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ORIGINAL PAPER

Multidrug-resistant Opportunistic and Pathogenic Bacteria Contaminate Algerian Banknotes Currency

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Abstract

Currency is one of the most exchanged items in human communities as it is used daily in exchange for goods and services. It is handled by persons with different hygiene standards and can transit in different environments. Hence, money can constitute a reservoir for different types of human pathogens. This study aimed to evaluate the potential of Algerian banknotes to shelter opportunistic pathogenic and multiresistant bacteria. To that end, 200 circulating notes of four different denominations were collected from various places and analyzed for their bacterial loads and contents. Besides, predominant strains were identified and characterized by biochemical and molecular methods, and their resistance profiles against 34 antibiotics were determined. Our results indicated that 100% of the studied banknotes were contaminated with bacteria. The total bacterial concentrations were relatively high, and different bacterial groups were grown, showing important diversity. In total, 48 predominant strains were identified as belonging to 17 genera. *Staphylococcus* and *Micrococcus* were the most prevalent genera, followed by *Bacillus*, *Pseudomonas*, and *Acinetobacter*. Antibiotic susceptibility testing showed that all the isolates harbored resistance to at least two molecules, and worrying resistance levels were observed. These findings prove that Algerian currency harbors opportunistic multiresistant bacteria and could potentially act as a vehicle for the spread of bacterial diseases and as a reservoir for antibiotic resistance genes among the community. Therefore, no cash payment systems should be developed and generalized to minimize cash handling and subsequent potential health risks.

Key words: currency, Algeria, opportunistic bacteria, antibiotic resistance, circulating resistance genes

Introduction

Microorganisms are continually present in our surroundings, and some of them are considered harmful as they possess the ability to invade and make humans ill. Therefore, their possible routes of transmission have been well studied, and it has been proven that inanimate objects or fomites can serve as a reservoir in the transmission of several human pathogens, including bacteria, viruses, and fungi (Lopez et al. 2013; Kraay et al. 2018; Stephens et al. 2019).

Currency is widely exchanged for goods and services among communities worldwide and exposed to microbial contamination. Money is handled by people with different levels of hygiene and can be stored on dirty surfaces. Indeed, unhygienic habits like improper hand washing after using the toilets, coughing, or sneezing on hands, and, then exchanging money leads to contamination of the next user. The potential of currency to act as a vehicle for communicable diseases has been investigated since the early 1970s (Abrams and Waterman, 1972) and continues to draw attention worldwide (Gabriel et al. 2013; Akoachere et al. 2014; Firoozeh et al. 2017; Abd Alfadil et al. 2018; Ejaz et al. 2018; Sunil et al. 2020).

Even though banknotes and coins both offer surface area to shelter microorganisms, it has been demonstrated that copper contained in coins acts as a limiting factor for their survival (Espírito Santo et al. 2010; Vriesekoop et al. 2010). On the contrary, banknotes are usually made of cotton, linen, or other textile fibers that can absorb humidity and provide fertile ground for microorganisms to adhere, develop, and persist. For instance, it has been reported that pathogenic bacteria like *Staphylococcus* spp., *Salmonella* spp., or *Escherichia coli* can persist on cotton-based banknotes for as long as 20 days (Vriesekoop et al. 2010), whereas the

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influenza virus was found to survive for up to 17 days (Thomas et al. 2008).

However, some countries have replaced paper banknotes with plastic polymer banknotes, which appear to be cleaner and to contain less microbial contamination (Vriesekoop et al. 2010).

Among bacteria that can be transmitted by fomites and most significantly by currency, opportunistic bacteria represent a matter of interest as they infect weakened hosts and are related to increased mortality rates worldwide (Lynch et al. 2007). Furthermore, this type of bacteria is difficult to eradicate as they are often multiresistant to antibiotics, leading to treatment failures.

In this context, we aimed this study to assess Algerian banknotes' safety by investigating their bacterial contents and determining their possible roles in transmitting pathogenic and opportunistic bacteria in the community and the spread of antibiotic resistance genes. To date, and to the best of our knowledge, bacterial contamination of Algerian currency is studied for the first time.

Experimental

Materials and Methods

Sampling collection. A total of 200 Algerian banknotes in four denominations (50 of each 200, 500, 1000, and 2000 Algerian Dinars, DZD) were randomly collected from various places: supermarkets, gas stations, coffee shops, pizzerias, restaurants, butchers, fish shops, bus stations and banks in Algiers City during the end of the year 2018 (November-December). In addition, for each denomination five new notes, collected from the Algerian Central Bank were included in the study as negative controls.

The samples were placed into sterile polyethylene bags using gloves to avoid further contamination. The latter were transported to the laboratory, where they were directly processed.

Isolation and numeration of the bacteria. To evaluate the bacterial diversity of the studied banknotes, different culture media were used, allowing the isolation and enumeration of the total cultivable bacteria and various specific bacterial groups.

Each banknote was dropped into 10 ml of Trypticase Soy Broth (TSB, Difco) and shaken for 30 minutes with a vortex agitator to dislodge the bacteria into the broth, as described by Kalita et al. (2013). Then, 0.1 ml of the obtained suspension and of its decimal dilutions (from 10^{-1} to 10^{-5}) were inoculated into the following solid media (Difco): Nutrient agar (for the enumeration of the total bacterial number), Columbia medium (allowing the growth of the exigent bacteria), Chapman agar (for the *Staphylococci* group), Bile-Esculin agar (for the *Enterococci* group), Hektoen agar (for the *Enterobacteriaceae* family and the other non-exigent Gram-negative bacteria) and Cetrimide agar (for *Pseudomonas* spp.). The plates were then incubated for 18 to 48 hours at 37°C.

