JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Characterization of carbapenemase-producing Gram-negative bacilli: first report of *blaNDM-1* in *Enterobacter cloacae*

Manel Debabza[1], Raoudha Dziri[2,3], Abdelbasset Mechai[1], Amel Bouguessa[4], Naouel Klibi[2], Hadda Imene Ouzari[2]

[1] Bioactive Molecules and Applications Laboratory, Faculty of Exact Sciences and Natural and Life Sciences, Echahid Cheikh Larbi Tebessi University (E.C.L.T.U)-Tebessa, Algeria
[2] Laboratory of Microorganisms and Active Biomolecules, Faculty of Sciences of Tunis, University of Tunis El Manar, El-Manar II, Tunisia
[3] University of Tunis El Manar, Laboratory of Epidemiology and Veterinary Microbiology, Group of Bacteriology and Biotechnology, Institute Pasteur of Tunis, BP 74, 13 Place Pasteur, Belvédère, 1002, Tunis, Tunisia
[4] Microbiology Laboratory, Faculty of Exact Sciences and Natural and Life Sciences, Echahid Cheikh Larbi Tebessi University (E.C.L.T.U)-Tebessa, Algeria

Abstract

Introduction: The spread of multidrug-resistant bacteria, particularly carbapenem-resistant Gram-negative bacilli (CR-GNB), has become a serious challenge for clinicians due to limited therapeutic options. The aim of the study was to investigate the prevalence of carbapenemase production among clinical isolates recovered from 352 samples collected in Tebessa hospital, Algeria.

Methodology: Bacterial isolates were identified by 16S RNA gene sequencing and susceptibility to antibiotics was determined by disk diffusion method. Carbapenem-resistant isolates were screened for carbapenemase production using modified carba Nordmann-Poirel test, modified Hodge test and imipenem-EDTA combined disc test. Extended-spectrum β-lactamases (ESBL) were detected using double-disk synergy test. Molecular characterization of carbapenemases and ESBL genes was performed by polymerase chain reaction (PCR) and sequencing.

Results: A total of 85 Gram-negative bacilli isolates were recovered mainly from urine samples and were identified as: *Klebsiella pneumoniae* (17.65%), *Serratia odorifera* (15.29%), *Escherichia coli* (12.94%), *Raoultella ornithinolytica*, *Enterobacter cloacae* (11.76%), *Serratia marcescens* (10.59%), *Morganella morganii* (7.06%), *Proteus mirabilis* (5.88%), *Acinetobacter baumannii* (4.70%) and *Pseudomonas aeruginosa* (2.35%). All strains were resistant or intermediate to imipenem and/or ertapenem. ESBL, carbapenemase and metallo-beta-lactamases (MBL) phenotypes were detected in 19 (22.35%), 9 (10.59%) and 2 (2.35%) GNB isolates, respectively. PCR results in nine carbapenemase-producing GNB strains chosen showed the presence of one to four carbapenemase genes (bla$_{GES}$, *bla*$_{SME}$, *bla*$_{NDM-1}$, *bla*$_{VIM}$, *bla*$_{GIM}$, *bla*$_{SPM}$, *bla*$_{OXA-48}$) in four strains; however, seven strains had at least one ESBL gene (*bla*$_{TEM-1}$, *bla*$_{CTXM-15}$, *bla*$_{SHV}$).

Conclusions: In this study, we report the first incidence of *bla*$_{NDM-1}$ gene in *Enterobacter cloacae* isolated from urine sample in Algeria.

Key words: Gram-negative bacilli; Extended-spectrum β-lactamases; Carbapenemases; Metallo-beta-lactamases; *Enterobacter cloacae*; *blaNDM-1*.

J Infect Dev Ctries 2023; 17(9):1300-1309. doi:10.3855/jidc.18031

(Received 05 February 2023 – Accepted 10 May 2023)

Introduction

Carbapenems are a class of antimicrobial agents used most frequently as last resort antibiotics for the treatment of infections with multidrug-resistant (MDR) Gram-negative bacilli (GNB), since they have the wide spectrum of bactericidal action and stability against most of the β-lactamases including ESBLs [1,2]. However, with the extensive use of these antibiotics, the number of carbapenem-resistant *Enterobacteriaceae* (CRE) are emerging and increasing rapidly [3]. Carbapenemase is the main determinant contributing to carbapenem resistance in *Enterobacteriaceae*. Indeed, the widespread use of these antibiotics has caused the expansion of resistant *Enterobacteriaceae* [4]. The concern about carbapenemase-producing Gram-negative bacilli (CP-GNB) that has now emerged is that it is often associated with the occurrence of MDR isolates for which there are few antimicrobial options available [5]. Carbapenemases are β-lactamases that hydrolyze carbapenems (imipenem, meropenem, and ertapenem). These β-lactamases are now extensively identified in GNB, particularly in *Enterobacteriaceae* [6].

Various carbapenemases have been reported in *Enterobacteriaceae* including *Enterobacter*, *Klebsiella*, *Escherichia coli*, *Serratia* [7] and other opportunistic

GNB such as *Acinetobacter* and *Pseudomonas* [8]. Nevertheless, carbapenem resistance in GNB may be due to other drug resistance mechanisms such as modification or loss of porins and efflux pumps [9].

One of the main reasons for the rapid spread of CRE through bacterial populations is that genes conferring resistance are carried on plasmids or on other highly movable genetic elements that are independently replicated and passed between bacterial cells and species [10]. Since the late 1990s, different types of carbapenemases have been recognized belonging to three molecular classes, Ambler classes A, B and D beta-lactamases [11]: class A carbapenemases such as Guiana extended spectrum β-lactamase (GES), *Serratia marcescens* enzyme (SME), sulfhydryl variable lactamase (SHV), *Klebsiella pneumoniae* carbapenemase (KPC), imipenemase/non-metallocarbapenemase-A (IMI/NMC-A) and *Serratia fonticola* carbapenemase (SFC); class B metallo-beta-lactamases (MBLs) such as imipenemase (IMP), Verona integrated-encoded MBL (VIM), Sao Paulo MBL (SPM), Germany imipenemase (GIM), New Delhi MBL (NDM) and Florence imipenemase (FIM); and carbapenem-hydrolysing class D β-lactamases or oxacillinases (OXA) such as OXA-48 enzymes [7,12].

Among the recent spread of multidrug-resistant bacteria, outbreaks of ESBLs and carbapenemase-producing GNB are a serious problem not only making treatment difficult but also worsening the prognosis of infected patients. In this background, the objective of our study is to assess the prevalence of antibiotic resistance and to characterize carbapenemase-producing strains among clinical GNB isolates collected in Tebessa hospital, Algeria, using phenotypic (modified Hodge test, Carba Nordmann-Poirel NP test, imipenem-EDTA combined disc test) and molecular (polymerase chain reaction - PCR and sequencing) tests.

