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Original Article

Prevalence and characterization of Carbapenem-Resistant Enterobacterales among inpatients and outpatients in Skikda, Algeria

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Abstract

Introduction: The spread of Carbapenemase-producing *Enterobacterales* (CPEs) has become a significant concern in Algeria, with limited data available on their presence in community settings. This research investigated the resistance mechanisms of carbapenem-resistant Enterobacterales (CREs) collected from hospitals and the community in Skikda city, Algeria, between December 2020 and June 2022.

Methodology: The study collected *Enterobacterales* strains resistant to ertapenem from inpatient and outpatient populations. An automated system was used for identification and antibiotic susceptibility testing. β-lactamase production was evaluated through phenotypic tests and confirmed by standard PCR. Lastly, the carbapenemase genes were sequenced using the Sanger method.

Results: 17 CRE were isolated, with 9 from inpatients and 8 from outpatients. These isolates belonged to four species: *Klebsiella pneumoniae* (n = 8), *Escherichia coli* (n = 6), *Enterobacter cloacae* (n = 1), and *Proteus mirabilis* (n = 1). Of 15 CPEs, 11 were extended-spectrum β-lactamases (ESBLs) positive, 5 were plasmid-mediated cephalosporinase (AmpC) positive, and 1 harbored all three β-lactamases. All metallo-β-lactamase-producing strains carried the New Delhi metallo-beta-lactamase gene (*bla*$_{NDM}$), including 5 NDM-1 and 7 NDM-5 variants. The presence of blaOXA-48 and blaOXA-244 was observed in one outpatient strain each. NDM was associated with Cefotaximase Munich (CTX-M) ESBL in 8 isolates, while Cephamycinase (CMY) was detected in 3 NDM-5-producing *E. coli.*

Conclusions: This research highlights the rising prevalence of carbapenemases NDM-1 and NDM-5 among inpatients and outpatients and supports the notion that OXA-48 is becoming increasingly widespread beyond Algerian hospitals.

Key words: Carbapenem-resistant *Enterobacterales* (cres); Algeria; inpatients, outpatients; *Enterobacterales*.

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Introduction

Enterobacterales are among the most common human pathogens, causing infections such as cystitis, pneumonia, pyelonephritis, septicemia, meningitis, peritonitis, and medical device infections. These agents, particularly *Escherichia coli and Klebsiella pneumoniae* are known to be the most frequent causes of both community- and hospital-acquired infections [1-2].

The global emergence of Multidrug-resistant (MDR) *Enterobacterales* is a growing concern and constitutes a major threat to public health. The widespread production of carbapenem-hydrolyzing β-lactamases (carbapenemases) is the leading cause of resistance to carbapenem antibiotics, leaving limited options for treating infections caused by these highly drug-resistant bacterial strains [3]. It is widely recognized that the carbapenem-resistance phenomenon can be attributed to the presence of three distinct classes of carbapenemases: Class A, including the KPC enzyme, Class B metallo-β-lactamases (MβLs), which encompass VIM, IMP, and NDM, and Class D represented mainly by the OXA-48-like enzyme [4-5].

The New Delhi MβL enzyme (NDM) is a highly problematic and rapidly spreading form of carbapenem resistance. NDM can cause resistance to a broad range of antibiotics, making it extremely difficult to treat. In recent years, cases of NDM have become endemic in the Arabian Peninsula, northern Africa, and the Balkans. Among the 25 different NDM variants, NDM-1 and NDM-5 are the two most commonly found in *Enterobacterales* [6].

The *bla*$_{OXA-48}$ gene has become increasingly widespread, particularly in Mediterranean countries, since its initial detection in a *K. pneumoniae* strain in

Turkey [5]. The OXA-48 class of carbapenemases, known as OXA-48-like, encompasses several variants that vary in amino acids but have not become as widespread as OXA-48. Some variants, including OXA-244 and OXA-232, have weaker hydrolytic activity against carbapenems than OXA-48 [7]. North African countries are hotspots for OXA-type carbapenemases [5]. NDM and OXA-48 are frequently identified in *K. pneumoniae* and *Escherichia coli*, but can also be found in other *Enterobacterales* species [8-9].

In Algeria, the emergence and spread of clinically significant CPE strains have been documented, with NDM-1, NDM-5, and OXA-48 enzymes being the most frequently identified in hospital and community settings [4,10–12]. To gain a comprehensive understanding of the presence and distribution of CPE strains in both hospital and community settings, particularly in smaller cities, this study was conducted in Skikda, Algeria. The aim was to characterize the *Enterobacterales* isolates that showed decreased susceptibility to carbapenems obtained from hospitalised and outpatients.

Methodology

Bacterial isolates and species identification

In this study, ertapenem-resistant isolates of *Enterobacterales* were collected from inpatient samples (urine and pus) obtained from the main hospital in Skikda and outpatient samples obtained from private laboratories. These specimens were taken from a variety of pathological sources. Isolates were obtained by aseptic plating of specimens on three different culture media: Nutrient agar, Hektoen, and CHROMagar, followed by incubation for 24 hours at 37°C. The susceptibility of the isolates to Ertapenem was determined using the established disc diffusion method on Mueller Hinton Agar.

Identification of the isolates was confirmed with automated systems, the API20E biochemical gallery (BioMérieux in Marcy-l'Étoile, France) and the Vitek® 2 Compact 15 automated system (BioMérieux).

