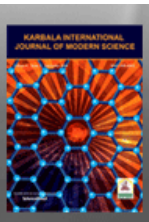

Karbala International Journal of Modern Science

Volume 8 | Issue 3 Article 25

Molecular characterization of ESBLs and AmpC β-lactamases in Bacteria Isolated from Currency Notes Circulating in Mosul City, Iraq

Mahmood Zeki Al-Hasso
Biophysics Dept. /College of Science / Mosul University, mahmoodalhasso@uomosul.edu.iq

Shakir Ghazi Gergees
Biology Dept. /College of Science / Mosul University Mosul/ Iraq

Zahraa Khairialdeen Mohialdeen
Biology Dept. /College of Science / Mosul University Mosul/ Iraq

Molecular characterization of ESBLs and AmpC β-lactamases in Bacteria Isolated from Currency Notes Circulating in Mosul City, Iraq

Abstract

The Iraqi currency notes circulating in Mosul city were evaluated for the occurrence of ESBLs and AmpC b-lactamaseproducing bacteria. Four hundred and twenty-two Gram-positive and negative bacterial isolates with different antimicrobial resistance profiles were recovered from 250 samples collected during the period from April to July 2021, among which 150 isolates (35.5%) were multi-drug resistant (MDR). The study found that 16.4% and 14.8% of Gram negative isolates were positive for ESBLs and AmpC phenotypic detection tests, respectively. Interestingly, 6.6% of the isolates were simultaneously positive for both tests. Molecular characterization was carried out using PCR technique to determine the prevalent ESBLs gene\s. The results revealed that blaTEM and blaCTX-M were the most prevalent ESBLs genes (28.6% and 26.2%, respectively). Moreover, 11.9% of the tested isolates appeared to have dual genes (blaTEM þ blaSHV). Multiplex PCR results showed the prevalence of blaFOX (52.6%) and blaCMY (21.1%) among AmpC genes detected. Remarkably, both types of b-lactamases were simultaneously detected in 76.5% of the phenotypically positive isolates. The present study suggests that the currency notes could act as a potential carrier of ESBLs and AmpC b-lactamaseproducing bacteria.

Keywords

ESBLs, AmpC β-lactamase, Iraqi Currency notes, Mosul (Iraq), Molecular characterization

Cover Page Footnote

We acknowledging Biophysics Dept. and Biology Dept. /College of Science/ Mosul University for their support.

RESEARCH PAPER

Molecular Characterization of ESBLs and AmpC β-lactamases in Bacteria Isolated From Currency Notes Circulating in Mosul City, Iraq

Mahmood Z. Al-Hasso [a,*], Shakir G. Gergees [b], Zahraa K. Mohialdeen [b]

[a] Biophysics Dept., College of Science, Mosul University, Mosul, Iraq
[b] Biology Dept., College of Science, Mosul University, Mosul, Iraq

Abstract

The Iraqi currency notes circulating in Mosul city were evaluated for the occurrence of ESBLs and AmpC β-lactamase-producing bacteria. Four hundred and twenty-two Gram-positive and negative bacterial isolates with different antimicrobial resistance profiles were recovered from 250 samples collected during the period from April to July 2021, among which 150 isolates (35.5%) were multi-drug resistant (MDR). The study found that 16.4% and 14.8% of Gram negative isolates were positive for ESBLs and AmpC phenotypic detection tests, respectively. Interestingly, 6.6% of the isolates were simultaneously positive for both tests. Molecular characterization was carried out using PCR technique to determine the prevalent ESBLs gene\s. The results revealed that bla_{TEM} and $bla_{CTX\text{-}M}$ were the most prevalent ESBLs genes (28.6% and 26.2%, respectively). Moreover, 11.9% of the tested isolates appeared to have dual genes ($bla_{TEM} + bla_{SHV}$). Multiplex PCR results showed the prevalence of bla_{FOX} (52.6%) and bla_{CMY} (21.1%) among AmpC genes detected. Remarkably, both types of β-lactamases were simultaneously detected in 76.5% of the phenotypically positive isolates. The present study suggests that the currency notes could act as a potential carrier of ESBLs and AmpC β-lactamase-producing bacteria.

Keywords: Bacterial contamination, Extended-spectrum β-lactamase, AmpC β-lactamase, Iraqi banknotes

1. Introduction

Currency notes (banknotes), issued by the financial authorities and distributed within communities, are undoubtedly the most public means for trading services and goods between people worldwide and mostly in third world countries [1,2]. They are one of the most regularly transferred objects from person to person during businesses and are being circulated by an enormous number of individuals with variable personal and environmental hygienic circumstances [3]. Inappropriate handling practices such as simultaneous handling of currency notes and foodstuffs, unhygienic behaviors (inappropriate hand washing, coughing, or sneezing on hands), wetting fingers with saliva during money counting, and storing currency notes in or on dirty places have led to the widespread contamination of currency notes with different species of microbes [4].

Currency's role in transmitting microbial infection is not a new concept. In England 1665, when plaque killed more than 60 000 individuals, the coins were presumed to be one of the plague dissemination factors [3]. Conducted studies, at that time, dealing with coins and notes contamination have been rather limited and insufficient. Nevertheless, after the incidence of some bacterial outbreaks, such reports had increased concern and shown that

Abbreviations: IQD, Iraqi dinar; CoNS, Coagulase- negative staphylococcus; ESBLs, Extended-spectrum β-lactamase; AST, Antimicrobial susceptibility testing; MDR, Multi-drug resistant strains; PBA, Phenylboronic acid,

Received 6 March 2022; revised 5 May 2022; accepted 9 May 2022.
Available online 1 August 2022

* Corresponding author.
E-mail addresses: mahmoodalhasso@uomosul.edu.iq, mahmoodalhasso@gmail.com (M.Z. Al-Hasso).

https://doi.org/10.33640/2405-609X.3239

currency can serve as a perfect site for the proliferation of microorganisms [5].

Banknotes might represent a potential risk to communities by acting as a tool in the transmission of opportunistic, pathogenic bacteria, and communicable diseases [6–9]. Several reports from many countries have shown that viable pathogenic organisms including bacteria, fungi, viruses, and even nematodes and protozoa can be isolated from currency notes [3,5–10]. Moreover, contaminated currency notes have been established as a possible reservoir for bacterial species that cause foodborne diseases such as food poisoning, shigellosis, typhoid fever, and gastroenteritis [4].

The detection of antimicrobial resistance in bacteria isolated from currency notes implies that currency notes may not only act as a vehicle for transporting pathogenic microorganisms but also as a reservoir for resistance determinants, like β-lactamase genes, which could be transferred from resistant strains to susceptible ones through horizontal gene transfer (HGT) worsening drug-resistance burden [4,8,10]. Therefore, surveillance and detection studies on this aspect are necessary to determine the occurrence and prevalence of such strains and genes on currency notes that might represent an extra source for the dissemination of multi-drug resistant (MDR) strains in the community.