After incubation, the grown colonies were counted and the total viable cells, expressed as colony-forming units (CFU) per banknote, were determined by the count plate method.

Bacterial identification. Different, representative, and predominant colonies grown in the enumeration plates were selected for each sampling place and then purified and isolated by repeated transfers on fresh agar plates of their initial culture media.

Isolates were analyzed by microscopic observations after Gram staining, and activities of oxidase, catalase, and nitrate reductase, VP-MR test, motility, indole production, citrate utilization, and respiratory type to Bergey's manual of systematic Bacteriology (Holt et al. 1994).

Based on the results of these preliminary tests and the initial isolation media, the biochemical identification of the isolates was carried out by the corresponding API system galleries (Biomérieux) using API 20E, API 20 NE, API 50CH, API Staph, or API Strep.

Moreover, Gram and catalase-positive strains retrieved from Chapman agar were submitted to additional tests comprising tube coagulase test with rabbit plasma (Biomérieux) and Pastorex Staph-Plus™ agglutination test (Bio-Rad). Strains that responded positively to both tests were identified as *Staphylococcus aureus*.

Finally, some isolates' taxonomic status was confirmed by 16s rRNA gene sequencing as described previously (Ben Dhia Thabet et al. 2004).

Phylogenetic analysis. The phylogenetic analysis and the phylogenetic tree constructions were conducted using the Molecular Evolutionary Genetics Analysis (MEGA) software, version 10.0 (Kumar et al. 2018).

Reference 16S rRNA gene sequences were uploaded from the Genbank database (https://www.ncbi.nlm.nih.gov/). Multiple alignments of the sequences were performed by CLUSTAL W (Thompson et al. 1994). Evolutionary distances were calculated using the maximum composite likelihood method and are in the units of the number of base substitutions per site (Tamura et al. 2004). The phylogenetic trees were reconstructed with the neighbor-joining algorithm (Saitou and Nei 1987). The resultant trees' topologies were evaluated by bootstrap analysis of the neighbor-joining dataset, based on 1,000 resamplings.

Hemolysis test. The ability to digest red blood cells was tested by spot inoculation of the isolated strains on Columbia agar supplemented with 5% sheep blood. The plates were incubated at 37°C for 24 to 36 hours and the potential clear zones (for β-hemolysis)

or changes of color (for α-hemolysis) around the bacterial spots were observed.

Determination of antibiotic resistance. The bacterial strains were tested for their resistance or susceptibility towards 34 different antibiotics by the Kirby-Bauer disk diffusion method on Mueller Hinton agar plates (Difco) using the following antibiotic disks (µg or International Unit „IU"/disk): amikacin (AK) 30 µg, amoxicillin + clavulanic acid (AMC) 30 µg, ampicillin (AMP) 10 µg, aztreonam (AZT) 30 µg, cefazolin (CZ) 30 µg, cefepime (FEP) 30 µg, cefotaxim (CTX) 30 µg, cefoxitin (CEF) 30 µg, ceftazidime (CAZ) 30 µg, cefuroxime (CXM) 30 µg, cephalotin (CH) 30 µg, chloramphenicol (C) 30 µg, ciprofloxacin (CIP) 5 µg, clindamycin (CN) 2 µg, daptomycin (DAP) 30 µg, ertapenem (ERT) 10 µg, erythromycin (E) 15 µg, fosfomycin (FOS) 50 µg, fusidic acid (AF) 10 µg, gentamicin (G) 10 µg, levofloxacin (LVX) 5 µg, linezolid (LIN) 30 µg, nitrofurantoin (NIT) 100 µg, oxacillin (OX) 5 µg, penicillin (P) 10 UI, piperacillin/tazobactam (TZP) 100 + 10 µg, rifampicin (RA) 5 µg, teicoplanin (TEC) 30 µg, tetracyclin (TE) 30 µg, ticarcillin + clavulanic acid (TCC) 75 + 10 µg, tigecycline (TIG) 15 µg, tobramycin (TM) 10 µg, trimethoprim + sulfamethoxazole (SXT) 1.25 + 23.75 µg and vancomycin (VA) 30 µg (Hi-media).

The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute. *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were used as control strains for antimicrobial susceptibility testing.

Results

A total of 220 banknotes, comprising 200, 500, 1000, and 2000 DZD denominations (200 samples consisting of 50 used banknotes for each denomination and 20 controls consisting of 5 mint banknotes for each denomination) were analyzed for their bacterial load and diversity. The predominant bacterial strains were identified, and their antibiotic resistance profiles were determined.

Bacterial enumeration. All circulating banknotes (100%) obtained from various sources were contaminated, whereas new notes from the central bank showed low or no growth (Table I).

Bacterial concentrations were related to the denominations as the most critical contamination was found among 200 DZD, 500 DZD, and 1000 DZD denominations, whereas 2000 DZD banknotes were moderately contaminated (Table I). Besides, we noted the banknotes' general bad physical state (moistness, smelliness, tearing), most significantly for 200 DZD, 500 DZD, and 1000 DZD denominations.

As expected, Columbia agar counts were the highest since the used medium is rich and allows the growth of various bacterial groups, even exigent ones. Besides, the mean for the total non-exigent flora (Nutrient agar) was relatively abundant (varying from $1{,}3 \times 10^3$ to $4{,}3 \times 10^4$ CFU/banknote).

Regarding the selective media, Chapman agar and Hektoen showed the most important rates of growth ($5{,}3 \times 10^3$ and $3{,}3 \times 10^3$, respectively), whereas Bile-Esculin and Cetrimide agar exhibited lower contamination levels.

