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Antimicrobial susceptibilities of various *Arcobacter* species

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Aim: To determine the antimicrobial susceptibilities of various *Arcobacter* strains isolated from domestic geese, which have great potential for the transmission of arcobacters to humans and animals by contamination of water sources.

Materials and methods: A total of 16 *Arcobacter* strains including *A. cryaerophilus* (7), *A. skirrowii* (7), and *A. butzleri* (2) were examined for their susceptibilities to 20 antibiotics using a disk-diffusion method.

Results: All *Arcobacter* isolates tested were resistant to cloxacillin, cefazolin, optochin, vancomycin, and fusidic acid, and most were susceptible to oxytetracycline, chloramphenicol (except for *A. butzleri*), nitrofurantoin, amikacin, enrofloxacin, ofloxacin, erythromycin, ampicillin sulbactam, and amoxicillin (except for *A. butzleri*). All *A. skirrowii* and most *A. cryaerophilus* isolates were susceptible to amoxicillin/clavulanic acid. All 3 *A. butzleri* strains tested were resistant to cephalothin, while most *A. skirrowii* strains and 3 strains of *A. cryaerophilus* were susceptible to this antibiotic. Both isolates of *A. butzleri* were susceptible to rifampicin. Variable results were obtained for the other antibiotics used in this study.

Conclusion: The incidence of antibiotic susceptibility in arcobacters varied among species, which suggests that suitable antibiotic(s) should be selected for the treatment of infectious disease(s) and/or when developing selective media for the isolation of a wide range of *Arcobacter* species.

Key words: *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter skirrowii*, antimicrobial susceptibility

1. Introduction

The genus *Arcobacter*, previously known as 'aerotolerant campylobacters', was initially isolated from aborted bovine and pig fetuses (1,2). In 1991, Vandamme et al. performed a comprehensive taxonomic study of all known *Campylobacter*-like organisms and proposed the genus name *Arcobacter*, which belongs to the epsilon subdivision of the *Proteobacteria*, called rRNA superfamily VI (3,4). Arcobacters are differentiated from campylobacters by their ability to grow in aerobic conditions and at lower temperatures such as 15–30 °C (5). The genus *Arcobacter* includes members considered to be emergent enteropathogens and potential zoonotic agents, presently including 12 formally accepted species and possibly additional species awaiting formal description (4–8).

Three species of *Arcobacter* spp., *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, have so far been associated with a variety of diseases in humans and animals. They have been isolated from various animals, including pigs, cattle, and sheep, in association with abortion,

reproductive problems, mastitis, gastric ulcers, and enteritis (4,9–12). The organisms have also been detected in water samples and clinically healthy farm animals, including several poultry species (7,13–17). *Arcobacter* has gained increasing attention as an emerging foodborne pathogen in humans, causing diarrhea and bacteremia (4,8,17–23). *A. butzleri* is recognized as a significant human pathogen by the International Commission on Microbiological Specifications for Foods (24). Although *A. butzleri* is the most commonly isolated species from humans, *A. cryaerophilus* and *A. skirrowii* have also been detected (4,12,17–23).

Arcobacter spp. have also been isolated from poultry carcasses, beef, pork, and water (7,25–27), but poultry meat is more frequently contaminated than red meat (27). Hence, foods of animal origin and water are regarded as the major sources of the transmission of *Arcobacter* to humans. *Arcobacter* species, like campylobacters, are fastidious microorganisms and require sensitive isolation method(s) and/or strategies for recovery, and the isolation

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media used to recover these microorganisms have usually been adapted from those developed for campylobacters (23,28). In addition, the strains of different *Arcobacter* spp., in particular those of *A. cryaerophilus* and *A. skirrowii*, are considered to range in susceptibility to various antimicrobial agent(s) commonly used in isolation media (29–31). There have been a limited number of reports determining the antimicrobial susceptibility of arcobacters, and these are mainly for *A. butzleri* (29,30,32–34). The aim of this study was to determine the antimicrobial susceptibilities of various *Arcobacter* spp. isolated from domestic geese, which have great potential for the transmission of arcobacters to humans and animals by contamination of water sources.

