The L2b real-time PCR targeting the pmpH gene of Chlamydia trachomatis used for the diagnosis of lymphogranuloma venereum is not specific to L2b strains

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Abstract

The French Reference Centre for chlamydiae uses two real-time PCRs targeting the pmpH gene of Chlamydia trachomatis to differentiate between L strains and variant L2b, responsible for a lymphogranuloma venereum outbreak in Europe. We compared the results obtained for 122 L2b C. trachomatis-positive specimens, using the two real-time PCRs, with the sequencing of the ompA gene. Only 91 specimens were confirmed as L2b. Our results demonstrate that the lymphogranuloma venereum outbreak is no longer dominated by the variant L2b, and that many L-positive specimens were misidentified as L2b with the method used, which raises the question of its specificity.

Over the past decade, lymphogranuloma venereum (LGV), a sexually transmitted infection caused by Chlamydia trachomatis genovar L, has emerged in Europe and North America as a leading cause of proctitis in men who have sex with men [1]. As a consequence, surveillance programmes were implemented in several European countries including France where a national LGV surveillance network has been in place since 2010 for the monitoring of LGV cases, set up by the French Reference Centre for Chlamydiae. The case definition of LGV requires the confirmation of the presence of LGV genovar in the C. trachomatis-positive specimen. Diagnosis of LGV infections is important because these invasive strains require a longer duration of treatment (3 weeks) than do infections caused by non-LGV genovars D–K (1 week). It is also required for preventing long-term consequences of infection, as well as restricting secondary spread to sex partners [1]. Almost all LGV cases are caused by the L2b variant [2–4], suggesting clonal spread. The L2b genovariant harbours an A/G substitution on the ompA gene at position 485 [5].

Several molecular diagnostic methods specific for LGV detection have been reported since the occurrence of this outbreak [6–10], most of them are targeting the pmpH gene because all the LGV-genovars possess a unique gap of 36 bp, which is absent in the other genovars. In our laboratory, we opted for two real-time PCRs, the first one exploits this unique 36-bp deletion in all LGV genovars [11], and the second one allows the identification of L2b variant by targeting a 9-bp insertion unique for L2b within the pmpH gene [10]. This rapid method allows us to provide daily results to clinicians and showed that the variant L2b was found in all cases of LGV. Because of the recent description of new L2 variants [12,13], as well as the co-circulation of variants [14], we decided to verify, by sequencing of the ompA gene, the clinical specimens that were L2b-positive.

The study was carried out on 122 C. trachomatis-positive clinical samples, collected between 2010 and 2015, comprising 119 rectal specimens, two specimens from inguinal lymphadenopathy, and one from genital ulceration. Only specimens with a high bacterial load corresponding to a cycle threshold of PCR ≤25 were selected. The specimens were collected within the national routine LGV surveillance system in France. All 122 specimens were typed as L2b-positive. Analysis of sequence variation of the ompA gene was performed to confirm the genotypes found. An 1100-bp fragment was amplified by a nested PCR using the previously described NLO and NRO primers, and PCTM3 and SERO2A primers [15], and sequenced in both directions. The DNA sequences obtained were compared with available
sequences of all C. trachomatis genotypes using the BLAST algorithm in GenBank and by sequence alignment with the ompA gene sequences of reference strains L2b/UUCH-I/proctitis (AM884177) and L2/434/Bu (AM884176). When discordant genotyping results were observed between real-time PCRs and ompA sequencing, a fragment of 586 bp of the pmpH gene was sequenced using primers pmpH_1443F-TTGGTTGTGACGGAAGGAC and pmpH_2009R-ATCCGCTACCCAAAGAGAG and compared by alignment with currently available chlamydial pmpH gene sequences from GenBank: L2/434/Bu (NC_010287.1), L2b/142189 (EF612788.1), L2b/UUCH-I (AM884177), L2b/190437 (EF534758), L1/440 (AY184167), L3/404 (AY184169), A (AY184155), B (AY184156), Ba (AY184157), C (AY184158), D (AY184159), Da (AY967759), E (AY184160), Swedish variant E (SW-E: FN652779), F (AY184161), G (AY184162), H (AY184163), I (AY184164), Ia (AY967760), J (AY184165) and K (AY184166).

