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First detection of an OXA-48-producing *Enterobacter cloacae* isolate from currency coins in Algeria

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ABSTRACT

Objectives: The aim of this study was to screen for the presence of β-lactamase-producing Gram-negative bacteria (GNB) from Algerian currency collected from food vendors in Batna city, Algeria.
Methods: During two periods (May 2018 and March–April 2019), a total of 408 coins and currency notes of different denominations of Algerian Dinar were randomly recovered from several food vendors. Samples were subjected to selective isolation of extended-spectrum cephalosporin- and carbapenem-resistant GNB. Bacterial species identification was performed using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). Antibiotic susceptibility testing was performed by the disk diffusion method. Carbapenemase and extended-spectrum β-lactamase (ESBL) genes were searched for by real-time PCR, standard PCR and sequencing. The clonal relationship of carbapenemase-producing isolates was investigated by multilocus sequence typing (MLST). The transferability of the detected carbapenemase-encoding gene was verified by conjugation experiments.
Results: Twelve cefotaxime- and/or carbapenem-resistant strains were isolated in this study and were identified as *Enterobacter cloacae*, *Raoultella ornithinolytica*, *Citrobacter freundii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas libanensis* and *Pseudomonas stutzeri*. The bla_{OXA-48} gene was detected in only one *E. cloacae* strain belonging to sequence type 108 (ST108), whilst the two *R. ornithinolytica* isolates harboured $bla_{CTX-M-27}$ and one *E. coli* strain carried $bla_{CTX-M-14}$. The detected bla_{OXA-48} gene was transferable by conjugation.
Conclusions: We report for the first time the detection of an OXA-48-producing *E. cloacae* isolate from money. This calls for consciousness development on the potential risks associated with poor handling of currency.

1. Introduction

Currency is used as a medium for goods, exchange and services in the entire global economic environment and has promoted trade worldwide [1,2]. The potential threat to public health that money might act as a vehicle for the transmission of potential pathogenic micro-organisms was suggested in the 1970s by Abrams and Waterman [3], given that money is handled and circulated by a large number of people, under different environmental and personal conditions [1,2]. Consequently, recent studies from different parts of the world have confirmed these theories and have shown that viable pathogenic organisms can be isolated on money surfaces, including bacteria, particularly Gram-negative bacteria (GNB), which may persist more than Gram-positive bacteria on inanimate surfaces, and viruses such as human influenza virus and coronavirus [2,4]. In addition, various studies show that currency could play a major role in the spread of bacteria resistant to commonly used antibiotics and thus pose a threat to the public health of currency handlers and the community [2].

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The β-lactam antibiotics have been among the most effective and widely used drugs for the treatment of bacterial infections both in human and veterinary medicine. In the past decade, resistance to β-lactams in GNB was mainly due to the production of extended-spectrum β-lactamases (ESBLs) and plasmid-encoded AmpC-type cephalosporinases. Furthermore, these determinants have been recognised to be of growing importance worldwide. Thus, carbapenems have become among the last-resort drugs in the treatment of severe infections caused by these bacteria. Unfortunately, widespread use of carbapenems has led to the emergence of carbapenem-resistant GNB [5,6].

Carbapenem resistance involves multiple mechanisms, however the most common is the production of carbapenemases. Carbapenem-hydrolysing β-lactamases are diverse enzymes belonging to different Ambler classes (A, B and D), among which the OXA-48 class D carbapenemase is mostly identified in enterobacterial species. OXA-48 hydrolyses carbapenems only at a low level, but does not hydrolyse broad-spectrum cephalosporins [6]. The bla_{OXA-48} gene was first identified in a clinical *Klebsiella pneumoniae* strain isolated in Istanbul, Turkey, in 2001. Since then, the OXA-48 enzyme has become endemic in many areas, particularly in the Mediterranean region, especially North African countries [7], where Algeria is known for the huge spread of this gene from different sources, including the community setting. In most parts of the world, it is believed that simultaneous handling of food and money contributes to the frequency of foodborne infectious agents, thus risking the safety of consumers in the community [1,8,9]. To the best of our knowledge, no studies have been published on carbapenemase-producing GNB from currency and no data are available. However, the World Health Organization (WHO) recommends that more studies be conducted in different sites, particularly in African countries, to assess the public-health effects of money contaminated by micro-organisms [3]. Therefore, the aim of this study was to screen for the presence of β-lactamase-producing GNB isolated from Algerian currency collected from food vendors in Batna city, Algeria.

