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Evaluation of Molecular Typing for Epidemiological Study of *Chlamydia trachomatis* Genital Infections

PATRICIA RODRIGUEZ,[1]* BERTILLE DE BARBEYRAC,[1] KENNETH PERSSON,[2] BRIGITTE DUTILH,[3] AND CHRISTIANE BEBEAR[1]

Laboratoire de Bactériologie, Université de Bordeaux 2, 146 rue Léo Saignat, F-33076 Bordeaux Cedex,[1] and Laboratoire d'Analyses Médicales, F-33000 Bordeaux,[3] France, and Department of Clinical Virology, Malmö General Hospital, S-214 01 Malmö, Sweden[2]

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Molecular typing and serotyping were compared for 150 *Chlamydia trachomatis* strains isolated from genital sources, belonging to 10 different serovars. Because of the general agreement of the two methods, molecular *ompl* genotyping was applied to the epidemiological study of *C. trachomatis* isolates from genital infections in Bordeaux (France), during a 29-month period. The most prevalent *ompl* genotypes were E (51.7%), F (17.3%), D (8.8%), and G (8.4%). Restriction enzyme analysis allowed identification of a serovar D variant (Dv), whereas serovar E strains were homogeneous.

Fifteen different serovars of *Chlamydia trachomatis* were initially recognized (4). Four of these serovars have been predominantly associated with endemic trachoma and designated A, B, Ba, and C. The serovars D through K have generally been isolated from the genital tract. Lymphogranuloma venereum is caused by the three serovars L1, L2, and L3. Three new serovars, Da, Ia, and L2a, have recently been described (14). Serotyping, with monoclonal antibodies recognizing antigenic determinants located on the major outer membrane protein (MOMP), is the reference method for typing *C. trachomatis* isolates (15). However, new methods, detecting nucleotide sequence differences in the MOMP gene (*ompl*) of the different serovars, have been described (1, 9, 11). A procedure previously developed (9) analyzed the restriction profile of *ompl* after amplification by polymerase chain reaction. Applied to reference strains, this procedure allowed identification of 13 of 15 serovars. The B and Ba serovars could not be separated by this method.

In this study, the results of restriction mapping of polymerase chain reaction-amplified *ompl* product and of serotyping were compared. We compared 150 clinical isolates selected for 10 serovars. The molecular typing method was extended to the three new serovars. Finally, we used this method for an epidemiological study of *C. trachomatis* genital infections. Furthermore, because of the high prevalence of isolates from serovar E, an attempt was made to find genomic differences in the *ompl* genes of these strains by restriction enzyme analysis.

Comparison of molecular typing and serotyping methods. One hundred fifty *C. trachomatis* clinical strains, isolated from genital specimens, were typed both by the serotyping method and by restriction fragment length polymorphism of *ompl* amplified by polymerase chain reaction (*ompl* genotyping). Serotyping was performed with serovar-specific monoclonal antibodies raised against the 15 reference serovars (6). Monoclonal antibodies with specificity towards Da, Ia, or L2a were not available. Genotyping was performed by *Alu*I and *Hpa*II-*Eco*RI-*Hin*fI restriction analysis of CT1-CT5-amplified *ompl*, according to the previously described procedure (9). Each *ompl* genotype was defined by testing a reference strain representing each of the 15 serovars. Comparison of the restriction profiles of each wild clinical isolate with those of the reference strains enabled us to assign a genotype to each clinical isolate.

The serotyping gave the following results: 19 B strains, 19 C, 11 D, 11 E, 9 F, 9 G, 10 H, 35 I, 13 J, 11 K, and 3 strains serotyped as F/G. The results obtained with the *ompl* genotyping were in agreement with serotyping for all the strains from the serovars D, E, F, G, H, J, and K.

The three serotyped F/G isolates presented the same restriction patterns as the G reference strain (9). They were then considered to be G strains, cross-reacting with the serovar F-specific monoclonal antibody, and not two different strains present in the same clinical specimen. This result confirmed a previous report of cross-reactivity of a G serovar with the serovar F monoclonal antibodies (12).

Discrepancies were observed for the B-, C-, and I-serotyped strains. The C strains reacted serologically with a C-specific monoclonal antibody and failed to react with two different monoclonal antibodies to the J serovar, which belongs to the C group (14). The B strains reacted with two different B-specific monoclonal antibodies. Sequencing now in progress of the MOMP genes from discrepant strains has disclosed strong similarity between these B strains and the prototype strain. The sequence of the C variant shows several differences from the C prototype sequence and may be more closely related to the J strains although serological markers and restriction fragment length polymorphism pattern set it apart from the J strains as well.

