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Arcobacter – an emerging food borne pathogen?

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Introduction:

Description of the genus *Arcobacter*:

Arcobacter was first isolated in 1977 from aborted foetuses of livestock animals, and referred to as ”atypical *Campylobacters*”, being aerotolerant and able to grow at 20°C. In 1985 all aerotolerant *Campylobacters* were considered a new species – *Campylobacter cryaerophila*.

In 1991-1992 *C. cryaerophila* was accepted as a separate genus and named *Arcobacter*. Four species (*A. butzleri, A. cryaerophilus, A. skirrowi* and *A. nitrofigilis*) were included in the new genus. Recently further species have been proposed (*A. cibarius, A. halophilus, Candidatus* A. sulfidicus and an ”*A. skirrowi* - like organism”), but they are not yet formally recognised. The *Arcobacters* belong to the rRNA superfamily VI of the *Proteobacteria*, similar to *Campylobacter, Helicobacter, Wolinella* and *Flexispira*. It seems likely that further research and improvement of isolation techniques will increase the number of *Arcobacter* species.

Arcobacters are 1 to 3 by 0.2 to 0.4 µm helical, curved or S-shaped rods, showing characteristically cork-screw movement by a single polar flagellum. They are gram negative, catalase and oxidase positive and grow on rich media under aerobic or microaerophilic conditions at 15° to 37°C. The upper temperature limit for growth seems to be around 40°C and the lower temperature limit is widely accepted as 15°C, although recently D'Sa & Harrison (2005) found some *A. butzleri* strains capable of growth at 10°C after 7 days in EMJH medium. At 37°C growth occurs at pH 5.5 to 8.5. Although being capable of aerobic growth, the optimum O_2 concentration is found to be 3 – 10 % oxygen. *Arcobacter* form round, 2–4 mm white/greyish, colonies on rich blood agars (e.g. BHI + 0.6 % yeast and 10 % blood), upon 3 days incubation at 30°C. *A. skirrowi* and a few *A. butzleri* show α-haemolysis. In broth cultures high cell densities of *Arcobacters* are seldom obtained. The key difference from *Campylobacter* is the ability to grow aerobically at 15 – 30 °C.

Clinical importance:

Except for *A. nitrofigilis*, the other 3 accepted species of *Arcobacter* are widely recognised as animal pathogens causing abortion or other reproductive failures in pig, cattle and sheep.

Also several publications report of isolation of *Arcobacter* from stool samples of sporadic cases of human enteritis, when no other enteric pathogen has been found. *A. butzleri* is the *Arcobacter* species most frequently isolated from human cases of illness and is most often associated with persistent, watery diarrhea. Besides *A. butzleri* also *A. cryaerophilus* have been isolated from human enteritis cases, whereas *A. skirrowi* and *A. nitrophigilis* is not yet considered as human enteric pathogens. However in 2004, *A. skirrowi* was isolated in a stool sample from a 72 year-old man with chronic

diarrhoea. This is the first and so far only reported case of *A. skirrowi* from human enteric cases. *A. nitrofigilis* have never been isolated from either animal or human cases of illness.

The so far only recognised outbreak of *Arcobacter* took place in a nursery/primary school in Rovigo in Italy 1983. A total of 10 school children aged 3 – 7 years suffered from abdominal cramps (no diarrhoea!) and 3 were hospitalized for approx. 1 week. *A. butzleri* were isolated from stool samples from all the patients (Vandamme, 1992). The timing of the cases strongly indicate person-to person spread and not a common food as vehicle for the outbreak.

Also a few cases of invasive infection caused by *A. butzleri* and *A. cryaerophilus* have been reported, all of the patients suffering from underlying diseases. Thus invasive *Arcobacter* infections seems to be exceptional rare and usually only in combination with serious underlying disease. The mechanism of pathogenicity or potential virulence factors are only poorly understood, but cytotoxic effect on Vero and HeLa cells have been demonstrated in a few studies. The toxin in question is however clearly different form CDT (Cytolethal Distending Toxin) in *Campylobacters*, as shown by absence of the Campylobacter-CDT-genes in *Arcobacter* spp isolated from cattle, poultry, water and human diarrhoea, when analysed by PCR-probes to the cdtA and cdtB gene found in *C. jejuni*.

Examination for *Arcobacter* in stool samples from cases of human gastroenteritis is not carried out on regular basis in any country. Thus, the prevalence of human cases caused by *Arcobacter* is not known, but based upon the published cases, it seems low. On the other hand, as the *Arcobacter* is not looked for on a routine basis, the prevalence may be grossly underestimated.

Recently a few studies on the prevalence of *Campylobacter* and *Campylobacter*-like organisms (CLO) including *Arcobacter* in stool samples have been published.