In vitro antibiotic susceptibility testing

To determine the minimum inhibitory concentrations (MICs) of Ertapenem-resistant *Enterobacterales* isolates, the Vitek® 2 Compact 15 automated system was utilized. The AST 365 card, which comprises a panel of antibiotics including Ampicillin, Amoxicillin + clavulanic acid, Piperacillin + tazobactam, Cefazolin, Cefoxitin, Cefotaxime, Ceftazidime, Imipenem, Ertapenem, Amikacin, Gentamycin, Ciprofloxacin, Chloramphenicol, Nitrofurantoin, Trimethoprim-Sulfamethoxazole, and Fosfomycin for *K. pneumoniae*, was used in the analysis. The obtained MIC values were interpreted based on the guidelines set by the Clinical Laboratory Standard Institute (CLSI) (CLSI 2020, Version of M02 M07 M11, 30th ed) [13]. The wild-type control strain *E. coli* ATCC 25922 was utilized, and the Broth Microdilution method was used to determine colistin's minimum inhibitory concentration (MIC) [13].

Phenotypic Characterization of Carbapenemase, ESBL, and AmpC production

The presence of carbapenemase was determined phenotypically using the Modified Carba NP test described previously by Bakour *et al* [14].

The inhibitory effect of ethylene-diamine-tetra-acetic acid (EDTA) on MβL activity was studied using the method of Yong *et al.* [15] with a slight

Table 1. Primers used in the PCR reaction.

Target gene	Primer sequence 5'- 3'	Amplicon Size (Pb)	Annealing Temperature (°C)	Reference
bla$_{NDM}$	F-CATTTGCGGGGTTTTTAATG R-CTGGGTCGAGGTCAGGATAG	1022	52	[42]
bla$_{OXA}$	F-TTGGTGGCATCGATTATCGG R- GAGCACTTCTTTTGTGATGGC	744	54	[43]
bla$_{KPC}$	F-ATGTCACTGTATCGCCGTCT R- TTTTCAGAGCCTTACTGCCC	893	55	[42]
bla$_{VIM}$	F- ATTGGTCTATTTGACCGCGTC R- ATGAAAGTGCGTGGAGAC	382	54	[44]
bla$_{IMP}$	F-CATGGTTTGGTGGTTCTTGT R-ATAATTTGGCGGACTTTGGC	448	53	[43]
bla$_{TEM}$	F-ATGAGTATTCAACAT TTC CG R-CCAATGCTTAATCAG TGA GG	840	50	[17]
bla$_{SHV}$	F-TTTATGGCGTTACCTTTGACC R-ATTTGTCGCTTCTTTACTCGC	1051	53	[45]
bla$_{CTX-M}$	F-TTTGCGATGTGCAGTACCAGTAA R-CGATATCGTTGGTGGTGCCATA	500	50	[17]
bla$_{CMY}$	F-ATGATGAAAAAATCGTTATGC R-TTGCAGCTTTTCAAGAATGCGC	1200	50	[17]

R: Reverse; F: Forward; bp: base pair.

modification - the imipenem disc was substituted with an ertapenem disc. The boronic acid test was used to identify Ambler class A carbapenemase KPC, as described by Tsakris *et al* [16].

The production of ESBL was evaluated using the Double Disc Synergy Test (DDST) [17]. This test was further conducted in a cloxacillin-supplemented medium [18]. The DDST involved placing the following antibiotic discs in a 2 cm inter-disk distance: cefotaxime, cefepime, aztreonam, ceftazidime, and cefoxitin, along with a disc of amoxicillin + clavulanic acid.

Molecular characterization of β-lactamase production

The extraction of DNA was carried out using the boiling lysis method, previously described by Feria *et al* [19].

The presence of the most prevalent genes encoding carbapenemase-hydrolyzing enzymes, such as $bla_{\text{OXA-48}}$, bla_{NDM}, bla_{KPC}, bla_{VIM}, and bla_{IMP}, as well as the common ESBL gene $bla_{\text{CTX-M}}$, and the major plasmid-mediated AmpC gene bla_{CMY}, were detected using standard simplex PCR technology and specific primers. The primer sequences for each targeted gene are listed in Table 1. In the case of strains showing resistance to colistin, the plasmid-mediated *mcr-1* gene was also targeted using the primer sequences and protocol described by Rebelo *et al* [20].

The amplification process was carried out using the following conditions: After a preliminary denaturation step at 95 °C for 15 minutes, 30 cycles of denaturation at 94 °C for 30 seconds, annealing for 30 seconds at a temperature determined by the primer sequence (as listed in Table 1), and elongation at 72 °C for 2 minutes were performed. The procedure was concluded with a final elongation step at 72 °C for 10 minutes. The amplified products were analyzed on a 1.5% agarose gel, stained with SYBR Safe DNA gel stain (Invitrogen, Spain), and visualized using a UV transilluminator (GEL Doc XR + Gel Documentation System from BIO-RAD, USA /Thermo Fisher Scientific).

The sequences of positive PCR products were obtained through purification and sequencing with a 3500 XL Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific in California, USA). The sequences were then compared to the Antibiotic Resistance Gene-ANNOTATION database (ARG-ANNOT) using the BLAST program available on the National Center for Biotechnology Information's website (www.ncbi.nlm.nih.gov).

Results

Bacterial isolates identification

17 CRE-positive isolates were identified using the disk diffusion method, with 11 isolates collected from urine and 6 from pus. Most isolated strains came from elderly patients (87.24%) aged 51 to 93. The patient information and strain data can be found in Table 2.

