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RESEARCH NOTE

First identification of an *Escherichia coli* clinical isolate producing both metallo-β-lactamase VIM-2 and extended-spectrum β-lactamase IBC-1

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ABSTRACT

An *Escherichia coli* strain with decreased susceptibility to carbapenems was isolated from a hospitalised patient in Athens, Greece. The strain was resistant to all β-lactams, including aztreonam, whereas the MIC of imipenem and meropenem was 0.5 mg/L. A positive EDTA–disk synergy test suggested the production of a metallo-β-lactamase. PCR experiments revealed the presence of the bla_{VIM-2}, bla_{IBC-1} and bla_{TEM-1} genes. Resistance to β-lactams was not transferable by conjugation. This is the first report of a clinical isolate of *E. coli* producing VIM-2, and the first report of the coexistence of bla_{VIM-2} and bla_{IBC-1} in a single clinical isolate.

Keywords bla_{IBC-1}, bla_{VIM-2}, carbapenemase, *Escherichia coli*, metallo-β-lactamase, resistance

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Metallo-β-lactamases (MBLs) of the VIM type are integron-borne enzymes that can be either chromosomally-encoded or plasmid-mediated. With the exception of aztreonam, they hydrolyse all

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β-lactams, including the carbapenems. They are susceptible to inhibition by divalent ion chelators such as EDTA, but not to β-lactamase inhibitors such as clavulanate. The VIM-2 enzyme has been described previously in Europe [1,2] and the Far East [3–7], mostly in *Pseudomonas aeruginosa* [1–3], but also in *Citrobacter freundii* [4], *Pseudomonas putida* [3], *Pseudomonas stutzeri* [5], *Serratia marcescens* [6] and *Acinetobacter baumannii* [7].

The IBC-1 enzyme is a novel integron-associated class A extended-spectrum β-lactamase that hydrolyses all β-lactams except the cephamycins and the carbapenems. It has been described in an *Enterobacter cloacae* isolate from Thessaloniki, Greece [8], and has been involved in an outbreak in a neonatal intensive care unit in the same area [9]. Its presence has also been documented in a clinical isolate of *Escherichia coli* from a patient in Athens, Greece [10].

This report describes a clinical isolate of *E. coli* with resistance to extended-spectrum cephalosporins, cefoxitin and aztreonam, as well as reduced susceptibility to carbapenems, associated with production of both VIM-2 and IBC-1. This is the first report of VIM-2 in *E. coli* worldwide, and the first report of the coexistence of the $bla_{\text{VIM-2}}$ and $bla_{\text{IBC-1}}$ genes in a single isolate.

The *E. coli* strain was isolated in 2001 from the bronchial secretions of a patient hospitalised in a tertiary care hospital in Athens, Greece. β-Lactam MICs were determined by standard methods [11]. A double-disk synergy test with imipenem- and EDTA-containing disks [12] was used to screen for MBL production.

Conjugation experiments were performed by both the broth and filter mating techniques, with *E. coli* K12 strain RC85 R^- (rifampicin-resistant; MIC > 128 mg/L) as the recipient [13]. Transconjugants were selected on agar supplemented with rifampicin (128 mg/L) and ceftazidime (16 mg/L). Isoelectric focusing of sonic extracts was performed on precast polyacrylamide gels with a pH 3–10 gradient. TEM-1 (pI 5.4), TEM-4 (pI 5.9) and TEM-3 (pI 6.3) enzymes, produced by reference strains, were used as standards with known pIs for β-lactamase characterisation.

PCR amplification was performed as described previously: with VIM-B (5′-ATGGTGTTTGGTC GCATATC-3′) and VIM-F (5′-TGGGCCATTC AGCCAGATC-3′) primers, which amplified a 510-bp (nucleotides 152–661) internal fragment of the bla_{VIM} gene (EMBL/GenBank accession no. AF 191564) [1]; with IBC-1A (5′- TGCATCG-GAAAAATTAACCT-3′) and IBC-1B (5′-AATTT-TACGAAATATGCG-3′) primers, which amplified a 400-bp (nucleotides 51–450) internal fragment of the $bla_{\text{IBC-1}}$ gene (GenBank accession no. AF 208529) [8]; and for the $bla_{\text{TEM-1}}$ gene [14]. PCR products were cloned and sequenced by standard molecular biology methods. PCR–restriction fragment length polymorphism (PCR-RFLP) analysis of the 510-bp PCR amplicon corresponding to the internal fragment of the bla_{VIM} gene was performed with *Sac*I restriction endonuclease to screen for the presence of a gene belonging to the $bla_{\text{VIM-1}}$ cluster. Previous unpublished observations from our group suggest that PCR-RFLP analysis can be used to differentiate between the two gene clusters containing $bla_{\text{VIM-1}}$ ($bla_{\text{VIM-1}}$, $bla_{\text{VIM-4}}$, $bla_{\text{VIM-5}}$) and $bla_{\text{VIM-2}}$ ($bla_{\text{VIM-2}}$, $bla_{\text{VIM-3}}$, $bla_{\text{VIM-6}}$, $bla_{\text{VIM-8}}$, $bla_{\text{VIM-9}}$, $bla_{\text{VIM-10}}$), as a *Sac*I restriction site is present at position 515 in genes belonging to the $bla_{\text{VIM-1}}$ cluster.