Enberg et al (2000) analysed 1376 stool samples from Danish patients for *Campylobacter* and *Campylobacter*-like organisms (CLO). Amongst the 135 isolates recovered were 78 C. *jejuni/C. coli* (58 %) and only 2 *Arcobacter* (1 *A. butzleri* and 1 *A. cryaerophilus*). Vandenberg et al (2004) found *Arcobacter* as the fourth most common CLO from 67,599 stool samples collected in an 8-year period in Belgium. CLO were found in 1906 samples and of these, 1471 were *C. jejuni*, 218 were *C. coli* and 77 were *Arcobacter* (67 *A. butzleri* and 10 *A. cryaerophilus*). Recently figures from France have been published (Prouzet-Maulèon et al, 2006). Of the 2855 strains of CLO send to the reference laboratory, 2114 were *C. jejuni*, 486 were *C. coli* and 29 were *A. butzleri*. From these studies, a rough estimate of the "true" incidence of *Arcobacter* can be made. If comparing the number of *Arcobacter* isolates to the number of *C. jejuni/C. coli* isolates, it can be estimated, that for each 100 cases of *Campylobacter* infection, 1

to 5 cases of *Arcobacter* should be found (2.6 in DK, 4.6 in Belgium and 1.1 in France).

It is stated in several publications that although the clinical significance of *Arcobacter* as a human, foodborne pathogen still needs to be fully elucidated, as well as the route of infection are still unknown, it is advisable to introduce a precautionary approach to eradicate or reduce *Arcobacter* in the food chain.

Methods:

Methods for isolation of *Arcobacter*:

Arcobacter was first isolated in 1977 from aborted foetuses of livestock animals, using EMJH P80 with 100 mg/liter 5-fluoro-uracil (Ellinghausen-McCullogh-Johnson-Harris Polysorbate 80), a medium normally used for culturing *Leptospira*. The following years several new enrichment broths were described, usually depending on various antibiotics and fungicides as selective ingredients (cefoperazone, cephalotin, vancomycin, novobiocin, trimethoprim, piperacillin, amphotericin, and teicoplanin) and microaerobic or aerobic incubation at 25° – 37°C. Also isolation agars developed from non-selective media with or without blood into sophisticated selective agars, often as modifications of established *Campylobacter* agars.
During this development, it was found that *Arcobacter* is susceptible to colistin, polymyxin B and rifampicin, and also partly susceptible to cefoperazone in concentrations used in *Campylobacter* media (32 mg/l). Especially *A. skirrowi* is difficult to culture on media containing antibiotics, partly due to a greater sensitivity to antibiotics and partly due to slow growth of this species.
During the last 2 decades many different enrichment media and solid as well as semisolid agars have been used for isolation of *Arcobacter*. In this paper, 5 different protocols widely used in comparative studies for detection of *Arcobacter,* are briefly discussed.

Collins et al (1996) used the original EMJH P80 medium (with 200 mg/liter 5-fluoro-uracil) for enrichment incubated aerobically for 9 days at 30°C. After enrichment samples were plated onto BHI supplemented with CVA supplement (20 mg/l cephalotin, 10 mg/l vancomycin, 5 mg/l amphotericin B) and 10 % bovine blood and incubated aerobically for 48 hours at 30°C.

The method of deBoer et al (1996) uses a so called Arcobacter Broth containing 28 g/l Brucella broth base supplemented with 32 mg/l cefoperazone, 75 mg/l piperacillin, 20 mg/l trimethroprim, 100 mg/l cycloheximide and 50 ml/l horse blood, and incubated for 48 hours at 24°C. Afterwards 20 µl of the enrichment was plated at the center of a

semisolid agar (Mueller-Hinton with 2.5 g/l agar, 32 mg/l cefoperazone, 75 mg/l piperacillin, 20 mg/l trimethoprim, 100 mg/l cycloheximide) and incubated at 24°C for 72 hours. Growth from the motility zone was sub-cultured on BHI-agar with 10 % sheep blood for 48 hours at 30°C. All incubations were done under aerobic conditions.

The method of Johnson & Murano (1999) uses JM-broth (containing 32 mg/l cefoperazone, 200 mg/l 5-fluoro-uracil, 0.25 % bile salts, 0.05 % thioglycollate, 3 % charcoal and 0.05 % pyruvate) incubated aerobic at 30°C for 48 hours. Afterwards JM plating medium (containing 5 % sheep blood, 32 mg/l cefoperazone, 0.05 % thioglycollate, and 0.05 % pyruvate) were inoculated using a loop and also incubated aerobic at 30°C for 48 hours.

