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# Filling the gaps in clinical proteomics: a do-it-yourself guide for the identification of the emerging pathogen *Arcobacter* by matrix-assisted laser desorption ionization-time of flight mass spectrometry

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ABSTRACT

Arcobacters are considered emerging gastrointestinal pathogens. Rapid, reliable and species-specific identification of these bacteria is important. Biochemical tests commonly yield negative or variable results. Molecular methods prove more reliable but are time consuming and lack specificity. Matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is a fast, cheap and robust technique that has revolutionized genus and species identification in clinical microbiology.

The performance of an in vitro diagnostic (RUO) spectral database of MALDI-TOF MS for the identification of human clinically relevant *Arcobacter* isolates was validated and compared to an in house created Reference Spectral database (RS) containing a representative set of deposited *Arcobacter* strains of zoonotic interest. A challenge panel of clinical, human and veterinary, unique Campylobacteraceae strains was used to test accuracy.

Using direct colony transfer, sensitivity with RS was significantly better than with RUO for *A. butzleri* and *A. cryaerophilus* identification (100% and 92% versus 74% and 16%). For *A. skirrowii*, sensitivity remained low (21% versus 0%). Reanalysis using formic acid overlay (on-target extraction) augmented sensitivity for the latter species to 64%. Specificity of RS database remained excellent without any misidentifications of human clinical strains including *Campylobacter fetus* and *C. jejuni/coli*.

The use of an enriched database for MALDI-TOF MS identification of *Arcobacter* spp. of human interest produced high-confidence identifications to species level resulting in a significantly improved sensitivity with conservation of excellent specificity. Misidentifications, which can have therapeutic and public health consequences, were not encountered.

## 1. Introduction

Arcobacters are emerging bacterial zoonotic agents, causing predominantly gastrointestinal infections in humans. Initially, two of the human relevant species were taxonomically classified in the genus *Campylobacter*: *Campylobacter cryaerophila* (Neill et al., 1985) and *Campylobacter butzleri* (Kiehlbauch et al., 1991). In the latter year, both species were transferred into a new genus, *Arcobacter*, within the family Campylobacteraceae (Vandamme et al., 1991). Since then, 25 additional species and one *Candidatus* species have been identified and new species are pending (Figueras et al., 2017). Arcobacters inhabit a wide variety of ecological niches and have been isolated from various hosts and environments (Collado and Figueras, 2011). Some *Arcobacter* species are commonly present in the digestive tract of healthy farm animals (Van Driessche et al., 2003). Although their prevalence varies geographically, the species most frequently reported are *Arcobacter butzleri*, *A. cryaerophilus* and *A. skirrowii* (On et al., 2002). They have been associated with enteritis and mastitis in animals, and may play a role in animal reproductive disorders, such as infertility and late term abortions in cattle, pigs and sheep (Ferreira et al., 2016). These same species are also reported in human infections throughout the world. An association of *A. butzleri* and *A. cryaerophilus* with enteritis, colitis and septicemia has been put forward in epidemiological studies, outbreaks and case reports, though *A. cryaerophilus* has also been recovered from stool of asymptomatic humans (Houf and Stephan, 2007). *A. skirrowii* has been implicated as causal agent of colitis in individual case reports

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**Table 1**
Main spectra present in the 3 spectral libraries: manufacturer-cleared IVD-RUO, in house RS- large and in house RS-small.

