Isolation and characterization of *Arcobacter* strains derived from human stool samples – results from a prospective German prevalence study (Arcopath)

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Aim

Arcobacter species are considered as emerging food- and waterborne pathogens associated with human diseases like gastroenteritis. However, reliable epidemiological data are missing and their role in human disease is still unclear. Thus, we performed a 13-month prospective *Arcobacter* prevalence study in stool specimen derived from German out- and inpatients. We further characterized the collected isolates regarding the genetic diversity, presence of virulence genes, cytotoxicity and antimicrobial susceptibility.

Methods

Isolation and identification

Isolation of *Arcobacter* was carried out using selective enrichment media. Suspected isolates were identified at species level using mPCR and verified by *rpoB* sequencing.

Antimicrobial susceptibility

Susceptibility testing of *Arcobacter* spp. isolates to erythromycin (EM), azithromycin (AZ), ciprofloxacin (CI), gentamycin (GM), ampicillin (AM) and tetracycline (TC) was performed using E-test.

ERIC PCR

Genetic diversity was determined by ERIC-PCR. Analysis of fragment pattern was performed using BioNumerics v7.1. Dendrogram was generated using Dice coefficient and UPGMA.

Detection of virulence genes

The occurence of 10 putative virulence genes was investigated by PCR. For *A. lanthieri* additional primers were used including also primers for detection of the cytolethal distending toxin genes *cdtABC.*

Cytotoxicity assay

Human colon adenocarcinoma cells HT-29/B6 were seeded in 96-well plates at a density of 3 x 10^5 cells/well and differentiated for 7 days. Cytotoxic effects were measured by the colorimetric WST-assay 48 h after bacterial inoculation with MOI of 100.

Results

Detection of *Arcobacter*

Arcobacter spp. were detected in 33 samples (0.85%) obtained from 3884 outpatients and in 3 samples (0.40%) from 752 inpatients. Overall, *A. butzleri w*as the most prevalent species followed by *A. cryaerophilus* and *A. lanthieri* (Fig. 1).

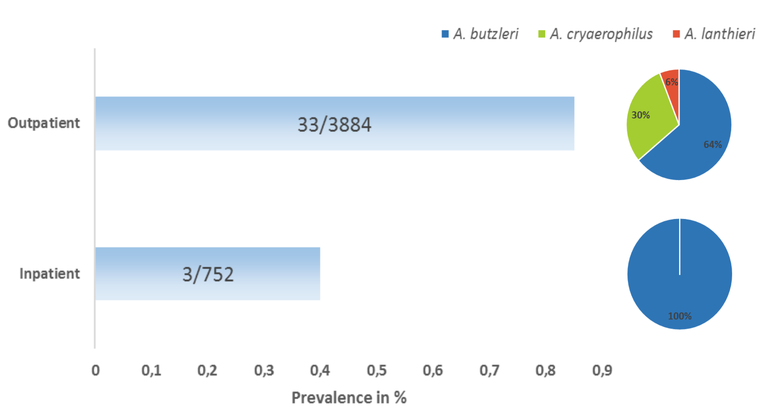

Figure 1: Detection of *Arcobacter* spp. in human stool samples in Germany. Prevalence and species distribution in out- and inpatients.

Antimicrobial susceptibility

Susceptibilities to EM, GM, TC and particularly to CI (in contrast to *C. jejuni)* were detected, whereas for AZ and AM bimodal distributions with elevated MICs for several isolates were observed (Fig. 2).

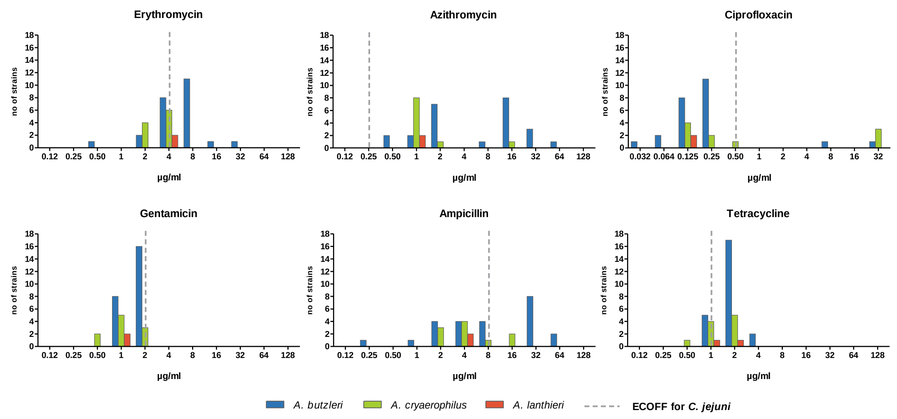

Figure 2: MIC distribution of *Arcobacter* isolates originating from human stool samples for six antimicrobial agents. E-test MICs were rounded up to the next upper two-fold dilution. Grey broken line: ECOFFs for *Campylobacter (C.) jejuni.*

