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Faecal contamination indicators, salmonella, vibrio and aeromonas in water used for the irrigation of agricultural products

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SUMMARY

The faecal contamination indicators (total coliforms, faecal coliforms, *Escherichia coli*, enterococci) and the genera *Salmonella*, *Vibrio*, *Aeromonas* were investigated in water samples used for irrigation. During 4 months, 52 samples were taken. The methods used were: multiple tube fermentation method for faecal contamination indicators and membrane filtration techniques for salmonella, aeromonas and vibrio. Two samples were positive for *Salmonella* spp., fourteen for *Aeromonas* spp. and no samples for *Vibrio* spp. No correlation was found between aeromonas and the indicators of faecal contamination. Regarding *Aeromonas* spp., 21·6% of the strains were adhesive and 12·6% cytotoxic: this confirms the possible role of aeromonas in human pathologies. These results are important to determine the quality of irrigation water in relation to human health. In fact, the spray or sprinkler irrigation produces bioaerosol, which can contaminate the crops that are likely to be eaten uncooked. In addition, the flood or furrow irrigation represents a risk to field workers.

INTRODUCTION

Biological contaminants in water are a concern because they represent a major health issue for humans and animals. They include, in fact, both pathogenic and opportunistic microorganisms responsible for various diseases in the host. Although there are valid, efficient epidemiological and microbiological methods, it has not been possible to associate a specific aetiological agent for a number of the infectious diseases reported in the last decade [1]. Furthermore many pathogens widely distributed in the aquatic environment, have never been directly correlated to the appearance of waterborne infections. However, reports of waterborne outbreaks associated with emerging pathogens such as *Cryptosporidium parvum*, *Escherichia coli* O157:H7, *Giardia* spp., and *Aeromonas* spp. are increasing [2–7].

Generally, a decline of pathologies due to traditional pathogens, and an increase of pathologies from emerging organisms have been recorded [8]. In recent years, special attention has been paid to bacteria such as aeromonas and vibrio, which are particularly adapted to the aquatic environment. During the last decade, strains of the genus *Aeromonas* have been associated with various human pathologies; they are known as causative agents of gastroenteritis and other diseases [7, 9]. The genus *Vibrio* includes species implicated in various pathologies: gastrointestinal and extra-intestinal diseases, infections of wounds, ear, and septicaemia [10–12].

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Water for irrigation could represent a source of microorganisms associated with a wide range of human diseases. In fact, through irrigation, the microorganisms present in the water can contaminate crops, then pass into the food chain and eventually infect humans.

In Italy, a Ministerial Decree [13] was issued by the Ministry of Agriculture and Forestry outlining policies concerning the quality of water for agricultural and zootechnical use. According to both Italian legislation and World Health Organisation (WHO) guidelines [13–15], the indicators of faecal contamination (total coliforms, faecal coliforms, *E. coli*, enterococci) and *Salmonella* spp. were investigated in water samples used for irrigation, taken from the Fucino Plain (Avezzano–Sulmona, Italy). In order to obtain a wider knowledge of the microbial characteristics of this water, we also investigated bacteria belonging to the genera *Vibrio* and *Aeromonas*, since some species represent a possible risk for human health. The adhesiveness and the cytotoxicity of the aeromonas isolates were also studied.

MATERIALS AND METHODS

Description of the sites and samplings

Giovenco is the major river in the Fucino Plain; it flows from the north-east and runs into the basin at Pescina. Giovenco and other rivers, typically flood streams, flow into a canal network running through the plain. The land reclamation canalization comprises approximately 260 km of canals and ditches, and it has been designed to preserve the delicate water balance of the area. This system represents the major irrigation resource, supported in low-water periods by drawing on groundwater; the test area is, in fact, rich in underground water, due to the presence of large carbonate aquifers.

According to the instructions of the Regional Agency for Agricultural Development Service (ARSSA) Technical Office, and as a result of satellite data, thirteen representative sites were set up (Fig. 1). Eight sites were in the various collection canals because these canals receive the water of the entire network (site nos. 1, 2, 3, 4, 5, 7, 8, 12); two were in the Giovenco river: one site was upstream of the city of Pescina (site no. 11), and the other downstream (site no. 6); two were in wells (site nos. 9 and 13); one was in a spring (site no. 10). The wells and spring were chosen because they are also a source of drinking water.

The collection of water samples was carried out monthly in the period of greatest demand, from July to October 2001.

The samples were collected with a sampler 'MARE LACUS' (International PBI, Milan, Italy). The water samples were transported in refrigerated bottles at 4 °C to the laboratory.

Bacteriological analysis

The indicators of faecal pollution (total coliforms, faecal coliforms, *E. coli* and enterococci) were determined by the multiple tube fermentation technique [13] using a five-tube series inoculated with undiluted and diluted (tenfold) water samples. Lactose broth (Oxoid, Garbagnate Milanese, Milan, Italy) and azide dextrose broth (Oxoid) were used in the presumptive phase for total, faecal coliforms, *E. coli* and enterococci respectively. Confirmation of presumptive reaction was done by inoculating aliquots (0·1 ml) from positive tubes in Brilliant Green lactose broth (Oxoid) for total coliforms, in EC broth (Oxoid) for faecal coliforms, in tryptone water broth (Oxoid) for *E. coli*, and in ethyl Violet azide dextrose broth (Oxoid) for enterococci.

