

Currency paper consists of a special blend of cotton and linen fibers. On the other hand polymer based currency has shown low bacterial count than cotton based currencies.

Currency notes are used for every type of commerce from buying milk at local store to buying even drugs, so the currency which is handled by a large number of people increases the possibility of acting as environmental vehicle for transmission of potential pathogenic microorganisms i.e. bacteria and fungi (Abrams and Waterman,1972). So, the infected currency is identified as potential public health hazard as pathogen spread by circulating banknotes (Empike and Oyro,2007).

People have unhygienic habits like keeping notes in shoes, socks, inside clothes that have been worn, under carpets and mattresses etc. can contaminate the notes. Wetting of fingers with saliva in mouth during money counting not only increase the contamination of money but also may increase the risk of self contamination.

Microorganisms on the skin could be transferred from cashiers, salespeople, and therefore the general public to the currency notes that they handle (Badvi *et al.*, 2013).

Microorganism present over notes are mainly *Staphylococcus*, *Salmonella* sp., *Shigella* sp., *E.coli*, *Pseudomonas aeruginosa* etc has been reported scientifically by Gosa Girma, (2014). Paper currency harbors a wide variety of hazardous microbial pathogen which can cause several infections and also become resistant in nature.

Due to the lack of information, there is unawareness in the public regarding proper handling and usage of money. Hands are the primary mode of transmission of infections. Therefore, hand hygiene is the most important measure to avoid the spread of infectious diseases.

Hand hygiene is the most important, simplest, and least expensive means of prevention of nosocomial infections (Ravi *et al.*, 2005).

To create the public awareness and hygiene after handling the money, this study is aimed to isolate some pathogenic microorganisms which can easily cause food poisoning or enteric diseases and the efficiency of commercially available hand sanitizers (like Dettol and Lifebuoy) on it to prevent its transmission by hands.

The concept of cleansing hands with an antiseptic agent probably emerged in the 19<sup>th</sup> century. As early as 1822, a French pharmacist demonstrated that solutions containing chlorides of lime or soda could eradicate the foul odors and that solutions could be used as disinfectants and antiseptics (John *et al.*, 2002).

According to several studies, sanitizers with minimum of 70% alcohol were suggested to kill 99.9% of the bacteria on hands (Eshun *et al.*, 2004). Antimicrobial property of sanitizer can be predicted on its effective ingredient. Each sanitizer has an effective ingredient, may be ethanol or isopropanol. Alcohols are broad spectrum disinfectant, kills pathogenic bacterium and fungi. The employment of alcohol based hand sanitizers might scale back the possibilities to unfold infections in the community. It has great demand in health care facilities, schools, food processing areas etc. (Aiello *et al.*, 2008; Bloomfield *et al.*, 2007; Allergranzi and Pittet,2007).

Alcohol based sanitizers found in the form of liquid, foam, and easy flowing gel. Alcohol kills microorganisms by destroying cell membranes and denaturation of proteins of bacterial cell. Gram negative bacteria like *E.coli* and *Salmonella* have thin layer of peptidoglycan cell wall surrounded by

an outer membrane which can be dissolved by alcohols thus more susceptible to sanitizers. Gram positive bacteria like *Staphylococcus* have thicker layer of peptidoglycan cell wall which are less susceptible to alcohol based sanitizers.

Information related to contaminated currencies with potential pathogenic microorganisms is very less in some developing countries. Lack of awareness leads to decline in public health policies in money handling. Government should replace the worn out old paper currencies and coins time to time to eradicate the infectious pathogens from currencies.

### **Materials and Methods :**

**Sample Collection :** In the present study, different value of currency like Rs 5 note and coin, Rs 10 note and coin and Rs 20 note were collected from person of vegetable vendor, Chicken shop, and Rickshaw driver. Samples were collected in sterile polythene bags and carried to the lab for further processing.

**Sample processing :** Each currency samples were rinsed in a beaker containing 10ml of normal saline and shaken for 1 to 2 minutes so that microbes adhered over the sample surface come out to normal saline and left soaked for 24 hrs (Prasai *et al.*, 2008 and Saadabi *et al.*, 2011). The wash of currency notes and coins were further used for detection of microbes.

