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Detection, characterization, and antibiotic resistance profiling of multidrug-resistant bacteria isolated from circulating currency in the Northeastern region of Bangladesh

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ABSTRACT

Objectives: The study aims to investigate the prevalence of multidrug resistant bacteria on paper and coin currency obtained from various occupational groups in Bangladesh and to identify the bacterial species present. The research further seeks to evaluate the antibiotic resistance patterns of the identified bacterial isolates.
Methods: 84 paper currency notes and 56 coins were collected from seven different sources. Bacterial contamination was assessed using standard bacteriological and biochemical tests to identify and characterize the bacteria. Antibiotic susceptibility of the isolated strains was evaluated using an antibiogram study.
Results: A total of 368 bacterial isolates were detected across the sampled currency, with 99% of the currency samples contaminated by bacteria. Paper currency exhibited a higher prevalence of contamination compared to coins. Gram-staining revealed 20% Gram-positive and 80% Gram-negative bacteria on notes, compared to 38% Gram-positive and 62% Gram-negative bacteria on coins. Bacterial contamination was most frequent in samples from fish sellers, followed by poultry sellers, fruit sellers, and restaurants. The most commonly identified bacteria were *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli*, and *Klebsiella aerogenes*. Antibiotic resistance testing revealed that all isolates were 100% resistant to amoxicillin, ampicillin, and penicillin G, while showing 100% sensitivity to azithromycin and gentamycin. Notably, 70.8% of the isolates were resistant to tetracycline, and 75% showed resistance to cefotaxime.
Conclusions: The widespread contamination of currency in Bangladesh with multidrug-resistant bacteria underscore the growing concern about antibiotic resistance. Preventative measures are essential to reduce cross-contamination between currency and food.

Introduction

Environmental contamination or pollution has become a major problem in recent years, causing significant damage to the earth and its inhabitants [1]. Interestingly, the environment also plays a crucial role in transmitting microorganisms to humans and others. Microbial contaminants can be transmitted in two ways: directly, through hand-to-hand contact, or indirectly, through food or other inanimate objects. These routes of transmission are particularly significant in developing countries, where poor hygiene and sanitation levels make it easier for contaminants to spread. There is well-documented evidence that paper currency can act as a vector for bacteria, due to its rough surface which can accumulate and proliferate a large number of bacteria [2,3]. People in Bangladesh are extensively using paper currency and coins as a medium of exchange objects that serve as potential carriers to transmit pathogenic bacteria [4]. The proliferation of antibiotic-resistant bacteria in the local community of Bangladesh is a major concern and a possible cause of severe infection outbreaks [5].

Paper currency is broadly exchanged worldwide between communities for merchandise and other basic needs [6]. However, currencies can become contaminated with normal flora and pathogens from sources such as skin, respiratory secretions, gastrointestinal tract, water, soil, and aerosols during handling as many people do not consider the cleanliness of their hands when handling currencies [2,7]. Additionally, it has been observed that older and damaged notes tend to accumulate more microbes than new ones [3].

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Over the last two decades, a large number of bacterial strains have been isolated from currency samples collected from various locations around the world. The most significant pathogens transmitted through contaminated currency include strains *E. coli, Klebsiella* spp., *Enterobacter* spp., *Vibrio* spp., *Pseudomonas* spp., *Salmonella* spp., *Shigella, Bacillus* spp., *S. aureus,* and *Campylobacter jejuni* [6–8]. These species have the potential to cause a wide range of illnesses such as food poisoning, wound and skin infections, pneumonia, tonsillitis, genital tract infections, respiratory, and gastrointestinal issues, as well as life-threatening diseases like meningitis and septicemia [7,9].

