Arcobacter: An Emerging Zoonotic Pathogen

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Abstract

Arcobacter species were first isolated from aborted bovine fetuses. These bacteria have been recognized as serious enteric bacterial pathogens since the early 2000s. These enteropathogens cause gastrointestinal disorders, mastitis, infertility, and abortion in animals and endocarditis, gastroenteritis, bacteremia, diarrhea, and peritonitis in humans. *Arcobacter butzleri, Arcobacter cryaerophilus,* and *Arcobacter skirrowii*, are the main strains responsible for human infections. They are transmitted to susceptible hosts via especially raw or inadequately cooked contaminated food (raw milk, poultry, pork, beef and vegetables) or water. These products are significant sources for *Arcobacter* contamination of the environment and the human food chain. Although *Arcobacter* infections are self-limiting, a treatment protocol including fluoroquinolones, tetracyclines, macrolides or aminoglycosides is usually applied depending on the severity and duration of the disease. However, emerging of high antimicrobial resistance in these pathogens affects the results of antibiotic therapy. Therefore, this increasing and alarming drug resistance has a triggering effect for new and alternative treatment options to effectively prevent and control the spread of *Arcobacter* spp. In this context, it is especially significant to determine treatment protocols based on the results of a standard antibiotic susceptibility test.

Keywords: *Arcobacter* infections, Zoonosis, Antimicrobial Resistance, Farm animals, Poultry

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Introduction

Arcobacters are global food-waterborne pathogens that cause diarrhea, abortion, mastitis in animals, and bacteremia and enteritis in humans (Ma et al., 2022). These bacteria can be found in many sources such as animal feces, the environment (Yesilmen et al., 2017), water (Baztarrika et al., 2024), and vegetables (Uljanovas et al., 2021). The genus *Arcobacter* is becoming significant day by day in terms of public health due to several species emerging enteropathogens, the emergence of new species, and most importantly its zoonotic potential. Also, this genus has been classified as a serious hazard to human health by the International Commission on Microbiological Specifications (Yesilmen et al., 2017).

In this chapter, etiological and epidemiological characteristics, virulence factors and pathogenesis, isolation and identification, especially emerging resistance to antimicrobial treatment, and general protection, control and prevention methods applied to prevent human infections depending on the routes of transmission in terms of food and water origin of *Arcobacter* species, which have become increasingly important in recent years due to their zoonotic characteristics, were emphasized.

1. History

Arcobacters were first identified in the late 1977s as spiral-shaped bacteria isolated from aborted fetuses of sheep, cattle, and pigs. These bacteria that initially named Campylobacter, were classified as “aerotolerant Campylobacter” because of their ability to grow in the presence of atmospheric oxygen after their first isolation in a microaerobic environment. As the result of comparative 16S rRNA sequencing and DNA-rRNA hybridization technique and then further immunodetection and further immunotyping, DNA-rRNA and DNA hybridization studies and SDS-PAGE fatty acid analysis on cellular proteins, the phylogenetic relationship among aerotolerant Campylobacters was determined and then the genus *Arcobacter* was proposed for these bacteria (Donachie et al., 2005; Celik, 2016).

2. Taxonomy

Arcobacter species have a taxonomy that is based on the sequence of the 16S rRNA gene in bacteria (Abdelbaqi et al., 2007). Based on the 16S rRNA gene sequences of the identified species, it has been revealed that they show an intraspecific sequence similarity ranging from 92% to 98% (Collado & Figueras, 2011). Housekeeping genes, such as the strongly conserved genes encoding DNA gyrase subunit A (gyrA), and DNA-dependent β and β' (rpoB-rpoC) RNA polymerase subunits not only determine relationships between species but also show a high rate of intraspecific variation with 16S rRNA genes, better revealing the differences and phylogenetic proximity between species (Abdelbaqi et al., 2007).

The class *Epsilonproteobacteria* was reclassified as *Campylobacteria* as the result of a recent comparative genomic analysis and the genus *Arcobacter* was included in the family *Arcobacteraceae* of the class *Campylobacteria* and the order *Campylobacterales* (Waite et al., 2017; 2018). Then, in 2018, Pérez-Cataluña et al. (2018a), proposed to reevaluate the taxonomy of the genus *Arcobacter* using phylogenetic and genomic analyses and divide it into seven different genera (*Arcobacter*, *Aliarcobacter*, *Haloarcobacter*, *Pseudoarcobacter*, *Poseidonibacter*, *Malacobacter*, and Candidate *“Arcomarinus” gen. nov*) (Zhou et al., 2022).

