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Isolation from Blood Culture of a *Leclercia adecarboxylata* Strain Producing an SHV-12 Extended-Spectrum Beta-Lactamase

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We report on the first isolation of an extended-spectrum beta-lactamase-producing *Leclercia adecarboxylata* strain from the bloodstream in a 58-year-old man with acute myeloid leukemia. The strain, resistant to ceftazidime, cefotaxime, and aztreonam, produces the SHV-12 β-lactamase, one of the most common variants found in Italian nosocomial isolates of *Enterobacteriaceae*.

Leclercia adecarboxylata, first described by Leclerc in 1962 as *Escherichia adecarboxylata* (5) and formerly also known as Enteric group 41, is a motile, gram-negative bacillus of the *Enterobacteriaceae* family (13, 15) and is rarely isolated from environmental and clinical specimens.

In clinical specimens, it has been found primarily as one of the components of polymicrobial wound infections (8, 15). Infections caused by *L. adecarboxylata* alone, as determined by the results of blood cultures, have been found only in immunocompromised patients (2, 10, 16).

L. adecarboxylata VR-01-1 was isolated in August 2001 from a blood culture of a 58-year-old man with acute myeloid leukemia admitted to the hematology unit of the Verona University Hospital. The strain was initially identified with biochemical tests by means of API ID32E (Biomérieux, Marcy l'Etoile, France). The identification was confirmed by the 16S rRNA gene sequence; the gene was amplified by using primers and the conditions reported by Woo et al. (17). The sequence was obtained with an ABI PRISM 377 automated sequencer (Perkin-Elmer) in accordance with the manufacturer's recommendations and compared with the known 16S rRNA gene sequences in GenBank, with no resulting detectable difference.

This isolate showed a behavior typical of extended-spectrum beta-lactamase (ESBL)-producing strains in the Kirby-Bauer test—namely, it presented resistance to ceftazidime, cefotaxime, aztreonam, and cefepime, and these resistances were reversed by clavulanic acid; this finding had never been described before in this species.

The isolate was also tested for its antimicrobial susceptibilities by broth microdilution in Müeller-Hinton medium at 37°C with a standard inoculum (9). The antimicrobials were all obtained from commercial sources. The MICs obtained for *L. adecarboxylata* VR-01-1 are reported in Table 1. The susceptibility pattern proved compatible with the presence of an ESBL that is capable of hydrolyzing ceftazidime, cefotaxime, and aztreonam, but not cephamycins, and that is susceptible to the common inhibitors.

The β-lactamase production was first confirmed by isoelectric focusing. Cells were harvested after overnight growth in brain heart infusion broth and collected by centrifugation, and the pellet was resuspended in physiological solution. The cell content was released by sonication with a Labsonic 2000 sonicator (B. Braun Melsungen AG, Melsungen, Germany). Isoelectric focusing was performed in a precast polyacrylamide (5%) gel containing ampholines (pH range, 3.5 to 9.0) (Amersham Pharmacia Biotech, Uppsala, Sweden) on a Bio-Phoresis apparatus (Bio-Rad, Hercules, Calif.). Enzyme activity was revealed by overlaying the gel with a paper filter soaked in 250 μM nitrocefin (Oxoid, Basingstoke, Hampshire, England). The strain showed only one band with a pI of 8.2 (data not shown).

The presence of bla_{TEM} or bla_{SHV} resistance genes was checked by PCR. The oligonucleotide primers used for the PCR assays were as follows. TEM-FW and TEM-REV, specific for bla_{TEM} (7), were 5′-ATAAAATTCTTGAAGACG AAA and 5′-GACAGTTACCAATGCTTAATCA, respectively; SHV-FW and SHV-REV, specific for bla_{SHV} (12), were 5′-GGGTTATTCTTATTTGTCGC and 5′-TTAGCGTTGCC AGTGCTC, respectively.

The PCR conditions were 94°C 1 for min, 58°C for 1 min, and 72°C for 1 min for 35 cycles. For direct sequencing, PCR products were purified with a Qiagen microspin apparatus (Qiagen GmbH, Hilden, Germany). *L. adecarboxylata* VR-01-1 showed an SHV-type gene which, after sequencing, was identified as an SHV-12 β-lactamase gene.