Bacterial identification. After enumeration, the counting plates were examined, and for each sampling place the dominant colonies were selected and purified for qualitative analysis. Thereby, 48 morphologically distinct bacterial strains were obtained and characterized (Table II). Out of the total, 19 strains were isolated from 200 DZD banknotes, 16 from 500 DZD banknotes and five and eight strains from 1000 and 2000 DZD banknotes, respectively.

The morphological study showed that 28 strains were Gram-positive (58.3%) including 23 cocci and

Table I
Enumeration of different bacterial groups isolated from Algerian banknotes.

Culture Media	The banknotes studied (CFU/banknote)				New banknotes (CFU/banknote)			
	200 DZD	500 DZD	1000 DZD	2000 DZD	200 DZD	500 DZD	1000 DZD	2000 DZD
Nutrient agar (Total non-exigent flora)	$4.3 \times 10^4 \pm 9 \times 10^3$	$2.6 \times 10^4 \pm 4 \times 10^3$	$1.5 \times 10^4 \pm 6 \times 10^3$	$1.3 \times 10^3 \pm 4 \times 10^2$	200 ± 16	150 ± 27	120 ± 12	80 ± 5
Columbia agar (Exigent and non-exigent bacteria)	$5.4 \times 10^5 \pm 8 \times 10^4$	$1.8 \times 10^5 \pm 4.3 \times 10^4$	$2.1 \times 10^4 \pm 3 \times 10^3$	$2.2 \times 10^3 \pm 8 \times 10^2$	100 ± 29	50 ± 6	0	0
Chapman agar (*Staphylococcus* and other Gram-positive cocci)	$5.3 \times 10^3 \pm 4 \times 10^2$	$6.7 \times 10^3 \pm 3 \times 10^2$	$6.6 \times 10^3 \pm 9 \times 10^2$	$8{,}5 \times 10^2 \pm 7 \times 10^1$	0	0	0	0
Hektoen agar (*Enterobacteriaceae* and other non-exigent Gram-negative rods)	$3.3 \times 10^3 \pm 7 \times 10^2$	200 ± 40	100 ± 20	0	0	0	0	0
Bile-Esculin agar (*Enterococcus* spp.)	200 ± 30	500 ± 20	300 ± 13	0	0	0	0	0
Cetrimide agar (*Pseudomonas* spp.)	80 ± 2	72 ± 4	75 ± 6	20 ± 3	0	0	0	0

Values are the means of three replicates

Table II
Strains isolated from Algerian banknotes currency and their characterization.

Notes	Strains	Biochemical identification (Api System)	Molecular identification	Resistance profiles	Hemo-lysis
200 DZD	B0P1	*Staphylococcus cohnii*	*S. equorum*	P, AZT, FOS, FA	none
	B1P3	*Staphylococcus epidermidis*	*S. epidermidis*	P, OX, AMP, AMC, CZ, CH, AZT,C, CN	none
	B1N2	*Staphylococcus aureus*	ND	P, OX, AMP, AZT, GN, FA	β
	B4M1	*Staphylococcus auricularis*	ND	AZT, FA, G, T, C	none
	B3M3	*Micrococcus* sp.	ND	P, OX, E	none
	B4P8	*Micrococcus* sp.	ND	P, AK	α
	B4N6	*Micrococcus* sp.	ND	P, SXT	none
	B5M4	*Micrococcus* sp.	ND	P, T, E	none
	B0H2	*Bacillus* sp.	*B. firmus*	P, OX, CZ, CH	β
	B5P5	*Bacillus* sp.	*S. luteloa*	P, OX, AMP	none
	B7G7	*Bacillus* sp.	*B. psychrodurans*	P, OX, AMP, FA, FOS	β
	B1M0	*Rothia mucilaginosa*	ND	P, OX, FA, V, CIP, C	none
	B1C1	*Pseudomonas aeruginosa*	*Ps. aeruginosa*	P, OX, AMP, AMC, TZP, TCC, CZ, CH, CEF, CXM, ERT, FA, TEC, VA, DAP	α
	B6C1	*Brevundimonas vesicularis*	*B. vesicularis*	P, OX, AMP, CZ, CEF, FA, G, TM, FA, TEC, VA, DAP, E, CN	none
	B7M1	*Acinetobacter lwoffii*	ND	P, OX, CZ, CH, FA, TEC, VA, DAP, T, CIP, LVX	α
	B7P2	*Moraxella* sp.	ND	P, OX, AMP, AMC, FA, TEC, VA, DAP, CN	none
	B6M5	*Shewanella putrefaciens*	ND	P, OX, AMP, AZ, CH, CZ, FOS, FA, TEC, VA, DAP, SXT	β
	B6J4	*Klebsiella ozaenea*	ND	P, OX, AMP, AMC, CH, CZ, G, FA, TEC, VA, DAP, T, E, NIT	α
	B5S3	*Enterobacter sakazakii*	ND	P, OX, AMP, AMC, CZ, CH, CEF, CXM, FA, TEC, VA, DAP	α
500 DZD	C1P1	*Staphylococcus arlettae*	*S. arlettae*	P, OX, AMP, CH, CZ, CEF, AZT, TEC, VA, E, G, TM, T, E, CN	none
	C5H1	*Staphylococcus lentus*	ND	P, AMP, AMC, AZT, G, AF	α
	C5G4	*Staphylococcus saprophyticus*	*S. saprophyticus*	P, AZT, T, E, CN	none
	C1M1	*Micrococcus* sp.	ND	P, E	none
	C4J3	*Micrococcus* sp.	ND	DAP, T, E	α
	C4P3	*Micrococcus* sp.	ND	P, T	none
	C5G1	*Enterococcus faecalis*	*E. faecalis*	OX,CZ, CH, CEF, CXM, FEP,TZP, TCC, AK, G, TM, CN, E	α
	C5P3	*Enterococcus faecium*	ND	P, OX, AMP, AMC, CH, CZ, CTX, CXM, FEP, ERT, AK, G, TM, FOS, CIP, LVX, T, E, CN	α
	C2H0	*Bacillus* sp.	ND	P, OX, AMP, E	β
	C5T1	*Bacillus* sp.	ND	P, OX, AMP, CZ	β
	C5C7	*Rothia mucilaginosa*	ND	P, OX, AK, G	none
	C1N3	*Pseudomonas fluorescens*	ND	P, OX, AMP, AMC, CZ, CH, CEF, CXM, ERT, FA, TEC, VA, DAP, G, E	none
	C2C5	*Pseudomonas mendocina*	*Ps. mendocina*	P, OX, AMP, AMC, CH, CZ, CEF, CXM, CTX, CAZ, ERT, G, FOS, FA, TEC, VA, DAP, CIP, LVX	α
	C5C11	*Pseudomonas fluorescens*	*Ps. fragi*	P, OX, AMP, AMC, CH, CZ, CTX, CXM, FEP, AZT, TZP, ERT, G, TM, FA, TEC, VA, DAP, CIP, T, E, CN	α
	C3K1	*Acinetobacter lwoffii*	ND	P, OX, AMP, AMC, CZ, CH, FA, TEC, VA, DAP	none
	C4R11	*Weeksella virosa*	ND	P, OX, CIP, AK, AZT, G, TM, FA, TEC, VA, DAP	none
1000 DZD	D1C1	*Micrococcus* sp.	ND	P, FA, C	none
	D1H1	*Enterobacter cloacae*	*E. cloacae*	P, OX, AMP, AMC, AZT, CZ, CH, CEF, CXM, CTX, CAZ, FEP, ERT, FA, TEC, VA, DAP, TIG, SXT	α
	D3P5	*Klebsiella oxytoca*	ND	P, OX, AMP, CZ, G, FA, TEC, VA, DAP, C, CIP, NIT	α
	D2P1	*Alcaligenes faecalis*	*A. faecalis*	P, OX, AMP, AMC, AZT, ERT, CAZ, FEP, G, FOS, FA, TEC, VA, CIP, DAP	none
	D4C5	*Acinetobacter lwoffii*	*A. lwoffii*	P, OX, AMP, CZ, CH, G, FA, TEC, VA, DAP, E, SXT	α

