scientific reports

Check for updates

OPEN

High increase of Nichols-like clade circulating *Treponema pallidum* subsp. *pallidum* in Barcelona from 2021 to 2023

Patricia Nadal-Barón[1,2]✉, Jesus Trejo-Zahinos[1,2], Maider Arando[3], Alicia Barberan-Masegosa[2], Marta Bernat-Sole[1], Arantxa Pérez-Ugarte[1,2], Ana Maria Villatoro[1], Pilar Alcubilla[1], Elena Sulleiro[1,2,4], Juan José Gonzalez-López[1,2,4], Andrés Antón[1,2,4], Mireia Alberny[5], Oriol Mitjà[6,7,8], Maria Nieves Larrosa[1,2,4] & Yannick Hoyos-Mallecot[1]

Worldwide, more than 90% of contemporary syphilis strains belong to SS14-like clade. This study aimed to describe the molecular profile of circulating *Treponema pallidum* subsp. *pallidum* (TPA) strains in Barcelona, Spain, from 2021 to 2023 building upon our report in 2015 which showed that 94.8% of typed strains belonged to the SS14 clade. Multilocus sequence typing (MLST) was conducted on TPA-positive samples obtained from swab samples by sequencing the tp0136, tp0548, and tp0705 loci. Strains were classified as Nichols-like or SS14-like clade. Macrolide and tetracycline resistance-associated mutations were determined through analysis of 23S rDNA and 16S rRNA gene sequences. Of the 96 typeable samples, 47.9% belonged to SS14-like and 52.1% to the Nichols-like. Fourteen haplotypes were identified, with ST26 representing 43.8% of the samples, distributed across 11 haplotypes in the SS14-like and 3 haplotypes in the Nichols-like. All the samples showed macrolide resistance-associated mutations, while none exhibited tetracycline-associated mutations. Our findings revealed a substantial shift in the proportion of TPA clades within the Barcelona population from 2021 to 2023, characterized by a higher proportion of Nichols-like strains compared to 2015 and international trends. The varying temporal and geographical trends underscore the need for regular surveillance to understand regional variations in syphilis and strengthen control programs.

Keywords Molecular epidemiology, Multilocus sequence typing, Phylogeny, Syphilis, *Treponema pallidum*

Syphilis, caused by *Treponema pallidum* subsp. *pallidum* (TPA), has reemerged as a substantial public health concern since the 2000s, alongside other sexually transmitted infections (STIs)[1]. Globally, the World Health Organization reported approximately 22.3 million cases of syphilis among individuals aged 15 to 49 years in 2020, with an annual incidence of 7.1 million new cases worldwide[2]. Within the European Union, the European Center for Disease Control and Prevention (ECDC) has documented a notable increase in syphilis incidence over the past two decades[3]. In Spain, the number of syphilis cases increased from 3886 cases (8.4 per 100,000 population) in 2015 to 8141 cases (17,1 per 100,000 population) in 2022[4]. Barcelona emerged as the region with the highest incidence, reaching 100.1 cases per 100,000 population in 2021[5,6].

The development of a new multilocus sequence typing (MLST) scheme with higher discriminatory power than previous schemes, along with improvements in whole genome sequencing methods, have enhanced our understanding of syphilis transmission dynamics[7–9]. After a bottleneck event in the 1990s and subsequent

[1]Department of Microbiology and Parasitology, Clinical Laboratories, Vall d'Hebron University Hospital, Passeig Vall d'Hebron 119-129, 08035 Barcelona, Spain. [2]Department of Genetics and Microbiology, Universitat Autonoma de Barcelona, Barcelona, Spain. [3]STI Unit Vall d'Hebron Drassanes, Infectious Diseases Department, Vall d'Hebron University Hospital, Barcelona, Spain. [4]Infectious Diseases CIBER (CIBERINFEC), Carlos III Health, Madrid, Spain. [5]Medical Management of Primary Care Services, Catalan Health Institute (ICS), Barcelona, Spain. [6]Skin Neglected Tropical Diseases and Sexually Transmitted Infections Section, Germans Trias i Pujol University Hospital, Badalona, Spain. [7]Fight Infectious Diseases Foundation, Badalona, Spain. [8]Disease Control and Surveillance Branch, National Department of Health, Port Moresby, Papua New Guinea. ✉email: patricia.nadal@vallhebron.cat

rapid population expansion in the 2000s, TPA strains predominantly fall into two deeply branching lineages: the SS14 clade and the Nichols clade, with multiple sub-lineages[8]. In our previous study conducted in 2015, we characterized TPA strains circulating in Barcelona using MLST, showing eleven different allelic profiles. We found that 94.8% of the typed strains belonged to the SS14 clade and 5.2% to Nichols-like clade, consistent with findings reported in other European studies[7,10] and global studies[8] at the time.

The aim of our study was to provide an updated perspective on the molecular epidemiology of TPA strains in Barcelona. We used MLST methodology on clinical specimens collected between 2021 and 2023 to elucidate any changes in strain distribution and genetic mutation associated with macrolide and tetracycline resistance and to compare our findings with those from 2015.

Results

Clinical characteristics of patients

Treponemal DNA was detected in 107 specimens corresponding to 107 syphilis episodes including 71 (66.3%) primary syphilis, 29 (27.1%) secondary syphilis, 3 early latent syphilis (2.8%), and 4 (3.7%) syphilis cases not classified. These specimens belonged to 105 different patients, two of whom experienced reinfection. Clinical characteristics of patients are presented in Table 1. Briefly, 88.8% of patients were men who have sex with men and 6.5% women. The median age was 35.5 years, with 24.3% of patients being people living with HIV and 29.9% using pre-exposure prophylaxis. One patient with secondary syphilis exhibited otic involvement.

