Epidem. Inf. (1987), **98**, 5–14
Printed in Great Britain

Epidemiological complexity of hospital aeromonas infections revealed by electrophoretic typing of esterases

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(*Accepted 15 July 1986*)

SUMMARY

An epidemiology analysis of a series of 12 *Aeromonas hydrophila* infections, including six of septicaemia, which occurred on several wards of one hospital during the summer of 1982 is presented. The hypothesis that the hospital water could be the source of these infections was supported by the isolation of 1–10 motile aeromonads per ml in most of the water samples collected from various points on the hospital water system. Electrophoretic esterase typing was used as an epidemiological screening method to determine the relationship between bacterial strains isolated from the patients and those from water samples. The epidemiology of *A. hydrophila* infection in the hospital was found to be complex. Amongst the 15 strains of *A. hydrophila* isolated from patients were 8 zymotypes, while amongst the 126 strains from the water samples there were 37. In some cases, several zymotypes were isolated simultaneously from the same tap water. On one ward, the same zymotype was found in 2 patients and in 2 water samples. The prophylactic measures taken in 1982–5 to avoid oral contamination of immunocompromised patients with infected hospital water have significantly reduced the number of cases of septicaemia. This success has constituted additional retrospective evidence for the water-borne origin of these infections.

INTRODUCTION

The motile aeromonads are gram-negative bacilli of the Vibrionaceae family which are frequently found in environmental water (Hazen *et al.* 1978) where they can produce a number of infections in animals and man. Their presence in drinking water is well established (Le Chevallier *et al.* 1982) and ingestion of such water may cause infections, including severe septicaemia, in hospitalized patients (Picard, Arlet & Goullet, 1984). However, the precise origin of the infections remains unclear (Cookson, Houang & Lee, 1984; Mellersh, Norman & Smith, 1984).

A series of 12 cases of *Aeromonas hydrophila* infections occurred in our hospital during the summer of 1982. Despite their clinical diversity (septicaemia, gastroenteritis, surgical infections) and distribution (3 medical wards and 2 surgical wards), they all displayed a number of common features. All the patients were immunocompromised; they had been in hospital for at least 2 days before the first symptoms of infection were detected; and all had used hospital water for both

drinking and ablutions. Consequently, the infections were not considered to be sporadic.

The aim of this work was to determine the origin of these infections through epidemiological investigation by isolation of *A. hydrophila* strains from samples of water taken from different parts of the hospital and by establishing a correlation between these strains and those isolated from the 12 infected patients. For this purpose, we used the electrophoretic typing of esterases, as it appeared to be a more discriminative method than biotyping (Picard & Goullet, 1984, 1985).

MATERIAL AND METHODS

Isolation of aeromonas

Patients. The major characteristics of each patient's cause of hospitalization and infection are shown in Table 1. Fifteen strains were isolated from pathological samples (urine, stool, blood and thoracic drainage) from the 12 patients.

Water samples. Ten batches of water samples were taken between 20 August and 26 November 1982, during and after the period when the 12 cases of infection occurred. Each 100 ml sample was taken from a cleaned, alcohol-flamed faucet and was immediately filtered through a Sartorius SM 139 membrane (Sartorius GmbH-PF 3243 D-3400 Göttingen, F.R.G.). The membranes were placed on tryptic soy agar and incubated at 30 °C for 24 h.

Identification of aeromonas

The aeromonas colonies were assigned to one of the three species: *A. hydrophila*, *A. caviae*, *A. sobria* (Popoff *et al.* 1981) according to the following criteria: (i) presence of oxidase; (ii) indole production; (iii) study of biochemical characters (API-20E). *A. hydrophila* was differentiated from *A. caviae* on the basis of VP positivity and gas production during glucose fermentation (Popoff & Veron, 1976).

Electrophoretic esterase typing

Typing was carried out on 141 strains of aeromonas including the 15 strains isolated from patients and 126 strains obtained from 72 water samples. A single strain was taken in 54 samples. From the remaining 18 samples 4 colonies were picked at random from the membrane and typed to evaluate the simultaneous presence of different aeromonas strains.