Methodology

Study setting and bacterial isolates

A total of 85 non-redundant GNB strains were isolated during the period between February and May 2018, at Tebessa hospital, from 352 clinical specimens including: urine (n = 281), pus (n = 28), dialysis fluid (n = 21), blood culture (n = 13) and cerebrospinal fluid (n = 9). Approximately 15000 patients were admitted at the outpatient department per year and more than 900 operations and invasive diagnostic therapeutic procedures were performed annually in this hospital. Samples taken from patients hospitalized in various services and from outpatients were cultured on Mac Conkey agar and cetrimide agar (Fluka, La Chapelle-sur-Erdre, Cedex, France). GNB identification was based on colony morphology and biochemical characteristics using API 20E, a semi-automatized assay (bioMérieux, Marcy l'Etoile, France). Isolates were frozen at -30 °C in brain-heart infusion broth with 15% glycerol until processed for further experimentation.

Antibiotic susceptibility testing

Antibiotic susceptibility testing of *Enterobacteriaceae* and non-fermentative GNB (NF-GNB) isolates was performed on Mueller-Hinton agar (BioMérieux, Marcy-l'Étoile, France) by standard disk diffusion method, using disk antibiotics (Liofilchem, Roseto degli Abruzzi TE, Italy) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [13]. *Klebsiella pneumoniae* ATCC700603 and *Escherichia coli* ATCC25922 strains were used as controls. The isolates were defined as MDR when they were resistant to at least three antibiotics from different classes [14]. The minimal inhibitory concentrations (MICs) of antibiotics were determined by the microdilution method recommended by EUCAST [13].

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

The double-disk synergy test (DDST) was carried out according to Jarlier *et al.* [15]. Third-generation cephalosporin disks, cefotaxime (CTX; 30 µg), ceftazidime (CAZ; 30 µg), ceftriaxone (CRO; 30 µg), or aztreonam disk (ATM; 30 µg) was placed 30 mm (center to center) from a central disk containing clavulanic acid (Amoxicillin-clavulanic acid - AMC 20/10 µg for *Enterobacteriaceae* and ticarcillin/clavulanic acid - TCC 75/10 µg for NF-GNB). ESBL production was suspected when the zone of inhibition around any of the four antibiotic disks was enhanced on the side of the disk containing clavulanic acid, resulting in a characteristically shaped zone referred to as a "champagne-cork".

Phenotypic carbapenemase detection

Isolates with decreased susceptibility (intermediate/resistant) to at least one of the carbapenems should be considered as carbapenem-resistant (CR) and suspicious for carbapenemase-producing (CP). Thus, isolates with a reduced sensitivity to ertapenem or to imipenem were screened for carbapenemase-producing strains by the Carba NP test and the modified Hodge test [13].

Modified Hodge test (MHT)

CR isolates were subjected to HT as was described in the CLSI guidelines [16]. Mueller-Hinton agar (MHA) plates were inoculated with an overnight culture of *E. coli* ATCC 25922 adjusted to one tenth turbidity of 0.5 McFarland. The plates were left for 15 minutes to dry and then ertapenem disc (10 µg) was placed at the center of the plate. Using a swab, overnight cultures of the tested isolates (3-5 colonies) were streaked from the edge of disc to the periphery of the plates and the plates were incubated at 37 °C for 24h. Carbapenemase-producer isolates were indicated by enhanced growth of *E. coli* around tested isolate, expressed as clover leaf like indentation, while no enhanced growth of *E. coli* indicates a non carbapenemase-producing isolate.

Carba NP test

The Carba NP test was performed following the protocol recommended by CLSI [16]. Briefly, bacteria were cultured overnight on MHA and then the bacterial mass was scraped off with a 1µL loop and suspended in a 1.5 mL Eppendorf tube containing 100 µL of 20mM Tris-HCl lysis buffer and mixed using a vortex device for 5 seconds. This lysate was mixed with 100 µL of an aqueous indicator solution consisting of 0.05% phenol red with 0.1 mmol/L $ZnSO_4$, previously adjusted to pH 7.8 and 6 mg/mL imipenem (reaction tube) and, as a control tube, the phenol red solution without antibiotic. Tubes were vigorously mixed for 5 to 10 seconds before incubation. Finally, tubes were incubated at 37 °C and monitored for 2 hours for colour change from red to orange/yellow in the tube containing antibiotic, which was interpreted as a positive result.

Imipenem-EDTA combined disc test

This test is used to detect carbapenemases class B (MBL) which are inhibited by EDTA. It was carried out for Carba NP test (+) and/or MHT (+) strains. Imipenem-EDTA combined disc test was conducted according to Prakash *et al.* [17]. The tested isolate was inoculated by swab on MHA, then a 10 µg imipenem (IPM) disk was placed on the plate at a distance of 20 mm from an Imipenem-EDTA (EIP) disk and the plates were incubated overnight at 37 °C. After incubation, the zone of inhibition of the imipenem and the imipenem-EDTA disks were compared. If the increase in the zone of inhibition with EIP disk exceeded 7 mm than IPM disk, the isolate was considered to be MBL positive.

Molecular characterization of ESBL and carbapenemase genes

Carbapenemase-producing isolates confirmed by phenotypic tests were subjected to PCR assay to ensure the presence of the following resistance genes: ESBLs genes: bla_{TEM} [18], bla_{SHV} [19], $bla_{CTX\text{-}MU}$ [20]; carbapenemases class A: bla_{KPC}, bla_{GES}, bla_{IMI} and bla_{SME} [21]; carbapenemases class D: $bla_{OXA\text{-}48}$ [22]. Isolates positive for imipenem-EDTA combined disc test were further tested for the presence of class B carbapenemases (metallo-β-lactamases) genes: bla_{IMP}, bla_{GIM}, bla_{SPM}, $bla_{SIM,}$ bla_{VIM} and bla_{NDM} [23].

Table 1. *Distribution of isolated Gram-negative bacilli (GNB) strains by species, wards, and type of sampling.*

Species	Wards							Type of sampling				
	Infectious	**Woman medicine**	**Phthisiology**	**Oncology**	**Internal Medicine**	**Dialysis**	**External patient**	**Urine**	**Pus**	**Blood culture**	**Dialysis fluid**	**Cerebro spinal fluid**
Enterobacteriaceae												
K. pneumoniae (n = 15)	4	0	0	4	2	1	4	14	1	0	0	0
R. ornithinolytica (n = 10)	0	1	0	6	0	1	2	9	0	0	1	0
E. cloacae (n = 10)	2	1	0	5	0	0	2	6	2	0	2	0
S. odorifera (n = 13)	3	0	0	0	4	0	6	10	0	1	2	0
S. marcescens (n = 09)	0	4	0	4	1	0	0	6	0	0	3	0
E. coli (n = 11)	3	0	4	1	2	1	0	7	0	1	0	3
P. mirabilis (n = 05)	1	0	0	1	0	0	3	2	1	1	1	0
M. morganii (n = 06)	2	0	0	0	2	2	0	3	1	0	2	0
Non-fermentative Gram-negative bacilli												
P. aeruginosa (n = 02)	2	0	0	0	0	0	0	2	0	0	0	0
A. baumannii (n = 04)	2	0	0	1	0	1	0	4	0	0	0	0
Total (n = 85)	19	6	4	22	11	6	17	63	5	3	11	3

Amplifications were performed on a T3000 thermocycler (Biometra, Doncaster, United Kingdom) and the PCR products were visualized in a 2% (W/V) Tris-Borate-EDTA agarose gel under ultraviolet illumination at a wavelength of 312 nm. All PCR products were sequenced and compared to reported sequences available in GenBank.