2. Materials and methods

2.1. Sampling

This study was carried out during two periods: May 2018 and March–April 2019. A total of 377 currency coin samples involving eight denominations and 31 currency note samples involving four denominations of Algerian Dinar were randomly collected from markets selling different products, including vegetables, fruit, fish and raw meat products, in Batna city, Algeria. Coins and notes were collected in sterile bottles and bags, respectively. All of the samples were transferred to the laboratory for analysis at 4 °C.

2.2. Screening for β-lactam-resistant gram-negative bacteria

Both sides of each currency note were wiped using a sterile cotton swab pre-dipped in brain–heart infusion (BHI) broth (BD Becton, Dickinson & Co., France) supplemented with 0.05% Tween 80 solution to ensure that the microbial content was dislodged. Swabs used for the same note category collected from the same market and at the same period were pooled (two to four notes per pool) and pre-enriched for 4 h at 37 °C in BHI broth. Currency coins were directly pre-enriched in bottles under the same conditions (eight to ten coins per pool). Thereafter, 1 mL of each pre-enriched sample was transferred into three tubes containing 10 mL of BHI broth supplemented with 64 μg/mL vancomycin and, respectively, three different antibiotics: (i) cefotaxime (2 μg/mL); (ii) ertapenem (2 μg/mL); and (iii) imipenem (9 μg/mL). After overnight incubation at 37 °C, a 10 μL aliquot from positive tubes was inoculated on MacConkey agar supplemented with the corresponding selective antibiotic combination. The obtained GNB isolates were identified using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Microflex; Bruker Daltonik, Bremen, Germany).

Table 1
Antibiotic susceptibility testing results and phenotypic and genetic characteristics of the isolates obtained in this study.

Strain	Source[a]	Species	Antibiotic susceptibility													Phenotypic and molecular characterisation[b]				
			AMX	FOX	CTX	CAZ	FEP	ATM	AMC	TCC	ETP	IPM	TOB	GEN	AMK	CIP	DDST	MHT	MCNP test	ESBL gene	Carbapenemase gene
C1	5DA[1]	*Raoultella ornithinolytica*	R	S	R	R	S	I	S	S	S	S	R	I	S	R	+	N/D	N/D	CTX-M-27	–
C2	5DA[1]	*Enterobacter cloacae*	R	R	R	R	S	S	S	S	S	S	I	S	S	R	–	N/D	N/D	–	–
C3	5DA[1]	*R. ornithinolytica*	R	S	R	R	S	I	S	S	S	S	R	S	S	R	+	N/D	N/D	CTX-M-27	–
C4	5DA[1]	*E. cloacae*	R	R	R	S	S	S	R	R	R	S	S	S	S	S	–	+	+	–	OXA-48
C5	100DA[2]	*E. cloacae*	R	R	R	R	S	R	R	R	S	S	S	S	S	S	–	N/D	N/D	–	–
C6	100DA[2]	*E. cloacae*	R	R	R	R	S	I	R	R	S	S	S	S	S	S	–	N/D	N/D	–	–
C7	100DA[2]	*Pseudomonas aeruginosa*	R	R	R	S	S	S	R	R	R	R	S	S	S	S	–	–	–	–	–
C8	10DA[3]	*Citrobacter freundii*	R	R	R	R	S	I	R	R	S	S	S	S	S	S	–	N/D	N/D	–	–
C9	10DA[3]	*C. freundii*	R	R	R	R	S	I	R	R	S	S	S	S	S	S	–	N/D	N/D	–	–
C10	10DA[4]	*Escherichia coli*	R	S	R	S	R	S	S	S	S	S	S	S	S	R	+	N/D	N/D	CTX-M-14	–
C 11	100DA[5]	*Pseudomonas libanensis*	R	R	R	S	S	R	R	R	R	R	S	S	S	S	–	–	–	–	–
C12	5DA[6]	*Pseudomonas stutzeri*	R	R	R	S	S	S	R	R	R	R	S	S	S	S	–	–	–	–	–

