ical relationship among strains. Thus, Greek strains from 2003 to 2004 cluster with strains from Asia of the same period, but differ from strains isolated in Greece in 2001. Further genetic studies are needed in order to gain a better insight into the genetic variability of EV strains and any relationship with pathogenicity, and to investigate any patterns of recombination, which is a frequent event during EV evolution.

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RESEARCH NOTE

Emergence of Proteus mirabilis carrying the bla_{VIM-1} metallo-β-lactamase gene

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ABSTRACT

Seven genetically related Proteus mirabilis clinical isolates from a hospital in Thessaloniki, Greece, exhibited decreased susceptibility to imipenem and carried a bla_{VIM-1} metallo-β-lactamase gene. PCR mapping revealed that bla_{VIM-1} was part of a class 1 integron that was probably located in the chromosome and also included the dhfr and aadA genes. This is the first description of the bla_{VIM-1} metallo-β-lactamase gene in P. mirabilis.

Keywords bla_{VIM-1} gene, carbapenem resistance, integron, metallo-β-lactamase, PCR mapping, Proteus mirabilis

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The carbapenems imipenem and meropenem are employed frequently in the treatment of serious nosocomial infections caused by Gram-negative bacteria, including extended-spectrum \(\beta\)-lactamase (ESBL)-producing enterobacteria. However, the emergence of clinical strains of various species producing carbapenem-hydrolysing metallo-\(\beta\)-lactamases (MBLs) of the VIM, IMP and SPM families has been reported [1,2]. VIM-producing strains of \(P\). aeruginosa and \(K\). pneumoniae are endemic in Greek hospitals [3,4]. Sporadic strains of \(E\). coli and \(E\). cloacae carrying \(bla\)\(_{\text{VIM}}\) genes have also been isolated in this setting [5–7]. The present report describes a cluster of \(P\). mirabilis isolates carrying the \(bla\)\(_{\text{VIM-1}}\) gene.

Seven \(P\). mirabilis isolates recovered from seven patients treated in the G. Papanikolaou general hospital in Thessaloniki during the period June 2004 to March 2005 were referred to the National School of Public Health with an unusual phenotype that included resistance to cefotaxime, ceftazidime and imipenem, as determined by the Vitek 2 system using the AST-N022 card (bioMe\'rieux, Marcy l’Etoile, France). Species identification was confirmed with the API 20E system (bioMe\'rieux). Molecular typing of the isolates was performed by pulsed-field gel electrophoresis (PFGE) following digestion of whole-cell DNA with \(\text{NotI}\) [8].

MICs of \(\beta\)-lactams were determined using the Etest method (AB Biodisk, Solna, Sweden). Susceptibility to non-\(\beta\)-lactam antibiotics was assessed by disk-diffusion [9]. Synergy between EDTA and imipenem was investigated with a double-disk test (DDT) [10] and MBL-detecting Etests (AB Biodisk). Conjugal transfer of resistance to \(\beta\)-lactams was attempted using the rifampicin- and streptomycin-resistant \(E\). coli strains 20R764 and 1R716 as recipients [11]. Plasmid DNA was prepared with a Plasmid Midi Kit (Qiagen, Hilden, Germany). Plasmid preparations were used to transform competent cells of \(E\). coli DH5\(\alpha\) [12].

Total DNA was extracted from the clinical isolates with a NucleoSpin tissue kit (Macherey-Nagel, Duren, Germany) and used as a template in PCR assays. Screening for \(bla\)\(_{\text{VIM}}\)-type genes was performed by PCR using the primers VIM-F and VIM-R [4]. The resulting 261-bp fragment was labelled with a digoxigenin labelling and detection kit (Roche Diagnostics, Mannheim, Germany) and used as a \(bla\)\(_{\text{VIM}}\)-specific probe in hybridisation experiments. PCRs for amplification of the entire \(bla\)\(_{\text{VIM-1}}\) gene were performed as described previously [13]. Mapping of class 1 integrons was performed by PCR using primers specific for conserved segments of class 1 integrons [14] and various antibiotic resistance genes, including \(aacA\), \(dhfrI\) and \(aadA\). Nucleotide sequences of PCR products were determined on both strands using an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

