. aureus* (58%) and *E. Coli*(23%) ; in the open market areas, *E. Coli* (34%) and *S. aureus* (36%) were found. The study concluded that the notes from hospitals were contaminated more with *S. aureus* than in open market, whereas *E. Coli* was present in open market areas hospitals. Antibacterial activity was performed by both Kirby Bauer disc diffusion technique and herbal extract of neem. Ethanolic extract of *Azadirachta indica* (neem) leaves(25,50,75,100%)was showed maximum level of Zone of Inhibition against the pathogens isolated from Currency notes at 100% concentration. Gentamycin was showed better than other antibiotics. Compared to antibiotic sensitivity test, ethanolic extract showed better results. Both the isolated microbes treated with UV treatment in that maximum level of inhibition was noticed in 20 minutes.

Keywords: bacterial contamination, currencynotes, *Azadirachta indica* *Escherichia coli*, *Staphylococcus aureus*. *Pseudomonas aeruginosa*

Introduction

Money is any medium of exchange that is widely accepted in payment for goods, services and in settlements of debts. Paper currency is widely exchanged for goods and services in countries worldwide. It also serves as a standard of value for measuring the relative worth of goods and services (Felgo and Nkansah 2010; Alwakeel and Naseer, 2011) [1, 2]. Modern scientific techniques have confirmed these theories and have shown that viable pathogenic organisms can be isolated on the surfaces of both paper and coin currency (Pradeep *et al*, 2012) [3]. Contamination of materials by pathogenic microorganisms is of public health concern as contaminated materials could be a source of Transmitting microbial pathogens (Ghamdi *et al* 2011) [4]. Movement of materials from hand to hand makes it more prone to contamination by pathogenic organisms. Polymer currency therefore, poses a serious threat to public health since communicable diseases could be contracted through fomites. Currency is handled by all categories of people and may be contaminated during coughing, sneezing, touching with hands and placement on dirty surfaces. Many people tongue-wet their fingers when counting money and contaminate their fingers as well as currency notes. So, it is obvious that gets on hands may be transferred to money and Vice-versa (Pal *et al* 2013) [5]. paper banknotes have a large surface area for bacterial attachment and would be a vector for transmission of potentially pathogenic microorganisms between Populations (Girmaet *al*, 2014) [6]. Hence, this study is undertaken to quantitatively assess the microbial

population present on the currency notes circulating in the open market area and hospitals in around Pattukkottai, Thanjavur district, Tamil Nadu, India and antibacterial activity was also performed by using commercial antibiotics and herbal extracts.

Sample collection and study design

Eight currency notes of each denomination (Rs. 10, Rs. 50, and Rs100) making it a total of 24 notes were collected, among which four notes of Rs. 10, Rs. 50, and Rs. 100, were obtained randomly from (Group I) open -air markets (food vendors, banks, Gas-filling stations) as well as from hospital cash counters (Group II). Persons handling the notes were asked to deposit them in sterile envelopes. They were compensated with other currency notes of the same denomination. The study samples were collected based on the level of usage and thus circulation. The currency notes were taken to the laboratory immediately for identification of microorganisms. (Saeed and Rasheed, 2011) [7].

Sample size

After demonetization the currency notes were chosen, such as ten, fifty, one hundred Indian currency notes.

Microbiological analysis

Isolation of microbes from currency notes sample

Standard procedures was under taken for the isolation of microbes from currency notes sample.

Susceptibility studies on the bacterial isolates (Prescott *et al.*, 2008) [8]

The Kirby-Bauer Disc Diffusion Method was used to test the in vitro susceptibility of the identified isolates to Ampicillin, Gentamycin, Tetracycline, Streptomycin. A sterile platinum loop was used to pick overnight bacterial colonies from the culture plate and emulsified in 4 ml of sterile pepton water. Using sterile swap, the surface of Mueller Hinton agar (Oxoid, Basingstoke, UK) was evenly inoculated with the suspension and allowed to air dry for 10 minutes. Using multichannel disc dispenser (Oxoid, Basingstoke, UK) antibiotics disc were deposited onto the surface of the inoculated medium and plates incubated at 37°C for 24 hours. Susceptibility of bacterial isolates was recorded as 'sensitive' or 'resistant'. Measuring the diameter of the zone of inhibition in mm.

Preparation of Ethanolic Extract of *Azadirachta indica* (neem) leaves (James *et al* 2014)[9]

Fresh leaves of *Azadirachta indica* (neem) leaves were collected. It was ensured that the collected leaves were healthy and uninfected. The leaves were washed under running tap water to eliminate dust particles. Leaves were air dried at room temperature. The solvent (250ml of ethanol) was poured into Soxhlet extract and condenser on an isomantle. The equipment was monitored due to the mix of running water and an electric appliance. The extract was recovered using a hot air evaporator.

Control of isolated Microbes by UV treatment

The isolated microbes were treated with different interval of UV treatment. The isolates were inoculated with nutrient broth separately. The inoculated broths were incubated at 37°C for 24 hours. After incubation all broths were read 600nm. Control also maintain (without UV treatment).

Statistical analysis

All the experiments were repeated as triplicates the results obtained in the present study were subjected to statistical analysis like mean X and standard deviation (SD) (Gupta, 2004) [10].