The identified species were *K. pneumoniae* (n = 9), *E. coli* (n = 6), *Enterobacter cloacae* (n = 1), and *Proteus mirabilis* (n = 1). *K. pneumoniae* was the dominant pathogen among outpatients, while *E. coli* was the most frequently encountered species among hospitalized patients (n = 5).

In vitro antibiotic susceptibility

The results from the Vitek-2 automated system showed that 11 out of the 17 CRE isolates were multidrug-resistant, meaning that 54% of the CPE were resistant to at least three different classes of antibiotic,

Table 2. Phenotypic and Genotypic Characteristics of Carbapenem-Resistant *Enterobacterales* (CREs) strains.

Source	Ward	Gender	Age	Specimen	ID	Phenotypic tests					Carbapenemase genes	Additional beta-lactamase genes
						MCNP	EDTA	Ab	ESBL	AmpC		
H	Nephrology	F	75	Urine	*E. coli*	+	+	-	-	+	$bla_{\text{NDM-5}}$	bla_{CMY}
H	Nephrology	F	82	Urine	*E. coli*	+	+	-	-	+	$bla_{\text{NDM-5}}$	bla_{CMY}
H	Surgery	M	57	Diabetic pus	*K. pneumoniae*	+	+	-	+	-	$bla_{\text{NDM-1}}$	$bla_{\text{CTX-M}}$
H	Surgery	F	71	Pus	*K. pneumoniae*	+	+	-	+	-	$bla_{\text{NDM-1}}$	$bla_{\text{CTX-M}}$
H	Nephrology	F	55	Pus	*E. cloacae*	-	-	-	-	+	None	None
H	ICU	M	60	Urine	*E. coli*	+	+	-	-	+	$bla_{\text{NDM-5}}$	bla_{CMY}
H	Neonatology	M	Nb	Pus	*E. coli*	+	+	-	+	+	$bla_{\text{NDM-5}}$	$bla_{\text{CTX-M}}$
H	Neonatology	F	Nb	Urine	*E. coli*	+	+	-	+	-	$bla_{\text{NDM-5}}$	$bla_{\text{CTX-M}}$
H	Nephrology	M	72	Urine	*K. pneumoniae*	+	+	-	+	-	$bla_{\text{NDM-5}}$	$bla_{\text{CTX-M}}$
C	Private	F	64	Pus	*K. pneumoniae*	+	+	-	+	-	$bla_{\text{NDM-1}}$	$bla_{\text{CTX-M}}$
C	Private	F	88	Urine	*K. pneumoniae*	+	+	-	+	-	$bla_{\text{NDM-1}}$	$bla_{\text{CTX-M}}$
C	Private	F	80	Urine	*K. pneumoniae*	+	+	-	+	-	$bla_{\text{NDM-1}}$	$bla_{\text{CTX-M}}$
C	Private	F	93	Urine	*K pneumoniae*	+	+	-	+	-	$bla_{\text{NDM-5}}$	$bla_{\text{CTX-M}}$
C	Private	M	82	Urine	*K. pneumoniae*	-	-	-	+	+	None	None
C	Private	M	72	Pus	*P. mirabilis*	+	+	-	+	-	bla_{NDM}	$bla_{\text{CTX-M}}$
C	Private	F	51	Urine	*K. pneumoniae*	+	-	-	+	-	$bla_{\text{OXA-244}}$	$bla_{\text{CTX-M}}$ / bla_{TEM} / bla_{SHV}
C	Private	F	7	Urine	*E. coli*	+	-	-	-	-	$bla_{\text{OXA-48}}$	bla_{TEM}

H: Hospital; C: Community; M: Male; F: Female; Nb: Newborn; MDR: Multi-Drug-Resistance; MCNP: Modified Carba Np; Ab: Acid boronic; EDTA: Ethylene diamine tetra-acetic acid; ESBL: Extended-spectrum beta-lactamase.

including fluoroquinolones, sulfonamides, and one or two aminoglycosides in addition to β-lactams.

Most community- and hospital-acquired CPE isolates were resistant to all tested β-lactams and β-lactams combined with β-lactamase inhibitors. However, one strain was susceptible to ceftazidime with a MIC value of 4μg/mL, and five isolates had intermediate resistance to imipenem with a MIC value of 2μg/mL. Additionally, 82.35%, 64.70%, 47.05%, and 47.06% of the isolates were resistant to ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin, and nitrofurantoin, respectively. On the other hand, only 23.53% of the strains showed resistance to amikacin with a MIC value of ≥ 64 μg/mL. Aminoglycosides and chloramphenicol were found to be the most effective antibiotics against both hospital and community-acquired CRE isolates, as shown in Figure 1 and Table 3.

Table 4 lists the MIC50 and MIC90 values of the antibiotics against the CRE isolates. The MIC values of ertapenem were ≥ 8 μg/mL for 12 CRE strains (70.59%), 4 μg/mL for two isolates (11.76%), and 2 μg/mL for 4 isolates (23.53%). For imipenem, the MIC values were ≥ 16μg/mL for 11 strains (64.7%), 8μg/mL for one isolate (5.88%), and 2 μg/mL for five isolates (11.76%).

Two *K. pneumoniae* isolates were resistant to colistin, MIC value of 4μg/mL, and isolates were also resistant to ertapenem and imipenem, MIC values of ≥ 8μg/mL and ≥ 16μg/mL, respectively.

Table 4. MICS of CRE isolates against 16 antibiotics.