Molecular identification of isolates

Bacterial isolates were grown in Luria-Bertani medium for 24 h at 30 °C. Thereafter, the genomic DNA was extracted as described by Wang *et al.* [24]. The 16S rRNA amplification was carried out using primers 27F and 1492R. PCR amplifications were carried out in a final volume of 50 µL, containing 25 µL of 2 × *Taq* PCR Master Mix (TransGen Biotech, Beijing, CHINA), 2 µL of each primer (10 µmol L^{-1}), 2 µL of template DNA (10 ng μL^{-1}), and 19 µL of ddH_2O. The cycling conditions were optimized with an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. The amplicons were sequenced and the gene sequences obtained were analysed and blast-searched in the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Bacterial isolates

In the present study, a total of 85 GNB with different colony morphologies were recovered from 352 clinical specimens including urine (63), dialysis fluid (11), pus (5), blood culture (3) and cerebrospinal fluid (3). Of these, 79 (92.94%) isolates were identified as members of *Enterobacteriaceae* family and 6 (7.06%) isolates were identified as non-fermentative Gram-negative bacilli (NF-GNB). The distribution of the isolates from various samples and wards is presented in Table 1.

The identification tests indicated that *Klebsiella pneumoniae*, was the most prevalent with 15 isolates (17.65%) followed by *Serratia odorifera* with 13 (15.29%), *E. coli* with 11 (12.94%), *Raoultella ornithinolytica* and *Enterobacter cloacae* with 10 (11.76%), *Serratia marcescens* with 9 (10.59%), *Morganella morganii* with 6 (7.06%) and *Proteus mirabilis* with 5 (05.88%) isolates. In addition, 4 strains (4.70%) of *Acinetobacter baumannii* and 2 strains (2.35%) of *Pseudomonas aeruginosa* were isolated.

The distribution of isolates from various clinical specimens showed that urine was the source of 63/85 (74.12%) of the isolates. Dialysis fluid (12.94%) was the second major source of isolates reflecting the relatively high frequency of GNB involved in bacteremia. The remaining isolates collected in our study were from pus, blood culture and cerebrospinal fluid.

Antibiotic susceptibility and phenotypic characterization

Antimicrobial resistance patterns of GNB isolates are summarized in Table 2. The results showed that most of the isolates were resistant to amoxicillin, amoxicillin/clavulanic acid, ticarcillin, cephalexin and cotrimoxazol. The isolates had a high resistance rate to

Table 2. Antibiotic resistance patterns of Gram-negative bacilli (GNB) strains.

ATB	Resistance pattern (%)									
	K. pneumoniae (n = 15)	*R. ornithinolytica* (n = 10)	*E. cloacae* (n = 10)	*S. odorifera* (n = 13)	*S. marcescens* (n = 09)	*E. coli* (n = 11)	*P. mirabilis* (n = 05)	*M. morganii* (n = 06)	*P. aeruginosa* (n = 02)	*A. baumannii* (n = 04)
AMX	15 (100)	10 (100)	10 (100)	13 (100)	9 (100)	9 (81.82)	5 (100)	6 (100)	/	/
AMC	9 (60)	6 (60)	10 (100)	12 (92.31)	9 (100)	6 (54.55)	3 (60)	6 (100)	/	/
TIC	15 (100)	10 (100)	8 (80)	8 (61.54)	8 (88.89)	8 (72.73)	4 (80)	4 (66.67)	1 (50)	3 (75)
PTZ	8 (53.33)	2 (20)	6 (60)	2 (15.38)	3 (33.33)	1 (9.1)	0 (0)	1 (16.67)	0 (0)	2 (50)
CL	13 (86.67)	9 (90)	10 (100)	12 (92.31)	9 (100)	9 (81.82)	3 (60)	6 (100)	/	/
FOX	6 (40)	1 (10)	10 (100)	4 (30.77)	9 (100)	2 (18.2)	0 (0)	1 (16.67)	/	/
CTX	8 (53.33)	6 (60)	5 (50)	3 (23.08)	6 (66.67)	1 (9.1)	1 (20)	2 (33.33)	/	4 (100)
CAZ	8 (53.33)	7(70)	4 (40)	4 (30.77)	6 (66.67)	1 (9.1)	2 (40)	3 (50)	1 (50)	4 (100)
ETP	14 (93.33)	7 (70)	3(30)	8 (61.54)	4 (44.44)	4 (36.36)	2 (40)	2 (33.33)	2 (100)	3 (75)
IPM	14 (93.33)	6 (60)	7 (70)	12 (92.31)	7 (77.78)	10 (90.91)	4 (80)	3 (50)	2 (100)	3 (75)
AK	0 (0)	1 (10)	0 (0)	0 (0)	2 (22.22)	1 (9.1)	0 (0)	0 (0)	0 (0)	1 (25)
GN	7 (46.67)	4 (40)	5 (50)	6 (46.15)	7 (77.78)	3 (27.27)	2 (40)	4 (66.67)	1 (50)	2 (50)
TOB	8 (53.33)	5 (50)	6 (60)	6 (46.15)	9 (100)	2 (18.2)	1 (20)	3 (50)	1 (50)	3 (75)
NA	13 (86.67)	8 (80)	9 (90)	7 (53.85)	5 (55.56)	2 (18.2)	3 (60)	2 (33.33)	/	/
CIP	9 (60)	6 (60)	7 (70)	5 (38.46)	4 (44.44)	2 (18.2)	2 (40)	1 (16.67)	1 (50)	3 (75)
OFX	8 (53.33)	5 (50)	5 (50)	4 (30.77)	4 (44.44)	3 (27.27)	2 (40)	1 (16.67)	/	/
NIT	6 (40)	7(70)	4 (40)	8 (61.54)	9 (100)	2 (18.2)	5 (100)	6 (100)	/	/
COT	9 (60)	6 (60)	6 (60)	10 (76.92)	8 (88.89)	6 (54.55)	3 (60)	5 (83.33)	/	4 (100)

ATB: Antibiotic; AMX: Amoxicillin; AMC: Amoxicillin/ Clavulanic acid; TIC: Ticarcillin; PTZ: Piperacillin+ tazobactam; CL: Cephalexin; FOX: Cefoxitin; CTX: Cefotaxime; CAZ: Ceftazidime; ETP: Ertapenem; IPM: Imipenem; AK: Amikacin; GN: Gentamicin; TOB: Tobramycin; NA: Nalidixic acid; CIP: Ciprofloxacin; OFX: Ofloxacin; NIT: Nitrofurantoin; COT: Cotrimoxazol.

Table 3. Results of phenotypic detection of extended-spectrum β-lactamases (ESBLs), carbapenemases and metallo-beta-lactamases (MBLs).