**Isolation and Screening of bacteria using selective media :** 0.1ml of each currency wash was added, using spread plate method, on the plates of Mannitol salt agar media and Eosin Methylene Blue agar media for selective growth of *Staphylococcus* and coliforms respectively. The plates were incubated for 24 to 48 hours at 37°C. Colonies were observed and further characterized.

**Identification and characterization :** The identification of bacteria was based on morphological characteristics and biochemical tests prescribed by Bergey's Manual of Systematic Bacteriology (Vos D. P. *et al.*, 2009). Cultural characteristics observed for each bacterial colony after 24h of growth included colony appearance; shape, elevation, margin, optical characteristics, colony surface and pigmentation. The preliminary identification of the bacterial isolates was done by performing Gram-staining technique (Gram, 1884) and observing cell shape and arrangements under the light microscope. The organism which were identified on the basis of Gram reaction and biochemical tests were further preceded for susceptibility test against sanitizers and hand washes.

**Agar well diffusion test to determine effect of hand sanitizers and hand wash against isolated culture:** Agar well diffusion method by Perez *et al.*, (1990) was followed to determine the susceptibility of isolated culture towards sanitizer and hand wash. A sterile cotton inoculating swab was dipped into the inoculum tube. Excess inoculum was removed by pressing the swab gently against the inside wall of inoculum tube above the liquid level. The swab was then streaked over the medium three times by rotating the plate at an angle of 60°. The inoculum was left to dry for few minutes at room temperature.

With the help of sterile 8mm cork borer, 2 equally spaced holes were bored in the nutrient agar plate. 100µl of the hand sanitizers as well as hand wash were then poured into each wells. For control plate, the well was filled with an equal volume of sterile water. This was done for the entire test organism and all hand wash and sanitizer sample. The plates were incubated for 24 hours at 37°C in an upright position. Plates were then

observed for zone of inhibition with various degrees of susceptibility and resistance. The test was

carried out in duplicates and the average of the readings was taken for zone of inhibition. The zone was measured in mm with ruler.

### Results and Discussion :

The study was done on some Indian Currency including paper notes and coin samples. Our study was limited up to isolation and identification of specific pathogenic bacteria only, so, selective agar media were used for the isolation.

Saadabi *et al.*, (2011) noticed that the isolated bacteria from different banknotes showed higher occurrence of *Staphylococcus aureus* and *Escherichia coli*.

From the analysis of 5 currency samples, large no. of colonies were obtained on Mannitol Salt Agar media which is named as S1, S2, S3, S4 and S5 on five different plates while on EMB agar media two different colonies were obtained, E1 and E2. The present study shows that paper currency has higher contamination than the coin sample. Prasai *et al.* (2008) also reported that paper currency was more contaminated than polymer notes and coins.

Mannitol Salt Agar media were used for the selective isolation of *Staphylococcus species*. The cultural and morphological characteristics of the isolates S1, S2, S3, S4, S5 on MSA plate from different sample was shown in Table 1. Eosin Methylene Blue Agar media were used for the selective isolation of coliform. Cultural and morphological characteristics of isolate E1 and E2 from currency sample on EMB media was shown in Table 2.

Strain E2 was selected for further identification due to its green metallic sheen characteristics on EMB agar which resembles with *E.coli* and thus E1 was eliminated.

Isolate S1, S2, S3, S4, S5 and E2 were analyzed further for biochemical tests and the result was shown in Table 3.

Based on cultural, morphological (Fig. 1) and biochemical tests the probable isolates S1, S2, S3, S4 and S5 could be *Staphylococcus aureus* and isolate E2 could be *Escherichia coli* (Fig. 2).

Coliform bacteria are the indicators of faecal contamination and *Staphylococcus aureus* is commonly present on the skin and in nasal passage. Barro *et al.*, (2006) also reported a prevailing presence of coliforms and *S. aureus* on currency. Money constitutes a reservoir of various bacteria as pathogenic *E.coli* which can survive for 11 days on the inert surface (Pomperayer & Gaylarde, 2000).

The purpose of hand sanitizer and hand wash is to set up hand hygiene and to control spread of infection. Hands are the primary medium of transmission and spread of diseases causing pathogen. Results after testing the effect of two sanitizers and two handwash (Fig. 3) showed that lifebuoy sanitizer is effective on both the isolate while dettol sanitizer was not effective on any of the isolates in our study. According to the study of Oke *et al.*, (2013) the dettol sanitizer was only effective against *P.aeruginosa* whereas it was not effective against *S.aureus* and *E.coli*. Between the two hand wash, Dettol hand wash is more effective than Care mate hand wash.

