

*Clostridium difficile* is a major cause of infectious diarrhea in hospitalized patients. *Clostridium difficile*-associated disease (CDAD) is of gaining importance now due to its increasing incidence and severity. However, little is known about the *C. difficile* infections in the Czech Republic. The aim of the study was to characterize *C. difficile* strains recently isolated (2008 to 2011) from patients hospitalized with gastrointestinal disease in four Prague health care institutions using molecular typing methods; PCR toxinotyping, PCR ribotyping and MLVA (multilocus variable number tandem repeat analysis). Among 273 *C. difficile* strains, we identified 8 toxinotypes (0, III, IV, V, VI, VIII, IX a XXIII) and 63 ribotypes, of which ribotypes 596 (23,4 % patient), 017 (13,9 %) and 176 (7 %) were the most frequent. According to PCR ribotyping, the situation in the Czech Republic is the most similar to the situation in Poland. Within ribotypes 017, 017/1 and 017/2 and ribotypes 596 and 596/1, 5 and 4 distinct clusters were identified by MLVA, none of which was institution-specific. Additionally, pathogenic *C. difficile* were isolated from piglet faeces (63,3 %) in a single piglet farm, evaluating the role of *C. difficile* as an emerging animal pathogen. All piglet isolates belonged to the toxinotype 0 and the ribotype AI-12.