Studies conducted in developed countries have produced a high level of attention on the possible role might the banknotes play as a vehicle for pathogen transmission and resulted in the formation of national agencies responsible for handling and withdrawal of damaged currency [7,10]. On the other hand, there is a significant shortage in the scientific data concerning bacterial currency contamination and β-lactamase profile in developing countries, including Iraq, where most of the available information is inadequate. Unfortunately, this might have contributed to the absence of general strategies dealing with circulation and proper handling of currency in these countries.

β-lactamases are considered the most common mechanism used by Gram-negative bacteria to resist β-lactam antimicrobials [11]. Extended-spectrum β-lactamases (ESBLs) represent an important group of β-lactamases belonging to class A of Ambler's molecular classification and subgroup 2be of Bush's functional classification [11,12]. ESBL-producing bacteria are characterized by their ability to resist a wide range of β-lactams i.e. penicillins, cephalosporins (including the third generation), and monobactams. They cannot hydrolyze cephamycins and are affected by clavulanate and other β-lactamase inhibitors. AmpC β-lactamases, on the other hand, are characterized by their ability to hydrolyze a wide diversity of β-lactam drugs except for cefepime and carbapenems. They can resist cephamycins (cefoxitin and cefotetan) in addition to penicillins and cephalosporins, and they are poorly affected by clavulanic acid [11–13]. They belong to group 3 of functional classification and class C of Ambler's molecular scheme [11,12]. β-lactamase-producing bacteria, usually associated with drug-resistant infections, are increasingly reported from different parts of the world as a global threat facing the treatment of bacterial infections [14–16].

Since no studies have been carried out, as far as we know, to evaluate the potential role of the Iraqi Dinar (IQD) in the transmission of MDR bacteria and detect the occurrence of ESBLs and AmpC β-lactamases in them, the present study aimed to analyze the role of the IQD as a potential carrier for the dissemination of MDR bacteria mainly β-lactamase producing strains in Mosul city. We hope that our results will assist in elevating health awareness during currency circulation in the community and participate in controlling the dissemination of resistant bacteria within the city.

2. Materials and methods

2.1. Collection of samples

From April to July 2021, a total of 250 samples of the Iraqi currency notes in five denominations (250, 500, 1000, 5000, and 10 000 Iraqi Dinar IQD) and under different physical conditions (dry, moist, dirty, acceptable, etc.) were collected from different places in Mosul city, Iraq. Sample collection was conducted randomly from different locations including supermarkets, hospitals, grocery stores, gas stations, restaurants, and banks. Additionally, ten new notes (for each denomination) obtained from Al-Rafidain Bank were included as a negative control. The banknotes were collected in sterile plastic bags and immediately transported to the laboratory for processing.

2.2. Isolation and identification of bacteria

Aseptically, each banknote was placed into sterile tubes containing 10 ml of Tryptone Soya Broth (TSB) (Himedia Co., India). The tubes were shaken for 30 min to displace the bacteria into the broth (Fig. 1). Subsequently, the banknotes were aseptically removed from the tubes with forceps. One hundred microliters of each sample were inoculated onto MacConkey agar, mannitol salt agar, and nutrient

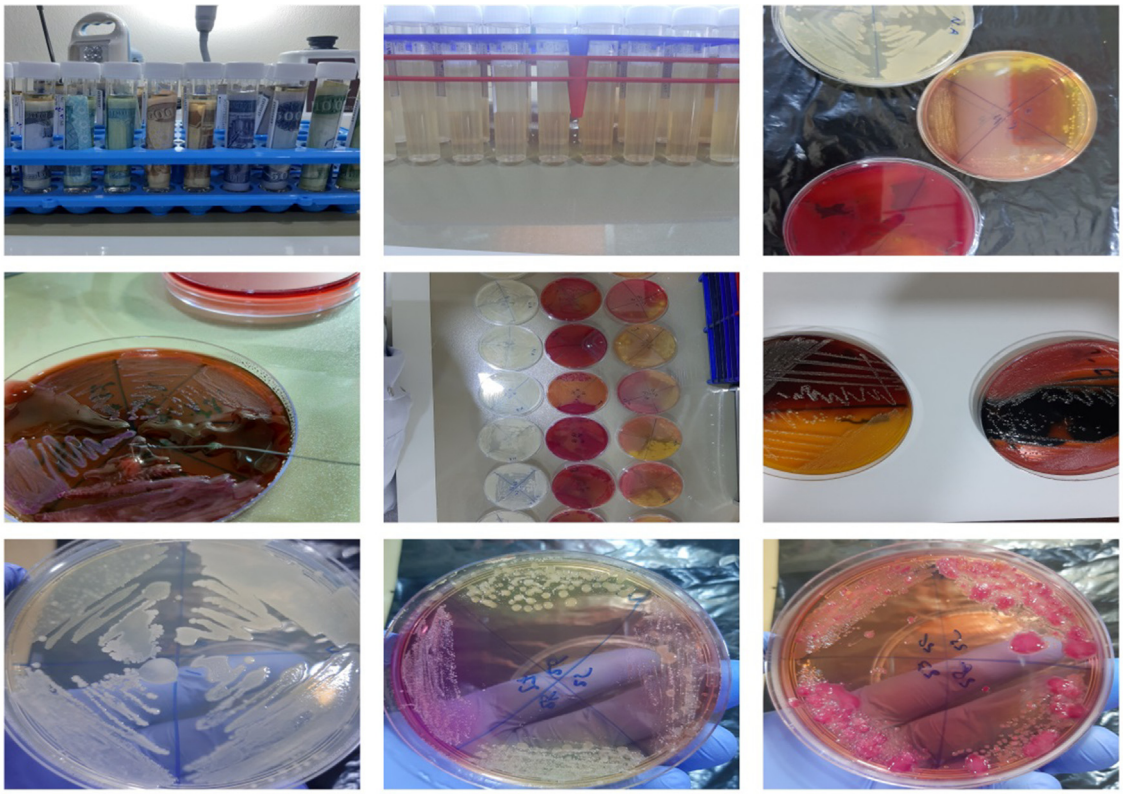

Fig. 1. Different stages of bacterial isolation from Iraqi currency notes.

agar (Himedia Co., India) plates and incubated aerobically for 24–48 h at 37 °C [1,10]. Small portions of the isolated bacterial colonies were streaked on corresponding media and incubated overnight at 37 °C to obtain pure cultures. Bacterial isolates were traditionally characterized according to their cellular and cultural morphology, Gram staining, and biochemical characters [17,18].