Results and Discussion

There is a possibility that currency notes might act as a medium for the transmission of potential pathogenic

microorganisms. Hence the present was undertaken to assess bacterial count and antibacterial activity was also done by Herbal extract of neem and synthetic leaves. Microbial examination was carried out for 24 currency notes in which all the currency notes were contaminated with microorganisms. All currency notes obtained from the open market (group I) and hospital (group II) were collected. Majority of samples were contaminated with bacteria. Identification showed the active participation of these species - *E. coli*, *Pseudomonas aeruginosa*, *S. aureus* were found. Group I total microbial population were estimated for Rs.10, Rs. 50, Rs. 100 notes collected from open -air market samples viz *S. aureus* (36%), *E. coli* (34%) and *P. aeruginosa* (21%). Group II total microbial population were estimated for Rs. 10, Rs. 50, Rs. 100 notes collected from hospital currency notes samples viz *S. aureus* (58%) *E. coli* (23%) and *P. aeruginosa* (20%). The active participation and maximum percentage of *S. aureus* (58%) were found in (Group I) hospital currency notes samples. Followed by *E. coli* (36%) and *P. aeruginosa* (21%) were found in (Group I) open market samples. Hospital currency notes samples (Group II) are highly contaminated with the microbial populations (Table-1).

The isolated bacterial species from the contaminated currency notes samples were tested for their susceptibility against antibiotics by disc diffusion method. Antibiotics such as Ampicillin, Streptomycin, Gentamycin, and Tetracycline. The results were present in (Fig-1). Gentamycin showed the maximum activity against the bacterial species present in currency notes samples.

Ethanolic extract of neem leaves showed strong as well as maximum level of zone of inhibition against pathogens isolated from currency notes in circulation. 100% concentration of neem leaves against showed better results on *E. coli*, followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Table 3) The isolated microbes were treated with different interval of UV treatment. The investigated results were presented in Table 2. The maximum level of inhibition was present in 20 minutes treatments broth tubes compare than other interval treatments.

Our results are correlated to the findings of Sunil *et al* 2020 [11] notes from hospitals were contaminated more with *S. aureus* than in open markets, whereas *E. coli* was found to be more in open market areas than in hospitals.

Table 1

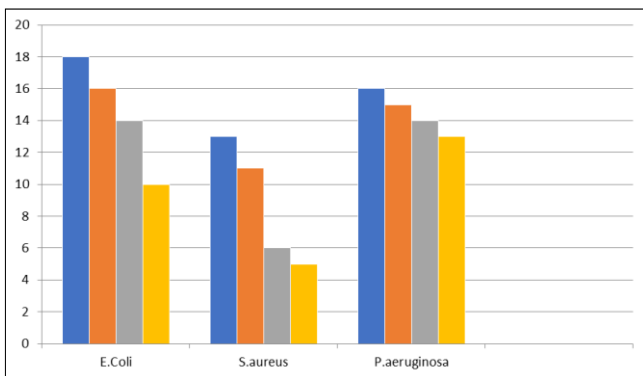
INCIDENCE OF MICROBIAL POPULATIONS								
Potential Pathogens	OPEN MARKET (GROUP I)				HOSPITAL (GROUP II)			
	Total Microbial Occurrence Percentage	Denomination Wise Microbial Occurrence Percentage			Total Microbial Occurrence Percentage	Denomination Wise Microbial Occurrence Percentage		
		Rs.10	Rs.50	Rs.100		Rs.10	Rs.50	Rs. 100
<i>S. aureus</i>	36	24	7	5	58	34	28	21
<i>E. coli</i>	34	21	8	5	23	11	7	5
<i>Pseudomonas aeruginosa</i>	21	9	12	-	20	-	12	8

Table 2: Control of isolated microbes by UV treatment

S. No	UV treatments (Minutes)	Inhibition of growth %		
		<i>E.coli</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>
1	10	30	45	60
2	15	80	90	90
3	20	100	100	100

Table 3: Ethanolic extract of Neem leaves against pathogens isolated from currency notes in Circulation

S. No	Ethanolic extracts of neem leaf (mg/ml)	Zone of Inhibition (mm)		
		<i>E.coli</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>
1	25	11	8	9
2	50	14	12	11
3	75	18	16	15
4	100	22	20	19

**Fig 1:** Antibiotic sensitivity pattern of isolated bacterial species from currency notes samples

Conclusion

The present study was carried out to isolate and identify the microorganisms in the locally available currency notes (Rs.10, Rs. 50 and Rs. 100).The samples were collected open-air market areas and hospitals in Pattukkottai, Thanjavur District, Tamil Nadu, India. Totally 3 bacterial species were isolated namely *Staphylococcus aureus*, *E. Coli*, and *Pseudomonas spp* and confirmed. Disc diffusion method was used to screen the antibacterial activity of both neem leaf extract and synthetic antibiotics. The currency notes collected from hospitals and open markets were more contaminated with pathogenic microorganisms From this study it can be concluded that Indian currency is commonly contaminated with pathogenic bacteria and this contamination may play a significant role in the transmission of infectious diseases. It is recommended to introduce plastic currency notes may be reduce the contamination. Behavioral practices such as using saliva while counting currency notes that could avoid to spread infection, regular disinfection of currency notes deposited in the banks using ultraviolet light or even by fumigation. It is also advised to wash hands in between handling of currency notes and food. Public education on proper handling and care of currency is advocated, in order to reduce currency contamination. Handling of currency notes between handwashing and food handling requires a repeat of the process to reduce the risk of contamination. Studies on the contamination of currency notes with pathogenic microorganisms are lacking in most of the developing countries. As the current study investigated the presence of microorganisms in lower denomination of currency notes in

circulation, further research including all denominations with a higher sample size is recommended.

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