First Report of a *Providencia stuartii* Strain Coproducing a Beta-Metalloenzyme NDM-1 Type and an Oxacillinase Type OXA-48

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Abstract

Introduction: The emergence and dissemination of carbapenemase-producers enterobacterias and their worldwide spread represents a major issue for both clinical practice settings and public health strategies. Their current extensive spread worldwide is an important source of concern due to limited therapeutic options and raised risk of mortality.

Materials and methods: Pus samples obtained from burned patients admitted to the plastic surgery department were analyzed. They were seeded on ordinary and enriched culture media. Depending on the the antimicrobial susceptibility testing, research of metallo-ß-lactamase was done on isolates by EDTA synergy testing, Hodg test and multiplex PCR.

Results: The identification objectified the presence of multiresistant *P. stuarti*. Imipenem CMI was high (CMI>8 mg). The resistance has also interested other antibiotics with the exception of aztrinam, amikacin and ciprofloxacin. The EDTA synergy testing was positive (φ>5 mm). The Hodge test was negative for both strains. The PCR have demonstrated the presence of resistance genes (*blaNDM-1, blaOXA-48*).

Conclusion: This study demonstrated that multidrug resistant strains are present in our country. Regular monitoring and documentation of carbapenem resistant is crucial in developing strategies to control infection due to these bacteria.

Keywords: *Providencia stuartii*, Metallo-ß-enzyme, Nosocomial infection, Burn

Introduction

The genus *Providencia* includes gram-negative bacilli that are responsible for a wide range of human infections. *Providencia stuartii* (*P. stuartii*) is the most common species that can cause infections in humans. It is an opportunistic pathogen observed in patients with severe burns or those with long-term urinary catheters.[1] They represent an emerging problem because of the increasing prevalence of beta-lactam extended spectrum resistance (ESBL) and the recent emergence of carbapenem resistance.[2] We report the first cases of isolation of *P. stuartii* producing a beta-metalloenzyme (MBL) type NDM-1 and oxacillinase type OXA-48 in two burned patients hospitalized at the plastic surgery department in Ibn Tofail Hospital, university hospital center Marrakesh.

Materials and Methods

Sampling

In front of local and general signs of infection (feverish peaks and high CRP), skin samples were taken from two patients, a 34-year-old woman and a 25-year-old man, admitted to the plastic surgery department at the same time, victims of explosion of butane bottle, with burned cutaneous areas of 32% and 41% respectively. The skin samples were sent to the laboratory of the hospital Ibn Tofail for bacteriological analysis.

Inoculation

Samples received at the laboratory were inoculated in selective and non-selective media and incubated at 37°C for at least 24 hours.

Identification

P. stuartii isolates were identified by studying the biochemical characteristics using the API20E system (bioMérieux, Marcy l'Etoile, France). The strains were subsequently sent to Ibn Roch Casablanca UHC for genotypic study by real-time PCR.

Antimicrobial susceptibility testing

Susceptibility to various classes of antimicrobial agents was determined by the disc diffusion method in accordance with the recommendations of the French Society of Microbiology guidelines. The test medium was Mueller-Hinton. The Minimum inhibitory concentrations were determined by an automated method using the Becton Dickinson BD Phoenix. The antibiotics disks used in this study.

Phenotypic tests

Synergy test EDTA: The EDTA synergy test was performed by placing the imipenem disks and an imipenem disk plus 10 µl of EDTA (Ethylene diamine tetraacetic acid) on Mueller agar. The test is positive when an increase in diameter greater than or equal to 5 mm between the zones of inhibition around the carbapenem disc and the combined disc IMP-EDTA is observed.

Hodge test: The modified Hodge test (MHT), which is recommended by CA-SFM as a confirmatory test for carbapenemase production was performed for each strain. A 0.5McFarland dilution of Escherichia coli in 5ml of broth was prepared. A 1/10 dilution was streaked on to a Meuller-Hinton agar plate. A 10 µg imipénème or ertapénème susceptibility disk was placed in the center of the test area. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. Two strains of *Klebsiella pneumoniae* were used as negative and positive controls. The plate was incubated overnight at 37°C for 24 h.

PCR (Polymerase chain reaction): The genotypic study was performed by multiplex PCR for the following carbapenemase genes *blaNDM, blaVIM, blaKPC* and *blaOXA-48*. The compositions of the primers for each gene studied are shown in Tables 1 and 2. Nucleic acid extraction was performed by kit marketed Mini Kit (QIAGEN). The real-time PCR was carried out by the Sybr Green technique on a CFX 96 real-time thermal cycler. The results of the PCR were interpreted by the quantization curve in relation to its threshold cycle (Ct) <25 cycles. For the confirmation of the results obtained two parameters have been added:

1. The threshold curve.

2. Migration of the amplifier on 1.5% agarose gel.

Results

On both samples, a monomorphic shoot with multi resistant *P. stuarti* was observed (Figures 1 and 2). In front of this profile the search for a metallo-β-enzyme was carried out and showed that the growth of the bacterium was inhibited around the IMP-EDTA disk with a diameter of inhibition greater than 5 mm (Figure 3). The minimal inhibitory concentrations (MIC) at imipenem are higher than 8 mg/L. Antibiotic resistance also affected other families of antibiotics with relatively high MICs (Table 3), with the exception of amikacin and ciprofloxacin. The Hodge test was negative for both strains. The PCR result with the primers tested showed amplification with blaNDM-1 and blaOXA-48 (Table 4).