The gene was cloned in the phagemid vector pPCR Script Cam SK^+ (Stratagene, La Jolla, Calif.). The entire SHV-12 gene was amplified by PCR with the primers SHV-CF (5′-GG GGAATTCTTATTTGTCGC) and SHV-CR (5′-CAGAATT CGCTTAGCGTTGCCAGT).

The PCR product was ligated with the phagemid vector pPCR Script Cam SK^+. This cloning vector has a chloramphenicol resistance gene and a lac promoter for gene expression. Ligated vectors were transformed in *Escherichia coli* XL10 ultracompetent cells by the ligation kit polishing protocol (Stratagene). Transformants were selected on a Luria-Bertani agar plate with 30 μg of chloramphenicol/ml and then checked by PCR and endonuclease digestion.

After ligating the SHV-12 PCR product of *L. adecarboxylata* VR-01-1 in the vector, we obtained the pAJ1 plasmid coding for SHV-12 β-lactamase.

The pAJ1 plasmid was transferred into the *E. coli* XL10 host

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TABLE 1. MICs for *L. adecarboxylata* and *E. coli* XL10 strains

Antimicrobial agent	MIC (μg/ml) for:		
	L. adecarboxylata	*E. coli* XL10/vector	*E. coli* XL10/pAJ1
Ampicillin	>128	4	>128
Ampicillin-sulbactam	4	4	8
Amoxicillin	>128	16	>128
Amoxicillin-clavularic acid	2	2	4
Cephaloridine	64	2	64
Penicillin G	>128	32	>128
Ceftazidime	32	0.12	32
Cefotaxime	4	<0.06	4
Cefoxitin	1	8	8
Cefotetan	0.12	0.25	0.25
Cefuroxime	16	4	16
Cefepime	0.5	0.25	1
Aztreonam	64	0.25	128
Ciprofloxacin	0.06	0.06	0.06
Gentamicin	0.25	0.12	0.12
Imipenem	0.12	0.12	0.12

cells, and mutants were selected on Luria-Bertani agar plates containing 30 μg of chloramphenicol/ml.

Both *L. adecarboxylata* VR-01-1 and the *E. coli* XL10/pAJ1 strain showed a band of pI 8.2 in the isoelectric focusing, while the *E. coli* XL10, harboring the vector alone, showed no such band, thus confirming the successful cloning.

Table 1 gives the MICs of a number of antimicrobial agents for *L. adecarboxylata* VR-01-1, *E. coli* XL10 harboring the plasmid pAJ1, and *E. coli* XL10 harboring only the vector pPCR Script Cam SK^+. The results show that the SHV-12 β-lactamase was responsible for increased MICs of ampicillin (>32 times), cephaloridine (32 times), penicillin G (>4 times), ceftazidime (256 times), cefotaxime (>64 times), cefuroxime (4 times), cefpirome (>32 times), and aztreonam (256 times). These MICs were the same in *L. adecarboxylata* VR-01-1 and *E. coli* XL10/pAJ1.

To investigate where the SHV-12 gene of *L. adecarboxylata* VR-01-1 was located, the plasmidic DNA was extracted from *L. adecarboxylata* with a Qiagen kit. We were able to amplify the SHV-12 gene from the plasmidic DNA of *L. adecarboxylata*, but all attempts to transfer the plasmid into *E. coli* XL10 or *E. coli* DH5α host cells by electroporation proved unsuccessful.

The world literature reports only nine cases of *L. adecarboxylata* over the past 10 years (1, 2, 3, 4, 6, 8, 14, 16), and most of them are quite recent.

The isolation of *L. adecarboxylata* VR-01-1 from the bloodstream of a patient with acute myeloid leukemia confirms that infections by *L. adecarboxylata* alone can be found only in patients whose immune defenses are compromised by an underlying medical condition, while in otherwise healthy adults, this microorganism is found primarily as just one of the components of a polymicrobial infection.

Testing antimicrobial agents in all the *L. adecarboxylata* strains reported to date has demonstrated pansensitivity. Thus, the presence of an ESBL in an isolate of this species and its location on a transposable element are disquieting findings which may be a prelude to the wider diffusion of this microorganism.

The identification of the *L. adecarboxylata* VR-01-1 ESBL as an SHV-12 β-lactamase confirms the widespread diffusion in Italy of this enzyme, which was one of the most common variants found in most hospitals and in several different species of *Enterobacteriaceae* in a recent Italian nationwide survey (11).

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