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Unseen Threats in Circulation: Pathogenic Bacteria and Antibiotic Resistance on Pakistani Currency Notes

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ABSTRACT

Microorganisms are ubiquitous, colonizing various environments, including the human body and frequently handled surfaces such as paper currency. Banknotes act as potential vectors for disease transmission, as they continuously circulate among individuals, increasing the risk of cross-contamination by pathogenic microorganisms. This study investigated the bacterial contamination of Pakistani currency notes, analyzing fifty (50) banknotes collected from diverse sources in District Abbottabad, including butcher shops, vegetable markets, fish markets, taxi drivers, university canteens, banks, students, and patients. Paper currency of 10, 20, 50, and 100 PKR denominations were randomly collected in sterile polyethylene bags and transported to the Microbiology Laboratory at Abbottabad University of Science and Technology for microbial analysis. Standard microbiological techniques were employed for the isolation, biochemical characterization, and identification of bacterial species. The results revealed the presence of various pathogenic bacteria, with the following distribution: Staphylococcus aureus: 33.3% (butcher shops), 20% (canteens), 13.3% (taxi drivers), 13.3% (fish markets), 13.3% (biryani shops), 6.6% (general stores). Bacillus spp.: 50% (butcher shops), 50% (general stores). Klebsiella pneumoniae: 18.5% (canteens), 14.2% (general stores). Vibrio spp.: 14.2% (butcher shops), 14.2% (fish markets). Escherichia coli (E. coli): 14.2% (butcher shops), 14.2% (fish markets). The findings indicate that Pakistani currency notes are frequently contaminated with pathogenic bacteria, posing a potential public health risk. Notably, lower-denomination banknotes exhibited higher contamination levels than higher-denomination notes, suggesting increased microbial exposure due to frequent handling and circulation. Strict hygiene measures and public awareness regarding currency handling are essential to minimize microbial transmission and safeguard human health.

INTRODUCTION

Commonly to as microorganisms or microbes, microscopic organisms are present in our surroundings and even within our bodies. Bacteria, fungi, viruses, algae, archaea, and protozoa are just a few of the enormous variety of organisms that fall under the category of "microbes." Some of these are well known, including fungi and bacteria. The vast invisible world that surrounds and inhabits us is made up of microscopic organisms called bacteria (Betsy and Keogh, 2005). Numerous environmental materials act as vehicles for the spread of microbial agents to humans, making the environment a significant factor in this process. Public health is affected when various objects become contaminated by potentially harmful microorganisms because contaminated materials may serve as conduits for the spread of these pathogens. In economic activities, money is used as a medium for debt settlement, deferred payments, and the exchange of goods and services (Sharma and Sumbali, 2014)

Immunocompromised individuals are susceptible to contracting an opportunistic infection by handling contaminated currency. Money is essential to human existence because it enables us to meet our needs for trade and economics (Anitha et al., 2020), around the world, currency notes are essential for goods and services. Despite the fact that paper currency, which is handled by many people, has a higher chance of serving as a vehicle for the spread of potentially harmful microorganisms, such as bacteria and fungi (Uneke and Ogbu, 2007, Venkatesh and Hiremath, 2021). Therefore, the contaminated currency is recognized as a possible public health risk since it spreads a pathogen through circulating banknotes (OLATUNJI, 2016). In addition to

skin burn and septicemia infection, a collaborative study conducted in the Microbiology Department of Karachi University in Pakistan found that contact with contaminated currency notes could result in diarrhea and urinary tract infections. The economic significance of paper notes as a source of microbial contamination was highlighted by their research findings. determined that the pathogenic bacteria Entrobacter sp., Staphylococcus sp., Citrobacter sp., Klebsiella sp., and Proteus sp. are susceptible to Amoxoftine, Gentamicin, Nalidixic acid, and Ofloxacin, but resistant to Tetracycline and Cotrimoxazole. With a 72% sample contamination rate, Peter Ender (2001) isolated S. aureus, Klebsiella pneumoniae, Enterobacter sp., and Pseudomonas sp. in the USA.

Paper money that is passed from person to person is likely to be contaminated with microorganisms that cause disease, particularly if it is handled with dirty hands or stored in an unclean environment. Therefore, since contact with fomites can spread infectious diseases, paper money poses a special risk to public health (Michaels, 2008, Dey, 2015) (Xu and Wunsch, 2005). Money is a frequently disregarded reservoir for enteric disease on which pathogenic microorganisms may thrive (OLUWATOYIN, 2022).

MATERIALS AND METHODS

Sample collection

Fifty samples of money were collected from different areas of district Abbottabad such as butchers or meat vendors, fish markets, canteens, stores that sell biryani, and general stores. Ten, twenty, fifty, and hundreds Pakistani currency notes were randomly selected and placed in sterile polyethylene bags.