For *Salmonella* spp. the membrane filtration method was employed [13]; the membranes were placed in selenite cystine broth (Oxoid) for 18 h at 37 °C and then were streaked onto Hektoen enteric agar (Oxoid) and salmonella–shigella agar (Oxoid). Suspected colonies were identified by the usual biochemical and serological tests [13].

Detection of *Aeromonas* spp. and *Vibrio* spp. was performed by the membrane filtration technique, as previously described [16, 17]. Water volumes of 0·1, 1 and 10 ml were filtered through 0·45 μm-pore-size filters (Millipore, Rome, Italy). The membranes were placed on m-aeromonas selective agar base (Havelaar) (Biolife, Milan, Italy) and incubated for 18–24 h at 28–30 °C for aeromonas and on thiosulphate–citrate–bile–sucrose agar (Oxoid) with 2% NaCl and incubated for 12–18 h at 30 °C for vibrio. The number of viable bacteria was estimated in c.f.u./100 ml of water. The strains found to be oxidase-positive, Gram-negative, resistant to vibriostatic agent O/129 (10 and 150 μg), motile, glucose and trehalose fermenting, nitrate reducing and did not grow at 6% NaCl, were identified as belonging to *Aeromonas* spp. with the API 20E system (bioMérieux, Rome, Italy). The strains were then typed to the phenospecies level using the following test: D-rhamnose,

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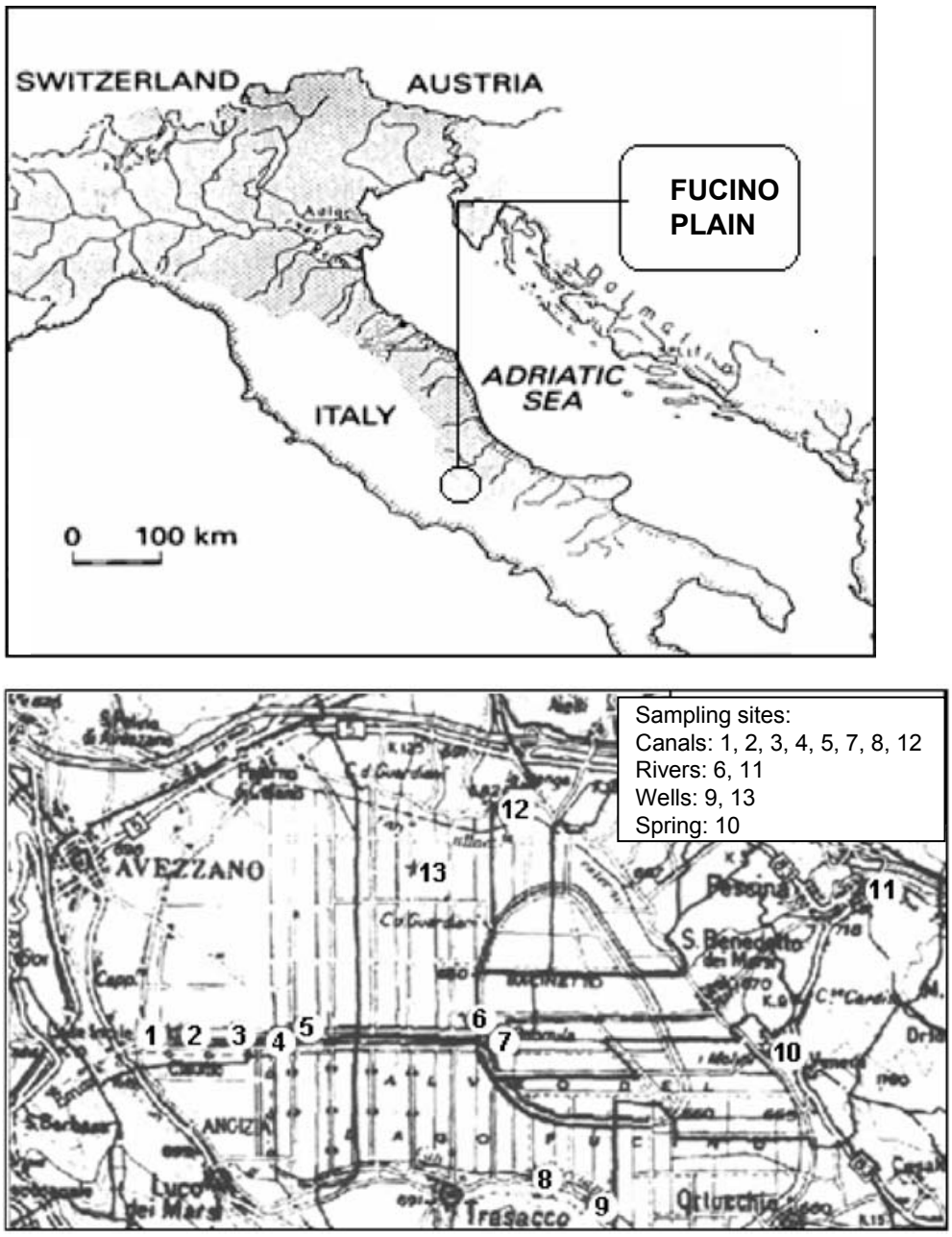

Fig. 1. Sampling sites on the Fucino Plain.

D-sorbitol, citrate and D-L-lactate for *A. hydrophila* (HGs 1–3); D-L-lactate, citrate, β haemolysis and pyrazinamidase activity for *A. caviae* (HGs 4–6); VP, arginine dehydrolase, D-cellobiose, ornithine decarboxylase for *A. sobria*/*veronii* (HGs 7, 8 and 10).

