First description of NDM-1-positive Klebsiella pneumoniae in the Tunisian community

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Letter to the Editor

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Sir,

Multidrug-resistant bacteria, especially carbapenemase-producing Enterobacteriaceae, are a major public health-threat worldwide. As part of a collaborative monitoring programme, our laboratory at the University of Bordeaux has received a collection of multidrug-resistant bacterial strains to further characterise their β-lactamase content. They were sent from private Tunisian diagnostic laboratories and were collected from community patients suffering from urinary tract infection.

In this context, multidrug-resistant isolate 18TA was collected in January 2018 in Sfax region from the urine of a 45-year old female with no previous hospitalisation during the preceding month and no history of recent foreign travel.

Strain 18TA had been initially classified as *Enterobacter* spp. by biochemical tests (API 10S gallery). Following matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonics) and confirmation by PCR amplification and sequencing of 16S rDNA, strain 18TA was re-identified as *Klebsiella pneumoniae*. Multilocus sequence typing (MLST) (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html) indicated that strain 18TA belonged to Sequence Type (ST), ST147.

Minimum inhibitory concentrations (MICs) of various antimicrobials were determined using a BD Phoenix™ 100 automated system (BD Diagnostic Systems, Le Pont-de-Claix, France) and the results were interpreted using BD EpiCenter™ software (BD Diagnostic Systems). The MICs for ciprofloxacin and colistin were also determined by the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing 2019 guidelines (https://www.sfm-microbiologie.org/2019/05/06/casfm-eucast-2019-v2/).

Strain 18TA was resistant to all tested β-lactams, including carbapenems (Table 1). The strain was also resistant to gentamicin, tobramycin, quinolones (nalidixic acid), fluoroquinolones (ciprofloxacin) and trimethoprim/sulfamethoxazole (SXT) and showed decreased susceptibility to tigecycline (MIC = 2 μg/mL). It remained susceptible to amikacin, fosfomycin and colistin (Table 1). The imipenem/ethylene diamine tetra-acetic acid (EDTA) combined disk diffusion test was positive since the inhibition zone increased by ≥7 mm with the imipenem/EDTA disk compared with the imipenem disk alone, suggesting the presence of a metallo-β-lactamase (MBL) [1]. In addition, the double-disk synergy test (between amoxicillin/clavulanic acid and broad-spectrum cephalosporins) showed the presence of an extended-spectrum β-lactamase (ESBL)-producing phenotype (data not shown).

The transferability of the β-lactam resistance determinant was assessed by conjugation assay using an azide-resistant (Az^R) mutant of *Escherichia coli* C600 as the recipient strain. Selection was performed on Mueller–Hinton agar plates supplemented with sodium azide (300 μg/mL) and ertapenem (4 μg/mL). A transfer frequency of ca. 10^{-4} transconjugants per donor was observed. Comparison of MICs between the transconjugant (Tc-18TA) and its recipient strain (C600 Az^R) showed increased resistance not only to the tested β-lactams but also to gentamicin, tobramycin and SXT (Table 1).