2.3. Antimicrobial susceptibility testing

Antimicrobial resistance profiles were determined using the Kirby-Bauer disk diffusion method and Mueller-Hinton agar (Himedia Co., India) as described and recommended by CLSI [19]. The tested antimicrobials were as follows: amikacin (AK) 30 μg, amoxicillin + clavulanic acid (AMC) 30 μg, ampicillin (AMP) 10 μg, cefotaxime (CTX) 30 μg, cephalexin (CN) 30 μg, chloramphenicol (C) 30 μg, ceftazidime (CAZ) 30 μg, ciprofloxacin (CIP) 5 μg, erythromycin (E) 15 μg, gentamicin (GEN) 10 μg, penicillin (P) 10 IU, tetracycline (TE) 30 μg, ceftriaxone (CRO) 30 μg, trimethoprim + sulfamethoxazole (SXT) 1.25 + 23.75 μg, and azithromycin (AZM) 15 μg (antimicrobial disks were supplied by Bioanalyse Co. Turkey). Standard strains *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *E. coli* ATCC 25922 were used as quality controls for the test. After incubation at 35 °C for 18 h and measuring the inhibition zones, the results were interpreted according to the standard criteria of CLSI [19]. Multiple Drug Resistant (MDR) strains were identified as those resistant to three or more antimicrobial groups [19].

2.4. Phenotypic detection of extended spectrum β-lactamases (ESBLs)

The occurrence of ESBLs in the recovered isolates (Gram-negative only) was detected using the CLSI method [19]. Briefly, isolates which shown resistance to third-generation cephalosporins with the following inhibition zone diameters: cefotaxime ≤27 mm, ceftazidime ≤22 mm, and ceftriaxone ≤25 mm were considered as potential producers of ESBLs. The results were confirmed by applying ceftazidime (30 μg) and cefotaxime (30 μg) disks alone and in combination with clavulanic acid (10 μg) to inoculated Mueller-Hinton agar plates. Plates were overnight incubated at 35 °C, an increase of ≥5 mm in the inhibition zone diameter for either antimicrobial tested in combination with

clavulanate vs the inhibition zone diameter of the antimicrobial alone was taken as a positive result for the occurrence of ESBLs. *E. coli* ATCC 25922 was used as quality control for the test [19].

2.5. Phenotypic detection of AmpC β-lactamase

Gram-negative isolates with cefoxitin inhibition zone diameter of ≤18 mm were considered as potential producers of AmpC β-lactamase [13,20,21]. These results were confirmed by the phenylboronic acid (PBA) disk confirmation test [13,20]. Briefly, Mueller-Hinton agar plates were inoculated with the tested isolates followed by the application of two disks of cefoxitin (30 μg) for each isolate one of them with 400 μg of phenylboronic acid and overnight incubated at 35 °C. An increase of ≥5 mm in the inhibition zone diameter in the presence of PBA compared with the zone diameter of cefoxitin disk alone was a confirmed result for the occurrence of AmpC enzymes [13,20–22].

2.6. Molecular characterization of extended-spectrum β-lactamase (ESBLs) genes

All isolates with positive results for phenotypic ESBLs detection test were molecularly investigated for the occurrence of ESBLs genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}) using conventional PCR technique. Pure isolates were aerobically cultured with shaking at 200 rpm in Trypton Soya Broth for 24 h at 30 C. The cells were harvested by centrifugation (12500 g, 10 min). Preparation of DNA templet was performed according to Presto Mini gDNA Bacteria Kit from Geneaid Biotech Ltd. (www.geneaid.com). Molecular detection was conducted using 96-well thermal cycler Optimus 96G, England. The PCR reactions were carried out in 25 μl final volume (2.5 μl of 10_X PCR buffer, 200 μM of deoxynucleotide triphosphates (dNTPs), 0.6 mg/μl $MgCl_2$, 0.5 units of Taq polymerase, 10 pmol of each primer, and 5 μl of sample DNA). Table (1) summarizes the amplicon sizes, primer sequences, and thermocycling conditions [8,23–26]. PCR products were examined by 1.5% agarose gel electrophoresis in 0.5x (TBE) buffer. Gene bands were visualized and confirmed with the aid of a UV transilluminator.

2.7. Molecular characterization of AmpC β-lactamase genes

A single colony of each isolate with a positive result of phenotypic AmpC detection test was inoculated into Tryptone Soya Broth (TSB) and aerobically incubated with shaking at 37 C for 20 h. Cells from 1.5 ml of culture were pelleted by centrifugation (12500 g, 5 min). Afterward, the bacterial cells were resuspended in 500 μl of distilled water. Cell lysis was conducted by heating for 10 min at 95 C and cellular debris was discarded. Two microliters of the supernatant were used as the DNA template source for amplification [21,26]. Multiplex PCR was used for gene amplification of AmpC genes using the method described by Pe'rez-Pérez and Hanson, [26]. PCR was performed using a DNA thermocycler Optimus 96G, England. Each reaction (50 μl final volume) contained 20 mM Tris–HCl, 1.25 U Taq DNA polymerase, 0.2 mM of each deoxynucleoside triphosphate, 50 mM KCl, 0.6 μM primers, and 1.5 mM $MgCl_2$. Two microliters of the template DNA were added to 48 μl of the master mix and coated with mineral oil. Cycling conditions and the primers used for PCR amplification are listed in Table 1. PCR products were examined by 1.5% agarose gel electrophoresis and visualized by UV transillumination.

2.8. Statistical analysis

Data was statistically analyzed using chi-square and Fisher's exact tests (SPSS software ver. 21) for the comparison of categorical data, p-values of ≤0.05 was considered as significant. Additionally, percentages were used for the expression of antimicrobial resistance profiles and phenotypic β-lactamase detection results.

3. Results and discussion

3.1. Bacterial contamination of currency notes

Our results showed that 100% of the Iraqi currency notes tested were contaminated with different bacterial species (Table 2, Fig. 1). This percentage was high in comparison with similar researches conducted in Saudi Arabia [27], Iran [6], Croatia [28], Cameroon [7], Pakistan [2], and Poland [10] reporting 72%, 77.7%, 78%, 94%, 97%, and 98%, respectively. However, our results were equal to the 100% rate of contaminated currency notes recovered in Erbil city, Iraq [29], Ethiopia [5], Sudan [8], India [9], Algeria [1], and Nigeria [4]. On the other hand, no bacteria were isolated from the new notes obtained from Al-Rafidain Bank in Mosul city which were considered as negative control and this finding was consistent with the results of other studies [8,10,29]. This might be associated with the fact that these notes had not been exchanged between people or exposed to usage and handling. Moreover, our results indicated that banknotes of the lower denominations were

Table 1. Primers used for the detection of ESBLs and AmpC genes.