Regarding vibrio, the isolates were identified by the following tests: colony shape and pigmentation on TCBS, Gram staining, cytochrome oxidase and catalase tests, motility, sensitivity to vibriostatic agent O/129 (10 and 150 μg), and the API 20E system.

Adhesiveness and cytotoxicity assays of *Aeromonas* spp.

The strains of *Aeromonas* spp. were tested for adhesiveness and cytotoxic activity.

Bacterial preparations

The *Aeromonas* spp. strains to be tested were inoculated in tryptone soya broth (TSB; Oxoid) supplemented with 0·6% (wt/vol) yeast extract and

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Table 1. *Frequency of indices of faecal contamination,* Salmonella *spp. and* Aeromonas *spp. from irrigation waters in the Fucino Plain*

Site no.	Month	TC MPN/100 ml	FC MPN/100 ml	*E. coli* MPN/100 ml	FS MPN/100 ml	*Salmonella* spp.	No. colonies	*Aeromonas* spp. phenospecies
1	July	$1{\cdot}6\times10^{3}$	$9{\cdot}2\times10^{2}$	$9{\cdot}0\times10^{0}$	$7{\cdot}9\times10^{1}$	Negative	$4{\cdot}8\times10^{3}$	*A. sobria*
	Aug.	$5{\cdot}5\times10^{2}$	$3{\cdot}5\times10^{2}$	$2{\cdot}0\times10^{0}$	$2{\cdot}1\times10^{1}$	Negative		
	Sep.	$1{\cdot}6\times10^{5}$	$5{\cdot}4\times10^{4}$	$9{\cdot}2\times10^{3}$	$1{\cdot}6\times10^{3}$	Negative		
	Oct.	$\geqslant2{\cdot}4\times10^{5}$	$\geqslant2{\cdot}4\times10^{5}$	$4{\cdot}0\times10^{1}$	$\geqslant2{\cdot}4\times10^{4}$	Negative		
2	July	$\geqslant2{\cdot}4\times10^{4}$	$1{\cdot}6\times10^{3}$	$1{\cdot}7\times10^{1}$	$2{\cdot}7\times10^{1}$	Negative	$3{\cdot}4\times10^{3}$	*A. sobria*/*A. caviae*
	Aug.	$3{\cdot}5\times10^{2}$	$2{\cdot}2\times10^{2}$	0	$1{\cdot}3\times10^{1}$	Negative		
	Sep.	$\geqslant2{\cdot}4\times10^{5}$	$\geqslant2{\cdot}4\times10^{5}$	$2{\cdot}6\times10^{2}$	$9{\cdot}2\times10^{2}$	Negative		
	Oct.	$\geqslant2{\cdot}4\times10^{5}$	$\geqslant2{\cdot}4\times10^{5}$	$9{\cdot}0\times10^{1}$	$1{\cdot}4\times10^{3}$	Negative		
3	July	$\geqslant2{\cdot}4\times10^{4}$	$7{\cdot}9\times10^{1}$	$9{\cdot}0\times10^{0}$	$3{\cdot}4\times10^{1}$	Negative		
	Aug.	$1{\cdot}6\times10^{3}$	$2{\cdot}4\times10^{2}$	0	$4{\cdot}0\times10^{0}$	Negative		
	Sep.	$\geqslant2{\cdot}4\times10^{5}$	$7{\cdot}9\times10^{2}$	$2{\cdot}0\times10^{1}$	$1{\cdot}7\times10^{1}$	Negative		
	Oct.	$5{\cdot}4\times10^{4}$	$3{\cdot}5\times10^{4}$	$9{\cdot}0\times10^{2}$	$1{\cdot}6\times10^{3}$	Negative	$2{\cdot}2\times10^{4}$	*A. hydrophila*/ *A. caviae*
4	July	$7{\cdot}9\times10^{1}$	$7{\cdot}9\times10^{1}$	$8{\cdot}0\times10^{0}$	$1{\cdot}3\times10^{2}$	Negative		
	Aug.	$2{\cdot}8\times10^{2}$	$3{\cdot}3\times10^{1}$	$2{\cdot}0\times10^{0}$	$2{\cdot}4\times10^{2}$	Negative		
	Sep.	$3{\cdot}3\times10^{2}$	0	0	0	Negative		
	Oct.	$1{\cdot}6\times10^{3}$	$1{\cdot}6\times10^{3}$	$2{\cdot}8\times10^{1}$	$1{\cdot}7\times10^{2}$	Negative	$3{\cdot}0\times10^{2}$	*A. caviae*
5	July	$5{\cdot}4\times10^{2}$	$5{\cdot}4\times10^{2}$	$4{\cdot}0\times10^{0}$	$2{\cdot}3\times10^{1}$	Negative		
	Aug.	$2{\cdot}2\times10^{2}$	$7{\cdot}0\times10^{1}$	0	0	Negative	$>3{\cdot}0\times10^{4}$	*A. sobria*
	Sep.	$\geqslant2{\cdot}4\times10^{5}$	$\geqslant2{\cdot}4\times10^{5}$	$3{\cdot}3\times10^{2}$	$1{\cdot}6\times10^{3}$	Negative		
	Oct.	$\geqslant2{\cdot}4\times10^{5}$	$\geqslant2{\cdot}4\times10^{5}$	$1{\cdot}4\times10^{2}$	$\geqslant2{\cdot}4\times10^{4}$	Negative		
