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Short Communication

First report of nosocomial infection caused by *Klebsiella pneumoniae* ST147 producing OXA-48 and VEB-8 β-lactamases in Tunisia

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ABSTRACT

The aim of this study was to determine the origin of virulence and multiresistance of a *Klebsiella pneumoniae* isolate from an abdominal wound infection of a patient with a gunshot injury in the thoracoabdominal region. The isolate was identified using biochemical tests and Phoenix™ automated system and was confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). MICs of each antibiotic were determined by Etest. Screening for carbapenemase production was performed by the modified Hodge test and was confirmed by PCR amplification. Virulence factors were also studied. Plasmid replicon typing was used to classify Incompatibility (Inc) plasmids harbouring the resistance genes. The transferability of each plasmid was determined by conjugation using *Escherichia coli* J53. Finally, multilocus sequence typing (MLST) was performed to determine the ST of the strain. The bacterial isolate was identified as *K. pneumoniae* and was named KPM2, carrying *entB*, *ybtS*, *mrkD* and *ycfM* virulence genes, but it did not overexpress OqxAB. Isolate KPM2 belonged to ST147 and was classified as resistant to all of the tested antibiotics with MICs above the clinical breakpoints. These resistances were due to production of OXA-48, CMY-2, TEM-1, CTX-M-15 and VEB-8 β-lactamases. Genetic and molecular studies showed that bla_{OXA-48} was embedded in transposon Tn*1999.2* and was carried by a conjugative IncL/M plasmid of ca. 60 kb; bla_{VEB-8} was harboured on a conjugative IncA/C plasmid of ca. 120 kb. This study confirmed that the resistance conferred by OXA-48 and VEB-8 contributed to the failure of antibiotic treatment and consequently death of the patient.

1. Introduction

Carbapenems are very often the last active therapy for treating multidrug-resistant (MDR) bacteria [1]. The emergence and worldwide spread of carbapenemase-producing Enterobacteriaceae is of great concern to public health services and is a major threat to the efficacy of carbapenem antibiotics such as imipenem, ertapenem and meropenem, which are the drugs of choice for the treatment of infections due to extended-spectrum β-lactamases (ESBL)-producing bacteria [2].

The most common ESBLs found in clinical isolates of Enterobacteriaceae belong to the TEM-, SHV- and CTX-M-derived β-lactamases [2]. However, other ESBLs have been reported in Enterobacteriaceae, including IBC-1 detected in an *Enterobacter cloacae* isolate in Greece [3], and VEB-1 in Enterobacteriaceae clinical isolates in Southeastern Asia such as *E. cloacae* and *Enterobacter sakazakii* in Bangkok, Thailand [4]. bla_{VEB-1} has been described as part of a gene cassette located in class 1 integrons of

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various structures [5]. Others variants of VEB-1 have been reported worldwide and a novel VEB variant, named VEB-8, was identified in a single *Escherichia coli* isolate from Libya that co-produced CMY-2 [4].

Various carbapenemases, such as NDM-, KPC- and OXA-types, have recently emerged and spread worldwide [6]. In North Africa, carbapenemases belonging to IMP- and VIM-types were previously reported in clinical and environmental isolates [7]. OXA-48, a class D carbapenemase, was first identified in a clinical *Klebsiella pneumoniae* isolated in Turkey in 2001 [8]. Subsequently, OXA-48 spread has been reported not only across Turkey but also in the Middle East, Europe and North Africa [9].

OXA-48 hydrolyses penicillins and at a lower level imipenem but is not active against third-generation cephalosporins, making its detection difficult.

MDR bacteria often show fluoroquinolone resistance due to mutations in the quinolone resistance-determining regions (QRDRs) as well as plasmid-mediated quinolone resistance (PMQR). Four major PMQR determinants have been described [10]: Qnr proteins; AAC(6′)-Ib-cr; QepA; and OqxAB efflux pumps. Interestingly, OqxAB has been also described as a virulence factor in *K. pneumoniae* [11].

The aim of the present study was to identify the antibiotic resistance and virulence determinants harboured by a nosocomial MDR *K. pneumoniae* strain isolated from a Libyan patient admitted to Military Hospital (Tunis, Tunisia).

