Carbapenem-Resistant *Pseudomonas aeruginosa*–Carrying VIM-2 Metallo-β-Lactamase Determinants, Croatia

To the Editor: Carbapenem-hydrolyzing enzymes of the VIM-type (six different variants are known: VIM-1, VIM-2, VIM-3, VIM-4, VIM-5, and VIM-6) are new molecular class B metallo-β-lactamases. These enzymes have recently been identified in carbapenem-resistant isolates of *Pseudomonas aeruginosa* and other gram-negative nonfermenters from European countries in the Mediterranean basin (Italy, France, Greece, Spain, Portugal, and Turkey), as well as in Far East countries (Korea, Taiwan, and Singapore) and the United States (1–3, Midilli et al., GenBank accession no. AY144612, Koh et al., GenBank accession no. AY165025). Similar to bla_{IMP}, bla_{VIM} genes are located on mobile gene cassettes inserted in the variable regions of integrons (1), a condition that provides a wide potential for expression and dissemination in gram-negative pathogens. VIM enzymes possess the broadest range of substrate hydrolysis and can degrade virtually all β-lactams, except monobactams (4).

According to a recent report, the overall resistance rate to imipenem in *P. aeruginosa* isolated from 17 representative laboratories in Croatia was 11% (range 0%–20%) (5). However, molecular basis of carbapenem resistance was not investigated.

In October 2000, two *P. aeruginosa* isolates with an unusual resistance profile were isolated from two Croatian patients (66 and 74 years of age, respectively) who underwent hysterectomies at the Split University Hospital. Both isolates were cultured from urine a week after surgery; a urinary catheter had been used for both patients who had become febrile and had signs and symptoms of urinary tract infection. Analysis of the macrorestriction profiles of chromosomal DNA of the two isolates by pulsed-field gel electrophoresis, carried out as described previously (6), indicated that the two isolates were clonally related (the two profiles were apparently identical). In routine antibiotic susceptibility testing, done by disk diffusion, both isolates showed a multidrug-resistant phenotype, including ureidopenicillins, piperacillin, piperacillin-tazobactam, ceftazidime, cefoperazone, cefepime, aztreonam, ciprofloxacin, gentamicin, netilmicin, imipenem, and meropenem.

MICs to imipenem and meropenem were high (>128 μg/mL). These findings suggested production of an acquired carbapenemase. In fact, crude extracts of the two isolates exhibited carbapenemase activity in a spectrophotometric assay (7) (imipenem hydrolyzing–specific activity was, in either case, >170 nmol/min/mg protein).

A colony blot hybridization, carried out as described with a bla_{IMP} and a bla_{VIM} probe (6), yielded a positive result with the latter probe. Polymerase chain reaction (PCR) amplification of the variable region of class 1 integrons, carried out as described previously by using primers designed on the 5′- and 3′-conserved segments of the integron (8), yielded a 4-kb amplification product from either isolate. Direct sequencing of these amplification products showed, in both cases, the presence of a bla_{VIM-2} allele located in a gene cassette inserted in the *attI* site of a class 1 integron.

The metallo-β-lactamase determinant was not transferred to *Escherichia coli* MKD135 or *P. aeruginosa* 10145/3 (9) in diparental mating experiments conducted on solid medium (the sensitivity of the assay was $\geq 1 \times 10^{-8}$ transconjugants per donor). Plasmid extraction was performed with several techniques, including lysis with sodium dodecyl sulfate (10) and alkaline lysis conducted with a conventional method (10) or with the Nucleobond BAC100 system (Macherey-Nagel, Duren, Germany). Extraction of whole genomic DNA was also performed, as described (8). Plasmid DNA was not detected in any of these preparations, either when analyzed by agarose gel electrophoresis or after Southern blot hybridization analysis with a bla_{VIM-2} probe generated with PCR amplification of the entire bla_{VIM-2} gene. In Southern blots, a hybridization signal was only detectable in correspondence of the band of chromosomal DNA.

To our knowledge, this isolation is the first one of clinical strains producing acquired metallo-β-lactamase in Croatia. A similar finding underscores the progressive emergence of these determinants in different geographic areas and emphasizes the need for an early recognition of these strains. In fact, monitoring dissemination of new antibiotic resistance determinants is essential to enforce adequate control measures and adjust guidelines for antimicrobial chemotherapy in different hospital settings.

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Rickettsia felis in the United Kingdom

To the Editor: *Rickettsia felis* is a bacterium transmitted by the cat flea (*Ctenocephalides felis*), which also acts as a reservoir by means of transovarial transmission (1–3). The distribution of *R. felis* is potentially as wide as that of its insect host, and to date, its presence has been confirmed in cat flea populations in North and South America and southern Europe (4,5). *R. felis* was first identified as a human pathogen in 1994 (6), and cases of "flea-borne spotted fever," which have signs and symptoms of febrile exanthema, have now been reported in the United States, Mexico, Brazil, France, and Germany (7,8). To our knowledge, reports on the presence of *R. felis*, or indeed any other spotted fever group rickettsia, in the United Kingdom have not been published.

To determine whether *R. felis* is present in the United Kingdom, we surveyed cat fleas collected from dogs and cats seen at veterinary practices in southern England and Northern Ireland. A total of 31 dogs and 79 cats from veterinary practices in Bristol, Dorset, London, Devon, Gloucestershire, Hampshire, and Antrim were included in our study. Fleas were collected by combing these animals for 10 minutes. All fleas from each animal were pooled in 70% ethanol. A total of 316 *Ct. felis* (Bouché, 1835), identified by using accepted morphologic criteria, were obtained, with each animal yielding one to five fleas. DNA was extracted from each of the 110 flea pools by using a standard silica cartridge method (QiaAmp DNA mini kit, QIAGEN Ltd., Crawley, West Sussex, U.K.) using the manufacturer's instructions for tissue DNA extraction. The presence of rickettsial DNA was determined by using the polymerase chain reaction (PCR) with oligonucleotide primers that target rickettesial *ompB* (5) or *gltA* (2) genes. Positive control material was cultured *R. felis*. Rigorous controls to limit contamination were carried out, including the use of separate, dedicated rooms for DNA extraction, PCR setup, and gel analysis. Amplification products obtained from *ompB* and *gltA* PCRs were analyzed by using DNA sequencing. Sequences obtained were edited by using BioEdit (available from: URL: http://www.mbio.mncsu.edu/BioEdit/bioedit.html). Similarity to published sequences was determined with the BLAST program (available from: URL: http://www.ncbi.nlm.nih.gov) hosted by the National Centre for Biotechnology Information.

Eighteen flea DNA pools were positive for spotted fever group rickettsia. All 18 yielded PCR products with both *ompB* and *gltA*-targeting PCRs. The *ompB* and *gltA* DNA sequences of all PCR products were 100% identical to those published for *R. felis*, thereby providing evidence for the presence of *R. felis* in fleas collected from >16% of the animals surveyed. PCR-positive fleas were collected from 4 dogs and 14 cats from Bristol, Hampshire, Dorset, and Northern Ireland. Taking into account the number of fleas in each pool, we estimate that 6% to 12% of the fleas collected were infected with *R. felis*.

This study represents the first description of a spotted fever group rickettsia endemic to the United Kingdom. The species detected, *R. felis,* has clear public health implications. The bacterium appears to be widely distributed within the country, infecting a geographically dispersed population of *Ct. felis*. Up to 12% of *Ct. felis* may be infected with *R. felis,* a flea that is by far the most common species of ectoparasite encountered on cats and dogs in the U.K. main-