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INVESTIGATIVE REPORT

Molecular Typing of *Treponema pallidum* in Denmark: A Nationwide Study of Syphilis

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The aim of this nationwide study is to determine the strain type diversity among patients diagnosed with syphilis by PCR during a 4-year period in Denmark. Epidemiological data, including HIV status, for all patients were obtained from the Danish national syphilis registration system. Molecular strain typing was based on characterization of 3 variable treponemal genes, *arp*, *tpr* and *tp0548*. A total of 278 specimens from 269 patients were included. Among the fully typeable specimens ($n = 197$), 22 strain types were identified, with 1 type, 14d/g, accounting for 54%. The majority (93%) of the patients reported acquiring syphilis in Denmark. Among patients with concurrent HIV, 9 full strain types were identified and no difference in strain type was found by HIV status ($p = 0.197$). In conclusion, the majority of patients were infected in Denmark and the HIV-infected syphilis patients were diagnosed with a wide spectrum of different strain types of *Treponema pallidum*. *Key words:* Treponema pallidum*; molecular typing; syphilis; HIV.*

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Treponema pallidum subspecies *pallidum*, the causative agent of syphilis, is a major cause of sexually transmitted infections. In western countries syphilis mostly occurs among men who have sex with men (MSM), but in the developing world the disease represents an extensive problem (1). During the last decade, Denmark has witnessed increasing rates of infectious syphilis, from almost eradicated in 1994 with 34 cases (2) to 352 cases in 2013 (3). According to Danish law, all cases are registered in a nationwide database. Most reported cases in Denmark are among MSM, many of whom have concurrent human immunodeficiency virus (HIV) infection (3). In an earlier study we demonstrated that almost 10% of Danish men with syphilis were diagnosed with HIV within the subsequent 5 years (4). Of particular public health concern is the increased risk of transmission and acquisition of HIV during syphilis infection (5).

Molecular typing is a useful tool for understanding epidemics, and molecular epidemiology can be used for preventing and controlling infectious diseases. Since 1998, *T. pallidum* has been characterized by means of the Center for Disease Control and Prevention's (CDC) molecular typing method based on 2 target genes, i.e. determination of the number of 60-base-pair (bp) repeats within the acidic repeat protein (*arp*) gene, and restriction fragment length polymorphism (RFLP) analysis of sequence differences in the *T. pallidum* repeat (*tpr*) subfamily II genes (*tprE*, *tprG* and *tprJ*) (6). Recently, this typing system was supplemented with sequence analysis of a variable region of the *tp0548* gene (7). The discriminatory capacity was markedly refined by combining the CDC subtype and the *tp0548* sequence type now yielding a strain type, using the nomenclature proposed by Marra et al. (7).

Macrolide resistance has been investigated in several countries where azithromycin is used as a treatment alternative, showing rates of resistance ranging from very low in southern Africa (8) to very high rates of the resistance-conferring A2058G mutation in the USA and Ireland (9–12) and 100% resistance in Shanghai, China (13). In Denmark syphilis is treated with penicillin or doxycycline and, to date, there have been no documented reports of treatment failures.

T. pallidum subtype diversity has been investigated in several countries (14–18). Nevertheless, no data exist on the diversity of strain types at a national level. As genital ulcers facilitate HIV transmission it is of pivotal importance to elucidate the epidemiological determinants underlying the high rates of syphilis. Our study aimed to determine the strain types among all Danish patients with syphilis diagnosed by PCR testing of material from genital ulcers over a 4-year period since the implementation of this method in Denmark in 2009. Moreover, we linked the *T. pallidum* strain type with epidemiological data (e.g. HIV status) from a nationwide database on syphilis.

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MATERIALS AND METHODS

Setting

Denmark has a population of 5.6 million people (19) with an estimated HIV prevalence of 0.07% (20) in the adult population. The Danish healthcare system is tax-funded. Patients suspected of having syphilis are primarily seen at specialized departments at hospitals or sexually transmitted disease (STD) clinics.