Preparation of bacterial extracts

Bacteria were grown at 30 °C in L broth (Lennox, 1955) with constant agitation for 18 h. After centrifugation the pellets were washed with 60 mM Tris-glycine buffer pH 8·7, resuspended in the same buffer and disrupted by intermittent sonic oscillations (Sonifier cell disrupter B 30, Branson Sonic Power Company, Dambury, Connecticut, USA) for 6 min at 4 °C, and the crude extract supernatants containing 40–60 mg of protein per ml were stored at −20 °C until use.

Electrophoresis

Horizontal slab electrophoresis in a composite polyacrylamide agarose gel (7 % and 1·4 % respectively) was performed according to the method described by Uriel

(1966) in a discontinuous Tris glycine buffer pH 8·7 (buffer for gel = Tris 0·075 M/glycine 0·06 M; buffer for batches = Tris 0·01 M/glycine 0·35 M) at a constant value of 7 V/cm, until the bromophenol blue marker had run 13 cm. To compare relative mobilities the bacterial extracts were inserted side by side into the same gel, and in some experiments the order of the extracts was changed.

Characterization of esterases

The esterases were stained within the gel (Lawrence, Melnick & Werner, 1960; Uriel, 1961). The substrates (0·2 mg/ml) used to detect and differentiate between the various esterase bands were α-naphthyl acetate, α-naphthyl butyrate, β-naphthyl acetate, β-naphthyl butyrate and indoxyl acetate in phosphate buffer 0·15 M, pH 7·4. Naphthanil diazo blue B was the dye coupler. Three types of esterases, major (M), slow (S) and fast (F), were identified by their electrophoretic mobility, their activity towards the substrates and their sensitivity or resistance to heat and to di-isopropyl fluorophosphate (Picard & Goullet, 1985).

Standardization of electrophoretic esterase typing

Strain ATCC 7946 (Popoff *et al.* 1981) was used to define a reference mobility scale for esterases. Electrophoretic mobility of a given variant was measured as the relative mobility value (M_R) which is the ratio between the distance measured from esterase M of *A. hydrophila* 7946 to the band of the variant and the distance measured from esterase M to esterase F of *A. hydrophila* 7946.

RESULTS

Clinical data

Two routes of contamination, intestinal and cutaneous, were distinguished for the 12 immunocompromised patients (Table 1).

Cutaneous route. In 2 patients, *A. hydrophila* was isolated from the thoracic drainage following pneumonectomy for bronchopulmonary carcinoma (cases 1 and 2), and in a third patient from a wound following foot surgery (case 3). These infections did not lead to septicaemia and recovery was complete. Epidemiological studies suggested that infection had resulted from the washing of the skin surrounding the surgical wounds during post-operative care.

Intestinal route (9 patients). Infections were associated with the ingestion of contaminated drinking water. In 2 cases, symptoms were confined to the intestinal tract and infection took the form of acute gastroenteritis which responded to antibiotic treatment (cases 4 and 5). In the remaining 7 cases, systemic disease occurred. One patient developed a urinary tract infection 24 h after drinking a single glass of hospital water (case 6); in the other 6, septicaemic illnesses (cases 7–12) were characterized by diffuse bilateral pulmonary changes, acute hypoxia and septic shock. Despite antibiotic therapy with aminoglycosides and cephalosporins four of the patients died, some in less than 24 h. Investigations pointed to contamination from drinking water in 7 cases, gastric lavage performed with hospital water during operation for a gastric haemorrhage in 1 case and a barium meal which had been prepared from a contrast agent diluted with tap water some hours before use in 1 case.

