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PCR AND SEQUENCING BASED METHODS FOR DETECTION AND TYPING OF PATHOGENIC MICROORGANISMS

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Gardaulan, Smittskyddsinstitutet, Nobels väg 18, Solna

Fredagen den 9 december 2011, kl 13:00

av
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Solna 2011

ABSTRACT

The potential use of bacteria and viruses as biological terror weapons makes certain highly pathogenic microorganisms a worldwide public health threat. In an outbreak investigation involving a possible deliberate spread of a biological warfare agent there is a need for a fast and reliable diagnostic method. Ideally, the same method should be usable for both bacteria and viruses. To distinguish between natural and deliberate spread, information on the prevalence/incidence of the organism/disease, ecology and natural mechanisms for spread is needed. In addition, knowledge of national and international subtypes reflecting both micro- and macro-evolution of the organisms is required for the analysis.

The field of molecular epidemiology has evolved during the last decades with the introduction of several different methods to type and characterize bacteria. There is now a plethora of different molecular techniques available to identify and discriminate between different strains of bacteria. In this thesis, rapid protocols based on pyrosequencing technology were developed and used for discrimination of *Bacillus anthracis* from closely related bacillus species. The technique was also used for identification and subtyping of *Francisella* species, including the human pathogen *Francisella tularensis* and its subspecies. Also a rapid generic protocol for detection and genotyping of infectious agents including bacteria, parasites, and viruses was developed.

Tularemia epidemics occur in limited geographical areas, and at variable intervals. The reasons for these irregularities are still unknown. There may be ecological niches in the affected areas, which harbor the bacteria between epidemics, or the bacterium may be reintroduced into an ecological system that permits its amplification. The establishment of a relevant typing system indentifying individual strains is of great importance .One of the most promising methods, MLVA was evaluated as a tool for practical use in epidemiological investigations of tularemia in Sweden. PFGE analysis was used as reference method. Typing data were combined with geomapping (GIS), in order to predict sources of infection and determine possible reservoirs for the different MLVA types. The results indicate that MLVA has the capacity to be used as a standalone typing method in outbreak investigations. It was possible to use this method directly on clinical specimens without isolating the bacterium by cultivation. In most cases, a certain MLVA type was not correlated to a strict geographic location, indicating that subtyping might be of limited use in surveillance and outbreak investigations of tularemia in endemic countries.