ERIC-PCR

Dendrogram analysis revealed high genetic diversity of *Arcobacter* spp. and species-specific clusters for most *A. butzleri* and both *A. lanthieri* strains, but not for *A. cryaerophilus* isolates (Fig. 3).

Detection of putative virulence genes

A large number of putative virulence genes was present in *A. butzleri* and *A. lanthieri*, while fewer virulence genes were detectable in *A. cryaerophilus* isolates (Fig. 3). Notably, the three CDT genes *cdtABC* were abundant in both *A. lanthieri* isolates (data not shown).

Cytotoxicity assay

Cytotoxic potential of *Arcobacter* spp. was assessed measuring the residual viability of HT/29-B6 cells 48h after infection. The majority of *A. butzleri* isolates induced moderate to high levels of cytotoxicity (Fig. 4a).
In addition, both *A. lanthieri* isolates exhibited a high degree of cytotoxicity on HT-29/B6 cells (Fig 4c).
In contrast, the majority of *A. cryaerophilus* isolates did not induce any cytotoxic effects (Fig. 4b).

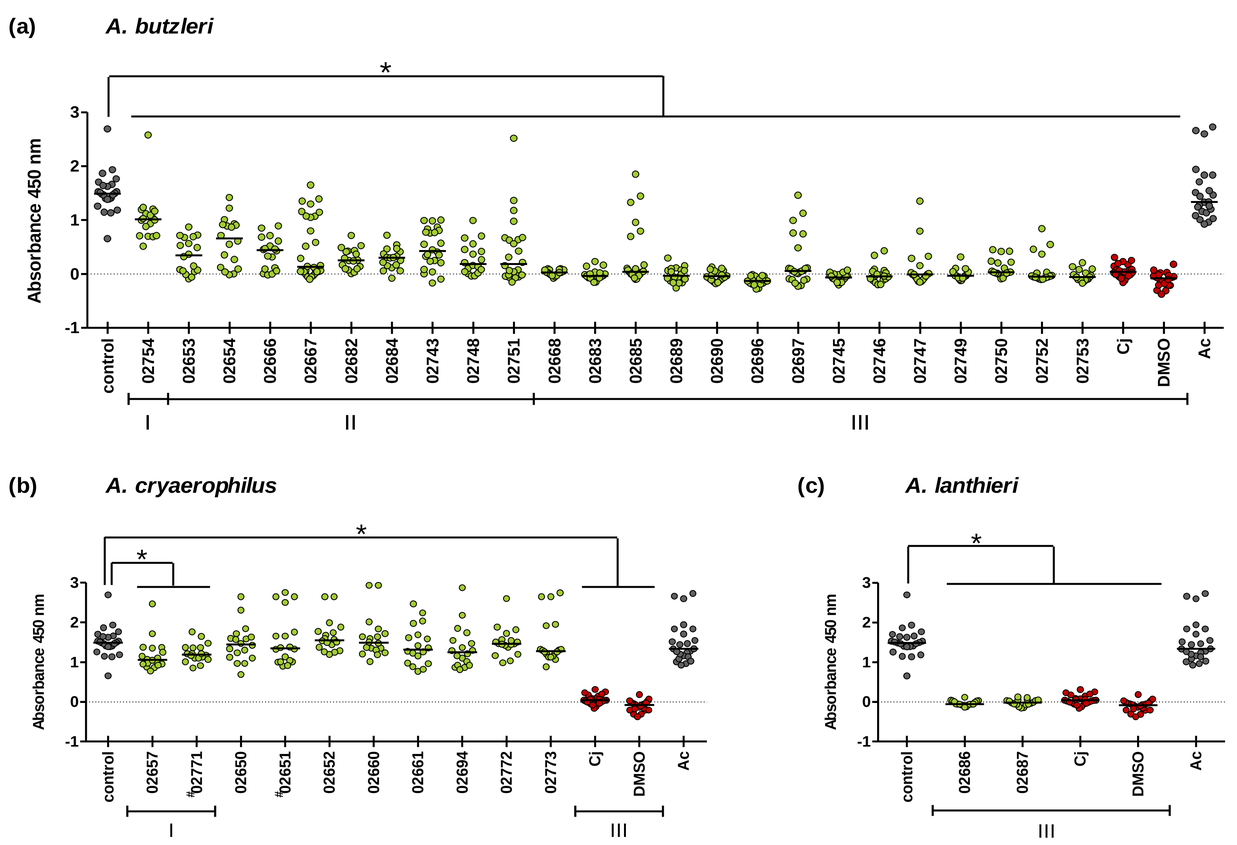

