Letters to the Editor

Expanded-Spectrum β -Lactamase PER-1 in an Environmental *Aeromonas media* Isolate from Switzerland[▽]

Aeromonas spp. are environmental reservoirs of resistance determinants to different classes of antibiotic molecules (1, 3, 7). Intrinsic resistance to β -lactams among this genus may arise from the expression of chromosomal β -lactamases and/or efflux pumps (5). Acquired β -lactamases conferring resistance to narrow-spectrum β -lactams, such as OXA-type enzymes and the penicillinases TEM-1 and SHV-1, have been identified in that genus as well as in a single report of the extended-spectrum β -lactamase (ESBL) TEM-24 (2, 4). The aim of this study was to evaluate the prevalence of ESBLs among *Aeromonas* sp. strains recovered from several rivers, lakes, and activated sludges from the southern part of the Swiss Alps.

Water samples were collected between 2002 and 2005 from the rivers Ticino and Vedeggio, from the lakes Cadagno and Lugano, and from activated sludges, all located in the Tessin county of Switzerland. The water samples were plated and growing bacteria identified by conventional biochemical methods (API-NE system; bioMérieux, Marcy-l'Etoile, France). All of the 50 *Aeromonas* sp. strains recovered were tested for ESBL detection by a synergy test using disks of ticarcillin-clavulanic acid, ceftazidime, cefotaxime, and aztreonam. The susceptibility testing of isolates was performed using Etest strips (AB Biodisk, Solna, Sweden).

A single isolate, A72, recovered from an activated sludge located in Bioggio, displayed an ESBL phenotype. This isolate was resistant to most β -lactams, except cephamycins and carbapenems (Table 1). PCR amplifications performed as described previously (9), using primers specific for ESBL-encoding genes, followed by sequencing identified the *bla*_{PER-1} gene. Genotyping of isolate A72, according to the results of *gyrB* sequencing (10), identified *Aeromonas media*, a waterborne species known to cause diarrhea in humans (6).

A 70-kb plasmid (pAM) harboring the *bla*_{PER-1} gene was transferred by conjugation into the *Escherichia coli* J53 recipient strain, using a selection made of ceftazidime (10 μ g/ml) and sodium azide (100 μ g/ml). Transconjugant *E. coli* J53(pAM) showed an

antimicrobial susceptibility pattern mirroring that of *A. media* A72 (Table 1).

PCR mapping performed as described previously (9) identified the *bla*_{PER-1} gene as part of a Tn1213 composite transposon, as previously found (9). In addition, this Tn1213 structure was bracketed by the *aphA6* and *strA* genes (encoding resistance to aminoglycosides) of transposon Tn5393d, as already observed in an *Alcaligenes faecalis* clinical isolate producing PER-1 recovered from Italy. In that latter isolate, the Tn5393d transposon was inserted into a Tn501 backbone carrying a mercury resistance transposon (8) that was not identified in *A. media* A72 by PCR mapping.

Our study constitutes the first identification of an ESBL-producing environmental *Aeromonas* sp. strain. The occurrence of *bla*_{PER-1} on a conjugative and broad-host-range plasmid, inside a genetic context similar to that described for an *A. faecalis* clinical isolate recovered in a neighboring country, may indicate that *Aeromonas* spp. may act as vehicles for spreading such an antibiotic resistance determinant. These findings also strengthen the role of *Aeromonas* spp. as reservoirs or vectors of antimicrobial resistance determinants of clinical relevance, as recently exemplified by the identification of the plasmid-mediated quinolone resistance *qnrS2* gene in *Aeromonas punctata* and *A. media* from the Seine River, Paris, France (1).

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REFERENCES

1. Cattoir, V., L. Poirel, C. Aubert, C. J. Soussy, and P. Nordmann. 2008. Unexpected occurrence of plasmid-mediated quinolone resistance determinants in environmental *Aeromonas* spp. *Emerg. Infect. Dis.* **14**:231–237.
2. Fosse, T., C. Giraud-Morin, I. Madinier, F. Mantoux, J. P. Lacour, and J. P. Ortonne. 2004. *Aeromonas hydrophila* with plasmid-borne class A extended-spectrum β -lactamase TEM-24 and three chromosomal class B, C, and D β -lactamases, isolated from a patient with necrotizing fasciitis. *Antimicrob. Agents Chemother.* **48**:2342–2343.
3. Goni-Urriza, M., M. Capdepuy, C. Arpin, N. Raymond, P. Caumette, and C. Quentin. 2000. Impact of an urban effluent on antibiotic resistance of riverine *Enterobacteriaceae* and *Aeromonas* spp. *Appl. Environ. Microbiol.* **66**:125–132.
4. Henriques, I. S., F. Fonseca, A. Alves, M. J. Saavedra, and A. Correia. 2006. Occurrence and diversity of integrons and β -lactamase genes among ampicillin-resistant isolates from estuarine waters. *Res. Microbiol.* **157**:938–947.
5. Hernould, M., S. Gagné, M. Fournier, C. Quentin, and C. Arpin. 2008. Role of the AheABC efflux pump in *Aeromonas hydrophila* intrinsic multidrug resistance. *Antimicrob. Agents Chemother.* **52**:1559–1563.
6. Janda, J. M., and S. L. Abbott. 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. *Clin. Infect. Dis.* **27**:332–344.
7. L'Abée-Lund, T. M., and H. Sørum. 2000. Functional Tn5393-like transposon in the R plasmid pRAS2 from the fish pathogen *Aeromonas salmonicida* subspecies *salmonicida* isolated in Norway. *Appl. Environ. Microbiol.* **66**:5533–5535.

TABLE 1. MICs of antimicrobial agents for *Aeromonas media* A72, *E. coli* J53 harboring natural plasmid p72, and *E. coli* reference strain J53

Antibiotic	MIC (μ g/ml)		
	<i>A. media</i> A72	<i>E. coli</i> J53 with plasmid pAM	<i>E. coli</i> J53
Amoxicillin	>256	>256	4
Amoxicillin + clavulanic acid	8	4	4
Ticarcillin	>256	>256	4
Ticarcillin + clavulanic acid	128	8	4
Cephalothin	>64	>64	8
Cefoxitin	2	2	2
Ceftazidime	>256	>256	0.06
Cefotaxime	>32	>32	0.06
Cefepime	4	8	0.06
Imipenem	0.25	0.25	0.12

8. Mantengoli, E., and G. M. Rossolini. 2005. Tn5393d, a complex Tn5393 derivative carrying the PER-1 extended-spectrum β -lactamase gene and other resistance determinants. *Antimicrob. Agents Chemother.* **49**:3289–3296.
9. Poirel, L., L. Cabanne, H. Vahaboglu, and P. Nordmann. 2005. Genetic environment and expression of the extended-spectrum β -lactamase *bla*_{PER-1} gene in gram-negative bacteria. *Antimicrob. Agents Chemother.* **49**:1708–1713.
10. Yanez, M. A., V. Catalan, D. Apraiz, M. J. Figueras, and A. J. Martinez-Murcia. 2003. Phylogenetic analysis of members of the genus *Aeromonas* based on *gyrB* gene sequences. *Int. J. Syst. Evol. Microbiol.* **53**:875–883.

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