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Banknotes as a Potential Source of Transmissible Diseases in the Al-Anbar Governorate, Iraq

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Background	Banknotes are handled by individuals with varying health and hygiene practices and are stored under diverse environmental and personal conditions. Paper currency is widely exchanged for commercial transactions and services, making it a potential vector for the transmission of bacteria.
Objective	To evaluate the bacterial contamination of banknotes in circulation in the Al-Anbar Governorate, Iraq.
Methods	The study was done between February and April 2023 and analyzed at the Microbiology Laboratories of the College of Medicine, University of Fallujah. A total of 84 samples were collected from seven banknote denominations across four locations. The bacteria were isolated and then identified by VITEK-2 then the antibiotic susceptibility test was done.
Results	Among the locations, the butcher shop had the highest number of positive samples 23 (32.1%), followed by the grocery store 21 (29.1%) and Rafidain Bank 18 (25%), while the pharmacy 10 (13.8%) had the fewest. Bacterial identification revealed a predominance of Gram-negative isolates over Gram-positive bacteria. The number of colony-forming units correlated with the locations of positive samples, with the grocery store being the most contaminated. Antibiotic susceptibility testing indicated that while antibiotics such as Imipenem and Ciprofloxacin may remain effective, significant resistance was observed for Ceftriaxone.
Conclusion	The study found that currency used in daily life may be a source of bacterial infection. The exchange of banknotes among individuals with different hygiene practices and occupations may facilitate the spread of diseases through contaminated currency.
Keywords	Antibiotic resistance, banknotes, currency contamination, disease transmission
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List of abbreviations: CLSI = Clinical laboratory standards institute, IQD = Iraqi Dinars, MHA = Mueller-Hinton agar, MRSA = Methicillin resistance *Staphylococcus aureus*

Introduction

B anknotes are handled by individuals with varying health and hygiene standards and are subjected to diverse environmental and personal storage conditions. Paper currency is frequently exchanged for the purposes of commerce and service provision, making it an ideal medium

for harbouring bacteria and other microorganism ^(1,2). It serves as a ubiquitous conduit for the transmission of pathogenic and potentially pathogenic microorganisms, both directly via hand-to-hand transactions and indirectly through contact with contaminated water or food ⁽³⁾.

A key factor in influencing the transmission of microorganisms through common items, such as banknotes, is their ability to persist on material surfaces ^(4,5). The microbial



contamination of banknotes is influenced by several factors, including the duration of circulation, handling practices, and the physical properties of the currency itself. The moistureabsorbent nature of banknotes further promotes the proliferation and survival of microorganisms ⁽⁶⁾.

Recent studies have sought to elucidate the composition of the microbial ecosystem on banknotes from various global regions ⁽⁷⁾. In one such study, researchers found that the most frequently isolated bacteria were coagulase-negative staphylococci, accounting for 43.6% of the total bacterial load. Other identified bacteria included Staphylococcus aureus (including Methicillin resistance Staphylococcus aureus (MRSA)), Streptococcus pyogenes, Acinetobacter spp., Escherichia coli (E. coli), Klebsiella spp., and Pseudomonas aeruginosa. Additionally, Enterococcus faecalis, and Enterococcus faecium, Enterococcus *durans* were identified as prevalent strains ^(4,8).

The prevalence of contaminants on currency varies regionally, influenced by the material composition of banknotes, which differs across countries ⁽⁹⁾. Abia and Ubomba-Jaswa (2019) observed that banknotes made from cotton-based materials carry a bacterial load approximately three times greater than those made from polymer. This difference is attributed to the structure of cotton fibres, which provide ample sites for bacterial attachment, a feature not present in polymer-based banknotes ⁽¹⁰⁾.

Individuals living in unsanitary conditions and engaging in poor hygiene practices, particularly those handling currency frequently, contribute significantly to the dissemination of pathogens, including faecal bacteria. Additionally, the storage of paper currency in materials such as polyethylene, cotton, and leather wallets, as well as in dark environments, promotes the proliferation of bacterial colonies on banknotes (11,12).

In Iraq, a country in the Middle East where paper currency is heavily relied upon for daily transactions due to limited infrastructure for online banking, the risk of bacterial transmission through banknotes is particularly high ⁽¹³⁾. The Central Bank of Iraq utilizes standard banknote paper for local transactions, including the circulation of United States of America (US) banknotes ⁽¹⁴⁾.