Sample processing

Sterile wet swabs by normal Saline was rubbed on surface of paper money and then streaked on freshly prepared nutrient agar and MacConkey agar media for bacterial strains isolation by placing at 37 degree centigrade for 24 hours (Adinortey et al., 2019).

Identification of bacteria:

The bacterial growth was identified by using Gram staining techniques and different biochemical tests and as described by (Moyes et al., 2009)

Gram staining

A common technique for distinguishing between gram positive and gram negative bacteria is gram staining. Gram-negative bacteria are pink, while Gram-positive bacteria are purple. Firstly, Cells were stained with crystal violet dye then iodine solution was added to form a complex. After adding a decolorizer like acetone or ethyl alcohol, the sample was finally stained red with safranin. Safranin does not interfere with the purple coloration of Gram-positive cells because it was less intense than crystal violet. Nevertheless, the decolorized Gram-negative cells had a pink stain (Moyes et al., 2009).

Biochemical tests

Following Gram staining, various biochemical tests were conducted to confirm the presence of distinct bacterial strains.

Catalase Test:

Hydrogen peroxide was broken down into oxygen and water by the enzyme catalase. When a small inoculum was added to hydrogen peroxide, the enzyme's presence in a bacterial isolate was demonstrated by the quick development of oxygen bubbles. The absence of catalase was demonstrated by the minimal or nonexistent production of bubbles (Reiner, 2010).

Coagulase Test

To differentiate between Coagulase Negative Staphylococcus (CONS) and Staphylococcus aureus (positive), the Coagulase test was applied. Coagulase, an enzyme produced by S. aureus, produced insoluble fibrin from plasma's soluble fibrinogen (Katz, 2010).

Oxidase Test

This test utilizes the presence of cytochrome oxidase in bacteria, which catalyzes the movement of redox dye and electron donors. The reaction reduced the reagent's tetramethyl-p-phenylene diamine dihydrochloride to a deep purple hue. This test produced positive results when used to screen for Pseudomonas, Vibrio, Neisseria, Brucella, and Pasteurella. Oxidase negative organisms are Enterobacteriaceae (Tarrand and Gröschel, 1982).

Indole Test

The indole test determine if an organism can create indole through tryptophan breakdown (MacWilliams, 2012). Tryptophan hydrolysis by tryptophanase may result in three byproducts: indole, pyruvate, and ammonium ions. Indole was discovered by watching the chemical reaction between indole and Kovac's reagent (isoamyl alcohol, para-dimethylaminobenzaldehyde, and strong hydrochloric acid) in an acidic environment. Indole reacts with para-dimethylaminobenzaldehyde in the intermediate to produce rosindole red coloring. The "rosindole" dye precipitates when it interacts with isoamyl alcohol. As a result, the residue and any remaining alcohol rose to the outside of the medium (MacWilliams, 2012).

Methyl-Red Test

When the methyl red indicator, which is added at the end of the incubation time, changes color it means that enough acid was produced during the fermentation of glucose and that the settings were kept favorable enough to keep the pH of an old culture lower a value of about 4.5 (Hamed and Alnazzal, 2023)

Motility test

The purpose of this test is to decide whether life forms

are motile through flagella. The location of the flagella varies with bacterial species (Shields and Cathcart, 2011). Bacterial motility is generally exhibited in semisolid agar media. Motile bacteria swarm on semi-solid agar substrate, leading to visible, diffuse growth. Bacterial motility can be seen as a growth zone that extends beyond the inoculation line (Shields and Cathcart, 2011).

Antimicrobial susceptibility testing

Disk Diffusion Susceptibility Testing

The disk diffusion susceptibility test determines the sensitivity or resistance of facultative anaerobic and pathogenic aerobic bacteria to various antibiotic drugs, assisting physicians in selecting the most effective treatment plan for their patients (Forbes et al., 2007). The disk diffusion (Kirby Bauer's) antimicrobial susceptibility test was performed on Mueller-Hinton agar (Merck, Germany) in this study. In accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI), this method was used to determine the susceptibility of UTI agents. Among the antibiotic disks are imipenem, cefixime, ciprofloxacin, moxifloxin, meropenum, and azithromycin (Forbes et al., 2007).

RESULTS

Isolation of bacteria from currency

A total of fifty samples were collected from currency notes and the sources were University canteen, Butchers, Fish shops, General stores, Drivers and Biryani shops (figure 1). After those sterile wet swabs by normal Saline were rubbed on surface of paper money then streak on nutrient agar plates. Of 50 samples, 26 were positive while 24 were negative. From positive samples, nineteen were Gram positive isolates and seven were Gram negative isolate identified. *Staphylococcus aureus*, Bacillus species, Klebsella species, *E. coli*, and vibrio species were among the bacteria that were isolated.