Antibiotic resistance is a growing concern for public health worldwide, as it renders antibiotic drugs ineffective in treating bacterial infections leading to increased morbidity and mortality [10]. The ability of bacteria to adapt and survive in the presence of antibiotics is a result of their complex genetic mechanism. Unfortunately, many individuals are unaware of the implications of antibiotic resistance and contribute to the problem through self-medication and irrational antibiotic use [11]. The misuse of antibiotics, whether by healthcare professionals or patients, is particularly prevalent in developing countries [12,13]. This highlights the need for increased education and awareness on the proper use of antibiotics to prevent the emergence of resistant strains and preserve the effectiveness of these life-saving agents [13].

Despite the prevalence of microbial contamination on coins and currency notes in Bangladesh, there is a lack of up-to-date research on this issue. Some of the previous studies have focused solely on identifying the presence of microorganisms on circulating currency, leaving important questions about multidrug-resistant microorganisms unanswered [4]. Therefore, the objectives of this study were to characterize different multi-drug resistant strains and understand their antibiotic resistance patterns from circulating currency samples.

Materials and methods

Collection and transport of samples

The currency sample collection was conducted from January 2018 to December 2020. A total of 140 samples were collected aseptically, including 84 paper notes of BDT 10, 20, and 100 and 56 metal coins of BDT 2 and 5. These samples were collected from various sources of Sylhet City (a Northeastern region of Bangladesh), including fish sellers, meat salesmen, poultry dealers, vegetable traders, fruit merchants, street-food vendors, and restaurant agents. The samples were collected in sterile zip-top bags, and sterility was maintained throughout the collection process. We confirmed that no cross-contamination occurred. The individuals were instructed to place the samples directly into the bags, and no one touched the samples with their bare hands during the collection process. The purpose of the study was explained to the individuals before collecting the sample. Additionally, 10 completely new note samples were collected from a commercial bank as controls. The collected samples were then transported to the microbiological laboratory for isolation, identification, and antibiotic sensitivity testing of pathogenic bacteria.

Colony counting

To determine the bacterial load in currency samples, colony-forming units per ml (CFU/ml) were used as a representative. Serial dilutions were performed to calculate CFU/ml. A 1 ml aliquot of the stock solution (solution 0) is introduced to tube 1 containing 9 ml of 0.9% saline, resulting in solution 1 (10^{-1}). The procedure was repeated by taking an aliquot of 1 ml of the newly generated solution 1 and transferring it into tube 2 (10^{-2}). This process of aliquoting and resuspending continues until the last tube (10^{-7}) is used, reducing the stock concentration by a factor of 10 at each stage. Subsequently, an aliquot sample of tube 5 (10^{-5}) and tube 7 (10^{-7}) was inoculated on nutrient agar plates using the spread plate method and incubated at 37°C for 24 hours. A negative control (with no bacterial culture) was also performed. The CFU/ml was calculated by taking the average of the number of counted colonies from two plates (10^{-5} and 10^{-7}). The CFU/ml was determined consistent with methods determined previously [14].

Bacteria culture and isolation

Each currency sample was dipped in sterile distilled water to collect maximum contaminants and transferred to Luria broth for overnight incubation at 37°C. After checking the turbidity, the organisms were streaked on Petri plates with nutrient agar (Luria Agar), MacConkey agar, blood agar, mannitol salt agar (MSA), Simmon's citrate agar (SCA), thiosulfate-citrate-bile salts-sucrose (TCBS) agar for *Vibrio spp.*, Hicrome™ *E. coli* agar, and *Salmonella-Shigella* (SS) agar for selective isolation of Gram-positive and Gram-negative organisms [15]. Bacterial growth was observed after 24 hours of aerobic incubation at 37°C. Pure colonies were obtained by streaking a small number of cells from a distinct colony on a nutrient agar plate.

Staining and biochemical tests

Each pure culture was identified and characterized based on colony morphology, Gram staining, and various biochemical tests, including catalase, coagulase, MSA, MacConkey Agar, SCA, oxidase, indole, and Methyl Red-Voges-Proskauer (MR-VP) tests, following standard protocols for species-level identification [16]. All media and reagents for biochemical tests were purchased from HiMedia Laboratories (India).