Bacterial species	ESBLs detection	Carbapenemases detection		MBLs detection
	DDST	Carba NP test	Modified Hodge test (MHT)	Imipenem-EDTA combined disc test
Klebsiella pneumoniae (n = 15)	3	0	0	0
Raoultella ornithinolytica (n = 10)	1	0	0	0
Enterobacter cloacae (n = 10)	4	3	3	1
Serratia odorifera (n = 13)	1	1	1	0
Serratia marcescens (n = 09)	3	2	1	0
Escherichia coli (n = 11)	2	0	0	0
Proteus mirabilis (n = 05)	1	0	0	0
Morganella morganii (n = 06)	1	0	0	0
Pseudomonas aeruginosa (n = 2)	0	0	0	0
Acinetobacter baumannii (n = 04)	3	3	2	1
Total (%) = 85 (100)	19 (22.35)	09 (10.59)	07 (08.23)	02 (2.35)

fluoroquinolones (ciprofloxacin), exceeding 64% for *K. pneumoniae*, *R. ornithinolytica*, *E. cloacae* and *A. baumannii*. In addition, the carbapenem resistance rate showed a worrying trend with 49/85 isolates (57.65%) for ertapenem and 68/85 isolates (80%) for imipenem. Besides, variable rates of resistance were noted for other antibiotics. Our results showed also that amikacin was the most active antibiotic with 80/85 sensitive strains (94.12%) for this antibiotic. Among the 85 GNB isolated, 58 (68.23%) strains exhibited MDR patterns to different classes of antibiotics.

The DDST results showed that 19/85 isolates (22.35%) were ESBL producers (Table 3). In the present study, all strains showed decreased sensitivity to at least one of the carbapenems tested (imipenem or ertapenem). Therefore, they were all screened for carbapenemase production by the Carba NP test and MHT. Results showed that Carba NP test was positive for 9/85 isolates (10.59%) and MHT was positive for 7/85 isolates (8.23%). The imipenem-EDTA combined disc test was positive for only two carbapenem-resistant strains (Figure 1), which indicates the presence of metallo-β-lactamase production in these strains.

Molecular characterization of ESBL and carbapenemase genes

In this study, 9 strains were detected as CP-GNB (Table 4) and they were subjected to molecular characterization for resistance genes. PCR for ESBL genes showed that seven strains contained bla_{TEM} ± bla_{CTX-M} genes: *S. marcescens* (two strains), *E. cloacae* (two strains) and *A. baumannii* (three strains) (Figure 2). In addition, bla_{SHV} was detected in three strains: *A. baumannii* (two strains) and *E. cloacae* (one strain). Sequencing analysis of resistance genes encoding ESBL producers revealed that these genes were bla_{TEM-1}, $bla_{CTXM-15}$ and bla_{SHV}. Moreover, the bla_{SHV} and

Table 4. Phenotypic and genotypic features of carbapenem-resistant Gram-negative bacilli clinical isolates.

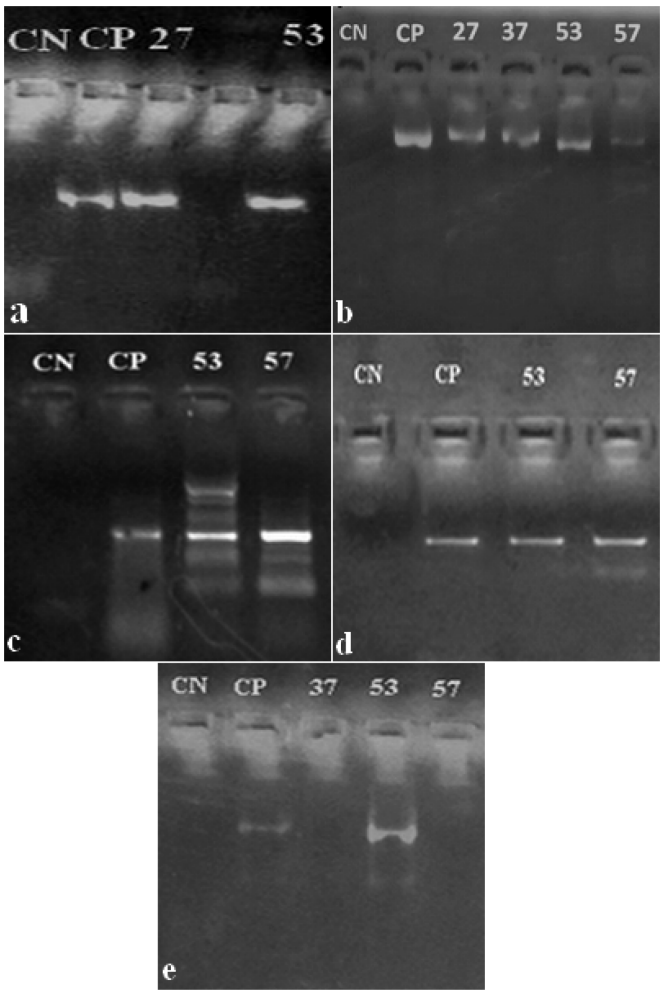
Strains	Sample source	Ward	Resistance profil	MIC (mg/L)	Phenotypic detection of carbapenemases and MBLs enzymes			ESBL and carbapenemases genes
					Carba NP test	Modified Hodge test (MHT)	Imipenem-EDTA combined disc test	
Serratia marcescens 27	Urine	*Womens* Health	AMX/AMC/TIC/CL/FOX/ CTX/CAZ/NIT	CTX 8, CAZ 16	+	-	-	$bla_{CTXM-15}$,bla_{TEM-1}
Serratia marcescens 30	Urine	*Womens* Health	AMX/AMC/TIC/CL/FOX/ CTX/CAZ/NIT	CTX 8, CAZ 16	+	+	-	$bla_{CTXM-15}$, bla_{TEM-1}
Acinetobacter baumannii 37	Urine	Oncology	TIC/CTX/CAZ/ETP/CIP/ COT	CTX 4, CAZ 12 ETP 1	+	-	-	bla_{TEM-1}
Enterobacter cloacae 53	Urine	External patient	AMX/AMC/TIC/PTZ/CL/ FOX/CTX/CAZ/IPM/ETP/GN/ TOB/NA/OFX/CIP/NIT/COT	CTX, CAZ ≥ 64, ETP ≥ 8, IPM ≥ 8	+	+	+	$bla_{CTXM-15}$, bla_{TEM-1}, bla_{NDM-1}, bla_{VIM} ,bla_{SME} , $bla_{OXA\ 48}$
Acinetobacter baumannii 57	Urine	External patient	TIC/PTZ/CTX/CAZ/IPM/ ETP/GN/TOB/CIP/COT	CTX ≥ 64, CAZ 16, ETP ≥ 8, IPM ≥ 8	+	+	+	bla_{TEM}, bla_{SHV}, bla_{NDM-1}, bla_{VIM}, bla_{GIM}, bla_{SPM}
Acinetobacter baumannii 60	Urine	External patient	TIC/PTZ/CTX/CAZ/GN/CIP	CTX ≥ 64, CAZ 16	+	+	-	bla_{TEM-1}, bla_{SHV}
Serratia odorifera 64	Urine	Oncology	AMX/AMC/TIC/FOX/CTX/CA Z/IPM/ETP	CTX 2, CAZ 4 ETP 2, IPM ≥ 8	+	+	-	bla_{GES}
Enterobacter cloacae 73	Pus	External patient	AMX/AMC/TIC/CL/FOX/ CTX/CAZ/ETP/ GEN/TOB/NA/OFX/CIP	CTX 4, CAZ 16, ETP 2, IPM ≥ 8	+	+	-	bla_{SME}
Enterobacter cloacae 75	Urine	External patient	AMX/AMC/TIC/CL/FOX/ CTX/CAZ/ETP/ GEN/OFX/CIP	CTX 4, CAZ 32, ETP 1	+	+	-	$bla_{CTXM-15}$, bla_{TEM-1}, bla_{SHV}

MIC: minimal inhibitory concentration; ATB: Antibiotic; AMX: Amoxicillin; AMC: Amoxicillin/ Clavulanic acid; TIC: Ticarcillin; PTZ: Piperacillin+ tazobactam; CL: Cephalexin; FOX: Cefoxitin; CTX: Cefotaxime; CAZ: Ceftazidime; ETP: Ertapenem; IPM: Imipenem; AK: Amikacin; GN: Gentamicin; TOB: Tobramycin; NA: Nalidixic acid; CIP: Ciprofloxacin; OFX: Ofloxacin; NIT: Nitrofurantoin; COT: Cotrimoxazol.