All seven isolates had been recognised as causes of hospital-acquired infections, and six of the seven patients had been hospitalised for \(\geq1\) month before the isolation of \(P\). mirabilis. The isolates were resistant to cefotaxime (MIC 128–256 mg/L) and ceftazidime (MIC 32 mg/L) by Etest. MICs of cefepime were 32 (one isolate), 16 (two isolates), 8 (two isolates) and 4 mg/L (two isolates). MICs of imipenem were 2–4 mg/L, but the MICs of meropenem were significantly lower (0.12 mg/L). Aztreonam was active against all isolates (MICs 1–2 mg/L). Disk-diffusion showed that the isolates were also resistant to tobramycin, netilmicin, amikacin, trimethoprim and sulphonamides. The EDTA-imipenem DDT showed clear synergy for all seven isolates that suggested MBL production (Fig. 1). In contrast, the MBL Etests were incon-
clusive, perhaps because of the relatively low MICs of imipenem. The bla\textsubscript{VIM}-specific PCRs were positive for all seven isolates. Electrophoresis of plasmid DNA preparations showed that all isolates possessed plasmids of c.80 kb, but conjugation and transformation experiments using various \(\beta\)-lactams for selection failed to yield \(\beta\)-lactam-resistant \(E.\ coli\) recipients.

PCR mapping revealed the presence of the same class 1 integron in all isolates. Assembly of the sequences of overlapping PCR products from one isolate showed that the structure of this integron was similar to that of the VIM-1-encoding integrons reported previously in \(E.\ coli\) and \(K.\ pneumoniae\) clinical isolates from Greece [4,6]. The variable region contained (5’ to 3’) genes for \(bla\textsubscript{VIM-1}, aacA7, dhfr\) and \(aadA\). The gene cassettes were preceded by a promoter sequence located at the 5’ end of an integrase 1 gene typical of an integron structure. The described segment was 100% homologous to a sequence carried by the VIM-1-encoding In-e541 integron carried on a plasmid in \(E.\ coli\) (nt 8284 – nt 12 110; GenBank accession no. AY339625).

PFGE analysis revealed four genomic fingerprints, A–D, with pattern A (four isolates) being predominant, that differed by three or fewer DNA fragments (Fig. 2). Hybridisation of the PFGE fingerprints with the \(bla\textsubscript{VIM-1}\) probe yielded a positive signal that corresponded to a c.450-kb fragment (data not shown). This finding, together with the failure of conjugation and transformation experiments, indicated that the \(bla\textsubscript{VIM-1}\) gene was chromosomally located.

Several studies have reported the emergence of \(P.\ mirabilis\) strains exhibiting resistance to newer \(\beta\)-lactam antibiotics because of the production of various class A ESBLs and AmpC-type cephalosporinases [15,16]. The present study describes the first occurrence of a VIM-type MBL in \(P.\ mirabilis\), and adds to the growing evidence of the ability of this microorganism to acquire potent \(\beta\)-lactamases. It also underscores the spreading capability of genes encoding MBLs. The VIM-1-encoding integron described here was probably located on the chromosome. Notably, this integron was similar to those carried by distinct plasmids from \(E.\ coli\) and \(K.\ pneumoniae\) strains isolated in Greek hospitals [4,6]. As these structures are not in themselves mobile, it can be hypothesised that the VIM-1-encoding integron is associated with elements capable of mediating its spread among various replicons.

PFGE analysis indicated a possible common ancestry of the \(bla\textsubscript{VIM-1}\)-positive \(P.\ mirabilis\) isolates according to established criteria [17]. It is therefore likely that the VIM-1-encoding integron was acquired by an index strain that became established in this setting and evolved over time. Isolation dates (between June 2004 and March 2005) indicated persistence of this strain in the hospital environment, but the respective infections occurred in a sporadic fashion in patients hospitalised for prolonged periods in three different wards.

The \(\beta\)-lactam resistance patterns of the \(P.\ mirabilis\) isolates described here generally reflected the hydrolysis spectrum of VIM-1, which includes all \(\beta\)-lactams except aztreonam [18]. However, carbapenem MICs were below the current resistant breakpoints for MBL-producing enterobacteria. Furthermore, the widely used MBL Etest method was ineffective. These properties may complicate identification of such isolates in the clinical laboratory, particularly when susceptibility testing is performed using a limited number of antibiotic dilutions. Applica-
tion of the imipenem-EDTA DDT may be an alternative option, but the limitations of an in-house technique must be considered.

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