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RESEARCH ARTICLE

ISOLATION, IDENTIFICATION AND CONTROL OF BACTERIA AND FUNGAL MICROORGANISMS FROM CONTAMINATED CURRENCY NOTES.

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Abstract

Money is very important to human life as it facilities the needs and currency notes are vital for goods and services worldwide. Paper currency is used repeatedly in exchange for goods and service and this are way the circulation of paper currency from one individual to another potentially spreads microorganisms. Contaminated different paper currency note samples were collected from hospital in Tiruchirappalli,Tamilnadu. The samples were analysed in microbiologically. Both gram positive and gram negative bacteria were found on currency notes. Predominant bacteria found in 25 currency notes were *Streptococcus pneumonia present (36%), Bacillus subtilis (24%), Pseudomonas aeruginosa (18%), Escherichia coli (12%) and Klebsiella pneumonia (10%).* fungi were *Aspergillus flavus (4%), Aspergillus fumigatus (8%), Aspergillus niger (4%)* and *Candida albicans (8%)*. DNA was separated by Agarose Gel Electrophoresis. The size of the DNA measured using molecular marker. The bands found at 9500 bp and 8000 bp respectively. The sensitivity tests were performed to detect the sensitivity of organisms against Standard disc placed. The maximum zone was observed in *Escherichia coli* and *Streptococcus pneumonia* against commercial antibiotics such as Chloramphenicol, Erythromycin .In fungi, the maximum and minimum zone of inhibition was observed in antibiotic clotrimazole and Amphotericin B respectively. The maximum level of inhibition was present in 20 minutes UV treatment. 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.

Introduction:-

Microbial contaminants may be transmitted, either directly, through hand –to –hand contact, or indirectly via food or other inanimate objects. These routes of transmission are of great importance in the health of many populations in developing countries, where the frequency of infection is a general indication of local hygiene and environmental sanitation levels. (Anderson, 1991; Struthers and Westran, 2003). Currency notes might act as environmental vehicles for the transmission of potential pathogenic microorganisms (Brady and Kelly 2000). Paper currency is used repeatedly in exchange for goods and service and this are way the circulation of paper currency from one individual to another potentially spreads microorganisms. If these currencies recontaminated by pathogenic bacteria, the rate of infectious diseases will continue to rise. Paper/polymer currency notes and coins may harbour various deadly pathogenic microorganisms. Currency in the form of notes and coins represents a universal medium for the transmission of bacteria in the environment and among humans (Xu *et al*.,2005). Paper currency, can be

contaminated by droplets during coughing, sneezing, touching with previously contaminated hands or other materials and placement on dirty surface. Pathogenic bacteria that may survive on the currency banknotes may serve as a potential source of enteropathogens causing food poisoning because food vendors handle and serve food and at the same time handle currency banknotes as they sell (Lamichhane *et al*.,2009).

Materials and methods:-

Sample collection

Contaminated 25 different paper currency note of different denominations such as 5, 10, 20.50,100 were collected from hospital in Tiruchirappalli,Tamilnadu. They were collected in sterile polyethylene bag and transported to the laboratory.

Isolation of microbes:-

Each currency note was aseptically transferred into individual 10 ml of sterile nutrient broth containing glass bottle for 24 Hours. The currency was removed and the resulting nutrient broth served as a test sample. The test samples were spread over Nutrient agar, Macconkey agar, Blood agar and PDA medium. All inoculated media were incubated aerobically at 24 hrs (37ºC) and PDA plates were incubated at 27 ºC for 72 hrs.

Bacteriological analysis:-

Isolation of various bacterial contaminants from currency notes was performed via standard techniques (Gilchrist,1993; Singh et al., 2002) and identified by assessing colony characteristics and Gram reaction ,Motility test and conducting biochemical tests including for Indole production ,Citrate utilization, Urease activity ;Triple sugar iron tests ,gas and hydrogen production tests, Catalase and Oxidase test, according to protocol described previously (Norris and Ribbons,1972).

Identification of fungi:-

The isolated fungal species were identified by cultural character and Lacto phenol cotton blue staining. The isolated fungal colonies aseptically transfer into clean glass slide and add one drop of Lacto Phenol cotton blue above the mixture put a cover slip and observe the slide at high power objectives.

Extraction and separation of dna:-

DNA were isolated from contaminated bacterial and fungal cultures and separated by using Agarose Gel Electrophoresis.

