

Isolation of *Aeromonas* species from clinical sources

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SYNOPSIS In a period of one year, in a general hospital, *Aeromonas hydrophila* was isolated from 13 patients and *Aeromonas shigelloides* from one patient. Eight of the patients had superficial infections, two had urinary tract infections, and four had bacteraemia. The association of *Aeromonas* bacteraemia with cirrhosis of the liver and malignant disease, which has been previously reported, was observed in three of the four bacteraemic patients. The key to laboratory diagnosis of this genus is the routine performance of the oxidase test in bacteriological procedures for the identification of Gram-negative bacilli.

Organisms belonging to the genus *Aeromonas* are seldom associated with human infections. Nevertheless, there are indications from sources in the United States and Australia that, in common with

other exotic Gram-negative bacteria, *Aeromonas* is being isolated with increasing frequency from clinical sources (von Graevenitz and Mensch, 1968; Cooper and Brown, 1968). During the period January 1971 to January 1972 *Aeromonas hydrophila* was isolated from 13 patients in Bexar County Hospital, San Antonio, Texas. Four of these patients had Aero-

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Case No.	Patient	Clinical Diagnosis	Source of Isolate	Associated Organisms	Concurrent Infection	Antibiotics before Isolation	Treatment	Clinical Response
1	F.C. 25 yr M	Possible cervical fracture, wound infection	Scalp wound	None	None	None	Debridement	Improved
2	R.D. 11 yr M	Infection of chest wound	Wound	<i>E. coli</i> <i>Edwardsiella tarda</i> <i>Clostridium</i> sp.	None	None	Cleaned	No followup
3	M.B. 72 yr M	Small bowel obstruction	Urine	None	None	None	None	No followup
4	C.T. 58 yr F	Hypoproteinaemia Carcinoma of uterus	Blood	None	Enteritis Pneumonia	None	Cephalothin and kanamycin	Initially good Cardiac arrest
5	A.H. 75 yr M	Acute blastic leukaemia	Blood	None	None	None	Cephalothin and kanamycin	Patient died
6	A.T. 40 yr M	Gram-negative sepsis Gastrointestinal bleeding, Laennec's cirrhosis	Blood	None	?	None	None	Patient died
7	S.P. 44 yr F	Massive decubitus ulcer, Gram-negative sepsis	Blood	Strep group A	Decubitus	Kanamycin, cephalothin, gentamicin	Tetracycline	Patient died
8	A.S. 59 yr M	Cirrhosis, possible carcinoma of caecum	Exudate	Strep group D	None	None	Oral neomycin penicillin, kanamycin	Patient died
9	J.P. 6 yr M	Cellulitis of foot (<i>Aeromonas shigelloides</i>)	Wound on foot	<i>Staph. aureus</i> Enterobacter A	None	None	Procaine penicillin, im	No followup
10	R.M. 68 yr M	Congestive heart failure, hypertension, cellulitis of legs	Leg lesion	Klebsiella, Strep group A	None	None	Cephalothin iv then erythromycin	Good
11	C.P. 5½ yr M	Cellulitis of foot	Exudate	None	None	None	Oral and im Penicillin	No followup
12	F.Y. 8 yr M	Abscess and cellulitis	Exudate	<i>Staph. epidermidis</i>	None	None	Penicillin im, incision and drainage	No followup
13	M.L. 17 yr F	Impetigo, abscess and cellulitis	Exudate from foot	Strep group A <i>Staph. aureus</i> , Klebsiella, <i>E. coli</i>	None	None	Incision and drainage	Improved
14	M.M. 5 mth M	Diarrhoea, dehydration	Urine	None	None	Ampicillin	None	Improved

Table I *Clinical summaries of 14 patients with Aeromonas infections*

monas bacteraemia. *Aeromonas shigelloides* was isolated from one other patient during that period.

Case Reports

Clinical and bacteriological summaries of these 14 patients are shown in Table I. To illustrate the clinical course of some of these infections seven of the patients are presented here in more detail. Permission for necropsy was not obtained in the four fatal cases.