β-lactamase gene	Primer sequences[a]	Amplicon size (bp)	Thermocycling conditions	Ref.
bla_{TEM}	5′-CATTTCCGTGTCGCCCTTATTC-3′ 5′-CGTTCATCCATAGTTGCCTGAC-3′	800	94 C, 10 min; 30 cycles of 94	Richter et al., 2020 [23]; Dallenne et al., 2010 [24]
bla_{SHV}	5′-AGCCGCTTGAGCAAATTAAAC-3′ 5′-ATCCCGCAGATAAATCACCAC-3′	713	C, 40 s, 58 C, 40 s, 72 C, 1 min; 72 C, 7min	
$bla_{CTX\text{-}M}$	5′-CGCTTTGCGATGTGCAG-3′ 5′- ACCGCGATATCGTTGGT-3′	550	94 C, 5 min; 35 cycles of 95 C, 30 s, 60 C, 30 s, 72 C, 30 s; 72 C, 5min	Yazdansetad et al., 2019 [25]
bla_{FOX}	'5-AAC ATG GGG TAT CAG GGA GAT G-3′ '5-CAA AGC GCG TAA CCG GAT TGG-3′	190	94 C for 3 min. 25 cycles of 94C for	Al-Hady and Adel, 2015 [21];
bla_{CMY}	'5-GCT GCT CAA GGA GCA CAG GAT-3′ '5-CAC ATT GAC ATA GGT GTG GTG C-3′	520	30s, 64 C for 30 s, 72 C for 1 min.	Perez-Perez and Hanson, 2002 [26]
bla_{ACC}	'5-AAC AGC CTC AGC AGC CGG TTA-3′ '5-TTC GCC GCA ATC ATC CCT AGC-3′	346	72 C for 7 min.	

[a] The first and second primers for each gene are forward and reverse primers, respectively.

significantly more contaminated in comparison with the higher denomination ones, as 298/422 isolates (70.6%) were isolated from low denomination notes (250 + 500+1000 IQD, collectively) while the rest 124 isolates (29.4%) were isolated from high denomination notes (5000 + 10 000 IQD, collectively) as shown in Table 2. This finding was consistent with other studies [1,4,8,10] since the lower denomination notes are more frequently colonized than those of the higher ones, and this was predictable as the circulation rate of lower denomination notes is usually higher. We also noticed that contamination of banknotes was related to the physical state of the notes (data not shown), as contamination was higher in the dirty banknotes than in the clean ones showing a direct correlation between the physical form of the notes and the bacterial contamination. This was in agreement with several studies which had reported similar results [7,8,10].

The high rates of contamination probably have resulted from the continuous usage of the banknotes by a large number of people with different hygienic levels during circulation at various exchanging points. This practice is mostly public and prevailing among almost all businesses in Mosul city due to the absence of electronic transactions which makes the use of banknotes very common, with consequent health threats. Additionally, notes are typically made of linen, cotton, or other types of fibers that can hold humidity and

Table 2. Bacterial species isolated in the study.

Bacterial species	Denominations (used)[a]:					Total (250)
	10 000 IQD (n = 50)	5000 IQD (n = 50)	1000 IQD (n = 50)	500 IQD (n = 50)	250 IQD (n = 50)	
Staphylococcus aureus	4	5	6	6	11	32 (7.6)
Coagulase-negative Staphylococcus (CoNS)	12	15	19	15	20	81 (19.2)
Bacillus spp.	7	6	11	13	16	53 (12.6)
Pseudomonas aeruginosa	9	11	12	11	15	58 (13.7)
Escherichia coli	11	14	16	17	21	79 (18.7)
Klebsiella spp.	9	12	13	16	19	69 (16.4)
Salmonella spp.	0	1	2	2	2	7 (1.7)
Shigella spp.	0	1	1	1	1	4 (0.9)
Enterobacter cloacae	1	1	4	1	3	10 (2.4)
Citrobacter freundii	0	0	2	2	4	8 (1.9)
Proteus mirabilis	2	3	5	3	4	17 (4)
Serratia marcescens	0	0	1	1	2	4 (0.9)
Total	55	69	92	88	118	422[b]

[a] No bacteria were isolated from the new currency notes (50 samples) included in the study.
[b] Significant difference between bacterial number isolated from low denomination notes (250 + 500+1000 IQD) and high denomination notes (5000 + 10 000 IQD) at p- value ≤ 0.05.

provide a productive area for bacteria to adhere, persist, and even multiply. For instance, pathogenic bacteria like *Escherichia coli*, *Staphylococcus* spp., and *Salmonella* spp. were reported to persist on cotton-based currency notes for more than 20 days [1,4].

In the present study, 422 bacterial isolates were recovered from 250 samples of the Iraqi banknotes which included 166 (39.3%) Gram-positive and 256 (60.7%) Gram-negative bacteria. Moreover, 159 (63.6%) notes showed growth of more than one bacterial species. The study isolated 81 (19.2%) Coagulase-negative *Staphylococcus*, 79 (18.7%) *E. coli*, 69 (16.4%) *Klebsiella* spp., 58 (13.7%) *P.aeruginosa*, 53 (12.6%) *Bacillus* spp., 32 (7.6%) *S. aureus*, 17 (4%) *Proteus mirabilis*, 10 (2.4%) *Enterobacter cloacae*, 8 (1.9%) *Citrobacter freundii,* and 4 (0.9%) *S.marcescens*. Interestingly, seven isolates of *Salmonella* spp. (1.7%) and four isolates of *Shigella* spp. (0.9%) were also recovered (Table 2). Some of these results are a little higher than that reported by Ejaz et al. [2], (i.e. 20.6% for Gram-positive bacteria, 14.5% for CoNS, 13.7% for *E.coli*, 11.5% for *Pseudomonas* spp., 0.8% for each of *Salmonella* spp. and *Shigella* spp), and others are lower (i.e. 79.4% for Gram-negative bacteria, 26% for *Klebsiella* spp., 5.3% for each of *Enterobacter* spp. and *Citrobacter* spp.). On the other hand, Kalita et al. [10], reported higher isolation rates for *Staphylococcus* spp. (43.6%), and *Bacillus* spp. (14%), and lower rates for *Pseudomonas* spp. (6%), and *E.coli* (1.3%) in comparison to our results. Gram-negative bacterial isolates belonging to *Enterobacteriaceae* and *P.aeruginosa* were prevailing in comparison to Gram-positive bacteria. *E.coli* was the most frequently isolated Gram-negative species; on the other hand, CoNS had the highest isolation rate among Gram-positive bacteria. Bacterial species recovered in the present study were very analogous to those reported in previous researches [1–10,27–35]. However, reports from Saudi Arabia [27,35], Ghana [33], Pakistan [32], Cameroon [7], Erbil city, Iraq [29], and India [34] indicated Gram-positive bacteria as the predominant microorganisms isolated from the contaminated currency notes. This variation in bacterial isolation rates may be attributed to the local differences in microbial profiles and hygienic levels and habits of the communities [4,8,10]. Furthermore, the prevalence of *Enterobacteriaceae*, in particular *Klebsiella* spp., *Escherichia coli,* and *Salmonella* spp. can be taken as an indicator of obvious fecal contamination of currency notes and subsequently reduced level of public health awareness (both personal and environmental) which might have a potential reflection and consequences for both nosocomial and community-acquired infections [1,8].