Table 1. *Principal clinical features of the 12 cases of* A. hydrophila *infection.*

No. of patient	Contamination route	Infection	Ward (No. of floor)	Underlying disease	Outcome	No. of zymotype
1	Cutaneous	Thoracic drainage	Thoracic surgery (IX)	Broncho-pulmonary carcinoma	Recovery	H31
2	Cutaneous	Thoracic drainage	Thoracic surgery (IX)	Broncho-pulmonary carcinoma	Recovery	H14
3	Cutaneous	Foot wound	Orthopaedic surgery (III)	Diabetes	Recovery	H19
4	Intestinal (barium meal)	Gastroenteritis	Haematology (XI)	Digestive carcinoma	Recovery	H14
5	Intestinal (gastric lavage)	Gastroenteritis	Hepatology (G*)	Cirrhosis	Recovery	H29
6	Intestinal	Urinary tract infection	Gastroenterology (VI)	Leukaemia	Recovery	H30
7	Intestinal	Septicaemia	Haematology (XI)	Leukaemia	Death	H14
8	Intestinal	Septicaemia	Hepatology (G*)	Leukaemia	Death	H5
9	Intestinal	Septicaemia	Haematology (XI)	Leukaemia	Death	H25
10	Intestinal	Septicaemia	Haematology (XI)	Refractory anaemia	Recovery	H25
11	Intestinal	Septicaemia	Hepatology (G*)	Cirrhosis	Recovery	H5
12	Intestinal	Septicaemia	Hepatology (G*)	Cirrhosis	Death	H17

* G ground floor.

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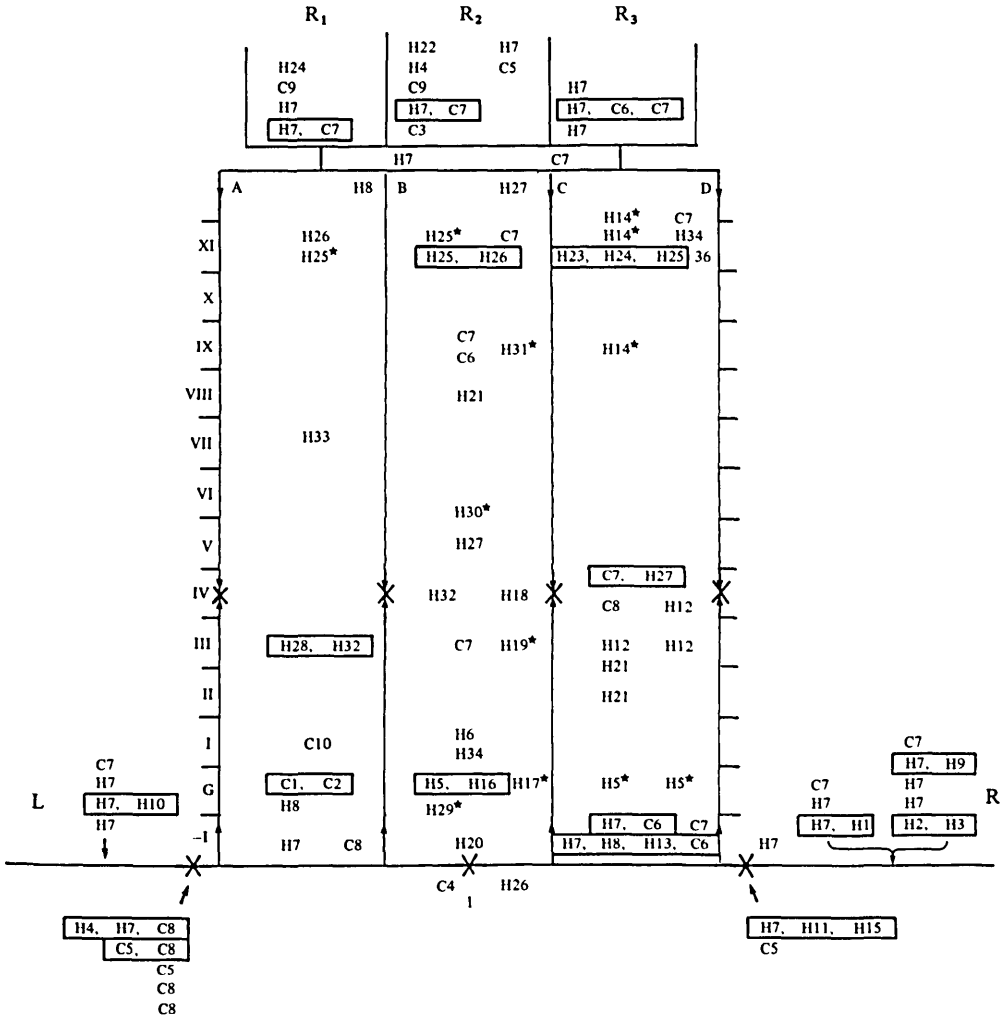