$bla_{CTX\text{-}M\text{-}15}$ combination was detected only in one strain identified as *E. cloacae*.

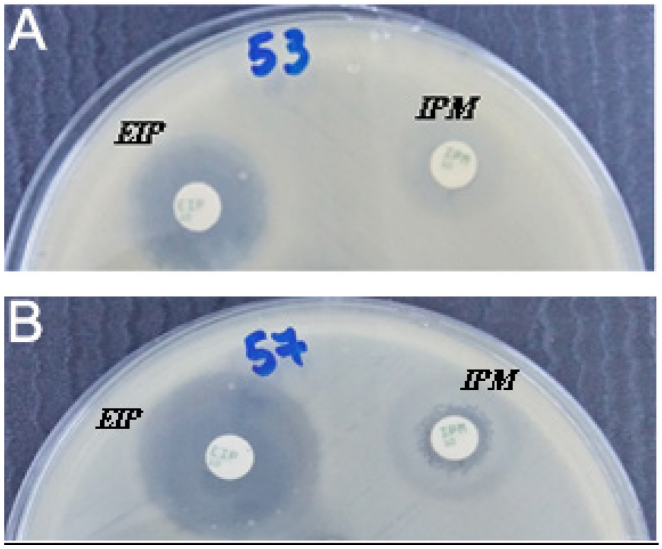
The CR-GNB strains were further tested to check if they carried carbapenemase genes. The PCR results (Table 4) showed that 4 out of 9 CP-GNB strains harbored at least one of the carbapenemase genes tested in our investigation. The genes detected were: bla_{GES} (one strain of *S. odorifera*) and bla_{SME} (two strains of *E. cloacae*) encoding class A carbapenemases; bla_{VIM} and bla_{NDM} (one strain of *E. cloacae* and one strain of *A. baumannii*) (Figure 2), bla_{GIM} and bla_{SPM} (one strain of *A. baumannii*) encoding class B carbapenemases (MBL); $bla_{OXA\text{-}48}$ (one strain of *E. cloacae*) encoding class D carbapenemases (Figure 2). Interestingly, we have noted that one strain of *E. cloacae* and one strain of *A. baumannii* harbored more than three types of carbapenemase genes.

Figure 2. PCR amplification of extended-spectrum β-lactamases (ESBLs) and carbapenemase genes from Gram-negative bacteria isolates.



a: $bla_{CTX\text{-}M}$ (593 pb), b: bla_{VIM} (390 pb), c: bla_{NDM} (620 pb), d: $bla_{OXA\text{-}48}$ (743 pb), e: bla_{TEM} (1150 pb). CP: positive control; CN: negative control; 27: *Serratia marcescens*; 37, 57: *Acinetobacter baumannii*; 53: *Enterobacter cloacae*.

Figure 1. Positive imipenem-EDTA combined disc test in metallo-beta-lactamase (MBL) strains.



A: *Enterobacter cloacae* 53; B: *Acinetobacter baumannii* 57; IPM: imipenem; EIP: imipenem + EDTA.

Discussion

Carbapenems are generally considered the most effective antibacterial agents for the treatment of multidrug-resistant bacterial infections. Unfortunately, with the widespread use of these antibiotics, CRE have increased dramatically over the last few years, and have been reported in many countries [2,25].

The present study investigated antibiotic susceptibility patterns and characterized ESBL and carbapenemase genes among isolates of GNB recovered from different clinical samples. Antibiotic susceptibility testing showed that out of 85 GNB isolated from urine, 58 (68.23%) were found to be MDR. This finding is higher than those reported at Annaba hospital - Algeria (35.53%) [26] and at Guelma hospitals (29%) [27]. The high prevalence of MDR-GNB reported worldwide may be attributed to higher selection pressure due to self-medication, misuse or overuse of carbapenems and third generation cephalosporins, frequent use of invasive devices and prolonged hospitalization [8].

Over the past decade, global data on ESBL-producing *Enterobacteriaceae* (ESBL-E) in North African countries have become extremely worrying and this region may well be one of the main epicenters of the global pandemic of ESBL. In the present study, the prevalence of ESBL-producing GNB was 22.35%, which is almost similar to that found in some North

African countries. In Algeria, data indicate a high prevalence of ESBL-E, where their prevalence has increased from 43.73% in 2014 to 44.16 % in 2015 [26]. In recent study from Egypt, ESBL-E prevalence among clinical strains varied between 38.8% and 70.6% both in hospital and community acquired urinary tract infections, and the most frequently detected beta-lactamase genes were $bla_{\text{CTX-M}}$ and bla_{TEM} [28]. In Tunisia, the presence of ESBL-E has been increasingly reported, and their prevalence ranged from 11.7 to 77.8% causing both community acquired and hospital acquired infections [29]. In Morocco, low prevalence rates between 1.3 and 7.5% have been found, of which $bla_{\text{CTX-M}}$ gene was the most prevalent [30]. A survey conducted in Libya showed a prevalence of ESBL-E between 6.7 and 32.6% in hospital samples and 13.4% in community samples [31].

Furthermore, the emergence of ertapenem or imipenem-resistant GNB has become a universal concern leaving very few therapeutic options, as these molecules have been considered as preferred agents for the treatment of patients with moderate-to-severe infections and risk factors for infection with multidrug-resistant GNB [26,32]. The prevalence of carbapenemase-producing GNB strains in our study was 10.59% which is comparable with previous reports in Algeria [33]. Due to the massive spread of CP GNB with overwhelming consequences in health care settings, the illogical and very common use of carbapenems has resulted in the emergence of CR-GNB strains [34].

Molecular identification of β-lactamases is considered to be a substantial trait for a reliable epidemiological investigation of antimicrobial resistance. Three types of ESBL were detected in our study: CTX-M-15, SHV and TEM-1. These findings corroborated a recent study [12] reporting ESBL-E co-harboring extended-spectrum β-lactamase genes in community-acquired urinary tract infection. The emergence of the $bla_{\text{CTX-M-15}}$ variant has been revealed to be attributed to the horizontal gene transfer of genetic elements and the clonal expansion of microorganisms [30]. Furthermore, the widespread and unnecessary use of ceftriaxone and cefotaxime has led to the emergence and spread of $bla_{\text{CTX-M}}$ resistance genes [25]. In concordance with previous reports, the co-existence of two or three different ESBL genes was detected in six of our isolates (Table 4). However, the most common combination of ESBL genes was between bla_{TEM}, bla_{SHV}, bla_{OXA} and $bla_{\text{CTX-M}}$, which was consistent with studies from Morocco [30] and Tunisia [35].

