In vitro susceptibility of *Arcobacter butzleri* and *Arcobacter cryaerophilus* to different antimicrobial agents

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Received 30 July 2002; accepted 10 September 2002

Abstract

Seventeen strains of *Arcobacter butzleri* and thirteen of *Arcobacter cryaerophilus*, were tested for their antimicrobial susceptibility to 26 antimicrobial agents. Among β-lactams agents in this study, imipenem was the most active agent against both *A. butzleri* and *A. cryaerophilus* isolates with MIC90 values of 2 and 4 mg/l, respectively. The most active cephalosporin tested was cefepime, although it was more active against *A. butzleri* (MIC90 8 mg/l) than *A. cryaerophilus* (MIC90 64 mg/l). Levofoxacin, marbofloxacin, enrofloxacin and ciprofloxacin were the best-performing fluoroquinolones against these species. Of the aminoglycosides, amikacin was the most active agent against both *A. butzleri* and *A. cryaerophilus* strains with MIC90 values of 64 and 16 mg/l, respectively. All isolates showed high levels of resistance to penicillins, macrolides, chloramphenicol, trimethoprim and vancomycin.

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Keywords: *Arcobacter butzleri*; *Arcobacter cryaerophilus*; Susceptibility

1. Introduction

The genus *Arcobacter* was first described by Ellis et al. in 1977 as containing Gram-negative, spirillum-like bacteria isolated from bovine and porcine foetuses [1]. These bacteria were allocated to the genus *Arcobacter* and differentiated into five species: *A. butzleri*, *A. cryaerophilus*, *A. nitrofigilis* and *A. skirrowii* [2–4]. Of these five species, only *A. butzleri* and *A. cryaerophilus* have been associated with human enteric diseases. In fact, Arcobacter has been isolated mainly from stool specimens of patients with mild diarrhoea and occasionally with severe diarrhoea [5–7]. Water is a potential source of *Arcobacter* spp. [8,9]. *A. butzleri* and *A. cryaerophilus* have been isolated from a drinking water reservoir in Germany [10], canal water in Thailand [11], river water in Italy [12], ground water sources and sewage [13]. Recently, we have isolated *Arcobacter* spp. from water and mussels of two brackish lakes near Messina, Ganzirri and Farò [14]. Despite water having been cited as a potential vehicle in acquiring diarrhoeal illness associated with these microorganisms, there have been only few reports concerning the susceptibility of these strains [15–17]. The aim of this study was to investigate the susceptibility of *Arcobacter* spp. environmental isolates to the most common antimicrobial agents used for the treatment of infectious diseases in human and in veterinary medicine, to determine the usefulness of these drugs in Arcobacter infections.

2. Materials and methods

2.1. Bacterial strains

Seventeen strains of *A. butzleri* and 13 *A. cryaerophilus* were isolated from brackish environment near
Messina. Biochemical and antibiotic susceptibility characteristics of the isolates were tested using the API CAMPY identification system (bioMérieux Italia S.p.A.). Phenotypic characters of the isolates were compared with those of *A. butzleri* ATCC 49616 and *A. cryaerophilus* ATCC 43157 and these species were included as reference strains. We confirmed phenotypic identification of Arcobacter strains by molecular identification methods. PCR with Arcobacter-specific primers was performed with all isolates [18]. Fresh isolates were stored in liquid nitrogen until assayed.

### 2.2. Antimicrobial agents

All antimicrobial agents were obtained from their respective manufacturers. All drug solutions were prepared immediately prior to use.

### 2.3. Susceptibility studies

The MICs for the investigated strains were determined in fluid media by a microdilution technique in duplicate according to National Committee for Clinical Laboratory Standards [19]. Serial twofold dilutions of antibiotics were prepared in 96-well microtitre plates using Mueller–Hinton broth. For the preparation of the inoculum the frozen bacteria strains were thawed at room temperature, subcultured to agar Mueller–Hinton medium and incubated for 2 h in a microaerophilic atmosphere (5% O₂, 10% CO₂, and 85% N₂) at 37 °C. Several individual colonies of each strain were suspended in saline solution and then further diluted to give a final concentration of approximately 10⁶ CFU/ml. The turbidity of the suspensions was adjusted to match that of 0.5 McFarland standard (≈ 10⁶ CFU/ml) and these suspensions were diluted 5:100 (5 × 10⁶ CFU/ml) with saline solution. Ten-microlitre aliquots of these suspensions were inoculated in each well of Nunclon MicroWell plates (Life Technologies, Milano, Italy), containing serial twofold dilutions of the drug tested in Mueller–Hinton broth (Oxoid). Each well contained 100 µl. All plates, including control plates containing no antimicrobial agent, were incubated for 24 h in a microaerophilic atmosphere. All the plates for MIC tests were further incubated for an additional day, to observe any changes in the MICs. The MIC was considered the lowest concentration of antimicrobial agents giving total inhibition of bacterial growth. Performing the tests in duplicate did not show any intrastrain variations. For the purpose of this study, breakpoints of ampicillin, amoxicillin, piperacillin, cefaclor, cefazolin, cefixime, ceftriaxone, cefepime, cefotetan, cefuroxime, imipenem, meropenem, norfloxacin, ciprofloxacin, levofloxacin, tobramycin, gentamicin, amikacin, clindamycin, vancomycin, chloramphenicol and trimethoprim were taken from the NCCLS recommendations [19]. The susceptibility breakpoint for marbofloxacin and enrofloxacin, was ≤ 1 and ≤ 2 mg/l, respectively, as recommended by the manufacturers [20]. The susceptibility breakpoint for difloxacin was ≤ 4 mg/l according to Barry et al. [21].

