Epidemiol. Infect. (1993), **110**, 633–639

Arcobacter cryaerophilus and thermophilic campylobacters in a sewage treatment plant in Italy: two secondary treatments compared

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(*Accepted 8 December 1992*)

SUMMARY

Microaerophilic organisms were monitored in sewage effluent undergoing two secondary treatments: air and oxygen-activated sludge. The mean numbers of *Arcobacter cryaerophilus* and thermophilic campylobacters detected in incoming sewage were 5639/100 ml and 1720/100 ml respectively.

Secondary treatment in air tanks reduced the population of *A. cryaerophilus* by 97·1% and of thermophilic campylobacters by 99·08%, whereas treatment in oxygen tanks reduced the bacteria 97·8% and 99·63% respectively, showing that oxygen-activated sludge treatment was more effective. Subsequent tertiary treatment with 2 p.p.m. chlorine dioxide evidenced the removal of *A. cryaerophilus* to 99·9% and eliminated thermophilic campylobacters.

Campylobacter jejuni and *C. coli* constituted 54·1% and 45·9% of 74 thermophilic campylobacter strains isolated. In air-activated sludge effluent *C. jejuni* was found more often, thus appearing more sensitive to oxygen.

The most probable number assay used for detection of campylobacters, blood medium for enrichment and blood-free medium for plating, also appeared to be fit for *A. cryaerophilus*, the high density of which in urban sewage may be due to inflows from slaughterhouses.

INTRODUCTION

Microorganisms belonging to the *Campylobacter* genus are asporogenous spirally curved Gram-negative bacteria which are motile and microaerophilic growing in a low-tension O_2 atmosphere (4–10%) in the presence of N_2 and CO_2.

Before the revision proposed by Vandamme and co-workers [1], these bacteria had been divided into three groups according to taxonomic structure. Group I contained *Campylobacter fetus*, *C. hyointestinalis*, *C. sputorum*, *C. jejuni*, *C. coli*, *C. lari*, '*C. upsaliensis*', *C. concisus* and *C. mucosalis*. Group II comprised *C. pylori* (*Helicobacter pylori* since 1989) [2] *C. fennelliae*, *C. cinaedi* and *Wolinella succinogenes*. Group III contained *C. nitrofigilis* and *C. cryaerophila*.

The bacteria are found in many habitats ranging from the purely environmental (*C. nitrofigilis*) to animal (*C. hyointestinalis* and *C. cryaerophila*) and human habitats. In humans the bacteria have been associated with gastro-duodenal ulcer (*H. pylori*), proctocolitis in homosexuals (*C. cinaedi* and *C. fennelliae*) [3–6] but mainly acute enteritis, in which *C. jejuni* is the most frequently detected biotype alongside *C. coli*, *C. lari*, '*C. upsaliensis*' and *C. hyointestinalis* [7–12]. The campylobacters responsible for diarrhoea are also called thermophilic, as they grow at 42 °C.

Campylobacters which also grow in normal air at 37° rather than 42 °C when subcultured, but not at the first isolation, are called aerotolerant. Among these there are Group II bacteria recently identified as *C. butzleri* [13], which were found in children with diarrhoea in Thailand [14] and in the United States [13]: and *C. cryaerophila*, which differs from *C. butzleri* in its ability to multiply at 25 °C, its catalase positivity and failure to grow in the presence of 3·5% NaCl.

Following DNA hybridization studies, Vandamme and co-workers [1] recently proposed a taxonomic revision of the *Campylobacter* family. *C. cryaerophila* now belongs to the new genus *Arcobacter* as does *C. nitrofigilis*, the nitrogen-fixing bacterium associated with plants. The genus *Arcobacter* differs from the genus *Campylobacter* as it can also grow in normal air at an optimal temperature of 30 °C, it does not produce H_2S and will grow in the presence of 2·5% NaCl. *Arcobacter cryaerophilus* has been detected in tissues and faeces from cattle, swine and poultry [15–19] and in man [20]. This bacterium has also been isolated from surface waters in Italy in a survey of the Savena river [21].

Very little is known about these bacteria in sewage, which generally contains human and animal wastes. In a previous study [22] we reported the occurrence of thermophilic campylobacters in sewage and their removal by treatment processes. The Bologna city sewage treatment plant has always used an activated sludge secondary treatment by air insufflation. Pure oxygen insufflation, as secondary treatment, was introduced in late 1990, and both treatments were in use until oxygen fully replaced air insufflation. During this period we took samples from both tanks to compare the two sewage treatments and assess results in relation to the subsequent tertiary treatment. The test parameter was the behaviour of strictly microaerophilic microorganisms such as thermophilic campylobacteria, and more oxygen-resistant germs like *A. cryaerophilus*.

