

Acute Renal Failure in an Infant Associated with Cytotoxic *Aeromonas sobria* Isolated from Patient's Stool and from Aquarium Water as Suspected Source of Infection

In May 1996, a previously healthy and normally developed 6-month-old female infant was hospitalized after a 7-day period of watery and finally bloody diarrhea. Upon admission, the child was in poor general condition. Laboratory tests and ultrasound examinations of the kidneys revealed acute renal failure with marked metabolic acidosis and moderate anemia. The child's creatinine level was 442 $\mu\text{mol/liter}$, and dialysis was instituted after 12 h. There was no clear-cut evidence of hemolysis or platelet involvement except a significantly elevated lactate dehydrogenase level. The patient remained in end-stage renal failure and after 16 months received a successful transplant from a pediatric donor in December 1997, without recurrence of renal failure or hemolytic-uremic syndrome (HUS).

Stool taken from the patient upon admission at the children's hospital was found negative for enteric bacterial pathogens such as *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., enteropathogenic *Escherichia coli*, and *Yersinia* spp. A bacterial stool culture showed cytotoxicity in the Vero cell test (2) but was found negative for Shiga toxin (Stx)-producing *E. coli* (STEC) or Stx-specific gene sequences when examined by an stx-specific PCR (7). However, verocytotoxic *Aeromonas sobria* was isolated from the patient's stool, but the stool was negative when examined for Stx by PCR and by the VTEC-RPLA assay (2). The diarrheal disease ceased 2 days after admission, and subsequent stools became negative for cytotoxic activity and for *A. sobria*.

Microbiological investigations as to the possible source of infection were carried out in the child's domestic environment. The child had been fed exclusively by formula milk, and *Aeromonas* organisms were not detected in food, in table water, or in stool samples taken from the parents. The microbiological investigations were then extended to water pipes, bathroom fixtures and an aquarium which was present in the family's

home. Only one *A. sobria* strain could be isolated from the drain of the bathtub; the aquarium water, however, yielded about 10^5 colonies of *Aeromonas* species per ml. Twenty different colonies were chosen and were all identified as *A. sobria*. On the basis of hemolytic properties, biochemical reactions, cytotoxicity tests, and morphological differences, three representative *A. sobria* strains from the aquarium, the strain from the bathtub, and the patient isolate were compared with *Aeromonas* reference strains (Table 1). The *A. sobria* isolates differed for verocytotoxic activity according to their growth temperature. All *A. sobria* strains except the isolate from the bathtub (CB6076) showed verocytotoxic activity after growth at 30°C, whereas only the patient strain (CB5869) and one strain from the aquarium (CB6179) showed clear cytotoxicity when grown at 37°C. As aerolysins are well known hemolytic cytotoxins present in different species of *Aeromonas*, a specific PCR for the aerolysin gene of *A. sobria* was developed on the basis of the published nucleotide sequence (6). The PCR was performed with primers SOBF (5' GCG ACC AAC TAC ACC GAC CTG 3') and SOBB (5' GGA CTT GTA GAG GGC AAC CCG 3'). Only *A. sobria* strains positive for cytotoxin production gave a positive PCR result (Table 1). The patient isolate (CB5869) and the isolate from the aquarium (CB6179) were compared by nucleotide sequencing of the two 288-bp PCR products. The nucleotide sequences of CB5869 and CB6179 (EMBL database accession no. AJ243046 and AJ243047, respectively) differed in five positions, indicating that the strains were not identical. All *A. sobria* strains were negative for the aerolysins of *A. hydrophila* and *A. caviae* when tested by PCR (8, 11) (Table 1) and also for *hlyA* gene sequence, which encodes another type of hemolytic cytotoxin found in some strains of *A. hydrophila* (5, 12) (Table 1).

Human infections with cytotoxic *Aeromonas* have occasionally been associated with renal failure and HUS (3, 4, 9, 10).

TABLE 1. Properties of *Aeromonas* isolates

Strain no.	Phenospecies	Source	Verocytotoxicity with bacteria grown at:		Hemolysis ^a	Aerolysin PCR specific for:	
			30°C	37°C		<i>A. sobria</i> (this work)	<i>A. hydrophila</i> (8)
CB5869	<i>A. sobria</i>	Patient's stool	+ ^b	+	+	+	- ^c
CB6179	<i>A. sobria</i>	Aquarium water	+	+	+	+	-
CB6180	<i>A. sobria</i>	Aquarium water	+	-	+	+	-
CB6181	<i>A. sobria</i>	Aquarium water	+	(+) ^d	+	+	-
CB6076	<i>A. sobria</i>	Drain, bathtub	-	-	(+)	-	-
CH570 ^e	<i>A. sobria</i>	Sushi	+	+	+	+	-
CH118 ^e	<i>A. hydrophila</i>	Unknown	+	+	+	-	+ ^f
CH505 ^e	<i>A. hydrophila</i>	Human stool	+	+	+	-	+ ^f
CH417 ^e	<i>A. caviae</i>	Human stool	-	-	+	-	-
CH504 ^e	<i>A. caviae</i>	Human urine	-	-	(+)	-	-

^a Hemolysis was tested on washed sheep blood agar (enterohemolysin agar) growth temperatures of 25 and 37°C.

^b +, positive.

^c -, negative.

^d (+), weakly positive.

^e Laboratory reference strain from the collection of the Robert Koch-Institute.

^f Strain also positive for *hlyA* (5)-specific DNA sequences by PCR.

Here we report the clinical and microbiological findings made for an infant with diarrhea-associated acute renal failure probably caused by a hemolytic and cytotoxic strain of *A. sobria*. The patient strain produced verocytotoxin at 37°C, indicating that toxin production has also occurred in the course of the infection. The end of the diarrheal disease was concomitant with the disappearance of *A. sobria* from the patient's stool. There was no evidence that the patient was infected by STEC, and the patient's serum did not contain antibodies to *E. coli* O157. The microbiological investigations suggest that aquarium water was the possible source of infection. *Aeromonas* spp. are fish pathogens found in aquatic environments worldwide (1). The infection could have occurred via the bathtub, which was contaminated with *A. sobria* and was frequently used for cleaning and rinsing the aquarium.

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