Concerning the serotyped I strains, only 1 of the 35 tested showed the same pattern as the I reference strain (ATCC I/VR 880). The other 34 isolates presented a typical H profile but did not react with four different serovar H-specific monoclonal antibodies. These I strains may belong to the new Ia serovar that has been reported to be more frequently isolated than serovar I strains (11). This hypothesis could probably have been confirmed by comparing these strains with the Ia reference strain with *Dde*I and *Fok*I digestions as was done later in the study.

Da, Ia, and L2a *ompl* genotype determination. Da (TW-448/H1), Ia (UW-202/H14), and L2a (UW-396/NS) reference strains from the Washington Research Foundation (Seattle, Wash.) were first tested to determine their *ompl* genotypes.

* Corresponding author.

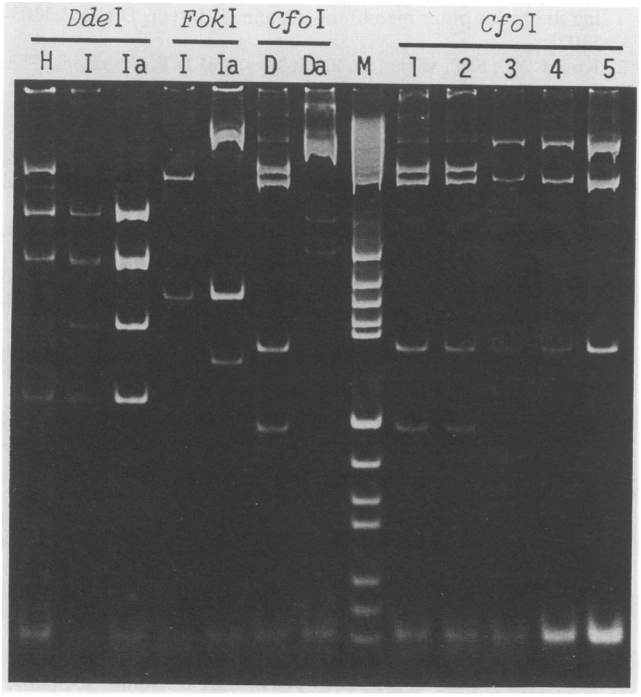

FIG. 1. Identification of H, I, Ia, D, Da, and Dv *ompl* genotype strains. Shown are *ompl* restriction profiles obtained on an 8% polyacrylamide gel of H, I, and Ia reference strains after *Dde*I digestion; I and Ia reference strains after *Fok*I digestion; and D and Da reference strains after *Cfo*I digestion. Lanes 1 to 5 show *Cfo*I analysis of five D-genotyped isolates, identifying D strains (lanes 1 and 2) and Dv strains (lanes 3 to 5). Lane M, molecular weight markers, pBR322 DNA digested with *Hae*III.

Da and L2a reference strains presented the same restriction profiles as serovar D and L2 reference strains, respectively. The Ia reference strain showed a typical serovar H reference strain profile (data not shown).

Specific restriction endonuclease digestions were performed on CT1-CT5-amplified fragments depending on the *ompl* genotype, as previously described (11). *Cfo*I restriction digestion of an *ompl* amplified fragment from D-typed strains allowed Da genotype identification as shown in Fig. 1. Ia genotype determination was performed in two steps. *Dde*I digestion of an *ompl* amplified fragment from H-typed strains allowed the differentiation between H and I or Ia types (Fig. 1). An Ia or I *ompl* genotype was then determined with *Fok*I digestion. L2 and L2a differentiation was done with *Nla*III digestion (data not shown).

Epidemiological study. The *ompl* genotyping method was then applied to all the *C. trachomatis* strains isolated from genital samples during a 29-month period (from June 1990 to October 1992). A total of 203 *C. trachomatis* isolates were collected from 120 women and 73 men. A total of 96 of these isolates were from the Department of Bacteriology, Pellegrin Hospital, Bordeaux, France, and 107 were from a private medical laboratory, Laboratoire d'Analyses Médicales, Bordeaux, France.

Typing of these strains revealed that the most prevalent *ompl* genotype was E (51.7%), followed by F (17.3%), D (8.8%), G (8.4%), H (3.9%), J (3.9%), and K (2.9%), as shown in Table 1. Only a few strains from types B, C, I, and Ia were identified, whereas no A, Ba, Da, L1, L2, L2a, or L3 strain was isolated. These results showed a distribution similar to that previously demonstrated for France and other countries (2, 5, 7, 10, 12, 13). Furthermore, a comparable distribution of *ompl* genotypes was observed for males and females, as well as between the strains isolated at the hospital and those in the private laboratory.

