Carbapenemase-producing Acinetobacter spp. in Cattle, France

To the Editor: Multidrug resistance in bacteria isolated from animals is an emerging phenomenon, mirroring what is happening among humans. During the past decade, expanded-spectrum β-lactamases in Enterobacteriaceae from humans \(^1\) and animals \(^2\) worldwide have been reported. Among humans, as a consequence of this high rate, use of carbapenems is increasing selection pressure; carbapenem-resistant gram-negative organisms are increasingly reported, including carbapenemase-producing Enterobacteriaceae and Acinetobacter spp. \(^3\).

The most commonly acquired carbapenemases identified in Acinetobacter spp. correspond to carbapenem-hydrolyzing class D β-lactamases \(^3\). In particular, the worldwide spread of OXA-23–producing \(A.\) baumannii is considered a serious threat; those strains are frequently involved in nosocomial outbreaks for which therapeutic options are extremely limited \(^3,4\). Our study objective was to evaluate the possible occurrence of carbapenemase-producing gram-negative bacteria in dairy cattle in France.

In August 2010, at a dairy farm 30 km from Paris, France, rectal swabs were collected from 50 cows. Samples were precultured in buffered peptone water and incubated for 18 h at 37°C. Cultures were inoculated by streaking 100 μL of the suspensions onto Drigalski agar plates (bioMérieux, Balmes-les-Grottes, France) containing 1 μg/mL of imipenem to select for carbapenem-resistant gram-negative isolates. Of the 50 samples, 9 produced growth on imipenem-containing plates. All colonies tested (10 colonies/sample) by using the API 20 NE (bioMérieux) system were identified as \(A.\) lwofii. Molecular techniques based on sequencing of the \(gyrA\), \(gyrB\), and \(rpoB\) genes \(^5\) enabled more precise identification and indicated that all isolates belonged to the \(Acinetobacter\) genomospecies \((\text{DNA group}) \ 15\)TU, which is known to be phylogenetically related to \(A.\) lwofii and which has been reportedly isolated from sewage, freshwater aquaculture habitats, trout intestines, and frozen shrimp \((6)\).

One colony per sample was retained for further investigation (isolates \(BY1\) to \(BY9\)). Susceptibility testing and MIC determinations were performed by disk-diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France) and Etest (AB bioMérieux, Solna, Sweden) \((7)\). All isolates except 1 were resistant to penicillins, combinations of penicillins and β-lactamase inhibitors, and carbapenems but susceptible to cefotaxime and of reduced susceptibility to ceftazidime. Isolate \(BY1\) showed higher MICs for carbapenems \((\text{Table})\). In addition, all isolates were resistant to tetracycline, kanamycin, and fosfomycin and remained susceptible to fluoroquinolones, chloramphenicol, gentamicin, amikacin, tobramycin, and sulfonamides. Susceptibility profiles of 3 \(Acinetobacter\) genomospecies \(15\)TU reference strains showed that they were fully susceptible to penicillins, carbapenems, tetracycline, and kanamycin.

Clonal diversity between the isolates was assessed by pulsed-field gel electrophoresis \((5)\), which showed 6 distinct genotypes. Isolate \(BY1\) corresponded to a single clone \((\text{data not shown})\), which indicated that the occurrence of \(Acinetobacter\) genomospecies \(15\)TU strains among these animals was not the result of dissemination of a single clone.

PCR detection and sequencing of genes that encode carbapenem-hydrolyzing class D β-lactamases \((5)\) showed that the 9 \(Acinetobacter\) genomospecies \(15\)TU isolates harbored a \(bla_{\text{OXA-23}}\) gene, whereas the 3 reference strains remained negative. Sequencing confirmed that all isolates expressed β-lactamase OXA-23, which is known to be widespread in \(A.\) baumannii.

Mating-out assays and plasmid electroporation assays were performed by using \(bla_{\text{OXA-23}}\)-positive \(Acinetobacter\) spp. isolates as donors and rifampin-resistant \(A.\) baumannii BM4547 isolates as a recipient strain \((5)\); however, these assays were unsuccessful. Plasmid DNA analysis \((5)\) gave uninterpretable results, with DNA degradations.

The genetic structures surrounding the \(bla_{\text{OXA-23}}\) gene were investigated by PCR mapping \((7)\), which identified transposon \(\text{Tn2008}\) in isolate \(BY2\) only. \(\text{Tn2008}\) is a major vehicle for the spread of the \(bla_{\text{OXA-23}}\) gene in \(A.\) baumannii in the People’s Republic of China \((8)\) and the United States \((9)\). In the other isolates, the \(\text{ISAba1}\) element of \(\text{Tn2008}\) had been truncated.

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by a novel insertion sequence termed ISAcsp2 (www-is.biotoul.fr).

The dairy farmer indicated that most animals from which OXA-23 producers had been identified had received antimicrobial drugs in the previous weeks. Although 1 animal had received amoxicillin-clavulanate, most of the others had been given oxytetracycline and neomycin to treat mastitis.

β-lactamase OXA-23 is a common source of carbapenem resistance in Acinetobacter baumannii (5). Infections with multidrug-resistant OXA-23–producing A. baumannii or A. junii have been reported from hospitals but not from the community. Our study showed that OXA-23–producers in particular, and carbapenemase producers in general, may be isolated from animals. Among the hypotheses that could explain the selection of this carbapenemase, use of penicillins or penicillin–β-lactamase inhibitor combinations could create selective pressure for β-lactamases because OXA-23 does confer, in addition to decreased susceptibility to carbapenems, a high level of resistance to those compounds. We have previously shown that A. radioresistens, an environmental species, was the progenitor of the bla_{OXA-23} gene (10). Studies are needed to determine to what extent and at which locations Acinetobacter genospecies 15TU and A. radioresistens might co-reside and therefore where the bla_{OXA-23} gene exchange might have occurred.