	Total	MSM	MSW	Cis-women
N, (%), [95% CI]	107	95 (88.8) [78.1–91.3]	5 (4.8) [95% CI 2.0-10.4]	7 (6.5) [3.2–12.8]
Median age	35.5 [IQR 30–43]	36.5 [IQR 30–43]	33.5 [IQR 29–34]	33.5 [IQR 29–38]
HIV positive*, n (%) [95% CI]	26 (24.3) [17.1–33.2]	26 (27.4) [20.0-38.1]	0	0
HIV Pre-exposure prophylaxis* (%) [95% CI]	32 (29.9) [22.0-39.1]	32 (33.6) [25.8–45.0]	0	0
Syphilis stage, n (%) [95% CI]				
Primary	71 (66.6) [56.6–75.2]	64 (67.4) [57.0-76.6]	2 (40.0) [11.7–76.9]	5 (71.4) [35.9–91.7]
Secondary	29 (27.1) [18.9–36.5]	25 (26.3) [17.8–36.3]	3 (60.0) [23.1-88.2-]	1 (14.3) [2.5–51.3]
Early latent syphilis	3 (2.8) [0.6-8.0]	3 (3.1) [1.1–8.8]	0	0
Unknown	4 (3.7) [1.5–9.2]	3 (3.1) [1.1–8.8]	0	1 (14.3) [2.5–51.3]
Type of lesión (%) [95% CI]				
Genital	67 (62.6) [53.2–71.2]	60 (63.1) [53.1–72.2]	3 (60.0) [23.0-88.2]	4 (57.1) [25.0-84.2]
Oral	4 (3.7) [1.5-9,2]	3 (3.1) [1.1–8.8]	0	1 (14.3) [2.5–51.3]
Anal	20 (18.7) [12.4–27.1]	17 (17.9) [11.5–26.9]	2 (40.0) [11.7–76.9]	1 (14.3) [2.5–51.3]
Skin	1 (0.93) [0.2–5.1]	1 (1.1) [0.2–5.7]	0	0
Absence of lesion	11 (10.3) [5.8–17.5]	11(11.6) [6.6–19.5]	0	0
Information no available	4 (3.7) [1.4–9.2]	3 (3.1) [1.1–8.8]	0	1 (14.3) [2.5–51.3]
Serology test				
Negative RPR	32 (29.9) [22.0-39.1]	31 (32.6) [24.0-42.6]	0	1 (14.3) [2.5–51.3]
Positive RPR	69 (64.5) [54.6–73.5]	59 (62.1) [51.6–71.9	5 (100) [56.5–100]	5 (71.4) [35.9–91.7]
Non RPR performed	6 (5.6) [2.1–11.8]	5(5.3) [1.7–11.9]	0	1 (14.3) [2.5–51.3]
Positive treponemal test	94 (93.1) [86.2–97.1]	83 (92.2) [84.6.2–96.8]	5 (100) [56.5–100]	6 (100) [54.1–100]
Previous syphilis story	39 (36.4) [27.9–45.0]	39 (42.4) [31.7–51.1]	0	0

Table 1. Clinical and demographic characteristics of patients with syphilis. *Information about HIV infection and the use of PrEP and serology tests was available for 101 patients. Abbreviations: MSM, men who have sex with men; MSW, men who have sex with women; HIV, human immunodeficiency virus; RPR, rapid plasma reagin test; CI, confidence interval; IQR, interquartile range.

Out of 107 positive cases, TPA DNA was detected in various clinical specimens including 67 (62.6%) genital swabs, 20 (18.7%) rectal swabs, 4 (4.0%) oral swabs, and 1 (0.93%) skin swab. Among the 11 patients without lesions (10.3%), treponemal DNA was detected in 3 rectal swabs (2.8%) and 8 oral swabs (7.6%). In patients in whom TPA was detected in oral swabs, active screening for TPA was conducted in pharyngeal specimens as patients were taking part in a clinical trial (Trep-AB, NCT05069974)[11]. The detection of TPA in rectal mucosa in absence of lesion was an accidental finding as the specimens were sent to the laboratory for *Neisseria gonorrhoea* and *Chlamydia trachomatis* screening. Upon detection of the latter in rectal localization, study of lymphogranuloma venerum (LGV) was performed using a multiplex NAAT (Allplex™ Genital ulcer Assay, Seegene, Seoul, Korea) which includes TPA among its targets. Information regarding the presence of lesions and the sample location was lacking in four episodes (3.7%).Serology test results were available for 101 (94.9%) patients upon diagnosis. Among them, 7 out of 101 (6.9%) tested negative for both treponemal and nontreponemal tests. Regarding patients without a history of previous syphilis (62/101, 61.4%), 24 (23.8%) had at least one positive treponemal test while nontreponemal test was negative. Among the patients with a previous history of syphilis (39/101, 38.6%), nontreponemal tests were negative in 8 out of 39 cases (20.5%).

Two patients experienced repeat episodes of syphilis. Genital ulcer samples were collected for initial and repeat episodes for one patient and rectal ulcer samples were collected for the other patient. The time between episodes was 11 and 7 months respectively. These cases were classified as reinfections, as both clinical cure and serological evidence (reduction in RPR titer) were observed between the first and second episodes. Specifically, the RPR titer decreased eight-fold in the first case and four-fold in the second.

Amplification success rate and typing of clinical samples based on TP0136, TP0548 and TP0705

A total of 90 specimens (84.1%) were successfully typed at all three loci and 6 (5.6%) could be partially typed in at least one locus (Table 2). Amplification success rate was similar for all three loci, TP0136 88.8%, TP0548 86.0%, and 88.8% TP0705. Oral swabs were the specimen type with the lowest typing success rate, with a rate of 75% for at least one locus. The median Ct value among fully typed samples was 28.4 [IQR 26.4-31.07], 29.7 [IQR 26.2–33.0] among partial typed samples and 33.7 [IQR 32.6–34.8] among nontypable samples.