Several behavioral practices may participate in the contamination of banknotes, i.e. improper hand washing, keeping banknotes underbody surfaces, using saliva to wet fingers when counting money, sneezing or coughing on hands, and storing money in dirty places during business [4,8,9]. Furthermore, paper banknotes offer a large surface that can bacteria adhere to and persist on for extended periods, especially if we take into account that the surface of the notes is usually irregular which facilitates bacterial adherence and persistence. Bacterial pathogens from the nose, skin, throat, or fecal origin are the most expected to be disseminated by the hands, emphasizing the urgent need for effective hand sanitary practices to be applied [4,9]. Methicillin-Resistant *S. aureus* (MRSA), *E. coli, Shigella* spp., and *Salmonella* spp. have obvious importance in food-borne infections especially when they correlated with the worldwide emergence of multidrug-resistant bacteria [9]. Noteworthy, all the recovered isolates are opportunistic or strictly pathogenic which makes them a serious threat among a population, mostly immunocompromised individuals [2,6,9].

3.2. Antimicrobial resistance profile

Bacterial isolates varied in their antimicrobial resistance profiles. In general, 91% of the isolates were resistant to ampicillin followed by amoxicillin-clavulanic acid (78.4%) and cephalexin (70.4%). On the other hand, amikacin, chloramphenicol, and ciprofloxacin were the most effective agents used in the study against the isolated bacteria with resistance rates of 11.1%, 13.5%, and 14.5%, respectively (Table 3). Furthermore, 81.3% of Gram-positive isolates were resistant to penicillin G and 59.6% were resistant to erythromycin. Some of these findings are consistent with the results of former studies like Djoudi et al. [1], who reported the resistance rates: 91.7%, 25%, 16.7%, and 14.7% for penicillin, ciprofloxacin, amikacin, and chloramphenicol, respectively. Also, Akoachere et al. [7] reported a 100% resistance rate to penicillin and 92.7% to ampicillin, while chloramphenicol, norfloxacin, and gentamicin were found to be the most effective agents used against the isolated bacteria. As well, the effectiveness of ciprofloxacin, amikacin, and chloramphenicol was indicated in further studies which were in agreement with our results [2,3,6–8]. Furthermore, our results found that 150 isolates (35.5%) were multi-drug resistant (MDR) strains. *P. aeruginosa*, *Bacillus* spp., *E.coli*, and *Klebsiella* spp. isolates were the most prevalent MDR strains in comparison to others (Table 4). This

Table 3. Antimicrobial resistance profiles of the isolated bacteria.

Bacterial species	No. (%) of resistant isolates to antimicrobials tested[a]														
	CTX	CAZ	C	AMP	AK	CRO	AMC	CN	CIP	TET	SXT	GEN	P[b]	E[b]	AZM[b]
S. aureus (n = 32)	13	9	0	28	0	6	24	21	0	8	4	6	25	20	19
CoNS (n = 81)	29	21	5	65	0	9	64	49	0	27	13	16	72	58	48
Bacillus spp. (n = 53)	19	18	1	47	0	12	44	39	0	23	15	20	38	21	17
P. aeruginosa (n = 58)	26	22	15	58	21	29	41	41	22	38	37	29	NT	NT	NT
E.coli (n = 79)	37	24	17	75	15	33	59	54	15	54	39	31	NT	NT	NT
Klebsiella spp. (n = 69)	39	29	18	69	11	40	65	61	23	60	44	37	NT	NT	NT
Salmonella spp. (n = 7)	3	1	1	6	0	3	5	6	1	2	4	3	NT	NT	NT
Shigella spp. (n = 4)	2	1	0	4	0	2	4	4	0	1	2	2	NT	NT	NT
E.cloacae (n = 10)	2	2	0	8	0	2	8	8	0	4	4	2	NT	NT	NT
C. freundii (n = 8)	3	2	0	7	0	3	7	6	0	4	3	3	NT	NT	NT
P.mirabilis (n = 17)	2	0	0	13	0	6	7	5	0	3	2	1	NT	NT	NT
S.marcescens (n = 4)	2	0	0	4	0	2	3	3	0	1	2	1	NT	NT	NT
Total (n = 422)	177 (41.9)	129 (30.6)	57 (13.5)	384 (91)	47 (11.1)	147 (34.8)	331 (78.4)	297 (70.4)	61 (14.5)	225 (53.3)	169 (40)	151 (35.8)	135 (81.3)	99 (59.6)	84 (50.6)

[a] CXT: cefotaxime, CAZ: ceftazidime, C: chloramphenicol, AMP: ampicillin, AK: amikacin, CRO: ceftriaxone, AMC: amoxicillin-clavulanic acid,CN: cephalexin, CIP: ciprofloxacin, TET: tetracycline, SXT: sulfamethoxazole, GEN: gentamicin, P: penicillin G, E: erythromycin, AZM: azithromycin.

[b] Tested for Gram positive isolates only (n = 166). NT: not tested.

indicates the occurrence of MDR strains in high percentage on the circulating currency notes in the city which might represent a serious threat to public health that calls for rapid actions to be taken to control their dissemination in the community [1,4].

3.3. Molecular characterization of ESBLs and AmpC β-lactamase genes

One of the main aims of the current study was to detect the occurrence of β-lactamases in the isolated bacteria from the Iraqi currency notes and to determine their types. Our results indicated that *out of 256 Gram-negative bacterial isolates tested,* 42 isolates (16.4%) *were ESBLs producers, Klebsiella* spp. *isolates gave the highest rate followed by P. aeruginosa* and *E.coli.* As for *AmpC enzymes,* 38/256 isolates (14.8%) *gave positive results for the detection test, P. aeruginosa* was the most producing species in comparison to others (Table 5). These results are close to the findings of Usman et al. [4], who reported a detection rate of (22.1%) for ESBLs production in Gram-negative isolates recovered from banknotes in Nigeria, *the enzymes were predominantly detected in* E.coli, *Klebsiella pneumoniae, and P. aeruginosa isolates as well* [4]. Also, Ahmed and Mashat [35] found in their study which was conducted in Saudi Arabia that (13.4%) of the isolated bacteria were ESBLs positive with E.coli being the prevalent species. Noteworthy, we couldn't find references that detected the occurrence of AmpC enzymes phenotypically or genotypically in bacteria isolated from currency notes, so we couldn't compare our results with others. Interestingly, 6.6% of the isolates were positive for both ESBLs and AmpC detection tests, *P. aeruginosa* isolates were the most prevalent strains followed by *Klebsiella* spp. and *E.coli*. Additionally, two isolates of *Salmonella* spp. were positive for the AmpC detection test, while only one isolate was an ESBLs producer (Table 5).