2. Materials and methods

2.1. Clinical data and past medical history of the patient

The patient was shot in the thoracoabdominal region during the revolution in Libya. During his admission to a Tunisian hospital, the patient had a fever and antibiotic treatment was initiated empirically before diagnosis. One month later, pus was sampled in order to screen for the causative agent. A MDR Gram-negative bacterium resistant to imipenem and colistin was isolated. At this time the patient was treated with imipenem (four injections of 50 mg/kg/day) in combination with amikacin and metronidazole without any clinical improvement. The treatment was changed to a combination regimen including ertapenem (1 g/day) and piperacillin/tazobactam (four injections of 200 mg/kg/day), but unfortunately the treatment was unsuccessful and the patient died from toxic shock.

2.2. Bacterial identification

The isolate was identified using biochemical tests and Phoenix™ automated system (Becton Dickinson, Oxford, UK) and was confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (IVD MALDI Biotyper; Bruker BioSpin SAS, Wissembourg, France).

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility was determined on Mueller-Hinton agar using the standard disk diffusion procedure. Minimum inhibitory concentrations (MICs) were determined using Etest strips (AB bioMérieux, Marcy-l'Étoile, France) and were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2014 breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_4.0.pdf).

2.4. Phenotypic detection of carbapenemases

The modified Hodge test was performed on a Mueller-Hinton agar plate with ertapenem with and without ethylene diamine tetra-acetic acid (EDTA), using *E. coli* ATCC 25922 as an indicator for detection of carbapenemases. *Klebsiella pneumoniae* ATCC BAA-1705 was used as a positive control and *K. pneumoniae* ATCC 8291T was used as a negative control.

2.5. Detection and identification of β-lactamase genes

β-Lactamase resistance genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}, bla_{VEB} and bla_{GES}) were amplified by PCR as previously described [12]. The primers used are listed in Table 1. Carbapenemase-encoding genes (bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-58-like}$ and $bla_{OXA-48-like}$) were screened using multiplex PCR as described previously [12]. PCR products were sequenced and the results were compared with reported sequences available in GenBank. The genetic environment of bla_{OXA-48} was determined by PCR mapping [8].

2.6. Virulence factors

Genes known to be linked to virulence (*ybtS*, *mrkD*, *entB*, *rmpA*, *kfu*, *allS*, *magA*, *ycfM* and *iroN*) were detected using PCR as described previously [13]. OqxA, OqxB and OqxR were also sequenced [11].

2.7. Conjugation experiments

Conjugation experiments were performed using *E. coli* J53 (sodium azide-resistant) as the recipient. Transconjugants were selected on brain–heart infusion agar supplemented with cefotaxime (16 mg/L) and sodium azide (100 mg/L).

Plasmid size was determined by Kieser extraction and electrophoresis using 0.7% agarose. *E. coli* NCTC 50192 harbouring 154, 66, 48 and 7 kb plasmids was used as a reference.

2.8. Plasmid replicon typing of plasmids harbouring bla_{OXA-48} and bla_{VEB-8}

Plasmid replicons harboured by the *K. pneumoniae* isolate and its transconjugants were determined using the PCR-based replicon

Table 1
Primers used for amplification and sequencing of β-lactamase-encoding genes.

Target	Primer name	Sequence (5′ → 3′)	Amplicon size (bp)
bla_{TEM} group	TEM-A	TAAAATTCTTGAAGACG	1074
	TEM-B	TTACCAATGCTTAATCA	
$bla_{CTX-M-1}$ group	CTX-M1-A2	CTTCCAGAATAAGGAATC	909
	CTX-M1-B2	CCGTTTCCGCTATTACAA	
bla_{CMY-2} group	CMY-2F	CGGACACCTTTTTGCTTTTAATTAC	1222
	CMY-2R	GAAAGAAAGGAGGCCCAATA	
bla_{OXA-48} group	OXA-48-F1	TGGATATTGCATTAAGCAAGG	876
	OXA-48-R1	TTTACAGCTTATATGACGGCTA	
bla_{VEB} group	VEB-F1	AACCAGATAGGAGTACAGACA	925
	VEB-R1	TTTATTTATTCAAATAGTAATTCCACG	