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First Description of Two Sequence Type 2 *Acinetobacter baumannii* Isolates Carrying OXA-23 Carbapenemase in *Pagellus acarne* Fished from the Mediterranean Sea near Bejaia, Algeria

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To determine the occurrence of carbapenem-resistant *Acinetobacter baumannii* in fish fished from the Mediterranean Sea near the Bejaia coast (Algeria), we studied 300 gills and gut samples that had been randomly and prospectively collected during 1 year. After screening on selective agar media, using PCR arrays and whole-genome sequencing, we identified for the first time two OXA-23-producing *A. baumannii* strains belonging to the widespread sequence type 2 (ST2)/international clone II and harboring aminoglycoside-modifying enzymes [*aac(6′)*-Ib and *aac(3′)*-I genes].

Acinetobacter baumannii is an opportunistic aerobic nonfermentative Gram-negative rod found ubiquitously in the environment (1). This bacterium has emerged as an important cause of nosocomial infections, most notably ventilator-associated pneumonia and bacteremia associated with high mortality, urinary tract infections, and endocarditis (2). Moreover, it is highly capable of developing resistance to antimicrobial agents (1). Over the last 10 years, an increase in carbapenem-resistant *A. baumannii* strains has been observed worldwide; in particular, we noted a high prevalence in different countries in the south of Europe (3). The most common mechanism of carbapenem resistance in *Acinetobacter* species is the production of acquired carbapenem-hydrolyzing OXA-type class D β-lactamases (4). They are represented worldwide by six gene clusters: intrinsic chromosomal OXA-51-like, of which there are over 70 variants, and the acquired OXA-23-like, OXA-24/40-like, OXA-58-like, OXA-143-like, and OXA-235-like β-lactamases (5–7). Worldwide dissemination of OXA-23-producing *A. baumannii* is now well established and, notably, has been observed among Algerian hospitals in recent years (8–10). While *A. baumannii* is isolated from patients and hospital environmental sources during outbreaks, the reservoir outside the hospital is not well delineated. Several investigators have suspected that the survival of *A. baumannii* in the environment (in particular in water) could contribute to the transmission of the organism during outbreaks (3). Moreover, recent reports have also described the presence of carbapenem-resistant *Acinetobacter* spp. from animals. The OXA-23 carbapenemase has been found in *Acinetobacter* spp. from cattle, horses, and cats (11–13). NDM-1 has also been reported in *Acinetobacter* spp. from food animals (chicken and pig farms) in China (14, 15). However, knowledge about carbapenemase-producing *Acinetobacter* spp. of animal origin remains very limited, making it difficult to assess its impact on public health.

Between 1 March 2012 and 28 February 2013, we randomly and prospectively screened a total of 300 samples from different fish fished in the Mediterranean Sea (2 km from the Bejaia coast, Algeria). Sampling was carried out from *Sardina pilchardus* ($n = 62$), *Engraulis encrasicolus* ($n = 38$), *Trachurus trachurus* ($n = 45$), *Sarpa salpa* ($n = 60$), *Pagellus acarne* ($n = 55$), and *Boops boops* ($n = 40$). The gills and gut of each fish sample were collected by opening the gut using a sterile scalpel following washing of the gut surface with sterile saline. Samples were placed in 1 ml sterile 0.9% saline and then vortexed. Cultures were inoculated by streaking 100 μl of the suspensions onto MacConkey agar plates supplemented with 2 μg/ml of ceftazidime and incubated 24 h at 37°C under aerobic conditions. One colony per sample was retained for further investigation. Bacterial identification was carried out using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Vitek MS; bioMérieux). Susceptibility testing was performed by the disk diffusion procedure (Bio-Rad) and E-tests (bioMérieux) according to the recommendations of the Antibiotic Committee of the French Society for Microbiology (http://www.sfm-microbiologie.org/page/page/showpage/page_id/90.html). Isolates were screened for carbapenemase production using the modified Hodge test (MHT) (16) and by the imipenem-EDTA method (17). Multiplex PCR detection and sequencing of genes that encode carbapenem-hydrolyzing class D β-lactamases were used (18). The presence of genes encoding the aminoglycoside-modifying enzymes (AMEs) was also detected using PCR (19). Finally, the genetic relationship was investigated by repetitive sequence-based PCR (rep-PCR) (using the DiversiLab system) and multilocus sequence typing (MLST) (20). Plasmid electroporation assays were performed with *A. baumannii* ATCC 19606. To identify the clonal lineage of the OXA-23-producing *A. baumannii* isolates, we used a previ-