In our study, the $bla_{\text{OXA-48}}$ and $bla_{\text{NDM-1}}$ genes have been detected in one strain identified as *E. cloacae.* To the best of our knowledge, this is the first report on potential *E. cloacae* isolates co-harbouring carbapenem-resistance genes $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ in Algeria. In agreement with our results, the co-harbouring of carbapenemase genes has been reported in several studies [12,34]. It should be noted that the coexistence of these carbapenemase genes is a therapeutic challenge to clinicians, due to restricted treatment options and the potential for global spread by horizontal transfer [36].

In Algerian hospitals, the OXA-48 carbapenemase gene was the most important mediator of carbapenem resistance in Enterobacteriaceae [37,38]. Therefore, few studies on OXA-48-producing *E. cloacae* have been reported in Algeria, the first one was reported in Guelma Hospital (Eastern Algeria) [27]. It has since been widely identified in different Algerian regions [37,39].

To date, the dissemination of $bla_{\text{OXA-48}}$ among GNB has been described in different countries around the world, since it was 57.1% in Algeria [12], 49% in Arabian Gulf [40], 53.3% in the United Arab Emirates [41], 49.2% in Egypt [42], and 86% in Turkey [43]. In Morocco, the $bla_{\text{OXA-48}}$ gene was reported in 5.24% of carbapenemase- producing *K. pneumoniae* [44], while in Tunisia, $bla_{\text{OXA-48}}$ was found in 67.2% of isolates [36]. Regarding the epidemiological situation in Algeria, it is important to note that $bla_{\text{OXA-48}}$ has been reported in different regions: Guelma [27], Batna [45], Ouargla [46], Annaba [33], Constantine, Setif and Tizi-ouzou [11], and Tlemcen, Oran and Algiers [47].

In this investigation, we also reported the isolation of NDM-1-producing *Enterobacter cloacae*. This species is an important nosocomial pathogen, which can cause various infections including urinary tract, respiratory tract, surgical site, biliary tract, sepsis, intravenous catheters, central nervous system and outbreaks in neonatal units [3]. *E. cloacae* carrying $bla_{\text{NDM-1}}$ is extremely rare and, to our knowledge, has not been described in Algeria to date. Moreover, NDM-1-positive *E. cloacae* has been found in India [48], China [3], South Africa [6] and Japan [49], indicating that the $bla_{\text{NDM-1}}$ gene has spread from *K. pneumoniae* to *E. cloacae*. Furthermore, $bla_{\text{NDM-1}}$ has been also reported in other *Enterobacteriaceae* such as *Raoultella ornithinolytica* [50] and *Escherichia coli* [51].

The widespread dissemination of $bla_{\text{NDM-1}}$ is mainly due to plasmids, integrons, insertion sequence common region (ISCR) and clonal outbreaks [52]. The $bla_{\text{NDM-1}}$ gene is mainly located on incompatible conjugative

plasmids and can be transmitted among different bacterial species, resulting in extensive drug resistance [10]. So far, most studies of NDM-1-positive isolates have focused on resistance mechanisms and transmission. However, it is not known whether bla_{NDM-1} affects the biological characteristics of the strains, such as growth ability and competitiveness.

Carbapenem-resistant GNB including *Enterobacteriaceae* are a matter of national and international concern because of their high levels of antimicrobial resistance and their association with high mortality. This study was not based on systematic surveillance of carbapenem resistance and so the sample size was small. However, our contribution can form the basis for further epidemiological studies of carbapenem-resistant GNB in order to promote appropriate antimicrobial therapy as well as to establish adequate infection control precautions and to stop the spread of these resistant bacteria.

Conclusions

This study reports for the first time the carbapenemase genes in Tebessa (eastern Algeria) hospital. It reports particularly the first incidence of bla_{NDM-1} in *Enterobacter cloacae* in Algeria. The detection of carbapenem-resistant and ESBL-producing GNB is of great concern and may significantly limit the efficacy of therapeutic options in hospital settings. There is thus an urgent need to implement strategies in Algerian hospitals to control the emergence of those microorganisms, including phenotypic and molecular tests to detect these bacteria. We emphasize that some measures must be taken to slow down the rising problem of such bacteria and to prevent nosocomial outbreaks which could lead to an endemic situation.

References

1. Cui X, Zhang H, Du H (2019) Carbapenemases in *Enterobacteriaceae*: detection and antimicrobial therapy. Front Microbiol 10: 1823. doi: 10.3389/fmicb.2019.01823.
2. Seman A, Mihret A, Sebre S, Awoke T, Yeshitela B, Yitayew B, Aseffa A, Asrat D, Abebe T (2022) Prevalence and molecular characterization of extended spectrum β-Lactamase and carbapenemase-producing *Enterobacteriaceae* isolates from bloodstream infection suspected patients in Addis Ababa, Ethiopia. Infect Drug Resist 15: 1367-1382. doi: 10.2147/IDR.S349566.
3. Yao J, Liu S, Du N, Niu M, Zhang M, Chen C, Li H, Du Y (2020) Genetic features of bla_{ndm-1} and characterization of the corresponding knockout mutant of *Enterobacter cloacae* produced by red homologous recombination. Jundishapur J Microbiol 13: e101645. doi: 10.5812/jjm.101645.
4. Pérez-Vazquez M, Oteo-Iglesias J, Sola-Campoy PJ, Carrizo-Manzoni H, Bautista V, Lara N, Aracil B, Alhambra A, Martínez-Martínez L, Campos J (2019) Spanish antibiotic resistance surveillance program collaborating group. Characterization of carbapenemase-producing *Klebsiella oxytoca* in Spain, 2016-2017. Antimicrob Agents Chemother 63: e02529-18. doi: 10.1128/AAC.02529-18.
5. Hamed, SL and Hasoon, NA (2019) Molecular characterization of carbapenemase-producing Gram-negative bacteria isolated from clinical specimens in Baghdad, Iraq. J Pure Appl Microbiol 13: 1031-1040. doi: 10.22207/JPAM.13.2.41.
6. Ebomah KE, Okoh AI (2021) *Enterobacter cloacae* harbouring bla_{NDM-1}, bla_{KPC}, and bla_{OXA-48}-like carbapenem-resistant genes isolated from different environmental sources in South Africa. Int J Environ Stud 78: 151-164. doi: 10.1080/00207233.2020.1778274.
7. Sawa T, Kooguchi K, Moriyama K (2020) Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. J Intensive Care 8: 13. doi: 10.1186/s40560-020-0429-6.
8. Haji SH, Aka STH, Ali FA (2021) Prevalence and characterisation of carbapenemase encoding genes in multidrug-resistant Gram-negative bacilli. PLoS One 16: e0259005. doi: 10.1371/journal.pone.0259005.
9. Nordmann P (2010) Gram-negative bacteria with resistance to carbapenems. Med Sci (Paris). 26: 950-959. [Article in French]. doi: 10.1051/medsci/20102611950.
10. Bolourchi N, Giske CG, Nematzadeh S, Mirzaie A, Abhari SS, Solgi H, Badmasti F (2022) Comparative resistome and virulome analysis of clinical NDM-1-producing carbapenem-resistant *Enterobacter cloacae* complex. J Glob Antimicrob Resist 28: 254-263. doi: 10.1016/j.jgar.2022.01.021.
11. Touati A, Mairi A (2020) Carbapenemase-producing Enterobacterales in Algeria: a systematic review. Microb Drug Resist 26: 475-482. doi: 10.1089/mdr.2019.0320.
12. Abderrahim A, Djahmi N, Loucif L, Nedjai S, Chelaghma W, Gameci-Kirane D, Dekhil M, Lavigne JP, Pantel A (2022) Dissemination of OXA-48- and NDM-1-producing Enterobacterales isolates in an Algerian hospital. Antibiotics (Basel) 11: 750. doi: 10.3390/antibiotics11060750.
13. EUCAST (2018). European Committee on Antimicrobial Susceptibility Testing Recommendations 2018; V.1.0 February. Available: https://www.eucast.org/: accessed 9 December 2022.
14. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268-281. doi: 10.1111/j.1469-0691.2011.03570.x.
15. Jarlier V, Nicolas MH, Fournier G, Philippon A (1988) Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev Infect Dis 10: 867-878. doi: 10.1093/clinids/10.4.867.
16. CLSI (2017) Performance standards for antimicrobial susceptibility testing. 27th edition. CLSI supplement M100.Wayne, PA: Clinical and Laboratory Standards Institute; 250 p.
17. Prakash MR, Veena M, Sharma A, Basavaraj KN, Viswanath G (2012) The prospective evaluation of four convenient