MATERIALS AND METHODS

The Bologna treatment plant receives waste from the mixed sewers of about 500000 inhabitants. Until a few months ago, grading and primary sedimentation were followed by a secondary treatment consisting of an air-activated sludge process. Eighteen months ago oxygen tanks for oxygen-activated sludge treatment were introduced (the Lindox process), offering the following key features compared with an air system: maintenance of a high sewage concentration of dissolved oxygen and suspended solids; smaller covered tanks (removing smell and aerosols): reduced production of excessive sludge.

The Bologna plant then used both air and oxygen secondary treatments during the transition period. Following secondary treatment the mixed activated sludge effluent (a mixture of the two treatment effluents) was disinfected with 2 p.p.m. chlorine dioxide.

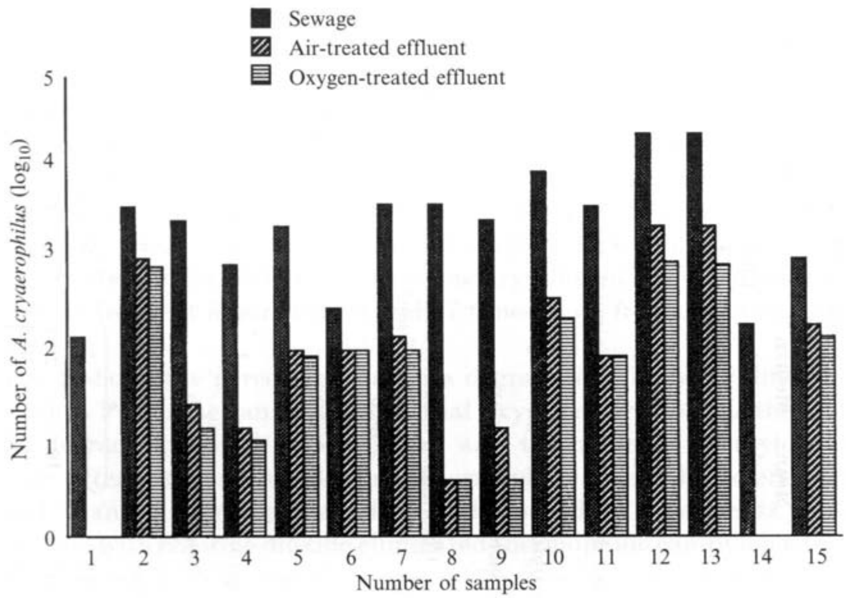

Fig. 1. Number of *Arcobacter cryaerophilus* ($\log_{10}$) isolated from 15 samples of incoming sewage, air-treated and oxygen-treated effluents.

Fifteen samples of each sort of sewage (incoming raw sewage, air-activated sludge effluent, oxygen-activated sludge effluent, mixed secondary effluent and chlorinated effluent) were examined for a total of 75 samples over a 12-month period (from February 1991 to February 1992).

The most probable number assay previously adopted [22] was used to detect thermophilic campylobacters and *A. cryaerophilus* as follows: enrichment in Preston broth (Oxoid) by direct inoculation and filtering on 0·45 μm Millipore membranes; microaerophilic incubation (gas generating kit, Oxoid) at 37° and 42 °C for 48 h; plating on CCDA agar (Oxoid) and microaerophilic incubation of plates at 37° and 42 °C for about 48 h; Gram staining of the suspect colonies, after which Gram-negative bacteria morphologically similar to the *Campylobacter* and *Arcobacter* genera underwent the following assays: oxidase, catalase, nalidixic acid and caphalothin sensitivity, nitrate reduction, H_2S production in FBP, H_2S production in TSI with lead acetate strip, DNA and hippurate hydrolysis, growth at 37 °C in normal air and at 25° and 42 °C under microaerophilic conditions. Biotyping was performed according to Lior's scheme [23].

RESULTS

The numbers of *A. cryaerophilus* and thermophilic campylobacter found in all samples of incoming sewage, air- and oxygen-treated secondary effluents are listed in Figs. 1 and 2 respectively.

Arcobacter cryaerophilus was detected in all 15 samples of incoming raw sewage (100% positivity) and in 13 of 15 (87%) of air- and oxygen-treated secondary effluents (Fig. 1) and in 10 of 15 (67%) of outgoing disinfected effluent.

