

Susceptibility of *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* to Antimicrobial Agents Used in Selective Media

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Several antimicrobial agents used in selective media for the isolation of *Arcobacter* were found to be inhibitory to strains belonging to this genus. All three species tested were susceptible to colistin and rifampin at concentrations used in selective media. *Arcobacter skirrowii* was the most susceptible species. 5-Fluorouracil, novobiocin, trimethoprim, and teicoplanin or vancomycin were found to be without any inhibitory effect on the strains tested at concentrations described for the isolation of *Arcobacter* species.

Arcobacter species rarely occur as the only or the dominant bacteria in different types of samples from animals, foods, and water. Two species, *Arcobacter butzleri* and *Arcobacter cryaerophilus* are associated with human diarrheal illness and bacteremia, but their pathogenic role is unclear. Although antimicrobial agents have to be used in enrichment broths and plating media (5, 7, 8) the exact susceptibility of arcobacters to these agents never has been specified. Limited data are available on the susceptibility of *A. butzleri* and the *A. cryaerophilus* subgroups to certain substances (10), and nothing is known on the activity of these agents against *A. skirrowii*.

The aim of the present study was to determine the MICs of the most common antimicrobial agents used for the isolation of the three animal-associated *Arcobacter* species, in order to determine the usefulness of the selective supplements in the isolation protocols for arcobacters and thermophilic campylobacters. Another goal of this study was to determine whether antimicrobial susceptibility patterns could be used to differentiate the three *Arcobacter* species that are recovered from humans and food animals.

A total of 111 strains of *Arcobacter* species were obtained from the BCCM/LMG Bacteria collection, Ghent University (Ghent, Belgium). These strains originated from human, food, animal, and environmental sources and belonged to the species *A. butzleri*, *A. cryaerophilus* subgroup 1 and 2, and *A. skirrowii* (Table 1). Twelve antimicrobial agents (Table 2) obtained as laboratory standard powders from Sigma, St. Louis, Mo. were dissolved and further diluted and added to Mueller Hinton II agar (Becton Dickinson, Cockeysville, Md.) as recommended by the National Committee for Clinical Laboratory Standards (NCCLS document M100-S7) (11). Plates were seeded by a Steers inoculum replicator (MAST, London, United Kingdom) with approximately 10^5 CFU of appropriately diluted strains grown overnight in brain heart infusion (Oxoid, Basingstoke,

United Kingdom). All incubations were at 30°C under microaerobic conditions created by evacuating 80% of the normal atmosphere and introducing a gas mixture of 8% CO₂, 8% H₂, and 84% N₂.

The MICs of the 12 antimicrobial agents tested (Table 2) did not show consistent differences between the species tested, although in general *A. skirrowii* isolates were more susceptible than were *A. butzleri* and *A. cryaerophilus*. All 111 isolates tested were highly resistant (MIC > 256 µg/ml) to the two antifungal agents, amphotericin B and cycloheximide, as well as to 5-fluorouracil. Most strains were resistant to novobiocin, and variable susceptibility was noted for piperacillin, trimethoprim, and vancomycin. MICs of piperacillin were ≤32 µg/ml for 17 *A. skirrowii* strains, but one strain was resistant (MIC of 256 µg/ml). Two strains of *A. cryaerophilus* subgroup 2 showed high resistance levels to bacitracin (MIC > 256 U/ml), and three strains of the same group showed decreased susceptibility to rifampin. Cefotaxime was more active than cefoperazone, the other cephalosporin tested.

These findings offer an explanation for certain observations reported in the literature and can be used to select or to design improved *Arcobacter* isolation protocols. Many of the *A. skirrowii* and *A. cryaerophilus* strains were found to be susceptible to piperacillin at a concentration of 64 µg/ml, a level which is only slightly lower than the 75 µg/ml added by de Boer et al. in their selective medium (5). This probably explains why *A. butzleri* was the only species isolated by these authors. Also, the inclusion of cefoperazone at 32 µg/ml in this medium as well as in others (2, 8, 9) may be detrimental to stressed or injured cells of *A. cryaerophilus* and *A. skirrowii*. The cefoperazone MIC levels are only marginally above this concentration. The results obtained in the present MIC study and in the one of Kiehlbauch et al. (10) suggest that the media with CVA and CIN selective supplements described by Collins et al. (4) may be useful for the isolation of *A. butzleri* and *A. cryaerophilus* but not for *A. skirrowii*. It can be concluded that the use of those supplements is not appropriate when the recovery of the all three *Arcobacter* species is pursued.

