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Original article

Prevalence and antimicrobial resistance of *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolates from retail meat in Lower Silesia region, Poland

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Abstract

Arcobacter butzleri and *A. cryaerophilus* are considered potential foodborne pathogens. Consumption of *Arcobacter*-contaminated food is regarded the most likely source of human poisoning. We investigated the prevalence and antimicrobial resistance of *Arcobacter* isolates in 210 retail meat samples. Seventy-nine *A. butzleri* and 6 *A. cryaerophilus* were isolated from pork, beef and chicken meat. Incidence of *A. butzleri* was found to be the highest in chicken meat (83%). Less of *A. butzleri* was isolated from beef (16%) and pork (14%). Most of the *A. butzleri* isolates were resistant to β-lactams, like ampicillin (85%), amoxicillin with clavulonic acid (63%), cefotaxime (66%) and macrolides, i.e., erythromycin (62%). In contrast, all except one *A. cryaerophilus* isolates were susceptible to erythromycin. Tetracycline and aminoglycosides showed the highest efficacy against *A. butzleri* and *A. cryaerophilus* since almost 80% of their population was susceptible to these agents. All, except one *A. cryaerophilus* and the majority of *A. butzleri* isolates (70%) were susceptible to fluoroquinolones. The incidence of multiresistant isolates was found in forty two (53%) *A. butzleri*, and one (16%) *A. cryaerophilus* isolates. Eight *A. butzleri* isolates were resistant to all antimicrobials tested. These results indicate significant incidence of potential foodborne zoonotic agents, i.e. *A. butzleri* and *A. cryaerophilus* including multiresistant isolates in retail meat in Poland.

Key words: *Arcobacter* spp., antimicrobial resistance, pork, beef, chicken meat

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Introduction

Genus *Arcobacter*, formerly known as „aerotolerant *Campylobacter*", includes motile, non-spore forming, Gram-negative bacteria (Neill et al. 1979). They form curved or S-shaped rods and have single polar flagellum at one or both ends of the cell (Ursing et al. 1994). Genus *Arcobacter* was included into the family *Campylobacteraceae* in 1991 (Vandamme and De Ley 1991), and currently consists of eighteen species (Levican et al. 2013) of which *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* have been associated with a variety of diseases in humans and animals (Vandamme et al. 1992a, On et al. 2002, Wybo et al. 2004). *Arcobacter* spp. can be differentiated from the *Campylobacter* by its ability to grow in aerobic conditions and at lower temperatures oscillating between 15-30°C (Ursing et al. 1994).

Arcobacter spp. has already been isolated from food, mainly from products of animal origin, with the highest prevalence in poultry, followed by pork and beef (Kabeya et al. 2004, Rivas et al. 2004, Gude et al. 2005). The bacteria have been detected in the intestinal tract of healthy domestic animals (Kabeya et al. 2003, Van Driessche et al. 2003), thus faecal contamination is regarded the main route of transmission of *Arcobacter* spp. into the food (Ho et al. 2008). Occurrence of these bacteria in meat has already been investigated in some countries, but not in Poland.

Arcobacter species were also isolated from drinking water reservoirs, sewage (Moreno et al. 2003) and from diseased animals, including cases of *mastitis*, septicaemia, *enteritis* and abortion (Logan et al. 1982, Vandamme et al. 1992b, Karpińska et al. 2009). The bacteria can attach to water pipes surfaces and resist low temperatures in the slaughterhouse environment (Assanta et al. 2002).

Recent evidence suggests that *Arcobacter* spp., especially *A. butzleri* and *A. cryaerophilus,* may be involved in human enteric diseases (Tee et al. 1988, Vandamme et al. 1992a, Lappi et al. 2013). Occasionally these species have also been found in cases of human extraintestinal diseases (Hsueh et al. 1997, Lau et al. 2002). Consumption of *Arcobacter*-contaminated food or water is regarded the most likely source of human poisoning (Ho et al. 2006). Infections caused by *Arcobacter* spp. are usually self-limiting, but in cases of chronic and severe conditions treatment is required (Vandenberg et al. 2004).