Antibiotics	MIC (μ/mL)		CRE Resistance rate (%)
	50%	90%	
AMP	≥ 32	≥ 32	100%
AUG	≥ 32	≥ 32	100%
TAZ	≥ 128	≥ 128	100%
CZ	≥ 64	≥ 64	100%
FOX	≥ 64	≥ 64	100%
CTX	≥ 64	≥ 64	100%
CAZ	≥ 64	≥ 64	94.11%
ERT	≥ 8	≥ 8	100%
IMP	≥ 16	R ≥ 16	70.59%
AK	≥ 64	4	23.53%
GEN	≥ 16	≥ 16	47.06%
CIP	≥ 4	≥ 4	82.35%
NIT	256	64	47.06%
FOS	≥ 256	≥ 256	44.44 % (*K. pneumoniae*)
CHL	≥ 64	8	11.76%
SXT	≥ 320	≥ 320	64.71%
COL	-	-	11.76%

MIC50% and MIC90% refer to the minimal inhibitory concentration of antibiotics required to inhibit 50% and 90% of the isolates, respectively. CRE: Carbapenemase-producing *Enterobacterales*. AMP: Ampicillin; AUG: Amoxicillin + clavulanic acid; TAZ: Piperacillin + Tazobactam; CZ: Cefazolin; FOX: Cefoxitin; CTX: Cefotaxime; CAZ: Ceftazidime; ERT: Ertapenem; IMP: Imipenem; AK: Amikacin; GEN: Gentamicin; CIP: Ciprofloxacin; FOS: Fosfomycin; NIT: Nitrofurantoin; CHL: Chloramphenicol; SXT: Trimethoprim-Sulfamethoxazole; COL: Colistin.

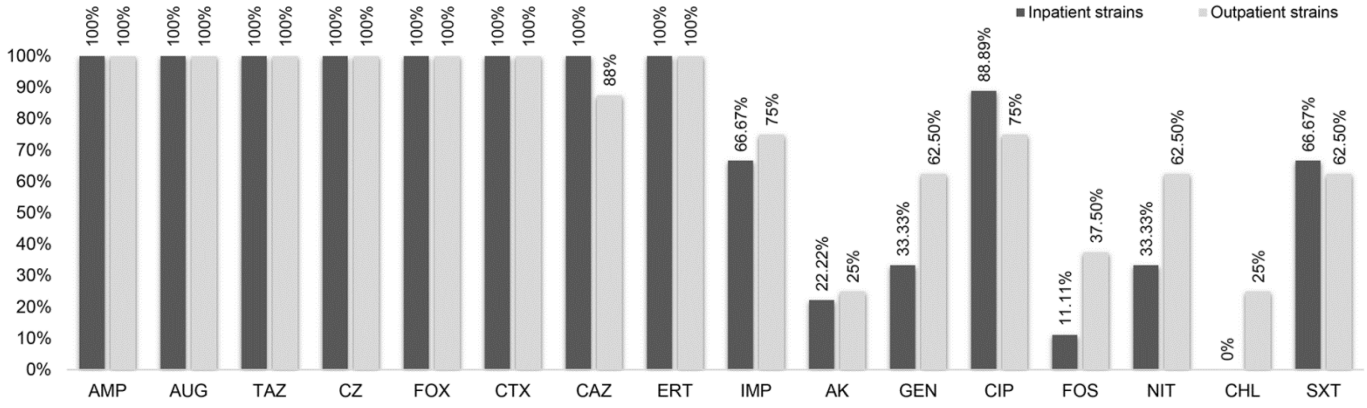
Phenotypic Characterization of Carbapenemase, ESBL, and AmpC production

The modified Carba-NP test confirmed carbapenemase production in 15 isolates. The EDTA assay, which suggests the presence of MβL, was positive for 13 of the 15 isolates. However, all strains tested negative for the boronic acid test. The DDST, with and without cloxacillin, showed that 10 CPE strains produced ESBL and 5 AmpC. In two strains, both ESBL and AmpC mechanisms were detected.

Molecular characterization of β-lactamase production

Among the 17 CREs, six β-lactamase encoding genes were identified, as listed in Table 2. The most frequently detected carbapenemase gene was *bla*$_{\text{NDM-5}}$

Figure 1. Rates of Antibiotic Resistance in Hospital and Community-Acquired Carbapenem-Resistant Strains.



AMP: Ampicillin; AUG: Amoxicillin + clavulanic acid; TAZ: Piperacillin + Tazobactam; CZ: Cefazolin; FOX: Cefoxitin; CTX: Cefotaxime; CAZ: Ceftazidime; ERT: Ertapenem; IMP: Imipenem; AK: Amikacin; GEN: Gentamicin; CIP: Ciprofloxacin; FOS: Fosfomycin; NIT: Nitrofurantoin; CHL: Chloramphenicol; SXT: Trimethoprim-Sulfamethoxazole; COL: Colistin.

(7 strains), followed by bla_{NDM-1} (5 strains). Other genes detected included bla_{OXA-48} (1 strain) and $bla_{OXA-244}$ (1 strain). The bla_{NDM-5} was present in five *E. coli* and one *K. pneumoniae*, while bla_{NDM-1} was exclusively found in *K. pneumoniae*. The bla_{OXA-48} and $bla_{OXA-244}$ genes were detected in one *E. coli* and one *K. pneumonia* strain, respectively. The targeted carbapenemase-encoding genes bla_{KPC}, bla_{VIM}, and bla_{IMP} were absent in all CRE strains.