Thermophilic campylobacter were isolated from all 15 samples of raw sewage

https://doi.org/10.1017/S0950268800051050 Published online by Cambridge University Press

Table 1. *Number of* Arcobacter cryaerophilus *and thermophilic campylobacter* (*MPN*) *in incoming sewage, activated sludge effluents and chlorinated effluent*

	Arcobacter cryaerophilus (MPN/100 ml)*					Thermophilic campylobacter (MPN/100 ml)				
	Mean	Median	S.D.	Min.	Max.	Mean	Median	S.D.	Min.	Max.
Incoming sewage	5639	2800	7810	150	24000	1720	1500	1362	90	4000
Air-activated sludge effluent	469	110	832	0	2400	21	0	54	0	210
Oxygen-activated sludge effluent	236	150	296	0	900	5	0	7	0	40
Mixed activated sludge effluent	581	400	639	0	2400	4	0	7	0	9
Chlorinated effluent	4	0	6	0	24	0	0	0	0	0

* MPN, Most probable number/100 ml, mean and median of 15 samples, S.D., standard deviation; min., minimum; max., maximum.

Table 2. *Removal* (%) *of* Arcobacter cryaerophilus *and thermophilic campylobacters by sewage treatment processes*

(Figures used are means of reduction values obtained on each sampling day.)

	Air-activated sludge effluent/incoming sewage	Oxygen-activated sludge effluent/incoming sewage	Mixed activated sludge effluent/incoming sewage	Chlorinated effluent/mixed activated sludge effluent	Chlorinated effluent/incoming sewage
Arcobacter cryaerophilus	97·1	97·8	96·6	97·8	99·9
Thermophilic campylobacters	99·08	99·63	99·8	100·0	100·0

Table 3. *Prevalence of thermophilic campylobacter species and biotypes from incoming sewage and activated sludge effluent*

	Campylobacter jejuni				*Campylobacter coli*				Total isolates	
	Biotype		Total		Biotype		Total			
	I	II	Number	%	I	II	Number	%	Number	%
Incoming sewage	14	10	24	70·6	8	2	10	29·4	34	100
Air-activated sludge effluent	5	2	7	43·7	6	3	9	56·3	16	100
Oxygen-activated sludge effluent	3	1	4	30·8	6	3	9	69·2	13	100
Mixed activated sludge effluent	4	1	5	45·5	4	2	6	54·5	11	100
Total	26	14	40	54·1	24	10	34	45·9	74	100

https://doi.org/10.1017/S0950268800051050 Published online by Cambridge University Press

(100%), but much less often in secondary effluents, dropping from 6 of 15 (40%) air-treated effluents to 4 of 15 (27%) oxygen-treated secondary effluents (Fig. 2); no campylobacter was found in the disinfected effluent.

Table 1 summarizes the average, standard deviation, minimum and maximum values and the median of the number of *A. cryaerophilus* and thermophilic campylobacters in the five types of sewage. Data analysis shows that the average number of *A. cryaerophilus* (5639) was more than three times higher than thermophilic campylobacters (1720). Average values of *A. cryaerophilus* and campylobacters differ widely in the secondary effluent: *A. cryaerophilus* was 22 times more frequent in air-treated and 47 times more frequent in oxygen-treated sewage.

Table 2 shows the percentage bacteria degradation following different sewage treatments. Percentage analysis shows that oxygen activation has the best results, as the degradation of *A. cryaerophilus* and thermophilic campylobacters was superior after oxygen insufflation. Thermophilic campylobacters were more reduced than *A. cryaerophilus* after both secondary treatments. Subsequent disinfection with chlorine dioxide eliminated thermophilic campylobacters but not all *A. cryaerophilus*, although there was a 97·8% reduction. Overall abatement of *A. cryaerophilus* (percentage reduction in chlorinated effluent compared with raw sewage) was 99·9%.

A total of 74 thermophilic campylobacter isolates was found (Table 3) with a prevalence of *C. jejuni* much more frequent in raw sewage (24 of 34, 71%). *Campylobacter coli* was the main isolate in both secondarily treated effluents: 9 of 16 (56%) in air-treated effluent and 9 of 13 (69%) in oxygen-treated effluent. Biotype I was most frequent in both species identified: 26 of 40 (65%) in *C. jejuni* and 24 of 34 (71%) in *C. coli*.