PATIENT NO. 1

F.C., a healthy 25-year-old man, dived into a swimming pool and struck his head on the bottom. He was admitted to hospital with a 4-in. laceration of the scalp and a suspected fracture of the cervical vertebrae. On the day after his admission the wound was purulent and a pure growth of *Aeromonas hydrophila* was obtained from the pus. At this time the patient's white cell count was 19 000 cmm with polymorphs predominating. The wound was extensively cleaned and the patient discharged the next day. He was not given antibiotics and his recovery was uneventful.

PATIENT NO. 2

R.D., an 11-year-old boy, received a superficial puncture of the chest from the branch of a dead tree while swimming in a lake. The following day the patient was admitted to hospital at which time the wound was inflamed and tender; his temperature was 99.8°F, and his white cell count was 20 000 per cmm. A heavy growth of *Aeromonas hydrophila* in mixed culture with moderate numbers of *Escherichia coli* and a few colonies of *Clostridium welchii* and *Edwardsiella tarda* was isolated from the wound. Surgical debridement was carried out and the patient made a good recovery without antibiotics.

PATIENT NO. 3

M.B., a 72-year-old man, was admitted to hospital with a diagnosis of intestinal obstruction secondary to chronic duodenal ulceration. The patient also had benign prostatic hypertrophy and was catheterized once. A urine culture at this time was sterile but over the next few days *Aeromonas hydrophila* in excess of 10⁸ organisms per ml of urine, together with insignificant numbers of *Escherichia coli*, were isolated from further samples of urine. The patient did not develop any signs and symptoms of urinary tract infection. Further catheterization was not required and within eight days the bacteriuria cleared completely without antibiotic therapy. The patient was released after a good response to conservative treatment of his intestinal complaint.

PATIENT NO. 4

C.T., a 58-year-old woman, came to hospital with a complaint of generalized oedema and severe diarrhoea of six months' duration. She had no previous history of cardiac, urinary, or liver disease. Shortly after admission a Foley catheter was inserted. After diuresis was induced the patient was found to be markedly hypoproteinaemic (serum proteins = 3.5 g per 100 ml) and 3 units of albumin were infused. Preliminary investigations suggested that the patient had a carcinoma of the uterus with spread to the colon and a secondary protein-losing enteropathy. Two blood cultures taken on admission were sterile but after two days in hospital she became febrile and rapidly comatose. A white cell count at this time was 23 000 per cmm, with 86% polymorphs, and a platelet count was 90 000 per cmm. A diagnosis of Gram-negative sepsis was made and two blood cultures taken on the third day after admission were positive for *Aeromonas hydrophila*. She was treated with cephalothin and kanamycin and showed some clinical improvement. On the second day in hospital she developed lower intestinal bleeding, following which 2 units of blood were given. When the antibiotic sensitivity pattern of the organism was known the patient was treated with gentamicin but she continued to have periods of extreme hypotension and died five days after admission.

PATIENT NO. 5

A.H. was a 75-year-old man known to have blast cell leukaemia and bronchiectasis. He was admitted to hospital with a diagnosis of Gram-negative bacteraemia. On admission he was febrile and disorientated and numerous petechiae were present on the skin. He was treated with cephalothin and kanamycin. Shortly after admission he became markedly hypotensive but improved initially with parenteral hydrocortisone. The following day, however, he began to have respiratory distress and died shortly after from cardio-respiratory arrest. Three blood cultures were taken before antibiotic therapy; all three were positive for *Aeromonas hydrophila*.

PATIENT NO. 6

A.T., a 40-year-old bartender, was admitted to hospital with upper gastrointestinal bleeding and melaena. He had a history of alcoholic liver disease and a previous episode of gastrointestinal haemorrhage. On admission the patient had retention of urine and a catheter was inserted. The source of gastrointestinal bleeding was found to be a large gastric ulcer. After transfusion of 4 units of blood the patient was treated conservatively and the gastric bleeding stopped the day after admission. The next day, however, he was febrile and had deep vein

thrombophlebitis in both legs. A white cell count at this time was 30 000 per cmm. Later the same day, following a marked decrease in urinary output, he became very restless and disorientated and he died suddenly. Antibiotic treatment was not given. Two blood cultures taken on the day he died were positive for *Aeromonas hydrophila*.