Six of the 8 hospital-associated strains carried bla_{NDM-5} and two carried bla_{NDM-1}. In contrast, among the 7 community strains, bla_{NDM-1} was the most frequently detected (3 strains), followed by bla_{NDM-5} (1 strain).

All 12 phenotypically confirmed ESBL-producing isolates carried the bla_{CTX-M} gene. The four non-ESBL *E. coli* isolates were AmpC producers, and three carried the bla_{CMY} gene. In two colistin-resistant *K. pneumoniae mcr-1* gene was not detected.

Discussion

This study aimed to analyze the prevalence of CRE strains among hospitalized patients and outpatients in Skikda, Algeria. The findings revealed the presence of CRE strains in both hospital and community settings. Extended hospital stays, exposure to antibiotics, invasive medical devices, and severe secondary infections are major risk factors for acquiring carbapenem-resistant strains in hospitals. On the other hand, self-medication and excessive use of antibiotics are considered the main factors contributing to the spread of these strains in the community, posing a significant challenge to public health [21].

The results of this study on tested samples suggested that NDM could be the most prevalent carbapenemase mechanism in both hospital and community groups, which contradicts previous studies in Algeria that have identified OXA-48 as the most commonly isolated class D β-lactamase [4,5]. The CPE strains in this study were resistant to third-generation cephalosporins (3GC) and carried either the bla_{CTX-M} or bla_{CMY} gene, which was confirmed through the phenotypic detection of ESBL or AmpC production. The lower occurrence of OXA-48 in this study can be attributed to its less resistance to 3GC, which is only present when associated with another β-lactamase such as ESBL or AmpC.

Table 3. Antibiotic Minimum Inhibitory Concentrations (MICs) and Resistance Patterns of Tested Carbapenem-Resistant *Enterobacteria* (CREs).

AMP	AUG	TAZ	CZ	FOX	CTX	CAZ	ERT	IMP	AK	GEN	CIP	FOS	NIT	CHL	SXT	COL	Resistance profile
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	2	≤ 2	≤ 1	≥ 4	≤ 16	≤ 16	16	≥ 320	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT- CIP-SXT
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	≤ 2	≤ 1	≥ 4	≤ 16	≤ 16	16	≥ 320	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-CIP-SXT
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	≥ 64	≥ 16	≥ 4	32	128	8	≥ 320	4	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-AK-GEN-CIP-NIT-SXT-COL
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	≥ 64	≥ 16	≥ 4	64	256	8	≥ 320	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT- IMP-AK-GEN- CIP-NIT-FOS-SXT
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	2	≤ 2	≤ 1	≥ 4	≤ 16	≤ 16	16	≥ 320	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-CIP-SXT
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	16	16	≥ 4	≤ 16	≤ 16	4	≤ 20	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-CIP
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	2	≥ 16	8	≤ 1	≥ 4	≤ 16	≤ 16	4	≤ 20	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-CIP
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	4	≥ 16	≥ 4	32	128	4	≥ 320	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-GEN-CIP-NIT-SXT
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	≥ 64	≥ 16	≥ 4	64	128	16	≥ 320	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-AK-GEN-CIP-FOS-NIT-SXT
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	16	4	≥ 4	32	128	16	≤ 20	4	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-CIP-COL
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	≥ 64	≥ 16	≥ 4	≥ 256	128	8	≥ 320	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-AK-GEN-CIP-FOS-NIT-SXT
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	≤ 2	≥ 16	≥ 4	≥ 256	I 64	≥ 64	≥ 320	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-GEN-CIP-FOS-CHL-SXT
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	4	8	4	≥ 16	≥ 4	32	256	≥ 64	≥ 320	NR	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT-IMP-GEN-CIP-NIT-CHL
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	2	2	2	≤ 1	≤ 0,25	≤ 16	64	4	≤ 20	2	AMP-AMC-TAZ-CZ-FOX-CTX-CAZ-ERT
≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	4	2	2	≤ 2	≤ 1	≤ 0,25	≤ 16	≤ 16	4	≥ 320	2	AMP-AMC-TAZ-CZ-FOX-CTX- ERT-SXT

NR: Naturally resistant; AMP: Ampicillin; AUG: Amoxicillin + clavulanic acid; TAZ: Piperacillin + Tazobactam; CZ: Cefazolin; FOX: Cefoxitin; CTX: Cefotaxime; CAZ: Ceftazidime; ERT: Ertapenem; IMP: Imipenem; AK: Amikacin; GEN: Gentamicin; CIP: Ciprofloxacin; FOS: Fosfomycin; NIT: Nitrofurantoin; CHL: Chloramphenicol; SXT: Trimethoprim-Sulfamethoxazole. COL: Colistin.

In the present study, NDM-1 and NDM-5 were identified as the only carbapenemases in the hospital setting, with NDM-5 being the most predominant. This finding aligns with previous studies in Algeria that have reported the circulation of both NDM-1 and NDM-5 in Algerian hospitals [22–25]. The detection of NDM-1 and NDM-5 in outpatients was also reported, with the detection of NDM-5 being the first in Algeria [10]. The presence of NDM-5 has been previously documented in non-hospitalized patients, healthy individuals, and animals in other countries, including Latin America [26] China [27], and Madagascar [28]. The presence of NDM-5 in the community could be due to various factors, including transmission from hospital patients or animals or even the environment, which could serve as a reservoir. NDM-5 has also been detected in Algeria in long-distance migratory birds [29], dogs [30], and raw milk [31].