Resistance to commonly used antibiotics observed among *Arcobacter* spp. emphasises the importance of research in this area (Houf et al. 2001, Kabeya et al. 2004, Son et al. 2007, Shah et al. 2012).

The aim of this work was to determine the prevalence of *A. butzleri* and *A. cryaerophilus* in pork, beef and chicken retail meat in Lower Silesia region, Poland and to determine the antimicrobial resistance of the isolates.

Materials and Methods

Isolation of *Arcobacter* spp.

Two hundred and ten of retail meat samples (seventy samples were taken from each pork, beef and chicken meat) were screened for the presence of *Arcobacter*. The samples were taken from four randomly selected supermarkets in Lower Silesia region, Poland. The sampling was performed twice a month during a six-month period in 2013. During every sampling session ca. 5 meat samples (pork – loin, ham; beef – chuck, rostbef; chicken – wing and drumstick with the skin) were purchased from each of the 4 supermarkets. The meat samples were packaged in sterile plastic bags and transported up to 2 hours to the laboratory. Ten grams of the meat sample were added into 90 ml of Arcobacter broth (Oxoid) with selective supplement containing cefoperazone, amphotericin B and teicoplanin (CAT, Oxoid). Additionally, novobiocin (32 mg/l), 5-fluorouracil (100 mg/l) and trimethoprim (64 mg/l) (Sigma) were added to the broth. The mixture was homogenized in a Lab Paddle Blender, Masticator. After 48-hours-incubation in aerobic atmosphere at 30°C, the bacteria were subcultured on Arcobacter agar plates (supplemented with mentioned above chemotherapeutics) and in parallel on agar plates with defibrinated sheep blood (Oxoid). Phenotypically suspected colonies (motile, Gram-negative, oxidase and catalase positive) were transferred to blood agar plates and incubated in aerobic conditions for 48 h at 30°C. One *Arcobacter* spp. isolate per sample was taken for further characterization. The isolates were preserved by freezing in Cryobank (Mast Diagnostics) at -80°C.

Preparation of bacterial DNA

Total DNA was isolated as described by Agersborg et al. (1997). Briefly, the bacteria from 1 ml overnight culture in Arcobacter broth (Oxoid) were pelleted by centrifugation and suspended in 200 µl of distilled water containing 1% Triton X-100. The mixture was boiled for 10 min and then the tubes were centrifuged for 5 min at 13 000 × g. The supernatant containing DNA was used in PCR.

Table 1. Antimicrobial resistance of *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolates derived from meat samples.

Source/Number of samples	Species	Number of isolates (% of samples)	Number of resistant isolates (%)							
			A*	AC	C	G	T	E	CI	N
Chicken meat/70	*A. butzleri*	58 (83)	53 (91)	38 (65)	41 (71)	11 (19)	11 (19)	34 (59)	15 (26)	16 (28)
	A. cryaerophilus	2 (3)	2 (100)	2 (100)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pork/70	*A. butzleri*	10 (14)	7 (70)	7 (70)	8 (80)	4 (40)	4 (40)	9 (90)	5 (50)	5 (50)
	A. cryaerophilus	1 (1)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Beef/70	*A. butzleri*	11 (16)	7 (64)	5 (45)	3 (27)	1 (9)	2 (18)	6 (54)	2 (18)	3 (27)
	A. cryaerophilus	3 (4)	0 (0)	0 (0)	1 (33)	0 (0)	1 (33)	1 (33)	1 (33)	0 (0)
Total/210	*A. butzleri*	79 (38)	67 (85)	50 (63)	52 (66)	16 (20)	17 (21)	49 (62)	22 (28)	24 (30)
	A. cryaerophilus	6 (3)	2 (33)	2 (33)	4 (67)	0 (0)	1 (17)	1 (17)	1 (17)	0 (0)

* A – ampicillin (10 µg/disc), AC – amoxicillin with clavulonic acid (20/10 µg/disc), C – cefotaxime (30 µg/disc), T – tetracycline (30 µg/disc), G – gentamicin (10 µg/disc), , E – erythromycin (15 µg/disc), CI – ciprofloxacin (5 µg/disc), N – norfloxacin (10 µg/disc).