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TABLE 1 Characteristics of carbapenem-resistant *A. baumanii* isolated from wild *P. acarne* fished from the Mediterranean Sea (2 km from Bejaia coast, Algeria)[a]

Strain	Isolation date (mo/day/yr)	Organ	Resistance phenotype antibiotics (MIC, μg/ml)[b]
IM1	11/04/2012	Gills	CTX (32), CAZ (32), FEP (32), IMP (>32), MEM (32), DOR (>32), AMK (2), GEN (16), KAN(>32), NET (1), OFX (>32), CIP (>32), SXT (8)
IM2	04/14/2012	Gut	CTX (32), CAZ (32), FEP (32), IMP (>32), MEM (32), DOR (>32), AMK (2), GEN (16), KAN(>32), NET (1), OFX (>32), CIP (>32), SXT (8)

[a] Both strain were ST2, harbored Tn*2006*, contained the β-lactamases OXA-23 and OXA-51, and carried the associated resistance genes *aac(6′)*-Ib and *aac(3′)*-I.
[b] CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; MEM, meropenem; DOR, doripenem; AMK, amikacin; GEN, gentamicin; KAN, kanamycin; NET, netilmycin; OFX, ofloxacin; CIP, ciprofloxacin; SXT, co-trimoxazole.

ously described PCR (21). Genomic DNA of *A. baumannii* IM2 was sequenced using a NextSeq 500 Illumina platform by Helixio (St-Beauzire, France).

Of the 300 samples analyzed, *A. baumannii* isolates IM1 and IM2 (0.7%) were recovered from two fish (*Pagellus acarne*). The two isolates were resistant to almost all the antibiotics tested, including carbapenems. They remained susceptible only to amikacin and netilmicin (Table 1). The phenotypic assays showed that the two isolates were positive according to the MHT but the activity of β-lactamases was not inhibited by EDTA. PCR detection showed that the two *A. baumannii* strains harbored the naturally occurring $bla_{\text{OXA-51}}$-like gene and the acquired OXA-carbapenemase $bla_{\text{OXA-23}}$-like gene. Sequencing confirmed that the isolates harbored the β-lactamase OXA-23. Moreover, we detected the presence of *aac(6′)*-Ib and *aac(3′)*-I in the two isolates. These two isolates and different human clinical strains isolated in France (Languedoc-Roussillon, southern France) and Algeria (Annaba, eastern Algerian) shared the same genotype (Fig. 1). Assays to transfer plasmids by electroporation were unsuccessful. MLST assigned the isolates to sequence type 2 (ST2). Multiplex PCR to identify clonal lineages was positive for group 1, showing that the strains belonged to the widespread ST2/international clone II. Whole-genome sequencing of IM2 indicated that the transposon Tn*2006* was the vehicle of the resistance gene $bla_{\text{OXA-23}}$. The 5,292 bp of this transposon were found in the genomic DNA of IM2 (in position 2759078, after a hypothetical-protein-coding gene), confirming the chromosomal insertion of Tn*2006* (GenBank accession no. KU168371).

