Phenotypic Characterization and Putative Virulence Factors of Human, Animal and Environmental Isolates of *Plesiomonas shigelloides*

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**ABSTRACT.** *Plesiomonas shigelloides* (a bacterium widely distributed in aquatic ecosystems causing both intestinal and extra-intestinal diseases) shows a host of putative virulence markers, such as hemolysins, cyto-toxins, production of exoenzymes associated with pathogenicity, adhesive ability and vacuolation of cell lines in vitro. Technical difficulties in detecting some of these virulence factors together with scantiness of epidemiological information, due to the lack of routine analysis for *P. shigelloides* as etiological agent of gastro-enteritis, lead to sporadic and occasional finding of these bacteria. All this casts doubt on the real virulence potential of *P. shigelloides* and fuels a debate about assignment of these bacteria to the list of human pathogens. Here we demonstrated the phenotypic diversity and the putative virulence markers by examining serotype biochemical and virulence properties of 60 strains of *P. shigelloides* isolated from human, animal and environmental samples in different countries, which showed the unpredictable occurrence of the above properties depending on various locations and diverse sources.

*Plesiomonas shigelloides* (*P.s.* ) is a Gram-negative, motile rod, non-sporulating and oxidase positive bacterium belonging to the family Enterobacteriaceae (Brenner et al. 2005). One biovar and more than 100 serotypes have been described. *P.s.* has been considered by Abbot et al. (1991) as a low potential pathogen and this definition has still been fuelling a debate about the true pathogenicity of this bacterium.

Ueda et al. (1999) found that *P.s.* ranked first (66.7 %) in the group of enteropathogens causing travelers’ diarrhea among the persons investigated between 1994 and 1996 at Kansai Airport Quarantine Station; in contrast, Shah et al. (2009), evaluating 51 published studies between 1973 and 2004 about the etiology of diarrhea in 57 different groups of travelers, reported a lower positive percentage of isolation of *P.s.* in Latin America/Caribbean (1.3 %), Africa (2.5 %), and Southern Asia (4.8 %).

The lack of routine analysis for *P.s.* in cases of gastroenteritis leads to only sporadic and occasional finding of this bacterium (Chan et al. 2003).

*P.s.* has also been reported as causative agent of various extra-intestinal infections including septicemia, meningitis, septic arthritis, osteomyelitis, peritonitis, cellulitis and pneumonia (Schneider et al. 2009). Bravo et al. (2000) also described an infection of *P.s.* with fatal outcome in a newborn.

Toxin production by *P.s.* includes heat-stable and heat-labile enterotoxins, cholera-like toxins, hemolysin, and cytotoxins (Levin 2008). Cell adhesion, cell invasiveness and apoptotic Caco-2 cells death and a range of other pathogenic putative factors have also been reported (Čižnár et al. 2004; Levin 2008).

Here we investigate the phenotypic diversity of *P.s.* and its putative virulence markers by examining serotypes, biochemical and virulence properties of 60 strains isolated from human, animal and environmental sources in different countries.

**MATERIAL AND METHODS**

*Strains.** All the strains used belong to the *Plesiomonas shigelloides* collection(s) of the authors.

*Culture conditions.** Sixteen strains of *P.s.* were stored frozen at −80 °C in nutrient broth containing 15 % of glycerol (V/V). Before analysis the strains were inoculated in fresh nutrient broth, cultivated at

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37 °C for 18 h and then streaked on blood agar containing 5% bovine erythrocytes and incubated at 37 °C for 18 h.

*Serology.* Serotyping was performed according to the international antigenic scheme (Aldová and Shimada 2000) by Dr Rosinsky (Department of Microbiology, State Public Health Institute, Komárno, Slovakia).

*Motility* was checked by deeply inoculating the strains with a sterile needle in tubes containing Motility Test Medium (Difco, USA) and incubated for 24–48 h at 37 °C.

*Growth at different NaCl concentrations.* The strains were challenged by increasing concentration of NaCl (3.0, 4.0 and 5% in pepton water, W/V) in order to test their ability to withstand harsh environmental conditions.

*Production of exoenzymes.* DNase was detected by inoculating *P. s.* on DNase Agar (Oxoid, UK). Nutrient Agar (Oxoid) containing 1% Tween 80 (Sigma, USA) and 1% Gelatin Bacteriological (Oxoid) were used for lipase and gelatinase tests, respectively. Elastase production was checked by inoculating the strains in Brain Heart Infusion Broth (Oxoid) supplemented with 10 µg/mL of Red Congo Elastine (Sigma). The inoculated tubes were incubated up to 7 d at 37 °C.

*Hemolysin* production was assayed by spot inoculation of the strains on 5% of both bovine and on a pool of human erythrocytes in Brain Heart Infusion Agar (Oxoid), respectively, and by recording erythrocyte lyses after a 24–48 h incubation at 37 °C.

*Biochemical fingerprinting.* The PhenePlate™ system (PhP-48; BioSys, Sweden) was used to carry out highly discriminatory biochemical phenotyping. The characterization is based on quantitative measurements of reaction products formed by the bacterial metabolism of several different substrates; it is performed in pre-prepared microplates. The approach assumes that bacterial isolates with identical genotypes, i.e. belonging to the same clone, share identical metabolic properties, whereas isolates with different genotypes exhibit also different in the involved reactions. The fingerprinting of isolates was done according to the manufacturer’s instructions. The data analysis of the biochemical fingerprinting of the isolates was performed using the PhenePlate™ software. The human strains H6 and H13 were not included in this analysis.

**RESULTS**

Strain characteristics are reported in Table I. All the environmental and animal strains were motile whereas 4 human strains (16%) were non motile. DNase activity was positive for the vast majority of strains. The highest positivity was recorded among the human strains (68% of them were strongly positive and 28% positive). Eleven% of animal strains were strongly positive, 83% were positive and 6% showed a weak DNase activity. No strongly positive reaction was recorded among environmental isolates; 82% of them were positive and 18% weakly positive.

In contrast, gelatinase was unequally distributed among strains; the human ones were all negative, whereas 22% of animal strains and 35% of environmental strains were positive.

About ½ of the human, 61% of animal and 71% of environmental isolates were hemolytic on bovine erythrocytes. In addition, ¼ of environmental strains showed a small β hemolysis just around the edge of the colonies. In contrast, 40% of human isolates of *P. s.* were positive and 20% showed a weak reaction on human erythrocytes. Similarly, 29 and 61% of environmental and animal strains were positive, whereas ¼ of environmental and 11% of animal isolates showed a weak reaction.

All isolates were negative for lipase and elastase activities. The growth in NaCl was positive for all strains up to 4% but it was negative for all strains at 5%.

The data analysis of the biochemical fingerprints allowed clustering of the strains according to their biochemical characteristics described by Kühn *et al.* (1997) (Fig. 1). The identity level (ID) was set at 0.975. All the isolates showing correlation coefficients equal to or higher than the set identity level (ID = 0.975) were attributed to the same PhP type.

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**Footnotes to Table I:***

*Strains are listed according to their serological characteristics following the international *P. shigelloides* antigenic scheme (Aldová and Shimada 2000).

9(++) – strong positive, (+) – positive, w = weak reaction, (−) – negative, si – single type (according to Khün *et al.* 1997); nd – not determined; C – Cuba, CZ – Czech Republic, FIN – Finland, S – Sweden, SK – Slovakia.

*Non-agglutinating for antigen H.  
*Non-agglutinating for antigen O.