Species identification of *Arcobacter* isolates

The isolates were identified as *A. butzleri* or *A. cryaerophilus* using multiplex PCR according to Houf et al. (2000). Amplification products were resolved in 1.5% agarose containing 0.5 µg/ml ethidium bromide and documented using GelDocXR System (Bio-Rad, Hercules, CA). Each PCR run was performed using DNA from the reference CCM 4826 *A. butzleri* and CCM 3933 *A. cryaerophilus* strains as positive controls, and ATCC 33560 *Campylobacter jejuni* as a negative control.

Antimicrobial resistance

Susceptibility of *Arcobacter* spp. isolates to ampicillin (10 µg/disc), amoxicillin with clavulonic acid (20/10 µg/disc), gentamicin (10 µg/disc), cefotaxime (30 µg/disc), tetracycline (30 µg/disc), erythromycin (15 µg/disc), ciprofloxacin (5 µg/disc) and norfloxacin (10 µg/disc) (all substances from Oxoid Ltd., United Kingdom) was tested by the disk-diffusion method. Briefly, the isolates were grown aerobically in *Arcobacter* broth (Oxoid) at 30°C for 48 h. After cultivation, a suspension of bacteria was prepared in physiological saline and the turbidity of inoculum was adjusted to McFarland 0.5. Bacteria were plated onto Mueller-Hinton agar plates (Merck). Thereafter, disks were placed onto the agar and the plates were incubated in aerobic atmosphere at 30°C for 48 h. To date, no recommendation of breakpoints values for *Arcobacter* spp. are available, thus according to Shah et al. (2012) classification of isolates as resistant or susceptible was based on breakpoints values recommended for *Enterobacteriaceae* (CLSI 2010). Reference *E. coli* ATCC 25922 strain served as control.

Results

Prevalence of *A. butzleri* and *A. cryaerophilus* in beef, pork and chicken meat

In total, 85 *Arcobacter* isolates including 79 *A. butzleri* and 6 *A. cryaerophilus* isolates, were obtained from 210 retail meat samples. The highest prevalence of *Arcobacter* spp. was found in chicken meat (60 samples, 86%), followed by beef (14 samples, 20.0%) and pork (11 samples, 15%). *A. cryaerophilus* was detected in 4%, 3% and 1% of beef, chicken and pork meat samples, respectively. *A. butzleri* isolates were found in 83% of chicken meat, 16% of beef and 14% of pork samples (Table 1).

Antimicrobial resistance of *A. butzleri* and *A. cryaerophilus* isolates

Most of the *A. butzleri* isolates were resistant to β-lactam antibiotics, i.e., ampicillin (85%), amoxicillin with clavulonic acid (63%), cefotaxime (66%) and macrolides, i.e., erythromycin (62%) (Table 1). Ampicillin resistance was found in 64%, 70% and 91%

Table 2. Number of multiresistant *Arcobacter butzleri* isolates from meat samples.

Source/Number of isolates	Number of *A. butzleri* isolates resistant to a given number of antimicrobials					Number of *A. butzleri* isolates resistant to at least 4 antimicrobials (%)
	4 antimicrobials	5 antimicrobials	6 antimicrobials	7 antimicrobials	8 antimicrobials	
Chicken meat/58	17	5	5	–	6	33 (57)
Pork/10	–	2	1	1	2	6 (60)
Beef/11	1	1	–	1	–	3 (27)
Total/79	18	8	6	2	8	42 (53)

A. butzleri isolates from beef, pork and chicken meat, respectively. Similarly, most of chicken (65%) and pork-derived (70%) *A. butzleri* isolates were resistant to amoxicillin with clavulonic acid (AC) and cefotaxime. In contrast, less than a half of *A. butzleri* isolates from beef showed resistance to AC (45%) and cefotaxime (27%). Among six *A. cryaerophilus* isolates only those from chicken meat were resistant to all β-lactams tested, while the others were susceptible to AC and ampicillin (Table 1).

The highest resistance to erythromycin was observed among *A. butzleri* isolates from pork (90%), followed by isolates from chicken meat (59%) and beef (54%). In contrast, all except one, *A. cryaerophilus* isolates were susceptible to erythromycin (Table 1).