2. Materials and methods

2.1. *Arcobacter* isolates

In the present study, a total of 16 *Arcobacter* isolates including 3 different species, *Arcobacter cryaerophilus* (n = 7), *A. skirrowii* (n = 7), and *A. butzleri* (n = 2), were used. The strains were isolated using a membrane filtration methodology with nonselective blood agar from cloacal swab samples collected from live domestic geese raised in Kars, Turkey, as previously described (14). The phenotypic characteristics of *Arcobacter* species were assessed based on Gram staining; productions of oxidase, catalase, urease, and alpha-hemolysis; and growth at different conditions (at 30 °C, at 37 °C, at 42 °C, aerobically, microaerobically, and anaerobically). The simultaneous identification of the *Arcobacter* isolates as *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* was performed by employing multiplex PCR. The amplification conditions and use of primers were followed as previously described by Houf et al. (35), and thermal cycles were performed in an MJ Mini Cycler (Bio-Rad, Hercules, CA, USA). The amplified products were observed by agarose gel electrophoresis and UV light illumination. The reference strain of *A. butzleri* (DCC25), kindly provided by M Waino and M Madsen from the Danish Institute for Food and Veterinary Research, Denmark, was included as a positive control throughout the study.

2.2. Antimicrobial agents

A total of 20 commercially available antibiotic disks were employed. The antibiotics and their concentrations ($\mu\text{g}/\text{disk}$) are shown in the Table. The antibiotics used in this study were purchased from Bayer (Germany) and Oxoid (Hampshire, UK).

2.3. Determination of antimicrobial susceptibility.

A disk-diffusion test was used for the determination of the antimicrobial susceptibility of the *Arcobacter* isolates as described elsewhere (36). Briefly, the isolates were grown microaerobically at 30 °C for 48 h. After cultivation, a suspension of each organism was made in physiological

saline and the turbidity of each inoculum was adjusted to McFarland 0.5. Bacteria from each suspension were inoculated onto blood agar that comprised 5% (v/v) defibrinated sheep blood in blood agar base no. 2 (Oxoid CM271) using a sterile cotton-tipped swab. Thereafter, each antibiotic disk was placed onto the agar and the plates were kept at 4 °C for about 20 min in order to allow the antibiotics to diffuse into agar. Incubation of the plates took place in a microaerobic atmosphere at 30 °C for 48 h and the diameter of the inhibition zones was measured with calipers. The susceptibility patterns (resistancy/sensitivity) of the strains were determined according to previously defined criteria (36).

3. Results

In the current study, a total of 16 isolates of various *Arcobacter* spp., including *A. cryaerophilus* (7), *A. skirrowii* (7), and *A. butzleri* (2), that were isolated from 90 samples from the cloacae of domestic geese were examined for their susceptibilities to 20 antibiotics. The results are summarized in the Table. All strains of the 3 *Arcobacter* spp. tested were found to be resistant to cloxacillin, cefazolin, optochin, vancomycin, and fusidic acid, and susceptible to oxytetracycline, nitrofurantoin, amikacin (except 1 strain of *A. skirrowii*), and ofloxacin (except 1 strain of *A. cryaerophilus*). All the *Arcobacter* isolates apart from 1 strain of *A. cryaerophilus* were susceptible to enrofloxacin and all strains of both *A. cryaerophilus* (except 1) and *A. skirrowii* showed susceptibility to amoxicillin; however, *A. butzleri* isolates including the reference strain were resistant to amoxicillin. Except for 1 strain of *A. cryaerophilus*, both *A. cryaerophilus* and *A. skirrowii* were resistant to ampicillin, whereas 1 of the 2 goose isolates of *A. butzleri* was found to be susceptible to this antibiotic. Most strains of *Arcobacter*, apart from 1 of both *A. cryaerophilus* and *A. butzleri* that showed an intermediate level of susceptibility, were susceptible to erythromycin and ampicillin sulbactam. All the *A. skirrowii* isolates were susceptible to amoxicillin/clavulanic acid, whereas 2 strains of both *A. cryaerophilus* and *A. butzleri*, including the reference strain, were resistant to this antibiotic. While all the strains of *A. cryaerophilus* examined were resistant to mezlocillin, 4 strains of *A. skirrowii* were found to be susceptible to this antibiotic. Four, 3, and 1 of the *A. skirrowii*, *A. cryaerophilus*, and *A. butzleri* strains, respectively, were found to be susceptible to cefuroxime, whereas 3 and 2 strains of *A. cryaerophilus* and *A. butzleri*, respectively, were resistant, and the remaining *Arcobacter* strains examined showed intermediate levels of susceptibility to this antibiotic. All 3 *A. butzleri* strains tested were resistant to cephalothin; however, most strains of *A. skirrowii* (6 out of 7) and 3 strains of *A. cryaerophilus* were susceptible to this antibiotic. Three *A. cryaerophilus* strains were determined to be resistant, and 1 strain of