Altogether, 14 different TPA sequence types (ST) were identified, obtaining a type diversity index (TDI) value of 0.16. Among typable samples ($n=96$), 52.1% of them belonged to the Nichols-like cluster (52.1%) with 3 different ST detected: ST26 (42/96, 43.8%), ST6 (7/96, 7.3%) and ST109 (1/96, 1.0% %) (Fig. 1). Among the 11 haplotypes related to SS14-like cluster (46/96,47.9%), the most common allelic profile was ST1 (26/96, 27.1%%), followed by ST28 (4/96, 4.2%) and ST3 (2/96, 2.1%). The frequency of the 7 remaining SS14-like haplotypes (Table 2) was 1.0% for each. All partially typed specimens belonged to SS14-like cluster (Fig. 1). No new allelic variants were identified. TPA phylogenetic analysis of allelic profiles found among fully typed samples is shown in Fig. 1.

In the two reinfection cases, one patient had both episodes of syphilis caused by TPA belonging to ST26, the most common haplotype observed in our study population. In contrast, for the other patient, the specimen from the first episode was partially typed as 1.X.1 (clade SS14-like), while the specimen from the second episode was fully typed and belonged to ST1.

Allelic profile	Typing	ST	Genetic group	No. of samples (%)
1.3.1	Complete	1	SS14	26 (27.0)
1.1.1	Complete	2	SS14	1 (1.0)
1.1.8	Complete	3	SS14	2 (2.1)
3.2.3	Complete	6	Nichols	7 (7.3)
1.5.1	Complete	8	SS14	1 (1.0)
6.3.1	Complete	13	SS14	1 (1.0)
9.7.3	Complete	26	Nichols	42 (43.8)
1.17.9	Complete	28	SS14	4 (4.2)
1.39.1	Complete	52	SS14	1 (1.0)
1.43.1	Complete	57	SS14	1 (1.0)
22.3.1	Complete	72	SS14	1 (1.0)
1.54.8	Complete	74	SS14	1 (1.0)
9.16.3	Complete	109	Nichols	1 (1.0)
1.77.1	Complete	116	SS14	1 (1.0)
X.3.1	Partial	NA	SS14	1 (1.0)
1.X.1	Partial	NA	SS14	4 (4.2)
1.1.X	Partial	NA	SS14	1 (1.0)

Table 2. Multilocus sequence typing (MLST) allelic profiles for 90 fully typed and 6 partially typed swab samples from patients with syphilis infection in Barcelona. Abbreviations: ST, sequence type.