PATIENT NO. 7

S.P., a 44-year-old quadriplegic woman, was admitted to hospital with large decubitus ulcers of the back and extremities and a history of fever and chills in the previous three weeks. The patient had had an indwelling catheter since a fracture of the cervical vertebrae occurred in a car accident four years previously rendering her quadriplegic. On admission she was febrile with a blood pressure of 60/40 mm Hg. Her white cell count was 26 000 per cmm with 93% polymorphs. Serum protein was 4.5 g per 100 ml. A diagnosis of Gram-negative endotoxin shock was made, blood cultures were obtained, and treatment with gentamicin was begun. Initial blood cultures were positive for group A beta-haemolytic streptococci and *Bacteroides oralis*; blood cultures taken after antibiotic therapy was begun were positive for *Aeromonas hydrophila*. On the second day tetracycline was substituted for gentamicin but despite this and supportive measures the patient continued to have episodes of hypotension and died four days after admission.

Bacteriology

Swabs of pus or exudates were plated out aerobically and anaerobically on 7.5% sheep blood agar plates and on McConkey's medium and were inoculated into thioglycollate tubes. Quantitative urine cultures were plated with a calibrated loop on blood agar and McConkey's medium. Blood cultures were performed by inoculation of a 100 ml bottle of Thiol¹ broth, a thioglycollate tube, and a tube containing 3.8%

¹Difco, Detroit, Michigan.

	<i>Aeromonas hydrophila</i>	<i>Aeromonas shigelloides</i>
O/F	F	F
Gas from glucose	+ or -	-
Gas from other carbohydrates	+(-)	-
Glucose acid	acid	acid
Lactose	-(acid)	acid(-)
Maltose	-(acid)	acid(-)
Sucrose	acid(-)	-
Xylose	-	-
Mannitol	acid	-
Aesculin	+(-)	-
Catalase	+(-)	+(-)
Oxidase	+	+
Urea	-(+)	-
Nitrate	+	+
Indole	+(-)	-
Methyl red	+(-)	+
Voges-Proskauer	- or +	-
TSI slant	Alk(acid)	Alk(acid)
butt	-	-
Gelatin	+(-)	-
Pigment	-	-
Growth at 35°C	+	+
37°C	+	+
42°C	+(-)	+(-)
Motility	+	+
KCN	+(-)	-(+)
Lysine decarboxylase	-(+)	+
Arginine dihydrolase	+	+(-)
Ornithine decarboxylase	-	- or +
Citrate	+(-)	-
Growth on McConkey's medium	+	+

Table II *Biochemical and physical properties of Aeromonas hydrophila and Aeromonas shigelloides*

() = minority reaction

+ or - = approximately 50% of isolates positive, 50% negative

sodium citrate. From this latter tube a pour-plate was prepared with 0.5% trypticase soy agar. *Aeromonas hydrophila* was isolated from aerobic and anaerobic cultures. The colonies resembled many other Gram-negative rods in colonial morphology but were recognized as possible *Aeromonas* species by the application of the oxidase test to colonies of all Gram-negative rods as a routine screening procedure on initial isolation. Twelve of the 13 isolates showed beta haemolytic colonies on blood

Antibiotic	Present Series		Results Summarized from Literature	
	No. of Isolates Tested	No. Resistant	No. Isolates Tested	No. Resistant
Penicillin	10	10 (100%)	25	25 (100%)
Ampicillin	10	10 (100%)	63	62 (98%)
Carbenicillin	3	3 (100%)	—	—
Streptomycin	10	6 (66.6%)	70	8 (11%)
Cephalothin	10	4 (40%)	52	38 (73%)
Gentamicin	3	1 (33.3%)	3	0
Tetracycline	10	0	71	1 (1.4%)
Polymyxin E	10	0	56	2 (4%)
Kanamycin	10	0	48	1 (2%)
Chloramphenicol	10	0	71	0

