Monitoring system for *Campylobacter* spp. based on Multilocus-Sequence-Typing (MLST)

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Problemstellung und Zielsetzung

Within the last few years *Campylobacter* spp. and especially *C. jejuni* became the major agent of food born gastroenteritis in humans in Switzerland and elsewhere. A lot of effort has been put in determining the ways of infection as well as identification and typing of *Campylobacter* and has elucidated possible risks and prevention measures and led to promising diagnostic methods. Despite these achievements, we do not have an up-to-date tool for the surveillance and monitoring of *Campylobacter* in Switzerland. Multilocus sequence typing (MLST) and typing based on the flagellin genes are methods currently used for *Campylobacter*, but they are still expensive and labour-intensive.

Material und Methoden

A sequence-based three-level characterisation system for *C. jejuni* and *C. coli*, which covers general identification, typing and determination of antibiotic resistance, was established. 16S rRNA and *rpoB* gene sequencing was applied for identification of isolates. Additionally, the current multilocus sequence typing (MLST) scheme was optimised. To increase resolution of this method we included the flagellin genes *flaA* and *flaB*. New primer sets that allow PCR amplification and sequencing were designed for the seven classical MLST targets as well as the flagellin genes. Finally, determination of macrolide and quinolone resistance was achieved by 23S rRNA and *gyrA* gene sequencing*,* respectively. Amplification and sequencing protocols were optimised to allow a fast and economic procedure. A total of 180 strains including *C. jejuni* and *C. coli* type strains and *C. jejuni* strains isolated from different species across Switzerland were investigated.

Ergebnisse und Bedeutung

Sequences of the seven MLST loci provided unambiguous information about genetic diversity among the strains. A total of 73 different sequence types (ST) were defined, 17 (23.3%) of which represented not yet described variants. The most frequent clonal complexes in *C. jejuni* isolated in Switzerland were ST-21 (23%) and ST-45 (14.6%), which also appear to be the most common types of *C. jejuni* worldwide. There was no association observed between host and sequence types. Cluster analysis of the seven MLST loci together with *flaA* or *flaB* provided nearly the same high resolution as AFLP analysis, what was not achieved using MLST alone. This shows that the established MLST scheme can be optimised. Macrolide resistance was not found in the tested strains, but quinolone resistance was detected in 31% isolates, what confirms the necessity of surveillance of resistance in *Campylobacter* and the prudent use of antibiotics both in veterinary and human medicine. The data and protocols generated in this study will serve as a basis for a comprehensive monitoring tool for *C. jejuni*.

Publikationen, Poster und Präsentationen

Kuhn, J. (2007) Phylogenetic Identification and Improved Multilocus Sequence Typing (MLST) of *Campylobacter jejuni*, Diplomarbeit der phil.-nat. Fakultät der Universität Bern

Korczak, B.M.; Kuhn, J.; Fehlmann, M.; Kuhnert, P. (2007) Online monitoring system for *Campylobacter jejuni* using an improved MLST scheme, 66th Annual assembly of the SSM, Interlaken, 1-2 March 2007

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