Antibiotic susceptibility test

The antibiotic susceptibility test was conducted by Kirby-Bauer Disk Diffusion Method on Muller-Hinton Agar [17]. Fourteen different antibiotics (amoxicillin, ampicillin, penicillin G, chloramphenicol, doxycycline, nalidixic acid, azithromycin, gentamycin, co-trimoxazole, tetracycline, cefotaxime, ciprofloxacin, meropenem, and polymyxin B), obtained from HiMedia Laboratories, were used to determine the resistance pattern of the isolated bacteria. These antibiotics were selected as a representative of the first, second, and third generation of antibiotics and are commonly prescribed in Bangladesh for the treatment of bacterial infections. According to the reference value of the Clinical and Laboratory Standards Institute and the European Committee on antimicrobial susceptibility testing interpretative chart, the zone of inhibition was measured [18].

Data analysis

All the data were analyzed using Excel 2021 and R Statistical Software (V4.1.2). The antibiotic resistance data were presented as percentages, representing the frequency of the isolated bacteria.

Results

Types of bacteria isolated from currency samples

A comprehensive analysis of 140 currency samples from seven different sources was conducted to assess bacterial contamination. The results showed that 99% of the currency samples were contaminated with various types of bacteria, with a higher prevalence observed in currency notes compared to coins (Figure 1).

The analysis identified 10 different types of bacteria present in the currency samples. To validate the results, 10 control samples (Currency Notes and Coins) were collected from a commercial bank and were found to contain normal flora only. The analysis revealed that the highest levels of bacterial contamination were present in currency samples collected from meat, fish, and fruit sellers. A total of 368 isolates were obtained from 140 currency samples. The most common isolates were *S.*

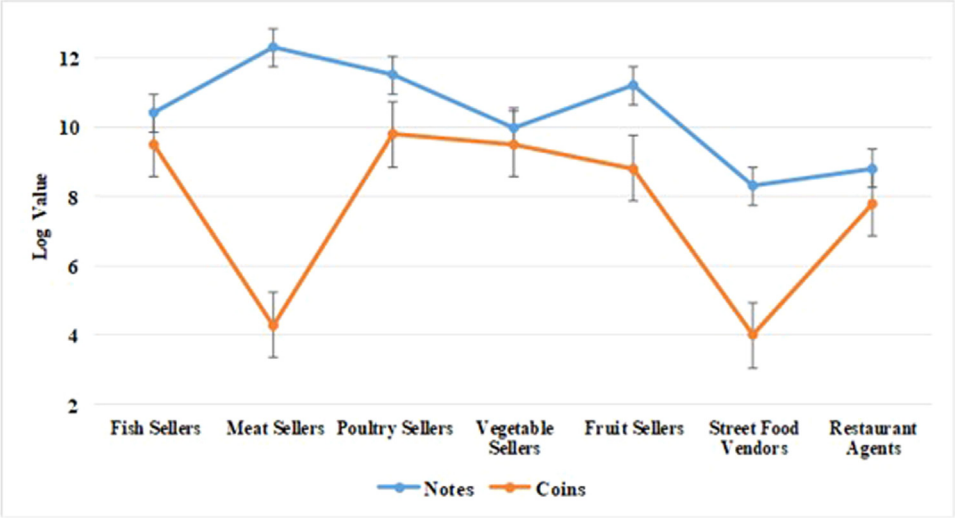


Figure 1. Bacterial concentration in currency samples. This line graph shows the bacterial concentration (colony forming units/ml) on currencies (note and coin) collected from different food vendors (fish seller, meat seller, poultry seller, vegetable seller, fruit seller, street food vendors, and restaurant agents). Note and coin are denoted by blue and orange lines respectively. Data represented as logarithmic value of mean ± SD.