Table III *Antibiotic resistance of Aeromonas in the present series and summarized from the literature*

agar. The biochemical reactions used for the identification of *Aeromonas hydrophila* and *Aeromonas shigelloides* are shown in Table II. Antibiotic sensitivity tests were carried out by the method of Bauer, Kirby, Sherris, and Turck (1966) on 10 of the 14 isolates. The results are shown in Table III where they are compared with the reported sensitivities of *Aeromonas* isolated from human sources.

Discussion

Priority in the earliest description of a member of the genus *Aeromonas* should probably be given to Sanarelli (1891) who described a bacterium which he named *Bacillus hydrophilus fuscus* (Ewing, Hugh, and Johnson, 1961). This organism is probably that which is now known as *Aeromonas hydrophila*. Organisms of this genus are Gram-negative, facultative anaerobes, motile with polar flagella. They are usually beta-haemolytic on blood agar and ferment carbohydrates with the production of acid and gas (Table II).

The nomenclature and classification of the genus are discussed in detail by Eddy (1960), Ewing *et al* (1961), Meeks (1963), Schubert (1967), and Gillardi (1967). Identification and classification of the *Aeromonas* group of organisms in our laboratory are based on the work of Ewing *et al* (1961), who recognized three species—*Aeromonas hydrophila*, *Aeromonas salmonicida*, and *Aeromonas shigelloides*. With one exception all the isolates described here belong to the species *Aeromonas hydrophila*; one strain was identified as *Aeromonas shigelloides*. The name *Plesiomonas shigelloides* has also been proposed for this species (Habs and Schubert, 1962).

This genus has long been known to be a pathogen for fish, reptiles, and amphibians (Wilson and Miles, 1964; Heywood, 1968). Isolates from human sources have been shown to cause sepsis and death in mice,

Source	No. of Isolates
Stool	66
Postoperative wounds, abscesses, superficial cellulitis	28
Blood	23
Urine	8
Sputum and/or throat	9
Nasopharynx	5
Bile	6
Intraabdominal abscesses, peritoneum, dialysis fluid	5
Total	150

Table IV Sources of 150 reported isolates of *Aeromonas*

rabbits, and guinea pigs (Kjems, 1955; Miles and Halnan, 1937; Pittman, 1953).

In the majority of the 150 isolates from human sources described in the literature (Table IV) no clinical information was given. Twenty-five cases of *Aeromonas* bacteraemia have been reported, 12 of which have included clinical data. These together with the four patients (Nos. 4, 5, 6, and 7) with bacteraemia described here are summarized in Table V). Among these 16 patients, five had hepatic cirrhosis and six had some form of acute leukaemia or lymphoma. Impairment of host defence mechanisms such as defective filtration by the cirrhotic liver (Conn, 1964) and the immunosuppressive effects of antileukaemic therapy (von Graevenitz and Mensch, 1968) has been suggested as playing a part in the pathogenesis of these bacteraemias. The four patients with *Aeromonas* bacteraemia described here died. From three of these patients *Aeromonas hydrophila* was obtained in pure culture from the blood; from one it was one of the organisms isolated from a polymicrobial bacteraemia. Three of the bacteraemic patients had marked hypoproteinaemia which may have been a further predisposing factor. The source of infection in these four patients could not be elucidated. Although all four had urinary catheters inserted, and it is tempting to consider this