AMX, amoxicillin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; AMC, amoxicillin/clavulanic acid; TCC, ticarcillin/clavulanic acid; ETP, ertapenem; IPM, imipenem; TOB, tobramycin; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; S, susceptible; I, intermediate; R, resistant; DDST, double-disk synergy test; MHT, modified Hodge test; MCNP, modified Carba NP; ESBL, extended-spectrum β-lactamase; DA, Algerian Dinar.

[a] 1, Pool 1 (containing 10 coins of 5 DA 1994 version); [2], Pool 2 (containing 10 coins of 100 DA); [3], Pool 3 (containing 10 coins of 10 DA 1994 version); [4], Pool 4 (containing 8 coins of 10 DA 1979 version); [5], Pool 5 (containing 10 coins of 100 DA); and [6], Pool 6 (containing 10 coins of 5 DA 1994 version).

[b] –, negative; +, positive; N/D, not determined.

2.3. Antibiotic susceptibility testing and minimum inhibitory concentration (MIC) determination

Antimicrobial susceptibility testing was performed on Mueller–Hinton agar (BD, France) by the standard disk diffusion method as recommended by the Antibiogram Committee of the French Society for Microbiology (CA-SFM 2018; https://www.sfm-microbiologie.org/wp-content/uploads/2018/12/CASFMV2_SEPTEMBRE2018.pdf). The following 14 antibiotics were tested: amoxicillin (20 μg); cefoxitin (30 μg); cefotaxime (30 μg); ceftazidime (30 μg); cefepime (30 μg); aztreonam (30 μg); amoxicillin/clavulanic acid (AMC) (20/10 μg); ticarcillin/clavulanic acid (TCC) (75/10 μg); ertapenem (10 μg); imipenem (10 μg); tobramycin (10 μg); gentamicin (10 μg); amikacin (30 μg); and ciprofloxacin (5 μg). In addition, MICs of imipenem were determined by Etest (bioMérieux, Marcy-l'Étoile, France). The results were interpreted according to the CA-SFM (2018) as well as Clinical and Laboratory Standards Institute (CLSI, 2015) breakpoints.

2.4. Phenotypic and molecular characterisation of extended-spectrum β-lactamase and carbapenemase production

The obtained isolates were tested phenotypically for ESBL production by the double-disk synergy test (DDST). The modified Carba NP (MCNP) test and the modified Hodge test (MHT) were applied to screen for carbapenemase activity [6]. Strains were analysed by real-time PCR and were further verified by standard PCR and sequencing using specific primers for ESBL-encoding genes (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) and carbapenemase genes (bla_{NDM}, bla_{VIM}, bla_{KPC} and bla_{OXA-48}).

2.5. Conjugation experiments

The transferability of the bla_{OXA-48} gene was determined by conjugation experiments between the donor isolate and sodium azide-resistant *E. coli* J53 as recipient strain. We separately inoculated the recipient isolate and donor carrying the bla_{OXA-48} gene overnight in BHI broth (10 mL) and then mixed the samples at a donor:recipient ratio of 1:10 followed by incubation for 4 h. Then, 10 μL of the resulting mixture was inoculated onto nutrient agar plates containing 200 μg/mL sodium azide and 2 μg/mL ertapenem and incubated for 18–24 h at 37 °C. The obtained transconjugant was subjected to antimicrobial susceptibility testing, MCNP test and MHT. Positive carbapenemase production was confirmed by OXA-48-PCR and sequencing.

2.6. Multilocus sequence typing (MLST)

MLST was performed for the OXA-48-producing *Enterobacter cloacae* isolate by targeting seven housekeeping genes (*dnaA*, *fusA*, *gyrB*, *leuS*, *pyrG*, *rplB* and *rpoB*). MLST data were analysed through the PubMLST scheme (https://pubmlst.org/ecloacae/).