Table 1: The countries and isolation sources of *Arcobacter* species (modified) (Shrestha et al., 2022)

Arcobacter Species	Isolation Sources	Country	Reference
A. cryaerophilus	Animal abortions	Ireland	Vandamme et al., 1991
A. butzleri	Feces (Human with diarrhea)	USA	Vandamme et al., 1992b
A. nitrofigilis	Roots of *Spartina alterniflora*	USA	Vandamme et al., 1992b
A. skirrowii	Diarrheic lamb, wild pig	USA	Vandamme et al., 1992b
A. halophillus	Hypersaline lagoon	USA	Donachie et al., 2005
A. cibarius	Broiler, skin	Belgium	Houf et al., 2005
A. mytili	Mussel	Spain	Collado et al., 2009
A. thereius	Aborted pig foetus, duck cloacal swab	Belgium	Houf et al., 2009
A. marinus	Dokdo island	Korea	Kim et al., 2010
A. molluscorum	Shellfish	Spain	Figueras et al., 2011
A. ellisii	Mussel	Spain	Figueras et al., 2011
A. defluvii	Sewage	Chile	Collado et al., 2011
A. trophiarum	Pig	Belgium	De Smet et al., 2011
A. bivalviorum	Shellfish	Spain	Levican et al., 2012
A. venerupis	Shellfish	Spain	Levican et al., 2012
A. suis	Pork meat	Spain	Levican et al., 2013
A. anaerophilus	Estuarine sediment	India	Sasi-Jyothsna et al., 2013
A. cloacae	Sewage, mussel	Spain	Levican et al., 2013
A. lanthieri	Pig and dairy cattle manure	Canada	Whiteduck-Léveillée et al., 2015
A. ebronensis	Mussel	Spain	Levican et al., 2015
A. aquimarinus	Seawater	Spain	Levican et al., 2015
A. pacificus	Seawater	China	Zhang et al., 2016
A. acticola	Seawater	Korea	Park et al., 2016
A. faecis	Human septic tank	Canada	Whiteduck-Léveillée et al., 2016
A. lekithochrous	Pecten maximus larvae and tank seawater	Norway	Diéguez et al., 2017
A. canalis	Water canal	Spain	Pérez-Cataluña et al., 2018b
A. caeni	Recycled wastewater	Spain	Pérez-Cataluña et al., 2019
A. lacus	Recycled wastewater	Spain	Pérez-Cataluña et al., 2019
A. antarcticus	Antartic intertidal sediment	Antarctica	Guo et al., 2019; On et al., 2021
A. vitoriensis	Wastewater	Spain	Alonso et al., 2020; On et al., 2021
A. vandammei	Porcine intestine	Belgium	Kerkhof et al., 2021
A. parvus	Squid	Korea	Kim et al., 2021; On et al., 2021
A. arenosus	Marine sediment	Korea	Baek et al., 2021; On et al., 2021
A. roscoffensis	Seawater	France	Pascual et al., 2023

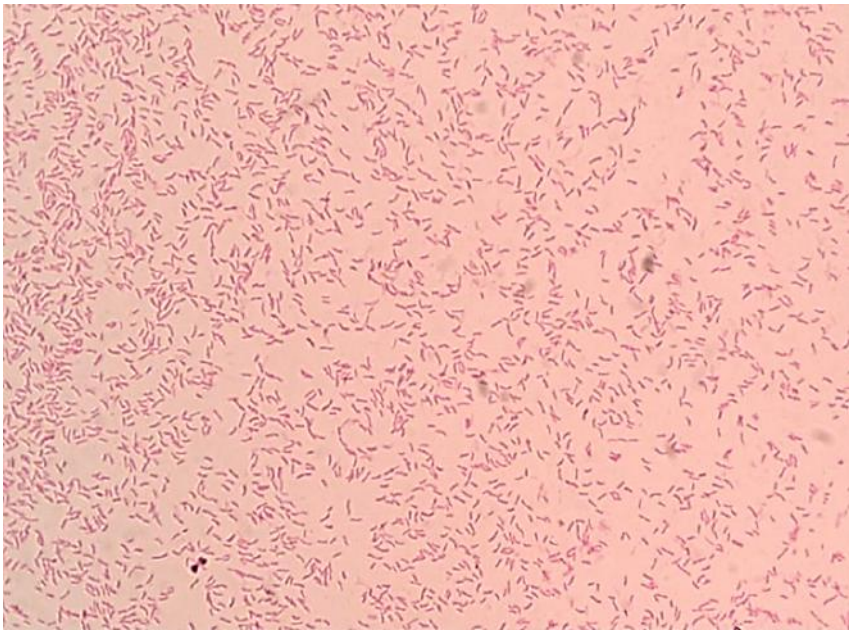

Fig. 1: Microscopic morphology of *Arcobacter* spp. after Gram staining (Celik, 2016).

The genus *Arcobacter* currently comprises a total of 34 species (Gokalp, 2023). *A. nitrofigilis* and *A. cryaerophilus* in 1991, *A. skirrowii* and *A. butzleri* in 1992, *A. halophilus* and *A. cibarius* in 2005, *A. mytili* and *A. thereius* in 2009, *A. marinus* in 2010, *A. ellisii*, *A. trophiarum A.*

molluscorum and *A. defluvii* in 2011, *A. venerupis* and *A. bivalviorum* in 2012, *A. anaerophilus*, *A. cloacae* and *A. suis* in 2013, *A. lanth*ieri, *A. ebronensis,* and *A. aquimarinus* in 2015, *A. pacificus* in 2016, *A. lekithochrous* in 2017, *A. canalis* in 2018, *A. acticola*, *A. antarcticus*, *A. caeni*, *A. faecis* and *A. lacus* in 2019, *A. arenosus*, *A. parvus*, *A. vandammei* and *A. vitoriens*is in 2021 (Table 1) (Shrestha et al., 2022; Gokalp, 2023) and *A. roscoffensis* in 2023 (Gokalp, 2023) were included in this genus.