Antibiotic sensitivity test:-

The commercially available antibiotic disc such as Cephalothin, Cephoxitin, Cefuroxime, Cefixime.
Chloramphenicol, Erythromycin, Clarithromycin, Amoxycillin, Clindamycin and Vancomycin are used for bacterial culture and Amphotericin B,Clotrimazone, Ketoconazole and Nystatin used for fungal culture.The antibiotic discs were purchased from high media chemical Pvt. Ltd, Mumbai.The antibacterial and antifungal activities were carried out by disc diffusion techniques. The sterile Mueller -Hinton agar plates were prepared. The bacterial and fungal isolated organisms *Escherichia coli, Klebsiella pneumoniae,Pseudomonas aeruginosa, Bacillus subtilis Streptococcus pneumonia and Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger and Candida albicans* were spread over the Mueller -Hinton agar plates by using separate sterile cotton buds. After the microbial lawn preparation the collected commercial antibiotic disc were placed on the organism inoculated plates with equal distance. All bacterial plates were incubated at 37^0 for 24 hours. All fungal plates were incubated at 28^0C for 72 hours. The plates were observed for the zone of inhibition

Control of isolated currency 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 treated with UV in different intervals such as 5, 10, 15 and 20 minutes. All bacterial broths were incubated at 37^0 C for 24 hours. All fungal broths were incubated at 28^0C for 72 hours. After incubation all broths were read 600nm.Control also maintain (without UV treatment).

Result and discussion:-

From the analysis of the 25 paper currency notes collected from hospital of Trichy, it was established that bacteria and fungi were present on the notes. Totally five different bacterial colonies were isolated from the currency notes. The isolated bacterial colonies were named as CB1CB2,CB3, CB4 and CB5.The currency note were highly contaminated from pathogenic bacteria such as *Streptococcus pneumonia* present (36%),*Bacillus subtilis*(24%),*Pseudomonas aeruginosa*(18%),*Escherichia coli* (12%) and *Klebsiella pneumoniae*(10%).Four different fungal colonies were noted. The colonies were named as CF1,CF2, CF3 and CF4.The percentage of fungi isolated from 25 different currency samples was denoted in(Plate–1,Table-1). Among the 25samples, 76% of samples not found fungal growth. *Aspergillus fumigatus* and *Candida albicans* contaminated in 8% of samples where as *Aspergillus flavus* and *Aspergillus niger* growth about 4% . Similarly results were reported (Singh and Thakur, 2002) The other isolates found in the present study were *Staphylococcus aureus*(20%) and *Proteus(*16%) which is approximately the same as reported in the previous reports (Gokta and Oktay, 1992) Moreover, less number of bacteria was observed in the present study than earlier recorded

The isolated DNA from bacterial and fungal cultures were separated by Agarose Gel Electrophoresis. The results were presented in (Plate-2, Plate-3) The size of the DNA measured using molecular marker. The bands found at 9500 bp and 8000 bp respectively. Hundred percent of the notes analyzed were found to be contaminated which is not observed in any of the previous studies. Currency notes of lower denominations (Rs.5, Rs.10) were the most contaminated and this is consistent with previous studies (Basavarajappa et al.,2005). This is expected, as lower denomination notes pass through more hands than the higher denomination.

The isolated bacterial species from the contaminated currency note sample were tested for their susceptibility against commercial antibiotics by disc diffusion method. The results were presented in (fig-1).The maximum antibacterial activity was noted in *Escherichia coli* and *Streptococcus pneumonia* against commercial antibiotics such as Chloramphenicol, Erythromycin, Clarithromycin, Clindamycin and Vancomycin At the same time minimum inhibitory activity was observed against *Bacillus subtilis* and *Pseudomonas aeruginosa*. All the bacterial isolates were resistant to Cephalothin, Cephoxitin, Cefuroxime,Cefixime and Amoxycillin. The highest antifungal activity was noted clotrimazole against all fungal isolates. At the same time Ketaconazole highly inhibit the growth of *Aspergillus fumigates* and *Aspergillus niger*. Moderate antifungal activity noted in Amphotericin -B and Nystatin. The climatic and environmental conditions of the tropics favour the thriving of many pathogenic microorganisms (Anderson,1999; Gwatkin,2000).