6	July	$7{\cdot}9\times10^{1}$	$3{\cdot}4\times10^{1}$	$1{\cdot}7\times10^{1}$	$1{\cdot}7\times10^{1}$	Negative	$1{\cdot}3\times10^{4}$	*A. hydrophila*/ *A. caviae*/*A. sobria*
	Aug.	$\geqslant2{\cdot}4\times10^{4}$	$9{\cdot}2\times10^{2}$	$2{\cdot}0\times10^{0}$	$9{\cdot}0\times10^{0}$	Negative		
	Sep.	$6{\cdot}3\times10^{2}$	$7{\cdot}0\times10^{1}$	$4{\cdot}0\times10^{1}$	$2{\cdot}0\times10^{0}$	Negative		
	Oct.	$\geqslant2{\cdot}4\times10^{4}$	$\geqslant2{\cdot}4\times10^{3}$	$7{\cdot}0\times10^{0}$	$1{\cdot}6\times10^{3}$	Negative		
7	July	$3{\cdot}5\times10^{2}$	$3{\cdot}5\times10^{2}$	$1{\cdot}7\times10^{1}$	$4{\cdot}6\times10^{1}$	Negative		
	Aug.	$9{\cdot}2\times10^{2}$	$1{\cdot}7\times10^{2}$	0	$7{\cdot}0\times10^{1}$	Negative	$1{\cdot}1\times10^{4}$	*A. hydrophila*
	Sep.	$6{\cdot}3\times10^{2}$	$6{\cdot}3\times10^{2}$	$7{\cdot}0\times10^{1}$	$3{\cdot}3\times10^{2}$	Negative		
	Oct.	$2{\cdot}6\times10^{3}$	$1{\cdot}7\times10^{2}$	0	$1{\cdot}7\times10^{1}$	Negative		
8	July	$2{\cdot}2\times10^{2}$	$2{\cdot}0\times10^{2}$	$2{\cdot}0\times10^{0}$	$7{\cdot}0\times10^{1}$	Negative	$1{\cdot}6\times10^{4}$	*A. caviae*
	Aug.	$1{\cdot}6\times10^{3}$	$1{\cdot}7\times10^{2}$	0	$2{\cdot}7\times10^{1}$	Negative		
	Sep.	$\geqslant2{\cdot}4\times10^{5}$	$\geqslant2{\cdot}4\times10^{5}$	$9{\cdot}0\times10^{1}$	$\geqslant2{\cdot}4\times10^{3}$	Negative		
	Oct.	$\geqslant2{\cdot}4\times10^{5}$	$\geqslant2{\cdot}4\times10^{5}$	$7{\cdot}0\times10^{1}$	$\geqslant2{\cdot}4\times10^{4}$	Negative	$>3{\cdot}0\times10^{4}$	*A. caviae*
9	July	0	0	0	0	Negative		
	Aug.	0	0	0	0	Negative	$3{\cdot}0\times10^{4}$	*A. sobria*
	Sep.	0	0	0	0	Negative		
	Oct.	0	0	0	0	Negative		
10	July	$\geqslant2{\cdot}4\times10^{4}$	$\geqslant2{\cdot}4\times10^{4}$	$1{\cdot}7\times10^{2}$	$1{\cdot}4\times10^{2}$	Negative		
	Aug.	$1{\cdot}6\times10^{3}$	$3{\cdot}4\times10^{1}$	0	$3{\cdot}0\times10^{1}$	Negative	$1{\cdot}9\times10^{3}$	*A. hydrophila*/*A. caviae*
	Sep.	$2{\cdot}8\times10^{3}$	$2{\cdot}8\times10^{3}$	$4{\cdot}0\times10^{1}$	$2{\cdot}3\times10^{2}$	*Salmonella*	$3{\cdot}4\times10^{3}$	*A. hydrophila*
	Oct.	$5{\cdot}4\times10^{4}$	$5{\cdot}4\times10^{4}$	$1{\cdot}4\times10^{3}$	$2{\cdot}8\times10^{2}$	Negative	$8{\cdot}3\times10^{3}$	*A. hydrophila*/*A. caviae*
11	July	$\geqslant2{\cdot}4\times10^{4}$	$\geqslant2{\cdot}4\times10^{4}$	$7{\cdot}0\times10^{0}$	$2{\cdot}2\times10^{2}$	Negative		
	Aug.	$1{\cdot}6\times10^{3}$	$3{\cdot}5\times10^{2}$	$2{\cdot}0\times10^{0}$	$5{\cdot}4\times10^{2}$	Negative		
	Sep.	$\geqslant2{\cdot}4\times10^{5}$	$\geqslant2{\cdot}4\times10^{5}$	$7{\cdot}0\times10^{1}$	$3{\cdot}5\times10^{4}$	Negative		
	Oct.	$2{\cdot}8\times10^{4}$	$4{\cdot}6\times10^{2}$	$2{\cdot}0\times10^{1}$	$2{\cdot}2\times10^{3}$	Negative		
12	July	$3{\cdot}5\times10^{2}$	$3{\cdot}5\times10^{2}$	$2{\cdot}0\times10^{0}$	$\geqslant2{\cdot}4\times10^{4}$	Negative		
	Aug.	$\geqslant2{\cdot}4\times10^{4}$	$\geqslant2{\cdot}4\times10^{4}$	$1{\cdot}4\times10^{3}$	$\geqslant2{\cdot}4\times10^{3}$	Negative		
	Sep.	$\geqslant2{\cdot}4\times10^{5}$	$9{\cdot}2\times10^{3}$	$1{\cdot}1\times10^{2}$	$5{\cdot}4\times10^{4}$	Negative		
	Oct.	$\geqslant2{\cdot}4\times10^{5}$	$\geqslant2{\cdot}4\times10^{5}$	$1{\cdot}1\times10^{2}$	$\geqslant2{\cdot}4\times10^{5}$	*S. arizona*		
13	July	0	0	0	0	Negative		
	Aug.	0	0	0	0	Negative		
	Sep.	0	0	0	0	Negative		
	Oct.	0	0	0	0	Negative	$1{\cdot}4\times10^{4}$	*A. caviae*

TC, Total coliforms; FC, faecal coliforms; FS, faecal streptococci.

