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Bloodstream infections caused by multidrug-resistant *Klebsiella pneumoniae* producing the carbapenem-hydrolysing VIM-1 metallo-β-lactamase: first Italian outbreak

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***Objectives*: To investigate the first Italian outbreak of bloodstream infections caused by multidrug-resistant (MDR) *Klebsiella pneumoniae* producing metallo-β-lactamase (MBL), which occurred in three wards of one large tertiary-care hospital in Genoa, Italy, from September 2004 to March 2005.**

***Methods*: MBL production was screened by an imipenem–EDTA disc synergy test and confirmed by a conventional hydrolysis test. Antibiotic susceptibility was determined by broth microdilution or disc diffusion. PFGE was used to study the genetic relatedness of isolates. PCR and sequencing were carried out to identify the β-lactamase genes and to analyse the genetic context of the MBL gene. Outer membrane protein (OMP) profiles were analysed by SDS–PAGE.**

***Results*: Nine cases of bloodstream infections caused by an MDR strain of *K. pneumoniae* producing the VIM-1 MBL and the SHV-5 extended-spectrum β-lactamase (ESBL) were identified. The isolates exhibited various carbapenem resistance levels (imipenem MICs ranged from 4 to 64 mg/L) and were resistant to other β-lactams, fluoroquinolones, trimethoprim/sulfamethoxazole and chloramphenicol. The isolate with the highest imipenem MIC also lacked the k36 OMP. The bla_{VIM-1} gene cassette was part of the variable region of a class 1 integron that also included an *aac(6′)-Ilc* cassette. The ESBL and MBL genes were transferable by conjugation.**

***Conclusions*: This is the first report on the emergence of an MDR strain of *K. pneumoniae* producing the VIM-1 MBL, causing an outbreak of bloodstream infections in an Italian hospital. The strain evolved through OMP alterations generating a mutant with increased carbapenem resistance.**

Keywords: carbapenem resistance, carbapenemases, extended-spectrum β-lactamases, class 1 integrons

Introduction

Carbapenems currently represent the drugs of choice for treatment of serious infections caused by multidrug-resistant (MDR) strains of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs). Recently, however, the emergence of carbapenem resistance mediated by the production of acquired carbapenemases has been increasingly reported among Enterobacteriaceae and is a matter of major clinical concern.[1]

Two major classes of acquired carbapenemases have been reported in Enterobacteriaceae, namely, the molecular class A serine-β-lactamases of the IMI-, SME- and KPC-types and the molecular class B metallo-β-lactamases (MBLs) of the IMP- and VIM-types.[1] Enterobacteriaceae producing acquired MBLs still remain uncommon worldwide, except in Greece.[2] Recently, sporadic isolates of *Enterobacter cloacae* and *Klebsiella pneumoniae* producing VIM-type MBLs have been described in Italy.[3,4]

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In this report, we describe the first Italian outbreak of bloodstream infections caused by an MDR strain of *K. pneumoniae* producing the VIM-1 MBL and the SHV-5 ESBL.

Materials and methods

Bacterial strains

Nine *K. pneumoniae* isolated from blood samples of nine inpatients at the S. Martino University Hospital (Genoa, Italy) from September 2004 to March 2005 were investigated in this work.

Antibiotic susceptibility testing and phenotypic screening for MBL production

Broth dilution and disc diffusion tests were performed according to the recommendations of CLSI.[5,6] To detect MBL production, a synergy test using imipenem and EDTA discs was used.[7]

β-Lactamase studies

Isoelectric focusing (IEF) was performed in polyacrylamide gels (pH range 3.5–9.5, GE Healthcare Bio-Science AB, Sweden), with crude cell extracts prepared by sonic disruption. MBL activity was determined by spectrophotometry essentially as described previously.[8]

Pulsed-field gel electrophoresis

Genomic DNA was prepared, digested with *Xba*I (New England Biolabs Inc., MA, USA) and subjected to PFGE with the CHEF DRII device (Bio-Rad, Milan, Italy), as described previously.[9]

Transfer of β-lactam resistance

Conjugation experiments were carried out between each test isolate and *Escherichia coli* K12 J53 Rif^R in broth medium. Transconjugants were selected on rifampicin (256 mg/L) and either imipenem (1, 2 or 4 mg/L) or aztreonam (1, 2 or 4 mg/L).