Figure 4: Viability of HT-29/B6 cells after inoculation with *Arcobacter* isolates. The level of toxicity was classified in low (I), moderate (II) and high cytotoxicity (III). At least three independent experiments were performed with six replicates each. Cj: *C. jejuni* 81-176, Ac: *A. cryaerophilus* ILSH 02659; * $p < 0.05$ (Mann-Whitney U-test) compared to control.

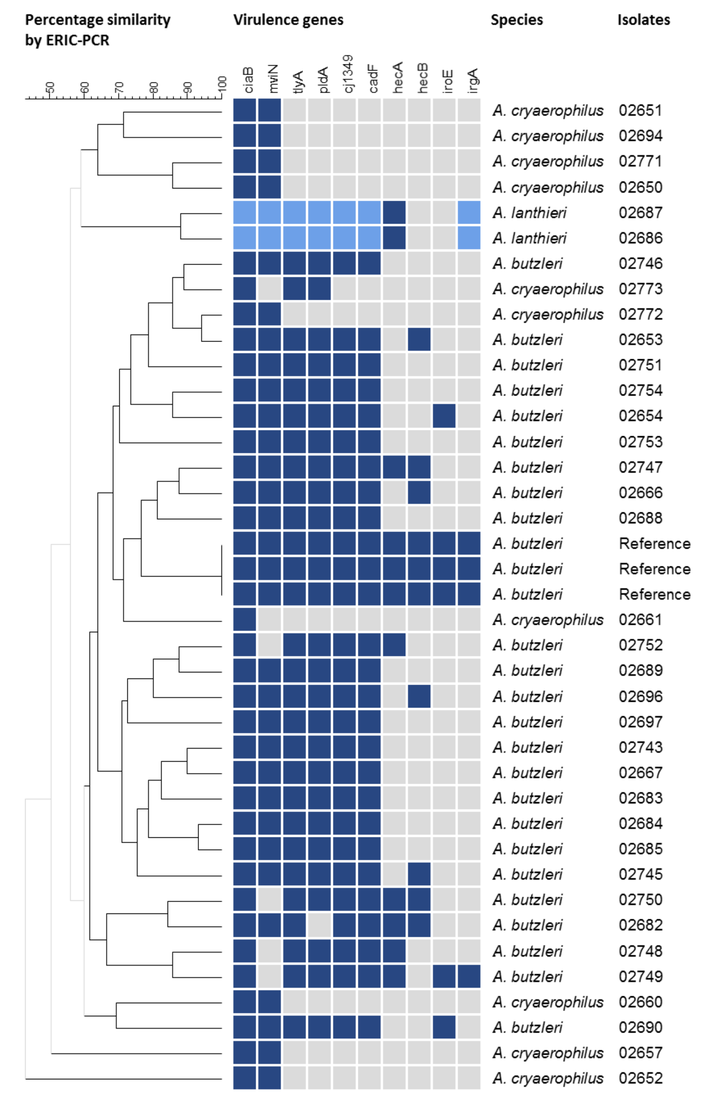

Figure 3: Genetic diversity of *Arcobacter* spp. and presence of virulence genes. The dendrogram is based on ERIC-PCR assay. Virulence genes: ■: detected by PCR; ■: not detected by PCR; ■: detected by *A. lanthieri* specific primers. *A. butzleri* (CCUG 30485) was included as reference strain.

Conclusion

Our study reveals i) an *Arcobacter* prevalence in German outpatients of 0.85 %, ii) antimicrobial susceptibilities towards commonly prescribed antibiotics, and iii) prominent *in vitro* cytotoxic effects of *A. butzleri* and *A. lanthieri*. Furthermore, the presence of the toxin genes *cdtA*, *cdtB*, *cdtC* in *A. lanthieri* may indicate the secretion of the exotoxin CDT as potential mechanism underlying cytotoxicity as opposed to *A. butzleri*. However, further investigation are required for a more in-depth evaluation of the role of *Arcobacter* in human disease.

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