

Review

Arcobacters as emerging human foodborne pathogens

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Abstract

Arcobacter spp. are increasingly being isolated from a wide range of foods especially those of animal origin, such as pork and poultry, and products derived from these. Although its role in the disease process remains to be fully elucidated, there have been several reports of the organism being implicated in human illness. This article reviews the procedures that have been developed for the isolation and identification of *Arcobacter* spp. which have lead to its detection in a wide range of animals and foods and discusses the role that this organism may have in the epidemiology of foodborne disease. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Arcobacter* spp.; Food; Human infections

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1. Introduction

Vandamme et al. (1991) proposed the genus *Arcobacter* within the RNA Superfamily VI to describe those organisms previously classified as ‘aerotolerant campylobacters’. The genus *Arcobacter* is included in the family *Campylobacteraceae* (Vandamme & De Ley, 1991a). There are four defined species: *Arcobacter butzleri*, *Arcobacter cryaerophilus* (Groups 1A and 1B), *Arcobacter skirrowii* and *Arcobacter nitrofigilis* (Vandamme et al.,

1992a), of which the first three have been associated with human illness. Arcobacters are gram-negative, non-sporing, curved rods which may appear as spiral. Unlike thermophilic, pathogenic campylobacters, they grow at 15°C but not at 42°C and are able to grow aerobically at 30°C. Minimum standards for describing new species within this family have been determined (Ursing, Lior, & Owen, 1994).

2. Isolation and identification

A number of isolation methods have been suggested for *Arcobacter* spp., some of which have been derived

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from those developed for campylobacters. CAT (cefoperazone, amphotericin, teicoplanin) agar supports growth of a wider range of arcobacters than mCCDA (modified cefoperazone, charcoal, deoxycholate agar) (Corry & Atabay, 1997). A pre-enrichment stage in either CAT broth or Arcobacter Enrichment Broth together with a filter method (Lammerding, Harris, Lior, Woodward, Cole, & Muckle, 1996) onto mCCDA or CAT agar has been suggested for optimum isolation from chicken carcasses (Atabay & Corry, 1997). A pre-enrichment in a Arcobacter Selective Broth (ASB), followed by plating onto semisolid Arcobacter selective medium using cefoperazone, trimethoprim and cycloheximide, has been suggested for isolation from raw poultry meats. The incorporation of piperacillin into the medium prevents the outgrowth of *Pseudomonas* spp. from raw meats at the isolation temperature of 24°C and results in isolation of *Arcobacter* spp. from 53 out of 220 poultry meat samples (De Boer, Tillburg, Woodward, Lior, & Johnson, 1996). A new commercial enrichment broth Arcobacter Broth has been developed in the UK which may be used supplemented with CAT or mCCD for isolation of *Arcobacter* spp. or *A. butzleri*, respectively. This medium supports good growth of *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* although *A. nitrofigilis* grows poorly. The growth characteristics in Arcobacter Broth compare favourably with those on media specifically designed for isolation of campylobacters (Atabay & Corry, 1998). Use of this medium allows high population densities to be achieved and eliminates the necessity for biphasic growth methods (Dickson, Manke, Wesley, & Baetz, 1996) which may be technically more difficult.

Although aerotolerance is a definitive characteristic which distinguishes *Arcobacter* spp. from *Campylobacter* spp., this is often not observed on initial isolation on media originally designed for the isolation of campylobacters (de Boer et al., 1996). Recently a new solid medium has been suggested for optimal aerobic growth at 30°C. The addition of 0.05% thioglycolic acid, 0.05% sodium pyruvate and 5% sheep's blood (pH 6.9) to a basal nutrient mix results in an effective medium for growth, with the added characteristic that a deep red colour develops around the colonies (Johnson & Murano, 1999a). An aerobic pre-enrichment with the so-called JM enrichment broth together with plating on this agar resulted in *Arcobacter* spp. being detected in 42 out of 50 broiler chicken samples compared with 15 with the de Boer et al. (1996) method (Johnson & Murano, 1999b).