Case No.	Authors	Year	Clinical Diagnosis	No. Positive Blood Cultures
1	Kjems	1955	Hepatic cirrhosis	3
2			Rheumatic fever	1
3	Conn	1964	Cirrhosis and peritonitis	1
4			Cirrhosis and peritonitis	1
5	Bulger and Sherris	1966	Lymphoblastic leukaemia	3
6	Dean and Post	1967	Acute myelogenous leukaemia	5
7	von Graevenitz and Mensch	1968	Cirrhosis and pneumonia	2
8			Chronic cholecystitis	1
9	Lopez <i>et al</i>	1968	Acute myelogenous leukaemia	4
10	Slotnick	1970	Leukaemia	2
11			Reticulum cell sarcoma	3
12			Bleeding ulcer	1
13	Present series	1972	Cirrhosis and gastrointestinal haemorrhage	1
14			Cancer of uterus	2
15			Quadriplegia: decubitus ulcers	1
16			Acute leukaemia	3

Table V Clinical diagnosis in 16 patients with *Aeromonas* bacteraemia

a likely source of the bacteraemia, none of them had clinical or bacteriological evidence of urinary tract infection by *Aeromonas*. The clinical picture of these four patients was indistinguishable from that due to other Gram-negative bacteria. Patients 4 and 5 developed the signs and symptoms of bacterial endotoxin shock with episodes of profound hypotension. *Aeromonas* infection of the urinary tract occurred in two patients (patients 3 and 14) from whom the organism was isolated in pure culture and in significant numbers. These two infections were thought to be acquired in hospital; patient no. 3 was catheterized and this was a probable source of infection. In patient no. 14 the source was not apparent although the use of ampicillin may have favoured the selection of *Aeromonas* as the infecting organism. Numerous hospital environmental samples over the past three years have not yielded a single isolation of *Aeromonas* among the many other Gram-negative species isolated.

Localized skin and soft tissue infections occurred mainly on the lower limbs; sometimes there was a preceding history of minor trauma. The single isolate of *Aeromonas shigelloides* was obtained from a patient who stepped on a rusty nail. The infections of the scalp and chest wall (patients 1 and 2) both occurred following swimming mishaps. The association of *Aeromonas* infections with exposure to contaminated water has been reported for pulmonary infections following drowning and for wound sepsis (von Graevenitz and Mensch, 1968).

There have been a number of reports of the association of *Aeromonas* species with gastrointestinal disease (Gillardi, 1967; Mohiedin, Gabr, El-Hefny, Mahmoud, and Abdallah, 1965; Lautrop, 1961) which has usually taken the form of mild to severe gastroenteritis. The significance of these associations is difficult to assess since up to 3.2% of normal people have been found to excrete *Aeromonas* in the faeces (Rosner, 1964; Paučková and Fukalová, 1968; von Graevenitz and Zinterhofer, 1970). The presence of the organism as a normal inhabitant of the gut would account for the isolation of *Aeromonas* from patients with peritonitis, postoperative wound infections and abscesses (Bras, Clearkin, Annamunthodo, and Caselitz, 1954; Bottone and Allerhand, 1970; Sonnenwirth, 1970). Cooper and Brown (1968) reported the association of *Plesiomonas* (*Aeromonas*) *shigelloides* with enteritis in children in Australia.

The results of antibiotic sensitivity tests (Table III) did not differ significantly from those previously reported by many authors. The resistance of *Aeromonas* to penicillin and ampicillin is probably due to the production of beta-lactamase (Bulger and Sherris, 1966). *Aeromonas* species are consistently

sensitive to chloramphenicol and tetracycline and Abrams, Zierdt, and Brown (1971) have suggested that these antibiotics be given in combination as soon as a tentative diagnosis of *Aeromonas* infection is made. However, in patients with *Aeromonas* septicaemia the use of an appropriate bactericidal antibiotic such as kanamycin should also be considered.

The occurrence of 14 instances of *Aeromonas* infections in one general hospital in one year is possibly fortuitous although in the previous two years only three or four isolates of this organism were obtained in our laboratory. However, *Aeromonas* species can readily be overlooked if the oxidase test is not performed on colonies of Gram-negative rods. We believe that many laboratories may fail to recognize the *Aeromonas* group for this reason and recommend the use of the oxidase test as a routine procedure in this area of diagnostic bacteriology.

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