Table 1
The number of isolates characterized from all the currency samples of different occupational groups (20 samples were collected from each of 7 occupational groups). Total 368 isolates were characterized, and 10 different types of bacteria were observed.

Isolated strains	Fish seller	Meat seller	Poultry seller	Vegetable seller	Fruit seller	Street food vendors	Restaurant agents	Total
S. aureus	7	2	2	10	14	11	7	53 (14%)
S. epidermidis	6	2	2	3	10	7	8	38 (10%)
S. pyogenes	1	0	1	0	1	0	0	3 (1%)
E. coli	10	4	8	8	5	4	10	49 (13%)
K. pneumoniae	8	6	7	6	4	4	5	40 (11%)
S. typhi	2	2	1	2	1	0	1	9 (3%)
S. typhimurium	8	7	12	7	4	4	12	54 (15%)
S. flexneri	7	2	6	3	3	6	10	37 (10%)
P. aeruginosa	4	4	12	9	8	0	0	37 (10%)
K. aerogenes	7	7	9	6	10	2	7	48 (13%)
Total	60	36	60	54	60	38	60	368 (100%)

typhimurium, followed by *S. aureus, E. coli, K. aerogenes, Klebsiella pneumoniae, Staphylococcus epidermidis, Shigella flexneri, Pseudomonas aeruginosa, S. typhi*, and *Streptococcus pyogenes*. The distribution is shown in Table 1.

Prevalence of bacteria among different occupational groups

In total, 368 bacterial isolates were characterized from various currency samples collected from meat, poultry, vegetable, fruit, street-food sellers, and restaurants (Table 1). Meat seller samples had 36 isolates, with *S. typhimurium* and *K. aerogenes* being the most frequent (19% each), while no bacteria were found in the coin samples. Poultry sellers yielded 60 isolates, with *S. typhimurium* and *P. aeruginosa* (20% each) being the most prevalent. Vegetable sellers had 54 isolates, led by *S. aureus* (18%) and *P. aeruginosa* (16%). Fruit seller samples showed 60 isolates, dominated by S. aureus (22%) and *K. aerogenes* (18%). Street-food sellers had 38 isolates, with *S. aureus* (29%) being the most frequent, and a lower prevalence of bacteria in coins compared to notes. Restaurant samples produced 60 isolates, with *S. typhimurium* (20%) and *E. coli* (17%) being the most common (Table 1).

Gram-staining of isolated bacteria

In terms of the Gram-staining results, 20% of the isolates from the note samples were Gram-positive and 80% were Gram-negative. In contrast, 38% of the isolates from the coin samples were Gram-positive and 62% were Gram-negative. The results show that the prevalence of Gram-positive bacteria was higher in coins compared to notes (Figure 2).

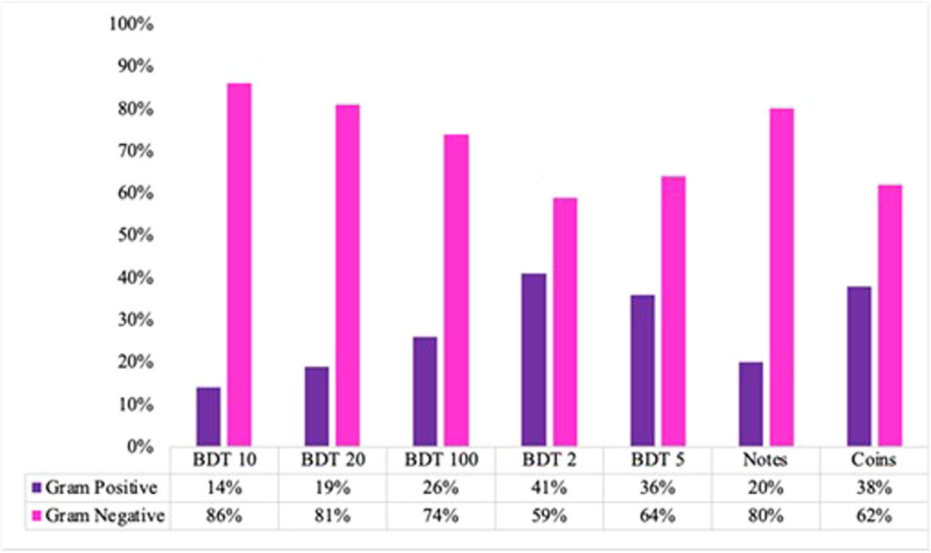
Bacteria characterized by different currency samples