DISCUSSION

Arcobacter cryaerophilus was found in all types of sewage with average values ranging from 5639/100 ml in incoming raw sewage to 4/100 ml in outgoing disinfected effluent.

Although they are air-tolerant, these microorganisms remain microaerophilic (microaerophilic conditions were required for isolation) and proved sensitive to pure oxygen treatment, as the degradation percentages obtained following oxygen activation were superior to those of air insufflation. However, these bacteria showed some resistance to 2 p.p.m. chlorine dioxide, as some *A. cryaerophilus* were still present in outgoing chlorinated effluent (4/100 ml).

Thermophilic campylobacters responded differently to the secondary and tertiary treatments. These bacteria are not aerotolerant, and being highly microaerophilic they were far less frequent in all types of substrate tested and absent in chlorinated effluent.

Oxygen treatment also proved more effective for campylobacters, above all *C. jejuni* (a reduction to 44% after air treatment and 30% after oxygen insufflation). These findings and previous data [22] show that *C. jejuni* is much more sensitive to the toxic effect of oxygen. The multiple-tube isolation procedure and traditional culture media for thermophilic campylobacters (blood medium

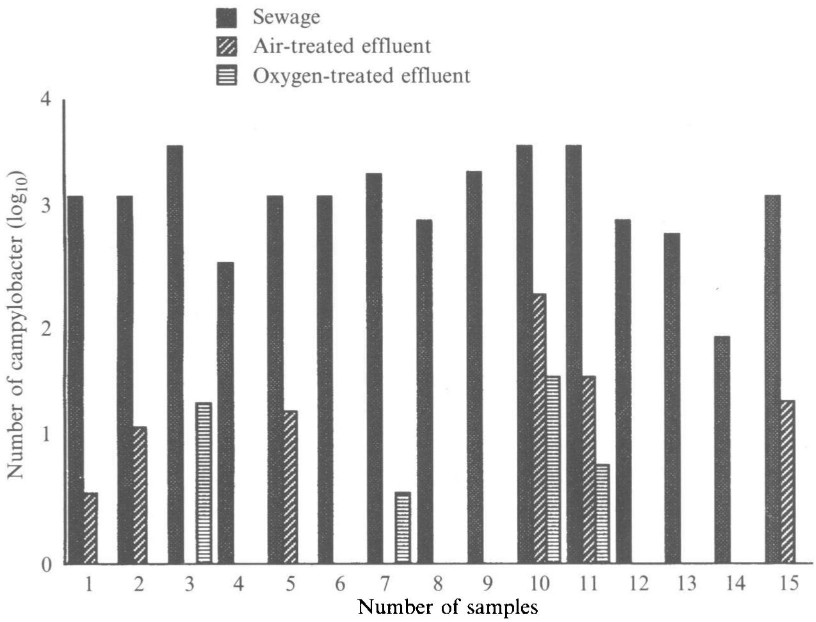

Fig. 2. Number of thermophilic campylobacter ($\log_{10}$) isolated from 15 samples of incoming sewage, air-treated and oxygen-treated effluents.

for enrichment by direct inoculation or membrane filtering and blood-free cefoperazone-supplemented medium for isolation) proved revealing, disclosing a high number of *A. cryaerophilus* in substrates subjected to severe stress.

Several considerations arise from the large number of *A. cryaerophilus* bacteria, typical of animal habitats, found in this plant's predominantly urban sewage with little livestock waste: the high number could be due to the daily high discharge from the municipal slaughterhouse (mainly cattle and pigs); these bacteria have seldom been reported in man [20], are of unknown pathogenesis and clinical significance, but could be more frequent than routine clinical tests have demonstrated to date. As Penner reported [24], this species may pass undetected in man, as the search for campylobacter in faeces does not require enrichment and plates are incubated at a temperature (42 °C) which does not allow growth of *A. cryaerophilus*.

In conclusion, our determinations of thermophilic campylobacters and *A. cryaerophilus* behaviour show that oxygen-activated sludge treatment in the Bologna sewage plant is superior to secondary air insufflation for the removal of these bacteria. Subsequent chorine dioxide disinfection eliminated thermophilic campylobacters but did not fully remove *A. cryaerophilus*.

ACKNOWLEDGEMENTS

This work was supported by grants from Ministry of University and Scientific Research Local Projects (60%), M.U.R.S.T. National Program of Research on Epidemiology and Prevention of Infectious and Non-Infectious Diseases (40%) and Emilia Romagna Region.

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