The present study detected OXA-48 in one outpatient, which aligns with previous reports of its emergence in community settings in Batna and Annaba, Algeria [11-12]. Additionally, we detected the first clinically significant isolate carrying the $bla_{OXA-244}$ gene in Algeria, specifically in a strain of *K. pneumoniae*. The OXA-244 carbapenemase, a variant of OXA-48 with a single substitution (Arg214Gly), is known to have decreased carbapenemase activity compared to OXA-48.[32] This variant of OXA has been widely reported in several countries, including Russia, Germany, France, the UK, Egypt, the Netherlands, Colombia, Turkey, and Lebanon, primarily in *E. coli*, but also in *K. pneumoniae* and *Enterobacter aerogenes* [32-33]. In Algeria, OXA-244-producing *E. coli* was detected in the Soummum River in Bejaia [34]. It is important to note that OXA-244-producing strains are known to have weak carbapenemase activity, making them difficult to detect and potentially contributing to their silent spread [32].

In the present study, NDM-producing strains were multi-drug resistant, unlike the OXA-48-like strains. This aligns with previous research which shows that NDM production is consistently associated with a multidrug-resistant phenotype [35]. On the other hand, OXA-type carbapenemases have limited activity against carbapenems and can only induce significant resistance when they are combined with an ESBL [36-37].

In our study collection, carbapenemase production was not detected in two isolates. Both isolates produced AmpC β-lactamase and one isolate was an ESBL producer. Isolates with AmpC production usually have ertapenem resistance due to hyperproduction of the enzyme [38].

This aligns with previous studies [10,39], suggesting that carbapenem resistance in these two strains is likely a result of a combination of ESBL or AmpC production and non-enzymatic resistance mechanisms [40-41].

Conclusions

The occurrence of CPE strains in both hospital and community settings is a cause for concern, as these bacteria are multi-drug resistant and challenging to treat. This study highlights the presence of NDM and OXA-48-like carbapenemases in Algeria, particularly in Skikda, where the NDM-1 and NDM-5 were found to be the most prevalent. The detection of the first human case carrying the $bla_{OXA-244}$ gene and the first NDM-5-producing *K. pneumoniae* in the community underscores the need for immediate action to control the spread of these infections.

The spread of MDR bacteria in the community is a serious threat to public health, as it can increase the risk of healthcare-associated infections and impede effective treatment. The presence of MDR bacteria in the community also increases the risk of their transmission from person to person, exacerbating the problem.

In conclusion, the occurrence of CPE strains in hospital and community settings is worrisome and requires immediate attention and action. Effective monitoring, improved infection control measures, and implementation of infection prevention strategies are crucial for preventing the spread of these bacteria and ensuring the safety of public health.

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Authors' contributions

AB collected the data, analyzed them, and wrote the article. ZC analyzed the microbiological data. HR and AB analyzed the molecular data AT analyzed the data and corrected the article.