3. Results

3.1. Bacterial strains

Twelve cefotaxime- and/or carbapenem-resistant isolates were obtained from six currency coin pooled samples (Table 1). However, none were isolated from currency notes. Of the 12 isolates, 9 were Enterobacteriaceae and 3 were non-fermenting bacteria. The obtained isolates were identified as follows: *E. cloacae* (4 strains); *Raoultella ornithinolytica* (2 strains); *Citrobacter freundii* (2 strains); *E. coli* (1 strain); *Pseudomonas aeruginosa* (1 strain); *Pseudomonas libanensis* (1 strain); and *Pseudomonas stutzeri* (1 strain).

3.2. Antimicrobial susceptibility testing and phenotypic detection of β-lactamase determinants

Antibiotic susceptibility tests showed that the 12 strains were resistant to almost all of the antibiotics tested as follows: amoxicillin (12/12; 100%); cefoxitin (9/12; 75%); cefotaxime (12/12; 100%); ceftazidime (7/12; 58%); cefepime (1/12; 8%), aztreonam (2/12; 17%); AMC (8/12; 67%); TCC (8/12; 67%); ertapenem (4/12; 33%); imipenem (3/12; 25%); tobramycin (2/12; 17%); and ciprofloxacin (4/12; 33%); however, they remained susceptible or intermediate to gentamicin and amikacin. Three isolates were positive for ESBL production by the DDST (2 *R. ornithinolytica* and one *E. coli*). The MHT and MCNP test were positive only for one *E. cloacae* strain, whilst no carbapenemase activity was detected in three carbapenem-resistant *Pseudomonas* isolates. Results of antibiotic susceptibility testing and phenotypic characterisation of β-lactamase production are summarised in Table 1.

3.3. Genetic characterisation

Screening for ESBL- and carbapenemase-encoding genes demonstrated that two *R. ornithinolytica* strains carried the $bla_{CTX-M-27}$ gene, one *E. coli* isolate harboured the $bla_{CTX-M-14}$ gene and one *E. cloacae* isolate carried the bla_{OXA-48} gene. None of the isolates were positive for TEM, SHV, KPC, or metallo-β-lactamase-type β-lactamases (NDM, VIM and IMP) (Table 1). MLST analysis showed that the OXA-48-producing *E. cloacae* isolate belonged to sequence type 108 (ST108).

3.4. Conjugation experiments

Conjugative transfer of the bla_{OXA-48} gene to *E. coli* J53 was successful, as the transconjugant obtained on the selective medium containing sodium azide and ertapenem was positive by the MCNP test. The transferability of the bla_{OXA-48} carbapenemase gene was confirmed by PCR and sequencing.

4. Discussion

To the best of our knowledge, this is the first report of OXA-48-carrying Enterobacteriaceae isolated from currency. The role of money in the transmission of infection is not a new concept. It dates back to 1665 in England when coin currency was commonly assumed to be one of the vehicles for the dissemination of plague that killed more than 60 000 people [10]. Studies regarding contamination of coins and paper have been few and rather limited. However, after outbreaks of some transmissible diseases, these reports have gained some serious consideration [11,12] and revealed that coins and paper currency can serve as an ideal breeding site for the multiplication of micro-organisms [1]. In this study, we isolated different GNB species, in agreement with several studies carried out on coin and paper currency collected from different sites including hospitals, public transport conductors and food vendors among different countries [1–3,9,13]. Most of the bacterial species identified here have been implicated in many life-threatening infections, which could pose a health risk particularly to immunocompromised individuals in the community. In addition to *P. libanensis* detected here for the first time from currency, the other GNB species have been already described with different levels of antibiotic resistance [3,8,9,13,14]. The obtained isolates could be from several sources depending on environmental conditions, age of money, handling, or the poor sanitary conditions and hygienic practices exercised by those manipulating currencies [2,3]. Detection of coliforms such *E. coli*, which can survive for 11 days on inert surfaces, could suggest faecal contamination of currency coins as a result of poor personal hygiene [1]. Banknotes

