BACTERIA

Arcobacter

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Glossary

Foodborne pathogens Microorganisms that are transmitted to humans via foods and cause diseases in humans.
Gastroenteritis Inflammation of stomach and intestinal tissues.
Identification Differentiation of bacteria at the species and/or subspecies level by characterizing by phenotypic and/or genotypic methods.
Isolation The growth of bacteria in/on a suitable growth medium to obtain the desired bacterial species.
Plating medium A solid growth medium solidified by addition of agar, that includes essential nutrients for bacterial growth.
Selective supplement Combinations of antimicrobial agents used to inhibit the unwanted bacteria in an isolation medium.

Background

The first isolation of an *Arcobacter* species was from bovine fetuses in the late 1970s. It was identified as an aerotolerant *Campylobacter* sp. (*Campylobacter cryaerophila*) because of its DNA base composition and general morphology, differing from other *Campylobacter* spp. because of its aerotolerance and its ability to grow at temperatures down to 15 °C. Similar bacteria were soon isolated from sources such as aborted bovine and porcine fetuses, cow's milk and bovine preputial sheath washings. *Campylobacter nitrofigilis* was isolated from a salt marsh, and appeared to be a nonpathogenic free-living species. After a comprehensive taxonomic study, the name *Arcobacter* was proposed in 1991 for this group, as a second genus in the family Campylobacteraceae. Consequently, the aerotolerant campylobacters became *Arcobacter cryaerophilus* and *Arcobacter nitrofigilis*, respectively. The genus *Arcobacter* later expanded to include two more members, *Arcobacter butzleri* and *Arcobacter skirrowii* isolated from various sources such as from human and animal diarrhea, aborted fetuses of animals, human blood, and from preputial fluids of bulls. Recently, another species, *Arcobacter cibarius*, was isolated from the skin of broiler carcasses and the same year, an obligately halophilic strain, called *Arcobacter halophilus*, was isolated from a hypersaline lagoon, indicating the ubiquitous distribution of *Arcobacter* spp. and a tendency to adapt to diverse environmental conditions. Three more species have been identified in the past few years: *Arcobacter mytili* has been isolated from mussels and brackish water; *Arcobacter thereius* from the liver and kidney of aborted pig fetuses and the cloaca of duck; and *Arcobacter marinus* from the marine environment. An unclassified organism, '*Candidatus* Arcobacter sulfidicus' producing filamentous sulfur has also been isolated from coastal seawater.

Characteristics of the Organisms

Like *Campylobacter* spp., *Arcobacter* spp. are Gram-negative, slightly curved, S-shaped, or helical rods 0.2–0.9 μm wide and 1–3 μm long. A characteristic darting or corkscrew-like motility is provided by a single polar, unsheathed flagellum. Arcobacters produce whitish or grayish smooth-rounded colonies varying in size, depending on the species. They produce oxidase and catalase and some strains are α-hemolytic. They can multiply at 15 and at 37 °C, but many are unable to multiply at 41.5 °C. Arcobacters can grow in aerobic conditions, but the best growth is observed under microaerobic conditions with 3–10% O_2, 5–10% CO_2, although *A. skirrowii* grows better when hydrogen is also present. They are unable to ferment carbohydrate but utilize organic acids and amino acids as carbon sources. Their most important distinctive features compared to *Campylobacter* spp. are their ability to grow in air at 30 °C and their growth at temperatures down to 15 °C.

Pathogenicity, Clinical Manifestations, and Virulence Factors

Three species of *Arcobacter*, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, are associated with various diseases in humans and animals. *Arcobacter butzleri* is the species most commonly reported as a cause of gastroenteritis and bacteremia in humans, with *A. cryaerophilus* and *A. skirowii* less frequent. Diarrhea is the most common symptom reported. They have been found particularly as a cause of diarrhea in malnourished children in developing countries. Although *Arcobacter* spp. and *Campylobacter jejuni* cause diarrhea with similar symptoms, *A. butzleri* infections are more frequently associated with a persistent,

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watery diarrhea, whereas *C. jejuni* more commonly causes bloody diarrhea. *Arcobacter butzleri* infections are also associated with abdominal pain with and without diarrhea, nausea and vomiting, or fever. However, some patients may remain asymptomatic. *Arcobacter skirrowii* has been implicated as a cause of chronic diarrhea.

Arcobacter butzleri, *A. cryaerophilus*, and *A. skirrowii* are associated with diseases in cattle, sheep, and pigs, such as enteritis, mastitis, and reproductive disorders, including abortions. *Arcobacter butzleri* is also associated with diarrhea in nonhuman primates, such as macaques.

Although arcobacters are associated with various diseases in humans and animals understanding of the mechanism(s) of pathogenicity of these microorganisms and their toxin production is still limited. Some strains have cytotoxins and cytolethal distending factors, some hemagglutinate human and animal erythrocytes, and some can adhere to cell lines and have invasive potential.

Epidemiology of *Arcobacter* spp.