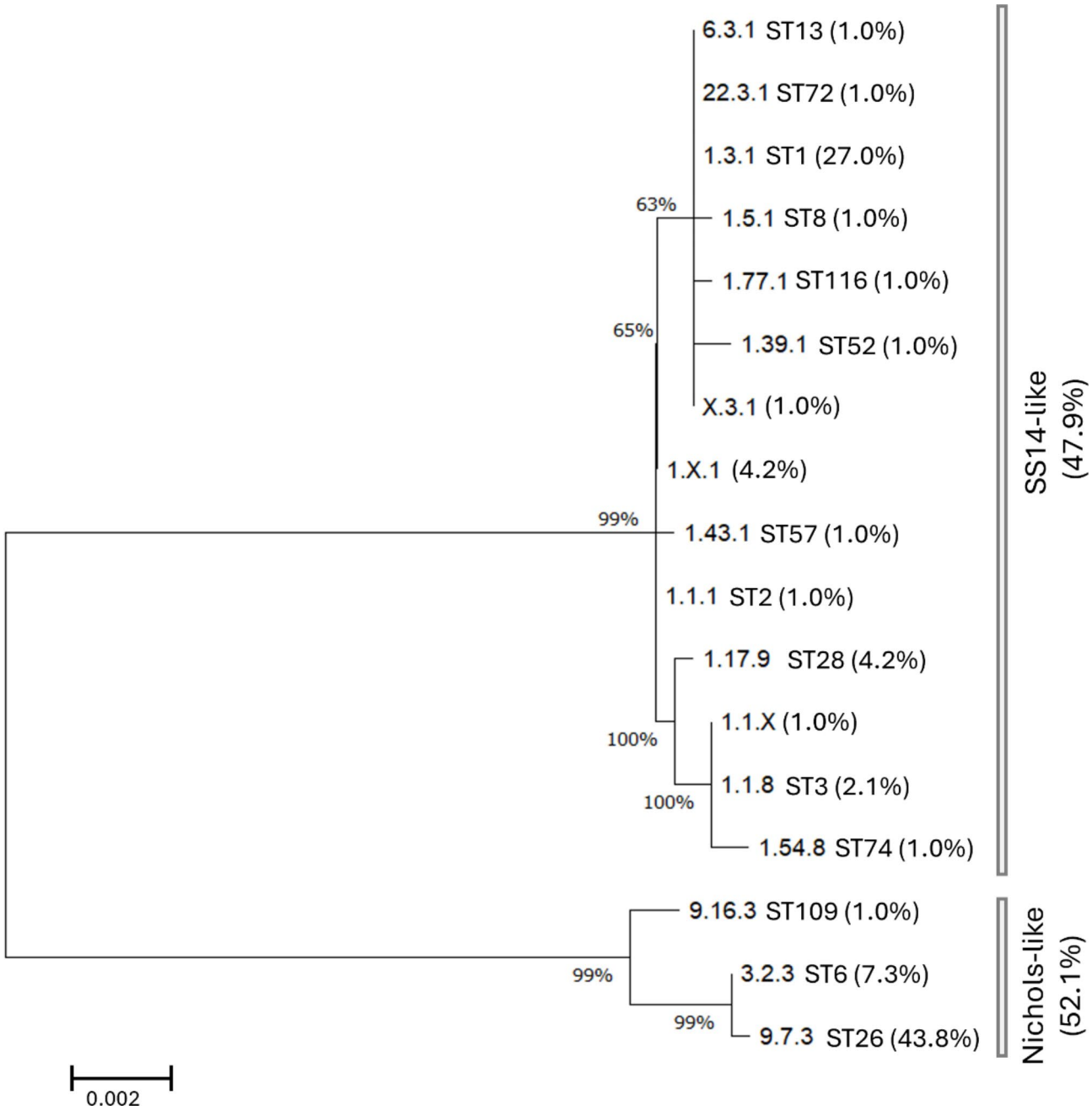

Fig. 1. Phylogenetic tree of the 14 TPA allelic profiles obtained from the 90 fully typed samples and the 6 partially typed samples identified in this study. The tree was constructed from concatenated sequences of typing loci (tp0136, tp0548 and tp0705) in MEGA11 using the maximum likelihood method with the bootstrap test (1000 replicates). The scale shows the number of substitutions per site. Bootstrap values are shown in nodes. Relative frequencies are shown in parentheses.

Proportion of macrolide and tetracycline resistance-associated genetic determinants in clinical samples

We investigated all 96 samples for mutations, but analysis was hindered by degradation from multiple freeze-thaw cycles after molecular typing (tp0136, tp548, and tp705) and low bacterial load in partially typed samples.

At least one copy of the 23S rRNA was sequenced in 78 of 90 (86.6%) fully MLST typed. The A2058G mutation associated with macrolide resistance was found in 100% of cases and no strains harboured the A2059G mutation. The sequence of the 16S rRNA was obtained from 80 of 90 (88.8%) fully typed samples and no mutations associated with tetracycline resistance were found.

Associations between TPA genetic variants and clinical characteristics of patients

No clade or allelic profile associations with patient age, sex, sexual orientation, syphilis stage, HIV status or PreP treatment were found (Table 3). Patients carrying Nichols-like strains ($n=8$) other than ST26 (ST3 and ST109) tended to be slightly older (47, IQR 30.4–51.3) than patients carrying ST26 (33.5, IQR 31.1–37.8) and SS14-like strains (35, IQR 29-43.3) but no significant differences were detected (p-value = 0.1519).

Discussion

In this report, we analysed the genetic diversity of TPA from positive samples collected from syphilis patients in Barcelona (Spain) spanning the years 2021 to 2023. It serves as a follow-up and enhancement of our previous report in 2015[12]. Notably, our findings revealed a substantial increase in the proportion of the Nichols-like strains (52.1%) compared to SS14-like strains (48.9%), representing a significant deviation from our own data from 2015, where Nichols-like represented only 5.2%, and from global studies, which typically report a proportion ranging between 5% and 20%[8,9,13].

Since the resurgence of syphilis in the early 2000s, various studies have consistently reported a proportion of Nichols clade strains that have remained relatively low and stable in several regions. According to the PubMLST database[14], in North America and Europe, the SS14-like clade is predominant over the Nichols-like with the proportion of Nichols remaining below 10%. Specifically, studies have reported a proportion of Nichols clade strains of 9% in France and Switzerland[7], 2% in the Czech Republic[10], and 5.2% in Spain[12]. In contrast, other regions of the world demonstrate a slightly higher prevalence of Nichols, ranging from 10 to 26% as observed in Argentina (26%)[15], Peru (21%)[16], Japan (15%)[17], Taiwan (20%)[18], Malawi[19] and Australia (26%)[20]. Notably, Madagascar stands as a unique case where there is a clear dominance of Nichols strain (100%)[13]. However, this finding is based on specimens from syphilis infections occurring between 2000 and 2007, and reports including recent specimens are not available.

Recent studies in Argentina indicate an increase in Nichols strains, rising from 26 to 37%, thereby challenging previous reports[21]. Concurrently, our regional study also highlighted a surge in Nichols strains from 5.2% in 2015[12] to 52.1% in 2021–2023 in a similar population.

In this study, we identified 14 different allelic profiles among 90 fully typed samples, yielding a TDI index of 0.16, which is in line with previous studies[12]. Remarkably, there was a clear variation in predominant allelic profiles, with ST26 (43.8%), ST1 (27.1%), and ST6 (7.3%) emerging as the most frequent in contrast to our previous study, where ST1 (55.6%), ST2 (11.1%), and ST28 (7.9%) had the highest frequency (Fig. 1). Variations in demographic and clinical characteristics are unlikely to explain the observed differences in molecular profiles. Compared to the previous report in 2015, both studies were conducted among a predominantly male population residing in Barcelona, particularly men who have sex with men (94.4% vs. 88.8%) and with comparable proportion of people living with HIV (36.3% VS. 24.3%)[12,22].