Zone of inhibition of lifebuoy sanitizer is 16mm on *S.aureus* and 19mm on *E.coli* and no zone for Dettol sanitizer were observed in our study.

Zone of inhibition of Dettol hand wash on *S.aureus* is 18mm and 30mm for *E.coli*. Care mate hand wash showed a zone of inhibition of 14mm on *S.aureus* and 24mm zone on *E.coli*.

Gram negative bacteria like *E.coli* and *Salmonella* have thin layer of peptidoglycan cell

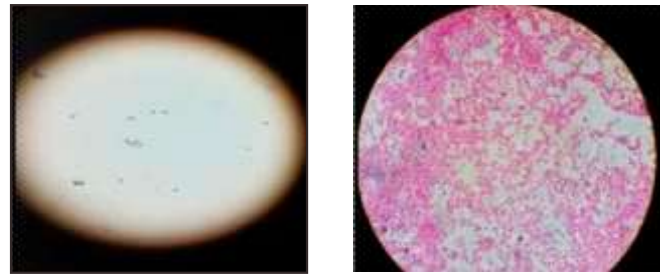
wall surrounded by an outer membrane which can be dissolved by alcohols thus more susceptible to sanitizers and hand wash. Gram positive bacteria like *Staphylococcus* have thicker layer of peptidoglycan cell wall which are less susceptible to sanitizers and hand wash.

The antimicrobial sensitivity test of isolated *Staphylococcus aureus* and *E. coli* against some hand sanitizers and hand washes revealed varying result shown in Table 4 and 5 respectively and in Fig. 5.

### Conclusion:

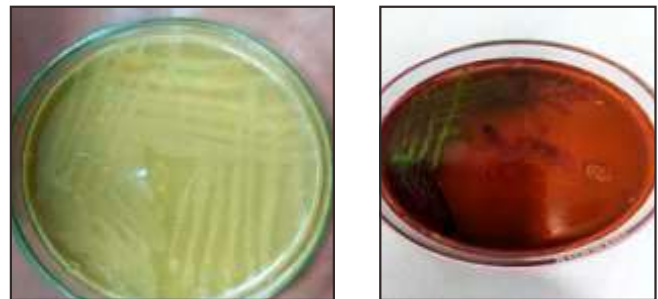
As currency notes are used on daily basis it gave shelter to potential pathogens and serves as a vector. The present study clearly demonstrates that the currency notes are commonly contaminated with pathogenic as well as non-pathogenic microbes. Some pathogenic microbes include *E.coli* and *Staphylococcus sp.* which cause a wide range of illness like diarrhea, food poisoning, urinary tract infection, skin infection etc. It was found that sanitizer and hand wash inhibit the growth of microbes. But not all the sanitizers affect the growth of microbes isolated from currency.

Till date, no outbreaks of illness have been associated with infection from money. However there was proof of the presence of pathogenic bacteria on currency which may cause diseases. This supports the requirement for strict hygienic practices for money handlers who also handle food and water. So, some of the recommendations are like money should be handled properly and should not be kept in dirty places, exposure of UV radiation on currency on regular basis, ultra sonicator can also be used to eradicate the microbes, hand wash and sanitizers can be used for hand hygiene, coins are preferred over paper notes as coins are less contaminated due to its composition etc.