Table 4. Multi drug resistant strains isolated from currency notes.

Bacterial species	Multi- Drug Resistant strains No. (%)
S. aureus (n = 32)	6
CoNS (n = 81)	16
Bacillus spp. (n = 53)	20
P. aeruginosa (n = 58)	28
E.coli (n = 79)	31
Klebsiella spp. (n = 69)	36
Salmonella spp. (n = 7)	3
Shigella spp. (n = 4)	2
E.cloacae (n = 10)	2
C. freundii (n = 8)	3
P.mirabilis (n = 17)	2
S.marcescens (n = 4)	1
Total (n = 422)	**150 (35.5)**

Table 5. Phenotypic detection tests of ESBLs and AmpC β-lactamases.

Bacterial species	ESBLs positive isolates No. (%)	AmpC positive isolates No. (%)	Isolates positive for both No. (%)
P. aeruginosa (n = 58)	11	14	7
E.coli (n = 79)	13	9	4
Klebsiella spp. (n = 69)	15	11	6
Salmonella spp. (n = 7)	1	2	0
Shigella spp. (n = 4)	0	1	0
E.cloacae (n = 10)	0	1	0
C. freundii (n = 8)	1	0	0
P.mirabilis (n = 17)	1	0	0
S.marcescens (n = 4)	0	0	0
Total (n = 256)	42 (16.4)	38 (14.8)	17 (6.6)

Molecular characterization results showed that 90.5% of the isolates were *positive for PCR detection of ESBLs genes. The most prevalent genes detected were* bla_{TEM} (28.6%) and $bla_{CTX\text{-}M}$ *(26.2%). Interestingly, 5/42 isolates (11.9%) had both genes* ($bla_{TEM} + bla_{SHV}$) *as shown in* Table 6 *and* Fig. 2. *These findings are in*

Table 6. Distribution of β-lactamase genes among bacterial isolates.

Distribution of ESBLs genes							
β-lactamase gene	*P. aeruginosa*	*E.coli*	*Klebsiella* spp.	*Salmonella* spp.	*C. freundii*	*P.mirabilis*	Total genes detected No. (%)
bla_{TEM}	3/11	3/13	5/15	1/1			12/42 (28.6)
bla_{SHV}	3/11	2/13	3/15		1/1	1/1	10/42 (23.8)
$bla_{CTX\text{-}M}$	2/11	5/13	4/15				11/42 (26.2)
$bla_{TEM}bla_{SHV}$	1/11	2/13	2/15				5/42 (11.9)
PCR negative	2/11	1/13	1/15				4/42 (9.5)
Distribution of AmpC genes							
β-lactamase gene	*P. aeruginosa*	*E.coli*	*Klebsiella* spp.	*Salmonella* spp.	*Shigella* spp.	*E.cloacae*	Total genes detected No. (%)
bla_{FOX}	7/14	4/9	5/11	2/2	1/1	1/1	20/38 (52.6)
bla_{CMY}	3/14	2/9	3/11				8/38 (21.1)
bla_{ACC}	1/14	1/9	2/11				4/38 (10.5)
$bla_{CMY} + bla_{ACC}$	1/14						1/38 (2.6)
PCR negative	2/14	2/9	1/11				5/38 (13.2)
Distribution of dual genes (AmpC + ESBLs)							
β-lactamase gene	*P. aeruginosa*	*E.coli*	*Klebsiella* spp.				Total genes detected No. (%)
$bla_{CTX\text{-}M} + bla_{FOX}$	2/7	1/4	2/6				5/17 (29.4)
$bla_{SHV} + bla_{FOX}$	3/7	2/4	3/6				8/17 (47.1)
PCR negative	2/7	1/4	1/6				4/17 (23.5)

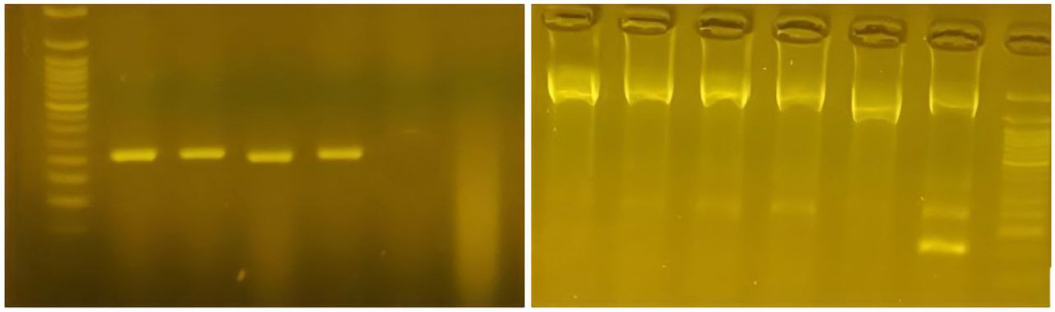

Fig. 2. PCR results for ESBLs genes (left) showing CTX-M gene from Klebsiella isolates (550 bp), and AmpC genes (right) showing ACC + CMY genes (346bp, 520 bp) in a single isolate of P.aeruginosa.