3. Phenotypic Characteristics of the Genus *Arcobacter*

Arcobacter spp. are 0.2–0.9 width and 0.5–3 µm length, Gram-negative, and spiral-shaped bacteria (Figure 1). They are unspored and move corkscrew-like via nonshielded polar flagella (Celik & Otlu, 2020).

Growth conditions of *Arcobacter* spp. vary depending on the strain as well as the species. Although the colony morphology on blood agar is species and strain dependent, it is generally round, white, gray, blue-gray or dirty yellow, 1-4 mm in diameter and convex (Fig. 2) (Celik, 2016).

Arcobacters differ from Campylobacters due to their ability to grow in aerobic conditions at temperatures between 15-30ºC (Kayman, 2012), and different structural formations in fatty acid profiles (Gonulalan & Ertas Onmaz, 2015). While *Arcobacter* species grow easily at 25 and 30ºC, their growth at 37 and 42ºC is variable. Optimal growth occurs in microaerobic environment (3-10% CO_2) and hydrogen is not needed during growth (Vandamme et al., 1992a). Optimal aerobic and anaerobic growth occur at 30ºC and 35-37ºC, respectively (Vandamme et al., 1992b). While the optimum pH for *A. butzleri* is 6-7, this limit is 7-7.5 for *A. cryaerophilus*. The pH limit values for other species vary between 5-8.5 (Irkin & Korukluoglu, 2009).

Arcobacter spp. have weak biochemical activities. They utilize organic acids and amino acids as carbon sources because they cannot ferment/oxidize carbohydrates. The bacteria mostly possess catalase and oxidase activity (Vandamme et al., 1991). They react negatively in voges proskauer, methyl red and urease tests. They do not form indole. They usually reduce nitrate to nitrite, hydrolyze indoxyl acetate, but cannot hydrolyze hippurate, esculin and starch (Table 2) (Savasan & Ciftci, 2003). *Arcobacter* species maintain their viability at very low temperatures such as 4ºC. However, their storage at -20ºC causes a reduction in their logarithmic phase. Temperatures of 55ºC and above inactive Arcobacters (Hilton et al., 2001).

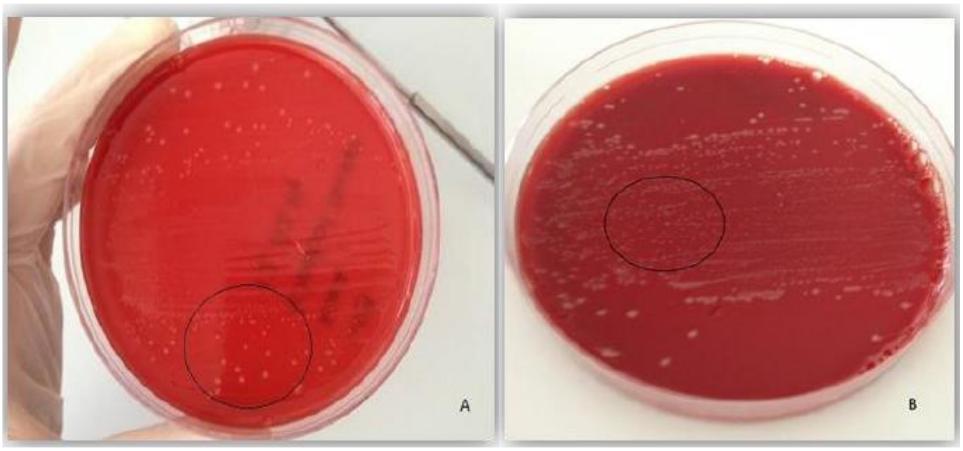

Fig. 2: Colony morphology of *Arcobacter* spp. on the blood agar plates (Plate A and B) (Celik, 2016).

Table 2: Phenotypic caharecteristics of *Arcobacter* species (modified) (Shrestha et al., 2022)