The use of a systematic range of biochemical tests, together with a probabilistic matrix comparing the characteristics of the unknown isolate with those of defined taxa, provides a method of identification of different species. However, these are only helpful if there is a match between the taxa in the scheme and the un-

known isolate. The original described scheme (Barrett, Patton, & Morris, 1988) and the commercially available scheme (API Campy, Biomérieux SA, France) only include 16 and 18 taxa, respectively. The latter, although effective for differentiation of *Campylobacter* spp., does not allow similar identification of *Arcobacter* at species level. *A. cryaerophilus* is the only one in the scheme and, even so, because of strain variability, it cannot be used for reliable identification (Jacob, Lior, & Feuerpfeil, 1993). SDS-PAGE of whole cell proteins has been shown to be successful in species level identification of *Arcobacter* spp. isolated from poultry at the abattoir (Ridsdale, Atabay, & Corry, 1998). Numerical analysis of phenotypic characters and use of an extensive probabilistic identification matrix confirms the differences between the three pathogenic *Arcobacter* spp. as a distinct group and *Campylobacter* spp. However, although *A. nitrofigilis* clusters with *C. curvis*, *C. rectus* and *C. showae* in another group, the former can be distinguished by aerobic growth at 25°C, making this characteristic an useful species-specific marker (On & Holmes, 1995; On, Holmes, & Sackin, 1996).

Identification of species within the family *Campylobacteriaceae* using standard biochemical tests is problematical because of the variability and atypical reactions of some strains resulting in mis-classification and possibly mis-identification, in epidemiological studies, of common sources of infection. In the latter case identification at species level is required making molecular typing techniques the method of choice. Species of *Arcobacter* are reliably identified using restriction length polymorphism or ribotyping involving the hybridization of *Pvu*II-digested chromosomal DNA with probes for the 16S rRNA gene (Kiehlbauch, Pliakaytis, Swaminathan, Cameron, & Wachsmuth, 1991; Wesley, Schroeder-Tucker, Baetz, Dewhirst, & Paster, 1995). Using primers targeted at these genes a PCR assay has been developed which has proved robust. The method may not only be used with purified DNA but also with crude cell lysate, thus reducing the time of identification to about 8 h compared with several days using conventional culturing techniques (Harmon & Wesley, 1996). A molecular identification scheme based on restriction profiles targeting the 23S rRNA gene, using three restriction endonucleases *Hpa*II, *Cfo*I and *Hin*fI allows differentiation of *A. butzleri* and *A. nitrofigilis* but not between *A. cryaerophilus* and *A. skirrowii* which have identical patterns with all restriction enzymes. The 23S rRNA gene-based PCR-RFLP identification generates conserved restriction profiles and because the 23S rRNA gene contains more recognition sites its use improves discrimination and specificity (Hurtado & Owen, 1997). Although PCR is a resource-laden method for routine isolation, in epidemiological studies use of a robust PCR will allow the source(s) and spread of the contamination to be identified.

3. Reservoirs in the environment

The first description of *Arcobacter* spp. (then referred to as aerotolerant *Campylobacter* spp.) was from isolates from aborted and normal porcine foetuses (Ellis, Neill, O'Brien, Ferguson, & Hanna, 1977), sows with reproductive problems and asymptomatic pigs (Neill, Ellis, & O'Brien, 1978, 1979).

In Brazil *A. cryaerophilus* has been isolated from sows with reproductive problems and from aborted porcine foetuses (De Oliveria, Baetz, Wesley, & Harmon, 1997) with 1B being found most commonly. However, in male pigs *A. cryaerophilus* 1A and 1B seem to be evenly distributed and are more common than *A. butzleri* (De Oliveria, Wesley, Baetz, Harmon, Kader, & de Uzeda, 1999). In one study gastric ulceration of swine was associated with *Arcobacter* spp., particularly *A. butzleri*, infection. The identification of the species involved seems to be dependent on the specificity of the PCR reaction used (Suarez, Wesley, & Larson, 1997).

Pork is routinely contaminated by *Arcobacter* spp., particularly *A. butzleri*, with varying prevalences at the processing plant. In one study of five such plants in Iowa, the inter-plant variation was between 90% and 0%. The variation between different plants depends on either the original primary source of the animals, or the hygiene practices at the abattoir itself, or both (Collins, Wesley, & Murano, 1996a).

Arcobacter spp. has been isolated from poultry with recovery rates ranging from 0% to 97% and although chickens are a source, eggs do not seem to be infected. In one study in Italy no *A. butzleri* was isolated from samples of either manually shelled eggs or retail chicken (Zanetti, Varoli, Stampi, & De Luca, 1996) whereas in another in The Netherlands 24.1% of 220 poultry meat products were positive for *Arcobacter*. In Canada, *A. butzleri* was recovered from 97% of poultry carcasses from five different processing plants (Lammerding et al., 1996). *A. butzleri* is not the only *Arcobacter* spp. present in poultry. *A. skirrowii* and *A. cryaerophilus* have also been isolated from chickens at the abattoir as well as on retail sale (Atabay, Corry, & On, 1998).