The majority of *A. butzleri* isolates from chicken meat (81%), beef (82%) and pork (60%) showed susceptibility to tetracycline. Similarly, most of *A. butzleri* isolates from chicken meat (72%), beef (73%) and pork (50%) were susceptible to fluoroquinolones (ciprofloxacin and norfloxacin). All, except one, *A. cryaerophilus* isolates were also susceptible to tetracycline and fluoroquinolones (Table 1).

Resistance to gentamicin was low among *A. butzleri* isolates from beef (9%) and chicken meat (19%), while the isolates from pork showed higher resistance to this antibiotic (40%). All *A. cryaerophilus* isolates were susceptible to gentamicin (Table 1).

The incidence of multiresistant isolates, defined as resistant to four or more antimicrobials, was reported in forty two (53%) *A. butzleri*, and one (16%) A. *cryaerophilus* isolates. The highest prevalence of multiresistant *A. butzleri* isolates was observed in population derived from pork (60%) and chicken meat (57%). Eight *A. butzleri* isolates (six from chicken meat and two from pork) were resistant to all tested antimicrobials (Table 2).

Discussion

The increasing occurrence of *Arcobacter* spp. in food as well as its considerable antimicrobial resistance enhances the research on significance of these bacteria for food safety. As yet, the incidence of *Arcobacter* spp. was only investigated in limited number of countries. In the present study, the prevalence of *Arcobacter* species in chicken meat (86%) was significantly higher than that in beef (20%) and pork (16%). The results are in agreement with observations of other authors, which showed that poultry meat is the most important source of *Arcobacter* (Kabeya et al. 2004, Rivas et al. 2004, Van Driessche and Houf 2007, Ho et al. 2008). In Japan, 23% of chicken, 7% of pork and 2% of beef retail meat samples were positive for *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* (Kabeya et al. 2004). Similarly, in Korea *A. butzleri* was detected in 19% of chicken meat, however it was not found in pork and beef (Lee et al. 2010). To date, a higher prevalence of *Arcobacter* spp. was observed in Australia, where 73% of chicken, 29% of pork and 22% of beef meat samples were found positive (Rivas et al. 2004). Studies conducted in Denmark and Holland also showed high prevalence (≥70%) of *A. butzleri* in chicken carcasses both from markets and abattoirs (Atabay et al. 2006, Ho et al. 2008). Ferreira et al. (2013) demonstrated that even 100% of broiler carcasses from a Portuguese slaughterhouse were positive for *A. butzleri*.

Studies on species distribution among *Arcobacter* spp. in meat indicate that *A. butzleri* occurs more frequently than A. *cryaerophilus* (Kabeya et al. 2004, Rivas et al. 2004, De Smet et al. 2010). In our study *A. butzleri* was predominant species in meat (93% of *Arcobacter* isolates), whereas *A. cryaerophilus* was only sporadically isolated from this source. According to some authors (Houf et al. 2001, Kabeya et al. 2004) the low recovery of *A. cryaerophilus* from meat may reflect its low prevalence in meat, but could also be explained by technical difficulties during isolation of these bacteria. Recovery of this species can be hampered e.g. by the presence of competing microflora or low resistance of *A. cryaerophilus* to some antibiotics used in selective media. In this study AB broth containing cefoperazone, known to potentially impact *A. cryaerophilus* growth, was also used. Therefore, possible bias in recovery rates of this species due to its growth inhibition could not be excluded.