Phenotypic charasteristics	*Arcobacter* species																																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
(Arotolerance) O_2 at 37ºC	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-	-	-	+	+	-	+	+	-	+	-	+	-	+	+	+	*	-	+
(Aerotolerance) CO_2 at 37ºC	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	-	+	+	*	+	-	+	-	+	+	+	*	*	+
(Aerotolerance) CO_2 at 42ºC	-	+	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	*	-	*	+	-	+	-	-	*	*	*
Growth in 4% (w/v) NaCl	-	-	+	+	+	-	+	-	+	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+
Growth in 1% (w/v) Glycine	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+	-	+	-	-	*	*	-	-	-	-	-	-	-	*	*	-
Growth on MacConkey agar	-	-	-	-	-	+	+	-	+	+	+	-	+	+	+	+	*	+	+	-	-	-	*	+	-	v	+	+	+	*	+	+	*
Growth on CCDA	+	+	-	+	-	-	-	-	-	-	+	+	+	+	+	-	*	+	+	-	+	*	*	+	-	-	+	+	+	*	*	-	*
Urease test	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	*	-	*	-	-
Catalase test	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	*	*	-
Hippurate hydrolysis	-	-	-	-	-	-	-	-	-	-	+	-	-	-	*	*	*	*	-	*	*	+	*	-	*	*	*	*	*	*	+	*	*
Indoxyl acetate hydrolysis	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	*	+	*	-	+	+	*	+	+	*	*
Voges-Proskauer test	-	-	-	-	-	-	-	-	+	-	-	-	-	-	*	*	*	*	+	*	*	*	*	+	-	*	*	*	+	*	*	*	*
Nitrate reduction	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	-	+	+	-	+	+	*	-	+	*	-
Nitrite production	+	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*	*	+	*	*	*	-	-	*	*	*	*	*	*	*	*	*
TTC reduction	-	+	-	-	+	+	+	-	-	-	-	-	-	-	*	*	*	*	+	*	*	*	*	+	+	-	-	-	+	-	w	*	*

Arcobacter species: 1: *A. cryaerophilus*, 2: *A. butzleri*, 3: *A. nitrofigilis*, 4: *A. skirrowii*, 5: *A. halophilus*, 6: *A. cibarius*, 7: *A. mytili*, 8: *A. thereius*, 9: *A. marinus*, 10: *A. molluscorum*, 11: *A. ellisii*, 12: *A. defluvii*, 13: *A. trophiarum*, 14: *A. bivalviorum*, 15: *A. venerupis*, 16: *A. suis*, 17: *A. anaerophilus*, 18: *A. cloacae*, 19: *A. lanthieri*, 20: *A. ebronensis,* 21: *A. aquamarinus*, 22: *A. pacificus*, 23: *A. acticola*, 24: *A. faecis*, 25: *A. lekinthochrous*, 26: *A. canalis*, 27: *A. caeni*, 28: *A. lacus*, 29: *A. vitoriensis*, 30: *A. vandammei*, 31: *A. parvus*, 32: *A. antarcticus,* 33: *A. arenosus.* '*': Not determined. '+': Positive, '-': Negative, 'v': 12–94% of strains positive, 'w': Weakly positive.

4. Epidemiology

i. Arcobacter Species in Water Sources

Water is an effective source in the transmission of *Arcobacter* species to animals and humans. Most of the members belong to this genus have been determined in various water sources around the world such as water channels, groundwater, surface water, water wells and rivers, lake water, irrigation water, activated sludge, flood and stormwater (Carson et al., 2024), sewage, and drinking water, seawater, marine sediments and estuaries (Celik, 2016). Even the presence of these bacteria (in particular, *A. butzleri, A. skirrowii*, and *A. cryaerophilus*) environmental waters has also been associated with fecal indicator bacterial contamination as well as the human fecal marker HF183 (Carson et al., 2024).

ii. Arcobacter Species in Foods

Arcobacter spp. have been isolated from various meat specimens including beef, pork, seafood, and poultry. The highest prevalence for *Arcobacter* species was observed in poultry meat (especially chicken meat), followed by beef and pork (Disli et al., 2024).

Arcobacter butzleri, followed by *A. cryaerophilus* was the most common *Arcobacter* species found in meat samples. Contact of contaminated animal feces with carcasses in slaughterhouses is an important factor in the transmission of *Arcobacter* species to meat (Disli et al., 2024). Intestinal contents may contaminate the carcass during and/or post-slaughter. This can pose a serious public health threat if contaminated carcasses are not cleaned, stored and cooked properly (Reddy & Zishiri, 2018).

Arcobacter species have also been detected in milk and dairy products (Fusco et al., 2020). In particular, *A. butzleri* is one of the *Arcobacter* species that can survive in UHT, pasteurized and raw milk and during the processing and storage of dairy products such as fresh village cheese, sheep ricotta cheese and buffalo mozzarella cheese (Yesilmen et al., 2014). However, *A. cryaerophilus* and *A. butzleri* can remain alive for six days in milk stored at 4ºC and 10ºC (Giacometti et al., 2014). One of the most effective factors in the persistence of Arcobacter species in raw milk is the deficiency of sanitation of this food source (Giacometti et al., 2013). Therefore, milk is one of the major sources of human infections caused by *Arcobacter* species (Giacometti et al., 2014).

Another food that poses a public health risk by carrying *Arcobacter* species is raw vegetables. These foods include fresh vegetables such as spinach, arugula, parsley, radish, lettuce (Abay et al., 2022), chard, and cabbage (Gonzalez et al., 2017). While the commonly found species in vegetables was A. *butzleri, the* species reported *in* green leafy vegetables was A. *cryaerophilus* (Buzzanca et al., 2024).