agreement with the results of Alfadil et al., [8] *who detected the genes* $bla_{\text{CTX-M}}$ *and* bla_{TEM} *in Gram-negative bacteria isolated from Sudanese banknotes, one of the isolates had both genes at the same time.* Furthermore, the present study indicated the occurrence of AmpC genes in 86.8% of the positive isolates for the phenotypic detection test. The genes bla_{FOX} and bla_{CMY} were the most prevalent genes detected (52.6% and 21.1%, respectively). *Interestingly,* an isolate of *P. aeruginosa* had two AmpC genes ($bla_{\text{CMY}} + bla_{\text{ACC}}$) which gave it more capabilities to resist β-lactams (Table 6, Fig. 2). Moreover, our results revealed the occurrence of both types of genes (ESBLs + AmpC) in (76.5%) of the isolates which were phenotypically positive for both enzymes, as (8) isolates had the two genes $bla_{\text{SHV}} + bla_{\text{FOX}}$ and (5) isolates had the two genes $bla_{\text{CTX-M}} + bla_{\text{FOX}}$ (Table 6), a similar result was reported by Upadhyay et al. [36] in *P.aeruginosa* clinical isolates. The high detection rates of ESBL and AmpC enzymes among the local strains may be due to the extensive use of β-lactam antimicrobials which are regularly prescribed for almost all types of nosocomial and community-acquired diseases and might contribute to the subsequent selection of multi-resistant strains. Consequently, this practice had led to the selection and dissemination of β-lactamase producing isolates with their potential threat to the therapeutic effectiveness of most of these antimicrobials. Infection by MDR strains can lead to treatment failure, due to limiting therapeutic options, and participate in hospitalization costs increase [3,4,37,38]. Moreover, it has been reported that different types of β-lactamase genes (as well as other resistance genes) from bacteria occurring on currency notes can be plasmid-, or integron-mediated and can rapidly disseminate among other MDR species including clinical strains [39–42]. For instance, a recent report from Algeria indicated the occurrence of $bla_{\text{CTX-M}}$ genes in Gram-negative strains isolated from local banknotes. Noteworthy, this gene family is known to be mediated by conjugated and highly transferrable plasmids [3].

To the best of our knowledge, this is the first study investigating the antimicrobial resistance profile and β-lactamase occurrence (ESBLs and AmpC) of isolated bacteria from currency notes in Mosul city, Iraq, and evaluating their role in the transmission of pathogenic and opportunistic bacteria. The detection of bacterial contamination with MDR determinants in currency notes is of great importance since it indicates what banknotes could represent to public health, especially to immunocompromised patients. This is worrisome, particularly in this period of a worldwide pandemic of SARS-CoV-2. Preventive measures and strategies directed at breaking the chain of transmission of bacterial infections and reducing the contamination level of banknotes are recommended. Such strategies could include public education on self and environmental sanitation and appropriate handling of banknotes, the usage of plastic currency, which can be easily washed, using electronic ATM cards, regular removal and withdrawal of damaged notes by authorities. Finally, periodical determination of antimicrobial resistance patterns in environmental microorganisms, particularly bacteria, is highly suggested. Inclusively, the present study demonstrated that Iraqi currency notes could play a significant role in transmitting antimicrobial-resistant bacteria and resistance genes as well, with the potential risk for dissemination among other species by horizontal gene transfer mechanisms.

4. Conclusion

The present study showed that Iraqi banknotes (IQD) circulating in Mosul city are highly contaminated with different kinds of antimicrobial-resistant bacteria and could serve as a carrier in transmitting serious infections and spreading antimicrobial-resistant strains and genes, particularly ESBLs and AmpC genes. Improper behavioral and hygienic practices when dealing with or handling money should be discouraged. Damaged and mutilated notes should be regularly withdrawn from circulation. Educational and awareness practices on the appropriate handling of banknotes should be presented publicly. Finally and most essentially, the usage of electronic transactions as alternatives for paper notes in business and trading should be encouraged.

4.1. Limitations and suggestions

The study had the limitation of not detecting the occurrence of other types of β-lactamase in the recovered isolates. We suggest conducting further studies investigating the prevalence of carbapenemase in the bacteria isolated from the Iraqi currency notes.

Conflict of interest

None.

Acknowledgment

The present work was supported by the department of Biophysics and the department of Biology, College of Science \ University of Mosul.

References

[1] L.N. Djouadi, N. Guezlane-Tebibel, K. Mansouri, H. Boumerdassi, Karim Arab, Marie-Laure Fardeau, and Farida Nateche, Multidrug-resistant opportunistic and pathogenic bacteria contaminate Algerian banknotes currency, Polish J Microbiol. 69 (2020) 491–501.

[2] E. Hasan, A. Javeed, M. Zubair, Bacterial contamination of Pakistani currency notes from hospital and community sources, Pak. J Med Sci. 34 (2018) 1225–1230.

[3] E. Bendjama, L. Loucif, W. Chelaghma, C. Attal, F.Z. Bellakh, R. Benaldjia, I. Kahlata, A. Meddoura, J.-M. Rolain, First detection of an OXA-48-producing *Enterobacter cloacae* isolate from currency coins in Algeria, J Glob Antimicrob Resist. 23 (2020) 162–166.

[4] M. Usman, J. Sani, A. Ibrahim, A. Olowo-okere, Microbial contamination of Naira notes circulating in Bauchi metropolis: prevalence, microbial load and detection of extended spectrum beta-lactamase producing Gram-negative bacteria, Afr J Clin Exper Microbiol. 22 (2021) 244–251.

[5] G. Girma, T. Ketema, K. Bacha, Microbial load and safety of paper currencies from some food vendors in Jimma Town, Southwest Ethiopia, BMC Res Notes. 7 (2014) 1–8.

[6] F. Firoozeh, E. Dadgostar, H. Akbari, M. Zibaei, S.M.S. Sadjjadian, M.M. Moshtaghi, A. Shakib, Bacterial contamination of Iranian paper currency and their antibiotic resistance patterns, Int J Enteric Pathog. 5 (2017) 106–110.

[7] J.F.T.K. Akoachere, N. Gaelle, M. Henry Dilonga, K. Theresa, Nkuo-Akenji, Public health implications of contamination of Franc CFA (XAF) circulating in Buea (Cameroon) with drug resistant pathogens, BMC Res Notes. 7 (2014) 1–13.

[8] N.A.A. Alfadil, M.M.A. Malik Suliman Mohamed, E.I.E. Nima, Characterization of pathogenic bacteria isolated from Sudanese banknotes and determination of their resistance profile, Inter J Microbiol. 2018 (2018) 1–8.

[9] S. Sunil, G.S. Panchmal, R.P. Shenoy, Vijaya Kumar, Praveen Jodalli, and Vinej Somaraj, Assessment of microbial contamination of Indian currency notes in circulation–an in vitro study, J Indian Assoc Public Health Dent. 18 (2020) 179–182.

[10] M. Kalita, M. Palusinska-Szysz, A. Turska-Szewczuk, S. Wdowiak-Wrobel, T. Urbanik-Sypniewska, Isolation of cultivable microorganisms from Polish notes and coins, Pol J Microbiol. 62 (2013) 281–286.

[11] K. Bush, A. George, Jacoby, Updated functional classification of β-lactamases, Antimicrob Agents Chemother. 54 (2010) 969–976.

[12] R. Perkins Ambler, The structure of β-lactamases, Philos Trans R Soc Lond B Biol Sci. 289 (1980) 321–331.

[13] S. Younas, E. Hasan, A. Zafar, A. Ejaz, R. Saleem, H. Javed, AmpC beta-lactamases in *Klebsiella pneumoniae*: An emerging threat to the pediatric patients, JPMA. 68 (2018) 893–897.

