Epidemiological Typing of Pathogenic Bacteria

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Bacterial species can be differentiated by rRNA gene restriction patterns generated after cleavage of total DNA with restriction enzymes and hybridization of the fragments with labelled 16S + 23S rRNA probes (ribotyping). We extended this methodology by development of RFLP probes based on other highly conserved sequences from common (eu)bacterial genes, ATPase, ribosomal protein S12, and elongation factor Ef-Tu, while preserving the generic approach.

RFLP probes were produced by PCR ampification using primers from highly conserved regions found in sequence alignment. A probe for the Campylobacter flagellin was also produced and applied in the same manner. RFLP analyses with these probes supplement the ribotyping data and add extra levels of discrimination, because the resolution level varies with the number and kind of restriction enzyme/probe combinations used. This DNA typing method successfully discriminated Campylobacter isolates on the strain level. The RFLP patterns obtained were reproducible and appeared to be stable.

RFLP-typing was applied to compare isolates from a *C. upsaliensis* outbreak in four day-care centres in Brussels and to investigate the possible route(s) of transmission of *C. coli* in pigs. In order to compare the RFLP-typing with the traditional serotyping according to Penner and Lior, and with the rapid PCR typing method called Random Amplified Polymorphic DNA (RAPD), 180 *Campylobacter* strains from various origins (humans, cattle, pigs, poultry) were collected, identified with our multiplex PCR assay for *C. jejuni/coli*, and subjected to the three typing methods in parallel. The resulting data were subsequently analyzed by nonlinear multivariate analysis.

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