The method of Houf et al (2001) makes use of a commercially available (Oxoid) Arcobacter Broth (containing 16 mg/l cefoperazone, 100 mg/l 5-fluoro-uracil, 10 mg/l amphotericin, 32 mg/l novo-biocin, 64 mg/l trimethoprim, 5 % lysed horse blood, 0.05 % thioglycollate, and 0.05 % pyruvate) incubated microaerobic at 28°C for 48 hours, followed by loop inoculation on Arcobacter Medium (containing 16 mg/l cefoperazone, 100 mg/l 5-fluoro-uracil, 10 mg/l amphotericin, 32 mg/l novo-biocin, 64 mg/l trimerophrim), which was incubated microaerobic at 28°C for 24, 48 and 72 hours.

The method of On et al (2002) includes CAT broth (containing 8 mg/l cefoperazone, 10 mg/l amphotericin, 4 mg/l teicoplanin and 5 % lysed horse blood) incubated microaerobic at 37°C for 24 hours. Afterwards the enrichment were membrane filtered onto blood agar (5 % defibrinated horse blood) and incubated microaerobic at 25° and 37°C and examined after 24, 72 and 100 hours. Shortly, the membrane filtering inoculation method uses 10 – 12 drops inoculated onto a 0.45µm or 0.65 µm membrane filter allowing the motile *Arcobacters* to swim through the filter and inoculate the medium. This membrane filter technique has previous been used as a ”selective tool” for isolation of *Arcobacter* as well as *Campylobacters* from fecal homogenates.

Johnson & Murano (1999) compared their method to the methods described by Collins and deBoer respectively. The JM method was found superior to the other two, as the JM method found 42 of 50 chicken positive for *Arcobacter* as compared to 24 positive using the method of Collins and only 15 positive using the method of deBoer. No species identification is reported, but an additional study concerning the ability of the JM methods to isolate all species of *Arcobacter* was undertaken. It was shown, that *A. nitrofigilis* was not as easily isolated as the other three species. This is however of little importance as *A. nitrofigilis* is considered apathogenic.

Houf et al (2001) found their method excellent for the isolation of *A. cryaerophilus* and *A. butzleri* from chicken neck skin samples, but the method failed to isolate *A. skirrowi* artificially inoculated into neck skin samples even after prolonged incubation. *A. skirrowi* could however be detected in the enrichment broth by means of a multiplex PCR analysis.

Ohlendorf & Murano (2002a) compared the JM method to the methods of deBoer and Collins analysing raw, ground pork. The JM method found 64 of 200 samples positive for *Arcobacter,* whereas the other methods found only 7 respectively 8 samples positive. A modification of the Collins methods, using PCR detection directly after enrichment increased the number of positive samples from 8 to 52 positive. Thus, it seems evident that the plating media used in the Collins method lacks either selectivity for *Arcobacter* or the ability to grow *Arcobacter*. It is noteworthy, however that even though the JM was superior to the other methods, it only found 32 % samples positive compared to an overall recovery at 45 % positive when combining all 3 methods.
Ohlendorf & Murano (2002b) also examined the detection limit of the methods using inoculated samples. They found the JM method being the most sensitive, capable of detecting 10 cfu/g *A. butzleri* in 4 of 4 samples and 10 cfu/g *A. cryaerophilus* in 3 of 4 samples.

Golla et al (2002) analysed samples from beef and dairy cattle for *Arcobacter* using the JM-method in comparison to the Collins method and modifications of the two methods. The JM-method was the best method isolating *Arcobacters* from 9 of 200 (4.5 %) samples compared to 5 of 200 (2.5 %) for the Collins method. A combination of the 2 methods (the JM enrichment + CVA agar) found 6 of 150 (4.0 %) samples positive. In total *A. butzleri* was found in 18 samples (9.0 %).
Recently, Scullion et al (2004) used 3 different methods (the method of On et al (2002), Houf et al (2001) and the JM method) when analysing retail raw poultry in Northern Ireland. The method of On et al found only 28 % of the samples positive, whereas the two other found 68 % positive, provided the enrichment broth was plated after 24 h as well as 48 h for the JM method. The JM method was the only one isolating *A. skirrowi* in the study and was also the method that recovered the most *A. cryaerophilus*. Combining the 3 methods, 92 % of the samples were found to harbour *Arcobacter*, showing that no method recovers all positive samples. The authors conclude that based on sensitivity, ease of use and the diversity of species recovered, the modified JM method should be the method of choice. In order to increase isolation rate, they recommend using the modified JM-method and the method of Houf et al in combination, provided the necessary resources can be found.
It can be concluded that at present, the most efficient method to isolate *Arcobacter* is the Johnson & Murano method. The method is the most sensitive and user-friendly, it isolates all 3 pathogenic species and can be completed in only 4 days. It must

however be noticed, that the JM method only detects around 70 – 80 % of all positive samples, emphasising the need for a combination of two selective enrichment (as for *Salmonella*), in order to obtain acceptable performance.
The use of the membrane filtration technique has been described as a mean to improve isolation of *Arcobacter*, thus inclusion of filtering in the JM method might be a way to increase the recovery rate for *Arcobacter*.