Specimens

PCR testing for *T. pallidum* is centralized at the Statens Serum Institut, where the method was implemented in 2009. The specimens in this study were taken for diagnostic purposes from patients with genital ulcers. We included 278 *T. pallidum* PCR-positive ulcer specimens from 269 patients diagnosed with syphilis during the period May 2009 to December 2013 in Denmark. An additional 50 specimens were *T. pallidum* PCR positive, but due to insufficient material these were not subjected to strain typing and were excluded from the study. The 3-component strain type was obtained by combining the *arp* repeat size, the *tpr* E, G and J RFLP pattern and the *tp0548* sequence type. For example, 14d/f refers to 14 *arp* repeats, *tpr* RFLP pattern "d", and *tp0548* sequence type "f" (6, 7) (see Appendix S1[1] for details).

Data sources

Under Danish law, newly acquired syphilis, i.e. primary, secondary and early latent syphilis, is a notifiable disease and the reporting is done by the treating physician. The patients are registered in the Danish national syphilis registration system, which was established in 1993 and is based at the Statens Serum Institut. The collected data include mode of infection, country of infection and result of prior HIV testing. Country of infection is based on the reporting of the patient, together with a clinical evaluation of the stage of infection in relation to the time of reported exposure. The unique 10-digit central person registry (CPR) number assigned to all individuals in Denmark at birth or upon immigration was used to link data from the Danish national syphilis registration system to patient specimens. Exemption for review by the ethics committee system and for obtaining informed consent was obtained from the Committee on Biomedical Research Ethics for the Capital Region.

Statistical analysis

Categorical data were compared using the χ^2 test or Fisher's exact test, where appropriate. The Mann-Whitney test was used for comparison of the bacterial load between typeable and non-typeable specimens. Differences with $p < 0.05$ (2-sided) were considered statistically significant. Data analysis was done using SPSS version 16.0 (SPSS Inc., Chicago, Il, USA).

RESULTS

In the period May 2009 to December 2013, 278 specimens that were positive by *T. pallidum* PCR were obtained from 269 patients with primary syphilis (7 patients returned with > 1 episode of syphilis during the study period). Most of the patients were men (94%), and when self-reported sexual orientation was stated, 206/239 (86%) of the men were MSM. The median age was 36 years (range 15–71 years). Ninety percent of patients were Danish citizen and 93% were infected in Denmark. Of the 278 specimens, 197 (71%) were fully strain-typed by the *arp*, *tpr* and *tp0548* assays and 63 (23%) specimens were partially typeable with at least 1 of the 3 assays. Eighteen (6%) of the specimens were negative in all 3 assays. We found a statistically significant lower bacterial load in the non-typeable specimens ($p < 0.001$).

T. pallidum *strain distribution*

A total of 7 *arp* sizes (6, 7, 8, 11, 14, 15 and 16) and 8 *tpr* RFLP patterns (b, d, e, f, j, k, l and p) were identified. Sequence analysis of the *tp0548* gene revealed 4 sequence types (c, d, f and g). By combining the 3 typing methods, 22 strain types were identified. The most common strain type was 14d/g (54%) followed by 14d/f (18%) and 14l/g (5%). The remaining strain types were rare (Table I).

Characteristics of patients with fully typeable specimens

Several strain types were detected in only one patient, suggesting that they were imported cases. For example, only 1 patient had strain type 14d/c and this patient reported being infected in Pakistan. Furthermore, only one patient had strain type 16d/d and this patient reported being infected in Brazil. Also, only 2 patients had strain type 6b/g and both reported being infected outside Denmark, in Thailand and Greenland, respectively. However, the majority of the patients reported being

Table I. *Strain type distribution of 197* T. pallidum *positive specimens typeable by the* arp, tpr *and* tp0548 *assays and HIV-infected patients, men who have sex with men (MSM) based on self-reported sexual orientation and patients infected in Denmark in each strain type. Strain types with 2 or more patients were considered a cluster*