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**Table. Antimicrobial drug MICs for Acinetobacter genospecies 15TU isolates from cows and reference strains, France, August 2010**

| Drug class               | MIC, µg/mL | Acinetobacter genospecies 15TU | Reference strain |
|-------------------------|------------|--------------------------------|-----------------|
|                         |            | BY1                           | BY2–BY9         | NIPH 2171 | NIPH 899 |
| Penicillins and combinations |            |                                |                 |
| Amoxicillin            | >256       | 128–256                        | 4               | 4         |
| Amoxicillin + CLA      | >256       | 128–256                        | 4               | 4         |
| Cephalosporins         |            |                                |                 |
| Cefoxitin              | 32         | 16–32                          | 8               | 6         |
| Cefotaxime             | 32         | 16–32                          | 8               | 6         |
| Ceftazidime            | 32         | 16–32                          | 16              | 16        |
| Cefepime               | 16         | 4–16                           | 4               | 4         |
| Monobactam (aztreonam) | 64         | 32                             | 32              | 16        |
| Carbapenems            |            |                                |                 |
| Meropenem              | 16         | 2–4                            | 0.5             | 0.5       |
| Imipenem               | >32        | 4–6                            | 0.25            | 0.25      |
| Doripenem              | 8          | 2–4                            | 0.5             | 0.5       |
| Cyclines               |            |                                |                 |
| Tetracycline           | >256       | >256                           | 0.5             | 0.5       |
| Tigecycline            | 0.064      | 0.047–0.064                    | 0.047           | 0.125     |
| Quinolones (ciprofloxacin) | 0.5       | 0.5                            | 0.25            | 0.25      |
| Aminoglycosides        |            |                                |                 |
| Gentamicin             | 0.5        | 0.25–0.5                       | 0.25            | 0.25      |
| Kanamycin              | >256       | >256                           | 0.5             | 0.5       |
| Sulfonamides           | 4          | 4                              | >256            |           |

*CLA, clavulanic acid (4 µg/mL).
Aedes albopictus Mosquitoes, Yucatan Peninsula, Mexico

To the Editor: We collected Asian tiger mosquitoes, Aedes albopictus (Skuse), in Cancun in the Yucatan Peninsula of Mexico in September 2011. This mosquito is a nuisance biter of humans and a vector of numerous arboviruses, including those causing dengue, yellow fever, and chikungunya (1).

Aedes albopictus mosquitoes, which are native to Southeast Asia, emerged in the continental United States in 1985 and thereafter spread rapidly across the southeastern United States and into northern Mexico (2,3). These mosquitoes have also been found in the states of Tamaulipas, Coahuila, and Nuevo Leon in northern Mexico, and south of Mexico in Guatemala and Belize (3–9). These findings are now complemented by our collection of Aedes albopictus mosquitoes from Cancun in Quintana Roo State, which with Yucatan and Campeche States compose the Yucatan Peninsula. A previous study of the mosquito fauna of Quintana Roo conducted in 2006 did not report any Aedes albopictus mosquitoes (10).

During September 2011, Aedes albopictus mosquitoes were collected from a cemetery in Cancun, which is located in the eastern part of the Yucatan Peninsula (21°8′53″N, 86°52′79″W) (Figure). The collection location was shaded by trees. Water in containers from which larvae were collected had an average temperature of 24.5°C and a pH of 8.5. The larval collection included ≈30 specimens of different developmental stages that were collected from vases and other artificial containers in the cemetery. The containers were examined as part of routine surveillance activities by Servicios Estatales de Salud de Quintana Roo. Larvae suspected to be those of Aedes albopictus mosquitoes were reared to adults for identification, and a colony of Aedes albopictus mosquitoes from Cancun was established.

F₀ or F₁ adult specimens were confirmed to be Aedes albopictus mosquitoes by species identification at Servicios Estatales de Salud de Quintana Roo (Quintana Roo, Mexico), Universidad Autónoma de Yucatan (Merida, Mexico), and Colorado State University (Fort Collins, CO, USA). The initial mosquito larval collection was composed of 26 Aedes albopictus, 3 Aedes aegypti, and 1 Culex sp. In addition, 6 Aedes albopictus female mosquitoes were collected from the cemetery by landing catches.

Finding Aedes albopictus mosquitoes in Cancun was not surprising because these mosquitoes have been found in nearby Belize (9). Cancun is also a major port for ships carrying tourists and goods that originate in areas to which Aedes albopictus mosquitoes are endemic, including Florida and Texas. Nevertheless, the introduction of Aedes albopictus mosquitoes into Cancun and the high potential for establishment and spread across the Yucatan Peninsula has major public health implications.

The Yucatan Peninsula is hyperendemic for dengue, with all 4 dengue virus (DENV) serotypes circulating in this region. Should Aedes albopictus mosquitoes persist in this region, they may spread and come to play a secondary role to Aedes aegypti mosquitoes as local vectors of DENV. Aedes albopictus mosquitoes may also change local virus transmission dynamics. For example, DENV transmission may be intensified in rural areas because Aedes albopictus mosquitoes are more likely than Aedes aegypti mosquitoes to be found in this setting. Aedes albopictus and Aedes aegypti mosquitoes also may differ in their potential for vertical transmission of DENV, which could

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