This study focuses on identifying bacterial strains that may be transmitted through banknotes in the Al-Anbar governorate, which serves as the primary region of investigation.

Methods

The study involved 84 samples, 7 Iraqi banknotes and four different locations. All collected samples were cultured and isolate the bacteria. Then, the bacterial growth was identified by VITEK 2. Finally, the antibiotic susceptibility test was performed to detect the antibiotic resistance of isolates.

Sample Collection

The samples used in this study were collected between February and April 2023 and analyzed at the Microbiology Laboratories of the College of Medicine, University of Fallujah. Local currency denominations sampled included 250 (Banknote 1), 1000 (Banknote 2), 5,000 (Banknote 3), 10,000 (Banknote 4), 25,000 (Banknote 5), and 50,000 (Banknote 6) Iragi dinars, along with 100 US dollars (Banknote 7). Three replicates of each currency were collected from four distinct locations: Rafidain Bank, a grocery store, a pharmacy, and a butcher shop, totalling 84 samples. The collection procedure involved using presterilised polyethylene self-sealing bags, aseptic conditions by allowing ensuring individuals to deposit banknotes directly into the bags, with equivalent currency provided on-site.

Isolation of bacteria

The collected samples were cultivated in a nutrient broth medium, supplemented with fluconazole (0.05 g/L) to inhibit fungal growth. The samples were then cultured using dip and blot techniques on solid nutrient media in large



plastic Petri dishes. Larger banknotes were sampled by swabbing both surfaces of the currency with a sterile cotton swab moistened in a 0.9% w/v sodium chloride saline solution (Figure 1).



Figure 1. Isolation of Bacteria A. Dip and blot techniques B. Swabbing method

Identification of Bacteria

The bacterial isolates were cultured on blood agar and MacConkey agar, followed by incubation at 37 °C for 24 hours. After incubation, a pure, distinct colony from each culture plate was selected for further analysis. For final confirmation, the VITEK-2 system, was employed to determine the bacterial identity.

Antibiotic Susceptibility test

Antibiotic susceptibility was assessed using the disc-diffusion method, as described by the Kirby-Bauer technique. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard and then cultured on Mueller-Hinton Agar (MHA) using a sterile cotton swab. After the medium dried, antibiotic discs were placed on the agar and the plates were incubated for 24 hours on 37°C. Antibiotic discs used in the analysis were procured from HiMedia Laboratories (India) and the procedure followed the Clinical Laboratory Standards Institute (CLSI) guidelines (15) The antibiotics tested included Ciprofloxacin (5 µg), Ceftriaxone (30 µg),

Imipenem (10 μ g), Doxycycline (30 μ g), and Azithromycin (30 μ g). Inhibition zones were measured using a ruler in millimetres (mm) ⁽¹⁶⁾.

Statistical analysis

The statistical analysis Statistical analysis was performed using GraphPad Prism 5 Software (San Diego, CA). The data were processed using the statistical package for the social sciences (SPSS) integrated into the software. T test and one way analysis of variance (ANOVA) were used for the statical significant at P value ≤ 0.05 .

Results

The study found that more than 70% of the samples were positive for bacterial growth on the initial culture (Figure 2). The butcher shop had the highest percentage of positive samples 23 (32.1%), followed by the grocery store 21 (29.1%) and Rafidain Bank 18 (25%), while the pharmacy 10 (13.8%) had the lowest number of positive samples compared to the other locations.





Figure 2. Number of positive samples in the study locations

Table 1 provides a detailed overview of thedistributionofvariousbanknotedenominations across four different locations:Rafidain Bank, a grocery store, a pharmacy, anda butcher shop.Each column represents a

different denomination of banknotes, ranging from lower denominations like 250 IQD to higher ones like 50,000 IQD, as well as 100 USD.