Analysis of the outer membrane proteins (OMPs)

OMP preparations were obtained after sonic disruption of bacterial cells grown in nutrient broth, followed by selective solubilization of cytoplasmic material with sodium *N*-lauroyl sarcosinate (2% w/v) and ultracentrifugation. The preparations were run on SDS–polyacrylamide gels (11%) and stained with Coomassie Blue.

Molecular analysis techniques

PCR was used for the detection of β-lactamase determinants (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{IMP} and bla_{VIM}) and for mapping of the variable region of class 1 integrons.[8,10] DNA sequencing was carried out on both strands of PCR amplification products, as described previously.[10]

Results

Description of the outbreak and characterization of the isolates

Between September 2004 and March 2005, a total of nine bloodstream infection episodes caused by MDR isolates of *K. pneumoniae* showing a putative MBL-positive phenotype according to an imipenem–EDTA synergy test were observed in nine inpatients at three wards [two intensive care units (ICUs) and the transplant surgery unit] of the S. Martino University Hospital (Genoa, Italy).

All isolates exhibited the same PFGE macrorestriction profile (data not shown), revealing the clonal nature of the outbreak.

Molecular analysis revealed the presence of the following β-lactamase genes in all isolates: bla_{TEM-1}, bla_{SHV-5} and bla_{VIM-1}. These findings were overall consistent with the IEF profiles obtained with crude extracts, which showed the presence of β-lactamase bands of both acidic pI (around 5.3, accountable for by TEM-1 and VIM-1) and alkaline (around 8.2, accountable for by SHV-5 and, possibly, by the resident SHV-type enzyme). Conventional spectrophotometric assays confirmed the presence of MBL activity in the crude extracts of all isolates (imipenemase specific activity, 27–33 nmol/min/mg protein; in all cases inhibitable by >80% after incubation with 1 mM EDTA).

From all patients, isolation of the *K. pneumoniae* occurred >72 h after hospital admission, indicating nosocomial acquisition. For eight patients, isolation of the *K. pneumoniae* occurred >72 h after ICU admission, suggesting that acquisition occurred in the ICU (Table 1). The majority of the patients had strong epidemiological links. Eight of nine were in the same ICU (ICU p4), although temporal links were evident for only seven of them. The patient in the transplantation surgery unit (E) was previously hospitalized in ICU p4, where they probably acquired the VIM-1-producing *K. pneumoniae*. A single patient (G) was infected with the same microorganism and in the same period of time but in a different ICU (ICU er). Contact between the patients in the two ICUs through the same medical equipment or medical staff was excluded. One patient (C), who was moved from ICU p4 to ICU er for 2 days and then back to ICU p4, possibly played a role in the dissemination.

All patients had underlying disease prior to admission: five had undergone recent liver or kidney transplantation, one had colon cancer, one had lung cancer and one had serious obesity.

Antimicrobial therapy prior to and after isolation of the MBL-producing *K. pneumoniae* is reported in Table 1. Four of the nine patients died, but only for two of them (Patients D and F), could the *K. pneumoniae* infection be considered as causative or contributory to death. In the other two patients, the *K. pneumoniae* infection was apparently eradicated following treatment with meropenem plus amikacin (Patient I) or treatment with piperacillin/tazobactam plus amikacin (Patient C), but the patients died because of a subsequent infection caused by an MDR *Pseudomonas aeruginosa* (septicaemia and pneumonia, respectively). The *P. aeruginosa* isolates from these patients were carbapenem-resistant and positive for the bla_{VIM} MBL gene by PCR.