Table II. Continued

Notes	Strains	Biochemical identification (Api System)	Molecular identification	Resistance profiles	Hemolysis
2000 DZD	DM4S1	*Staphylococcus aureus*	ND	P, OX, CZ, CH, AZT, G, TM	α
	DM5C1	*Staphylococcus epidermidis*	ND	P, OX, AMP, AMC, AK, G, TM, FOS, FA, TEC, VA, CIP, T, E, SXT, CN, RA, NIT	none
	DM3C3	*Micrococcus* sp.	*M. luteus*	AZT, NIT	none
	DM3C5	*Rothia dentocariosa*	ND	P, SXT, NIT	none
	DM1C2	*Klebsiella ozaeane*	*K. oxytoca*	P, OX, AMP, AMC, CH, CZ, CEF, CTX, CXM, FEP, AZT, ERT, AK, G, TM, FOS, FA, TEC, VA, DAP, C, CIP, LVX, T, TIG, E, CN	α
	DM2C3	*Serratia marscesens*	*S. rubidaea*	P, OX, AMP, AMC, CZ, CH, CEF, CTX, CAZ, ERT, FA, TEC, VA, DAP, C, CIP, LVX, SXT	none
	DM1J1	*Acinetobacter lwoffii*	ND	P, OX, CZ, CH, FA, TEC, VA, DAP, CIP, SXT	none
	DM4G6	*Chryseobacterium indologenes*	ND	P, OX, CZ, CH, CXM, CAZ, FEP, AZT, ERT, AK, G, TM, FA, TEC, VA, DAP, SXT	none

ND – not determined

five spore-forming rods whereas 20 strains were Gram-negative rods or coccobacilli (41.7%).

Biochemical identification showed that the 48 isolated strains belonged to 17 different genera. 16S rRNA gene sequencing allowed us to confirm and specify the taxonomical statutes for 18 of the isolates. The latters were submitted to phylogenetical analysis, and the resulting trees for Gram-positive and Gram-negative bacteria are shown in Fig. 1a and 1b, respectively.

The 16S rRNA sequences for the 18 strains have been registered to the NCBI Genbank database and the accession numbers attributed are as follows: B6C1: MT786742; B1C1: MT786743; C2C5: MT786744; C5C11: MT786745; D1H1: MT786746; D2P1: MT786747; D4C5: MT786748; DM1C2: MT786749; DM2C3: MT786750; B0P1: MT786751; B0H2: MT786752; B5P5: MT786753; B7G7: MT786754; C1P1: MT786755; C5G1: MT786756; C5G5: MT786757; B1P3: MT786758; DMC3: MT786759.

The isolation frequencies for each genus are presented in Table III. The most predominant groups retrieved from Algerian banknotes were catalase-positive and

Table III
Diversity of bacterial strains isolated from Algerian banknotes.

