Serovar distribution of urogenital *C trachomatis* isolates in Germany

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Abstract

Using monoclonal antibodies in a micro-immunofluorescence test the distribution of *C trachomatis* serovars isolated in the STD outpatient clinic of the Department of Dermatology of the University of Heidelberg was determined. Of 56 isolates investigated the most frequent was serovar E with 35.7%, followed by serovar D with 28.6% and serovar F with 26.8%. This shows that the distribution of *C trachomatis* in Germany is very similar to that in other Western countries.

Introduction

Antigens can be taken as a criterion for the classification of microorganisms and can give information about the biological behaviour of the infectious agent and the spread of the infection. Starting with the toxicity-prevention test,^{1,2} followed by the two-step,³ later one-step micro-immunofluorescence test (IFT) using polyclonal antibodies,⁴ typing of *C trachomatis* serovars is carried out today with monoclonal antibodies.^{5,6} On the basis of polyclonal or monoclonal micro-IFT, 15 serovars of *C trachomatis* including trachoma biovars A-K and the lymphogranuloma venereum biovars L1-L3 have been defined.^{5,7,8}

So far, urogenital *C trachomatis* isolates collected in the United States,^{9,10} Scandinavian countries,¹¹⁻¹³ the Netherlands¹⁴ and earlier in England¹⁵ have been serotyped. In contrast, nothing is known about the distribution of *C trachomatis* serovars in Germany. Using monoclonal antibodies in a micro-IFT, we studied the distribution of *C trachomatis* serovars isolated in the STD outpatient clinic of the Department of Dermatology of the University in Heidelberg. In addition, the site of the infection has been compared with the serovars isolated.

Patients, materials and methods

Collection of isolates A total of 61 transport media found positive by cell culture upon investigation of urogenital specimens were stored frozen at -70°C. The specimens had been collected from female and male patients seen consecutively in the STD outpatient clinic at the Department of Dermatology, University of Heidelberg. Specimens from known male homosexuals and known male or female partners as well as specimens of patients with indication of other simultaneous infections were not included.

Preparation of antigens Frozen specimens were thawed for the inoculation in cell culture and cultivation of *C trachomatis* isolates. Briefly, McCoy cells were cultured on glass cover slips in Roosevelt Park Memorial Institute (RPMI) medium 1640 supplemented with 10% fetal calf serum. After growth to monolayers, cell cultures were treated with cycloheximide at a final concentration of 1 µg/ml. Cultures were then inoculated with 0.2 ml transport medium, centrifuged at 3000 g for one hour, medium changed after two hours, and then incubated for up to 72 hours. To detect inclusions, cells were fixed in methanol for ten minutes and 20 µl of fluoresceine isothiocyanate conjugated monoclonal antibodies (Syva-Merck, Darmstadt, Germany) applied. After being incubated in a moist chamber for 15 minutes at 37°C cover slips were rinsed with distilled water, air dried, mounted and examined with a Zeiss fluorescence microscope.

In order to enrich *C trachomatis* organisms, sub-passages were carried out in vial cultures. Briefly, one culture vial of duplicate cultures was sonicated and dependent on the infectivity of the monolayers (observed by staining the other of the duplicate cultures), the culture medium was inoculated at different ratios. When infectivity, usually after four to nine passages of culture, had reached at least 70%, the medium was removed, the infected cells scraped and the culture vials washed off by adding 0.5 ml phosphate buffered saline containing 0.02% formalin. After centrifugation at 11 000 g for 15 minutes the pellet was resuspended in 0.02-0.03 ml of 3% formalinised yolk sac suspension obtained from Washington Research Foundation, Seattle, USA and vortexed.

Typing of isolates A panel of 16 monoclonal antibodies with mono- or polyspecificity for *C trachomatis* prototype strains were obtained from the Washington Research Foundation, Seattle, USA. Prior to use for typing isolates, the pattern of reactivity⁵ was checked with *C trachomatis* prototype strains (obtained from Washington Research Foundation, Seattle, USA), which had been enriched and processed for antigen preparation according to the protocol for the isolates.

Anti-mouse polyvalent immunoglobulin antibodies conjugated with fluoresceine isothiocyanate (Sigma, München, Germany) were used as a second antibody. The appropriate dilution of this conjugate was determined prior to use and Evans blue added as counter stain in a final concentration of 0.05%.