DNA based methods:
Also several PCR-based or hybridisation-based methods have been described to detect or identify *Arcobacters* (see Forsythe 2006 for an overview). This includes primers/probes for detection of genus *Arcobacter* as well as for differentiation of *Arcobacter* to species level. To the authors' knowledge, to date, no real-time PCR have been published and no commercial PCR methods are available for the detection of *Arcobacter* in foods. This is probably only a matter of time, if legislation should be altered and the examination for *Arcobacter* becomes mandatory for certain groups of food.

Epidemiological investigation:
Provided successful isolation from food or environmental samples, *Arcobacter* can easily be differentiated into different species and different subtypes of species using DNA based typing methods. Usually these methods are targeting the conserved genes in the organism, as 16S and 23S rDNA. The methods includes a range of widely accepted typing techniques as RAPD, Ribotyping, PFGE, RFLP, AFLP, multiplex PCR, nested PCR and ERIC-PCR (Enterobacterial repetitive Intergenic Consensus-PCR). However, no method has up to now been established as the "method of choice" in epidemiological investigation, making it difficult to monitor the epidemiology of *Arcobacter* on a European or a world-wide basis (Forsythe 2006).

Arcobacter in the food chain

Prevalence of *Arcobacters* in food and production sites:

The true prevalence of *Arcobacters* is largely unknown, due to lack of an 100 % efficient isolation method. Furthermore it is difficult to compare data from different studies as the isolation methods varies from study to study. But the many studies carried out during the last two decades provide a pretty good picture of the *Arcobacter* occurrence in livestock and meat.
Several studies report of high prevalence of *Arcobacters* in feces from cattle, sheep, pigs, horses and poultry, and especially poultry seems to be a significant reservoir for *Arcobacter*.

Arcobacter has also been isolated from the intestine of these livestock animals, although there are conflicting reports whether *Arcobacter* is a true habitant of the intestinal microflora of poultry. As can be expected, the reported prevalence varies strongly depending of the actual isolation method. Also the age of the animal, the geographical region and the sampling method seems to influence the prevalence of *Arcobacter* in livestock.

In pig feces different studies report of prevalences between 16 – 85 %. For feces of cattle, prevalences of 9.5 to 71 % have been reported from 4 different studies. A single study reports a prevalence of 16.1 % for sheep feces and 15.4 % for horse feces respectively. Several studies report of prevalences up to 100 % for chicken carcasses and neck skin sample. For ducks and turkeys, prevalence of 50 % and 24 % respectively has been reported when analysing carcasses or neck skin samples. Interestingly the incidence in chicken and duck caecal samples is significantly lower, than in carcass samples indicating that cross-contamination takes place during slaughter of the poultry.
Beef and pork seems less frequently contaminated with *Arcobacter* than poultry. The incidence of *Arcobacter* in pork/ground pork has been reported to be from 0.5 % up to 32 %, whereas incidence in beef was reported from 1.5 to 5 %. Recently a Mexican study from 2003 reported prevalences in pork meat and beef of 51.5 % and 28.8 % respectively.
Arcobacters have been found in levels of up to 10^4 cfu/g in neck skin samples of chicken and in levels of 10^2 cfu/g in feces from sheep, cattle and pigs.

As for *Campylobacter*, also water (ground water, surface water, and sewage) has been found to be contaminated with *Arcobacter* and must be considered a likely source of human *Arcobacter* infection. Furthermore it has been found that *Arcobacter* easily attach to stainless steel, copper and plastic used for water pipes and survive on these surfaces for days. Also knifes and slaughter equipment is frequently found to be contaminated with *Arcobacters*.

Prevention measures:
As for many other human pathogens, the best way to reduce *Arcobacter* in meat is to improve hygiene during slaughter. Also cold storage is reported to reduce viability of *Arcobacter*. Freezing reduces the number of *Arcobacter* by 1 – 2 log, but freezing alone is not sufficient to reduce risk to an acceptable level.
As for *Campylobacter*, growth is inhibited by rather low concentration of NaCl (2 % - 3 %), and very low concentration of sodium tri-polyphosphate (0.02 %) inhibits growth of *Arcobacter*.
The termotolerance of *Arcobacter* is in one study found to be comparable to *Campylobacter*, as a D_{55}-value at 1.1 minute for cells harvested from the exponential

growth phase have been reported. Another study reported of a 3 times higher termotolerance for *A. butzleri* compared to *Campylobacter*. Nevertheless, a standard cooking temperature recommended for cooked meat products (i.e. core temp. at 70° for few seconds) is sufficient to eliminate *Arcobacter*.
Obviously more knowledge of how to avoid *Arcobacter* in the food chain is strongly needed.

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