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

Table-1

ISOLATION OF BACTERIAL SPECIES FROM HOSPITAL CURRENCY NOTES

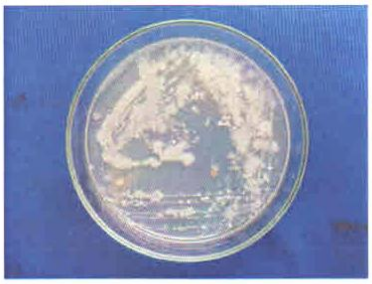
S.No	Isolated Bacterial Strains	% of occurrence
1	Escherichia coli	36
2	Klebsiella pneumoniae	24
3	Pseudomonas aeruginosa	18
4	Bacillus subtilis	12
5	Streptococcus pneumonia	10

TABLE - II
CONTROL OF ISOLATED MICROBES BY UV TREATMENT

No.	UV Treatments (mins)	Inhibition of Growth %								
		Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Streptococcus pyogenes*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus niger*	*Candida albicans*
1	5	-	-	-	-	-	-	-	-	-
2	10	55	30	60	45	60	-	-	-	-
3	15	90	80	100	90	90	60	50	75	80
4	20	100	100	100	100	100	100	100	100	100

PLATE-1
ISOLATION OF BACTERIA

Escherichia coli



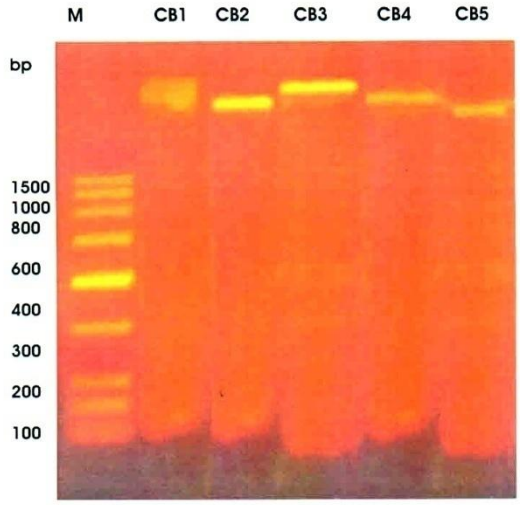
Klebsiella pneumoniae

Pseudomonas aeruginosa

Bacillus subtilis

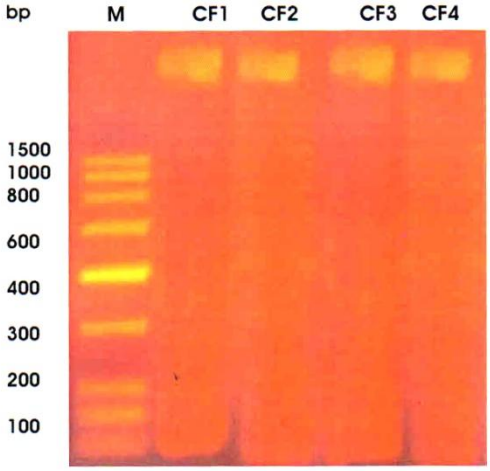
Streptococcus pneumoniae

PLATE – II
BACTERIAL DNA SEPERATION (AGROSE GEL ELECTROPHOROSIS)



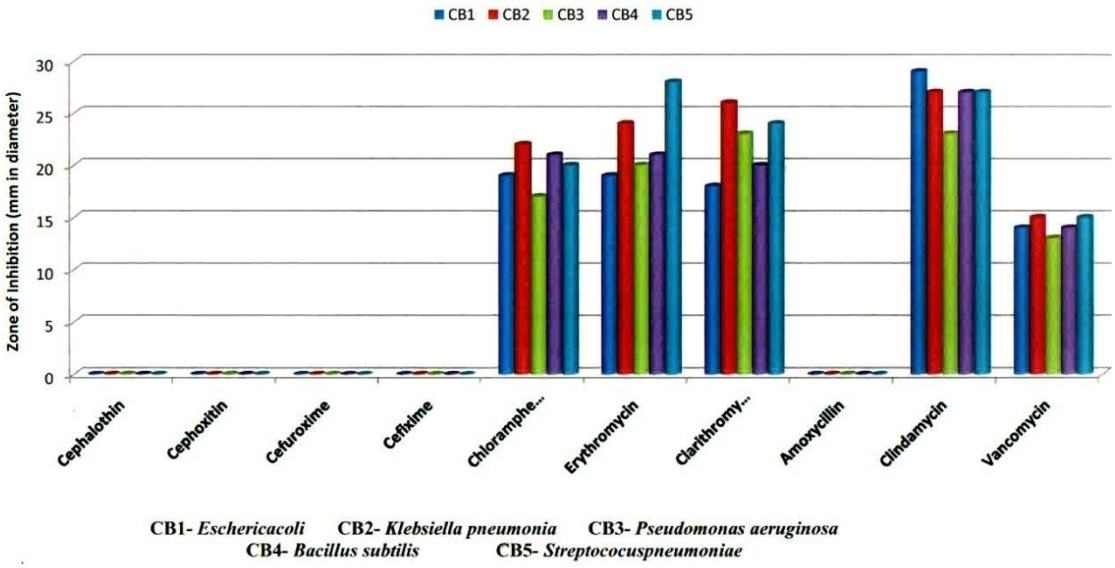
M - Marker
CB1 - *Bacillus subtilis*
CB2 - *Escherichia coli*
CB3 - *Klebsiella pneumonia*
CB4 - *Pseudomonas aeruginosa*
CB5 - *Streptococcus pneumonia*