Based on the published PubMLST data[23], all the allelic profiles identified in this study have been previously identified in studies carried out worldwide but exhibit considerable variation in proportions, particularly when compared with closely related regions in Europe[14] (Table 4). However, an update on the current circulating TPA strains is needed as recent research has included samples from 2000 to 2020 (Table 4)[7–10,12,13,20,24].The proportion of ST1 identified in our study (27%) was lower than that reported in other European and North American studies, ranging from 40 to 60%. Nevertheless, this haplotype continues to be the SS14 clade dominant allelic

	Total	ST1	Other SS14-like clade	ST26	Other Nichols-like clade	*p*-value
N (%)	96	26 (27.1)	20 (20.8)	42 (43.7)	8 (12.1)	NA
Median age [IQR]	35 [33-37.1]	34.5 [31–38]	35.5 [29-48.7]	33.5 [31.1–37.8]	47 [30.4–51.3]	0.1519
Men (%)	89 (92.7)	24 (92.3)	18 (90.0)	39 (92.8)	8 (100)	0.8370
MSM (%)	84 (87.5)	23 (88,4)	17 (85.0)	36 (85.7)	8 (100)	0.9390
MSW (%)	5 (5.2)	1 (3.8)	1 (5.0)	3 (85.7)	0	
Women (%)	7 (7.2)	2 (7.7)	2 (10.0)	3 (7.1)	0	
HIV (%)	22 (22.9)	7 (26.2)	3 (15.0)	10 (23.8)	2 (25.0)	0.8051
PreP	27 (28.1)	8 (30.7)	6 (30.0)	9 (21.4)	4 (50.0)	0.3990
Syphilis stage						
Primary	64 (66.6)	20 (76.9)	12 (60.0)	24 (57.1)	8	0.4002
Secondary	26 (27.1)	4 (15.4)	7 (35.0)	15 (35.7)	0	
Early latent	3 (3.1)	1 (3.8)	1 (5.0)	1 (2.4)	0	
Not classified	3 (3.1)	1 (3.8)	0	2 (4.8)	0	

Table 3. Distribution of allelic profiles with patient characteristics: age, sex, sexual behaviour, people living with HIV, use of HIV pre-exposure prophylaxis and syphilis stage. Allelic profiles comprising less than 8% of the alleles were grouped under their genetic group. The category other SS14-like clade includes ST2, ST3, ST8, ST13, ST28, ST52, ST57, ST72, ST74, ST116, and the partially typed strains assigned to the SS14-like clade and the category other Nichols-like clade includes ST6 and ST109. Abbreviations: MSM, men who have sex with men; MSW, men who have sex with women; HIV, human immunodeficiency virus; PreP, HIV pre-exposure prophylaxis.

		Genetic group	SS14-like				Nichols-like	
	Sample collection year	*N**	1.3.1 ST1 (%)	1.1.1 ST2 (%)	1.1.8 ST3 (%)	1.17.9 ST28 (%)	3.2.3 ST6 (%)	9.7.3 ST26 (%)
Barcelona	2021–2023	90	28.9	1.1	2.2	4.4	7.8	46.7
Barcelona	2015	64	54.7	10.9	6.3	7.8	6.3	1.6
Argentina	2015–2019	34	26.5	26.5	2.9	0	0	35.3
France	2011–2016	128	61.7	14.1	3.9	1.6	0.8	4.7
Czech Republic	2004–2020	224	49.1	11.2	14.7	0	0	3.1
UK	2013–2018	17	41.2	11.8	0	0	11.8	0
Portugal	2009–2014	25	52.0	28.0	0	0	0	0
Peru	2018–2019	6	50.0	33.3	0	0	0	0
Cuba	2012–2016	73	84.9	1.4	0	0	0	0
Canada	2001–2021	28	57.1	3.6	3.6	7.1	3.6	0
China	2016–2019	55	0	1.8	41.8	0	7.3	0
Japan	2019–2020	52	9.6	3.8	76.9	0	5.8	1.9
The Netherlands	2006–2020	220	40.9	19.1	2.7	0.5	2.3	7.3

Table 4. Comparison of reported TPA allelic profile frequencies between this study and studies conducted in other continents. The data were obtained from the PubMLST database[14]. Only the frequencies of fully typed strains were used for calculations and strains with percentages higher than 5% in the reports performed in Spain (2015 and 2021–2023) were included. *Only fully typed samples included. Abbreviations: ST, sequence type, UK, United Kingdom.

profile. Among the other common SS14 clade haplotypes frequently detected in global studies, ST2 is anecdotal here (1.0%) while its proportion ranges 15–30% among European samples; ST28 has similar proportion to those reported, and ST3, common in Asia[17,25], continues to be a minority (2.1%). Only the most frequent profiles overlap among studies while haplotypes with a prevalence less than 5% tend emerge and fade over time and different regions[10,26].

In this study, despite the Nichols clade being the majority, we identified only 3 different STs (Fig. 1), which is surprising considering the higher nucleotide diversity observed in strains belonging to the Nichols clade than in those in the SS14 clade[9]. This is noteworthy given our sample size (107) and the variety of SS14 allelic profiles detected (Fig. 1), and our regional scope may contribute to explaining this phenomenon. Additionally, it is surprising that the proportion of ST6 remained stable over the years and closely related regions. As expected, the Nichols-like clade haplotypes reported here overlap with those detected in Europe, America and Asia (Table 4) and are completely different from those identified in Madagascar specimens[13].

While specific studies have indicated an association of Nichols strains with slightly older patients compared to those carrying SS14-like strains, we did not observe a significant association between clades or allelic profiles and patient characteristics (Table 3)[26]. To emphasize, the most frequent circulating TPA strains in women, ST26 (3/7 42.8%) and ST1 (2/7, 28.5%) did not differ from those in men. This findings aligns with recent research demonstrating a similar distribution of circulating TPA strains among groups with varying age, sex, sexual orientation, syphilis stage, or HIV status[19,21].

The most prevalent allelic profiles are consistently identified across various countries, indicating the widespread dissemination of specific syphilis strains at the supranational level. According to PubMLST[23]and recent research findings, the prevailing syphilis strains globally are identified as ST1, ST2, ST3, and ST26. These strains consistently emerge in diverse geographic regions, as evidenced by numerous reports[10,21,25,27,28]. This observation prompted an inquiry into whether these strains possess adaptive features that contribute to their sustained prevalence over time compared to other strains.