Figure 1

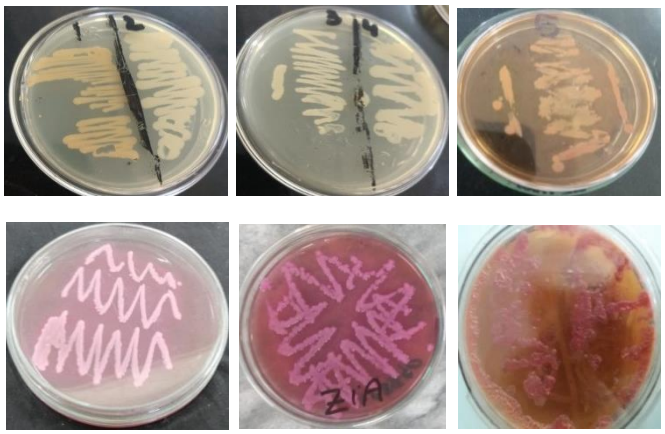
Isolation of bacteria from currency

Morphological characterization

Bacterial isolates were then characterized by morphology, by using different mediums that nutrient agar and MacConkey medium. On nutrient agar gram positive isolate produces yellow and white colonies that are present on the surface of the medium. MacConkey medium is used as a differential medium for gram negative isolates. All gram-negative isolate produced pink colonies (figure 2).

Figure 2

Morphological characterization of bacterial isolates



Gram staining

The isolated cultures were used for Gram staining which showed that the organisms are Gram negative and gram positive (table 1). Bacterial isolates appear road, cocci, singly or in pairs, or sometimes even in chains. Under microscopy slide A, B, show gram negative isolates and C, D show gram positive bacteria (figure 3).

Figure 3

Microscopy of bacterial isolates

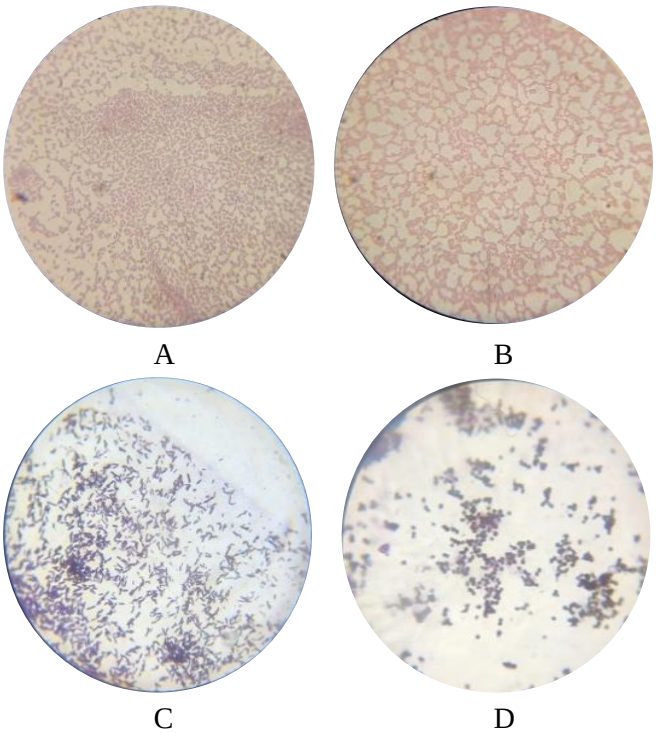


Table 1
Gram's staining of bacterial isolates

Organism	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Gram Test	+	+	+	+	+	+	+	+	+	+	+	+	+
Shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	cocci	cocci