Arcobacter species have been isolated from various fish and many shellfish. *A. molluscorum* and *A. mytili*, *A. bivalviorum*, *A. ebronensis*, and *A. ellisii* from mussels, A. *venerupis*, *A. molluscorum A. b*ivalviorum, *A. halophilus,* and *A. marinus*, were isolated from oysters. The most common species observed in bivalve species is *A. butzleri*. Therefore, consumption of especially undercooked or raw shellfish contaminated with *A. butzleri* can cause infections in humans (Eser, 2022).

iii. Arcobacter Species in Animals

In animals Arcobacters generally cause diarrhea and mastitis. *A. butzleri* causes enteritis or diarrhea in farm animals such as horses, cattle, and pigs. *A. skirrowii* causes hemorrhagic colitis or diarrhea in cattle and sheep (Ho et al., 2006). Toxin production by *A. cryaerophilus* and *A. butzleri* in cases where no other abortion agent is present, and the presence of *Arcobacter* species in the internal organs of the aborted foetus provide data on the role of *Arcobacter* species in causing abortion in animals. *A. cryaerophilus* is the dominant species isolated from aborted animals. In contrast, *A. skirrowii* and *A. butzleri* have been reported less frequently (Ramees et al., 2017). *A. thereius* was isolated from the kidneys and liver of aborted pig fetuses (Houf et al., 2009).

Arcobacter spp. have also been isolated from healthy animals. Species the most frequently isolated from healthy animals are *A. butzleri* and followed by *A. cryaerophilus* (Van Driessche et al., 2003). Healthy cattle and some other animals are known to be reservoirs for *Arcobacter* species, as in poultry (Van Driessche et al., 2005). In cattle, *Arcobacter* spp. is present in vaginal swabs, preputial sheath washings feces, mastitic milk, and various internal organs. Isolation rate of *Arcobacter* spp. in fecal material is especially high in dairy cows (Kabeya et al., 2003).

Pigs are also recognized as an important reservoir of *Arcobacter* spp. However, Arcobacters cause infertility, reproductive disorders and gastric ulcers in pigs (Eser, 2022). *A. butzleri* has been associated with gastroenteritis and *A. thereius* with abortion cases in pigs. However, due to the isolation of *A. trophiarum* and *A. cibarius* from pig manure and pig farm waste, there is a view that pigs are asymptomatic carriers for these agents (Collado & Figueras, 2011; Shange et al., 2019).

Isolations of *A. cryaerophilus* and *A. butzleri* are high in sheep as well as pigs and cattle. These hosts are also considered as reservuars of *Arcobacter* species, but it has also been reported that *A. skirrowii* can cause diarrhea and hemorrhagic colitis (Shange et al., 2019).

Poultry with high isolation rates of *Arcobacter* species not only harbor multiple species, but also carry versatile genotypes of some species. These animals are recognized as potential reservoirs for *Arcobacter* species. Geese and ducks, which are waterfowl, can be an important source of *Arcobacter* spp. contamination in stagnant freshwater. Along with turkeys, these aquatic birds are carriers of *A. cryaerophilus, A. skirrowii,* and *A. butzleri* (Eser, 2022).

Pet animals such as cats and dogs are also considered as reservuars of *Arcobacter* species (Fera et al., 2009). In addition, regarding the prevalence of *Arcobacter* spp. in non-domesticated and wild animals, isolation of *Arcobacter* species from the digestive tracts of raccoon (Hamir et al., 2004), galapos tortoise, white rhinoceros, gazelle, rhea and alpaca has been reported (Wesley et al., 2003).

Both vertical and horizontal transmission of *Arcobacter* species in animals has been reported (Celik et al., 2022). Related to vertical transmission in sows has been reported *A. cryaerophilus* has the ability to penetrate the intestine and placenta and is transferred to the offspring. Similar information is not available for cattle, sheep and poultry. In relation to, it has been demonstrated that breeding hens carrying *Arcobacter* spp. in the gastrointestinal tract and mucosa of the oviduct magnum are unable to transmit *Arcobacter* spp. to offspring (Shange et al., 2019).

iv. Arcobacter Species in Human

Arcobacter spp. have been isolated from blood and feces in humans and symptoms can change from diarrhea to septicemia. An acute diarrhea case is more watery compared to campylobacteriosis and lasts 3-15 days, sometimes lasting for more than two weeks or even up to two months, or it may follow a persistent or recurrent course. This situation is usually accompanied by nausea and abdominal pain, and some patients also vomiting, chills, malaise, experience fever. Also, symptoms of vomiting, nausea, fever or abdominal pain, without diarrhea have been associated with the presence of the bacteria. The worrying grup is immunocompromised patients (Gabucci et al., 2023). Infections in these patients can result in endocarditis, peritonitis, and bacteremia (Uljanovas et al., 2021). The most important risk is recurrence of the infection (Gabucci et al., 2023). The first evidence for this is the diagnosis of peritonitis caused by *Arcobacter* spp. in an elderly peritoneal dialysis patient with renal failure and a peritoneal dialysis catheter (Yap et al., 2013). The first *Arcobacter* species reported in humans was *A. cryaerophilus*. While *A. cryaerophilus* and *A. butzleri* are the most common encountered species in clinical outbreaks, *A. skirrowii*, *A. thereius* and *A. cibarius* species have also been associated rarely (Buzzanca et al., 2024).

