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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

We report a case of necrotizing fasciitis with probable in vivo transfer of a TEM-24 plasmid-borne extended-spectrum β-lactamase (ESBL) gene from *Enterobacter aerogenes* to *Aeromonas hydrophila*. The patient was an 87-year-old female with a leg lesion following a trauma. She had a history of rheumatoid polyarthritis treated by 10 mg of prednisone per day, refractory anemia, and chronic venous insufficiency of the lower limbs. Within 5 days, the infection grew worse and the initial amoxicillin-clavulanic acid antibiotic therapy was replaced with ceftriaxone-metronidazole (1 to 1.5 g daily). Surgical debridement revealed extensive necrosis, and 3 days later, the lesion evolved toward typical necrotizing fasciitis (1, 6), leading to a second surgical intervention for above-knee amputation followed by complete healing.

Routine bacteriological procedures revealed (i) *Escherichia coli* NI-202 susceptible to most β-lactam compounds, (ii) *E. aerogenes* NI-203 resistant to all β-lactam antibiotics except imipenem, (iii) *A. hydrophila* NI-204 resistant to ceftazidime, and (iv) *A. hydrophila* NI-205 susceptible to ceftazidime (Table 1). Pulsed-field electrophoresis confirmed that *A. hydrophila* NI-204 and NI-205 derived from a single clone. For β-lactamase analysis, the *E. aerogenes* isolate was grown in brain heart infusion broth with and without cefoxitin or ceftazidime induction (10 μg/ml) at 37°C before analytical isoelectric focusing with crude sonic cell extracts on polyacrylamide gels (2, 4). Two bands of β-lactamase activity were detected with iodine gel with cefazolin (500 μg/ml) as the substrate, which was suggestive of the production of an inducible cephalosporinase (pI 8.8) and an ESBL (pI 6.5). *Aeromonas* isolates were grown at 30°C with cefoxitin (10 μg/ml), imipenem (1 μg/ml), or tobramycin (1 μg/ml) induction (5). Analytical isoelectric focusing with penicillin and cefazolin as substrates revealed three bands (pI 7, 7.8, and 8.2) probably corresponding to previously described cephalosporinase-, imipenemase-, and oxacillinase-type inducible β-lactamases (5, 11, 12). *A. hydrophila* NI-204 produced an additional enzyme similar to *E. aerogenes* NI-203 ESBL (pI 6.5).

Taking into account resistance to ceftazidime, pI determination, and local epidemiology, the ESBL was presumed to be the plasmid-mediated TEM-24 βlactamase (2–4, 7). The plasmid was transferred from *E. aerogenes* NI-203 to *A. hydrophila* NI-205 and to *E. coli* C1a at a high frequency (10^{-4}). Recipient strains (NI-206 and NI-207, Table 1) presented the same acquired resistance pattern. After plasmid extraction and gel electrophoresis, both wild-type strains (*E. aerogenes* NI-203, *A. hydrophila* NI-204) and recipient strains (*A. hydrophila* NI-206, *E. coli* C1a NI-207) showed a common 180-kb band, as previously characterized with *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and recently *A. caviae* (4, 7–9). The capacity of *Aeromonas salmonicida* to maintain either or both of the *Pseudomonas* and *Enterobacteriaceae* R factors has already been observed (10). PCR amplification with TEM family-specific primers was applied to *E. aerogenes* NI-203 and *A. hydrophila* NI-204 and showed a deduced protein sequence with 100% identity to that of TEM-24 (3, 7).

This report demonstrates probable in vivo transfer of ESBL TEM-24 from *E. aerogenes* to the genus *Aeromonas*. It was observed in a wild-type strain of *A. hydrophila* simultaneously producing the class A, B, C, and D β-lactamases.

TABLE 1. β-Lactamases, plasmid content, and MICs (μg/ml) of the clinical isolates and transconjugant strains