Fig. 1. See text for description. Hospital water supply showing the location of the zymotypes isolated from patients (*) and from water samples. The zymotypes in 'boxes' were simultaneously isolated from a single sample. The drinking water system is shown in solid lines. G; ground floor.

Organisms isolated from patients and from the water supply system

The 15 strains isolated from the 12 patients were identified as *A. hydrophila*.

The principal characteristics of the water supply system from which aeromonas were isolated are shown in Fig. 1. This system (installed in 1936 when the hospital was constructed) comprised two main supply pipes (L & R) from the town water supply. The L and R pipes are connected by an underground distribution pipe (1). This primary line directly supplies the four wings (A, B, C, D) on the first to fourth floors of the hospital. Water is pumped from distribution pipe (1) to three reservoirs (R_1, R_2, R_3), located directly under the roof of the 12th floor of the hospital. This arrangement constitutes the upper system supplying the fifth to eleventh floors.

Water samples were taken at all the outlet points on the system: L and R supply pipes, on each of the 12 floors of wings A, B, C, D and the three water reservoirs.

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Table 2. *Electrophoretic mobilities (M_R) of the major (M), fast (F) and slow (S) esterases characterizing the 34 zymotypes of* A. hydrophila (*a*) *and the 10 zymotypes of* A. caviae (*b*)

Table 2*a*

No. of zymotype	H1	H2	H3	H4	H5*	H6	H7	H8	H9	H10	H11	H12	H13	H14*	H15	H16	H17*
No. of strains	1	1	1	2	3	1	38	4	1	1	1	3	1	4	1	2	1
Esterase M, M_R value	55	50	44	39	22	33	28	28	28	28	28	19	22	17	33	14	11
Esterase S, M_R value	†	−55	−55	−55	−22	−33	−28	−28	−28	−28	—	—	11	—	−22	—	—
Esterase F‡, M_R value	167	167	167	158	167	167	155	155	155	164	178	114	175	139	136	164	164
	136	133	133	78	150	64	86	125	136	155	158		133	111	92	114	130
					133			86	86	105	133		89		53	94	108
										86	117						86
											105						
											61						
											3						

No. of zymotype	H18	H19*	H20	H21	H22	H23	H24	H25*§	H26	H27	H28	H29*	H30*	H31*	H32	H33	H34
No. of strains	1	1	1	3	2	2	3	4	3	3	1	1	1	2	2	1	3
Esterase M, M_R value	11	5	11	8	5	3	3	3	3	3	3	3	3	0	14	11	0
Esterase S, M_R value	—	—	—	—	—	—	—	−55	—	—	—	—	—	—	—	—	—
Esterase F, M_R value	—	136	150	139	136	153	147	133	133	136	105	128	136	—	136	136	161
		103	117	111	103	122	114	108	108	103		89	80		122	108	133
			103						97						108		
															72		
															50		

Table 2*b*

No. of zymotype	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
No. of strains	1	1	1	1	4	5	15	9	2	2
Esterase M, M_R value	64	58	55	39	39	36	33	33	33	33
			44							
Esterase S, M_R value	−22	−22	−22	—	—	—	—	—	−22	—
Esterase F, M_R value	89	86	128	122	100	130	130	130	122	—
			114	100	83	89	83	83	83	
				83	72	67	17	69	58	
				72	11	53	11	17		
				11			3	11		
								3		

* Zymotypes isolated from patients. † Undetected enzyme band.
‡ Esterase F exhibits several bands. § Zymotype isolated from water samples and from patients.