[14] K. Thomson, Extended-spectrum β-lactamase, AmpC, and carbapenemase issues, J Clin Microbiol. 48 (2010) 1019–1025.

[15] David Livermore, β-lactamases in laboratory and clinical resistance, Clin Microbiol Rev. 8 (1995) 557–584.

[16] P. Bradford, Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistant threat, Clin Microbiol Rev. 14 (2001) 933–951.

[17] P. Gary, D. Church, G. Hall, W. Janda, E. Koneman, Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 7th ed., Wolters Kluwer, Philadelphia, PA, 2017.

[18] F. Jean, MacFaddin, Biochemical Test for Identification of Medical Bacteria, Williams & Willkins, Inc., Baltimor, 1980.

[19] Clinical and Laboratory Standards Institute CLSIPerformance Standards for Antimicrobial Susceptibility TestingCLSI document M100, M02, M07, and M11, in: Thirty first informational supplement update, Clinical and Laboratory Standards Institute, Wayne, PA, 2021.

[20] J. Pitout, P. Le, K. Moore, D. Leigh Church, D. Gregson, Detection of AmpC β-lactamases in *Escherichia coli, Klebsiella* spp., *Salmonella* spp. and *Proteus mirabilis* in a regional clinical microbiology laboratory, Clin Microbiol Infect. 16 (2010) 165–170.

[21] S. El-Hady, L. Adel, Occurrence and detection of AmpC β-lactamases among *Enterobacteriaceae* isolates from patients at Ain Shams University Hospital, Egyptian J Med Hum Genet. 16 (2015) 239–244.

[22] R. Manohar Shoorashetty, T. Nagarathnamma, J. Prathibha, Comparison of the boronic acid disk potentiation test and cefepime–clavulanic acid method for the detection of ESBL among AmpC-producing *Enterobacteriaceae*, Indian J Med Microbiol. 29 (2011) 297–301.

[23] L. Richter, E. Du Plessis, S. Duvenage, L. Korsten, Occurrence, phenotypic and molecular characterization of extended-spectrum-and AmpC-β-lactamase producing *Enterobacteriaceae* isolated from selected commercial Spinach supply chains in South Africa, Front Microbiol. 11 (2020) 1–10.

[24] C. Dallenne, A. Da Costa, D. Decré, C. Favier, G. Arlet, Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in *Enterobacteriaceae*, J Antimicrob Chemother. 65 (2010) 490–495.

[25] S. Yazdansetad, M. Alkhudhairy, R. Najafpour, E. Farajtabrizi, R. Al-Mosawi, M. Saki, E. Jafarzadeh, F. Izadpour, Atefah Ameri, Preliminary survey of extended-spectrum β-lactamases (ESBLs) in nosocomial uropathogen *Klebsiella pneumoniae* in north-central Iran, Heliyon. 5 (2019), e02349.

[26] F.J. Pérez-Pérez, N.D. Hanson, Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR, J Clin Microbiol. 40 (2002) 2153–2162.

[27] S. Alwakeel, L. Nasser, Bacterial and fungal contamination of Saudi Arabian paper currency and cell phones, Asian J Biol Sci. 4 (2011) 556–562.

[28] D. Belić, M. Ilinić, S. Burian, J. Talapko, D. Drenjančević, Dirty croatian money: how big is the threat? SEEMEDJ. 1 (2017) 5–11.

[29] M. Sawsan, Abdulla, Isolation and identification of causative agents from some Iraqi Banknote currency, Ibn Al-Haitham, J Pure Appl Sci. 26 (2013) 75–81.

[30] S.U. Ahmed, S. Parveen, T. Nasreen, B. Feroza, Evaluation of the microbial contamination of Bangladesh paper currency notes (Taka) in circulation, Adv Biol Res. 4 (2010) 266–271.

[31] C. de Carvalho, M.J. Caramujo, Bacterial diversity assessed by cultivation-based techniques shows predominance of *Staphylococccus* species on coins collected in Lisbon and Casablanca, FEMS Microbiol Ecol. 88 (2014) 26–37.

[32] R. Ali, S. Zaheer Abbas, Z. Hussain, K. Hussain, A. Hayat, A. Khan, Bacteriological analysis and antibiogram of Pakistani paper currency notes in circulation in Karachi, Sindh, Pakistan, Inter J Sci Res Environ Sci. 3 (2015) 370–376.

[33] P. Feglo, M. Nkansah, Bacterial load on Ghanaian currency notes, Afr J Microbiol Res. 4 (2010) 2375–2380.

[34] K. Pal, N.S. Das, S. Bhattacharya, Bacteriological profile of Indian currency circulating in a tertiary care hospital in rural Bengal, IJRRMS. 3 (2013) 23–30.

[35] O. Ahmed, B. Mashat, Occurrence of ESBL, MRSA and VRE pathogens in contaminated banknotes in Makkah, Saudi Arabia, Glob Adv Res J Microbiol. 4 (2015) 27–30.

[36] S. Upadhyay, M.R. Sen, A. Bhattacharjee, Presence of different beta-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing AmpC beta-lactamase enzyme, J Infect Dev Ctries. 4 (2010) 239–242.

[37] P. Mohajeri, G. Darfarin, A. Farahani, Genotyping of ESBL producing Uropathogenic *Escherichia coli* in west of Iran, Inter J Microbiol. 2014 (2014) 1–6.

[38] P. Mohajeri, A. Farahani, M. Mehdi Feizabadi, B. Norozi, Clonal evolution multi-drug resistant *Acinetobacter baumannii* by pulsed-field gel electrophoresis, Indian J Med Microbiol. 33 (2015) 87–91.

[39] A. Anthony, O. Anthony, The in vitro effect of vancomycin on multidrug resistant *Staphylococcus aureus* from hospital currency notes, Afr J Microbiol Res. 5 (2011) 1881–1887.
[40] A. Sarwar, I. Ahmad, A. Amin, M. Saleem, Paper currency harbours antibiotic-resistant coliform bacteria and integron integrase, J Appl Microbiol. 130 (2021) 1721–1729.
[41] F.S. Ahmad, H. Veisi, M. Shahin, M. Getso, A. Farahani, Frequency of quinolone resistance genes among extended-spectrum β-lactamase (ESBL)- producing *Escherichia coli* strains isolated from urinary tract infections, Tropical Med Health. 47 (2019) 1–7.
[42] R. Ranjbar, A. Farahani, Study of genetic diversity, biofilm formation, and detection of Carbapenemase, MBL, ESBL, and tetracycline resistance genes in multidrug-resistant *Acinetobacter baumannii* isolated from burn wound infections in Iran, Antimicrob Resist Infect Control. 8 (2019) 1–11.