A total of 252 isolates were obtained from the examination of 10, 20, and 100 Taka notes, while 116 isolates were identified from 2 Taka and 5 Taka coin samples. The results showed that in the case of 10 Taka notes, 21% of the isolates were identified as *S. typhimurium*, followed by 14% each of *K. pneumoniae* and *K. aerogenes*, and 12% *E. coli*. For the 20 Taka notes, 21% of the isolates were identified as *E. coli*, followed by 14% *K. pneumoniae*, 12% *S. typhimurium*, and 12% *K. aerogenes*. Finally, in the case of 100 Taka notes, 18% of the isolates were identified as *S. typhimurium,* and 17% were identified as *E. coli* (Figure 3).

In contrast, 54 isolates were obtained from 2 Taka coins, and 62 isolates were obtained from 5 Taka coins. The results showed that in the case of 2 Taka coins, the highest proportion (20%) of the isolates was identified as *S. aureus*, followed by 17% *S. epidermidis*. In the case of 5 Taka coins, 26% of the isolates were identified as *S. aureus*, followed by 18% *P. aeruginosa* and 16% *K. aerogenes* (Figure 3). As previously mentioned, the prevalence of Gram-positive bacteria was higher in the coin samples compared to the note samples.

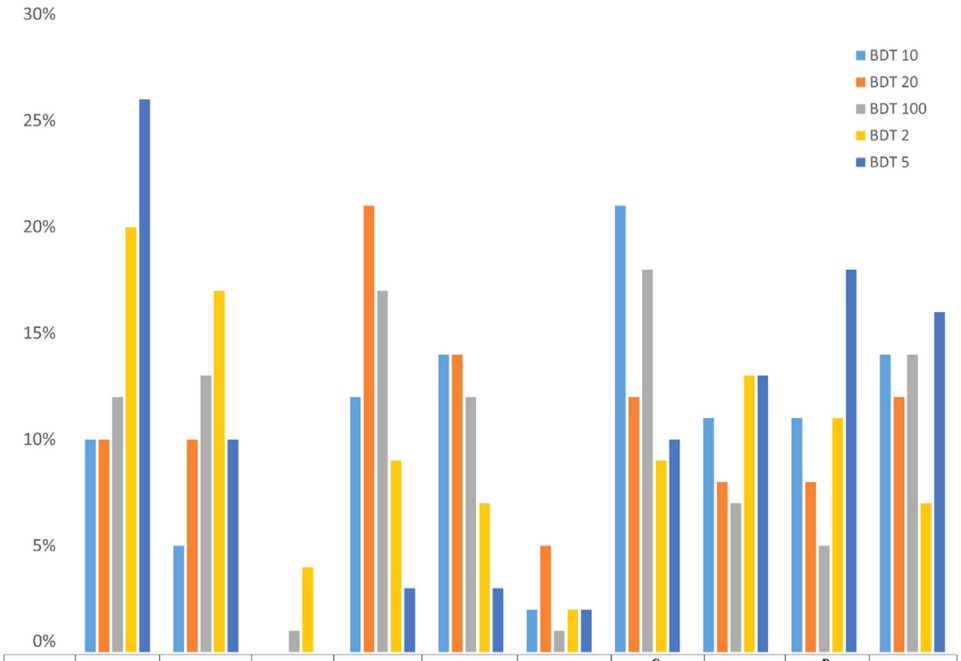
Antibiotic susceptibility test

The susceptibility of 368 bacterial isolates to 14 antibiotics from seven different classes was tested (Table 2). The isolates exhibited complete resistance to amoxicillin, ampicillin, and penicillin G. Resistance was also observed against some IInd and IIIrd generation antibiotics, including co-trimoxazole, tetracycline, cefotaxime, and to a lesser extent, ciprofloxacin and meropenem. Notably, all species were sensitive to azithromycin and gentamycin, suggesting their effectiveness. High