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TABLE 1. Origins of the collection strains used in this study

| Species | Total no. of strains | No. of strains with indicated origin | | | | | | |
|------------------------------------|----------------------|--------------------------------------|--------|---------|-------|--------|-------|------|
| | | Human | Bovine | Porcine | Ovine | Equine | Water | Food |
| <i>A. butzleri</i> | 47 | 30 | 2 | 4 | | 1 | 9 | 1 |
| <i>A. cryaerophilus</i> subgroup 1 | 6 | | 2 | 2 | 2 | | | |
| <i>A. cryaerophilus</i> subgroup 2 | 40 | 4 | 5 | 29 | 1 | 1 | | |
| <i>A. skirrowii</i> | 18 | | 15 | 2 | 1 | | | |

TABLE 2. Distribution of MICs for 47 *A. butzleri*, 46 *A. cryaerophilus*, and 18 *A. skirrowii* collection strains of human, animal, and environmental origin

| Antimicrobial agent | Species | No. of strains with MIC ($\mu\text{g/ml}$) of: | | | | | | | | | | | | | | | |
|-------------------------|------------------------------------|--|------|------|------|-----|----|----|---|----|----|----|----|-----|-----|------|----|
| | | ≤ 0.06 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | >256 | |
| Amphotericin B | <i>A. butzleri</i> | | | | | | | | | | | | | | | | 47 |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | | | | | | | | 6 |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | | | | | | | | | | | 40 |
| | <i>A. skirrowii</i> | | | | | | | | | | | | | | | | 18 |
| Bacitracin ^a | <i>A. butzleri</i> | | | | | | | | | | 1 | 16 | 16 | 14 | | | |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | 1 | 1 | 2 | 2 | | | | |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | | | | 3 | 11 | 10 | 14 | | | | 2 |
| | <i>A. skirrowii</i> | | | | | | | 2 | 1 | 1 | 3 | 1 | 10 | | | | |
| Cefotaxime | <i>A. butzleri</i> | | | | | | | | | 2 | 7 | 21 | 17 | | | | |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | 2 | 1 | 3 | | | | | |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | | 3 | | 3 | 7 | 18 | 8 | 1 | | | |
| | <i>A. skirrowii</i> | | | | 5 | | | 6 | 4 | 1 | | 1 | | 1 | | | |
| Cefoperazone | <i>A. butzleri</i> | | | | | | | | | | | | | 2 | 4 | 7 | 34 |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | | | | | 3 | 2 | 1 | |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | | | | | 1 | 6 | 13 | 16 | 2 | | 2 |
| | <i>A. skirrowii</i> | | | | | | | | | | | 3 | 11 | 4 | | | |
| Colistin ^a | <i>A. butzleri</i> | | | 8 | 5 | 15 | 14 | 5 | | | | | | | | | |
| | <i>A. cryaerophilus</i> subgroup 1 | | | 1 | | 2 | 3 | | | | | | | | | | |
| | <i>A. cryaerophilus</i> subgroup 2 | 3 | | 4 | 4 | 9 | 19 | 4 | 1 | | | | | | | | |
| | <i>A. skirrowii</i> | 13 | | 4 | | 1 | | | | | | | | | | | |
| Cycloheximide | <i>A. butzleri</i> | | | | | | | | | | | | | | | | 47 |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | | | | | | | | 6 |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | | | | | | | | | | | 40 |
| | <i>A. skirrowii</i> | | | | | | | | | | | | | | | | 18 |
| 5-Fluorouracil | <i>A. butzleri</i> | | | | | | | | | | | | | | | | 47 |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | | | | | | | | 6 |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | | | | | | | | | | | 40 |
| | <i>A. skirrowii</i> | | | | | | | | | | | | | | | | 18 |
| Novobiocin | <i>A. butzleri</i> | | | | | | | | | | | | | | | | 2 |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | | | | | 1 | 2 | | 3 |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | | | | | | | 1 | 12 | 15 | | 12 |
| | <i>A. skirrowii</i> | | | | | | | | | | | | 6 | 3 | | | 9 |
| Piperacillin | <i>A. butzleri</i> | | | | | | | | | | | | | 1 | 3 | 21 | 23 |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | | | | | 1 | 2 | 3 | |
| | <i>A. cryaerophilus</i> group 2 | | | | | | | | | | | 1 | 4 | 11 | 19 | | 5 |
| | <i>A. skirrowii</i> | | | | | | | | | 2 | | 5 | 10 | | | 1 | |
| Rifampicin | <i>A. butzleri</i> | | | | | | 4 | 19 | 9 | 15 | | | | | | | |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | 1 | | 2 | 3 | | | | | | | |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | 6 | 16 | 9 | 6 | | | 3 | | | | |
| | <i>A. skirrowii</i> | | | | | | 7 | 6 | 4 | 1 | | | | | | | |
| Trimethoprim | <i>A. butzleri</i> | | | | | | | | | | | | | | | | 5 |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | | | | | | | | 5 |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | | | | | | | | 3 | 33 | | 4 |
| | <i>A. skirrowii</i> | | | | | | | | | | | | 1 | 1 | 11 | 5 | |
| Vancomycin | <i>A. butzleri</i> | | | | | | | | | | | | | | | | 47 |
| | <i>A. cryaerophilus</i> subgroup 1 | | | | | | | | | | | | | | | | 6 |
| | <i>A. cryaerophilus</i> subgroup 2 | | | | | | | | | | | | | 1 | | 1 | 38 |
| | <i>A. skirrowii</i> | | | | | | | | | | | | | 7 | 3 | | 8 |

^a Units for MIC are units per milliliter.