As well as chickens other poultry have been reported as harbouring the organism. A survey of mechanically separated turkey samples has suggested that this meat may be highly contaminated by *Arcobacter* spp. (77%) or *A. butzleri* (56%). As in the case of pig processing plants there was variation in prevalence. One plant yielded 96% of samples contaminated with *Arcobacter* species (80% of which were *A. butzleri*) whereas *Arcobacter* spp. was isolated from 44% of samples from another, 59% of these positive for *A. butzleri*. Using PCR-based fingerprinting to identify the organisms there was a diversity in DNA patterns within all plants sampled suggesting that there probably is a multiplicity of contaminating sources rather than one major source (Manke, Wesley, Dickson,

& Harmon, 1998). In a study of ten duck carcasses at the abattoir *A. butzleri* was isolated from two, *A. skirrowii* from one and *A. cryaerophilus* from five. Out of four flocks investigated one was positive for *A. butzleri* and one for *A. cryaerophilus* (Ridsdale et al., 1998).

Despite its common isolation from poultry carcasses, *A. butzleri* is infrequently isolated from caecal samples suggesting that contamination may be post-slaughter (Atabay & Corry, 1997; Harrass, Schwarz, & Wenzel, 1998). However, natural infections of live poultry do occur. In one study, *Arcobacter* spp. was isolated from 14.3% of cloacal swabs from 405 birds of which 1% were *A. butzleri*. As with pigs, variations in isolation rate from poultry occur which seems to be dependent on the environmental conditions of the originating flock. However, unlike piglets, *per os* experimental infections with *A. butzleri* are not readily established in poultry. Only in the highly inbred Beltsville White turkeys was *A. butzleri* recovered from cloacal swabs or tissues of a majority of the experimentally infected birds (Wesley & Baetz, 1999).

Water probably has a significant role in the transmission of *Arcobacter* spp. both to animals and to humans. *A. butzleri* has been isolated from canal water in Thailand (Dhamabuttra, Kamol-Rathanakul, & Pienthaweechai, 1992). In a two year study of water treatment plants in Germany the organism was isolated from all stages of the processing, although more frequently from raw compared with treated water. Serotype 1 was the predominant type isolated followed by serotypes 17, 19 and 2 (Jacob, Woodward, Feuerpfeil, & Johnson, 1998). This corresponds to other studies in poultry (Lammerding et al., 1996; Marinescu, Collignon, Squinazi, Woodward, & Lior, 1996a) and clinical samples (Vandamme, Pugina, Benzi, van Etterijk, Vlaes, Kersters, Butzler, Lior, & Lauwers, 1992b) which have showed similar prevalences of serotypes. *A. butzleri* has been isolated from a well-water source in USA. However, the organism seems sensitive to chlorine inactivation so that infection probably occurs because of improper chlorination procedures or post-treatment contamination (Rice, Rodgers, Wesley, Johnson, & Tanner, 1999). *A. butzleri* has been found in various types of sewage sludge from treatment plants in Italy. Frequencies varied from 41% in digested sludges (values of 7649 MPN/g) to 80% in thickened and activated sludges. In comparison *C. jejuni* and *C. coli* were only isolated from primary sludge and at counts of 278 and 1403 MPN/g, respectively suggesting that land application of anaerobically digested sludge may cause high risk of infection (Stampi, Deluca, Varoli, & Zanetti, 1999).

4. *Arcobacter* and human infections

The organism has been associated with animal diseases including abortion, mastitis and diarrhoea

(Wesley, 1996). *A. butzleri* infection occurs in non-human primate populations (Anderson, Kiehlbauch, Anderson, McClure, & Wachsmuth, 1993) although there is no evidence at present to suggest that handling of such animals provides a means of human infections. In the case of a chronic diarrhoeic macaque (*Macaca mulatta*) no human infections were reported in people associated with the animal even though it was showing symptoms for ten months (Higgins, Messier, Daignault, & Lorange, 1999).