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3% (wt/vol) casamino acids, and incubated at 28 °C for 18–24 h. One loop of the culture was streaked on tryptone soya agar (TSA; Oxoid) and incubated at 28 °C overnight.

Adhesion assay

The bacteria were inoculated into 5 ml of brain heart infusion broth (BHI; Oxoid), and incubated at 37 °C overnight. Bacteria were harvested by centrifugation (3000 rpm for 30 min) and the pellet was resuspended in 5 ml of Eagle's Minimum Essential Medium (MEM) (Sigma, Milan, Italy) to obtain an absorbance (600 nm) of 0·07 (5×10^6 c.f.u./ml).

As the efficiency of the toxin production depends on the incubation temperature [7, 18], for the cytotoxicity test the bacteria were grown in BHI at 28 and 37 °C for 18 h with agitation. The bacterial suspension was centrifuged at 10000 rpm for 30 min at 4 °C. The supernatant was filtered through cellulose nitrate filter (0·45 μm pore size) and stored at −20 °C until required.

The HEp-2 and Vero cell lines were used for adhesion and cytotoxin production respectively. The cells were cultured in MEM with 10% foetal bovine serum (Sigma). The adhesion property was tested as previously described [17]. The adhesive capacity was expressed as a percentage of cells with more than 10 bacteria per cell.

Cytotoxicity assay

Serial twofold dilutions of filtrate starting from 1:2 to 1:256 were incubated for 24 h at 37 °C and readings were taken for 7 days [16, 19]. A sample was considered cytotoxic when at least 50% of the cultured cells rounded up. The cytotoxic titre was defined as the reciprocal of the highest dilution of the filtrate that produced a cytopathic effect.

For adhesiveness and cytotoxicity assays the ATCC 7966 strain was used as positive control.

RESULTS

The indicators of faecal contamination and S*almonella* spp. (Table 1) were absent in site nos. 9 and 13. With regard to the other sites, in July, the lowest values of total coliforms were found in site nos. 4 and 6 ($7{\cdot}9 \times 10^1$/100 ml), while the highest indices were found in site nos. 2, 3, 10, 11 ($\geqslant 2{\cdot}4 \times 10^4$/100 ml). Faecal coliforms showed lower values compared to the total coliforms in site nos. 2 and 3 ($1{\cdot}6 \times 10^3$/100 ml and $7{\cdot}9 \times 10^1$/100 ml respectively); on the other hand, in site nos. 4, 5, 6, 7, 8, 10, 11, 12 we found similar values.

The Most Probable Number (MPN) indices found for streptococci were variable: in site nos. 2, 3, 5, 6 we found low values, ranging from $1{\cdot}7 \times 10^1$ to $3{\cdot}4 \times 10^1$/100 ml; in site nos. 1, 7, 8, 10, 11 indices were slightly higher, ranging from $4{\cdot}4 \times 10^1$ to $2{\cdot}0 \times 10^2$/100 ml; in site no. 12 the value was $\geqslant 2{\cdot}4 \times 10^4$/100 ml.

In August total coliforms increased in site nos. 4, 6, 8, ranging from $2{\cdot}8 \times 10^2$ to $2{\cdot}4 \times 10^4$/100 ml and they decreased in site nos. 1, 2, 3, 10, 11. The values of faecal coliforms were higher than in July in site nos. 3, 6, 12 (from $2{\cdot}4 \times 10^2$ to $\geqslant 2{\cdot}4 \times 10^4$/100 ml). The streptococci indices, compared to the values of July, decreased generally, ranging from 0 (site no. 5) to $\geqslant 2{\cdot}4 \times 10^3$/100 ml (site no. 12).

In September and October the microbiological values increased in most of the sampling sites.

Regarding *E. coli*, in the sites where it was detected, the lowest values were found in August ($2{\cdot}0 \times 10^0$/100 ml) and the highest in September and October, ranging from $2{\cdot}0 \times 10^1$ to $9{\cdot}2 \times 10^3$/100 ml and from $7{\cdot}0 \times 10^0$ to $1{\cdot}4 \times 10^3$/100 ml respectively.

With regard to salmonella, two strains were isolated: *Salmonella* spp. in September in site no. 10, and *S. arizona* in October in site no. 12 (Table 1).

No *Vibrio* spp. were found, but bacteria belonging to the genus *Aeromonas* were isolated (Table 1). These microorganisms were found in eleven sampling sites (84·6%); the highest frequency was found in site no. 10 with positive samples in August, September and October.

The number of aeromonads ranged between 10^2 and 10^4 c.f.u./ml in October (site no. 4) and August and October (site nos. 5 and 8 respectively).

All together, 111 strains were isolated; *A. caviae* was the predominant phenospecies (48·64%), followed by *A. sobria* (35·13%) and *A. hydrophila* (16·22%) (Fig. 2).