This study highlighted for the first time that wild fish in the Mediterranean Sea can be contaminated with carbapenem- and aminoglycoside-resistant *A. baumannii* isolates belonging to the worldwide clone ST2. The emergence and spread of several outbreak or sporadic *A. baumannii* strains producing OXA-23-like enzymes have been reported around the world, and these strains were assigned to international clonal lineage I or II (4). Over a long period, the $bla_{\text{OXA-58}}$ carbapenemase gene has been predominant among carbapenem-resistant *A. baumannii* isolates in various Mediterranean countries. Since 2009, a replacement of $bla_{\text{OXA-58}}$ with $bla_{\text{OXA-23}}$ has been reported, and it became the most prevalent carbapenemase-encoding gene circulating in the Mediterranean region (5). The replacement of OXA-58 by OXA-23 might be explained by the selective advantage associated with the higher carbapenemase activity of OXA-23 (22). Recently, different reports showed the dissemination of multidrug-resistant pathogenic bacteria in food products and in food-producing animals (5, 23, 24). Comparison of human and animal carbapenem-resistant *Acinetobacter* isolates is important to enhance the knowledge of the potential routes of transfer of these bacteria and resistance genes in different ecosystems. Few studies have been published describing the dissemination of carbapenem-resistant *Acinetobacter* isolates in food animals and wild animals (11–15). All these points and the clonality between a panel of clinical strains showed the possible exchange place between the *A. baumannii* populations in infected humans and water. We suggest that these isolates were most likely derived from contamination of the fish from human sewage via river water and a growing amount of waste from land urban, industrial, and agricultural operations that has

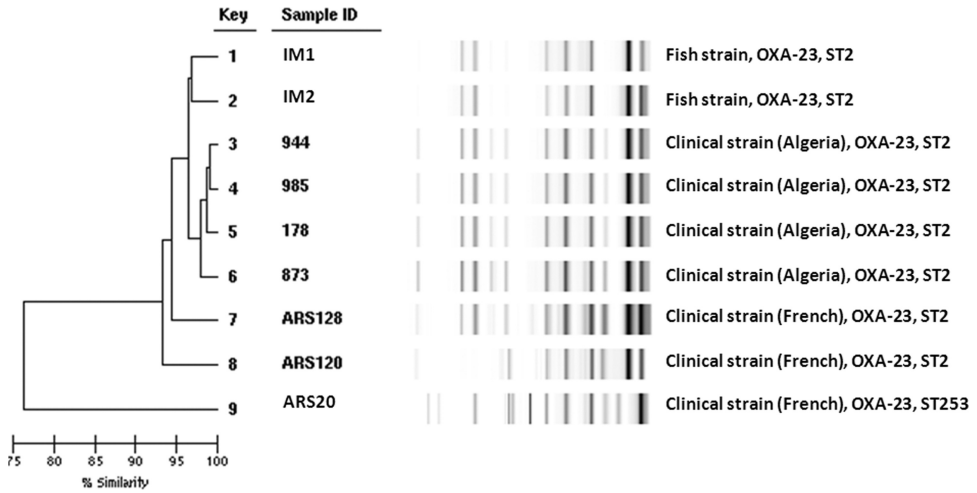

FIG 1 Dendrogram of Rep-PCR and MLST of the two OXA-23-producing *Acinetobacter baumannii* strains isolated from wild fish in the Mediterranean Sea, 3 representative human clinical strains isolated from a French hospital, and 3 representative human clinical strains isolated from an Algerian hospital. For the purpose of predicting different clones, the top match feature at 95% similarity was used.

been discharged untreated into the sea near the coast in regions of the Mediterranean Sea.

In conclusion, our study highlighted the idea that OXA-23-producing *A. baumannii* may be isolated from wild animals in rare cases. These findings emphasize the ability of these isolates to spread in the environment. More studies should be performed in the future to track the evolution of carbapenem-resistant *Acinetobacter* isolates and their frequencies in different ecosystems.

Nucleotide sequence accession number. Sequence information for *A. baumannii* strain IM2 transposon Tn*2006* was deposited in GenBank under accession no. KU168371.

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