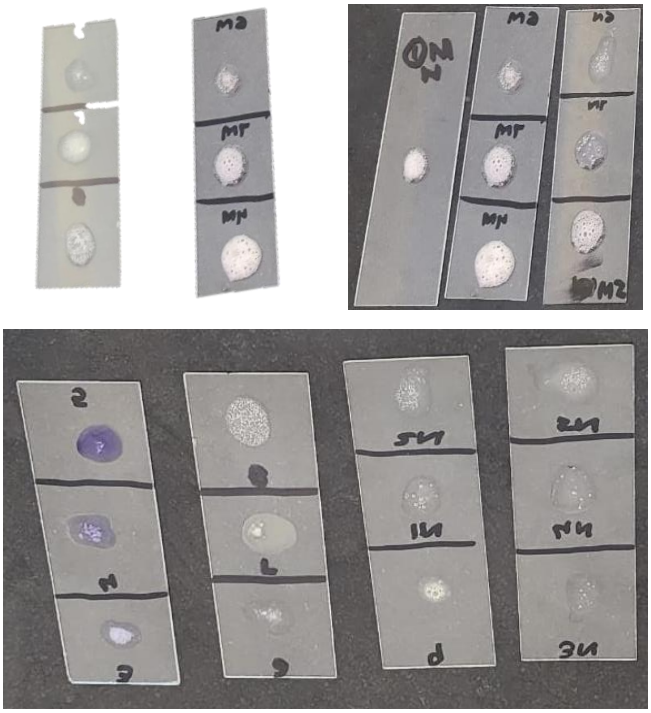
Organism	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26
Gram Test	=	=	=	=	=	=	=	+	+	+	+	+	+
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Cocci	cocci	Rod	Rod	Rod	Rod

Biochemical characterization of bacterial isolate

Catalase test

Catalase test shown that all bacterial isolates were positive. Bubbles of oxygen were produced when a drop of hydrogen peroxide was added to its isolates (figure 4).

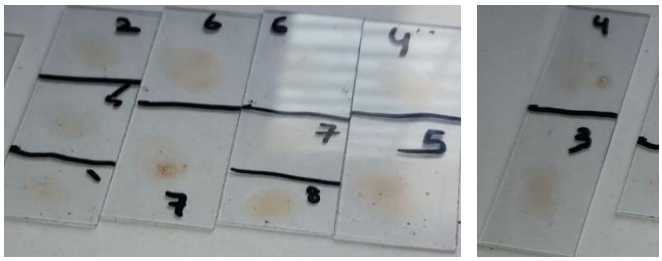
Figure 4
Catalase test results for all bacterial isolates



Coagulase test

The results of the coagulase test for fifteen isolated bacteria showed positive to produce aggregates and for seven bacteria show negative results, they were unable to form aggregates or clumps (figure 5).

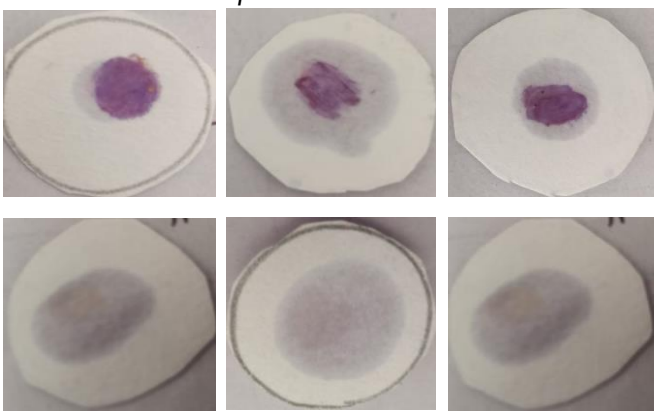
Figure 5
Coagulase test for all bacterial isolates



Oxidase test

The all-isolated bacteria were tested for the oxidase test. Out of twenty-six, nineteen were negative they were unable to produce any color and seven were positive show blue colour (figure 6).

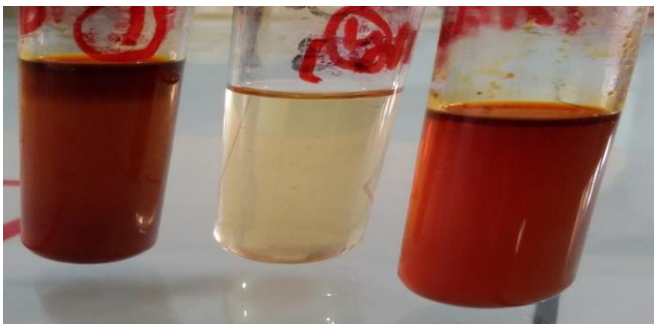
Figure 6
Oxidase test results for all bacterial isolates



Indole Test

The all-isolated bacteria were tested for the indole test. Out of twenty-six, twenty-four were negative they were unable to produce any ring and two were positive which show red ring (figure7).