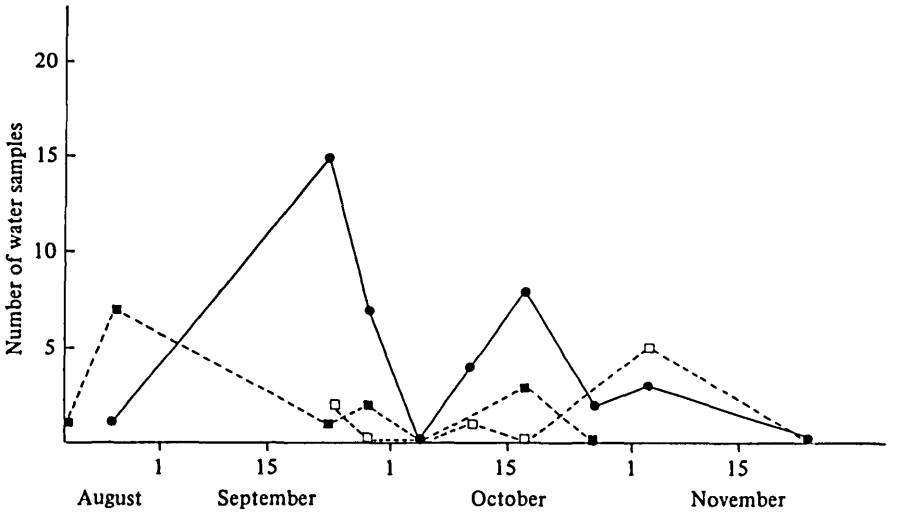

Fig. 2. Variations in the isolation frequency of three more frequent aeromonas zymotypes over the sampling period. ●—●, Zymotype H7; ■--■, zymotype C7; □--□, zymotype C8.

Sixty-eight per cent of the samples showed the presence of *A. hydrophila* or *A. caviae* at levels varying between 1 and 10 bacteria/ml.

Electrophoretic esterase typing

Of the 141 strains of aeromonas typed, 100 isolates (including the 15 from patients) belonging to the species *A. hydrophila* were distributed among 34 zymotypes (Table 2*a*), while 41 isolates belonging to *A. caviae* displayed 10 zymotypes (Table 2*b*). Some of these zymotypes were isolated more frequently than others: 23 zymotypes were found at least twice among the strains; zymotypes C7, C8 and H7 were the most frequently encountered (H7 in 38 isolates).

Zymotypes are reported on the water supply plan (Fig. 1). Some were isolated from several water samples at intervals of time from 4 to 90 days. The zymotypes H7, C7 and C8 persisted for 69, 58 and 40 days, respectively (Fig. 2); zymotype C7 appeared to be succeeded by C8. For the 18 samples in which 4 colonies of aeromonas were typed, 2 distinct zymotypes were simultaneously isolated in 13 cases, 3 in 4 cases and 4 on a single occasion. In 10 cases an identical zymotype was isolated at several locations in the water supply on the same day, e.g. zymotype C7 was isolated at 7 points on 26 August and zymotype H7 was isolated at 7 points on 23 September.

The 15 organisms isolated from 12 patients were distributed among 8 zymotypes (Table 1). Zymotype H5 was found in 2 septicaemic cases. Zymotype H14 was found in 1 septicaemic case, in 1 case of gastroenteritis and in another with an infected thoracic wound. A third zymotype (H25) isolated from patients with septicaemia was also isolated from 2 water samples taken in the ward where the patients were hospitalized. Distinct zymotypes isolated for each of the other patients were not found in the water samples. Thus, correlation between bacteria from the patient and those in the water was established for only 2 patients.

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DISCUSSION

In this study the distinct consequence of the cutaneous as opposed to the intestinal route of infection by *A. hydrophila* is clearly demonstrated.

