Dirty notes are usually moist and thus provide a good surface for Bacterial growth. The level of contamination and risk of microbial transmission via Currency Notes are associated with levels of community hygiene and economic status of the country (Saadabi et al.,2010; Fonseca et al.,2015). The infected Currency is, therefore, identified as potential public health hazards as pathogens spread by circulating bank notes (Emikpe and Oyero;2007). Health care associated infections are one of the most serious patients' safety issues in health care today. Pathogenic microorganisms that survive on the Currency Notes may serve as potential sources of Entero-pathogens that cause infections and potential sporadic cases of food borne diseases (Tagoe et al., 2011).

Various microorganisms have been isolated from money worldwide including *Enterococcus sp., Staphylococcus aureus, Escherichia coli* and *Klebsiella sp.* These pathogenic or potentially pathogenic bacteria cause a wide variety of diseases like food poisoning, skin infections, respiratory diseases, gastrointestinal diseases and can also cause life threatening diseases such as Meningitis and Septicemia. Every year over 1 million people worldwide are affected by Meningitis and cause around 1,70,000 deaths globally every year (Murray et al.,2012 ; Paireau et al.,2016; Mclntyre, 2012) whereas Septicemia affects more than 30 million people worldwide every year potentially leading to 6 million deaths. So, keeping in mind about the health issues and hygiene, this research seeks to determine the microbial contamination of Indian old and new notes with its pathogenic nature and the risk factor.

Materials and Methods :

Isolation of Microorganisms - The study was conducted in the months of July and August. 8 Indian Currency Notes including new and old of INR ₹ 10, ₹ 20, ₹ 50 and ₹ 100 (2 notes each) were collected. These notes were collected in the sterile plastic bags and transported to the laboratory. Isolation was done with the help of cotton swab dipped in 1% of peptone water. Swabbing was done from the corners to central parts and was then transferred to sterilized Nutrient. Culture plates were incubated for 48 hours at 37°C. Isolation of various bacterial strains and fungal colonies were performed via standard techniques (Gilchrist 1993; Singh et al., 2002).

Identification of Bacteria- Different colonies were sub cultured on Nutrient Agar and incubated for 48 hours. The isolates were characterized on the basis of their Morphology, Staining and Biochemical Tests.

Table 1 shows the Bacteria isolated from Paper Currency. They are named with upper case Alphabets to minimize error while performing Gram Staining and different Biochemical tests

Table 1. Table showing isolated bacterial strains

S. No.	Bacteria found	Symbols used
1.	₹ 20 (old) yellow bacteria	A
2.	₹ 20 (old) white bacteria	B
3.	₹ 50 (old) yellow bacteria	C
4.	₹ 10 (old) cream bacteria	D
5.	₹ 10(new) white bacteria	E
6.	₹ 10 (old) cream bacteria	F
7.	₹ 50 (old) white bacteria	G
8.	₹ 10 (old) white bacteria	H
9.	₹ 100 (old) white bacteria	I
10.	₹ 100 (new) white bacteria	J
11.	₹ 100 (new) yellow bacteria	K

Gram Staining tests were performed to distinguish bacterial species as Gram positive and Gram negative bacteria. With the help of inoculation loop, drops of cultured microorganism were transferred on slide and were spread to form a thin film. After heat fixing, it was stained by crystal violet

solutions (primary stain) for 2 minutes then was rinsed with water. Gram iodide solution was applied on it for 1 min followed by washing with water. Then slide was put in alcohol (decolourizer) and then was flooded with safranin (counterstain). It was allowed to react for 60 seconds and was washed with water and observed under microscope.

Identification of fungal colonies- Lactophenol Cotton Blue Stain formulated with Lactophenol serves as a mounting fluid and Cotton Blue is an acid dye that stains chitin present in cell wall of fungi. It was taken on a clean slide and fragments of fungal colony were put on it. It was teased by needle, a coverslip was placed on it and was observed under Microscope.

Four Biochemical tests were carried out namely Indole test, Methyl Red test, Voges-Proskauer (VP) test and Citrate test.

Indole test was performed on bacterial species to determine the ability of the organism to convert Tryptophan into Indole. Bacterial cultures were inoculated in the prepared Tryptophan broth. The culture was incubated at 37°C for 48 hours. When the medium became turbid, 0.5 ml of Kovac's reagent was added to the medium and results were observed. Formation of red colour indicates positive Indol test.