| IVD-RUO Bruker reference library | RS-large in house reference Strains library | RS-small dereplicated in house reference Strains library |
|---|---|---|
| *A. butzleri* 347 98 NVU | | |
| *A. butzleri* 460 98 NVU | | |
| | *A. butzleri* LMG 15577 | |
| *A. butzleri* CCUG 10373 | *A. butzleri* LMG 6620 | |
| | *A. butzleri* LMG 10223 | |
| | *A. butzleri* LMG 9906 | *A. butzleri* LMG 9906 |
| | *A. butzleri* LMG 9869 | |
| | *A. butzleri* LMG 14714 | *A. butzleri* LMG 14714 |
| *A. butzleri* DSM 8739$^T$ | *A. butzleri* LMG 10828$^T$ | *A. butzleri* LMG 10828$^T$ |
| | A. butzleri LMG 9910 | |
| | *A. butzleri* LMG 9939 | |
| | *A. butzleri* LMG 11120 | |
| | *A. butzleri* LMG 10828$^T$ | |
| | *A. butzleri* LMG 11632 | |
| | *A. butzleri* LMG 10900 | |
| *A. cibarius* DSM 17680$^T$ | | |
| *A. cryaerophilus* QBR 3911 QBC | | |
| *A. cryaerophilus* QBR 3998 QBC | | |
| *A. cryaerophilus* T277 CPB | | |
| *A. cryaerophilus* V441 CPB | | |
| *A. cryaerophilus* CCUG 1780 T NVU | | |
| | *A. cryaerophilus* LMG 9947 | *A. cryaerophilus* LMG 9947 |
| | *A. cryaerophilus* LMG 10228 | *A. cryaerophilus* LMG 10228 |
| | *A. cryaerophilus* LMG 9865 | *A. cryaerophilus* LMG 9865 |
| | *A. cryaerophilus* LMG 9867 | |
| | *A. cryaerophilus* LMG 10216 | |
| | *A. cryaerophilus* LMG 10209 | |
| *A. cryaerophilus* DSM 7289$^T$ | *A. cryaerophilus* LMG 24291$^T$ | *A. cryaerophilus* LMG 24291$^T$ |
| | *A. cryaerophilus* LMG 10242 | |
| | *A. cryaerophilus* LMG 10244 | |
| | *A. cryaerophilus* LMG 10221 | |
| | *A. cryaerophilus* LMG 10224 | *A. cryaerophilus* LMG 10224 |
| | *A. cryaerophilus* LMG 10216 | |
| | *A. cryaerophilus* | |
| | *A. cryaerophilus* LMG 10233 | |
| | *A. cryaerophilus* LMG 10215 | |
| | *A. cryaerophilus* | |
| | *A. cryaerophilus* LMG 10217 | *A. cryaerophilus* LMG 10217 |
| | *A. cryaerophilus* LMG 10222 | |
| | *A. cryaerophilus* LMG 10218 | |
| | *A. cryaerophilus* | |
| | *A. cryaerophilus* LMG 10212 | |
| | *A. cryaerophilus* LMG 10213 | |
| | *A. cryaerophilus* LMG 10244 | |
| | *A. cryaerophilus* LMG 9863 | *A. cryaerophilus* LMG 9863 |
| | *A. cryaerophilus* LMG 9948 | |
| | *A. cryaerophilus* LMG 9065 | |
| | *A. cryaerophilus* LMG 10210 | |
| | *A. cryaerophilus* LMG 10211 | |
| | *A. cryaerophilus* LMG 10228 | |
| *A. skirrowii* DSM 7302$^T$ | *A. skirrowii* LMG 6621$^T$ | |
| *A. skirrowii* ATCC 51132$^T$ | *A. skirrowii* LMG 6621$^T$ | *A. skirrowii* LMG 6621$^T$ |
| | *A. skirrowii* LMG 9801 | *A. skirrowii* LMG 9801 |
| | *A. skirrowii* LMG 14985 | *A. skirrowii* LMG 14985 |
| | | *A. skirrowii* LMG 9912[a] |
| | | *A. skirrowii* LMG 10234[a] |
| Total number of strains in spectral databases | | |
| 13 | 46 | 15 |

Data represent MSPs of strains from the Bruker Reference Library 3.2.1.0 ($n$ = 13), MSPs of strains from the home made spectral database RS-large ($n$ = 46) and MSPs of the dereplicated home made spectral database RS-small ($n$ = 15), [a] two strains added after dereplication from the large in house reference library, species Type strains are designated by superscript T. The name of the MSP consists of the abbreviation of the culture collection and strain number. For the in house libraries, RS large and small, LMG reference numbers are used throughout the list. Corresponding reference numbers from other culture collections can be retrieved from www.straininfo.net.
CCUG, Culture Collection of the University of Göteborg;
ATCC, American Type Culture Collection;
DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen;

(Wybo et al., 2001). *Arcobacter* is presumably transmitted to humans by contaminated food and water, with poultry and pork products identified as important reservoirs (Collado and Figueras, 2011). Two extensive studies identified *Arcobacter* as the fourth most common *Campylobacter*-like organism in stool of patients with gastrointestinal disease (Vandenberg et al., 2004, Prouzet-Mauléon et al., 2006) and one study ranked *Arcobacter* fourth as bacterial pathogen causing diarrheal illness (Van den Abeele et al., 2014). The three *Arcobacter* species of human relevance are Gram-negative, slender, spirally curved rods, able to grow in air and at temperatures ranging from 25 up to