Genera	Number of strains for each denomination				Total (frequency %)
	200 DZD	500 DZD	1000 DZD	2000 DZD	
Staphylococcus	4	3	0	2	9 (18.75)
Micrococcus	4	3	1	1	9 (18.75)
Bacillus	2	2	0	0	4 (8.33)
Pseudomonas	1	3	0	0	4 (8.33)
Acinetobacter	1	1	1	1	4 (8.33)
Rothia	1	1	0	1	3 (6.25)
Klebsiella	1	0	1	1	3 (6.25)
Enterococcus	0	2	0	0	2 (4.16)
Enterobacter	1	0	1	0	2 (4.16)
Serratia	0	0	0	1	1 (2)
Brevundimonas	1	0	0	0	1 (2)
Shewanella	1	0	0	0	1 (2)
Moraxella	1	0	0	0	1 (2)
Weeksella	0	1	0	0	1 (2)
Alcaligenes	0	0	1	0	1 (2)
Chryseobacterium	0	0	0	1	1 (2)
Sporosarcina	1	0	0	1	1 (2)
Total	19	16	5	8	N = 48

catalase-negative Gram-positive cocci represented by the genera *Staphylococcus* and *Micrococcus* (18.75%, each) followed by *Bacillus*, *Pseudomonas,* and *Acinetobacter* (8.33%, each). The remaining strains were identified as belonging to the following genera: *Rothia*, *Klebsiella*, *Enterococcus*, *Serratia*, *Brevundimonas*, *Shewanella*, *Moraxella*, *Weeksella*, *Alcaligenes*, *Chryseobacterium*, and *Sporosarcina.* There were no significant differences observed in the bacteria distribution between the denominations studied.

Among *Staphylococcus* spp., two strains (B1N2 and DM4S1) showed coagulase production and responded positively to the Pastorex Staph-Plus™ agglutination test. These results confirmed the prior biochemical identification as *S. aureus* (Table II).

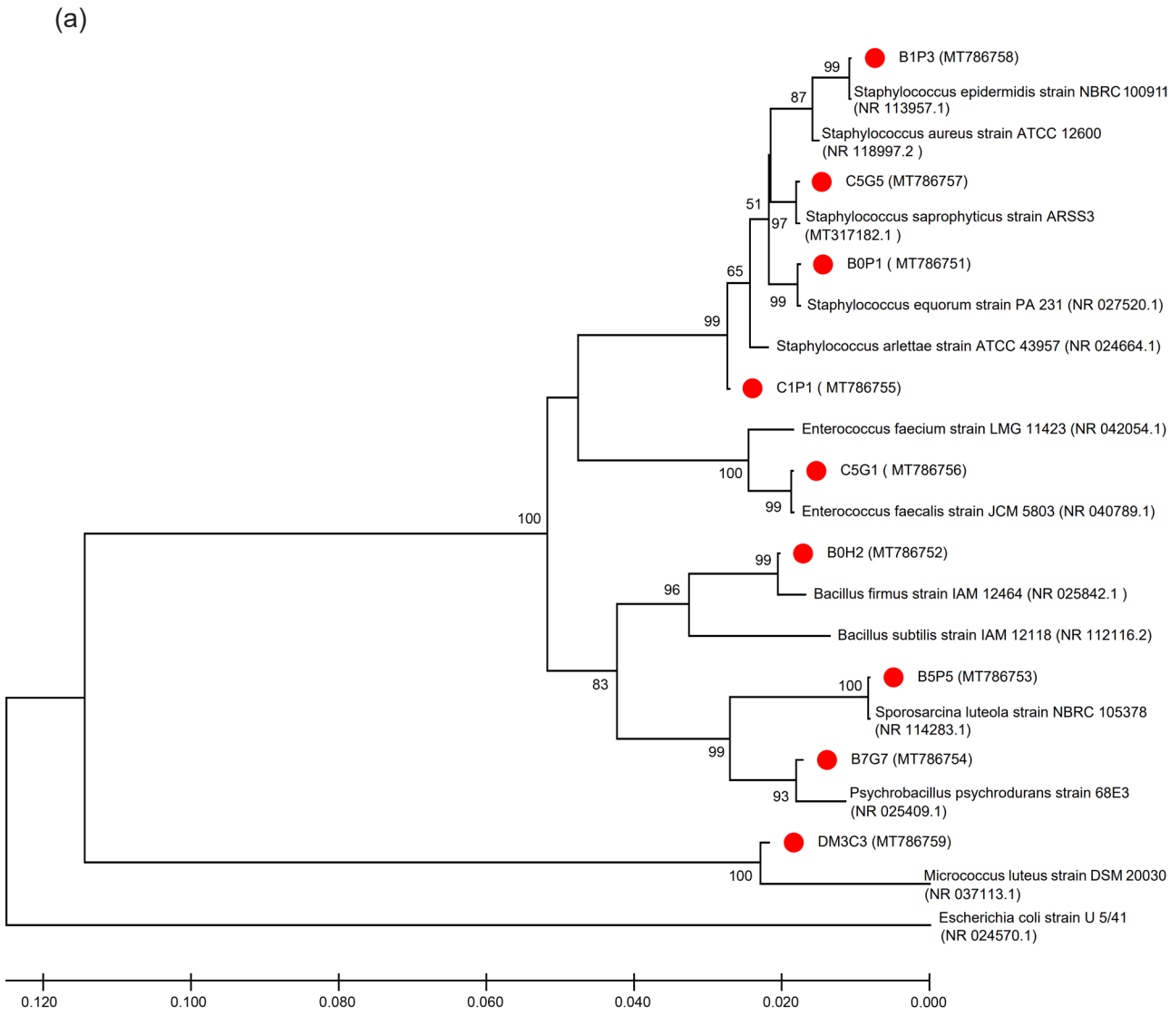
Hemolysis test. The results of the hemolysis test are presented in Table II. In total, 22 strains (45.8%) were able to digest blood cells and 16 (33.3%) showed α-hemolysis, whereas 6 (12.5%) were β-hemolytic.