Total genomic DNA of strain 18TA was screened using different multiplex PCR amplifications for various β-lactamase genes ($bla_{TEM\text{-}like}$, $bla_{SHV\text{-}like}$, $bla_{OXA\text{-}1\text{-}like}$, $bla_{CTX\text{-}M}$ groups 1, 2, 9, 18 and 25, $bla_{OXA\text{-}48\text{-}like}$, bla_{KPC} and bla_{GES} and the MBL genes bla_{VIM}, bla_{IMP} and bla_{NDM}) as described previously [2]. Amplification results following agarose gel electrophoresis analysis showed the presence of group 1 $bla_{CTX\text{-}M}$ and bla_{NDM} genes together with $bla_{TEM\text{-}like}$, $bla_{SHV\text{-}like}$ and $bla_{OXA\text{-}1\text{-}like}$ genes. Except for the bla_{SHV} gene that was found in strain 18TA but not in the transconjugant Tc-18TA and that was attributed to the chromosomally-encoded species-specific enzyme of *K. pneumoniae*, the four other β-lactamases were also found in transconjugant Tc-18TA (Table 1). Amplification of the entire *bla* genes was performed and subsequent sequencing ((Custom DNA sequencing; Eurofins Genomics GmbH, Ebersberg, Germany) showed the presence of the narrow-spectrum β-lactamase genes $bla_{TEM\text{-}1B}$ and $bla_{OXA\text{-}1}$ associated with the $bla_{CTX\text{-}M\text{-}15}$ ESBL gene and the $bla_{NDM\text{-}1}$ MBL gene both in 18TA and Tc-18TA (Table 1). Furthermore, amplifications searching for *aac(3)-IIa* (gentamicin and tobramycin resistance) and *sul1* and *dfrA1* (sulfamethoxazole and trimethoprim resistance, respectively) were positive both in 18TA and Tc-18TA. These genes were also present in Kp3771, a ST147 NDM-1-producing *K. pneumoniae* strain recently recovered from a patient hospitalised in an intensive care unit of University Hospital Tahar Sfar in Tunisia [3].

NDM-1-positive *K. pneumoniae* strains have been previously described in Tunisia, but only from hospitalised patients [1–5]. The current study reports the first description of *K. pneumoniae* carrying the carbapenemase NDM-1 in the Tunisian community

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Table 1
β-Lactamase (*bla*) gene content and antimicrobial susceptibility of *Klebsiella pneumoniae* 18TA and its *Escherichia coli* (Ec) transconjugant Tc-18TA.

Strain	*bla* gene content	MIC (μg/mL)[a]														
		TZP	FEP	ATM	IPM	MEM	ETP	GEN	TOB	AMK	SXT	NAL	CIP	FOS	TGC	COL
18TA	$bla_{SHV\text{-}like}$ $bla_{TEM\text{-}1B}$ $bla_{OXA\text{-}1}$ $bla_{CTX\text{-}M\text{-}15}$ $bla_{NDM\text{-}1}$	>64	>16	>16	>8	>8	>1	>4	>4	≤4	>4	>16	128	32	2	1
Tc-18TA[b]	$bla_{TEM\text{-}1B}$ $bla_{OXA\text{-}1}$ $bla_{CTX\text{-}M\text{-}15}$ $bla_{NDM\text{-}1}$	**>64**	**>16**	**>16**	**4**	**4**	**>1**	**>4**	**>4**	≤4	**>4**	≤4	0.0625	≤16	≤0.25	0.25
Ec C600 Az^R	–[c]	≤4	≤1	≤0.25	≤0.25	≤0.25	≤0.25	≤1	≤1	≤4	≤1	≤4	0.0625	≤16	≤0.25	0.25

MIC, minimum inhibitory concentration; TZP, piperacillin/tazobactam; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; ETP, ertapenem; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; SXT, trimethoprim/sulfamethoxazole; NAL, nalidixic acid; CIP, ciprofloxacin; FOS, fosfomycin: TGC, tigecycline; COL, colistin; Az^R, azide-resistant.
[a] MICs were determined by BD Phoenix™ automated system. Strain 18TA and the transconjugant Tc-18TA were resistant to all β-lactams tested in the panel; only MICs to TZP, FEP, ATM, IPM, MEM and ETP are given. MICs for CIP and COL were also determined by the broth microdilution method.
[b] In transconjugant Tc-18TA, the β-lactamase genes transferred by conjugation are reported, as well as the co-transferred resistances which are indicated in bold characters.
[c] Not present.

setting and confirms that the NDM-1-positive ST147 *K. pneumoniae* clone has become endemic in this country [5]. Detection of this clone is of concern, therefore implementation of adequate infection control measures and uninterrupted active surveillance programmes both in hospital and community settings are crucial to prevent the dissemination of the NDM-1 enzyme in this region.

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Competing interests

None declared.

Ethical approval

Not required.

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