However, after analysis of D genotypes by *Cfo*I digestion of CT1-CT5-amplified product, two restriction patterns were observed, as shown in Fig. 1. A total of 12 of the 18 D-typed strains presented the D reference profile whereas 6 strains showed an unexpected profile (Fig. 1, lanes 3 to 5), different from that obtained with either the D or the Da reference strains. Similar observations were reported by Sayada et al. (12), with *Cfo*I on a different amplified *ompl* fragment. Strains from the present study were then designated D variant (Dv) strains. This variant group of serovar D represented 33% of the D-typed isolates. Recent data (3) reported nucleotide sequences of variable domains of two D variants (D^- and D*). These sequences do not suggest classification of our Dv strains as D^- or D*. Sequencing of amplified *ompl* would demonstrate nucleotide substitutions detected by *Cfo*I digestion.

For 8 of the 193 patients tested, two *C. trachomatis* strains were isolated sequentially during a period of from 2 to 15 months. Six of them were infected twice by the same *ompl* genotype strains: four patients by E strains, one by D strains (the two strains were identified as Dv), and one by G strains. Different *ompl* genotype strains were isolated from the two other patients: from the first, H and F strains were identified after a 5-month period, and the last patient was infected first with a Dv strain and 9 months later with an E strain. Although the *ompl* genotyping method was verified to detect artificial mixtures of different serovars (data not shown), no case of simultaneous multiple *ompl* genotype infection could be detected within the studied population. Furthermore, multiple specimens from different sites were collected simultaneously, from four female patients. In all the cases, the same *ompl* genotypes were identified for all the sites from the same patient.

Two *ompl* genotype B and two *ompl* genotype C strains were isolated among the 203 strains. They were identified as B and C types by serotyping although they presented the atypical B and C restriction profiles mentioned earlier. At present, no typical B or C strains, similar to the reference

TABLE 1. *ompl* genotype distribution of 203 genital *C. trachomatis* strains isolated in Bordeaux, France, from June 1990 to October 1992

Specimen source	No. of specimens with *ompl* genotype (% of total):											
	B	C	D	Dv	E	F	G	H	I	Ia	J	K
Male urethra (n = 75)	1 (1.3)		5 (6.6)	5 (6.6)	34 (45.3)	11 (14.6)	10 (13.3)	5 (6.6)			2 (2.6)	2 (2.6)
Endocervix (n = 128)	1 (0.8)	2 (1.6)	7 (5.5)	1 (0.8)	71 (55.5)	24 (18.7)	7 (5.5)	3 (2.3)	1 (0.8)	1 (0.8)	6 (4.7)	4 (3.1)
Total (n = 203)	2 (1)	2 (1)	12 (5.9)	6 (2.9)	105 (51.7)	35 (17.3)	17 (8.4)	8 (3.9)	1 (0.5)	1 (0.5)	8 (3.9)	6 (2.9)

strains, have been isolated from clinical specimens in Bordeaux.

***ompl* genotype E subtyping.** Because of the high prevalence of some *ompl* genotypes that cause infection, restriction enzyme analysis was applied to 41 *C. trachomatis* E strains: the ATCC reference strain (VR 348B) and 40 genital strains isolated in Bordeaux (19 strains) or in Malmö, Sweden (21 strains). The CT1-CT5-amplified fragment was digested by 15 different enzymes, chosen after computer analysis of the serovar E *ompl* sequence (8)—*Ava*I, *Dde*I, *Eco*RI, *Hha*I, *Hin*cII, *Hin*fI, *Hpa*II, *Mbo*I, *Pvu*II, *Rsa*I, *Sau*3AI, *Taq*I (Bethesda Research Laboratories, Gaithersburg, Md.), *Bam*HI, *Xho*I (Promega Corporation, Madison, Wis.), and *Hae*III (Pharmacia, Milwaukee, Wis.)—following the manufacturers' instructions. The restriction fragment length polymorphism profiles were then analyzed with polyacrylamide gels.

Unfortunately, no difference between reference and wild strains, and between isolates from France and those from Sweden, was observed. These results demonstrated a high level of conservation of *ompl* between the E strains, even among strains from different geographical origins. Putative nucleotide substitutions should be detected with a more sensitive method, such as direct sequencing.

In conclusion, molecular biology procedures allow typing of *C. trachomatis* as well as immunological techniques, as shown by the comparison of *ompl* genotyping and serotyping. The restriction enzyme analysis method reported in this study is not sufficient to determine subtypes among *ompl* genotype E clinical strains. However, genotyping allows identification of two groups in the D serovar strains by using *Cfo*I restriction hydrolysis. Variant identification will allow more detailed epidemiological studies of *C. trachomatis* infections, especially by giving more information about the strains sequentially isolated from the same patient. Furthermore, detection of *ompl* sequence variations is of great interest because of the importance of MOMP as a vaccine candidate. Molecular typing, which requires only commonly used molecular biology equipment and techniques, constitutes a promising method for detecting variations among *C. trachomatis* strains.

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