Locations	Banknote 1 (250) IQD	Banknote 2 (1000) IQD	Banknote 3 (5000) IQD	Banknote 4 (10000) IQD	Banknote 5 (25000) IQD	Banknote 6 (50000) IQD	Banknote 7 (100) \$
Rafidain Bank	110	172	90	50	44	39	16
Grocery store	550	620	601	560	582	400	138
Pharmacy	480	350	322	280	234	200	100
Butcher shop	200	300	290	268	380	550	400
Total	1340	1442	1303	1158	1240	1189	654

Table 1: Number of colonies forming among each banknote

Gram-negative bacteria were more prevalent than Gram-positive and 41 (91.1%) isolates out of 45 Gram-negative over Gram-positive bacteria. The most prevalent bacteria were *Pseudomonads*, with 16 (36%) isolates, including three distinct species: *P. aeruginosa*, *P. fluorescens*, and *P. cepacia*. This was followed by *Pantoea species*, with 13 (29%) isolates, including *Pantoea agglomerans*, and 9 (21%) *Klebsiella pneumoniae* isolates, along with three other *E. coli* species, following with four isolates of Gram-positive bacteria *Staphylococcus aureus* as determined by the Vitek 2 system (Figure 3).





Figure 3. The percentage numbers of diagnosed bacteria strain

Table 2 highlights the importance of antibiotic susceptibility testing in guiding treatment choices. It suggests that while antibiotics such

as Imipenem and Ciprofloxacin may remain effective, others, like Ceftriaxone, face substantial resistance challenges.

No.	Ceftriaxone	Imipenem	Ciprofloxacin	Doxycycline	Azithromycin
A1	R	28	34	20	R
A2	R	28	28	24	R
A3	R	20	24	16	R
B1	R	24	34	20	R
B2	22	20	28	R	14
B3	R	26	38	30	24
C1	R	24	26	30	18
C2	R	22	20	20	R
C3	18	20	32	22	R
D1	16	24	R	28	24
D2	R	26	28	20	R
D3	16	20	26	14	R
E1	R	20	30	16	24
E2	R	20	30	R	18
E3	16	24	30	R	18

Table 2. Antibiotic resistance and sensitivity towards isolated strains (inhibition zone (mm))



Discussion

Gram-negative bacteria are often more prevalence than Gram-positive bacteria due to their ability to survive in the moist environments and ability to develop multidrug resistance. However, most studies on the antimicrobial resistance reported that up to 60-70% are nosocomial infections with Gramnegative bacteria ^(16,23). Sharing money papers is considered one of the sources of bacterial contamination. In our results, Rafidain Bank showed a relatively balanced distribution of banknotes across denominations, with a notable emphasis on Banknote 2. However, there were fewer higher denominations (Banknotes 4, 5, and 6) compared to Banknotes 1 and 2. The grocery store had a significantly higher quantity of banknotes across all denominations compared to other locations, which aligns with previous findings (17) most Banknote 3 was the common denomination at the grocery store, followed closely by Banknote 2. Notably, the grocery store had a significant number of Banknotes 5 and 6, indicating potential large transactions. pharmacy primarily handled The lower denominations, with Banknote 1 and Banknote 2 being the most common. There was a gradual decrease in the quantity of banknotes as the denomination increased, reflecting typical transaction amounts at a pharmacy. The butcher shop had a relatively balanced distribution of banknotes, although Banknote 6 was the least common, suggesting fewer highvalue transactions in this setting.

The study demonstrated that the grocery store was the most contaminated site compared to other locations. This is likely due to the high volume of visitors, as the grocery store offers essential daily items at affordable prices ⁽¹⁸⁾. Furthermore, lower-denomination currency, which is circulated more frequently among individuals with varying hygiene practices, was found to be the most contaminated among the sampled banknotes ⁽¹⁹⁾. However, when we compared the materials used to produce the banknotes, it became clear that Banknote 7



The resistance challenge of antibiotic highlights the growing concern of this pattern, necessitating careful selection of treatments based on susceptibility testing ^(10,21). These results clearly indicate the danger of bacteria transmitted through banknotes, as many isolated bacteria exhibited resistance to a wide range of antibiotics. This result was agreed with what Al-Abassi, 2010 found as most of the bacteria were resistance to the antibiotics ⁽²²⁾.

In conclusion, the study found that currency used in daily transactions may serve as a source of infection. Sharing banknotes among individuals with differing hygiene practices and occupations may contribute to the spread of infectious diseases. The material used in banknote production plays a critical role in bacterial transmission, with lowerdenomination banknotes being the most contaminated due to their frequent use and exchange across various locations within the Al-Anbar province.

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Conflict of interest

The author has no conflicts of interest to disclose.

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