Classification of *Plesiomonas shigelloides*

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Introduction

*Plesiomonas shigelloides* represents Gram-negative, motile, facultative anaerobic and oxidase positive bacteria. Plesiomonads are very important enteric bacteria and only one valid species is recognized in the genus *Plesiomonas* at present. The species *P. shigelloides* was described as a member of the family *Vibrionaceae* but it was later reclassified as an enteric pathogen belonging to the family *Enterobacteriaceae*. Waters with low salinity are predominant sources of plesiomonads, but they are also occurring in different poikilothermic as well as homothermic animals and may be responsible for serious gastrointestinal and extra-intestinal infections of animals as well as humans. The alimentary way is crucial for the infection transmission.

The aim of our study was a phenotypic (ENTER0Test 24, Biolog system) and genotypic (automated ribotyping) identification and classification of *P. shigelloides* strains isolated from animals, humans and the environment.

Material and methods

Bacterial strains

A set of 45 *P. shigelloides* strains isolated from different human and veterinary materials worldwide (mainly faeces) was analysed. Reference strains *P. shigelloides* (CCM 1991, CCM 1996, CCM 5860 and CAPM 5861) were obtained from the Czech Collection of Microorganisms (http://www.sci.muni.cz/ccm/) and from the Collection of Animal Pathogenic Microorganisms (Brno, Czech Republic).

Biotyping

The bacteria were classified to the species level by using two commercial identification systems, ENTER0Test 24 (Erba Lachema) and by Biolog GN2 Micro Plate kits (Biolog). Moreover, several conventional tube and plate tests (e.g. catalase, oxidase, OF test, gas from glucose, motility, DNase etc.) were additionally carried out to confirm the genus determination.

Genotyping

Multiplex PCR of 23S rRNA gene (González-Rey et al. 2000) and hugA gene (Herera et al. 2006) was used for a species confirmation of strains at the molecular level. The automated ribotyping performed with EcoRI restriction endonuclease using the Riboprinter system (DuPont Quaiac) was used for a more detailed characterization of the strains.

Results

The phenotypic variability found among the investigated isolates by ENTER0Test 24 was low; all analysed strains revealed almost coherent results mostly corresponding with the species description. Surprisingly, the majority of the isolates (91 %) showed positive DNase activity. In contrast, the ribotyping with the commercial kit GN2 Micro Plate identified only 27 strains as *P. shigelloides* and 11 strains were misidentified as members of the genus Vibrio. However, these strains were confirmed as *P. shigelloides* by a multiplex PCR technique. Remaining 7 strains were unidentified using the Biolog GN2 kit, but the most closest entry offered by the Biolog identification database was *P. shigelloides* in all cases.

Similarly, the genetic heterogeneity revealed by the automated ribotyping within the investigated group was rather high. Numerical analysis of the ribotype patterns separated all strains into a few clusters and a few strains formed clearly differentiated singletons. Automated identification provided by the Riboprinter system identified only strain P2268 as *P. shigelloides*; remaining strains were not assigned to the species level. No correlation between the biotyping and the automated ribotyping results was found (Fig 1. and Fig 2.).

Conclusions

Our results confirmed that *P. shigelloides* strains are phenotypically very diverse. These data clearly showed inconvenience of Biolog GN2 kits to reliably identify plesiomonads – of 45 strains only 27 (60%) were identified correctly.

The biotyping by Biolog system misidentified eleven *P. shigelloides* strains as members of the genus *Vibrio*. A multiplex PCR technique based on 23S rRNA and hugA genes was used for the confirmation of the misidentified strains as *P. shigelloides* (e.g. P2282, P2284, P2287, P2291, P2302, P2303).

The majority of strains (91 %) showed an ability to hydrolise DNA. This trait represents a putative virulence factor, but *P. shigelloides* is generally known as a DNase negative species (Altwegg, 2005).

Automated ribotyping revealed high genetic heterogeneity within the analysed strains which implies this technique as a useful tool for typing of plesiomonads but showed ribotyping as unsuitable for their identification.

The investigated strains originate from different sources and geographically distant areas. They could probably represent various biotypes and/or ecotypes of *P. shigelloides*.

References


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