Discussion

The *Providencia* genus has five species, of which *P. stuartii* is the most frequently isolated from infections and the most resistant to antibiotics.[2] The high-level resistance to beta-lactamines in this species is mainly caused by the overexpression of its natural beta-lactamase type AmpC, but also by the acquisition of extended-spectrum beta-lactamases of the TEM-, SHV- or CTX-M type as well as than VEB-1 and PAR-1.[2]

The geographical distribution of resistant strains differs around the world; those that produce a KPC-type carbapenemase (class A) have been more frequently observed in the United States, Greece and Israel, OXA-48 (class D) is more common in the Mediterranean region including Morocco[3] while those producing beta-lactamases of type VIM and IMP class B were generally found in Asia and in the northern part of the Mediterranean basin.[4] Among these enzymes metallocarbapenemases are particularly worrying because they hydrolyze almost all classes of beta-lactams except aztreonam. Initially observed in strains of *Pseudomonas aeruginosa* and *Acininitobactere* spp., this resistance propagated in the Enterobacteriaceae family during the 1990s and 2000s.[4]

Discovered in 2008 in Sweden from an Indian patient, metallo-β-lactamase NDM-1 has already been identified on the majority of continents in 2010 and was linked in all cases with a stay in the subcontinent Indian.[5] Several epidemics or sporadic cases have been described in hospital or community settings.[6] This type of enzyme has been mainly isolated from *K. pneumoniae*, *Enterobacteriaceae* and recently from *P. stuartii*, and at various locations around the world.[7-10]

In our study, genotyping the two strains of *P. stuartii* isolated revealed the coexistence of the *blaNDM-1 and blaOXA-48* genes. Other international studies have reported the coexistence of these two resistance genes and even several genes in both *P. stuartii.*[2,9,10] In Morocco the presence of beta-metalloenzyme-producing bacteria associated with OXA-48 has been reported in *Klebsiella pneumoniae* in Taza, Casablanca and Rabat. [3,11,12] To our knowledge, our study is the first to report the case of isolation of *P. stuartii* strains bearing *bla-NDM-1* gene in Morocco, a region considered endemic for OXA-48. This is consistent with the fact suggesting the current spread of metallo-β-lactamase especially in clinical strains throughout the North African Countries.[3,11,13]

The level of carbapenem resistance is variable. The plasmids carrying the *blaNDM-1* gene very often contain several other resistance genes, which ultimately leads to multi-resistance or even total resistance of the strains.[14] Strains isolated in this study showed resistance to all antibiotic families tested except aztreonam, tobramycin, amikacin and ciprofloxacin.

The hodge test in our study was negative whereas the genotyping of the strains shows the existence of beta-metallolo enzyme and *oxacillinase* genes. This result has also been reported by other studies, which means that this test is almost abandoned because of false negatives.[13]

The coexistence of these determinants of resistance in nosocomial strains is a threatening situation. The search for the clonal relationships between the *P. stuartii* isolates was not made in our study, but the phenotypic similarities of the strains suggest that it is the same strain that has spread from the first to the second patient. The rapid detection and the technical and geographical isolation of the two patients made it possible to limit the spread of this strain within the burn unit of the plastic surgery department and thus to prevent an epidemic.

The production of beta-metallo-lactamase in *P. stuartii* has been associated with an adverse evolution, most often fatal.[8,10] In our context, the evolution of both patients was favorable thanks to the combination of amikacin and ciprofloxacin in parallel with local care.

Conclusion

P. stuartii remains among the multiresistant strains prevalent in hospitals. In addition, the NDM-1 type metallo-β-lactamase producing strains represent an emerging threat in Morocco. The antimicrobial selection pressure could allow the silent propagation of the *blaNDM-1* gene in Morocco. Thus, it seems necessary to start a serious and immediate management of this problem by implementing multidisciplinary strategies to optimize the detection and reduce the diffusion of strains producing MBL.

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Table 1: Composition of the primers of the *OXA-48* gene.

Primers	**Composition of the primers**	**Number of base pairs**	**Size of the gene**
Sense primers (5’ ? 3’)	GCTTGATCGCCCT CGATT	18	281 pb
Anti-sense primers (3’ ? 5’)	GATTTGCTCCGTG GCCGAAA	20	

Table 2: Oligonucleotide sequences of the primers used.

