Molecular tools for epidemiological investigations into Legionella pneumophila environmental diffusion: applications for the prevention

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Abstract

Microbiological typing is a useful tool in the epidemiological investigations of infectious diseases, given that it allows for the identification of specific clones among a set of isolates. In the last ten years several studies have demonstrated how genotyping methods can be useful in *Legionella* spp investigations in hospital setting (e.g., epidemic events). Pulsed field gel electrophoresis and amplified fragment length polymorphisms are the current typing methods of choice, even though multilocus sequence typing will probably be the gold standard of the future.

Introduction

Legionellosis is considered an emerging public health problem. *Legionella* spp is an ubiquitous microorganism present in natural aquatic environments and able to colonize building water systems.[1,2] The problem is highlighted in health structures where immunocompromised patients are strongly predisposed to serious infection.[3,5] In the few last years, the Multicentric Italian Group has shown that most of the sanitary buildings are colonized by *Legionella* spp, and this is probably due to several aspects related to the age of the buildings, inadequate maintenance of the water distribution system and the creation of biofilms.[6] When a nosocomial or community acquired legionellosis occurs, the identification of the sources of infection is often difficult, even if it is required in order to implement more specific preventive control measures.[7,8] In this way, microbiological typing is an real and useful tool in the epidemiological investigations of infectious diseases, given that it allows for the identification of specific clones among a set of isolates.[9] The adoption of a typing method for an epidemiological investigation of an epidemic event is often useful for the definitive identification of the source of infection and the way of transmission. Traditional methods for microbiological typing were based on phenotypic investigations, but genotyping methods rapidly exceeded them. In fact, the latter are more accurate given that are based on the determination of polymorphic sites in the whole bacterial genome. In the last ten years several studies have demonstrated how genotyping methods can be useful in *Legionella* spp investigations in hospital setting (e.g., epidemic events), as well as in other situations (source of contamination in homes or hotels).[10,11] Among the most recent techniques automated ribotyping, pulsed field gel electrophoresis (PFGE) and amplified fragment length polymorphisms (AFLP), have been applied to *Legionella* spp typing[12-14] These techniques allow *fingerprinting* of the isolates to be obtained, so that they can be matched in order to establish similarities. At the moment PFGE and AFLP are the most used methods, due to their high discrimination power.[12,14]

Description of the Project

In the context of the Italian Multicentric Study Group on Legionellosis, the Unit in Rome is involved in genotyping by PFGE *Legionella pneumophila* isolates collected from various sites in Italy.

This experience allowed us to see a high genetic heterogeneity among all the *L. pneumophila* isolates collected. Most patterns were unique and corresponded to a single *L. pneumophila* strain.[15] These were sporadic isolates mainly recovered from private homes and related in some cases to community-acquired legionellosis infections. On the other hand, identical clones were observed in the same building (e.g., hospitals) over long time periods, as well as identical PFGE patterns in apparently unrelated isolates of *L. pneumophila*. In some cases of nosocomial infection, it was also possible to find the source of infection by comparison of environmental and

clinical isolates of *Legionella pneumophila,* so that more specific decontamination interventions were adopted.

Conclusions

The systematic adoption of a molecular typing method in the evaluation of clonality in *L. pneumophila* isolates collected in different situations seems to be a powerful tool in epidemiological investigations. In this context, a major development in the field of molecular biology that will continue to have a clear impact on bacterial epidemiology in the future is nucleic acid sequencing. Multilocus sequence typing (MLST) is one example: this method is based on the sequencing of multiple genes for each isolate and allows for unique sequence typing (ST) profile[16,17] to be obtained. This method has an intrinsic major reproducibility compared to others based on "band patterns" as PFGE or AFLP, and results can be easily validated, stored and shared electronically (at national and international level for the comparison of the genetic data). This method will probably become, in the future, the gold standard for bacterial epidemiological investigations.

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