In the *A. caviae* phenospecies, *A. caviae* biotype *caviae* (HG 4) and *A. caviae* biotype *media* (HG 5) were identified, with a frequency of 22·22 and 11·11% respectively; in the *A. sobria* phenospecies, *A. veronii* biotype *sobria* (HG 8) was identified in 53·84% of the strains; in the *A. hydrophila* phenospecies, *A. hydrophila* biotype *hydrophila* (HG 1) was identified in 50·00% of the strains.

With regard to virulence factors, 21·62% (24/111) of the strains were found to adhere to the cells: six

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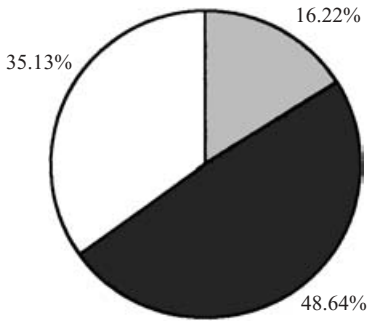

Fig. 2. Relative abundance (%) of *Aeromonas* spp. phenospecies isolates from irrigation waters in the Fucino Plain. □, *A. sobria*; ▨, *A. hydrophila*; ■, *A. caviae*.

(33·33%) belonged to the *A. hydrophila* phenospecies, isolated in site nos. 7 and 10 in August, six (11·11%) to the *A. caviae* phenospecies found in site no. 13 in October, and twelve (30·77%) to the *A. sobria* phenospecies from site no. 5 in August.

Four of six *A. hydrophila* strains and twelve *A. sobria* strains were also found to be cytotoxic. The *A. hydrophila* strains showed a titre of 64 and the *A. sobria* strains a titre of 128; there were no differences in the production of toxin between the incubation temperatures considered, 28 and 37 °C (Table 2, Fig. 3).

DISCUSSION

It is known that surface water can transmit microbial pathogens directly and indirectly. Among the indirect ways, irrigation of the crops with the possible passage of the agents into the food chain can be included.

In our investigation we found faecal contamination indicators in almost all of the sites. Of all the sites tested the wells never showed any faecal contamination indicators. At the remaining sites, the concentration of faecal contamination indicators fluctuated throughout the test period.

Considering the data for the 4 months overall, we found that several sites had exceeded the Italian legislative limits ($2{\cdot}0 \times 10^4$ MPN/100 ml for total coliforms; $1{\cdot}2 \times 10^4$ MPN/100 ml for faecal coliforms; $2{\cdot}0 \times 10^3$ MPN/100 ml for enterococci). In particular, site nos. 10, 11 and 12 were the most polluted. An interesting result is the constant contamination of the spring (site no. 10).

The highest microbial charges were found in September and October; these results are probably due to the leaching action of the rain that had fallen before the sampling.

Table 2. *Characteristics of virulence of* Aeromonas *spp. isolated from irrigation waters in the Fucino Plain*

Phenospecies	Sampling station	Total isolates (*n*)	Adherent strains (*n*)	Cytotoxin strains (*n*)
A. hydrophila	3	3	0	0
	6	3	0	0
	7	3	3	1
	10	9	3	3
Total		18	6	4
A. caviae	2	6	0	0
	3	3	0	0
	4	3	0	0
	6	6	0	0
	8	18	0	0
	10	9	0	0
	13	9	6	0
Total		54	6	0
A. sobria	1	6	0	0
	2	3	0	0
	5	12	12	10
	6	9	0	0
	9	9	0	0
Total		39	12	10
Total		111	24	14

At present, in Italy the current legislation regarding drinking water [20] and sewage [21] includes tests for *E. coli*. In our survey, *E. coli* was isolated in several sites, in some cases with a quite high MPN index, therefore underlining the importance of this parameter as a specific indicator of faecal pollution. This microorganism is therefore important in a habitat where it is necessary to discriminate between environmental and potentially pathogenic flora belonging to humans or animals.

Bacteria of the genus *Aeromonas* were also identified in our investigation; the microorganism was isolated in almost all the sampling sites but it was not present in those sites every month. According to other investigations [22–24], no correlation was found between the indices of faecal contamination and aeromonas. On this subject, an interesting result is the isolation of *Aeromonas* spp. without positive indices of faecal contamination in the wells (site nos. 9 and 13). This event can be explained by the fact that these bacteria are autochthonous in the aquatic environment and do not have a diffusion linked to specific faecal contamination.

The species identified belonged to the phenospecies *A. hydrophila*, *A. caviae* and *A. sobria*. *A. caviae* phenospecies was found more frequently (48·6%);