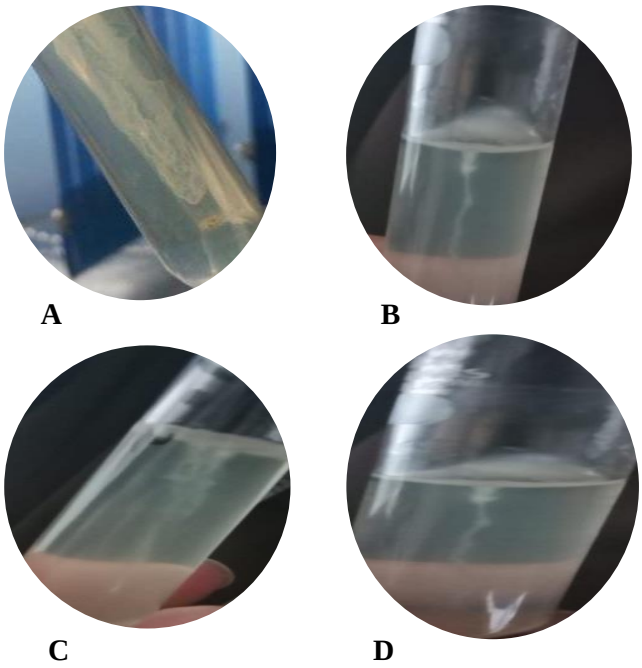
Figure 7
Indole test results for all bacterial isolates



Motility tests

The motility tests were tested for all isolated bacteria. Seven were positive out of twenty-six which showing diffusing from the line of inoculation (A, B) and twenty were negative there were no diffusing observed from the line of inoculation (C, D) (figure 8).

Figure 8
Motility test for Bacterial isolate



Methyl Red Test
The culture provides a satisfactory result for the MR test when the red color of the culture medium results from the fermentation of glucose, which occurs at or below pH 4.4. Yellow in common culture indicated a negative MR test show in (A) and (B) picture and C was used as a positive control (Fig 9).

Figure 9
Methyl red test for Bacterial isolate

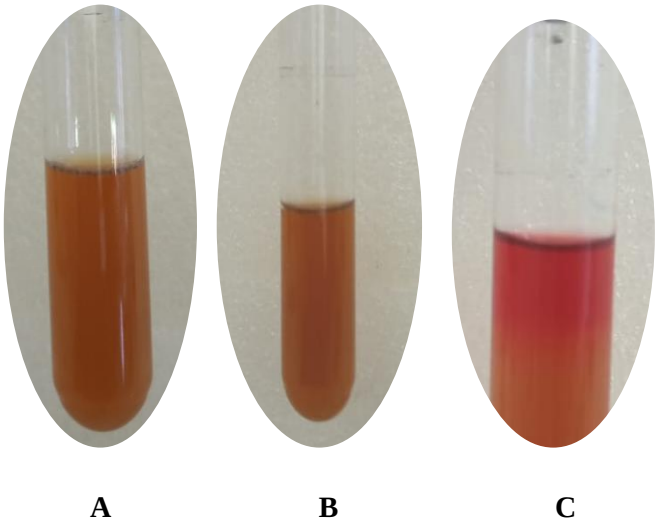


Table 2

S. No.	Gram staining	shape	Catalase	oxidase	coagulase	Motility	MR test	indole	Species
1	+ve	Cocci	+	-	+	-	-	-	S. aureus
2	+ve	Rods	+	+	-	+	-	-	Bacillus spp

Show the Gram-Positive isolates

Table 3
Show the Gram negative isolates

S. No.	Gram Staining	Shape	Catalase	Oxidase	Coagulase	Indole	MR Test	Motility Test	Species
1	-ive	Rods	+ive	-ve	-	-	-	-	E.coli
2	-ive	Rods	+ive	+ive	-	+	-	+	Vibrio spp.
3	-ive	Rods	+ive	-ve	-ve	-	-	-	Klebsiella pneumonia

Table 4
Show percentage of total isolates

Sample no	Isolates	Butchers	Uni Canteen	Drivers	Fish market	Biryani shop	General store
1	S .aureus	5(33.3%)	3 (20%)	2 (13.3%)	2 (13.3%)	2 (13.3%)	1 (6.6%)
2	Bacillus	2(50%)	0	0		0	2(50%)
3	Vibrio spp.	1(14.2%)		0	1(14.2%)	0	0
4	Klebsiella pneumoniae	0	2(18.5%)	0	0	0	1(14.2%)
5	E.coli	1(14.2%)	0	0	1 (14.2%)	0	0

Figure 10: Showed the prevalence rate of *S. aureus.* Isolated from different sources i.e. Fish market, university canteen, Drivers, Biryani shop, butchers' shop and general store. The highest rate of prevalence was observed among butchers' shop (33.3%).