### 3. Results

Table 1 shows the range of MICs of the 26 compounds tested against 30 isolates of *Arcobacter* spp. and the concentrations required to inhibit 50 and 90% of the isolates (MIC₉₀ and MIC₉₀₀).

A high level of antimicrobial resistance to ampicillin, amoxycillin and piperacillin was found for Arcobacter isolates. The most active cephalosporin tested was cefepime; it was highly active against all *A. butzleri* isolates (MIC₉₀ 8 mg/l). The MIC₉₀ value of cefepime against *A. cryaerophilus* isolates was 64 mg/l. Imipenem was the most active agent against both *A. butzleri* and *A. cryaerophilus* isolates with MIC₉₀ values of 2 and 4 mg/l, respectively. Strains of *A. butzleri* were slightly more susceptible to meropenem than strains of *A. cryaerophilus* with MIC₉₀ values of 8 and 64 mg/l, respectively. Of the two species tested, *A. butzleri* was less susceptible to fluoroquinolones. The activity of levofloxacin against *A. butzleri* isolates was comparable with that of enrofloxacin and superior to that of norfloxacin, ciprofloxacin, difloxacin and marbofloxacin. Levofloxacin and marbofloxacin were the best-performing fluoroquinolones against *A. cryaerophilus* isolates based on MIC₉₀ and MIC₉₀₀ values. Of the aminoglycosides, the activity of tobramycin was similar to that of gentamicin and lower than that of the amikacin; all *A. cryaerophilus* strains were fully susceptible to amikacin (MIC₉₀ 16 mg/l), whereas *A. butzleri* strains showed lower levels of susceptibility (MIC₉₀ 64 mg/l). High levels of resistance to clindamycin, chloramphenicol, trimethoprim and vancomycin were found among *Arcobacter* isolates tested. For rokitamycin, no breakpoint is available, MIC₉₀ values for *A. butzleri* and *A. cryaerophilus* were more than 128 mg/l.

### 4. Discussion

The isolation of *Arcobacter* spp. from water sources underscores the potential role of these organisms as water-borne pathogens. The aim of the present study was to provide information about the activity of the most common antimicrobial agents against Arcobacter isolates, so that suitable antimicrobials may be chosen for severe Arcobacter-infections.

The MICs for penicillins were high for most of the strains tested. The high level resistance to penicillins in *Arcobacter* seen in our study agrees with results obtained by Atabay et al. [16]. The mechanism of
bacterial resistance to penicillins and other β-lactam antibiotics involves alteration in one or more of the penicillin binding proteins causing their lowered affinity to β-lactam antibiotics. Among β-lactam agents in this study, the most effective compound against Arcobacter isolates was imipenem. Meropenem also had good in vitro activity; both agents are stable to the most common β-lactamases, including those with extended spectrum activity against third-generation cephalosporins, have a good penetration into Gram-negative bacilli and displayed a post-antibiotic effect [22].

The fluoroquinolones tested showed good activity against isolates of *A. butzleri* and *A. cryaerophilus*; there was good susceptibility to levofloxacin, marbofloxacin, enrofloxacin and ciprofloxacin. The development of new fluoroquinolones has been impressive during recent years, accounting for their wide use in clinical practice; they are antimicrobial drugs which generally have a high volume of distribution, low serum protein binding, limited biotransformation and excellent bioavailability [23,24].

Amikacin was the most active aminoglycoside against both *A. butzleri* and *A. cryaerophilus* isolates.

All or approximately half of the isolates evaluated were resistant to more than one class of antimicrobial agent notably macrolides, chloramphenicol, trimethoprim and vancomycin. However, macrolide resistance is an increasing worldwide problem, in fact mutations could occur on 23S rRNA or on ribosomal proteins. Resistance to chloramphenicol is determined by the synthesis of chloramphenicol acetyltransferase and resistances to trimethoprim and vancomycin are the result of the presence of altered forms of appropriate enzymes.

The carbapenems remain an excellent choice in the treatment of severe Arcobacter infections. Another possible β-lactam choice is cefepime and the fluoroquinolones could be second line anti-Arcobacter drugs. Amikacin remains a useful antibiotic in the treatment of systemic Arcobacter infections.

### References