methods for detecting MBLs in the clinical isolates. J Clin of Diagn Res 6: 1196-1199.
18. Belaaouaj A, Lapoumeroulie C, Caniça MM, Vedel G, Névot P, Krishnamoorthy R, Paul G (1994) Nucleotide sequences of the genes coding for the TEM-like beta-lactamases IRT-1 and IRT-2 (formerly called TRI-1 and TRI-2). FEMS Microbiol Lett 120: 75-80. doi: 10.1111/j.1574-6968.1994.tb07010.x.
19. Pitout JD, Thomson KS, Hanson ND, Ehrhardt AF, Moland ES, Sanders CC (1998) Beta-lactamases responsible for resistance to expanded-spectrum cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* isolates recovered in South Africa. Antimicrob Agents Chemother 42: 1350-1354. doi: 10.1128/AAC.42.6.1350.
20. Batchelor M, Hopkins K, Threlfall EJ, Clifton-Hadley FA, Stallwood AD, Davies RH, Liebana E (2005) bla$_{(CTX-M)}$ genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. Antimicrob Agents Chemother 49: 1319-1322. doi: 10.1128/AAC.49.4.1319-1322.2005.
21. Hong SS, Kim K, Huh JY, Jung B, Kang MS, Hong SG (2012) Multiplex PCR for rapid detection of genes encoding class A carbapenemases. Ann Lab Med 32: 359-361. doi: 10.3343/alm.2012.32.5.359.
22. Poirel L, Héritier C, Tolün V, Nordmann P (2004) Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48: 15-22. doi: 10.1128/AAC.48.1.15-22.2004.
23. Ellington MJ, Kistler J, Livermore DM, Woodford N (2007) Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother 59: 321-322. doi: 10.1093/jac/dkl481.
24. Wang X, Wang C, Li Q, Zhang J, Ji C, Sui J, Liu Z, Song X, Liu X (2018) Isolation and characterization of antagonistic bacteria with the potential for biocontrol of soil-borne wheat diseases. J Appl Microbiol 125: 1868-1880. doi: 10.1111/jam.14099.
25. Ibrahim ME, Algak TB, Abbas M, Elamin BK (2021) Emergence of bla$_{TEM}$, bla$_{CTX-M}$, bla$_{SHV}$ and bla$_{OXA}$ genes in multidrug-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* in Saudi Arabia. Exp Ther Med 22: 1450. doi: 10.3892/etm.2021.10885.
26. Toumi S, Meliani S, Amoura K, Rachereche A, Djebien M, Djahoudi A (2018) Multidrug-resistant Gram-negative bacilli producing oxacillinases and metallo-β-lactamases isolated from patients in intensive care unit - Annaba hospital - Algeria (2014- 2016). J App Pharm Sci 8: 107-113. doi: 10.7324/JAPS.2018.8717.
27. Bouguenoun W, Bakour S, Bentorki AA, Al Bayssari C, Merad T, Rolain JM (2016) Molecular epidemiology of environmental and clinical carbapenemase-producing Gram-negative bacilli from hospitals in Guelma, Algeria: multiple genetic lineages and first report of OXA-48 in *Enterobacter cloacae*. J Glob Antimicrob Resist 7: 135-140. doi: 10.1016/j.jgar.2016.08.011.
28. Mohamed ES, Khairy RMM, Abdelrahim SS (2020) Prevalence and molecular characteristics of ESBL and AmpC β -lactamase producing *Enterobacteriaceae* strains isolated from UTIs in Egypt. Antimicrob Resist Infect Control 9: 198. doi: 10.1186/s13756-020-00856-w.
29. Kharrat M, Chebbi Y, Ben Tanfous F, Lakhal A, Ladeb S, Othmen TB, Achour W (2018) Extended spectrum beta-lactamase-producing *Enterobacteriaceae* infections in hematopoietic stem cell transplant recipients: epidemiology and molecular characterization. Int J Antimicrob Agents 52: 886-892. doi: 10.1016/j.ijantimicag.2018.05.006.
30. El bouamri MC, Arsalane L, Zerouali K, Katfy K, El kamouni Y, Zouhair S (2015) Molecular characterization of extended spectrum-lactamase-producing *Escherichia coli* in a university hospital in Morocco, North Africa. Afr J Urol 21: 161-166. doi: 10.1016/j.afju.2015.02.005.
31. Ahmed SF, Ali MM, Mohamed ZK, Moussa TA, Klena JD (2014) Fecal carriage of extended-spectrum β-lactamases and AmpC-producing Escherichia coli in a Libyan community. Ann Clin Microbiol Antimicrob 13: 22. doi: 10.1186/1476-0711-13-22.
32. Kamel NA, El-Tayeb WN, El-Ansary MR, Mansour MT, Aboshanab KM (2018) Phenotypic screening and molecular characterization of carbapenemase-producing Gram-negative bacilli recovered from febrile neutropenic pediatric cancer patients in Egypt. PLoS One 13: e0202119. doi: 10.1371/journal.pone.0202119.
33. Bourafa N, Chaalal W, Bakour S, Lalaoui R, Boutefnouchet N, Diene SM, Rolain JM (2018) Molecular characterization of carbapenem-resistant Gram-negative bacilli clinical isolates in Algeria. Infect Drug Resist 11: 735-742. doi: 10.2147/IDR.S150005.
34. Mohamed A, Daef E, Nafie A, Shaban L, Ibrahim M (2021) Characteristics of carbapenem-resistant Gram-negative bacilli in patients with ventilator-associated pneumonia. Antibiotics (Basel) 10: 1325. doi: 10.3390/antibiotics10111325.
35. Dziri R, Talmoudi A, Barguellil F, Ouzari HI, El Asli MS, Klibi N (2019) Huge diversity of TEM and SHV β-Lactamases types among CTX-M-15-producing *Enterobacteriaceae* species in Tunisia. Microb Drug Resist 25: 1149-1154. doi: 10.1089/mdr.2018.0445.
36. Ben Helal R, Dziri R, Chedly M, Klibi N, Barguellil F, El Asli MS, Ben Moussa M (2018) Occurrence and characterization of carbapenemase-producing *Enterobacteriaceae* in a Tunisian hospital. Microb Drug Resist 24: 1361-1367. doi: 10.1089/mdr.2018.0013.
37. Mairi A, Pantel A, Sotto A, Lavigne JP, Touati A (2018) OXA-48-like carbapenemases producing *Enterobacteriaceae* in different niches. Eur J Clin Microbiol Infect Dis 37: 587-604. doi: 10.1007/s10096-017-3112-7.
38. Yousfi M, Touati A, Muggeo A, Mira B, Asma B, Brasme L, Guillard T, de Champs C (2018) Clonal dissemination of OXA-48-producing *Enterobacter cloacae* isolates from companion animals in Algeria. J Glob Antimicrob Resist 12: 187-191. doi: 10.1016/j.jgar.2017.10.007.
39. Bendjama E, Loucif L, Chelaghma W, Attal C, Bellakh FZ, Benaldjia R, Kahlat I, Meddour A, Rolain JM. (2020) First detection of an OXA-48-producing *Enterobacter cloacae* isolate from currency coins in Algeria. J Glob Antimicrob Resist 23: 162-166. doi: 10.1016/j.jgar.2020.09.003.
40. Alhazmi W, Al-Jabri A, Al-Zahrani I (2022) The molecular characterization of nosocomial carbapenem-resistant Klebsiella pneumoniae co-harboring bla$_{NDM}$ and bla$_{OXA-48}$ in Jeddah. Microbiology Research 13: 753-764. doi: 10.3390/microbiolres13040054.
41. Moubareck CA, Mouftah SF, Pál T, Ghazawi A, Halat DH, Nabi A, AlSharhan MA, AlDeesi ZO, Peters CC, Celiloglu H, Sannegowda M, Sarkis DK, Sonnevend Á (2018) Clonal emergence of *Klebsiella pneumoniae* ST14 co-producing OXA-48-type and NDM carbapenemases with high rate of colistin resistance in Dubai, United Arab Emirates. Int J