Antibiotic susceptibility. The 48 strains were tested for their antibiotic susceptibility towards 34 antibiotics belonging to 15 different families. The antibiotypes of the 48 isolates are shown in Table II**,** and the antibiotic resistance frequencies are presented in Table IV.

All isolates presented antibiotic resistance towards 2 (strains B4P8, B4N6, C1M1, C4P3, and DM3C3, all identified as *Micrococcus* spp.) to 27 molecules (strain DM1C2 identified as *Klebsiella oxytoca*) at the same time (Tab. II).

Penicillin (P) and oxacillin (OX) showed the most important resistance frequencies. However, as Gram-negative bacteria are naturally resistant to these molecules, only Gram-positive bacteria resistance frequencies should be noted. Thus, 24/28 and 15/28 Gram-positive strains were found to be resistant to P and OX, respectively.

Generally, high resistance frequencies for aminopenicillins (54.1% and 33.3% for ampicillin (AMP) and amoxicillin + clavulanic acid (AMC), respectively) and for 1st generation cephalosporins (50 % and 43.8% for cefazolin (CZ) and cephalotin (CH), respectively) were observed whereas other β-lactams showed better efficiency than the latter.



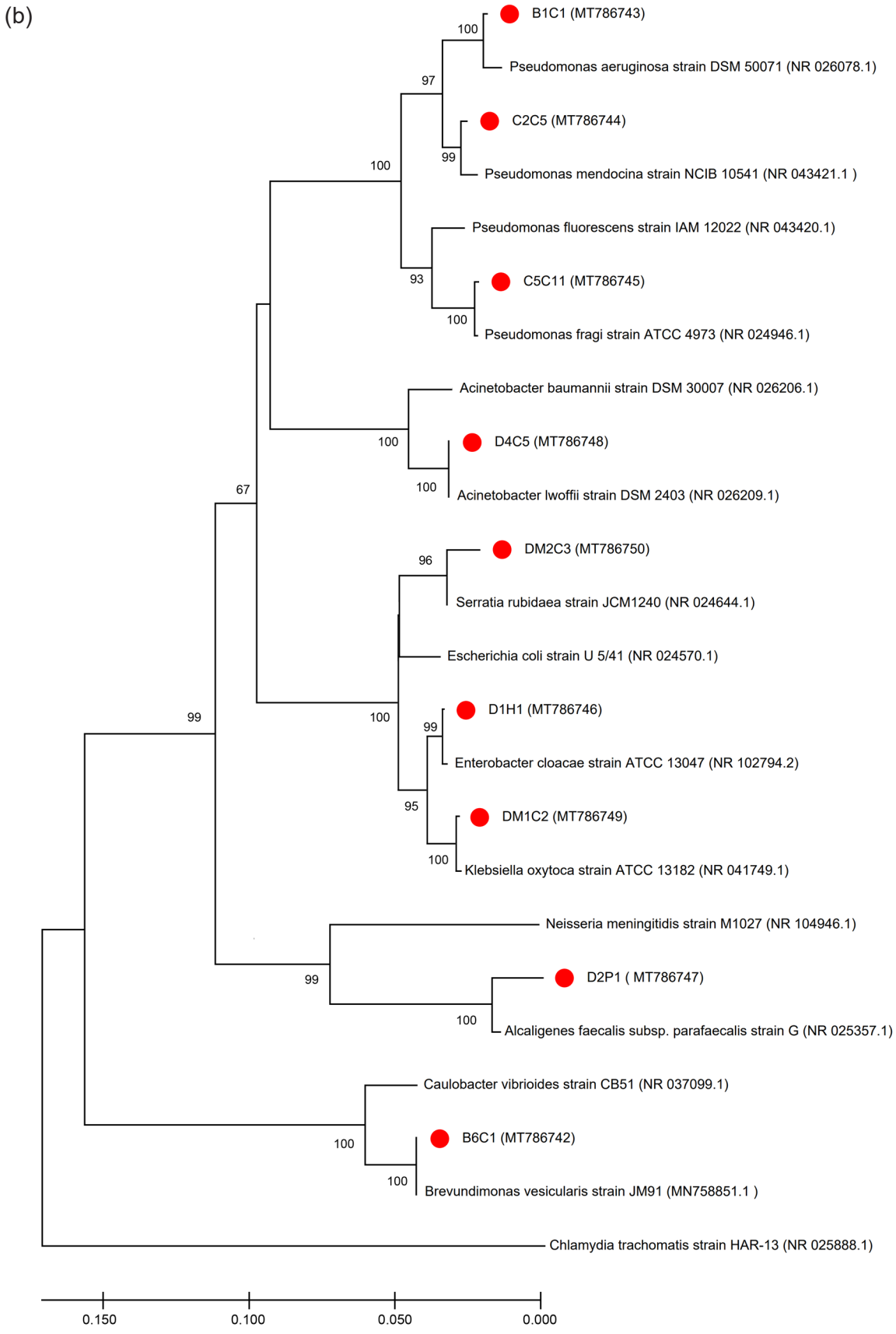


Fig. 1. Phylogenetic relationships between strains isolated from Algerian banknotes and reference bacterial strains using 16S rRNA sequences: (a) Gram-positive strains; (b): Gram-negative strains. The evolutionary distances were computed using the Tamura-Nei method and are in the units of the number of base substitutions per site. The phylogenetic trees were constructed using the Neighbor-joining algorithm. Bootstrap values (1,000 replicates) are indicated at the nodes.