Strain type	Cases *n* (%)	HIV *n* (%)	MSM *n* (%)	Denmark *n* (%)
6b/g	2 (1.0)	0 (0)	1 (50)	0 (0)
6d/g	2 (1.0)	1 (50)	2 (100)	2 (100)
7f/g	1 (0.5)	0 (0)	1 (100)	1 (100)
8d/f	1 (0.5)	0 (0)	1 (100)	1 (100)
14b/f	3 (1.5)	0 (0)	1 (33)	3 (100)
14b/g	7 (3.6)	2 (29)	7 (100)	6 (86)M=1
14d/c	1 (0.5)	0 (0)	0 (0)	0 (0)
14d/d	1 (0.5)	M=1	M=1	M=1
14d/f	35 (17.8)	4 (11) M=2	25 (71) M=1	23 (66) M=8
14d/g	107 (54.3)	18 (17) M=6	87 (81) M=8	87 (81) M=18
14e/g	4 (2.0)	0 (0)	2 (50) M=1	3 (75) M=1
14f/f	3 (1.5)	0 (0)	2 (67)	3 (100)
14f/g	7 (3.6)	3 (43) M=1	7 (100)	6 (86)
14j/g	3 (1.5)	0 (0) M=1	2 (67)	2 (67) M=1
14k/f	1 (0.5)	1 (100)	1 (100)	1 (100)
14k/g	2 (1.0)	2 (100)	2 (100)	2 (100)
14l/c	1 (0.5)	M=1	M=1	M=1
14l/f	1 (0.5)	1 (100)	1 (100)	1 (100)
14l/g	10 (5.1)	3 (30)	10 (100)	6 (60) M=3
14p/g	3 (1.5)	0 (0)	3 (100)	3 (100)
15d/d	1 (0.5)	0 (0)	M=1	M=1
16d/d	1 (0.5)	0 (0)	0 (0)	0 (0)

M: patients with missing data.

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infected in Denmark and overall, the clustering of the patients confirmed this. Among the patients reporting being infected in Denmark, 16 full strain types were identified. Moreover, among the patients with full strain types, 10 of the clusters/singletons were constituted of MSM exclusively (Table I). Women were represented in 5 different clusters. For example, in one cluster including 3 patients infected with strain type 14b/f, a transmission chain could be clearly demonstrated as 2 women were diagnosed with this uncommon strain type only a few weeks apart in the same region of Denmark.

Syphilis and HIV coinfection

Information on HIV status was available from 256/269 (95%) patients. Of these patients, 48/256 (19%) were HIV-infected at time of syphilis diagnosis. Only one was female (and non-Danish). Of the patients with full strain types, information on HIV status was available from 185/197 (94%) and 35/185 (19%) had concomitant HIV infection. The patients with concurrent HIV at time of syphilis diagnosis were all MSM and presented 9 different full strain types (Table I). We did not find a difference in strain type by HIV status when analysing specimens with full strain type ($p = 0.197$). In comparison, when looking at clusters containing only a few patients, both HIV-infected and HIV-uninfected MSM were represented, indicating shared sexual networks (Table I). For example, strain type 6d/g was diagnosed in 2 MSM with discordant HIV status in the Copenhagen area in the same month.

Re-infection

Full strain type was identified in 4 patients with more than one episode of syphilis within the study period. One patient with concurrent HIV returned with a second syphilis episode after 5 months, both episodes with the uncommon 14f/g strain type, but the latter episode was acquired in Great Britain. Another patient had fully typeable specimens from 3 episodes of syphilis with 6-month-intervals; the very common strain type 14d/g was identified during the 2 first episodes while strain type 14l/g was identified during the latter episode. This patient had 4 episodes of primary syphilis within the study period. Moreover, a patient with concurrent HIV had 2 episodes of syphilis with an interval of 2 years; both infections were with the very common 14d/g strain type. Yet another patient had 2 episodes of syphilis with an interval of 2 years; again both infections were with the very common 14d/g strain type and the patient reported acquiring the infection in Denmark at both occasions.

Temporal distribution of strain types

The specimens for this study were collected over 4 years and the distribution of strains was investigated from 2010 to 2013 (Fig. S1[1]). For purpose of analysis, the specimens were divided into 2 groups of 24 months (2010–2011, 2012–2013). No significant change in the strain type distribution was identified over the 2 periods ($p = 0.183$).

DISCUSSION

The population-based design allowed us to study *T. pallidum* at a national level by linking strain type with epidemiological data from patients with PCR-positive syphilis during a 4-year period. Of the 278 cases of infectious syphilis diagnosed by amplification of *T. pallidum* DNA in genital ulcer specimens, 71% of the specimens were fully typeable with both the *arp*, *tpr* and *tp0548* assays.

In this population, 22 different strain types were identified. Others have suggested that settings in which syphilis is endemic have higher levels of strain diversity (8, 18, 21) and the strain diversity in this study was surprisingly high. However, with the use of the 3-component strain typing system we expected to demonstrate additional diversity. When applying the 2-component subtyping system to our specimens we could only demonstrate 14 different subtypes, which is in line with the discriminatory capacity demonstrated by Marra et al. (7).