Antimicrob Agents 52: 90-95. doi: 10.1016/j.ijantimicag.2018.03.003.
42. Khalifa HO, Soliman AM, Ahmed AM, Shimamoto T, Hara T, Ikeda M, Kuroo Y, Kayama S, Sugai M, Shimamoto T (2017) High carbapenem resistance in clinical Gram-negative pathogens isolated in Egypt. Microb Drug Resist 23: 838-844. doi: 10.1089/mdr.2015.0339.
43. Iraz M, Özad Düzgün A, Sandallı C, Doymaz MZ, Akkoyunlu Y, Saral A, Peleg AY, Özgümüş OB, Beriş FŞ, Karaoğlu H, Çopur Çiçek A (2015) Distribution of β-lactamase genes among carbapenem-resistant *Klebsiella pneumoniae* strains isolated from patients in Turkey. Ann Lab Med 35: 595-601. doi: 10.3343/alm.2015.35.6.595.
44. Barguigua A, Zerouali K, Katfy K, El Otmani F, Timinouni M, Elmdaghri N (2015) Occurrence of OXA-48 and NDM-1 carbapenemase-producing *Klebsiella pneumoniae* in a Moroccan university hospital in Casablanca, Morocco. Infect Genet Evol 31: 142-148. doi: 10.1016/j.meegid.2015.01.010.
45. Loucif L, Kassah-Laouar A, Saidi M, Messala A, Chelaghma W, Rolain JM (2016) Outbreak of OXA-48-producing *Klebsiella pneumoniae* involving a sequence type 101 clone in Batna University Hospital, Algeria. Antimicrob Agents Chemother 60: 7494-7497. doi: 10.1128/AAC.00525-16.
46. Yagoubat M, Ould El-Hadj-Khelil A, Malki A, Bakour S, Touati A, Rolain JM (2017) Genetic characterisation of carbapenem-resistant Gram-negative bacteria isolated from the university hospital Mohamed Boudiaf in Ouargla, southern Algeria. J Glob Antimicrob Resist 8: 55-59. https://doi.org/10.1016/j.jgar.2016.10.008.
47. Benamrouche N, Lafer O, Benmahdi L, Benslimani A, Amhis W, Ammari H, Assaous F, Azzam A, Rahal K, Tali Maamar H (2020) Phenotypic and genotypic characterization of multidrug-resistant *Acinetobacter baumannii* isolated in Algerian hospitals. J Infect Dev Ctries 14: 1395-1401. https://doi.org/10.3855/jidc.12348.
48. Khan AU, Nordmann P (2012) NDM-1-producing *Enterobacter cloacae* and *Klebsiella pneumoniae* from diabetic foot ulcers in India. J Med Microbiol 61: 454-456. https://doi.org/10.1099/jmm.0.039008-0.
49. Gotoh K, Hagiya H, Iio K, Yamada H, Matsushita O, Otsuka F (2022) Detection of *Enterobacter cloacae* complex strain with a bla_{NDM-1}-harboring plasmid from an elderly resident at a long-term care facility in Okayama, Japan. J Infect Chemother 28: 1697-1699. https://doi.org/10.1016/j.jiac.2022.08.019.
50. Khajuria A, Praharaj AK, Grover N, Kumar M (2013) First report of bla_{NDM-1} in *Raoultella ornithinolytica*. Antimicrob Agents Chemother 57: 1092-1093. https://doi.org/10.1128/AAC.02147-12.
51. Thapa A, Upreti MK, Bimali NK, Shrestha B, Sah AK, Nepal K, Dhungel B, Adhikari S, Adhikari N, Lekhak B, Rijal KR (2022) Detection of NDM Variants (bla_{NDM-1}, bla_{NDM-2}, bla_{NDM-3}) from carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae*: First report from Nepal. Infect Drug Resist 15: 4419-4434. https://doi.org/10.2147/IDR.S369934.
52. Du N, Liu S, Niu M, Duan Y, Zhang S, Yao J, Mao J, Chen R, Du Y (2017) Transmission and characterization of bla NDM-1 in *Enterobacter cloacae* at a teaching hospital in Yunnan, China. Ann Clin Microbiol Antimicrob 16: 58. https://doi.org/10.1186/s12941-017-0232-y.

Corresponding author

Associate Professor Abdelbasset Mechai
Bioactive Molecules and Applications Laboratory, Faculty of Exact Sciences and Natural and Life Sciences,
Echahid Cheikh Larbi Tebessi University (E.C.L.T.U)-Tebessa 12002, Algeria
Tel: 00213663028130
Fax: 00213037765010
Email: abdelbasset.mechai@univ-tebessa.dz

Conflict of interests: No conflict of interests is declared.