Table IV
Antibiotic resistance in Algerian banknotes isolates.

Families	Antibiotics	Number of Gram-positive resistant strains (N = 28)	Number of Gram-negative resistant strains (N = 20)	Number of Total resistant strains Frequencies (%)
β-lactams	P	24	20*	44 (91.7)
	OX	15	20*	35 (72.9)
	AMP	10	16	26 (54.1)
	AMC	4	12	16 (33.3)
	CZ	7	17	24 (50)
	CH	6	15	21 (43.8)
	CEF	2	8	10 (20.8)
	CXM	2	10	12 (25)
	CTX	1	5	6 (12.5)
	CAZ	0	5	5 (10.4)
	FEP	2	5	7 (14.6)
	TZP	1	2	3 (6.2)
	TCC	1	1	2 (4.2)
	AZT	9	6	15 (31.2)
	ERT	1	9	10 (20.8)
Aminosides	AK	5	3	8 (16.7)
	G	8	10	18 (37.5)
	TM	5	5	10 (20.8)
Phosphonic acids	FOS	4	4	8 (16.7)
Fusidanines	FA	7	20*	27 (58.3)
Glycopeptides	TEC	2	20*	22 (45.8)
	VA	2	20*	22 (45.8)
Lipopeptides	DAP	1	20*	21 (43.8)
Oxazolidinones	LIN	0	0	0 (0)
Phenicols	C	4	3	7 (14.6)
Fluoroquinolones	CIP	3	9	12 (25)
	LVX	1	4	5 (10.4)
Tetracyclines	T	8	4	12 (25)
	TIG	0	2	2 (4.2)
Macrolides	E	12	7	19 (39.6)
Lincosamides	CN	6	4	10 (20.8)
Rifamycines	RA	1	2	3 (6.2)
Nitrofurantoines	NIT	3	2	5 (10.4)
Sulfamides	SXT	3	6	9 (18.8)

* – natural resistance for Gram-negative bacteria

Gentamycin (G) and erythromycin (E) showed poor efficiency (39.6% and 37.5% of resistant strains, respectively), whereas no resistance was found against linezolid (LIN).

Regarding Gram-positive bacteria, specific antibiotics (for which Gram-negative bacteria are naturally resistant), seven strains were resistant to fusidic acid (FA), two to vancomycin (VA) and teicoplanin (TEC), and one to daptomycin (DAP).

Finally, we noted that overall antibiotic resistance was more important for Gram-negative strains than for Gram-positive ones (Table IV).

Discussion

In this study, the overall bacterial contamination (100% of the banknotes) was higher than that of similar studies conducted in Iran (Firoozeh et al. 2017),

Croatia (Belić et al. 2017), Cameroon (Akaouchere et al. 2014), and Pakistan (Ejaz et al. 2018) reporting 77%, 78%, 94%, and 97% of contaminated banknotes, respectively, but comparable to the 100% rate of contaminated notes retrieved in Sudan (Abd Alfadil et al. 2018), and in India (Sunil et al. 2020). The high rates of bacterial contamination found in Algerian currency could be explained by necessary money handling as most commercial services are paid in cash. Indeed, Algeria's banking system is poorly developed, and keeping cash in houses is part of the Algerian practices. Also, it is to be noted that Algerian bills are made of a cotton-linen mix, which offers a good surface for bacterial attachment and development, especially when the latter is moist and damaged, as it has already been suggested (Vriesekoop et al. 2010). Besides, keeping money underbody surfaces and other poor hygiene practices like inproper hand washing or counting banknotes with fingers wet with saliva may play a role in the dirtiness degree.

Furthermore, it appears that the lower the denomination, the higher the bacterial load. The same observation has been made in similar studies (Akoachere et al. 2014; Girma et al. 2014; Ejaz et al. 2018) and explained that lower denominations are more often exchanged than higher ones. However, it has to be noted that 2000 DZD banknotes were issued and sent for the first time to circulation in 2011, whereas the other analyzed denominations circulate since the 1990s. The longer the banknotes remain in circulation, the greater are their chances to be soiled. Thereby, 2000 DZD notes were generally cleaner and less or not damaged when compared to 200 and 500 DZD denominations in a bad physical condition (moist and smelly). More generally, bacterial loads are a function of the banknotes' physical conditions since in the present study, bacterial counts were higher on dirty and damaged notes than on relatively clean ones, as has already been demonstrated (Kalita et al. 2013; Akoachere et al. 2014).

The total non-exigent bacterial loads were relatively significant when compared to similar studies (Kalita et al. 2013; Mändar et al. 2016), showing that Algerian currency could act as a vector for the transmission of bacterial agents in the community. Also, growth was noted on all selective media, demonstrating important bacterial diversity. Notably, Hektoen and Chapman media showed important growth rates suggesting important contamination with *Enterobacteriaceae* and *Staphylococcus* spp. These two bacterial groups are known to be implied in food-borne diseases and could certainly pose a health risk since the studies banknotes were partly collected from food vendors. Moreover, the presence of *Enterobacteriaceae* (Hektoen agar) and *Enterococcus* spp. (Bile-Esculin agar) suggests possible fecal contamination of the banknotes due to lack of hygiene like not washing hands properly after using the toilets.

Qualitative analysis, in which 48 different strains were isolated, indicated that Gram-positive bacteria were more common than Gram-negative. These results are consistent with earlier reports (Akoachere et al. 2014; Girma et al. 2014; Firoozeh et al. 2017; Ejaz et al. 2018).