Based on the MICs obtained in this study and the activity of teicoplanin being comparable to that of vancomycin, the CAT supplement (cefoperazone at 8 µg/ml, amphotericin B at 10 µg/ml, and teicoplanin at 4 µg/ml) used by Atabay et al. (1) appears to be appropriate for the recovery of the three *Arcobacter* species. However, this medium does not sufficiently suppress contaminating flora in poultry neck skin (6). The same remark holds true for 5-fluorouracil used in EMJH medium.

Another problem concerns the selectivity of *Campylobacter* selective isolation procedures. It is not clear whether or not *Campylobacter*-like bacterial growth appearing on these plates may be identified as *Campylobacter* without further testing because arcobacters are not fully inhibited. All three *Arcobacter* species are susceptible to colistin (polymyxin E) and rifampin, two important components of these media. Considering that polymyxin B has the same antimicrobial activity as colistin, arcobacters can be considered to be susceptible to polymyxin B as was found for *A. butzleri* and *A. cryaerophilus* (10). However, from results obtained in the present investigation as well as in the study by Kiehlbauch et al. (10), it appears that only the Butzler medium (4) containing colistin at 10 U/ml and rifampin at 10 µg/ml fully inhibits arcobacters. The concentrations of polymyxin B and rifampin are lower in other media, such as Blaser-Wang, Skirrow, and Preston media, and probably only marginally effective.

In conclusion, only enrichments in EMJH medium with 5-fluorouracil or in *Arcobacter* broth with the CAT supplement fully support growth of the three *Arcobacter* species tested. However, none of the presently available supplements fully suppresses the accompanying flora in biological samples. As shown by the MICs in the present study, *A. skirrowii* is the most susceptible species, and this can be an explanation for the low recoveries reported to date for this species. Antimicrobial

agents that can be used in selective media include 5-fluorouracil, novobiocin, trimethoprim, and cefoperazone at appropriate concentrations.

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REFERENCES

1. Atabay, H. I., and J. E. L. Corry. 1998. Evaluation of a new *Arcobacter* enrichment medium and comparison with two media developed for enrichment of *Campylobacter* spp. *Int. J. Food Microbiol.* **41**:53–58.
2. Bolton, F. J., D. N. Hutchinson, and D. Coates. 1984. Blood-free selective medium for isolation of *Campylobacter jejuni* from feces. *J. Clin. Microbiol.* **19**:169–171.
3. Butzler, J. P. and M. B. Skirrow. 1979. *Campylobacter* enteritis. *Clin. Gastroenterol.* **8**:737–765.
4. Collins, C., I. Wesley, and E. Murano. 1996. Detection of *Arcobacter* spp. in ground pork by modified plating methods. *J. Food Prot.* **5**:448–452.
5. de Boer E., J. Tilburg, D. Woodward, H. Lior, and W. Johnson. 1996. A selective medium for the isolation of *Arcobacter* from meats. *Lett. Appl. Microbiol.* **23**:64–66.
6. Houf, K., A. Tutenel, L. De zutter, J. Van Hoof, and P. Vandamme. Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. *FEMS Microbiol. Lett.* **193**:89–94.
7. Johnson, L. G., and E. A. Murano. 1999. Comparison of three protocols for the isolation of *Arcobacter* from poultry. *J. Food. Prot.* **62**:610–614.
8. Johnson, L. G., and E. A. Murano. 1999. Development of a new medium for the isolation of *Arcobacter* spp. *J. Food. Prot.* **62**:456–462.
9. Karmali, M. A., A. E. Simor, M. Roscoe, P. C. Fleming, S. S. Smith, and J. Lane. 1986. Evaluation of a blood-free, charcoal-based, selective medium for the isolation of *Campylobacter* organisms from feces. *J. Clin. Microbiol.* **23**:456–459.
10. Kiehlbauch, J. A., C. N. Baker, and I. Wachsmuth. 1992. In vitro susceptibilities of aerotolerant *Campylobacter* isolates to 22 antimicrobial agents. *Antimicrob. Agents Chemother.* **36**:717–722.
11. National Committee for Clinical Laboratory Standards. 1997. Approved standard M7–A4. Scheme for preparing dilutions of antimicrobial agents to be used in agar dilution susceptibility tests. Suggested modifications of standard methods for susceptibility testing of some fastidious and special problem bacteria (M100–S7). NCCLS, Wayne, Pa.