PLATE – III
FUNGAL DNA SEPERATION (AGROSE GEL ELECTROPHOROSIS)



CF1 - *Aspergillus flavus*
CF2 -*Aspergillus fumigatus*
CF3 -*Aspergillus niger*
Cf4 -*Candida albicans*

FIG – I
ANTIBIOTIC SENTIVITY TEST AGAINST BACTERIA



CB1- ***Eschericacoli*** **CB2-** ***Klebsiella pneumonia*** **CB3-** ***Pseudomonas aeruginosa***
CB4- ***Bacillus subtilis*** **CB5-** ***Streptococuspneumoniae***

Conclusion:-

Paper currency is commonly contaminated with bacteria and this may play a role in the transmission of potentially harmful organisms. According to our results cash should not be handled by children and should be kept away from food and cosmetics. Great care should be taken for facilitates the handling of money to avoid cross contamination. Plastic banknotes are strongly recommended. We recommend that currency notes must be handled with caution.

References:-

1. **Anderson, R.M., 1991.** Infectious disease of humans, dynamics and control Oxford University Press, New York.
2. **Anderson, R. 1999.** Inhibition of Staphylococcus aureus and spheroplasts of gram negative bacteria by an antagonistic compound produced by a strain of Lactobacillus plantarum, *Int. J. Food. Microbiol*.,3:149-160.
3. **Basavarajappa, K.G., Rao, P.N and Suresh, K., 2005.** Study of bacterial,fungal, and parasitic contaminaiton of currency notes in circulation. *Indian J. Pathol. Microbiol*.,48: 278-279.
4. **Brady, G. and J. Kelly., 2000.** The assessment of the simultaneous handling of food and money in the food industry. Central Goldfields Shire Council,Money Survey; Dunn, Son and stone.
5. **Gilchrist, M.J.R (1993)**. Microbiological culturing of environment and medical device surfaces.in H.Eisenberg (ed), clinical microbiology procedures hand book (p.11.10.4).Washington, DC: American society for microbiology.
6. **Goktas, P. and G. Oktay., 1992.** Bacterial Examination of Paper Money. Microbiological Bulletin.,26(4): 344-8.
7. **Gwatkin, D.R and Guillot, M., 2000.** The burden of disease among the global poor Washington, DC: the world bank.
8. **Lamichhane, J., Adhikary, S., Gautam, P., Maharjan, R and Dhakal, B., 2009.** Risk of Handling Paper Currency in Circulation Chances of Potential Bacterial Transmittance. *Nepal. J. Sci. Technol.,* 10: 161-166.
9. **Norris, J.R. and Ribbon D.W., (ed) 1972.** Methods in Microbiology, Academic press.London. 211-276.
10. **Singh,D.V.,K.Thakur,A.Goel.2002.** Microbiologial Surveillance of currency. *Indian journal of medical microbiology* 20(1):53
11. **Struthers, J.K and R.P. Westran., 2003.** Clinical Bacteriology. ASM Press, Washington, DC.
12. **Xu, J., Moore, J.E and Millar, B.C., 2005.** Ribosomal DNA identification of the culturable bacterial flora on monetary coinage from 17 currencies. *J. Environ. Health.,* **67**: 23-25.