Arcobacter spp. infections in humans are frequently due to raw consumption or inadequate cooking of food products (meat, milk, seafood) or consumption of contaminated water (Uljanovas et al., 2021). In addition, fecal contamination of at various stages of food production is considered one of the main routes of transmission. In addition, maintaining their viability for longer periods of timeforming biofilms in environments in the food production chain, and developing resistance to agents used in sanitation together with disinfectants have been reported. Because of all these characteristics, *Arcobacter* species stands out as a recognized and dominant human pathogen that poses a potential threat to the health of consumers, especially immunocompromised individuals (Gabucci et al., 2023). Furthermore, several studies have pointed to the pathogenic potential of *Arcobacter* species such as *A. faecis, A. vitoriensis* and *A. lacus*, even if not associated with human pathology (Baztarrika et al., 2024).

5. Virulance Factors of *Arcobacter* Species

Currently, information on the virulence properties and pathogenic mechanisms (adhesion, cytotoxicity, invasion) of *Arcobacter* strains is limited (Šilha et al., 2019). However, in vitro studies have shown that, in addition to *Arcobacter* species develop resistance to various antimicrobial agents and cause disruptions in the intestinal epithelial barrier, they adhere and invade different intestinal (IPI-2I, Caco-2, Caco-2/HT29-MTX, HT29, IPEC-J2, and HT29/B6) and extraintestinal cell lines (HeLa, INT407, Hep-2) and cause cytotoxic effects on some of them (Brückner et al., 2020). Comparative genomic analyses and next-generation sequencing have identified several virulence genes important for the virulence of *Arcobacter* species. Ten of them are commonly used as virulence markers in *Arcobacter* species (Baztarrika et al., 2024). These potential virulence genes include *ciaB*, which encoding the *Campylobacter* invasive antigen that involves in host cell invasion; *cadF* and *cj1349*, encoding outer membrane proteins that enable intercellular communication with intestinal epithelial cells and adhere to fibronectin; *mviN*, encoding the sigma factor required for expression of the flg operon, which enables flagella synthesis and the essential protein *mviN* required for peptidoglycan synthesis; *pldA* phospholipase gene encoding the outer membrane phospholipase protein *pldA* associated with hydrolyzation acyl ester bonds and lysis of erythrocytes; *tlyA* hemolysin gene; *irgA* gene encoding the outer membrane receptor required for the iron regulatory protein enterobactin; the *hecA* gene encoding the *hecA* protein which is a part of the filamentous hemagglutinin family, and the *hecB* gene encoding the hemolysin activation protein (Douidah et al., 2011), and the *iroE* gene, which is active in iron assimilation (Baztarrika et al., 2024). Apart from these *A. butzleri* contains also PorA antigen on its outer membrane which is important factor in the virulence and host adaptation of the microorganism (Isidro et al., 2020), and *A. lantheri* has cytotoxin (CDT) genes encoding *cdtABC* (Brückner et al., 2020).

As can be understood from the above mentioned, potential virulence factors of *Arcobacter* species are mostly associated with cell invasion, toxin production, and induction of immune responses. In fact, researches have shown that *A. butzleri* has adhesion, invasion and cytotoxicity and can induce the expression of interleukin-8 (IL-8, proinflammatory cytokine) in IPI-2I and Caco-2 cell lines (Uljanovas et al., 2023). IL-8 stimulation, a proinflammatory cytokine, is also a virulence factor in *A. cryaerophilus, A. skirrowii* and *A. cibarius* as well as *A. butzleri* (Collado & Figueras, 2011).

6. Pathogenesis of *Arcobacter* Species

Although the pathogenicity of *Arcobacter* spp. is still unclear, so various in vivo and in vitro studies have tried to understand these characters (Collado & Figueras, 2011).

A 20 kDa hemagglutinin, which is thought to interact with a glycan receptor containing D-galactosidase involved in adhesion in *Arcobacter* spp. has been characterized as sensitive to proteolytic digestion and inactivation at 80°C (Gonulalan & Ertas Onmaz, 2015). A lectin-like molecule that binds to erythrocytes via a glycan receptor containing D-galactosidase has also been reported to be part of this structure (Tsang et al., 1996). Mammalian cell-associated cytotoxin activity was detected in *A. butzleri* strain NCTC 12481 (Hilton et al., 2001).

Arcobacter strains are known that have adhesion and invasion abilities as well as cytotoxicity in human Caco-2 cells, and IPI-21 cells. In addition, the factors decrease the expression of claudin protein, which is responsible for tight junctions between intestinal epithelial cells and barrier sealing properties (Sertcelik, 2021). Decreased levels of these TJs proteins lead to epithelial barrier disorders and apoptosis of epithelial cells and consequently to leaky diarrhea (Taslı, 2023).