Antibiotic susceptibility

All *K. pneumoniae* isolates exhibited the same multiresistance pattern and were susceptible or intermediate to carbapenems, with the exception of that from the last patient included in the study (Patient I), which was resistant to imipenem and meropenem (Table 2). Imipenem hydrolytic activity of the extract from this isolate did not reveal significant differences from those of the extracts from other isolates (data not shown). Synergy between clavulanate and carbapenems or other β-lactams was weak or absent, except for aztreonam, in agreement with the

Table 1. Clinical characteristics of the nine patients with bla_{VIM-1}-positive *K. pneumoniae* strains isolated, in order of acquisition

Patient	Clinical events	Ward	Date of admission in ICU (dd.mm.yy)	Date of isolation of first bla_{VIM-1}-containing *K. pneumoniae* (dd.mm.yy) origin and number	Primary infection	Subsequent infection and pathogen	Antimicrobial agents used for therapy prior to isolation of bla_{VIM-1}-containing *K. pneumoniae*	Antimicrobial agents used for therapy after isolation of bla_{VIM-1}-containing *K. pneumoniae*	Outcome (death/ discharge)
A	kidney transplantation	ICU p4	02.09.04	03.09.04, blood, 6885	bacteraemia	surgical wound infection, *K. pneumoniae*	piperacillin/tazobactam	piperacillin/tazobactam	discharge
B	complications after abdominal surgery for obesity	ICU p4	13.10.04	23.10.04, blood, 6887	bacteraemia	pneumonia, *K. pneumoniae*, *P. aeruginosa*	cefazolin, piperacillin/ tazobactam, amoxicillin/ clavulanate, levofloxacin	levofloxacin plus meropenem, levofloxacin plus meropenem plus amikacin, meropenem plus trimethoprim/sulfamethoxazole, aztreonam plus amikacin, piperacillin/tazobactam, piperacillin/tazobactam plus levofloxacin	discharge
C	complications after abdominal surgery, colon cancer	ICU p4	11.10.04	26.10.04, blood, tracheal aspirate, 6911	bacteraemia and pneumonia	pneumonia, *K. pneumoniae*, *P. aeruginosa*	piperacillin/tazobactam, ceftazidime	ceftazidime plus levofloxacin, meropenem plus levofloxacin, levofloxacin, piperacillin/ tazobactam plus amikacin	death
D	complications after surgery, lung cancer	ICU p4	01.11.04	15.11.04, blood, tracheal aspirate, 6940	bacteraemia and pneumonia	NA	ampicillin/sulbactam, piperacillin/tazobactam, piperacillin/tazobactam plus levofloxacin	piperacillin/tazobactam plus levofloxacin	death
E	kidney transplantation	transplant surgery unit	04.12.04	13.12.04, blood, 6952	bacteraemia	urinary tract infection, *K. pneumoniae*, *P. mirabilis*	meropenem	meropenem	discharge
F	liver transplantation	ICU p4	13.01.05	26.01.05, blood, 6984	bacteraemia	NA	cefazolin, piperacillin/ tazobactam, meropenem	meropenem	death
G	NA	ICU er	24.01.05	03.02.05, blood, 6997	bacteraemia	NA	NA	NA	NA
H	liver transplantation	ICU p4	06.01.05	16.02.05, blood, 7010	bacteraemia	abdominal infection, *K. pneumoniae*	ceftazidime, levofloxacin, meropenem plus levofloxacin, piperacillin/ tazobactam, trimethoprim/ sulfamethoxazole	piperacillin/tazobactam, piperacillin/tazobactam plus levofloxacin, meropenem plus levofloxacin, meropenem plus amikacin, meropenem plus ciprofloxacin, meropenem plus trimethoprim/sulfamethoxazole, piperacillin/tazobactam plus ciprofloxacin, piperacillin/ tazobactam plus amikacin	discharge
I	liver transplantation	ICU p4	28.01.05	01.03.05, blood, 7023	bacteraemia	surgical wound infection and sepsis, *P. aeruginosa*	NA	meropenem, meropenem plus amikacin	death

NA, not available.