The Methyl Red Test (MR test) identifies bacteria producing stable acids by mechanisms of mixed acid fermentation of glucose. These bacteria are called Methyl Red positive. The bacterial culture was inoculated in the prepared glucose phosphate broth and incubated at 37°C for 48 hours. After 48 hours 5 drops of MR reagent were added. Development of red colour was taken as positive. MR negative organisms produced yellow colour.

VP test detects Acetoin in a bacterial broth culture. Bacterial cultures were inoculated in the glucose phosphate broth and were incubated at 37°C for 48 hours. After 48 hours, 0.6 ml VP-I (-Naphthol & Ethanol) and 0.2 ml VP-II (Potassium Hydroxide & Distilled Water) were added and shaken well. The test tubes were allowed to stand for 15 minutes. The red colour appearance indicates positive VP test.

The Citrate Test detects the ability of an organism to use Citrate as the sole source. Bacterial colonies were inoculated into slope of Citrate Agar and incubated at 37°C for 24 to 48 hours. If the organism has the ability to utilize Citrate, the medium changes its colour from green to blue.

Spore testing for bacteria: A smear of the culture was prepared, air dried and then heat fixed. The slide was placed over a beaker of boiling water with bacterial film on upper side. After the large droplets were condensed on the lower side of the slide, the slide was flooded with Schaeffer and Fulton's stain A for 3-6 minutes. It was steamed in water bath and was rinsed under tap water. It was counterstained with Schaeffer and Fulton's stain B for 30 seconds. The slides were washed with water, dried and properly observed under microscope. No spore forming bacteria were observed.

Results and Discussion :

On the basis of Morphological characters, Gram staining and Biochemical testing, different types of bacteria were isolated. These were: *Klebsiella pneumonia, Enterobacter aerogenes, Escherichia coli, Staphylococcus pneumonae, Staphylococcus epidermidis* and *Staphylococcus aureus* (Table 4). Some very infectious fungal colonies like *Mucor, Aspergillus flavus, Aspergillus niger* and *Penicillium species* (Table 2 and 3) were also found.

Table 2. Table showing microorganisms on old currency notes

OLD CURRENCY NOTES	BACTERIAL COLONIES	FUNGAL COLONIES
Rs.10/-	**D.** White bacteria **H.** Cream bacteria	*Aspergillus flavus* *Aspergillus niger*
Rs.20/-	**A.** Yellow bacteria **B.** White bacteria	No fungal colonies found
Rs.50/-	**C.** Yellow bacteria **G.** White bacteria	No fungal colonies found
Rs.100/-	**I.** White bacteria	No fungal colonies found

Table 3. Table showing microorganisms on new currency notes

NEW CURRENCY NOTES	BACTERIAL COLONIES	FUNGAL COLONIES
Rs.10/-	**E.** White bacteria **F.** Cream bacteria	*Aspergillus flavus* *PenciliumSp.*
Rs.20/-	No bacterias could be isolated due to dense fungal colonies	*Mucor Sp.* *Aspergillus niger*
Rs.50/-	No bacterias could be isolated due to dense fungal colonies	*Mucor Sp.*
Rs.100/-	**J.** White bacteria **K.** Yellow bacteria	*Aspergillus niger* *Aspergillus flavus*

Table 4. Table showing results of biochemical testing

Bacterial colonies	Indole test	Methy l Red test	Vp1 and Vp2	Citrate test	Bacterial colonies
A. Yellow bacteria	-VE	-VE	+VE	+VE	*Klebsiella pneumonae*
B. White bacteria	-VE	-VE	-VE	+VE	*Klebsiella pneumonae*
C. Yellow bacteria	-VE	+VE	-VE	+VE	*Staphylococcus Pneumonia*
D. Cream bacteria	-VE	-VE	+VE	+VE	*Klebsiella pneumonae*
E. White bacteria	-VE	+VE	-VE	+VE	*Enterobacter aerogenes*
F. Cream bacteria	-VE	-VE	+VE	+VE	*Klebsiella pneumonae*
G. White bacteria	-VE	-VE	+VE	-VE	*Staphylococcus epidermidis*
H. White bacteria	-VE	-VE	-VE	+VE	*Enterobacter aerogenes*
I. White bacteria	-VE	+VE	+VE	+VE	*Staphylococcus aureus*
J. White bacteria	+VE	+VE	-VE	-VE	*Escherichia coli*
K. Yellow bacteria	-VE	-VE	+VE	-VE	*Enterobacter aerogenes*