Using a capillary, 16 dots of approximately 0.2 µl of the different antigen suspensions were placed on a slide. At room temperature the slides were air dried and fixed in acetone for 15 minutes.

Diluted monoclonal antibody-suspensions (0.1 ml) were applied to the slides to cover the antigen. After 30 minutes incubation at 37°C the slides were rinsed, washed four times, in turns, with phosphate buffered saline and then distilled water and air dried. Afterwards 0.1 ml of the diluted conjugate was applied to the slide and incubated for 30 minutes at 37°C in a moist chamber. Again, the slides were washed four times and air dried. The slides were inspected with a Zeiss fluorescence microscope at a magnification of 400. The serovar was determined according to the reaction pattern originally established by Wang *et al.*⁵

Results

Out of 61 frozen specimens which originally had been found positive by routine investigation with cell culture, in 56 cases *C trachomatis* could successfully be recultivated.

Typing of these isolates revealed that the most prevalent serovar was E (n=20; 35.66%), followed by D (n=16; 28.57%) and F (n=15; 26.77%).

Figure 1 Serovar distribution of urogenital *C trachomatis* isolates (n=56).

Figure 2 Serovar distribution of *C trachomatis* isolates collected from the urethra (n=28).

Serovar G (n=2; 3.6%) and K (n=3; 5.39%) were less often found (fig 1). Serovars associated with trachoma (A, B, Ba, C) and lymphogranuloma venereum-biovars L1, L2 and L3 as well as serovars H, J, I, were not isolated.

When the serovars were compared with the site of collection of the specimens, a similar distribution of serovars was found. Among 28 urethral isolates, serovar E was most common (n=10; 35.74%). Serovars D and F could be found in equal numbers in the urethral specimens (n=9; 32.13%) (fig 2). Twenty one endocervical specimens consisted in equal numbers of serovar E and D (n=6; 28.6%) and serovar F was isolated in 23.8% (n=5). In addition, serovar G and K could each be detected in 9.5% (n=2) of the cervical specimens (fig 3).

Discussion

This study shows that the distribution of *C trachomatis* in Germany is principally similar to the situation found in previous studies in the United States,^{9,10} in Scandinavian countries,¹¹⁻¹³ in the Netherlands¹⁴ and in England.¹⁵ In our study Serovar E (35.7%) was most prevalent, followed by serovar D (28.6%) and F (26.8%) representing 91.1% of the isolates investigated. In previous studies using the polyclonal micro-IFT which often did not allow the clear distinction of serovars D and E on the one hand,

Figure 3 Serovar distribution of *C trachomatis* isolates collected from the cervix (n=21).

and serovars F and G on the other, the portions of E/D were approximately between 40% and 60%, the portions of F/G between 20% and 30% of the isolates and the portions of the remaining isolates were below 10%.^{10 12 16-18} A high prevalence (95%) of D/E and F/G similar to our results was found by Dwyer *et al.*¹⁵ The portions of serovars D, E and F determined with monoclonal antibodies ranged from 63% to 80%.^{5 6 13 15} As outlined by Kuo *et al.*¹⁰ the preponderance of serovars D, E and F may be the result of the selective pressure by socioeconomic and environmental factors rather than by differences in the pathogenic characteristics of the serovars.

In five of 61 specimens which originally were found positive for *C. trachomatis* using the cell culture, *C. trachomatis* could not be recultivated. This recovery rate of 91.8% was similar to that described by Newhall *et al.*⁶ and seems high enough to exclude an essential bias with respect to the portions of distinct serovars which might be recultivated with less efficacy. Infections caused by multiple serovars could not be detected. Since the rate of those infections is approximately 2%¹⁹ the relatively low number of specimens investigated in our study may be responsible for this finding.

The fact that most of the urogenital chlamydiae infections are caused mainly by only a small portion of serovars limits the use of serotyping of *C. trachomatis* isolates as a tool of epidemiological investigations such as contact tracing. Auxotyping of *C. trachomatis* isolates has been investigated,^{20 21} but is difficult to establish owing to the obligatory intercellular replication of chlamydiae. In addition, the use of serotyping on a large scale is still hampered by the fact that relatively high amounts of antigens are necessary before typing can successfully be carried out by the micro-IFT. These technical inconveniences may be overcome by the use of polymerase chain reaction followed by cleavage with endonucleases.²²

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