Table. Susceptibility of *Arcobacter cryaerophilus*, *A. skirrowii*, and *A. butzleri* isolates to various antimicrobial agents.*

Antimicrobial agent	<i>Arcobacter cryaerophilus</i> (n = 7) ^b			<i>Arcobacter skirrowii</i> (n = 7) ^b			<i>Arcobacter butzleri</i> (n = 3) ^{b, c}		
	R	I	S	R	I	S	R	I	S
Ampicillin, 10 ^a	6	0	1	5	0	2	2	0	1
Amoxycillin, 25	1	0	6	0	0	7	3	0	0
Oxytetracycline, 30	0	0	7	0	0	7	0	0	3
Nitrofurantoin, 300	0	0	7	0	0	7	0	0	3
Erythromycin, 15	0	1	6	0	0	7	0	1	2
Amoxicillin/clavulanic acid (2/1), 30	2	0	5	0	0	7	2	0	1
Cloxacillin, 5	7	0	0	7	0	0	3	0	0
Amikacin, 30	0	0	7	0	1	6	0	0	3
Cefazolin, 30	7	0	0	7	0	0	3	0	0
Ampicillin sulbactam, 20	0	1	6	0	0	7	0	1	2
Optochin, 5	7	0	0	7	0	0	3	0	0
Ofloxacin, 10	0	1	6	0	0	7	0	0	3
Mezlocillin, 75	7	0	0	1	2	4	2	1	0
Cefuroxime, 30	3	1	3	0	3	4	2	0	1
Enrofloxacin, 5	1	0	6	0	0	7	0	0	3
Cephalothin, 30	3	1	3	0	1	6	3	0	0
Chloramphenicol, 30	0	0	7	2	1	4	2	0	1
Vancomycin, 30	7	0	0	7	0	0	3	0	0
Fusidic acid, 10	7	0	0	7	0	0	3	0	0
Rifampicin, 30	5	0	2	4	1	2	1	0	2

R: resistant; I: intermediate level of resistance; S: susceptible.

*: Zone of inhibition was measured in mm and the results were assigned as R, I, or S using previously defined criteria (36).

^a: Concentrations of respective antibiotics that are given in µg/disk.

^b: Total number of isolates tested.

^c: Including a reference strain of *A. butzleri*.

both *A. cryaerophilus* and *A. skirrowii* were found to have an intermediate level of susceptibility to cephalothin. Although all the strains of *A. cryaerophilus* were found to be susceptible to chloramphenicol, 2 of the *A. skirrowii* and *A. butzleri* strains tested showed resistance to this antibiotic. Most *A. cryaerophilus* (5 of 7) and *A. skirrowii* (4 of 7) isolates tested were resistant to rifampicin. Interestingly, while the reference strain of *A. butzleri* included in the present study was found to be resistant to rifampicin, both goose isolates of this species were susceptible to this antibiotic.