Figure 10
Graphical representation of S. aureus

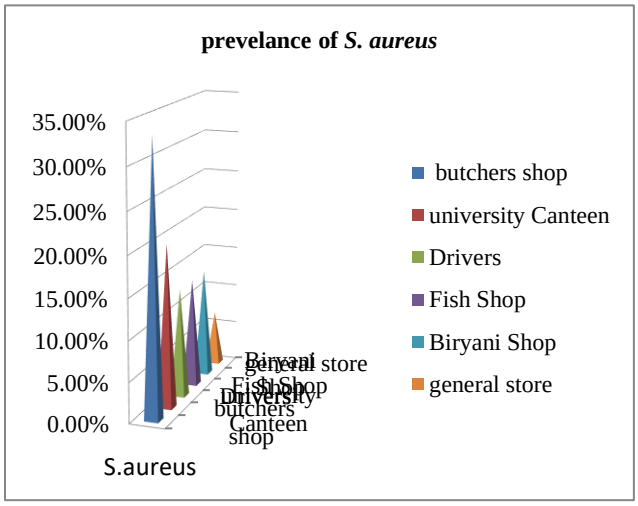


Figure 11: Showed the prevalence rate of K pneumonia. Isolated from different sources i.e. Fish market, University canteen, Drivers, Biryani shop, butchers shop and general store. the highest rate of prevalence was observed among university canteen (18.5%).

Figure 11
Graphical representation of K. Pneumoniae

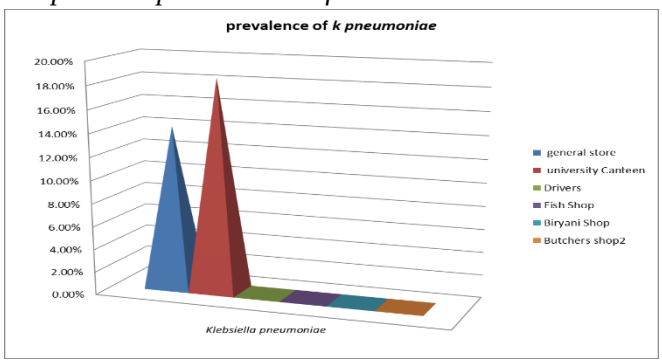


Figure 12: Showed the prevalence rate of Vibrio spp. Isolated from different sources i.e. Butchers shop, Fish market, University canteen, Drivers, Biryani shop and general store the highest rate of prevalence was observed among fish shop and butchers shop (14.2%).

Figure.12
Graphical representation of Vibrio spp

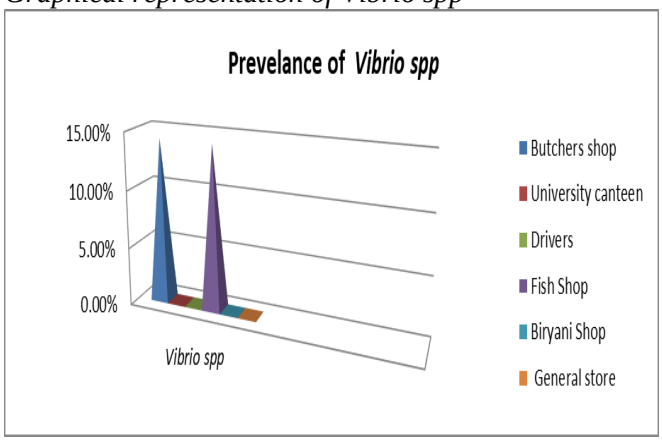


Figure 13: Showed the prevalence rate of Isolated from different sources i.e. Butchers shop, Fish market, University canteen, Drivers, Biryani shop and general store the highest rate of prevalence was observed among butcher's shop and fish shop (14.2%).

Figure 13
Graphical representation of E. coli

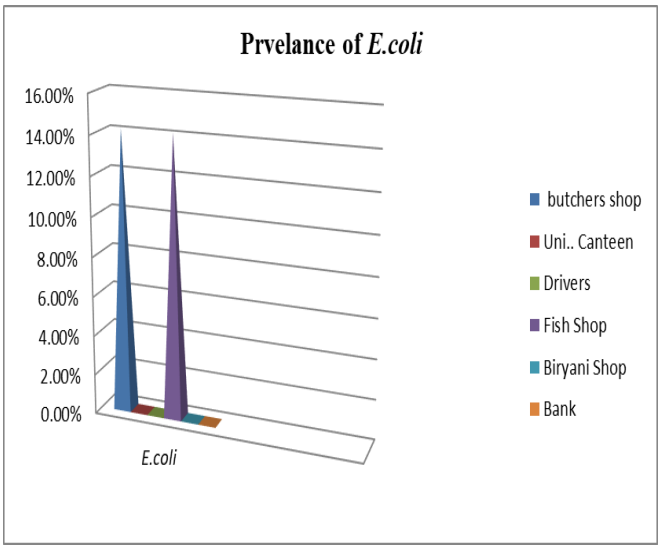
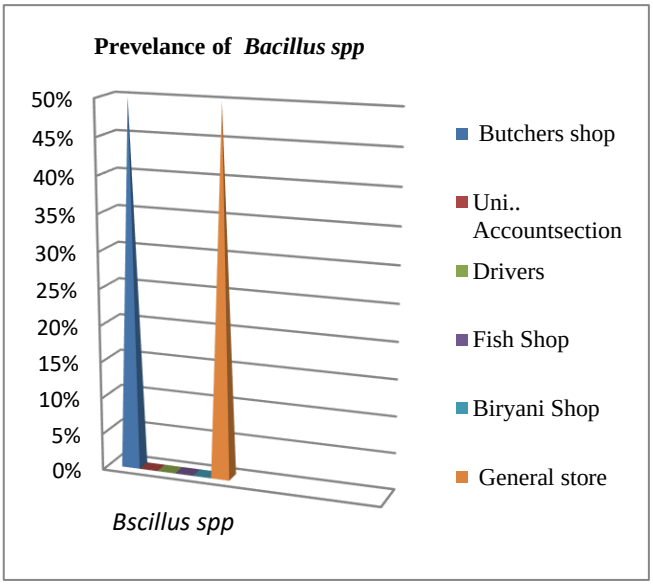