In this study, along with the non pathogenic bacteria, many pathogenic bacteria such as *Klebsiella pneumonae, Escherichia coli* and *Staphylococcus aureus* were isolated as shown in the Table 4. Pathogenic bacteria may cause several diseases in immune suppressed people like old age people and new born babies. Without even our noticing, we come across such dangerous microorganisms that are pathogenic to human beings and expose ourselves with all kinds of threats. Igumbor et al, (2007) tested 240 bank notes for microbial contamination. All the notes were contaminated by bacteria and fungi. 12 different bacterial species were isolated and the most common were *Staphylococcus aureus, Escherichia coli* and *Klebsiella* sp. and only one fungus *Candida albicans* were isolated.

Another big problem is that currency notes are also impressive potential carrier for fungal strains. With bacterial colonies various fungal colonies were also isolated. Fungal colonies are very outspreading, their spores could transfer in seconds. Several infections like skin diseases, ringworms and food poisoning are caused by the fungus. Some diseases caused by fungus are Aspergillosis, Candidiasis, Blastomycosis and fungal eye infections. Fungal infections are highly painful and hard to cure as they are very rigid.

A study done by Ahmed et al, (2010) in Bangladesh suggested that paper Currency Notes were commonly contaminated with pathogenic microorganisms and this contamination played a significant role in the transmission of potentially harmful microorganisms for different diseases.

Saadabi et al, (2017) performed a similar study on Saudi Bank Notes and isolated different bacterial species and the most common were *Staphylococcus aureus, Escherichia Coli, Klebsiella sp.* and *Streptococcus sp.* He also isolated 9 fungal colonies and the most common were *Aspergillus sp.* and *Penicillium sp.*

It was found that, handling Currency Notes properly is very important. With this growing globalization and busy lifestyle we tend to forget that our hands are homes for billions of such microbes which cause innumerable skin infections, dysentery, loose motion and several other infections.

It is very important that we keep babies, specially toddlers and infants away from notes and coins. With above test results, it can be said that, using Currency Notes every now and then without care should be stopped. Proper hand sanitization should be done. Banks should use proper disinfectants on notes before circulating it. Moreover polymer mixed note should be used as they are comparatively less cable for harbouring bacterial colonies. The most important step we could take is, if possible, we can use cashless apps for transaction or else if we come across really dirty and unclean Bank notes, we can take initiative and change it through Bank. It is very important that we people should create awareness among ourselves regarding handling of paper Currency notes.

Conclusion :

The aim of this research was to show people that the Currency Notes we keep in our pockets, wallets, wardrobe, cupboards, sometimes on beds and shelves are so infected and filled with pathogenic microorganisms. We should completely take care of how we use paper notes. Moistening paper notes while counting with tongue should be strictly avoided. We should avoid having it on the bed and dining tables. Avoid keeping it with clothes and food items or in other households. They should be made aware of how the Paper Currency Notes carry several harmful microorganisms and it could affect their health. The initiative should be taken to make every class aware of its pros and cons and how it should be handled so that minimum of infections could take place.

We came to a conclusion that it is our misconception that only old Paper Currency can be infectious and contaminated. Even the new Paper Currency could be contaminated with some serious pathogens. Hence, it is very important that we take proper care about hygiene when handling Paper Currency. Paper Currency circulates through all strata of people. Many of them not living in hygienic conditions contaminating the notes with some serious infections. As this contaminated money circulates, we unconsciously expose ourselves to risks. We should now and then exchange currency notes from banks if they are not in good condition. New notes are washed with disinfectants that inhibit bacterial and fungal colonies to flourish. Moreover, it has come to light that polymer based Paper Currency carries less bacterial colonies than paper based, as Paper Currency Notes are mixed with cotton which provide suitable surfaces for bacterial and fungal growth. Further research can be done on how effective these polymer mixed Paper Currency are with cotton mixed Paper Currency.

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