	BDT 10	BDT 20	BDT 100	BDT 2	BDT 5	Notes	Coins
Gram Positive	14%	19%	26%	41%	36%	20%	38%
Gram Negative	86%	81%	74%	59%	64%	80%	62%

Figure 2. Gram-positive and Gram-negative bacterial profiles from all samples. The following bar diagram shows the percentages of Gram-positive (purple) and Gram-negative (pink) bacterial percentages in different currency notes (BDT 10, BDT 20, and BDT 100) and coins (BDT 5 and BDT 2) collected from different occupational groups.



	S. aureus	*S. epidermidis*	*S. pyogene*	*E. coli*	*K. pneumoniae*	*S. typhi*	*S. typhimurium*	*S. flexneri*	*P. aeruginosa*	*K. aerogene*
BDT 10	10%	5%	0%	12%	14%	2%	21%	11%	11%	14%
BDT 20	10%	10%	0%	21%	14%	5%	12%	8%	8%	12%
BDT 100	12%	13%	1%	17%	12%	1%	18%	7%	5%	14%
BDT 2	20%	17%	4%	9%	7%	2%	9%	13%	11%	7%
BDT 5	26%	10%	0%	3%	3%	2%	10%	13%	18%	16%

Figure 3. Percentage of bacterial contamination per currency samples. The histogram represents different bacterial contamination percentages in currency notes (BDT 10, BDT 20, and BDT 100) and coins (BDT 5 and BDT 2) collected from different occupational groups.

resistance rates were noted for tetracycline (up to 70.8% in *K. aerogenes*) and variable resistance to cefotaxime (10% in *E. coli* to 75% in *K. pneumoniae*). Meropenem and polymyxin B showed minimal resistance, with polymyxin B having only 2% resistance in *E. coli* and 3.7% in *S. typhimurium* (Table 2). Low resistance rates were observed for doxycycline, nalidixic acid, chloramphenicol, and ciprofloxacin, indicating their potential effectiveness. Overall, while beta-lactam antibiotics are largely ineffective, ciprofloxacin, meropenem, and polymyxin B remain viable treatment options.

Discussion

The results of this study indicate that currency samples can indeed act as vectors for microorganism transmission, corroborating previous research that highlights the role of money in carrying bacteria, viruses, and fungi [2–4]. This is of particular concern as money is constantly shifting hands and is often not disinfected before being used. With 368 isolates characterized from 140 currency samples (notes and coins), our findings reveal a greater abundance of bacteria on notes compared to coins, likely due to the paper's rough surface which accumulates bacteria more readily over time [19]. Coins, composed of metals with antimicrobial properties, tend to harbor fewer bacteria [20]. Besides, the prevalence of Gram-negative bacteria was greater in currency samples compared to Gram-positive bacteria. Interestingly, the number of Gram-positive bacteria was higher in coins compared to notes. The significance of such discrepancy is not well understood.

Bacteria belonging to different species such as S. aureus, S. epidermidis, S. pyogenes, E. coli, K. pneumoniae, S. typhi, S. typhimurium,

Table 2
Antibiotic resistance percentage of isolated bacterial strains against 14 different antibiotics.