7. Diagnosis of *Arcobacter* Species

i. Isolation of *Arcobacter* spp.

Although different pre-enrichment and isolation methods have been developed and are currently used for optimal isolation of *Arcobacter* spp. from different sources (animal food products, milk, rectal and oral fluid, water) there is no nowadays standardized method. (Shrestha et al., 2022). For the purpose of isolation of *Arcobacter* spp., Arcobacter selective broth (ASB I), Arcobacter enrichment medium, Johnson and Murano broth (JMB), and Ellinghausen- McCullough-Johnson-Harris-Polysorbate 80 medium (EMJH P80), Arcobacter medium supplemented

with 2.5% NaCl and Arcobacter selective broth (ASB II) are used (Rahman et al., 2020). JMB supplemented with cefoperazone, amphotericin, teicoplanin (CAT) supplement is also used for enrichment. Cefoperazone, 5-fluorouracil, trimethoprim, piperacillin, amphotericin B, cycloheximide, novobiocin, and teicoplanin added to the media used for pre-enrichment inhibit the growth of undesirable microorganisms and help the selective growth of *Arcobacter* spp. Following pre-enrichment, media such as Johnson-Murano agar (JMA), Cephalotin-vancomycin-amphotericin B (CVA) agar, modified charcoal cefoperazone deoxycholate agar (mCCDA) with CAT supplement (Nguyen et al., 2023) are used for cultivation to isolate *Arcobacter* spp. *A. butzleri* also grows on standard agars such as chocolate agar, MacConkey agar, and blood agar at 37°C and under standard conditions such as atmosphere enriched with 5% CO_2 (Binder et al., 2023).

The membrane filtration method is also used for isolation. This method is generally applied after pre-enrichment stage (Figure 3). *Arcobacter* species can also be isolated from fecal samples by direct inoculation on a selective solid medium (Kayman, 2012).

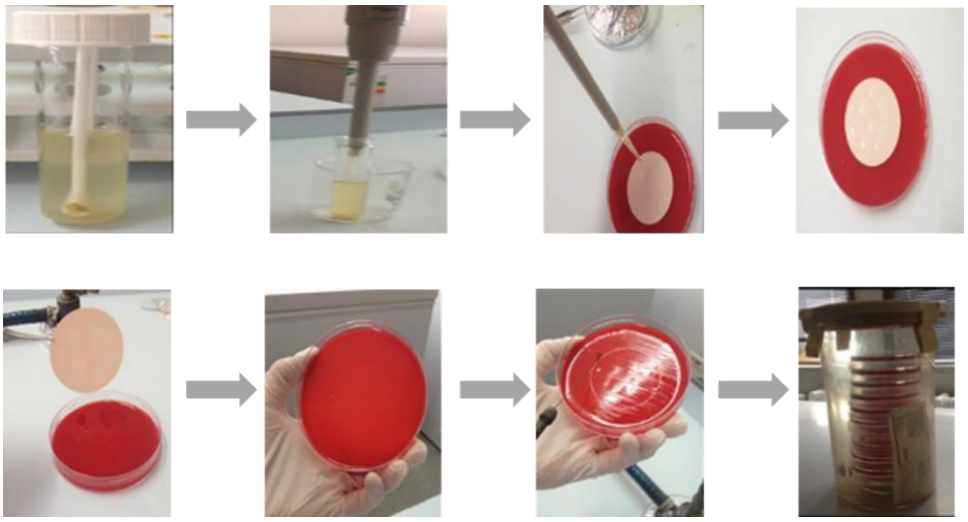

Fig. 3: Membrane filtration method for the isolation of *Arcobacter* spp (Celik, 2016).

ii. Identification of *Arcobacter* spp.

a. Phenotypic Identification

Growth conditions of *Arcobacter* spp. vary depending on the strain as well as the species. Although the colony morphology on blood agar is species and strain dependent, it is generally round, white, gray, blue-gray or dirty yellow, 1-4 mm in diameter and convex (Picture 1) (Celik, 2016). Colony morphology, Gram staining, motility examination and biochemical tests are used for phenotypic identification of suspected *Arcobacter* spp. isolates obtained from pure culture. *Arcobacter* spp. have Gram-negative cell wall structure and stain pink in Gram staining. Biochemical tests used in identification are oxidase and catalase tests, indoxyl acetate and hippurate hydrolysis and urease activity tests (Pérez-Cataluña et al., 2018; Celik and Otlu, 2020).

b. Molecular Identification

Many different methods are used to distinguish one *Arcobacter* strain from another, to examine transmission routes and to track outbreak sources such as conventional PCR, multiplex-PCR, ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus), Real Time PCR, PFGE (Pulsed Field Gel Electrophoresis), AFLP (Amplified Fragment Length Polymorphism), MALDI TOF MS (Matrix Assisted Laser Desorption Ionization-time-of-flight Mass Spectrometry), and FISH (Fluorescence in situ Hybridisation), MLST (Multilocus Sequence Typing) (Collado & Figueras, 2011).