offer a large surface to be contaminated by micro-organisms, which can persist on it for longer periods. In addition, the surface of paper currency is irregular, thus facilitating adherence by different types of micro-organisms [3]. However, in our study, none of the antibiotic-resistant strains were isolated from our Algerian paper-based banknotes, which could be explained by the limited number of note samples analysed (31 samples) and the absence or low bacterial load of antibiotic-resistant GNB strains as well as the age of the paper collected, since newer paper notes contain less microbes [2,3]. Algerian coins are composed of nickel, AISI 430 steel and cooper, and these compounds have been shown to possess antimicrobial activity, which may explain the low rate of bacterial contamination observed in our study [3,15].

There are several investigations confirming that antibiotic-resistant bacteria may contaminate money and that this might play an important role in their spread. Few studies have focused on antibiotic resistance among GNB recovered from currency. In our study, we followed the selective isolation approach, which allowed us to isolate 12 drug-resistant GNB. Two of them harboured the $bla_{\text{CTX-M-27}}$ gene (both *R. ornithinolytica*) and one *E. coli* strain carried the $bla_{\text{CTX-M-14}}$ gene. To the best of our knowledge, this is the first description of CTX-M-27-producing Enterobacteriaceae isolates in Algeria. In this context, only one report describing the presence of the $bla_{\text{CTX-M}}$ gene in *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. aeruginosa* and *Acinetobacter baumannii* from banknotes has been published in Sudan, where, different from our study, they evaluated the bacterial contamination rate of 135 banknotes showing a contamination rate of 100% [14]. The $bla_{\text{CTX-M-27}}$ gene was first described in an *E. coli* strain isolated in 2001 from a patient hospitalised in Grenoble, France, which also co-harboured the $bla_{\text{TEM-1}}$ gene [16]. After this report, several studies showed that CTX-M-27 is emerging worldwide, especially in Europe and Japan [17]. Furthermore, the $bla_{\text{CTX-M-14}}$ gene has already been detected in multidrug-resistant *E. coli* isolated from fresh ground beef in Algiers [18]. Recently, Nabti et al. reported the isolation of four *E. coli* strains that co-harboured the $bla_{\text{CTX-M-14}}$ and $bla_{\text{OXA-1}}$ ESBL genes or $bla_{\text{CTX-M-14}}$ with the $bla_{\text{TEM-1}}$ β-lactamase gene from outpatients and inpatients in Setif University Hospital [19]. Worryingly, our study is the first to report the detection of OXA-48-producing *E. cloacae* from currency coin. Moreover, it is of special interest that the OXA-48-like enzyme has been identified in Algeria, a part of North Africa, where the OXA-48-type carbapenemase is considered endemic. Indeed, various studies on Enterobacteriaceae producing OXA-48-like enzymes have been published in Algeria involving clinical strains recovered from various hospitals located in different regions in Algeria, including outbreaks [6,7], as well as from companion and wild animals, river water and fresh vegetables [7]. More recently, the $bla_{\text{OXA-48}}$ gene has also been detected in the Algerian community [5]. The OXA-48-producing *E. cloacae* isolate identified in the current study was shown to belong to ST108. This high-risk international clone (ST108) has been previously associated with CTX-M-9 and SHV-12 co-production as well as TEM-3 and SHV-12 production in clinical *E. cloacae* strains recovered from different European countries including France, Greece, Italy and Luxembourg [20]. In addition, this study reports the first detection of *E. cloacae* ST810 clone in Algeria.

In conclusion, our findings showed that Algerian currency may play an important role in the transmission of carbapenem-resistant GNB, particularly those producing the silent menace OXA-48. These data constitute a starting point for further research; nevertheless, more studies are needed to determine the potential role of currency in the dissemination of these high levels of resistance to β-lactams in the community. Thus, it calls for consciousness development at all levels, especially among personnel working in food establishments, on the possible health risks associated with poor handling of currency.

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Ethical approval

Not required.

Conflict of interests

None declared.

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