The most common strain type was 14d/g, followed by 14d/f. Previous studies have shown that 14d is the predominant subtype in various geographical regions (6–8, 11, 14, 15, 22, 23) and 14d/g may be a more virulent or transmissible strain. It is interesting to note that the strain type 6b/g, not previously described, was found in 2 patients who reported infection outside Denmark, i.e. Greenland and Thailand. In addition, the distribution of the patients who reported acquisition of syphilis outside Denmark showed that these imported cases did not result in circulating clones. However, since patients diagnosed with syphilis by serological testing were not included in the study, we cannot rule out that more patients have become infected with imported strains. Of note, many of the uncommon strain types in this study have also been identified as subtypes in Scotland (22), indicating possible links across Europe. Finally, we identified 3 strain types that, to our knowledge, have not previously been reported (6b/g, 6d/g and 7f/g).

The majority of the patients were MSM and a few medium-sized clusters consisted of only MSM, indicating localized transmission networks. The marked increase in syphilis among MSM has been attributed mainly to increased sexual risk behaviour in response to the improved effect of antiretroviral therapy on HIV (24, 25). Rather than a general increase in sexual risk behaviour among all MSM, the re-establishment of a risk-taking core group of MSM may have enabled a higher level of endemicity, causing continuous syphilis circulation (26). In a previous study, we investigated the risk of re-infection with syphilis and found that HIV-infected men had a markedly higher risk of re-

infection with syphilis relative to HIV-uninfected men (4), suggesting that the risk behaviour that resulted in an HIV infection was continued after seroconversion. In the present study we did not find an association between HIV status and strain type, suggesting that HIV sero-sorting, i.e. trying to establish knowledge about HIV status concordance before practicing unprotected sex, was not practiced to any large degree. Similarly, a study from London, UK, found no association between HIV status and either full *T. pallidum* subtype or type according to the *tp0548* sequence alone (23), further supporting this theory. Declining rates of HIV sero-sorting could also explain the declining rates of HIV coinfection among MSM who are diagnosed with syphilis; MSM with syphilis and HIV coinfection have unprotected sex with HIV-uninfected MSM, but only transmit syphilis because they are effectively treated for their HIV infection (27).

The proportion of concurrent HIV in our study was lower than the 32% recently reported among MSM diagnosed with syphilis in Denmark (3). This probably reflects that patients with HIV are screened for syphilis yearly. Consequently, patients with concurrent HIV are overrepresented among patients diagnosed by serological testing compared with the patients comprised by this study who were diagnosed by PCR testing of material from genital lesions.

Our data did not provide evidence of occurrence of treatment failures in Denmark, and even though several patients had more than one episode of syphilis with the very common 14d/g strain type, we assume that each new chancre represents a re-infection and not a treatment failure. However, because patients are also diagnosed by serological testing and because patients would not necessarily present genital ulcers in case of relapse due to treatment failure, we cannot definitively conclude that treatment failures do not occur in Denmark.

We did not detect a shift in the strain type distribution during the study. However, 4 years is a relatively short period when investigating a temporal distribution.

The strengths of our study are the nationwide design, including all Danish patients with PCR-positive syphilis within the study period, and the epidemiological data on all patients. Furthermore, the study was based in a setting in which all healthcare services are publicly funded and free, and this universal access probably results in fewer undiagnosed cases. Moreover, many studies investigating *T. pallidum* have used only 2 typing methods. Since the 3-component strain type system was introduced, the discriminatory capacity has improved markedly and we applied this enhanced typing system.

This study has certain limitations. First, the study only included patients diagnosed with PCR-positive genital lesions. Consequently, as two-thirds of Danish syphilis patients are diagnosed by serological testing at later stages (28) our results might be biased. This is accentuated by the fact that more HIV-infected patients are diagnosed with syphilis by serological testing at their yearly check-ups. However, our patient characteristics (e.g. age and sexual orientation) were very similar to Danish syphilis patients diagnosed through an 11-year period in a study including both patients diagnosed by serological and PCR testing (4). Certainly, when investigating transmission chains from imported cases, inclusion of all syphilis patients, and not just the minority diagnosed by PCR testing, would have been preferable. Secondly, a relative high number of specimens were not fully typeable. This could be caused by DNA degradation during storage of the specimens. The specimens were collected during a 4-year period, resulting in some specimens having been stored for up to 3 years. Others have encountered the same problem, and Müller et al. (8) demonstrated that only 85% of *T. pallidum*-positive specimens were positive after re-testing stored specimens. In our study, the non-typeable specimens had significantly lower bacterial load in the diagnostic PCR, and our success rate above 70% is comparable to others using the 3-component system with success rates of 41%, 63% and 77%, respectively (23, 15, 22).