4. Discussion

There have been a limited number of reports on the susceptibility of *Arcobacter*, and these are mainly for *A. butzleri* (29–34). *A. cryaerophilus* and *A. skirrowii* have, in general, been found to be more susceptible to the antimicrobial agents than *A. butzleri*. *A. skirrowii* was found to be the most susceptible in this study, which

is in agreement with the findings reported in previous studies (30,33). All isolates of the 3 species of *Arcobacter* examined were resistant to cloxacillin, cefazolin, optochin, vancomycin, and fusidic acid, and most were resistant to ampicillin. In contrast to the findings of the current study, Kabeya et al. reported that all *Arcobacter* strains tested in their study were susceptible to ampicillin (33). This study implies that this antibiotic might not be appropriate for the treatment of *Arcobacter* infections. Although most strains of *A. butzleri* have been reported to be resistant against cephalothin and cefuroxime in earlier studies (32,33), the majority of the *A. cryaerophilus* and *A. skirrowii* strains tested in this study were found to be susceptible to those antibiotics. This is important since cephalosporins, and in particular cefoperazone, are usually used in *arcobacter*-selective media in order to suppress accompanying flora due to their good penetration into gram-negative bacilli (29,37). Thus, caution should be exercised when devising and/or using this group of antibiotics in selective media

due to existence of susceptible strains of *Arcobacter* other than *A. butzleri*. In addition, cefoperazone-susceptible strains of *A. butzleri* have occasionally been reported in earlier studies (30,32,33).

Amoxicillin, oxytetracycline, nitrofurantoin, erythromycin, amoxicillin/clavulanic acid, ampicillin sulbactam, ofloxacin, enrofloxacin, and amikacin were the most active antibiotics against the *A. cryaerophilus* and *A. skirrowii* strains tested. Therefore, these antibiotics may be preferred for the treatment of disease(s) caused by these species in humans and animals, but the existence of resistant strains should be born in mind, as some isolates were resistant to some of those antibiotics (see Table for details). However, amoxicillin/clavulanic acid and ampicillin resistances (at the rate of 20% and 78%, respectively) among *A. butzleri* isolates have recently been reported (31). Although chloramphenicol was reported to be very active against *A. butzleri* in an earlier study (32), it is interesting that in this study 2 strains of both *A. skirrowii* and *A. butzleri* were found to be resistant to this antibiotic.

Most isolates of the 3 *Arcobacter* species examined were found to be susceptible to the fluoroquinolones ofloxacin and enrofloxacin. However, 1 *A. cryaerophilus* isolate was resistant to enrofloxacin, and 1 showed an intermediate level of resistance to ofloxacin, suggesting that resistant strain(s) of *Arcobacter*, in this instance *A. cryaerophilus*, do exist in the environment. Fluoroquinolones are active

against many pathogenic bacteria and thus have wide use in the treatment of several infectious diseases. However, with the introduction of fluoroquinolones either as feed additives or therapeutically both in human and veterinary medicine, fluoroquinolone-resistant strains of some bacterial species such as *Campylobacter jejuni* subsp. *jejuni* have emerged (38). Fluoroquinolone resistance does not yet seem to be a big problem for *Arcobacter* isolates, but the presence of resistant strains should not be ruled out as encountered in the current study and as reported by Lerner et al. (20).

Arcobacters were reported to be susceptible to aminoglycosides and tetracycline (4,29,31,39,40). The present study also demonstrated that all *Arcobacter* isolates were sensitive to amikacin and oxytetracycline, with the exception that 1 *A. skirrowii* showed an intermediate level of resistance to amikacin. These findings suggest that tetracycline along with aminoglycosides may be other drugs of choice for the treatment of *Arcobacter* infections in humans and animals.

The results of this study suggest that various *Arcobacter* isolates vary in their susceptibilities to several antibiotics. This should be taken into account when selecting antibiotic(s) and/or antibiotic combinations for the treatment of infections caused by these organisms and when devising media for the isolation of a wide range of *Arcobacter*.

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