Figure 14 Showed the prevalence rate of *Bacillus Spp.* Isolated from different sources i.e. Butchers shop, Fish market, University canteen, Drivers, Biryani shop and General store the highest rate of prevalence was observed among general store and butchers shop (50%).

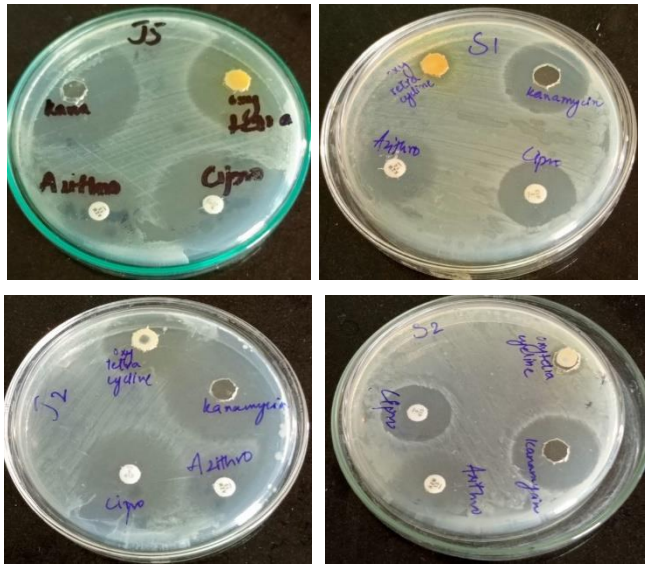
Figure 14
Graphical representation of Bacillus spp



Antimicrobial susceptibility testing

Sensitivity testing was done to ascertain the isolated strains' pattern of antibiotic sensitivity. The highest antimicrobial sensitivity was recorded against the antibiotics of ciprofloxacin, kanamycin, oxytetracycline and azithromycin.

Figure 16
Antibiotic susceptibility results for bacterial isolates



DISCUSSION

The study described here demonstrated that currency may be a vector that plays a significant role in the spread of harmful microorganisms in the community by isolating bacterial agents from currency notes. Currency notes may be contaminated by microorganisms such as Streptococcus and Staphylococcus strains, which are typically nonpathogenic but can cause urinary tract infections and severe food poisoning in humans. Other pathogenic bacteria can cause a variety of illnesses, from pneumonia and toxic shock syndrome (TSS) to opportunistic infections of the skin and other tissues. *Klebsiella pneumoniae* is one of the virulent pathogenic bacteria that has been isolated. It can cause common pneumonia as well as infections of the urinary tract and wounds, especially in immunocompromised people. Similar to this, *S. aureus* is a pathogenic and nosocomial bacterium that infects skin and other tissues opportunistically. The findings showed that the highest rate of contamination on the paper notes occurred when bacteria played a somewhat significant role in contamination. Similar contamination patterns were linked to the coins, where 93% and 58% of bacteria and fungi were isolated, respectively.

A total fifty samples were collected from currency notes and the sources were canteen, Butchers, Fish shops, General stores, Drivers and Biryani shops. After those sterile wet swabs by normal Saline were rubbed on surface of paper money then streak on nutrient agar plates. Of 50 samples, 26 were positive while 24 were negative. From positive samples, nineteen were Gram positive isolates and seven were Gram negative isolate identified. The isolated bacteria were *Staphylococcus aureus, Bacillus spp, Klebsella spp, E. coli* and *vibrio spp.* Similar study was conducted by (MacWilliams, 2012) which shows out of 100 sample collection from butchers shop, dairy shops, drivers, hospital, hotels 56 were positive while 44 were negative.