Cutaneous infection, frequently post-operative, did not lead to serious illness and never led to septicaemia. Twelve infections of thoracic drains following surgery for bronchopulmonary cancer, including 2 of our cases and 10 others which were studied retrospectively from 1973, all recovered, often spontaneously. In contrast, the environmental water contamination of wounds described in the literature frequently had more serious outcomes. Infected wounds (Hanson *et al.* 1977), cellulitis (McCracken & Barkely, 1972), gangrene (Quinot *et al.* 1982) and septicaemia have all followed minor injuries such as simple cutaneous abrasions (Wolff, Wiseman & Kitchens, 1980).

Intestinal infection had the most serious consequences in the form of gastroenteritis, for which aeromonas has already proved to be frequently responsible (Janda *et al.* 1983; Burke *et al.* 1984*b*), and of septicaemia with considerable mortality from respiratory complications. In addition to the 4 fatal cases reported here 9 others diagnosed between 1973 and 1982 have been recorded (Picard, Arlet & Goullet, 1984). The immunocompromised condition of the patients in previously reported cases of septicaemia (Wolff, Wiseman & Kitchens, 1980; Cookson, Houang & Lee, 1984) was also observed in the present study. Three of our four patients who died had leukaemia. Most patients had disorders which favoured the penetration of the digestive tract, such as lesions of the intestinal mucosa resulting from chemotherapy (Wolff, Wiseman & Kitchens, 1980) or hepatic cirrhosis (Conn, 1964). The relative enteropathogenicity of some strains of *A. hydrophila* could also have contributed to the course of the infection (Sanyal, Singh & Sen, 1975).

To support the hypothesis that the hospital water supply was the origin of aeromonas infections, we compared isolates from water samples with those from patients. Only a limited number of typing methods for *Aeromonas* spp. have been published. Cookson, Houang & Lee (1984), Burke *et al.* (1984) and Janda, Reitano & Bottone (1984) used biotyping, described by Popoff & Veron (1976); Adams, Atkinson & Woods (1983) used haemagglutinin typing. In our laboratory we have developed a typing method based on esterase electrophoretic patterns. This is a discriminating and reproducible technique which has been effective in epidemiological and pathophysiological studies of *Escherichia coli* septicaemia (Goullet & Picard, 1984).

In order to standardize aeromonas esterase typing results obtained by different clinical laboratories, we propose an electrophoretic scale based on the readily available reference strain *A. hydrophila* ATCC 7946. Our results show the great epidemiological complexity of *A. hydrophila* infections in hospitals. While similar zymotypes have been found in numbers of samples from a variety of sources taken on the same or different days, up to four zymotypes were demonstrated in a single water sample. The zymotypes most frequently encountered in the water samples (H7, C7, C8) were not isolated from the patients, whereas, of the three zymotypes which were common to several patients, one only was detected in two water samples. We found that many distinct strains coexisted in the hospital water supply. Possibly some strains only rarely found in water may have been responsible

for the infections because of particular though unidentified pathogenic factors (Sanyal, Singh & Sen, 1975).

Short- and long-term preventative measures were implemented to avoid contamination of patients by *A. hydrophila*. Short-term measures consisted of efforts to protect the immunocompromised patients from contact with water from the hospital system. Contact by way of the gastrointestinal tract was avoided by giving patients at risk mineral water to drink and by using sterile water to make up medical preparations, such things as gastric lavage and radio-opaque solutions. Cutaneous contamination was prevented by washing the patient with boiled water to avoid contamination of the wound dressing by aeromonas. Longer-term measures involved modifications of the hospital water system by disconnecting the reservoirs on the 12th floor, which were a major source of contamination.

The number of septicaemia cases decreased in the period following the initiation of prophylactic measures. One case of septicaemia occurred each year from 1983 to 1985 and probably resulted from specific infringements of the prophylactic measures, e.g. one cirrhotic patient was given, in error, a gastric lavage using hospital water.

The reduction in the number of septicaemia cases following prophylactic measures constitutes additional retrospective evidence for the water-borne origin of these *Aeromonas hydrophila* infections.

The authors thank Madame C. Gaillard and Madame N. Hautier for technical assistance.

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