Insertion Sequence 1515 in the ply Gene of a Type 1 Clinical Isolate of Streptococcus pneumoniae Abolishes Pneumolysin Expression

Fabien Garnier,1* Rajendra Prasad Janapati,2 Emmanuelle Charpentier,2 Geoffrey Masson,1 Carole Grélaud,1 Jean François Stach,3 François Denis,1 and Marie-Cécile Ploy1

Laboratoire de Bactériologie-Virologie-Hygéine, CHU Dupuytren, EA 3175, Limoges, France; Max F. Perutz Laboratories, University of Vienna, Vienna, Austria; and Laboratoire de Microbiologie, Centre Hospitalier de Guéret, Guéret, France

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A serotype 1 Streptococcus pneumoniae strain isolated by blood culture from a woman with pneumonia was found to harbor insertion sequence (IS) 1515 in the pneumolysin gene, abolishing pneumolysin expression. To our knowledge, this is the first report of an IS in the pneumolysin gene of S. pneumoniae.

Streptococcus pneumoniae is a bacterial pathogen frequently isolated from young, elderly, and immunocompromised subjects. Pneumococci are divided into 90 serotypes based on their capsular polysaccharide (1). Serotype 1, one of the most prevalent invasive serotypes, is associated with complicated pneumonia, empyema, peritonitis, and salpingitis (4).

Pneumolysin (Ply), a pneumococcal virulence factor (3) classically defined as a pore-forming toxin inhibited by cholesterol, is produced by virtually all clinical isolates of S. pneumoniae (2). The enzyme is cytotoxic for ciliated epithelial cells, and its cytotoxic effects can directly inhibit phagocyte and immune cell function (8). Ply-deficient pneumococci exhibit a reduced capacity for (i) nasopharynx colonization, (ii) lower respiratory tract infection, (iii) intrapulmonary growth, (iv) survival in the bloodstream, and (v) cerebrospinal fluid invasion (7).

The Ply amino acid sequence is generally highly conserved among pneumococci, but some clinical isolates have been shown to bear mutations in the corresponding gene, ply. These mutations are found predominantly in serotypes 1, 7, and 8. A recent study (4) showed that more than 50% of serotype 1 strains harbored ply mutations. Analysis of these strains by multilocus sequence typing (MLST) revealed that two sequence types (ST), ST227 and ST306, predominated. ST306 harbored mutations at six amino acid positions (Y150H, T1721, K224R, A265S, and ΔK271), whereas ST227 harbored a single mutation (D380N). Ply activity was conserved in ST227 isolates, whereas ST306 isolates were unable to form pores in erythrocyte membranes (4).

Here, we report the insertion of an insertion sequence (IS) into the coding sequence of the ply gene in a type 1 clinical isolate of S. pneumoniae.

The serotype 1 S. pneumoniae strain Lim45, which belongs to our clinical strain collection, was isolated by blood culture from a woman with acute infection of the right upper pulmonary lobe. This isolate was identified using the Gram and optochin tests and subsequently used as positive control in the development of a method for identifying S. pneumoniae species based on the amplification by PCR of an internal fragment of the ply gene. However, in strain Lim45, PCR amplification of genomic DNA with primers Ia (5′-ATTTCGTAACAGTACTCAAC-3′) and Ib (5′-GATTCCTGTCTTTTACAGTC-3′) targeting an internal fragment of ply (9) yielded a 1,220-bp DNA fragment instead of the expected 348-bp product (data not shown). Sequence analysis of this amplified fragment with both strands (Perkin-Elmer Applied Biosystems, Les Ulis, France) revealed the presence of an 871-bp foreign DNA fragment inserted within the ply coding sequence. The insert corresponded to IS1515, which was first described for the capsule-encoding gene capIE of a serotype 1 S. pneumoniae strain (6). Primers LysI (5′-CCGACTTCTTATCTGACCC-3′) and LysII (5′-TTTGTCGCAAGCATTC-3′) were designed to amplify and sequence the entire ply gene of strain Lim45. Sequence analysis of the 2,460-bp amplicon showed that (i) IS1515 was inserted at nucleotide position 430 of the ply coding sequence and was flanked by two direct AAT repeats, indicating duplication of the target sequence, and that (ii) the ply coding sequence of Lim45 harbored nine mutations, only one of which induced an amino acid change, D380N, which was described as characteristic of ST227 (4). Surprisingly, MLST, performed as described elsewhere (4), showed that strain Lim45 belongs to ST228 rather than to ST227. These two ST have two allelic variations, gdh and gki, which differ by two and ten nucleotides, respectively (www.mlst.net). Comparison of the nucleotide sequence of the ply gene harboring IS1515 (2,290 bp) with the 38 ply allele sequences listed in the NCBI databank revealed 100% identity with the sequence of the ply-14 allele (accession number EF413948, unpublished reference).

Multiple copies of IS1515 (from 1 to 13) have been detected in the genome of most type 1 S. pneumoniae strains but not in most other serotypes (6). To determine the number of copies of IS1515 in the genome of strain Lim45, we used Southern analysis of EcoRI-digested chromosomal DNA, using as a probe an internal IS1515-specific fragment obtained by PCR amplification with primers IS1 (5′-TATCAGATATTACAGACCAGC-3′) and IS1II (5′-CTACGTCAATAGCCACA-3′), as described elsewhere (5). As one EcoRI cleavage site is present in IS1515 but not in the DNA fragment used as a specific probe, the number of hybridization bands should reflect the

* Corresponding author. Mailing address: Laboratoire de Bactériologie-Virologie-Hygéine, EA 3175, CHU Dupuytren, 2 avenue Martin Luther King, 87042 Limoges cedex, France. Phone: 33 55 05 63 48. Fax: 33 55 05 67 22. E-mail: fabien.garnier@unilim.fr.

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IS1515 copy number, which was nine in strain Lim45 (data not shown).

To determine whether IS1515 affected the synthesis of the ply gene product, we used Western blotting analysis of crude bacterial lysates probed with an anti-Ply polyclonal antibody. As shown in Fig. 1, no Ply was detected in strain Lim45, whereas a control strain with no Ply was detected in strain Lim45, bacterial lysates probed with an anti-Ply polyclonal antibody. This suggests that IS1515 can abolish the synthesis of the ply gene product in strain Lim45.


REFERENCES