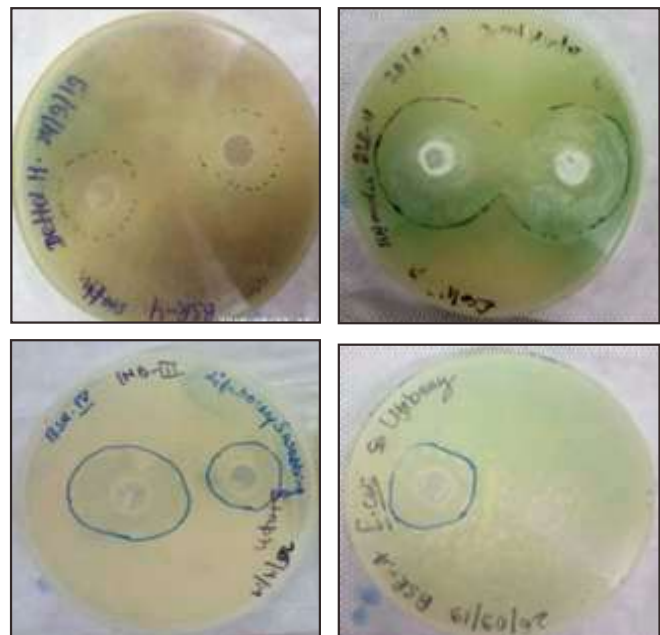
(a) Gram +ve coccus (b) Gram -ve rods

**Fig. 1. Microscopic view of isolated bacteria under 100x magnification**



(A) *Staphylococcus* on MSA Media (b) *E. coli* on EMB media

**Fig. 2. Pure culture of isolated bacteria**



**Fig. 3. Antimicrobial sensitivity test of isolates towards sanitizers and hand wash**

**Table 1. Cultural and morphological characteristics of isolates from different currency samples on MSA media**

S.No.	Sample (INR)	Isolate	Media	Cultural characteristics				Morphological characteristics	
				Color	Texture	Margin	Elevation	Gram stain	Shape
1.	5Rs note	S1	MSA	Yellow	smooth	entire	Raised	-ve	Coccus
2.	10Rs note	S2	MSA	Yellow	smooth	entire	Raised	+ve	Coccus
3.	20Rs note	S3	MSA	Yellow	smooth	entire	Raised	+ve	Coccus
4.	5Rs coin	S4	MSA	Yellow	smooth	entire	Raised	+ve	Coccus
5.	10Rs note	S5	MSA	Yellow	smooth	entire	Raised	+ve	Coccus

(-ve=positive)

**Table 2. Cultural and morphological characteristics of isolates on EMB media**

S.No.	I.solate	Media	Cultural characteristics				Morphological characteristics	
			Color	Texture	Margin	Elevation	Gram stain	Shape
1.	E1	EMB	Pink	Mucoid	Regular	Raised	-ve	Rod
2.	E2	EMB	Green metallic sheen	Smooth	Regular	Flat	-ve	Rod

**Table 3. Biochemical test for different isolates**

S.No.	Biochemical tests	Isolate					
		S1	S2	S3	S4	S5	E2
1.	Glucose fermentation	+ve	+ve	+ve	+ve	+ve	+ve
2.	Lactose fermentation	+ve	+ve	+ve	+ve	+ve	+ve
3.	Mannitol fermentation	+ve	+ve	+ve	+ve	+ve	+ve
4.	Sucrose fermentation	+ve	+ve	+ve	+ve	+ve	+ve
5.	Indole	-ve	-ve	-ve	-ve	-ve	+ve
6.	Methyl red	+ve	+ve	+ve	+ve	+ve	+ve
7.	Voges-Proskauer	-ve	-ve	-ve	-ve	-ve	-ve
8.	Citrate	+ve	+ve	+ve	+ve	+ve	-ve
9.	Urease	+ve	+ve	+ve	+ve	+ve	-ve

(+ve=positive; -ve=negative)

**Table 4. Inhibition zone of sanitizers & handwash against *S.aureus***

Sl. No.	Samples	Zone of inhibition (mm)		Mean value $\bar{X} = \sum X \div N$
		Reading 1	Reading 2	
1.	Lifebuoy sanitizer	16.3	15.7	16
2.	Dettol sanitizer	No zone	No zone	No zone
3.	Dettol handwash	17.9	18.1	18
4.	Caremate handwash	13.8	14.2	14

$\bar{X}$ =arithmetic mean,  $\sum X$ = sum of individual observation, N= no.of observation

**Table 5. Inhibition zone of sanitizer & handwash against *E.coli***

Sl. No.	Samples	Zone of inhibition (mm)		Mean value $\bar{X} = \sum X \div N$
		Reading 1	Reading 2	
1.	Lifebuoy sanitizer	18.9	19.1	19
2.	Dettol sanitizer	No zone	No zone	No zone
3.	Dettol handwash	29.8	30.2	30
4.	Caremate handwash	23.8	24.2	24

$\bar{X}$ =arithmetic mean,  $\sum X$ = sum of individual observation, N= no.of observation

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