The rise of Nichols-like strains in Argentina and our region has been driven by the allelic profile ST26; however, as of now, no fitness advantages have been described. Other possible explanations aside from evolutionary advantages may include changes in sexual networks or outbreaks that may temporarily favour the local spread of Nichols-like clade, sampling bias, or antibiotic resistance. The independent and spontaneous acquisition of macrolide resistance in multiples sub-lineages is believed to have played a role in the expansion of strains belonging to the SS14 clade[20,29]. In our previous study, while the A2058G mutation appeared in 96.6% of SS14 clade strains, it only appeared in 40% of Nichols strains, all of which belonged to the same haplotype (ST6). Currently, all identified Nichols strains in our study exhibit the A2058G mutation which could potentially make them as likely as SS14 strains to spread, particularly among a population with macrolide antibiotic pressure. A similar trend has recently been observed in Argentina, with a high frequency of Nichols-like strains and increased levels of macrolide resistance in TPA[21]. Although macrolides are no longer recommended for syphilis treatment, resistant strains remain highly prevalent suggesting that resistance may be driven by antibiotic use for other infections[30]. Additionally, no tetracycline resistance-associated mutations were found in the studied region of 16 S rRNA, as occurred in previous studies[31,32]. Close monitoring of circulating strains of TPA and tetracycline and azithromycin resistance-associated mutations is crucial, particularly following the progressive availability and use of doxycycline for STI prevention (DoxyPEP) which may impose additional selective pressure[33].

This study is not without limitations. Firstly, our specimen collection was confined to Barcelona, providing limited information about the trends of TPA strains in the rest of Spain, although this region has the highest incidence of syphilis in Spain[5,6]. Future investigations should aim to include specimens from diverse regions across Spain. Secondly, data regarding the TPA strains circulating between the time of our last report and the present are currently unavailable. Thirdly, the inclusion of few specimens from women (6.5%) and the absence of specimens isolated from cerebrospinal fluid raise concerns about potential gaps in understanding strains associated with congenital syphilis and neurosyphilis. Nevertheless, we assert that our sample size is adequate for comprehending the circulating TPA strains in our region, considering the infrequency of these pathologies in our area and that the prevalence of syphilis in women in our region (11.5%) is close to the percentage of women in this study[5,6].

The strengths of this study lie in the fact that all included samples were clinical, and patient characteristics were available, allowing us to gain insights into syphilis transmission networks in our region. Our substantial sample size enabled us to demonstrate that no specific strains are associated with any population group. This study included a considerable sample size collected from various sources, including two tertiary hospitals (Hospital Universitari Vall d'Hebron and Hospital Universitari German Trias I Pujol), 2 specialized STI centres (BCN-Checkpoint and Drassanes Vall d'Hebron Centre) and 16 primary community care facilities from Barcelona, which encompass a population of 1 million inhabitants. Additionally, we successfully isolated strains from early latent syphilis and secondary syphilis using oral and rectal swabs from patients who did not present with ulcers.

In conclusion, our study revealed a noteworthy shift in the epidemiology of circulating TPA strains in Barcelona, characterized by an elevated proportion of Nichols-like strains and distinctions from neighbouring regions. Given that Barcelona is a highly dynamic city with significant population mobility, this research underscores the imperative for periodic surveillance of circulating TPA strains. Such ongoing monitoring is vital for adjusting public health policies and enhancing the efficacy of STI prevention and control programs such as DoxyPep.

Materials and methods

Clinical samples collection

Clinical swab samples were collected between October 2021 and September 2023 from patients with suspected syphilis at various healthcare facilities including the Sexually Transmitted Infection (STI) Unit of Drassanes Vall d'Hebron Centre for International Health and Transmissible Diseases, sixteen primary health care centres in Barcelona, the Barcelona CheckPoint STI Unit, Hospital Universitari Vall d'Hebron and Hospital Universitari Germans Trias I Pujol.

The diagnosis of syphilis was based on clinical signs, dark-field microscopy, serology (treponemal and nontreponemal tests) and/or nucleic acid amplification tests. All samples were sent to the Microbiology Department of Hospital Universitari Vall d'Hebron for the detection of treponemal DNA. Clinical and demographic data including patient age, sex, sexual orientation, HIV status, use of pre-exposure prophylaxis, type of clinical material, syphilis stage and syphilis serology results were collected from clinical health records when available. The syphilis stage was classified according to the criteria in the European guideline on the management of syphilis by the International Union Against STIs (IUSTI)[34].

DNA isolation and treponemal DNA detection

Nucleic acid extraction from genital, oral, skin and anal swabs was performed using eMAG® (bioMerieux, Marcy l'Etoile, France) using 300 µL of sample and eluting in 100 µL of RNase-free water according to the manufacturers' instructions. TPA molecular detection was performed by using a commercial assay targeting the *polA* gene (Allplex™ Genital Ulcer Assay, Seegene, Korea).

Multilocus sequence typing of *T. pallidum* and detection of genetic resistance-associated determinants to macrolides and tetracyclines

MLST was performed on positive PCR laboratory-confirmed samples. A partial region of the *tp0136*, *tp0548* and *tp0705* genes was amplified by nested-PCR and sequenced by Sanger sequencing as described by Grillova et al.in 2018[7]. Sequence analyses and phylogenetic and molecular evolutionary analyses were conducted using MEGA software v.11.0.13. TP0136 and TP0548 were aligned to the reference sequences TPA Nichols (CP004010.2) and TPA SS14 (CP004011.1) to determine the clade. Phylogenetic trees were constructed using the maximum likelihood method with bootstrapping (1000 replications, cutoff value of 50%) and the Tamura-Nei model. The TDI was calculated as the ratio between the number of different types detected and the number of fully typeable samples[31].