Parameter or drug(s)	*E. aerogenes* NI-203	*A. hydrophila* NI-204	*A. hydrophila* NI-205	*A. hydrophila* NI-206	*E. coli* C1a	*E. coli* C1a NI-207
pI	6.5, 8.8	6.5, 7, 7.8, 8.2	7, 7.8, 8.2	6.5, 7, 7.8, 8.2	None	6.5
Plasmid size (kb)	180	180		180		180
PCR TEM	+	+	−	−	−	+
Amoxicillin	>256	>256	>256	>256	2	>256
Amoxicillin + clavulanic acid	8	8	8	16	2	2
Ticarcillin	>256	>256	>256	>256	8	>256
Ticarcillin + clavulanic acid	64	32	64	16	8	8
Piperacillin	256	4	2	4	2	64
Piperacillin + tazobactam	16	1	1	1	2	2
Cefoxitin	>256	16	16	2	2	2
Cefepime	1	0.25	0.06	0.25	≤0.03	1
Cefepime + clavulanic acid	0.125	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03
Cefuroxime	>256	2	1	2	1	1
Cefpirome	2	0.5	0.03	0.5	0.03	1
Cefotaxime	>32	0.5	0.06	0.5	0.06	1
Ceftazidime	>256	32	0.125	32	0.125	256
Ceftazidime + clavulanic acid	>4	0.06	0.06	0.06	0.25	0.25
Imipenem	0.5	4	4	8	0.5	0.5
Amikacin	8	8	2	8	0.25	16
Tobramycin	8	16	2	16	0.25	16
Sulfamethoxazole-trimethoprim	>32	>32	0.25	>32	0.5	>32
Ciprofloxacin	>32	≤0.03	≤0.03	≤0.03	0.125	0.125

REFERENCES

1. **Angel, M. F., F. Zhang, M. Jones, J. Henderson, and S. W. Chapman.** 2002. Necrotizing fasciitis of the upper extremity resulting from a water moccasin bite. South. Med. J. **95:**1090–1094.
2. **Bradford, P. A.** 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. **14:**933–951.
3. **Chanal, C., M.-C. Poupart, D. Sirot, R. Labia, J. Sirot, and R. Cluzel.** 1992. Nucleotide sequences of CAZ-2, CAZ-6, and CAZ-7 β-lactamase genes. Antimicrob. Agents Chemother. **36:**1817–1820.
4. **Dumarche, P., C. De Champs, D. Sirot, C. Chanal, R. Bonnet, and J. Sirot.** 2002. TEM derivative-producing *Enterobacter aerogenes* strains: dissemination of a prevalent clone. Antimicrob. Agents Chemother. **46:**1128–1131.
5. **Fosse, T., C. Giraud-Morin, I. Madinier, and R. Labia.** 2003. Sequence analysis and biochemical characterization of chromosomal CAV-1 (*Aeromonas caviae*), the parental cephalosporinase of plasmid-mediated AmpC 'FOX' cluster. FEMS Microbiol. Lett. **222:**93–98.
6. **Furusu, A., N. Yoshizuka, K. Abe, O. Sasaki, K. Miyazaki, M. Miyazaki, Y. Hirakata, Y. Ozono, T. Harada, and S. Kohno.** 1997. Aeromonas hydrophila necrotizing fasciitis and gas gangrene in a diabetic patient on hemodialysis. Nephrol. Dial. Transplant. **12:**1730–1734.
7. **Giraud-Morin, C., and T. Fosse.** 2003. A seven-year survey of *Klebsiella pneumoniae* producing TEM-24 extended-spectrum β-lactamase in Nice University Hospital (1994–2000). J. Hosp. Infect. **54:**25–31.
8. **Marchandin, H., H. Jean-Pierre, C. De Champs, D. Sirot, H. Darbas, P. F. Perigault, and C. Carriere.** 2000. Production of a TEM-24 plasmid-mediated extended-spectrum β-lactamase by a clinical isolate of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **44:**213–216.
9. **Marchandin, H., S. Godreuil, H. Darbas, H. Jean-Pierre, E. Jumas-Bilak, C. Chanal, and R. Bonnet.** 2003. Extended-spectrum β-lactamase TEM-24 in an *Aeromonas* clinical strain: acquisition from the prevalent *Enterobacter aerogenes* clone in France. Antimicrob. Agents Chemother. **47:**3994–3995.
10. **Olsen, R. H., and C. D. Wright.** 1976. Interaction of *Pseudomonas* and *Enterobacteriaceae* plasmids in *Aeromonas salmonicida.* J. Bacteriol. **128:** 228–234.
11. **Rasmussen, B. A., and K. Bush.** 1997. Carbapenem-hydrolyzing β-lactamases. Antimicrob. Agents Chemother. **41:**223–232.
12. **Walsh, T. R., L. Hall, A. P. MacGowan, and P. M. Bennett.** 1995. A clinical isolate of *Aeromonas sobria* with three chromosomally mediated inducible β-lactamases: a cephalosporinase, a penicillinase and a third enzyme displaying carbapenemase activity. J. Antimicrob. Chemother. **35:**271–279.

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