Gene	**Composition of the primers**	**Number of base pairs**	**Tm**	**Size of the gene (pb)**
KPC	5’CATTCAAGGGCTTTCTT GCTGC3’	22	60.93	538
	3’ACGACGGCATAGTCATT TGC5’	20	58.99	

VIM	5’ GATGGTGTTTGGTCGCATA 3’ 3’CGAATGCGCAGCACCA G5’	19 17		55.61 59.54	390
NDM	5’GGTTTGGCGATCTGGTT TTC3’ 3’CGGAATGGCTCATCACG AT5’	20 20		60 62	621

Table 3: Antibiotics tested and MIC.

Code	Antibiotics	Pro 1	MIC	Pro 2	MIC
AM	Ampicilline	R	>8	R	>8
AMX	Amoxicilline	R		R	
TIC	Ticarcilline	R	>16	R	>16
PIP	Pipéracilline	R		R	
AMC	Amoxicilline+acide clavulanique	R	>8/2	R	>8/2
Tic	Ticarcillin-Clavulanate		>16/2		>16/2
	Piperacillin-Tazobactam		>16/4		>16/4
CF	Céfalotine	R	>32	R	>32
CXM	Céfuroxime	R		R	
	Céftriaxone	R	>4	R	>4
CTX	Céfotaxime	R		R	
CAZ	Céftazidime	R	>8	R	>8
FEP	Céfépime	R	>8	R	>8
FOX	Céfoxitine	R	>32	R	>32
	Cefixime	R	>2		>2
ATM	Aztreonam	S	2	S	2
IMP	Imipénème	I	>8	R	>8

ERT	Ertapeneme	R	>1	R	>1
GEN	Gentamicine	R	>4	R	>4
TM	Tobramycine	S		S	
AN	Amikacine	S	≤ 4	S	≤ 4
CIP	Ciprofloxacine	S		S	
SXT	Triméthoprime/Sulfaméthoxazol	R	≤ 1/19 S	R	≤ 1/19 S
CS	Colistine	R		R	
R=resistant, S=sensible, I=intermediate, Less than or equal (≤)					

Table 4: Result of the amplification by PCR in real time.

Strains tested	**Pro 1**	**Pro 2**
NDM-1	POSITIVE	POSITIVE
VIM	NEGATIVE	NEGATIVE
OXA-48	POSITIVE	POSITIVE
KPC	NEGATIVE	NEGATIVE

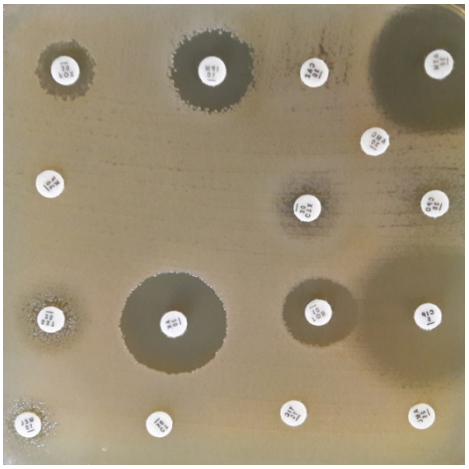

Figure 1: Antibiogram of the strain *Providencia stuartii* (Pro1).

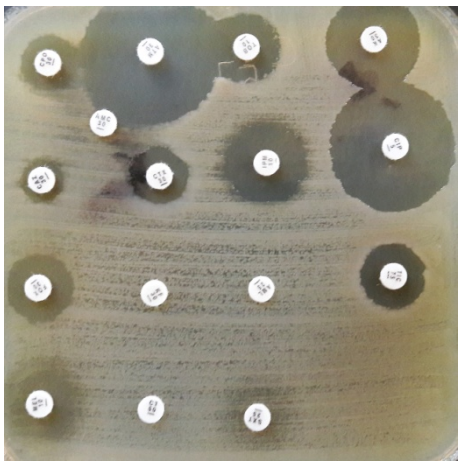

Figure 2: Antibiogram of the strain *Providencia stuartii* (Pro2).

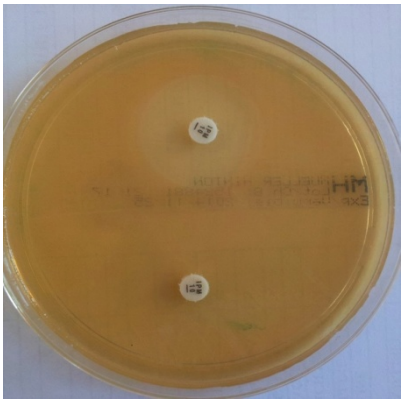

Figure 3: IMP-EDTA Synergy Test of the Pro1 strain.