Bacterial Species	Amoxicillin	Ampicillin	Penicillin G	Azithromycin	Gentamycin	Co-Trimoxazole	Tetracycline	Cefotaxime	Meropenem	Polymyxin B	Doxycycline	Nalidixic Acid	Chloramphenicol	Ciprofloxacin
	Percentage of resistance (%)													
S. aureus	100	100	100	0	0	37.7	47	69.8	1.88	0	18.8	15	3.7	3.7
S. epidermidis	100	100	100	0	0	13	26.3	39.47	0	0	7.89	7.89	0	0
S. pyogenes	100	100	100	0	0	33	33	66.6	0	0	0	33	0	0
E. coli	100	100	100	0	0	40.8	51	10	0	2	10	18	0	0
K. pnemoniae	100	100	100	0	0	25	52.5	75	0	0	5	10	0	0
S. typhi	100	100	100	0	0	22	66.6	33	0	0	22	22	0	0
S. typhimurium	100	100	100	0	0	24	68.5	46	1.85	3.7	18.5	27.7	0	0
S. flexneri	100	100	100	0	0	32.4	70	56.7	0	0	13.5	8	0	0
P. aeruginosa	100	100	100	0	0	27	21.6	43	0	0	13.5	13.5	0	0
K. aerogenes	100	100	100	0	0	35.4	70.8	50	0	0	20.8	22.9	0	0

S. flexneri, P. aeruginosa, K. aerogenes, and V. cholerae were identified from the currency samples of different occupational groups which were reported in several other studies worldwide as well [3,4,9]. These species can cause various illnesses, ranging from minor such as diarrhea and food poisoning to life-threatening diseases like meningitis and septicemia [4,9]. In our study, S. typhimurium, followed by S. aureus, E. coli, and K. aerogenes was the most prevalent bacteria identified from note and coin samples, all of which have the potential to cause severe health issues and outbreaks of diarrhea. Besides, the frequency of bacterial contamination was highest in samples collected from fish sellers, followed by poultry sellers, fruit sellers, and restaurants. This could be due to the handling of raw fish and the lack of proper hygiene practices, which increases the chances of bacteria transfer to the currency used by the fish sellers. Poultry sellers also showed a high frequency of bacterial contamination, which is in line with previous studies that have reported the presence of bacteria such as Salmonella and E. coli in poultry product [21]. Fruit sellers and restaurants were also found to have a significant amount of bacterial contamination, which could be attributed to the handling of fresh produce and the presence of food-borne bacteria [22]. These findings emphasize the critical need for stringent hygiene practices and effective contamination control measures in the food industry to mitigate bacterial spread and safeguard public health [23,24].

The widespread resistance to first-generation antibiotics, such as amoxicillin, ampicillin, and penicillin G, suggests a worrying trend that reflects global patterns of rising antibiotic resistance. Studies conducted worldwide have similarly reported a sharp decline in the efficacy of these antibiotics due to their overuse and misuse in both medical and agricultural settings [25,26]. Our results, indicating 100% resistance to these antibiotics, align with findings from other developing countries, where resistance to these first-line drugs is rampant due to unregulated use [26,27]. However, a positive observation from this study is the complete sensitivity of bacterial isolates to azithromycin and gentamycin. These antibiotics remain highly effective, suggesting their continued potential for treating infections caused by these resistant organisms. This is consistent with earlier studies that highlight azithromycin's efficacy against many Gram-negative bacteria (including Enterobacteriaceae) and provides coverage of many Gram-positive organisms [28]. Gentamycin, an aminoglycoside, is often used in clinical settings due to its broad spectrum of activity, and its 100% sensitivity in this study confirms its viability as a treatment option [29].

The most alarming aspect of the results is the resistance observed against second- and third-generation antibiotics. The resistance of *S. aureus* and *E. coli* to tetracycline and co-trimoxazole reflects a broader issue in antimicrobial resistance, where overuse of these antibiotics has led to reduced efficacy [30]. In our study, *S. aureus* demonstrated moderate resistance to co-trimoxazole (37.7%) and tetracycline (47%). The resistance levels of *S. aureus* and other isolates to these antibiotics are increasing in both hospital and community settings [31]. The resistance patterns of *E. coli* are particularly concerning, as it showed high resistance to co-trimoxazole (40.8%) and tetracycline (51%). This is consistent with global trends where *E. coli* isolates, especially those causing urinary tract infections, exhibit high resistance to commonly used antibiotics, making treatment more challenging [30]. Notably, *E. coli* exhibited no resistance to ciprofloxacin and meropenem, which is encouraging, as these drugs remain effective for treating infections caused by multidrug-resistant strains.