The current method of choice for *T. pallidum* typing, consisting of 3 components, is time-consuming and, thereby, not applicable for routine testing. A recent study compared the CDC typing system with sequenced-based molecular typing in a group of patients with 2 or more parallel specimens (i.e. taken at the same time). The study found differences in treponemal genotypes detected in whole blood and swab specimens, suggesting important differences between compartments. In the majority of the patients they found discrepancies within the *arp* and *tpr* loci using the CDC typing system (29). However, under experimental conditions, Pillay et al. (6) showed that the CDC subtype was stable with repeated rabbit passages of the Nichols strain. This was confirmed by Marra et al. (7), who additionally demonstrated that neither the Sea 81-4 nor the Chicago C strain changed with repeated rabbit passages. Whether this inconsistency is caused by differences between human infections and experimental infections of rabbits or is due to the fact that skin and blood represent 2 immunologically distinct compartments has yet to be explored. Further studies on advanced high-throughput technologies are highly needed before molecular typing can be implemented in routine STD surveillance and a sequenced-based typing system with promising results has been proposed recently (16, 29, 30).

To our knowledge no other studies have investigated *T. pallidum* strain type diversity at a national level. The molecular typing methods, combined with epidemiological data, shed some light on the current syphilis endemicity in Denmark. However, the picture remains complex, especially the interaction with HIV infection.