Among the 122 analysed specimens, 91 were confirmed to be of genovar L2b according to their ompA sequences. Interestingly, 30 specimens had an ompA genotype identical to the L2 reference strain L2/434/Bu (Fig. 1; Table 1). One specimen, collected from a human immunodeficiency virus-positive man who had sex with men with typical symptoms of rectal LGV (anal discharge, anal mucosal ulceration, tenesmus), had the ompA sequence of genovar Da. This last specimen was confirmed as L2b-positive by real-time PCR twice and by sequencing of the pmpH gene. Analysis of the pmpH gene of the 30 L2-positive specimens showed the presence at positions 1885–1893 of the 9-bp insertion supposed to be unique for L2b (Fig. 2, example of specimen TG93), which explains the positive amplification by real-time PCR, and demonstrates that this 9-bp insertion is no longer specific to the L2b genovar. Our results show that the LGV outbreak is no longer dominated by the L2b variant and suggest a potential genotype shift in the last 5 years (Table 1). Analysis of a large sampling is needed to confirm these findings.

This epidemiological situation characterized by the co-circulation of two LGV variants has been previously described [14]. In one case, ompA sequencing matched genotype Da whereas pmpH amplification was positive for L2b strain, suggesting either a mixed infection or a recombinant strain. Both hypotheses stand, previous studies reported LGV cases with mixed invasive and non-invasive genotypes [11,16,17]. Besides, a mixed infection could facilitate the selection of new recombinant variants, such as the hyper-virulent strain described by Somboonna et al. [18], which is the result of recombination events between L2 and D genotypes, as well as the variant described by Rodriguez-Dominguez et al. with genetic exchange between genotypes belonging to LGV and members of the G genotype [16]. Chlamydia trachomatis is a flexible bacterial species where recombination is relatively frequent, and where genes under selective immune pressure such as the ompA or the pmpH genes, have undergone mutational changes resulting in new variants with more virulence or more transmissibility traits. Hence, to date many L2-ompA variants (a to g) were described [12,13], and our results suggest a recombination event in the pmpH gene of our L2-positive specimens with L2b strains. This might reflect a bacterial skill for evading the immune system. The dynamic of the sexual behaviour among men who have sex with men contributed to the global spread of LGV. This epidemic situation increases the probability for the same host to be infected with two different strains, which could create an adequate environment for inter-strain recombination [16].

In the light of these results, some changes should be undertaken regarding the diagnosis of LGV infection. The detection of L genotypes should be maintained but not specifically the L2b variant, first because the LGV outbreak is no longer dominated by this variant, and second because many L-positive specimens were misidentified as L2b with the method used since the target is no longer unique for L2b variant.

**TABLE 1. Repartition of the genotypes involved in the 122 L2b selected cases between 2010 and 2015 according to the ompA sequencing**

<table>
<thead>
<tr>
<th>Year</th>
<th>L2b</th>
<th>L2</th>
<th>Non-L</th>
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<td>17</td>
</tr>
<tr>
<td>2014</td>
<td>12</td>
<td>8</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>2015</td>
<td>10</td>
<td>6</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>30</td>
<td>1</td>
<td>122</td>
</tr>
</tbody>
</table>

**FIG. 1.** Partial ompA gene sequences (position 400 to 500) of three L2-confirmed clinical specimens, Co5, TG93 and SL296, aligned against L2b/UUCH-I (AM884177) and L2/434/Bu (AM884176). Highlighted in grey is the A/G substitution at position 485 specific of L2b variant.

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FIG. 2. Partial pmpH gene sequence of the L2-positive clinical specimen TG93 aligned against L2b/142189 (EF612788.1), L2b/190437 (EF534758), L2/434/Bu (NC_010287.1, L1/440 (AY184167), L3/404 (AY184169), H (AY184163) and G (AY184162). Highlighted in grey are the nine nucleotides specific of L2b strains, but also found in the L2-positive clinical specimen.

Acknowledgements

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References