References

1. Iredell J, Brown J, Tagg K (2016) Antibiotic resistance in *Enterobacteriaceae*: mechanisms and clinical implications. BMJ 8: 352: h6420. doi: 10.1136/bmj.h6420.
2. Paczosa MK, Mecsas J (2016) *Klebsiella pneumoniae*: Going on the offense with a strong defense. MMBR 80: 629–661. doi: 10.1128/MMBR.00078-15.
3. Carmeli Y, Akova M, Cornaglia G, Daikos GL, Garau J, Harbarth S, Rossolini GM, Souli M, Giamarellou H (2010) Controlling the spread of carbapenemase-producing Gram-negatives: therapeutic approach and infection control. Clin Microbiol Infect 16: 102–111. doi:10.1111/j.1469-0691.2009.03115.x.
4. 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.
5. Mairi A, Pantel A, Ousalem F, Sotto A, Touati A, Lavigne JP (2019) OXA-48-producing *Enterobacterales* in different ecological niches in Algeria: clonal expansion, plasmid characteristics and virulence traits. J Antimicrob Chemother 74: 1848–1855. doi: 10.1093/jac/dkz146.
6. 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 (blaNDM-1, blaNDM-2, blaNDM-3) from carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae*: first report from nepal. Infect Drug Resist 15: 4419–4434. doi: 10.2147/IDR.S369934.
7. Emeraud C, Girlich D, Bonnin RA, Jousset AB, Naas T, Dortet L (2021) Emergence and polyclonal dissemination of OXA-244-producing *Escherichia coli*, France. Emerg Infect Dis 27: 1206–1210. doi: 10.3201/eid2704.204459.
8. Findlay J, Perreten V, Poirel L, Nordmann P (2022) Molecular analysis of OXA-48-producing *Escherichia coli* in Switzerland from 2019 to 2020. Eur J Clin Microbiol Infect Dis 41: 1355–1360. doi:10.1007/s10096-022-04493-6.
9. Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z (2019) NDM metallo-β-lactamases and their bacterial producers in health care settings. Clin Microbiol Rev 32: e00115-18. doi:10.1128/CMR.00115-18.
10. Abderrahim A, Djahmi N, Loucif L, Nedjai S, Chelaghma W, Kirane DG, Dekhil M, Lavigne JP, Pantel A (2022) Dissemination of OXA-48- and NDM-1-producing *Enterobacterales* isolates in an Algerian hospital. Antibiotics 11: 750. doi: 10.3390/antibiotics11060750.
11. 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.
12. Loucif L, Chelaghma W, Helis Y, Sebaa F, Baoune RD, Zaatout W, Rolain JM (2018) First detection of OXA-48-producing *Klebsiella pneumoniae* in community-acquired urinary tract infection in Algeria. J Glob Antimicrob Resist 12: 115–116. doi: 10.1016/j.jgar.2017.12.017.
13. Clinical and Laboratory Standards Institute (CLSI) (2020) Performance standards for antimicrobial susceptibility testing. 30th ed CLSI supplement M100 Available: https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf. Assessed: 2.3.2024.
14. Bakour S, Garcia V, Loucif L, Brunel J-M, Gharout-Sait A, Touati A, Rolain JM (2015) Rapid identification of carbapenemase-producing *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* using a modified Carba NP test. New Microbes New Infect 7: 89–93. doi: 10.1016/j.nmni.2015.07.001.
15. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y (2002) Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of *Pseudomonas spp*. and *Acinetobacter spp.* J Clin Microbiol 40: 3798–3801. doi: 10.1128/JCM.40.10.3798-3801.2002.
16. Tsakris A, Kristo I, Poulou A, Themeli-Digalaki K, Ikonomidis A, Tsakris A, Kristo I, Poulou A, Themeli-Digalaki K, Ikonomidis A, Petropoulou D, Pournaras S, Sofianou D (2009) Evaluation of boronic acid disk tests for differentiating KPC-possessing *Klebsiella pneumoniae* isolates in the clinical laboratory. J Clin Microbiol 47: 362–367. doi: 10.1128/JCM.01922-08.
17. Kiiru J, Kariuki S, Goddeeris BM, Butaye P (2012) Analysis of β-lactamase phenotypes and carriage of selected β-lactamase genes among *Escherichia coli* strains obtained from Kenyan patients during an 18-year period. BMC Microbiol 12: 155. doi:10.1186/1471-2180-12-155.
18. Drieux L, Brossier F, Sougakoff W, Jarlier V (2008) Phenotypic detection of extended-spectrum β-lactamase production in *Enterobacteriaceae*: review and bench guide. Clin Microbiol Infect 14: 90–103. doi: 10.1111/j.1469-0691.2007.01846.x.
19. Féria C, Ferreira E, Correia JD, Gonçalves J, Caniça M (2002) Patterns and mechanisms of resistance to beta-lactams and beta-lactamase inhibitors in uropathogenic *Escherichia coli* isolated from dogs in Portugal. J Antimicrob Chemother 49: 77–85. doi: 10.1093/jac/49.1.77.
20. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Guyomard AP, Granier SA, Escobar C, Kumar SM, Villa L, Carattoli A, Hendriksen RS (2018) Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Eurosurveillance 23: 1–11. doi: 10.2807/1560-7917.ES.2018.23.6.17-00672.
21. Saleem Z, Saeed H, Hassali MA, Godman B, Asif U, Youssaf M, Ahmed Z, Riaz H, Raza SA (2019) Pattern of inappropriate antibiotic use among hospitalized patients in Pakistan: a longitudinal surveillance and implications. Antimicrob Resist Infect Control 8: 188. doi: 10.1186/s13756-019-0649-5.
22. Sassi A, Loucif L, Gupta SK, Dekhil M, Chettibi H, Rolain JM (2014) NDM-5 carbapenemase-encoding gene in multidrug-resistant clinical isolates of *Escherichia coli* from Algeria. Antimicrob Agents Chemother 58: 5606–5608. doi: 10.1128/AAC.02818-13.
23. Abderrahim A, Djahmi N, Pujol C, Nedjai S, Bentakouk MC, Gacemi DK, Dekhil M, Sotto A, Lavigne JP, Pantel A (2017) First Case of NDM-1-Producing *Klebsiella pneumoniae* in Annaba University Hospital, Algeria. Microb Drug Resist 23: 895–900. doi: 10.1089/mdr.2016.0213.
24. Nabti LZ, Sahli F, Olowo-Okere A, Benslama A, Harrar A, Mwenebitu DL, Diene SM, Rolain JM (2022) Molecular characterization of clinical carbapenem-resistant *Enterobacteriaceae* isolates from Sétif, Algeria. Microb Drug Resist 28: 274–279. doi: 10.1089/mdr.2021.0123.
25. Khaldi Z, Nayme K, Bourjilat F, Bensaci A, Timinouni M, Khelil A (2022) Detection of ESBLs and carbapenemases among *Enterobacteriaceae* isolated from diabetic foot infections in Ouargla, Algeria. J Infect Dev Ctries 16: 1732–1738. doi: 10.3855/jidc.16660.