The transmission of *Arcobacter* spp. to humans is considered to occur mainly through contaminated food and drinking water; close contact with pets; and perhaps through person-to-person-transmission. *Arcobacter* spp. have been isolated from the intestinal tract of healthy mammals including cattle, pigs, sheep, dogs, and horses, and also from pork, beef, lamb, poultry meat, and shellfish. Like campylobacters, arcobacters are more prevalent on poultry meat than red meat and thus, poultry carcasses may be a major reservoir of these bacteria. Four species of *Arcobacter*, *A. butzleri*, *A. cryaerophilus*, *A. cibarius*, and *A. skirrowii* have so far been recovered from poultry carcasses. Although arcobacters are common on chicken carcasses, in contrast to *Campylobacter* spp., they are rarely isolated from the intestinal contents of these birds probably because birds have a relatively high body temperature ($\geq 41\ ^{\circ}C$). However, *Arcobacter* spp. have been isolated from the cloacal swabs taken from various types of poultry, including chickens, turkeys, ducks, and geese. Eggs are not regarded as a source of arcobacters.

In contrast to the situation with campylobacters, contamination of poultry carcasses with arcobacters is considered to occur from the processing and slaughterhouse environment rather than the intestinal tract of the birds. Arcobacters are able to survive and multiply in the warm and wet environment of processing plants, and form biofilm on processing lines and surfaces. They have also been detected in piggery effluent, and effluent-irrigated soils, and in farm environments including surface water. Whether or not arcobacters have been detected in animals and from different farms could be affected by a number of factors, including the isolation/identification methods used and sample size. *Arcobacter* spp. are also found in various aqueous environments, including drinking water reservoirs, canal waters, river or surface water, ground water, and sewage. *Arcobacter butzleri* is sensitive to chlorine, so consumption of so nonchlorinated drinking water or contact with contaminated water sources may be a source of infection with these bacteria. *Arcobacter* infection may be more common in developing countries with poor water supplies.

Isolation, Identification, and Detection of *Arcobacter* spp.

Isolation

Arcobacter spp. were initially isolated from bovine fetuses using a semisolid *Leptospira* medium (Ellinghause–McClullough–Johnson–Harris) containing 5-fluorouracil. There is still no standard isolation method, but a number of isolation media and protocols have been devised in order to detect the three most common arcobacters (*A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*) in various types of sample. These methods generally exploit the ability of arcobacters to grow at lower temperatures ($\leq 30\ ^{\circ}C$) and in aerobic conditions in order to avoid isolating camplyobacters. Prolonging incubation of plating media up to 7 days aids detection of less robust species such as *A. cryaerophilus* and *A. skirrowi*, for which inclusion of hydrogen in the incubation atmosphere may be helpful. Basal media originally developed for other bacterial pathogens such as cefsulodin–irgasan–novobiocin agar base, and modified campylobacter cefoperazone deoxycholate agar base have been used in combination with different selective agents, for example, cefoperazone, amphotericin B, and teicoplainin (CAT) and 5-fluorouracil with some success for the isolation of arcobacters. They can also be recovered from various types of samples by using different isolation strategies that depend on their high motility and small size. In this context, arcobacters can be recovered by dispensing a small volume of a suspension of the samples, either before or after enrichment, onto a 0.45 or 0.65 μm pore size membrane filter laid on a plate of selective and/or nonselective agar medium for about 30 min before removing the filter and spreading the liquid remaining evenly over the agar. This allows the highly motile arcobacters and campylobacters to penetrate though the filter, whereas leaving other bacteria behind, and also allows detection of strains of *Arcobacter* more sensitive than most to selective agents. For instance, Atabay and Corry in 1997 were able to recover three species of *Arcobacter*, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, the latter two for the first time, from chicken carcasses using blood agar and this isolation protocol after enrichment. At present, a commercially marketed arcobacter enrichment broth (Oxoid), which incorporates peptone, yeast extract, and sodium chloride and CAT supplement as selective agents, is available. A number of other media and protocols have been devised to recover arcobacters by using different combinations of antibacterial agents and/or incorporating indicators such as color change and swarming on a semisolid medium. For instance, Johnson and Murano in 1999 developed an enrichment and plating medium (JM) to isolate *A. butzleri*, *A. cryaerophilus*, and *A. nitrofigilis* from poultry. They used cefoperazone, thioglycollate, bile salts, and pyruvate in both media. In addition the liquid broth contained 5-fluorouracil., and activated charcoal in enrichment broth and sheep blood in plating agar were used as detoxifying agents. A deep red color around colonies, possibly due to the thioglycollate, was observed with JM plating medium. Houf *et al.* in 2001 described a new liquid and a similar solid medium, both incorporating amphotericin B, cefoperazone, 5-fluorouracil, novobiocin, and trimethoprim as selective agents, with Oxoid arcobacter enrichment broth as basal medium. Their media were less effective for isolating *A. skirrowii* than *A. butzleri* and *A. cryaerophilus* from poultry

samples. The review articles written by Philips in 2001 and Corry *et al.* in 2003 provide more detailed information regarding the isolation media and methods used for arcobacters.