The bacterial isolates were then characterized by morphology, by using Nutrient Agar (NA) medium. After applying the Gram-staining method, microscopic examination demonstrated that bacterial isolate appear road, cocci, singly or in pairs, or sometimes even in chains. Similar investigation by (Moyes et al., 2009) which shows bacterial pathogens isolated from currency note appear rods, cocci and sometimes even in chains. In the current study, tests for catalase, coagulase, oxidase, methyl red, indole and motility were carried out. Catalase test shown that all bacterial isolates were positive. Bubbles of oxygen were produced when a drop of hydrogen peroxide was added to its isolates. Similarly study was conducted by (Ding et al., 2008) shows positive catalase test for all bacteria isolated from currency. The results of the coagulase test for fifteen isolated bacteria showed positive to produce aggregates and for seven bacteria show negative results, they were unable to form aggregates or clumps. The all-isolated bacteria were tested for the oxidase test. Out of twenty-six, nineteen were negative they were unable to produce any color and seven were positive show blue colour. Similar study was conducted by (Ding et al., 2008) which shows pathogenic bacterial isolates from contaminated curreny notes 45 isolates were coagulase positive,10 coagulase negative, 35 were oxidase positive while 25 were oxidase negative.

The all-isolated bacteria were tested for the indole test. Out of twenty-six, twenty-four were negative they were unable to produce any ring and two were positive which showed red ring. The motility tests were tested for all isolated bacteria. Seven were positive out of twenty-six which showing diffusing from the line of inoculation and twenty were negative there were no diffusing observed from the line of inoculation. The culture provides a satisfactory result for the MR test when the red color of the culture medium results from the fermentation of glucose, which occurs at or below pH 4.4. similar study was conducted by (Udayashankar et al., 2011) which shows out of 100 samples 50 were indole positive, 50 were indole negative, 30 shows positive results for motility test while 60 shows positive results towards methyl red test.

The five mains bacterial isolates from pathogenic currency were *S. aurous*, *Bacillus*, *E. coli*, *K. pneumonia* and vibrios species. Alike study by Akhtar *et al.*, (2004) which shows the main bacterial isolates from pathogenic currency were *S. aurous*, *E. coli*, *K. pneumonia*, *Vibrios cholera*, Clostridium, *Bacillus cerus*, *Salmonella typhi* and *Shigella*. The highest rate of prevalence of *S. aurous* was observed among butcher's shop (33.3%). The highest rate of prevalence of *K. pneumonia* was observed among university canteen (18.5%). The highest rate of prevalence of vibrio was observed among fish shop and

butchers shop (14.2%). The highest rate of *E. coli* prevalence was observed among butcher's shops and fish shop (14.2%). The highest rate of bacillus prevalence was observed among general stores and butchers shop (50%). Alike study by Akhtar *et al.*, (2004) which shows prevalence of *S. aureus* and clostridium were highest from butchers and fish shops (56%), prevalence of *K. pneumonia* was higher in hotels and cafes (46%), highest rate of prevalence of vibrio and Salmonella typhi and *E. coli* was observed among fish shop and butchers shop (19.2%). The highest rate of bacillus and clostridium prevalence was observed among general store and butchers shop (70%).

Sensitivity testing was done to ascertain the isolated strains' pattern of antibiotic sensitivity. The highest antimicrobial sensitivity was recorded against the antibiotics of ciprofloxacin, kanamycin, oxytetracycline and azithromycin. Similar study was conducted by Ding *et al.*, (2015) which shows pathogenic bacteria isolated from currency shows highest antimicrobial sensitivity against antibiotics of ciprofloxacin, imipenem, azithromycin and meropenum.

CONCLUSION

This study highlights currency notes as potential reservoirs and vectors for pathogenic bacteria, contributing to disease transmission. The isolation of *Staphylococcus aureus, Bacillus spp., Klebsiella pneumoniae, Escherichia coli,* and *Vibrio spp.* from Pakistani banknotes, especially in high-risk environments like butcher shops and fish markets, confirms their susceptibility to microbial contamination. Lower-denomination notes showed higher contamination due to increased handling. Biochemical characterization and antibiotic susceptibility testing revealed high sensitivity of the isolates to ciprofloxacin, kanamycin, oxytetracycline, and azithromycin. The study emphasizes the need for hygiene practices, awareness campaigns, digital transactions, and banknote disinfection. Further research on fungal and viral contamination is recommended to mitigate microbial transmission through cash handling.

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