The most predominant bacterial genera were *Staphylococcus* and *Micrococcus* (18.75%, each), both naturally found in the human skin and mucous membranes microbiota suggesting hand-borne contamination of the banknotes and, hence, frequent cash exchanges in the community. Coagulase-negative staphylococci and *Micrococcus* spp. are related to opportunistic infections, including community-acquired ones in immunocompromised subjects (Seng et al. 2017; Shi et al. 2017; Ianiello et al. 2019). In addition, strains B1N2 and DM4S1 were identified as *S. aureus*, a well-known pathogen responsible for a broad range of infections but particularly famous for causing food poisoning (Sergelidis and Angelidis 2017). Since *S. aureus* can survive on paper notes for more than 19 days (Gabriel et al. 2013), the presence of this bacterium poses a severe health problem.

The presence of *Bacillus* spp. and *Sporosarcina luteola*, which are spore-forming organisms, could suggest soil contamination even though *Bacillus* spp. are recognized as potential food-borne opportunistic agents (Logan 2012).

The occurrence of *Rothia* spp. confirms possible contamination of the banknotes with saliva as these microorganisms are known as colonizers of the human oral cavity causing peridontal diseases. However, the members of this genus are also implied in opportunistic infections (Ramanan et al. 2014).

More generally, all the recovered bacteria are considered opportunistic or strictly pathogens and represent a threat of infectious diseases among the population, particularly in immunocompromised subjects. These conclusions agree with similar and recent reports from other parts of the world (Firoozeh et al. 2017; Ejaz et al. 2018; Sunil et al. 2020).

To estimate one of their virulence aspects, the isolates were submitted to hemolysis test. The results indicated that 45.8% of the strains were able to produce hemolysins and could be considered potentially dangerous. Indeed, these virulence factors are responsible for cell lysis and destruction of tissues causing damages in the colonized host.

Furthermore, all 48 strains presented antibiotic resistance to at least two molecules simultaneously and showed high resistance levels.

The β-lactams family, notably penicillin and 1st generation cephalosporins, showed the highest rates of

resistance. This observation is also pointed out by analogous studies reporting low efficiency of penicillins and old cephalosporins (Akoachere et al. 2014; Firoozeh et al. 2017; Abd Alfadil et al. 2018; Ejaz et al. 2018). It can be explained by the fact that these molecules have a broad spectrum of activity and are frequently prescribed for a large panel of community-acquired infections leading to the selection of resistant bacterial strains (Melander et al. 2000). Resistance to penicillins and cephalosporins is often due to the production of extended-spectrum-β-lactamases (ESBL), which are generally plasmid-mediated and can rapidly spread among bacteria. Accordingly, a recent study conducted in Algeria describes the presence of ESBL producing Gram-negative bacteria isolated from currency and reports that the implied determinants were members of the $bla_{\text{CTX-M}}$ genes family known to be carried by highly transferrable plasmids (Bendjama et al. 2020).

Nonetheless, we noticed a high resistance frequency towards ertapenem (ERT), a carbapenem molecule, considered the last-resort antibiotic, particularly against ESBL producing Gram-negative bacteria. In this work, two highly multiresistant Gram-negative strains identified as *Klebsiella oxytoca* (DM1C2) and *Serratia rubideae* (DM2C3) were found to be ERT resistant. These findings are corroborated by Bendjama et al. (2020), who report the isolation of a carbapenem-resistant strain of *Enterobacter cloacae* from Algerian currency. It has to be noted that the reported strain carried the carbapenemase $bla_{\text{OXA-48}}$ gene, which is known to be endemic in Mediterranean countries. The existence of carbapenem-resistance genes in the community and more specifically in Gram-negative bacteria is to take very seriously since it could lead to therapeutic failures.

Likewise, glycopeptides are considered as the last-resort treatment for multiresistant Gram-positive bacteria related infections. Yet, here, we report two vancomycin (VA) and teicoplanin (TEC) resistant Gram-postitve strains identified as coagulase-negative staphylococci (C1P1 and DM5S3). Consequently, the dissemination of the implied resistance genes among other bacteria found in the community could lead to a significant health problem.

More generally, multiresistant bacteria found in currency harbor antibiotic resistance genes, often carried by transferrable elements. This idea is supported by a Pakistani study that describes various antibiotic resistance genes and integrons in bacteria isolated from paper currency (Sarwar et al. 2020).

Overall, this study demonstrates that Algerian currency is a reservoir for various antibiotic resistance genes that could spread among phylogenetically related or not related bacteria by horizontal transfers and, hence, play a role in the emergence of antibiotics multi-resistance.

Conclusions

This study shows that Algerian currency banknotes are highly contaminated with opportunistic and pathogenic antibiotic-resistant bacteria and could act as vehicles for infectious diseases in the community and play a role in disseminating antibiotic resistance genes. Given that most commercial transactions are paid in cash in Algeria, our banknotes are very frequently exchanged and handled by the population, leading to more and more bacterial contamination and greater exposure to infections. For these reasons, poor hygiene practices should be discouraged, particularly in restaurants and food shops. Furthermore, no cash transactions should be encouraged by generalizing e-payments and bank cards, and damaged or old banknotes should be frequently replaced. At the time of writing, the urge to implement these recommendations is very well illustrated by the Covid-19 pandemic. Indeed, in the battle against the new Coronavirus, it is strongly advised to avoid cash and favor electronic and bank card payments when no cash transaction systems are poorly or not available at all in Algeria.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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