Table 2. Susceptibility profiles of the nine bla_{VIM-1}-containing *K. pneumoniae* clinical isolates

Isolate (patient)	MIC (mg/L)															Other resistance markers[a]
	AMX	AMX/CLA	PIP	PIP/TZB	CTX	CTX/CLA	CAZ	CAZ/CLA	ATM	ATM/CLA	IPM	IPM/CLA	MEM	MEM/CLA	FEP	
6885 (A)	>256	128	>256	>256	>64	32	>256	128	>256	2	4	1	1	0.5	32	CIP, SXT, CHL
6887 (B)	>256	128	>256	>256	>64	32	>256	128	>256	2	4	1	1	0.25	32	CIP, SXT, CHL
6911 (C)	>256	128	>256	>256	>64	64	>256	128	>256	2	4	1	1	1	32	CIP, SXT, CHL
6940 (D)	>256	128	>256	>256	>64	32	>256	64	>256	4	8	4	1	1	32	CIP, SXT, CHL
6952 (E)	>256	128	>256	>256	>64	64	>256	128	>256	1	4	2	1	0.25	32	CIP, SXT, CHL
6984 (F)	>256	128	>256	>256	>64	64	>256	64	>256	2	4	2	1	0.5	32	CIP, SXT, CHL
6997 (G)	>256	128	>256	>256	>64	32	>256	64	>256	4	4	2	1	0.5	32	CIP, SXT, CHL
7010 (H)	>256	128	>256	>256	>64	32	>256	128	>256	2	4	2	1	1	32	CIP, SXT, CHL
7023 (I)	>256	128	>256	>256	>64	>64	>256	128	>256	32	64	16	16	8	>64	CIP, SXT, CHL

AMX, amoxicillin; CLA, clavulanic acid; PIP, piperacillin; TZB, tazobactam; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; FEP, cefepime; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; CHL, chloramphenicol.
[a]Resistance has been determined using the disc diffusion test.

β-lactamase profile of these isolates which included both an ESBL and an MBL.

Analysis of OMPs

Electrophoresis of OMPs showed two major OMPs of 36 and 35 kDa in all *K. pneumoniae* isolates except that from Patient I (showing high carbapenem MICs), which lacked the k36 OMP.

Genetic context of bla_{VIM-1}

The genetic context of bla_{VIM-1} was investigated in the index isolate 6885. The MBL gene was carried on a mobile gene cassette inserted into the variable region of a class 1 integron. The integron variable region contained two cassettes: the bla_{VIM-1} cassette in the first position and an *aac(6′)-IIc* cassette in the second position.

Transfer of β-lactam resistance

Conjugal transfer of resistance traits to imipenem and aztreonam was assayed with all isolates. *E. coli* transconjugants selected with aztreonam exhibited both an ESBL and an MBL phenotype and carried both β-lactamase determinants, whereas those selected with imipenem showed only an MBL phenotype. These results showed that both the ESBL and the MBL determinants were transferable by conjugation and that they were likely carried on different conjugative plasmids. Further plasmid characterization was not carried out in this work.

Discussion

In this report, we have described the first outbreak of bloodstream infections caused by MBL-producing *K. pneumoniae* in Italy. The outbreak was caused by a single strain producing VIM-1 MBL and also an SHV-type ESBL. Interestingly, the structure of the variable region of the VIM-1-encoding integron was different from that carried by a VIM-1-producing *E. cloacae* previously detected in Genoa,[4] although it was identical to that previously described in a VIM-1-producing *E. cloacae* from Greece.[11] This suggests the possibility of a common origin of those integrons, although epidemiological links between the first patient and Greece or Greek nationals could not be ascertained.

Although the outbreak involved three different wards, the epidemiological links among patients revealed a crucial role of one of the ICUs in the dissemination of the MBL-producing strain. Because in this case the VIM-1 integron was carried on a transferable plasmid, the containment of this resistance is expected to be more difficult.

Most VIM-1-producing *K. pneumoniae* isolates included in this study did not show a frank carbapenem resistance phenotype, and their carbapenem MICs were still in the susceptible range. This fact complicates the detection of MBL-producing Enterobacteriaceae and may delay the enforcement of infection control measures, including isolation of these patients. Only one isolate was resistant to carbapenems because of the concomitant loss of the k36 OMP. This finding underscores the notion that carbapenem resistance can easily emerge by mutation in this OMP gene in MBL-producing klebsiellae, even if carbapenem MICs remain initially in the susceptible range.

Our data confirm the need for a systematic screening to detect MBL-producing strains among isolates circulating in high-risk wards such as ICUs.

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Transparency declarations

None to declare.

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