K. pneumoniae, known for its resistance due to extended-spectrum β-lactamase production, also showed susceptibility to meropenem and ciprofloxacin, consistent with findings from other studies. extended-spectrum β-lactamase-producing *K. pneumoniae* is a growing threat in both hospital and community settings, but carbapenems like meropenem are still considered the treatment of choice for these resistant strains [32]. However, the emergence of carbapenem-resistant *K. pneumoniae* in certain parts of the world is a reminder that the current efficacy of meropenem could be compromised in the near future

if resistance continues to spread [30,32]. This suggests that pathogenic bacteria are adapting and evolving to overcome the effects of even the most advanced antibiotics. The presence of resistance to these antibiotics is concerning as it may limit treatment options and result in prolonged illness, and increased risk of morbidity and mortality [33]. To effectively address the antibiotic resistance crisis, it is crucial to quantify the pathways and address the mechanism involved in the environmental evolution and transmission of resistance [34]. Future studies could complement our findings by performing antibiograms on individual isolates and detecting and sequencing resistant genes to provide a detailed resistance map.

The issue of bacteria becoming resistant to the most recent antibiotics and more dangerous due to factors like unawareness, carelessness, and improper handling of money is a growing concern in many countries, particularly in developing nations. Therefore, this study recommends emphasizing the necessity of strict hygiene guidelines when handling cash to reduce the risk of bacterial contamination and transmission. Promoting digital payment methods and paperless transaction systems (card, internet banking, and/or mobile banking) can significantly reduce physical currency exchange as well as contamination, particularly in high-risk environments such as markets and restaurants. To stop multidrug-resistant bacteria from emerging and spreading, sensible antibiotic use policies must be put into place, backed by public and healthcare professional education. The creation of novel antibiotics, molecular studies of resistance mechanisms, and continuous monitoring of antimicrobial resistance patterns are also essential. To protect public health, infection control procedures must be strengthened, and public awareness campaigns should stress the value of hand cleanliness and handling cash safely.

Conclusion

In conclusion, this study reveals that first-generation antibiotics, such as amoxicillin, ampicillin, and penicillin G, are ineffective against the bacterial isolates found on currency notes. Conversely, newer antibiotics like gentamycin, meropenem, and polymyxin B remain effective treatment options, especially against drug-resistant strains. The findings highlight the critical need for ongoing monitoring of antimicrobial resistance and susceptibility testing before treatment. Additionally, the presence of pathogenic bacteria on currency notes underscores the necessity for stringent public health measures to mitigate microorganism transmission, particularly in high-risk settings like currency handling. The increasing resistance to third-generation antibiotics is a significant concern, emphasizing the need for further molecular research to understand resistance patterns and improve public health strategies.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical approval statement

This study did not involve the use of human subjects, animal models, or human tissues. The research focused solely on bacterial isolates obtained from circulating currency and exempt from ethical approval as no direct human or animal involvement occurred.

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Author contributions

Study conception and design: MAM, MA, and MAKA; drafting of the manuscript: MAM, AI, AS, and MAKA; Sample and data collection: AI, AS, and MRI; data analysis and interpretation: MA, SI and MAKA, laboratory testing: MAM, A.I., A.S, SF, MRI; critical appraisal and review: SI, MA and MAKA. All the authors approve the final version of the manuscript to be submitted.

Data availability statement

All data supporting the findings of this study are available within the article. Any additional data that supports the results reported in this article can be made available upon reasonable request.

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