8. Treatment and Emerging Antibiotic Resistance

Arcobacter spp. infections are self-limiting like *Campylobacter* infections and most of them can heal spontaneously without any antibiotic treatment. However, antibiotic treatment is recommended when necessary in case of severity and prolonged duration of the disease, as well as in immunocompromised individuals (Sertcelik, 2021). Within the therapeutic management, tetracyclines, fluoroquinolones, aminoglycosides, macrolides, and β-lactam antibiotics in combination with β-lactamase inhibitors are mostly recommended as appropriate treatment strategy these infections (Uljanovas et al., 2021).

However, a recent meta-analysis revealed emerging resistance in *Arcobacter* spp. to fluoroquinolones, macrolides, tetracyclines, and penicillins (Uljanovas et al., 2021). Fluoroquinolone resistance has been reported to be caused by *gyrA* mutations. Fluoroquinolone-resistant *Campylobacter*-like organisms were classified as part of 12 antibiotic-resistant priority pathogens that pose the greatest threat to human health by the the World Health Organization (WHO). More than 50 genes are known to be associated with tetracycline resistance, especially in *Arcobacter* of environmental origin (Paintsil et al., 2023). High resistance rates to quinolones in *Arcobacter* spp. are also noteworthy. Resistance to the third-generation antibiotic cefotaxime in *A. butzleri* shows that the susceptibility of this bacterium to new antimicrobials is decreasing. In addition, the development of multidrug resistance in *Arcobacter* is also important (Gabucci et al., 2023).

In addition, antibiotic resistance in *Arcobacter* is mostly associated with decreasing of antibiotic effectiveness in case of infection or the presence of efflux pumps that can be effective in conferring these properties to a wide range of antibiotics, possible horizontal gene transfer of antibiotic resistance genes to other bacteria, specific antimicrobial resistance genes, genetic variants and the presence of orthologs (Buzzanka et al., 2024).

Antibiotic susceptibility testing is used to determine the sensitivity/resistance profiles of *Arcobacter* spp. isolates from animals, humans, and the environment to various classes of antibiotics. However, there is no standardized procedure for determining antimicrobial susceptibility for these bacteria, even though the procedures recommended by and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) for the *Campylobacter* or *Enterobacterales* are normally used for *Arcobacter* spp. This situation shows that *Arcobacter* spp. should be included in internationally recognized official procedures (Buzzanca et al., 2024).

9. Ways of Protection, Control and Prevention

Considering that *Arcobacter* spp. are water and foodborne pathogens, it is important to implement practices for these sources in terms of protection and control methods. These include good cooking practices, food acid treatment (1-2% citric and lactic acid), slaughter hygiene and carcass control, effective sanitation of water sources and monitoring of their contamination, Hazard Analysis and Critical Control Point (HACCP) and good manufacturing practices (Gabucci et al., 2023). Prevention of contamination of a food, especially raw meat, before and post appropriate heat treatment is very effective and important in the control of pathogenic bacteria in foods. In this regard, heating food to an internal temperature of 70°C and irradiating it with 0.27-3.3 kGy (the amount of energy absorbed by ionizing radiation per unit mass of matter) for 10 seconds can also inactivate *Arcobacter* spp. As known, *Arcobacter* species are temperature sensitive, and are easily inactivated at temperatures of about 55°C and above. Therefore, heat treatment of animal foods is effective in inactivating the agent and storage conditions may be accompanied to this. Food storage conditions can also negatively affect *Arcobacter* spp. In this respect, it is important to store meat at or around 4°C (Sertcelik, 2021).

These bacteria, which cause diarrhea by maintaining their viability for a long time (about 35 days) especially in unchlorinated drinking water, are destroyed within 5 minutes by chlorination (Andersen et al., 2007). Many chemicals such as 3.5-4% salt concentrations (except *A. halophilus*) and 0.02% trisodium polyphosphate are effective in the inhibition of *Arcobacter* spp., especially in foods. Optimization of hygiene practices in slaughterhouses is one of the most effective ways to reduce *Arcobacter* spp. in poultry and red meat, an important source of animal food (D'sa & Harrison, 2005).

Apart from those mentioned above, another alternative way to inhibit *Arcobacter* species is to use herbal extracts with natural antimicrobial properties. These include essential oils such as orange oil (Nannapaneni et al., 2009), cinnamon, bay, clove, rosemary, (Fisher et al., 2007; Irkin et al., 2011), allspice, garlic, licorice, bergamot, blackberry, cumin, coriander, ginger, thyme, fennel, sage, black pepper, peppermint and chamomile (Fisher et al., 2007).

Conclusion

It is very important to continue comprehensive research on Arcobacters and thus fully understand the ecology of Arcobacters, the complex structure of host/*Arcobacter* interactions, the virulence factors associated with the pathogenesis of these bacteria, and response of the immune system to these microorganisms. In light of all these issues, determining the mechanisms that enable Arcobacters to maintain their viability and the mechanisms and sources of resistance developed in these bacteria against both antimicrobials and adverse environmental conditions will play an important role in developing effective prevention and control strategies and thus reducing the incidence of human infections

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