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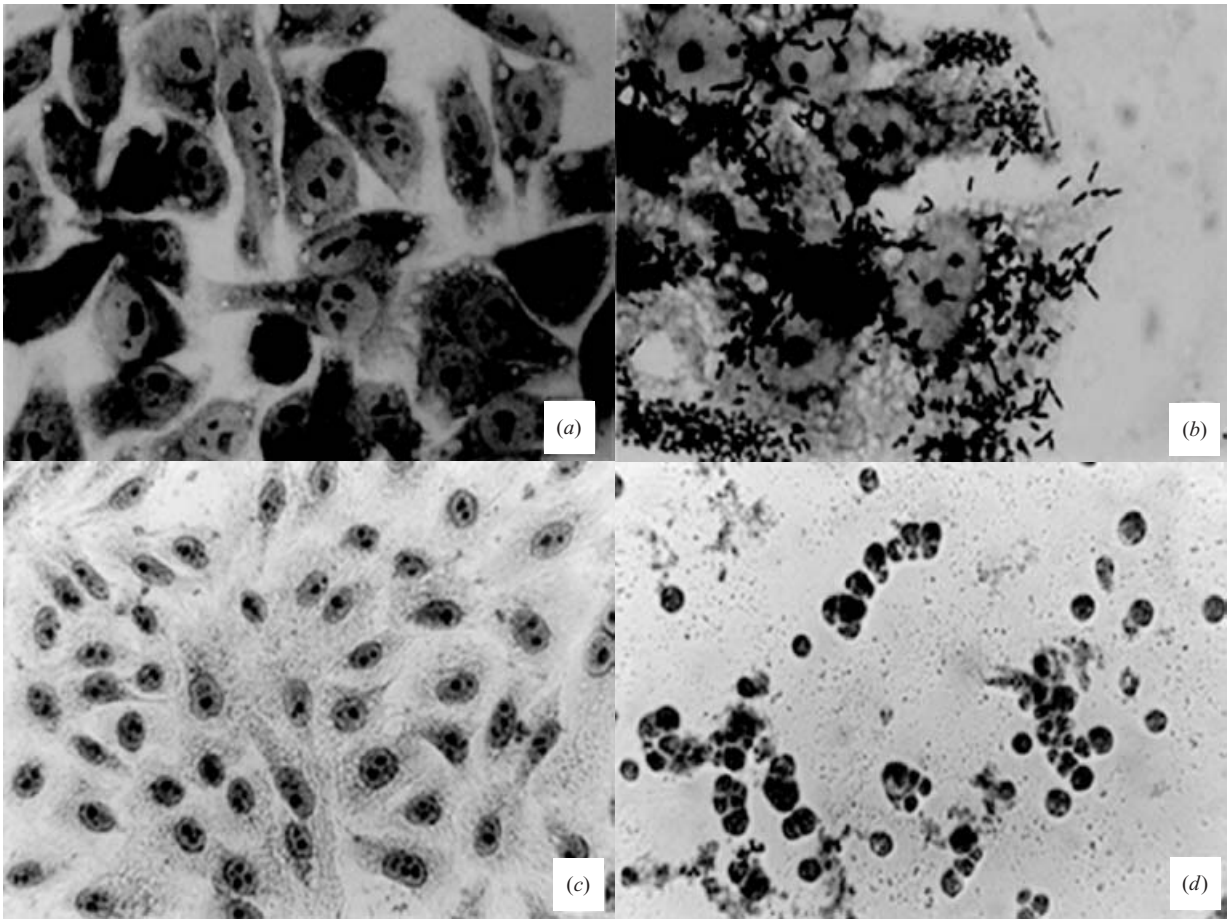

Fig. 3. Adhesiveness and cytotoxic activity of *Aeromonas* spp. isolated from irrigation waters in the Fucino Plain. Adhesion of isolates to Hep-2 cells (*b*) compared to control (*a*); cytotoxic effect of bacteria on Vero cells (*d*) compared to control (*c*).

this species, in general, predominates in sewage-contaminated water, seawater and brackish water [7, 16, 23, 25, 26]. *A. caviae* is also the species most commonly found in vegetables (~60%) in Japan [27].

A. hydrophila (HG 1) was found in spring water (site no. 10), this is in agreement with other authors [7], who refer to its prevalence in this type of water.

With regard to the virulence factors considered (adhesiveness and cytotoxicity), these were more widespread in *A. hydrophila* and *A. sobria* compared to *A. caviae*, according to other authors [7, 28, 29], who have reported that environmental strains of *A. caviae* are less adhesive. Adhesiveness was, in fact, found in all three phenospecies, with a lower frequency in *A. caviae*. Cytotoxic activity was shown in *A. hydrophila* and *A. sobria*, and the latter, as is also reported in literature [7], among the species producing the highest titres of toxins (titre 128). All cytotoxic strains were adhesive, and therefore the simultaneous presence of these virulence factors confirms the role of such microorganisms as potential pathogens. However the existence of strains without virulence factors shows that these properties are strain-specific and not species-specific. The research of *Aeromonas* spp., supported by the determination of virulence factors, is therefore important to better outline the role of environmental aeromonads as possible human pathogens.

In conclusion, irrigation water represents a possible risk for human health. In fact, this water can cause contamination of the soil, where vegetables are grown. Furthermore, the spray or sprinkler irrigation produces bioaerosol, which contaminates the crops that are eaten uncooked. In addition, the flood or furrow irrigation represents a risk to field workers. Therefore periodic bacteriological monitoring of the aquatic systems is recommended. In addition to the faecal indicator organisms, it is important to detect environmental microorganisms. These bacteria have an epidemiological behaviour different from the traditional enteric bacteria, and they could be involved in human pathology.