Identification

Arcobacters are not easy to culture, and they are unable to ferment or utilize carbohydrates, so that only a few biochemical tests are available for characterization and speciation, so identification of *Arcobacter* species using phenotypic tests is difficult. In addition, their close resemblance to campylobacters can lead to misidentification in routine laboratories. Catalase activity, indoxyl acetate hydrolysis, cadmium chloride susceptibility, nitrate reduction, α-hemolysis, growth on MacConkey agar and in the presence of 3.5% NaCl and 1% glycine are the most useful tests to differentiate *Arcobacter* spp. phenotypically. Whole-cell protein profiling using sodium dodecyl sulfate polyacryamide gel electrophoresis or whole-cell fatty acid profiling has also been used successfully. In addition, there is a phenotypic identification scheme based on the probabilistic identification of campylobacters and arcobacters, but these are difficult to perform for a routine laboratory and aberrant results can be encountered. Therefore, several nucleic acid-based methods such as multiplex-polymerase chain reaction (PCR), and real-time PCR, 16S rDNA sequencing and microarray techniques can be utilized accurately and definitively for the differentiation of *Arcobacter* to the species level. The primers and oligonucleotide probes used for the identification and detection of arcobacters are mainly based on a part of the 16S rRNA and/or 23S rRNA genes. A species-specific multiplex-PCR assay is widely used, which involves five primers targeting for simultaneous detection and identification of *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*. Because this m-PCR enables differentiation of only three species of arcobacters, a new 16S rDNA-restriction fragment length polymorphism method has been developed to differentiate the six species of *Arcobacter* of interest in human and/or animal health.

Molecular Detection

Although cultural methods are a well-established way of isolating pure cultures of living bacteria, the methods available for *Arcobacter* spp. are slow and labor-intensive, and not as sensitive as those used, for example, for *Salmonella* spp. Therefore, PCR-based detection–identification of *Arcobacter* spp. is often used after enrichment culture, instead of or as well as selective plating. The current PCR protocols generally target 16S or 23S rDNA sequences.

Two TaqMan-based real-time PCR methods have recently been developed using primer-probe sets developed for the *rpoB/C* and 23S rDNA sequences of *A. butzleri* and *A. cryaerophilus*, respectively. These have been used for detecting arcobacters from environmental samples, without the need for prior enrichment, with a detection sensitivity of 5–7 CFU per reaction. Use of several different real-time PCR assays with different genomic DNA targets will enable the development of better detection–identification schemes for a wider range of *Arcobacter* spp.

Control/Preventive Measures for *Arcobacter* spp.

As previously mentioned, *Arcobacter* spp. are similar to *Campylobacter* spp. except that (like *Salmonella*) they are able to multiply at lower temperatures and in air, and thus might multiply in domestic and catering kitchens, or after cross-contamination onto ready-to-eat foods. Campylobacters sometimes cause outbreaks of infection from contaminated water or milk, and a significant proportion of human cases are linked to contaminated raw poultry. Although arcobacters are common in untreated water and on raw poultry meat, human infection from these sources does not seem to be very common and the authors have very little information concerning any other sources of human infection with *Arcobacter* spp. Consumers probably do not need to be educated specifically with respect to the risks of infection with arcobacters, as they are a relatively rare infection compared to *Campylobacter* and *Salmonella*, and precautions to avoid these two more common infections should be effective against arcobacters.

In general, *Arcobacter* spp. are susceptible to various treatments including heating and food preservatives such as nisin, and lactic and citric acids, although they are somewhat more resistant than *Campylobacter* spp. to freezing, desiccation, irradiation, and disinfectants. Therefore, they do not seem likely either to survive well, or multiply in chilled processed foods. However, some field isolates of *Arcobacter* are resistant to antibiotics commonly used for the treatment of bacterial diseases in humans and animals (e.g., chloramphenicol, vancomycin, methicillin, sulfamethoxazole-trimethoprim, erythromycin, ciprofloxacin, and ampicillin) so *Arcobacter* infections might be difficult to treat.

Bacteria in biofilms or attached to surfaces are well known to be more difficult to inactivate using disinfection or heat than planktonic bacteria. The ability of *Arcobacter* spp. to colonize food processing environments indicates that they are more likely than campylobacters to contaminate foods from this source. For this reason, and their tendency to be resistant to antibiotics, they should not be ignored as possible emerging pathogens.

Research Needs

Reliable, standardized, and optimal methods are required to recover all species of *Arcobacter* including sublethally damaged strains from food, environmental, and clinical samples in addition to faster and more reliable detection methods. Currently few countries monitor human clinical samples for arcobacters, and their significance as human pathogens is not known. More information is needed concerning their antimicrobial resistance mechanisms. Studies concerning their mechanisms of pathogenicity are also needed. In addition, the ecology and epidemiology of *Arcobacter* spp. needs to be elucidated in more detail.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Safety of Food and Beverages: Poultry and Eggs

Further Reading

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