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REFERENCES

1. Kuznik A, Lamorde M, Nyabigambo A, Manabe YC. Antenatal syphilis screening using point-of-care testing in Sub-Saharan African countries: a cost-effectiveness analysis. PLoS Med 2013; 10: e1001545.
2. Axelsen N. Syphilis 1998–1999. EPI-NEWS 2000; 34.
3. Cowan S, Hoffmann S. Syphilis 2013. EPI-NEWS 2014; 34.
4. Salado-Rasmussen K, Katzenstein TL, Gerstoft J, Cowan SA, Knudsen TB, Mathiesen L, et al. Risk of HIV or second syphilis infection in Danish men with newly acquired syphilis in the period 2000–2010. Sex Transm Infect 2013; 89: 372–376.
5. Farhi D, Dupin N. Management of syphilis in the HIV-infected patient: facts and controversies. Clin Dermatol 2010; 28: 539–545.
6. Pillay A, Liu H, Chen CY, Holloway B, Sturm AW, Steiner B, Morse SA. Molecular subtyping of Treponema pallidum subspecies pallidum. Sex Transm Dis 1998; 25: 408–414.
7. Marra C, Sahi S, Tantalo L, Godornes C, Reid T, Behets F, et al. Enhanced molecular typing of treponema pallidum: geographical distribution of strain types and association with neurosyphilis. J Infect Dis 2010; 202: 1380–1388.
8. Muller EE, Paz-Bailey G, Lewis DA. Macrolide resistance testing and molecular subtyping of Treponema pallidum strains from southern Africa. Sex Transm Infect 2012; 88: 470–474.
9. Mitchell SJ, Engelman J, Kent CK, Lukehart SA, Godornes C, Klausner JD. Azithromycin-resistant syphilis infection: San Francisco, California, 2000–2004. Clin Infect Dis 2006; 42: 337–345.
10. A2058G Prevalence Workgroup. Prevalence of the 23S rRNA A2058G point mutation and molecular subtypes in Treponema pallidum in the United States, 2007 to 2009. Sex Transm Dis 2012; 39: 794–798.
11. Grimes M, Sahi SK, Godornes BC, Tantalo LC, Roberts N, Bostick D, et al. Two mutations associated with macrolide resistance in Treponema pallidum: increasing prevalence and correlation with molecular strain type in Seattle, Washington. Sex Transm Dis 2012; 39: 954–958.
12. Lukehart SA, Godornes C, Molini BJ, Sonnett P, Hopkins S, Mulcahy F, et al. Macrolide resistance in Treponema pallidum in the United States and Ireland. N Engl J Med 2004; 351: 154–158.
13. Martin IE, Gu W, Yang Y, Tsang RS. Macrolide resistance and molecular types of Treponema pallidum causing primary syphilis in Shanghai, China. Clin Infect Dis 2009; 49: 515–521.
14. Katz KA, Pillay A, Ahrens K, Kohn RP, Hermanstyne K, Bernstein KT, et al. Molecular epidemiology of syphilis – San Francisco, 2004–2007. Sex Transm Dis 2010; 37: 660–663.
15. Grange PA, Allix-Beguec C, Chanal J, Benhaddou N, Gerhardt P, Morini JP, et al. Molecular subtyping of Treponema pallidum in Paris, France. Sex Transm Dis 2013; 40: 641–644.
16. Flasarová M, Pospíšilová P, Mikalová L, Vališová Z, Dastychová E, Strnadel R, et al. Sequencing-based molecular typing of treponema pallidum strains in the Czech Republic: all identified genotypes are related to the sequence of the SS14 strain. Acta Derm Venereol 2012; 92: 669–674.
17. Castro R, Prieto E, Aguas MJ, Manata MJ, Botas J, Pereira FM. Molecular subtyping of Treponema pallidum subsp. pallidum in Lisbon, Portugal. J Clin Microbiol 2009; 47: 2510–2512.
18. Martin IE, Tsang RS, Sutherland K, Anderson B, Read R, Roy C, et al. Molecular typing of Treponema pallidum strains in western Canada: predominance of 14d subtypes. Sex Transm Dis 2010; 37: 544–548.
19. Statistics Denmark. Available from: http://www.dst.dk. [Accessed 9 Nov 2014].
20. Lohse N, Hansen AB, Jensen-Fangel S, Kronborg G, Kvinesdal B, Pedersen C, et al. Demographics of HIV-1 infection in Denmark: results from the Danish HIV Cohort Study. Scand J Infect Dis 2005; 37: 338–343.
21. Pillay A, Liu H, Ebrahim S, Chen CY, Lai W, Fehler G, et al. Molecular typing of Treponema pallidum in South Africa: cross-sectional studies. J Clin Microbiol 2002; 40: 256–258.
22. Cole MJ, Chisholm SA, Palmer HM, Wallace LA, Ison CA. Molecular epidemiology of syphilis in Scotland. Sex Transm Infect 2009; 85: 447–451.
23. Tipple C, McClure MO, Taylor GP. High prevalence of macrolide resistant Treponema pallidum strains in a London centre. Sex Transm Infect 2011; 87: 486–488.
24. Sterne JA, Hernán MA, Ledergerber B, Tilling K, Weber R, Sendi P, et al. Long-term effectiveness of potent antiretroviral therapy in preventing AIDS and death: a prospective cohort study. Lancet 2005; 366: 378–384.
25. Lohse N, Hansen AB, Pedersen G, Kronborg G, Gerstoft J, Sørensen HT, et al. Survival of persons with and without HIV infection in Denmark, 1995–2005. Ann Intern Med 2007; 146: 87–95.
26. Spielmann N, Münstermann D, Hagedorn HJ, an der Heiden M, Houareau C, Gunsenheimer-Bartmeyer B, et al. Time trends of syphilis and HSV-2 co-infection among men who have sex with men in the German HIV-1 seroconverter cohort from 1996–2007. Sex Transm Infect 2010; 86: 331–336.
27. Truong HM, Kellogg T, Klausner JD, Katz MH, Dilley J, Knapper K, et al. Increases in sexually transmitted infections and sexual risk behaviour without a concurrent increase in HIV incidence among men who have sex with men in San Francisco: a suggestion of HIV serosorting? Sex Transm Infect 2006; 82: 461–466.
28. Søborg B, Cowan S, Hoffmann S. Syphilis 2012. EPI-NEWS 2013; 34.
29. Mikalova L, Pospisilova P, Woznicova V, Kuklova I, Zakoucka H, Smajs D. Comparison of CDC and sequence-based molecular typing of syphilis treponemes: tpr and arp loci are variable in multiple samples from the same patient. BMC Microbiol 2013; 13: 178.
30. Grillová L, Pětrošová H, Mikalová L, Strnadel R, Dastychová E, Kuklová I, et al. Molecular typing of Treponema pallidum in the Czech Republic during 2011 to 2013: increased prevalence of identified genotypes and of isolates with macrolide resistance. J Clin Microbiol 2014; 52: 3693–3700.