Characterization of *Arcobacter butzleri* isolates from poultry and slaughterhouse environment

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The genus *Arcobacter* is an emerging pathogen associated with several clinical symptoms, namely diarrhoea, abdominal pain or bacteraemia. This genus is widely distributed and has been isolated from environmental, animal, food and human samples, being poultry considered the major reservoir.

In this study, forty three *Arcobacter butzleri* strains were isolated from poultry of three flocks from different farms and environment samples at a Portuguese slaughterhouse, also three reference strains were included. All isolates were confirmed at species level by multiplex PCR and genomic DNA fingerprints of all isolates were determined using Pulsed Field Gel Electrophoresis (PFGE) after enzymatic digestion with *SmaI* and the isolates with undistinguishable *SmaI* patterns were further analyzed with a second restriction enzyme, *SacII*. Phenotypic resistance profiles to nine antibiotics were assessed by broth microdilution method. Biofilm formation assays were performed in the 36 out of the 43 *A. butzleri* isolates, either presenting different pulsetypes or similar PFGE patterns but different origin or even different resistance patterns.

PFGE patterns obtained using restriction enzymes *SmaI* and *SacII* revealed genetic diversity, with 32 distinct PFGE patterns. A high percentage of *A. butzleri* isolates was found to be resistant, recording four different resistance profiles. Twenty four of the 43 isolates presented a phenotypic resistance to ciprofloxacin, in contrast to the great susceptibility against gentamicin and chloramphenicol. Among the 36 selected *A. butzleri* isolates, 13.9% were categorized as moderately adherent, while 58.3% were defined as weakly adherent.

Overall, the results showed a high degree of genetic heterogeneity among *A. butzleri* isolates, along with high levels of resistance to several antibiotics. Biofilm formation ability of *A. butzleri* can possibly favour dispersion and cross-contamination along the slaughterhouse processing line. These findings may represent a contribution to get insight the survival and even persistence mechanisms of this organism in the environment and on its relevance as a potential hazard for foodborne infections.

Keywords *Arcobacter butzleri*; Poultry; Pulsed Field Gel Electrophoresis; Antimicrobial resistance; Biofilm

Characterization of *Clostridium difficile* 027 strains from an outbreak in a Portuguese hospital

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C. difficile infection (CDI) is the cause of an intestinal disease mediated by two potent cytotoxins, TcdA and TcdB. Symptoms of CDI can range from asymptomatic colonization or mild diarrhea, to life-threatening inflammatory lesions such as pseudomembraneous colitis, toxic megacolon or bowel perforation. In part because of the recent emergence of so-called hypervirulent strains, especially (but not exclusively) those belonging to ribotype 027, *C. difficile* is now considered a main nosocomial enteric pathogen.

Hypervirulent epidemic strains have been associated with more severe disease conditions, with higher relapse rates and increased mortality. Health care-associated CDI develops in hospitalized patients undergoing antibiotic treatment because *C. difficile* can colonize the gut if the normal intestinal microbiota is disturbed. However, *C. difficile* is also emerging as an important pathogen in the community, as well as in animal husbandry. The organism is an obligate anaerobe, and has the ability to form spores. Spores are extremely resilient and can accumulate and remain viable in the environment or in the host for long periods of time. Spores that remain latent in the gut are responsible for the recurrence of *C. difficile*-associated disease (CDAD) when antibiotic therapy is stopped. At least some of the hypervirulent epidemic strains show a greater sporulation capacity *in vitro*, as well as robust toxin production.

The first detection of *C. difficile* 027 hypervirulent epidemic strains implicated in a hospital outbreak in Portugal dates from January 2012, involving 12 patients, with a crude mortality rate of 50%. Here we report on the genetic characterization of those strains as well as the antibiotic resistance profile, toxin production, and rate and efficiency of spore formation. In parallel, *C. difficile* 027 non-outbreak strains isolated from other Portuguese health care facilities are also investigated.

Keywords *C. difficile* 027; nosocomial enteric pathogen; hypervirulent strains; spores; antibiotic resistance