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ORIGINAL ARTICLE

Comparative molecular and antibody typing during the investigation of an outbreak of Legionnaires' disease

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Abstract An outbreak of Legionnaires' disease with 113 confirmed cases was reported in the town of Mataró, Spain, in August 2002. In this study, we compared three different typing methods and characterized the clinical isolates by comparing them with other clinical isolates with the same ST from our own database to further characterize the outbreak. In the outbreak, a total of 16 clinical (nine patients) and 32 environmental (from four environmental sources) *Legionella pneumophila* isolates were analyzed by pulsed-field electrophoresis (PFGE), sequence-based typing (SBT), and monoclonal antibody typing (MAb). We compared the MAb and SBT profiles of the outbreak clinical isolates and other unrelated clinical isolates showing the same ST profile. We obtained seven different PFGE and SBT profiles and six MAb patterns from the outbreak isolates. PFGE and SBT showed 100 % concordance during the outbreak. SBT proved to be highly discriminatory, particularly with the addition of the new *neuA* gene. One PFGE, SBT (ST-37), and Philadelphia profile was observed among the clinical isolates. Using PFGE, this ST37 Philadelphia profile was closely related to other unrelated clinical isolates. These findings suggest that the ST37 Philadelphia profile could be a virulence marker in our area. The combination of the three methodologies was useful to further characterize and obtain additional information on a very explosive outbreak. Despite the minor discrimination of PFGE versus SBT, the two genetic methods are recommended in outbreak investigations. Further studies are currently underway in this area to obtain more definitive conclusions.

Keywords *Legionella* · PFGE · SBT · MAb · Typing · Outbreak · Virulence

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Introduction

Since *Legionella* was first identified in 1976, the diagnosis of Legionnaires' disease has increased considerably. Different studies have demonstrated how *Legionella pneumophila* has become one of the leading causes of community-acquired pneumonia in adults, accounting for 6–14 % of cases requiring hospitalization [1, 2]. Transmission of *Legionella* occurs most frequently with the inhalation of aerosols containing the microorganism, although micro-aspiration of contaminated potable water has also been implicated, especially in hospitalized patients [3].

Several environmental sources have been associated with *Legionella* outbreaks, including cooling towers, water distribution systems of homes, hotels, and ships, ornamental fountains, and whirlpool spas. Colonization of cooling towers with production of aerosols has also been identified as one of the major sources of community outbreaks of *Legionella* infection [4–8].

During an outbreak, appropriate typing methods are needed to establish the link between environmental and clinical isolates in epidemiological investigations. Pulsed-field gel electrophoresis (PFGE) is considered to be one of the most efficient methods for subtyping *L. pneumophila* strains and previously was the gold standard methodology in the epidemiological investigation of *Legionella pneumophila* outbreaks because of its high discriminatory power. Recently, however, the European Working Group for Legionella Infections (EWGLI) proposed sequence-based typing (SBT) as the new gold standard methodology [9]. SBT is a powerful epidemiological method, being both rapid and easy to perform, and provides unambiguous results [10]. SBT has shown to be useful in the investigation of prevalence and distribution of DNA sequence types among clinical and environmental *L. pneumophila* isolates [11–14]. However, only a few studies have demonstrated the usefulness of this technique in the investigation of nosocomial and community outbreaks using the combination of seven genes [15–19].

A community outbreak of *L. pneumonia* involving more than 154 people, 113 of whom had confirmed Legionnaires' disease, was detected in the district of Cerdanyola, Mataró (Catalonia, Spain). This explosive outbreak was investigated in an epidemiological, environmental, and molecular study during August 2002.

Epidemiological and molecular data identified a cooling tower as the direct cause of the community outbreak [20]. The aim of the present study was to further characterize this explosive outbreak comparing three different methods for the subtyping of *L. pneumophila* [monoclonal antibody (MAb) typing, PFGE, and SBT] and to characterize the clinical isolates, comparing them with other clinical isolates with the same subtype (ST) from our own database.

Materials and methods

Bacterial isolates

Bacterial isolates from the community outbreak We included a total of 16 clinical *Legionella pneumophila* serogroup (sg.) 1 isolates from nine patients admitted to the hospital for pneumonia-like symptoms and 32 isolates from four environmental sources collected during the epidemiological investigation in the 2002 community outbreak in Mataró [20]. All the clinical isolates and 28 environmental isolates were previously typed by PFGE. Furthermore, we analyzed the total of 48 isolates using a combination of three of the most commonly used typing methods for *Legionella* investigation: MAb typing, PFGE, and SBT.

Other clinical isolates We selected eight unrelated clinical isolates related to other community outbreaks showing the same ST as the outbreak clinical isolates [21]. Four of these isolates were epidemiologically and PFGE linked to cooling towers and three to a hot-water supply; one had an unknown origin.

MAb typing of *Legionella pneumophila*

MAb typing, performed by indirect immunofluorescence assay with the "Dresden MAb panel" [22], was used to determine the phenotypic subgroup.

Pulsed-field gel electrophoresis of *Legionella pneumophila*

Genomic DNA was prepared using a previously described protocol with some modifications [23]. Restriction digestion of genomic DNA with 50 U Sfi I (New England Biolabs, England, UK) was performed according to the manufacturer's recommendations. Fragments of DNA were separated in a 1 % agarose gel prepared and run in 0.5× Tris–borate-EDTA buffer (pH 8.3) in a contour-clamped homogeneous field apparatus (CHEF DR II system; Bio-Rad, Ivry sur Seine, France) with a constant voltage of 5 V cm^{-1} and increasing pulse times (5.6–50.6 s) at 14 °C for 25 h. The lambda ladder PFGE marker (New England Biolabs) was included as a molecular weight marker.

Pattern analysis was performed using the Gel Compar II software (Applied Maths, Kortrijk, Belgium) using the Dice band-based similarity coefficient and the UPGMA as the clustering method with a tolerance of 1 %. Isolates with a PFGE pattern that differed by one band or more were considered to belong to different genotypes and were designated with capital letters.

Sequence-based typing of *Legionella pneumophila*

Genomic DNA was extracted from the study isolates using the Chelex extraction technique (Bio-Rad Laboratories, Hercules, CA, USA). The seven target genes (*flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, and *neuA*) were amplified using the primers and amplification protocol provided by the EWGLI (v. 4.2). The primers and the polymerase chain reaction (PCR) conditions were the same as those previously described [10, 23]. Amplified products were sequenced in both directions using the ABI PRISM BigDye Terminator v. 3.1 Cycle Sequencing kit in the ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). The sequences were analyzed using BioEdit v. 7.0.9 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). SBT allele numbers were assigned to each strain based on the EWGLI database [9].