The clinical isolate of *E. coli* was resistant to ampicillin, ampicillin–sulbactam, piperacillin, piperacillin–tazobactam, cefalothin, cefoxitin, cefotaxime, ceftriaxone, ceftazidime and aztreonam. It was also resistant to trimethoprim–sulphamethoxazole, ciprofloxacin and tobramycin, and intermediately resistant to amikacin, but retained susceptibility to gentamicin and tetracycline. MICs of imipenem and meropenem for the isolate were 0.5 mg/L, whereas those of ceftazidime, cefotaxime, cefepime and aztreonam were > 512, 16, 2 and 32 mg/L, respectively. The isolate gave a positive result in the EDTA–disk synergy test, and isoelectric focusing showed three β-lactamase bands with pI values of *c.* 5.4, 5.8 and 6.9, corresponding to TEM-1, VIM-2 and IBC-1, respectively. Neither imipenem nor ceftazidime resistance was transferred by conjugation. PCR-RFLP analysis of the bla_{VIM} amplicon suggested the presence of a $bla_{\text{VIM-2}}$ variant. PCR amplification and nucleotide sequence analysis revealed that the clinical isolate carried $bla_{\text{TEM-1}}$, $bla_{\text{VIM-2}}$ and $bla_{\text{IBC-1}}$.

The spread of MBLs in Enterobacteriaceae is a matter of concern in Greece. Outbreaks caused by VIM-1-producing *Klebsiella pneumoniae* strains have been described in several hospitals in Athens [15], and a sporadic strain of *E. coli* carrying $bla_{\text{VIM-1}}$ was isolated at a tertiary hospital in Athens [16]. MBLs of the VIM type, which are well-established in *P. aeruginosa* strains in Greek hospitals [2,17,18], have now been introduced into Enterobacteriaceae.

Clinical isolates of *Ent. cloacae* and *E. coli* producing IBC-1 have, until now, been reported only from Greece, and the class 1 integrons carrying these genes have been well-characterised [8,10]. The present study provides the first description of the coexistence of bla_{VIM-2} and bla_{IBC-1} in a single isolate. VIM- and IBC-encoding genes appear to be located in mobile gene cassettes inserted into integrons, which accounts for their enhanced spread. It is possible that a single integron is the vehicle for both genes in the *E. coli* isolate reported in the present study. Repeated attempts to transfer either gene to an *E. coli* recipient were unsuccessful, suggesting that these genes were located either on non-transferable plasmids or on the chromosome, as was the case for bla_{VIM-1} and bla_{VIM-3} in *P. aeruginosa* [5,19]. The carbapenem MICs for the *E. coli* isolate studied were below the resistance breakpoint, but it has been noted previously that MBLs confer only a low level of resistance to carbapenems in Enterobacteriaceae unless another mechanism, such as impaired outer-membrane permeability, is also present. Aztreonam resistance in the isolate was probably associated with IBC-1, since monobactams are stable to hydrolysis by carbapenemases. Furthermore, the cefepime MIC was consistent with previous reports of the weak hydrolytic activity of both IBC-1 and VIM-2 against cefepime [1,10].

The difficulties in detecting MBL-producing strains in the clinical microbiology laboratory, together with the mobile nature of the gene cassettes carrying the VIM-type enzymes, may facilitate their dissemination. The coexistence of MBLs and non-MBL extended-spectrum β-lactamases in the same strain complicates the situation further. These observations highlight the challenge for clinical microbiologists and infectious disease specialists in detecting these strains, so that infection control measures and appropriate treatment can be implemented in a timely manner.

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RESEARCH NOTE

Results of two worldwide surveys into physician awareness and perceptions of extended-spectrum β-lactamases

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ABSTRACT

An omnibus survey of microbiologists (n = 400) and a survey of participants (n = 49) in the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programme were conducted to determine the awareness and prevalence of extended-spectrum β-lactamases (ESBLs), and the regularity and method of screening. Of the omnibus survey participants, 69% screened regularly for ESBLs, compared with 83% of MYSTIC participants. In both surveys, ESBLs were more common in *Klebsiella pneumoniae* (73% and 79%, respectively) and *Escherichia coli* (63% and 81%, respectively) than in other bacteria. The surveys demonstrated that awareness of, and testing for, ESBLs is inconsistent.

Keywords ESBLs, *Escherichia coli*, *Klebsiella pneumoniae*, meropenem, MYSTIC, susceptibility testing

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In order to make informed prescribing decisions, clinicians need accurate information on the likely antibiotic resistance profile of the organism causing the infection. Surveillance studies, such as the Alexander Project, SENTRY and the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programme, have been useful in providing accurate prevalence rates of specific bacterial resistance caused by different mechanisms, such as extended-spectrum β-lactamases (ESBLs) [1–3]. Infections caused by ESBL-producing pathogens may be associated with increases in mortality, duration of hospital stay and hospital costs [4,5]. However, awareness of the clinical importance of ESBL-producing strains may vary considerably among clinicians and microbiologists, with continued surveillance and testing not performed widely, and especially when the prevalence of resistance is low. Therefore, two global surveys, an omnibus survey and a survey of participants in the MYSTIC programme, were conducted to determine the degree of awareness of ESBLs, the methods and frequency of ESBL screening, and the reasons for not screening.

The omnibus survey comprised a panel of microbiologists who participate regularly in telephone interviews conducted by ISIS Research (Putney, London, UK), an independent market research agency. The respondents were not given advance notice of the questions, and so were asked to give an estimate when asked for percentages. All participants in the MYSTIC programme were sent a questionnaire. The two surveys were performed during February and March 2002. Both surveys included similar questions relating to perception of the incidence of

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