A. butzleri has been isolated from patients with severe diarrhoea (Lerner, Brumberger, & Preac-Mursic, 1994; Marinescu, Collignon, Squinazi, Derimay, Woodward, & Lior, 1996b) and from blood, including a case of neonatal bacteraemia (On, Stacey, & Smyth, 1995). The first description of a clinical case of *A. cryaerophilus* 1B human infection was in a homosexual male with diarrhoea (Tee, Baird, Dyall-Smith, & Dwyer, 1988). Clinical isolates have been reported in children with diarrhoea (Taylor, Kiehlbauch, Tee, Pitarangsi, & Echeverria, 1991) although the definitive association between human illness and pathogenicity of this organism remains uncertain. A case of an uremic patient with bacteraemia caused by *A. cryaerophilus* 1B suggests that this organism is able to cause invasive infections (Hsueh, Teng, Yang, Wang, Chang, Ho, Hsieh, & Luh, 1997) although the patient concerned had an underlying illness which may have contributed to the progress of *A. cryaerophilus* infection.

The importance of *Arcobacter* spp. in the causation of human illness remains to be determined. This is probably because optimal isolation techniques have not yet been established and routine primary screening procedures used for *Campylobacter* spp. do not allow recovery of *Arcobacter* spp., which do not grow at 42°C (Marinescu et al., 1996b). The symptoms of *Arcobacter* infection are similar to campylobacteriosis and may be transient in nature making infection rates difficult to assess.

A study in Belgium carried out between 1991 and 1994 which screened all routine stool specimens submitted for detection of enteropathogens found that less than 0.1% were positive for *Arcobacter* (8 out of 21 527 samples). However, the method included non-selective isolation medium and microaerophilic conditions, which may not be the optimum conditions for isolation of the organism. Using the same isolation procedure no *Arcobacter* isolates were obtained from 879 samples from 468 elderly patients during a one year study (Lauwers, Breynaert, Van Etterijck, Revets, & Mets, 1996).

A porcine model has proved valuable in evaluating the pathogenicity of both *Campylobacter* spp. (Babakhani, Bradley, & Joens, 1993) and *Helicobacter* spp. (Eaton, Brooks, Morgan, & Krakowka, 1991; Eaton, Morgan, & Krakowka, 1992). *Arcobacter* spp. are able to colonise neonatal piglets as indicated by faecal

shedding and re-isolation from tissues such as liver, kidney, ileum and brain although no gross pathology is observed. *A. butzleri* is particularly able to colonise piglets and use of this model for investigating the pathogenesis of this organism in humans may prove to be useful (Wesley, Baetz, & Larson, 1996).

An in vitro study suggests that *A. butzleri* produces cytotoxic effects on Hela cells and Intestine 407 cells and that the strains that do not, produce a cytotoxic effect instead. This effect on cells in vitro probably reflects the potential virulence of this organism in vivo (Musmanno, Russi, Lior, & Figura, 1997).

5. Control

Although *Arcobacter* and *Campylobacter* are closely related this does not necessarily mean that treatments designed to eliminate the latter from various foods, particularly those of animal origin, are as effective inactivating or removing *A. butzleri*. For example, *A. butzleri* is more resistant than *C. jejuni* to irradiation treatment. The average D_{10} value is 0.27 kGy compared with 0.19 kGy for *C. jejuni*. Doses of irradiation currently allowed for pork in USA (0.3–1.0 kGy) provides an effective method of reducing, if not completely eliminating, *A. butzleri* from pork (Collins, Murano, & Wesley, 1996b). Treatment with sprays of organic acids has been shown to be effective at reducing *Salmonella* and *Campylobacter* spp. on pork carcasses (Epling, Carpenter, & Blankenship, 1993) and incorporating nisin and/or sodium lactate into fresh pork sausage has been suggested in order to reduce bacterial contamination especially *Staph. aureus* and *Salmonella kentucky* (Scannell, Hill, Buckley, & Arendt, 1997). Sodium lactate is not effective in reducing growth of *A. butzleri* in pure cultures (Phillips, 1999) but this does not mean that it is not effective in the complex ecosystems represented by food systems.

Implementation of hazard analysis and critical control points (HACCP) programs is important in the control of all foodborne pathogens, including *A. butzleri* and other species. However, changes in food processing, food handling practices and the development of novel food products, together with consumer influences will ensure that emerging foodborne pathogens will continue to be an important public health concern (Meng & Doyle, 1997).

6. Conclusion

The role of *Arcobacter* spp. is not, as yet, defined in terms of human foodborne illness. However, as more sophisticated detection, isolation and identification techniques are developed more information will be

gathered on its epidemiology and sources both in the environment and in food. Thus, although presently *A. butzleri* itself, or *Arcobacter* spp. in general, do not appear to play a major role in human foodborne disease, it may certainly be added to the ranks of the potential 'emerging foodborne pathogens.'

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