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REFERENCES

1. Morris RD, Levin R. Estimating the incidence of waterborne infectious disease related to drinking water in the United States. In: Reichard EG, Zapponi GA, eds. Assessing and managing health risks from drinking water contamination: approaches and applications. Wallingford, UK: IAHS Press, 1995: 75–88.
2. Gold WL, Salit IE. *Aeromonas hydrophila* infections of skin and soft tissue: report of 11 cases and review. Clin Infect Dis 1993; **16**: 69–74.
3. *Escherichia coli* O157:H7 outbreak at a summer camp – Virginia, 1994. MMWR 1995; **44**: 419–421.
4. Lake-associated outbreaks of *Escherichia coli* O157:H7 – Illinois, 1995. MMWR 1996; **45**: 457–459.
5. Anderson Y, Tan I, Mohamad-Kamel A. Occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in raw treated water treatment plants in Selangor, Malaysia. Water Res 1997; **31**: 3132–3135.
6. Kelly P, Baboo KS, Nbudani P, et al. *Cryptosporidiosis* in Adults in Lusaka, Zambia, and its relationship to oocyst contamination of drinking water. J Infect Dis 1997; **176**: 1120–1123.
7. Kirov SM. *Aeromonas* and *Plesiomonas* species. In: Doyle M, Beuchat L, Montiville T, eds. Food microbiology. Fundamentals and frontiers. Washington DC: ASM Press, 1997: 265–287.
8. Schwartz DA, Bryan RT. Infectious disease pathology and emerging infections. Arch Pathol Lab Med 1996; **120**: 117–124.
9. Janda JM, Abbott SL. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Clin Infect Dis 1998; **27**: 332–344.
10. Mouzin E, Mascola L, Tormey MP, et al. Prevention of *Vibrio vulnificus* infections. Assessment of regulatory educational strategies. JAMA 1997; **278**: 576–578.
11. Jackson JK, Murphree RL, Tamplin ML. Evidence that mortality from *Vibrio vulnificus* infections results from single strains among heterogeneus populations in shellfish. J Clin Microbiol 1997; **35**: 2098–2101.
12. Stroms MS, Paranjpye RN. Epidemiology and pathogenesis of *Vibrio vulnificus*. Microbes Infect 2000; **2**: 177–188.
13. DM 23 marzo 2000 approvazione dei 'Metodi ufficiali di analisi delle acque per uso agricolo e zootecnico'. Suppl 'G.U.' no. 87, 13 aprile 2000.
14. Delibera del Comitato Interministeriale per la Tutela delle Acque dall'Inquinamento (CITAI) del 4/2/77 di cui all'art. 2, lettera b), d) ed e), della legge 10 maggio 1976, no. 319, recante norme per la tutela delle acque dall'inquinamento. Suppl 'G.U.' no. 48, 21 febbraio 1977.
15. Blumenthal UJ, Mara DD, Peasey A, et al. Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. Bull WHO 2000; **78**: 1104–1116.
16. Fiorentini C, Barbieri E, Falzano L, et al. Occurrence, diversity and pathogenicity of mesophilic *Aeromonas* in estuarine waters of the Italian coast of the Adriatic sea. J Appl Microbiol 1998; **85**: 501–511.
17. Barbieri E, Falzano L, Fiorentini C, et al. Occurrence, diversity and pathogenicity of halophilic *Vibrio* spp. and non-O1 *Vibrio cholerae* from estuarine waters along the Italian Adriatic coast. Appl Environ Microbiol 1999; **65**: 2748–2753.
18. Mateos D, Anguita J, Naharro G, et al. Influence of growth temperature on the production of extracellular virulence factors and pathogenicity of environmental and human strains of *Aeromonas hydrophila*. J Appl Bacteriol 1993; **74**: 111–118.
19. Handfield M, Simard P, Couillard M, et al. *Aeromonas hydrophila* isolated from food and drinking water: hemagglutination, hemolysis, and cytotoxicity for a human intestine cell line (HT-29). Appl Environ Microbiol 1996; **62**: 3459–3461.
20. D.L.vo 2/2/01 no. 31 'Attuazione della direttiva 98/83/CE relativa alla qualità delle acque destinate al consumo umano'. Suppl no. 41 'G.U.' no. 52, 3 marzo 2001.
21. D.L.vo 11/5/99 no. 152, recante: 'Disposizioni sulla tutela delle acque dall'inquinamento e recepimento della direttiva 91/271/CEE concernente il trattamento delle acque reflue urbane e della direttiva 91/676/CEE relativa alla protezione delle acque dall'inquinamento provocato dai nitrati provenienti da fonti agricole'. Suppl no. 101/L 'G.U.' no. 124, 29 maggio 1999.
22. Araujo RM, Arribas RM, Pares R. Distribution of *Aeromonas* species in water with different levels of pollution. J Appl Bacteriol 1991; **71**: 182–186.
23. Pianetti A, Baffone W, Bruscolini F, et al. Presence of several bacteria in the Metauro and Foglia rivers (Pesaro-Urbino, Italy). Water Res 1998; **32**: 1515–1521.
24. Dumontet S, Krovacek K, Svenson S, et al. Prevalence and diversity of *Aeromonas* spp and *Vibrio* spp in coastal waters of southern Italy. Comp Immunol Microbiol Infect Dis 2000; **23**: 53–72.
25. Farmer JJ III, Arduino MJ, Hickman-Brenner FW. The genera *Aeromonas* and *Plesiomonas* In: Balow A, Trüper HG, Dworkin M, Harder W, Schleifer KH, eds. The prokaryotes, 2nd edn. New York: Springer-Verlag, 1992: 3012–3028.
26. Ashbolt NJ, Ball A, Dorsch M, et al. The identification and human health significance of environmental aeromonads. Water Sci Technol 1995; **31**: 263–269.
27. Nishikawa Y, Kishi T. Isolation and characterisation of motile *Aeromonas* from human, food and environmental specimens. Epidemiol Infect 1988; **101**: 213–223.
28. Grey PA, Kirov SM. Adherence to Hep-2 cells and enteropathogenic potential of *Aeromonas* spp. Epidemiol Infect 1993; **110**: 279–287.
29. Kirov SM. Adhesion and piliation of *Aeromonas* spp. Med Microbiol Lett 1993; **2**: 274–280.