A region of both copies of the 23S rRNA gene was amplified following previously described nested PCR conditions[35] and genetic macrolide resistance-associated determinants were identified by detecting A2058G and A2059G mutations (positions according to the 23S rDNA gene of *Escherichia coli*, accession no. V00331) via Sanger sequencing. Genetic resistance-associated determinants to tetracyclines were identified by detecting mutations within the 965–968 and 1058 region of 16S rRNA (positions according to 16 S rDNA gene of *E. coli*, accession no. V00331)[31] .

Statistical analysis

All statistical analyses were performed using Stata/IC V.16.1 (Stata Corp). Associations between two categorical variables were determined by the $\chi 2$ test or Fisher's test (if the data did not meet normal distribution with Shapiro-Wilk test); median comparison was performed using the Mann–Whitney U test and multiple comparisons were performed with ANOVA. Differences with $p < 0.05$ (two-sided) were considered statistically significant.

Data availability

Data is provided within the manuscript or supplementary information (Supplementary Table 1). All alleles found in this study were identical to previously reported sequences, which can be found in the PubMLST database (https://pubmlst.org/organisms/treponema-pallidum)[14].

Received: 10 June 2024; Accepted: 25 September 2024
Published online: 08 October 2024

References

1. Mitjà, O. et al. Epidemiology and determinants of reemerging bacterial sexually transmitted infections (STIs) and emerging STIs in Europe. *Lancet Reg. Health Eur.* **34**, 100742. https://doi.org/10.1016/j.lanepe.2023.100742 (2023).
2. World Health Organization. Global health sector strategies on, respectively, HIV, viral hepatitis and sexually transmitted infections for the period 2022–2030. Annex 2: Measurement framework (2021).
3. European Centre for Disease Prevention and Control & Syphilis *ECDC Annu Epidemiol Rep 2019* (2022).
4. Unidad de vigilancia de VIH, ITS y hepatitis B y C. Vigilancia epidemiológica de las infecciones de transmisión sexual (Centro Nacional de Epidemiología, Instituto de Salud Carlos III/División de Control de VIH, ITS, Hepatitis virales y Tuberculosis, Dirección General de Salud Pública, 2022).
5. Vigilància epidemiològica de. les Infeccions de Transmissió Sexual a Catalunya—Informe 2021 (2021).
6. Centro Nacional de Epid emiología, I nstituto de Salud Car los III/Plan Nacional sobre el Sida, Dirección General de Salud Pública. Vigilancia epidemiológica de las infecciones de transmisión sexual, 2019 (2021).
7. Grillová, L. et al. Molecular characterization of Treponema pallidum subsp. pallidum in Switzerland and France with a new multilocus sequence typing scheme. *PLoS One* **13**, e0200773. https://doi.org/10.1371/journal.pone.0200773 (2018).
8. Beale, M. A. et al. Global phylogeny of Treponema pallidum lineages reveals recent expansion and spread of contemporary syphilis. *Nat. Microbiol.* **6**, 1549–1560. https://doi.org/10.1038/s41564-021-01000-z (2021).
9. Arora, N. et al. Origin of modern syphilis and emergence of a pandemic Treponema pallidum cluster. *Nat. Microbiol.* **2**, 16245. https://doi.org/10.1038/nmicrobiol.2016.245 (2016).
10. Vrbová, E. et al. MLST typing of Treponema pallidum subsp. pallidum in the Czech Republic during 2004–2017: clinical isolates belonged to 25 allelic profiles and harbored 8 novel allelic variants. *PLoS One* **14**, e0217611. https://doi.org/10.1371/journal.pone.0217611 (2019).
11. Ubals, M. et al. Oral linezolid compared with benzathine penicillin G for treatment of early syphilis in adults (Trep-AB Study) in Spain: a prospective, open-label, non-inferiority, randomised controlled trial. *Lancet Infect. Dis.* https://doi.org/10.1016/S1473-3099(23)00683-7 (2024).
12. Fernández-Naval, C. et al. Multilocus sequence typing of *Treponema pallidum* subsp. *pallidum* in Barcelona. *Future Microbiol.* **16**, 967–976. https://doi.org/10.2217/fmb-2021-0037 (2021).
13. Lieberman, N. A. P. et al. Treponema pallidum genome sequencing from six continents reveals variability in vaccine candidate genes and dominance of Nichols clade strains in Madagascar. *PLoS Negl. Trop. Dis.* **15**, e0010063. https://doi.org/10.1371/journal.pntd.0010063 (2021).
14. Grillova, L. et al. A public database for the new MLST scheme for *Treponema pallidum* subsp. *pallidum*: surveillance and epidemiology of the causative agent of syphilis. *PeerJ* **6**, e6182. https://doi.org/10.7717/peerj.6182 (2019).
15. Gallo Vaulet, L. et al. Molecular typing of Treponema pallidum isolates from Buenos Aires, Argentina: frequent Nichols-like isolates and low levels of macrolide resistance. *PLoS One* **12**, e0172905. https://doi.org/10.1371/journal.pone.0172905 (2017).
16. Flores, J. A. et al. Treponema pallidum pallidum genotypes and macrolide resistance status in syphilitic lesions among patients at 2 sexually transmitted infection clinics in Lima, Peru. *Sex. Transm. Dis.* **43**, 465–466. https://doi.org/10.1097/OLQ.0000000000000465 (2016).
17. Nishiki, S. et al. Phylogenetic and genetic characterization of Treponema pallidum strains from syphilis patients in Japan by whole-genome sequence analysis from global perspectives. *Sci. Rep.* **11**, 3154. https://doi.org/10.1038/s41598-021-82337-7 (2021).
18. Wu, B-R. et al. Multicentre surveillance of prevalence of the 23S rRNA A2058G and A2059G point mutations and molecular subtypes of Treponema pallidum in Taiwan, 2009–2013. *Clin. Microbiol. Infect.* **20**, 802–807. https://doi.org/10.1111/1469-0691.12529 (2014).
19. Seña, A. C. et al. Clinical and genomic diversity of *Treponema pallidum subsp. pallidum*: a global, multi-center study of early syphilis to inform vaccine research. *medRxiv*. https://doi.org/10.1101/2023.07.19.23291250 (2023).
20. Taouk, M. L. et al. Characterisation of Treponema pallidum lineages within the contemporary syphilis outbreak in Australia: a genomic epidemiological analysis. *Lancet Microbe* **3**, e417–e426. https://doi.org/10.1016/S2666-5247(22)00035-0 (2022).
21. Morando, N. et al. High frequency of Nichols-like strains and increased levels of macrolide resistance in Treponema pallidum in clinical samples from Buenos Aires, Argentina. *Sci. Rep.* **12**, 16339. https://doi.org/10.1038/s41598-022-20410-5 (2022).
22. Arando, M. et al. Early syphilis: risk factors and clinical manifestations focusing on HIV-positive patients. *BMC Infect. Dis.* **19**, 727. https://doi.org/10.1186/s12879-019-4269-8 (2019).
23. Jolley, K., Bray, J. E. & Maiden, M. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *PubMLST* (2018). https://pubmlst.org/bigsdb?db=pubmlst_tpallidum_seqdef (accessed 12 February 2024).
24. Pinto, M. et al. Genome-scale analysis of the non-cultivable Treponema pallidum reveals extensive within-patient genetic variation. *Nat. Microbiol.* **2**, 16190. https://doi.org/10.1038/nmicrobiol.2016.190 (2016).
25. Liu, D. et al. Molecular characterization based on MLST and ECDC typing schemes and antibiotic resistance analyses of Treponema pallidum subsp. pallidum in Xiamen, China. *Front. Cell. Infect. Microbiol.* **10**, 618747. https://doi.org/10.3389/fcimb.2020.618747 (2021).
26. Pospíšilová, P. et al. Multi-locus sequence typing of Treponema pallidum subsp. pallidum present in clinical samples from France: infecting treponemes are genetically diverse and belong to 18 allelic profiles. *PLoS One* **13**, e0201068. https://doi.org/10.1371/journal.pone.0201068 (2018).
27. Noda, A. A., Rodríguez, I. & Šmajs, D. Genotyping of Treponema pallidum in Cuba (2018–2019): increased circulation of recombinant genotype and no New Treponema pallidum subspecies endemicum infection among Syphilis patients. *Sex. Transm Dis.* **47**, e39–41. https://doi.org/10.1097/OLQ.0000000000001217 (2020).
28. Venter, J. M. E. et al. Treponema pallidum macrolide resistance and molecular epidemiology in Southern Africa, 2008 to 2018. *J. Clin. Microbiol.* **59**, e02385–e02320. https://doi.org/10.1128/JCM.02385-20 (2021).
29. Grimes, M. 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.* **39**, 954–958. https://doi.org/10.1097/OLQ.0b013e31826ae7a8 (2012).
30. Machalek, D. A. et al. Prevalence of mutations associated with resistance to macrolides and fluoroquinolones in Mycoplasma genitalium: a systematic review and meta-analysis. *Lancet Infect. Dis.* **20**, 1302–1314. https://doi.org/10.1016/S1473-3099(20)30154-7 (2020).