26. Costa A, Figueroa-Espinosa R, Gaudenzi F, Lincopan N, Fuga B, Ghiglione B, Gutkind G, Conza J (2021) Co-occurrence of NDM-5 and RmtB in a clinical isolate of *Escherichia coli* belonging to CC354 in Latin America. Front Cell Infect Microbiol 11: 654852. doi: 10.3389/fcimb.2021.654852.
27. Shen Z, Hu Y, Sun Q, Hu F, Zhou H, Shu L, Ma T, Shen Y, Wang Y, Li J, Walsh TR, Zhang R, Wang S (2018) Emerging carriage of NDM-5 and MCR-1 in *Escherichia coli* from healthy people in multiple regions in China: a cross sectional observational study. EClinicalMedicine 6: 11–20. doi: 10.1016/j.eclinm.2018.11.003.
28. Milenkov M, Rasoanandrasana S, Rahajamanana LV, Rakotomalala RS, Razafindrakoto CA, Rafalimanana C, Ravelomandranto E, Ravaoarisaina Z, Westeel E, Petitjean M, Mullaert J, Clermont O, Raskine L, Samison LH, Endtz H, Andremont A, Denamur E, Pradel FK, Lefevre LA (2021) Prevalence, risk factors, and genetic characterization of extended-spectrum beta-lactamase *Escherichia coli* isolated from healthy pregnant women in Madagascar. Front Microbiol 12: 786146. doi: 10.3389/fmicb.2021.786146.
29. Loucif L, Chelaghma W, Cherak Z, Bendjama E, Beroual F, Rolain JM (2022) Detection of NDM-5 and MCR-1 antibiotic resistance encoding genes in *Enterobacterales* in long-distance migratory bird species *Ciconia ciconia*, Algeria. Sci Total Environ 25: 814. doi: 10.1016/j.scitotenv.2021.152861.
30. Yousfi M, Mairi A, Bakour S, Touati A, Hassissen L, Hadjadj L, Rolain JM (2015) First report of NDM-5-producing *Escherichia coli* ST1284 isolated from dog in Bejaia, Algeria. New Microbes New Infect 8: 17–18. doi: 10.1016/j.nmni.2015.09.002.
31. Yaici L, Haenni M, Saras E, Boudehouche W, Touati A, Madec JY (2016) blaNDM-5-carrying IncX3 plasmid in *Escherichia coli* ST1284 isolated from raw milk collected in a dairy farm in Algeria. J Antimicrob Chemother 71: 2671–2672. doi: 10.1093/jac/dkw160.
32. Hoyos-Mallecot Y, Naas T, Bonnin RA, Patino R, Glaser P, Fortineau N, Dortet L (2017) OXA-244-producing *Escherichia coli* isolates, a challenge for clinical microbiology laboratories. Antimicrob Agents Chemother 61: e00818-17. doi: 10.1128/AAC.00818-17.
33. European Centre for Disease Prevention and Control (2021) Increase in OXA-244-producing *Escherichia coli* in the European union/European economic area and the UK since 2013 – first update. Available: https://www.ecdc.europa.eu/sites/default/files/documents/OXA-244-producing-E-coli-in-EU-EEA-since-2013-first-update. Assessed: 2.3.2024.
34. Tafoukt R, Touati A, Leangapichart T, Bakour S, Rolain JM (2017) Characterization of OXA-48-like-producing *Enterobacteriaceae* isolated from river water in Algeria. Water Res 120: 185–189. doi: 10.1016/j.watres.2017.04.073.
35. Annavajhala MK, Gomez-Simmonds A, Uhlemann AC (2019) Multidrug-resistant *Enterobacter cloacae* complex emerging as a global, diversifying threat. Front Microbiol 10: 1–8. doi: 10.3389/fmicb.2019.00044.
36. Evans BA, Amyes SGB (2014) OXA β-lactamases. Clin Microbiol Rev 27: 241–263. doi: 10.1128/CMR.00117-13.
37. Codjoe FS, Donkor ES (2017) Carbapenem resistance: a review. Med Sci 6: 1. doi: 10.3390/medsci6010001.
38. European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2017) EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Available: https://www.scribd.com/document/383499955/EUCAST-Detection-of-Resistance-Mechanisms-170711. Accessed: 19.7.2023.
39. Barguigua A, El Otmani F, Talmi M, Zerouali K, Timinouni M (2012) Emergence of carbapenem-resistant *Enterobacteriaceae* isolates in the Moroccan community. Diagn Microbiol Infect Dis 73: 290–291. doi: 10.1016/j.diagmicrobio.2012.03.01.
40. Yamachika S, Sugihara C, Kamai Y, Yamashita M (2018) Correlation between penicillin-binding protein 2 mutations and carbapenem resistance in *Escherichia coli.* J Med Microbiol 62: 429–436. doi: 10.1099/jmm.0.051631-0.
41. Doumith M, Ellington MJ, Livermore DM, Woodford N (2009) Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter spp*. clinical isolates from the UK. J Antimicrob Chemother 63: 659–667. doi: 10.1093/jac/dkp029.
42. Diene SM, Bruder N, Raoult D, Rolain J-M (2011) Real-time PCR assay allows detection of the New Delhi metallo-β-lactamase (NDM-1)-encoding gene in France. Int J Antimicrob Agents 37: 544–546. doi: 10.1016/j.ijantimicag.2011.02.006.
43. Mellouk FZ, Bakour S, Meradji S, Al-Bayssari C, Bentakouk MC, Zouyed F, Djahoudi A, Boutefnouchet N, Rolain JM (2017) First detection of VIM-4-producing *Pseudomonas aeruginosa* and OXA-48-producing *Klebsiella pneumoniae* in Northeastern (Annaba, Skikda) Algeria. Microb Drug Resist 23: 335–344. doi: 10.1089/mdr.2016.0032.
44. Nordmann P, Boulanger AE, Poirel L (2012) NDM-4 metallo-β-lactamase with increased carbapenemase activity from *Escherichia coli*. Antimicrob Agents Chemother 56: 2184–2186. doi: 10.1128/AAC.05961-11.
45. Yagi T, Kurokawa H, Shibata N, Shibayama K, Arakawa Y (2000) A preliminary survey of extended-spectrum beta-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Japan. FEMS Microbiol Lett 184: 53–56. doi: 10.1111/j.1574-6968.2000.tb08989.x.

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