Restriction endonuclease patterns of the \( \text{omp1} \) gene of reference \textit{Chlamydia trachomatis} strains and characterization of isolates from Cameroonian students

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Eighteen reference strains of \textit{Chlamydia trachomatis} were differentiated by \( \text{omp1} \) PCR- and nested PCR-based RFLP analysis, using two restriction digestions, one with \textit{AluI} and the other with the three enzymes \textit{HpaII}, \textit{EcoRI} and \textit{Hinfl}. \textit{AluI} digestion allowed the differentiation of 12 different profiles after \textit{CT1/CT5} PCR and 13 different profiles after the nested PCR. The triple hydrolysis permitted the identification of 15 different patterns. In all, 16/18 reference strains were clearly identified. These reference patterns were successfully used to genotype 34 of 35 (28 strains and 7 clinical specimens) samples from infected students, collected during a screening programme in Yaoundé (Cameroon). Genotypes D, Da, E, F, G and J were found. The most prevalent \( \text{omp1} \) genotype was E (\( n = 14; 40 \% \)), followed by F (\( n = 7; 20 \% \)). As RFLP patterns of reference strains are essential for typing clinical isolates, they will greatly facilitate \textit{C. trachomatis} characterization in many resource-limited laboratories.

Introduction

\textit{Chlamydia trachomatis} is the causative agent of a variety of diseases and syndromes, including trachoma, urogenital infections and lymphogranuloma venereum, depending on the biovar and serovar of the strains involved. Currently, 19 human serovars and numerous variants have been identified with the use of polyclonal and monoclonal antibodies against the major outer-membrane protein (MOMP). Serovars A–C are most often associated with trachoma and serovars L1–L3 are associated with lymphogranuloma venereum, while serovars D–K are primarily associated with urogenital and neonatal infections (Morré et al., 1998a; Ossewaarde et al., 1994; Sturm-Ramirez et al., 2000). Various studies have shown the feasibility of typing clinical isolates by PCR-based RFLP analysis of the amplified \textit{omp1} gene encoding MOMP (Frost et al., 1991; Rodriguez et al., 1991; Sayada et al., 1991), avoiding the costly production of monoclonal antibodies. This method was originally performed on cell cultures of cervical and urethral specimens. Nowadays, the direct typing of cervical specimens by a nested \textit{omp1} PCR-RFLP assay is used for the differentiation of isolates into genotypes (Lan et al., 1993, 1994). Nevertheless, typing of \textit{C. trachomatis} is not routine in many African laboratories. Up to now, only one study showing the genotypic diversity of genital \textit{C. trachomatis} in sub-Saharan Africa has been performed (Sturm-Ramirez et al., 2000). Although RFLP analysis of an unknown strain consists of comparing the patterns with those of reference strains, patterns of all reference strains have not yet been published. Therefore, the aim of this study was to present RFLP patterns of 18/19 \textit{omp1}-amplified reference strains, in order to promote typing of \textit{C. trachomatis} by RFLP analysis in many laboratories with limited resources. In addition, this study was intended to describe the distribution of \textit{C. trachomatis} genotypes in urogenital specimens, collected from Cameroonian students residing in Yaounde.

Methods

According to the omp1 nucleotide sequence of C. trachomatis serovar D/UW-3/Cx (Stephens et al., 1998).
granuloma venereum) were clearly identified. In addition, the genotypes corresponding to the three main groups based on genetic relatedness and serological reactivity (Yuan et al., 1989) were clearly identified. Genotypes B, Ba, D, Da, E, L1 and L2 in the B complex, genotypes F and G in the intermediate group F/G and genotypes C, A, H, I, Ia, J, K and L3 in the C complex.

Fig. 2. Reference patterns from PCTM3/SERO2A PCR-based RFLP genotyping of 17 Chlamydia trachomatis reference strains (genotypes L2 and L2a had similar profiles) cleaved with AluI (top) and with HpaII+EcoRI+HinfI (bottom). Lanes M, size markers (pBR322 DNA digested with HaeIII).

The accessibility of reference patterns facilitated the typing of strains circulating among Cameroonian students. The prevalence of urogenital chlamydial infection in the screening study was 3.78% (48/1267). Thirty-five Cameroonian positive specimens were used in this typing study, of which 28 strains were isolated on McCoy cells and typed by PCR-based RFLP analysis. The remaining seven specimens were subject to direct omp1 amplification by nested PCR and typed. The isolation of strains in cell culture is expensive and not easy to perform, but allows good amplification of omp1. Direct amplification of omp1 from specimens by nested PCR is not always successful. In our study, of the 20 non-cultured specimens, only seven could be typed after nested-PCR omp1 amplification. In all, of the 35 strains from students from which the omp1 gene was successfully amplified, RFLP analysis allowed genotyping of 34 (97.1%). The genotypes found were E (n = 14; 40%), F (n = 7; 20%), G (n = 5; 14.3%), D (n = 3; 8.6%), Da (n = 3; 8.6%) and J (n = 2; 5.7%). One strain could not be identified. Sequence analysis found it to be a genotype J strain, with three nucleotide substitutions, in one case involving an AluI restriction site. The omp1 sequence of our triple-mutant J strain was not identical to those of variants Ja and Jv, respectively described by Dean et al. (2000) and Morré et al. (1998a). The patterns were very reproducible and comparison between clinical and reference patterns was easy. The same size markers have to be used for good comparison with reference patterns. PCR-based RFLP analysis has been shown in many studies, including ours, to be a simple, sensitive, inexpensive and reproducible method for typing of urogenital Chlamydia trachomatis in clinical specimens (Ikehata et al., 2000; Morré et al., 1998a).

This study was the first to characterize Cameroonian C. trachomatis isolates. RFLP analysis found a heterogeneous population of C. trachomatis in this student population. These results are in agreement with those reported in other areas (Brunham et al., 1996; Sturm-Ramirez et al., 2000; van de Laar et al., 1996; van Duynhoven et al., 1998). The predominance of class B genotypes (57%) in this study and in many others may suggest a real biological advantage over other genotypes (Sturm-Ramirez et al., 2000). The distribution of C. trachomatis genotypes was comparable in males and females, with genotype E (respectively 43.8 and 36.8%) predominating. The most frequent types, E and F, have been described previously (Jurstrand et al., 2001; Morré et al., 1998b; Poole & Lamont, 1992). Genotypes D/Da, F and G were also common in both sexes. The observed distribution of genotypes has also been described in many studies (Batteiger et al., 1989; Morré et al., 2000; van De Laar et al., 1996; van Duynhoven et al., 1998).

The interest of our study was to obtain reference RFLP patterns after amplification of the C. trachomatis omp1 gene either from cell culture isolates or directly from clinical specimens. With the availability of these reference patterns, PCR-based RFLP typing could be used in laboratories with limited resources to characterize C. trachomatis circulating in such areas, since ordering reference strains is expensive and performing reference patterns is time-consuming. It is up to each laboratory to choose the best method, depending on its resources.

Acknowledgements

We thank the Agence Universitaire de la Francophonie for granting a PhD fellowship, Y. Idrissi for technical assistance and A. Kfutwah for English correction.

References


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