31. Giacani, L. et al. Enhanced molecular typing of Treponema pallidum subspecies pallidum strains from 4 Italian hospitals shows geographical differences in strain type heterogeneity, widespread resistance to Macrolides, and lack of mutations Associated with doxycycline resistance. *Sex. Transm. Dis.* **45**, 237–242. https://doi.org/10.1097/OLQ.0000000000000741 (2018).
32. Wu, B-R. et al. Surveillance study of Treponema pallidum harbouring tetracycline resistance mutations in patients with syphilis. *Int. J. Antimicrob. Agents* **44**, 370–372. https://doi.org/10.1016/j.ijantimicag.2014.06.009 (2014).
33. Kong, F. Y. S., Kenyon, C. & Unemo, M. Important considerations regarding the widespread use of doxycycline chemoprophylaxis against sexually transmitted infections. *J. Antimicrob. Chemother.* **78**, 1561–1568. https://doi.org/10.1093/jac/dkad129 (2023).
34. Janier, M. et al. 2020 European guideline on the management of syphilis. *J. Eur. Acad. Dermatol. Venereol.* **35**, 574–588. https://doi.org/10.1111/jdv.16946 (2021).
35. Matějková, P. et al. Macrolide treatment failure in a case of secondary syphilis: a novel A2059G mutation in the 23S rRNA gene of Treponema pallidum subsp. pallidum. *J. Med. Microbiol.* **58**, 832–836. https://doi.org/10.1099/jmm.0.007542-0 (2009).

Author contributions

YHM and PNB conceived and led the design of this project. PNB, ABM, AMV and PA performed technical work. PNB, MA and MA collected and reviewed clinical data. PNB analysed the results and wrote the initial draft with YHM. PNB, JTZ, MA, ABM, MBS, APU, AMV, PA, ES, JJGL, AA, MA, OM, MNL and YHM reviewed and approved the final draft.

Competing interests

The authors declare no competing interests.

Ethics statement

This study was approved by the Ethics Committee of the Hospital Universitari Vall d'Hebron (PR(AG)571/2023). The study was conducted according to the principles expressed in the Declaration of Helsinki, as well as national regulations regarding research. The Ethics Committee of the Hospital Universitari Vall d'Hebron granted a waiver of direct informed consent because of the retrospective nature of the study, the use of anonymized residual samples, and the infeasibility of conducting the research under other conditions.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-74355-y.

Correspondence and requests for materials should be addressed to P.N.-B.