Resistance of studied here *A. butzleri* isolates from chicken meat and pork was high to ampicillin (91% and 70%, respectively), followed by cefotaxime (80% and 71% resistant isolates in pork and chicken, respectively) and amoxicillin with clavulonic acid (70% and 65% resistant isolates in pork and chicken, respectively). Incidence of β-lactam resistant *A. butzleri* was lower in beef, accounting for 64% of ampicillin, 45% of amoxicillin with clavulonic acid and 27% of cefotaxime-resistant isolates. A high rate of ampicillin resistance was already found in *A. butzleri* isolates recovered from broilers in Portugal (98%) and Turkey (64%) (Atabay and Aydin 2001, Ferreira et al. 2013). Resistance of *A. butzleri* to cephalosporins (cefotaxime), usually added into selective media to supress the growth of accompanying microorganisms, has been reported to occur frequently in this species (Atabay and Aydin 2001, Kabeya et al. 2004). In turn, cephalosporin resistance of *A. cryaerophilus* has been reported to be less frequent (Houf et al. 2001, Kabeya et al. 2004). Nevertheless, we detected 2 of *A. cryaerophilus* and 27 of *A. butzleri* isolates susceptible to cefotaxime, despite culture medium we used was complemented with other cephalosporin, i.e., cefoperazone. Since similar observation has been reported by other authors (Kabeya et al. 2004, Shah et al. 2012, Ünver et al. 2013), it seems that some *Arcobacter* isolates can be successfully isolated from studied material, irrespectively of their antibiotic susceptibility. However, taking into account the same reason, i.e., cephalosporin susceptibility, it is also plausible that other isolates were not recovered from studied material, thus care should be taken in reporting on actual incidence of this species.

In this study significant part of *A. butzleri* population (62%) was resistant to macrolides (erythromycin), what is in agreement with previous reports (Fera et al. 2003, Shah et al. 2012). In contrast, only 1 out of 6 *A. cryaerophilus* isolates was found to be resistant to this antibiotic. Similarly, Unver et al. (2013) demonstrated that among 7 *A. cryaerophilus* strains only 1 was resistant to erythromycin.

All, except one, *A. cryaerophilus*, and the majority of *A. butzleri* isolates (70%) were susceptible to fluoroquinolones. Studies held in Turkey, Malaysia and USA also revealed a high efficacy of fluoroquinolones against *Arcobacter* spp. (Son et al. 2007, Shah et al. 2012, Unver et al. 2013). In turn, significant part of *A. butzleri* population (56%) examined in Portugal were found to be ciprofloxacin-resistant (Ferreira et al. 2013).

Tetracycline and aminoglycosides (gentamicin) showed the highest efficacy against *A. butzleri*, and *A. cryaerophilus* since almost 80% of their population was susceptible to these agents. *A. butzleri* was as yet reported to be highly sensitive to tetracycline and aminoglycosides, hence they were recommended as the first choice agents in the treatment of *Arcobacter* spp. infections in humans and animals (Abay et al. 2012, Shah et al. 2012, Unver et al. 2013).

The incidence of multiresistant isolates (defined as resistant to 4 or more antimicrobials) was significantly higher in *A. butzleri* than in studied *A. cryaerophilus* population, since 42 *A. butzleri* and only one *A. cryaerophilus* isolates were multiresistant. The highest prevalence of multiresistant phenotype was observed within pork (60%) and chicken-derived (57%) *A. butzleri* isolates. Moreover, 10% of *A. butzleri* population was resistant to all used antimicrobials. Multiresistance among *Arcobacter* spp. was reported to be variable in its populations (Kabeya et al. 2004, Son et al. 2007, Shah et al. 2012). In US 72% of isolates from broiler carcasses were resistant to two or more antimicrobials (Son et al. 2007). Shah et al. (2012) demonstrated that 35% of *A. butzleri* isolates originating mainly from beef were resistant to at least three antibiotics. Diversity of incidence of multiresistant isolates within *Arcobacter* species was already observed in Japan. There 73% of *A. butzleri* and only 13% of *A. cryaerophilus* population was resistant to two or more antimicrobials (Kabeya et al. 2004).

In this study we report for the first time the prevalence and antimicrobial resistance of *Arcobacter* spp. in retail meat in Poland. Incidence of *A. butzleri* was found to be the highest in chicken meat. Less of *A. butzleri* was isolated from beef and pork. *A. cryaerophilus* was only sporadically found in studied meat samples. Most of *A. butzleri* isolates were resistant to β-lactams and macrolidies. The tetracycline and aminoglycosides were highly efficient against both *A. butzleri* and *A. cryaerophilus*. Multiresistant phenotype was found to be more frequent within *A. butzleri* than *A. cryaerophilus* population. Significant part of *A. butzleri* isolates was resistant to four or more antimicrobials. These results indicate significant incidence of potential foodborne zoonotic agents i.e., *A. butzleri* and *A. cryaerophilus*, including multiresistant isolates in retail meat in Poland.

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