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Identification of *Treponema pallidum* in the oral cavity of patients with early syphilis who reported unprotected oral sex practices: prevalence and associated factors

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Introduction

1. The incidence of syphilis has been on the increase globally in both developed and less developed countries, especially among men who have sex with men (MSM).
2. High PCR detection rates (60-90%) of *T. pallidum* from the genital, oral mucosal or skin lesions of patients presenting with primary syphilis and secondary syphilis have been reported, which suggests that higher rates of syphilis transmission may occur during oral sex.
3. The oral cavity is an important but easily forgotten portal of entry for sexually transmitted diseases (STDs) to occur
4. Whether *T. pallidum* can be identified in patients with syphilis who do not present with oral mucosal lesions has rarely been systematically studied.

Patients and Methods

1. Study period: December 2010 and June 2014.
2. Study subjects: patients aged 20 years or older who presented to the clinics with the diagnosis of any stage of syphilis, RPR titers ≥1:4 and positive TPPA
3. The stages of syphilis were defined according to the Sexually Transmitted Diseases Treatment Guidelines of the U.S Centers for Disease Control and Prevention.

Collection of clinical specimens

1. All appropriate clinical specimens were collected with the use of swabs (CultureSwab EZ; BD, Franklin Lakes, NJ) before the patients received antibiotic treatment for syphilis. In patients with oral lesions involving the mucosa of the oral cavity (the gingiva, soft palate, hard palate, mouth floor, and tongue) or the lips (Figure 1)

Laboratory investigations

1. Treponemal DNA was extracted from clinical specimens using the Qiagen DNA minikit (Qiagen, Gmbh, Hildens, Germany) according to the manufacturer's protocol. The presence of *T. pallidum* was determined by the amplification of the *polymerase I* gene (*pol A*)
2. The detection of macrolide resistance mutations (A2058G or A2059G) in the 23S rRNA gene was performed using PCR-restriction fragment length polymorphism (RFLP).
3. Molecular typing was performed based on 3 treponemal genes: the number of 60-bp repeats in the acidic repeat protein (*arp*) gene; the PCR-RFLP patterns of *T. pallidum* repeat (*tpr*) genes including *tprE*, *tprG*, *tprJ,* and *tprC*; and the sequence of a short region (131-215 bp) in the *tp0548* gene.
4. Serological tests for syphilis included the RPR test (BD Macro-VueTMRPR Card tests, USA) and *T. pallidum* particle agglutination test (FTI-SERODIA-TPPA. Fujirebio Taiwan Inc., Taoyuan, Taiwan)

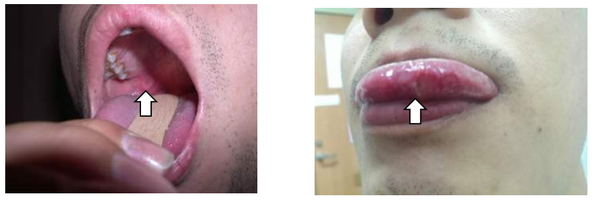

Figure 1. Chancre of the right buccal and tongue regions

Results

1. 240 patients with 267 episodes of syphilis were enrolled, including 242 episodes (90.6%) occurring in HIV-infected patients (Table 1).
2. 64.5% of the oral swab specimens were tested positive for *T. pallidum* by PCR assays in patients with secondary syphilis or both primary and secondary syphilis, regardless of oral ulcers.
3. In patients with early latent syphilis and late latent syphilis who had visually intact oral mucosa, *T. pallidum* DNA was detected in 28.0% and 40.0% of the oral swab specimens, respectively.
4. Factors associated with detection of treponemal DNA in the oral swab specimens in patients without oral ulcers are shown in Table 2.
5. Yield rates of PCR assays to detect *pol A* (screening), *arp*, *tpr*, and *tp0548* genes in the oral swab specimens from patients with or without oral ulcers are shown in Figure 3
6. Of the 113 amplifiable specimens, 45 (40.0%) were tested positive for all *tpr*, *arp*, and *tp0548* genes yielding 7 full subtypes. As for the distribution of genotypes, 14f/f was the most common subtype (28/45, 62.2%), followed by 14b/c (9/45, 20%), 14a/f (3/45, 6.7%), 10b/a (2/45, 4.4%), 13b/a (1/45, 2.2%), 14b/a (1/45, 2.2%), and 14b/f (1/45, 2.2%) (Figure 3).

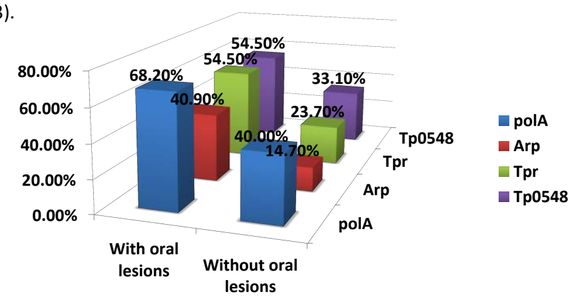

Figure 2. Yield rates of polymerase-chain-reaction assays to detect *polymerase A* (polA), *arp*, *tpr*, and *tp0548* genes from the oral swabs in patients with or without oral lesions

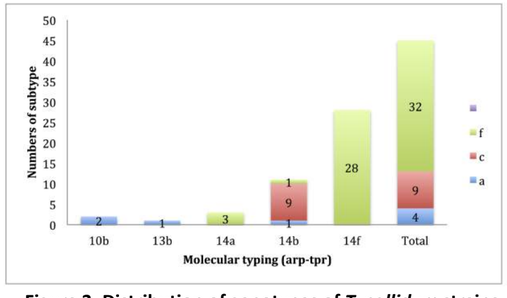

Figure 3. Distribution of genotypes of *T. pallidum* strains

Table 1. Clinical characteristics of the 240patients with 267 cases of syphilis

	All cases (n=267)	Primary (n=38)	Secondary (n=76)	Primary + Secondary (n=21)	Early latent (n=125)	Late latent (n=5)	Neurosyphilis (n=2)
Age, mean (SD), years	33.1 (7.2)	34.3 (6.9)	30.0 (7.3)	33.4 (7.5)	34.5 (6.7)	32.4 (2.9)	40.5 (0.71)
MSM	267 (100)	38 (100)	76 (100)	21 (100)	125 (100)	5 (100)	2 (100)
Oral ulcers	22 (8.2)	17 (44.7)	0 (0)	4 (19.0)	0 (0)	0 (0)	1 (50.0)
PCR-positive	113 (42.3)	13 (34.2)	49 (64.5)	13(61.9)	35 (28.0)	2 (40.0)	1 (50.0)
[b]Oral sex	238 (89.1)	35 (92.1)	67 (88.2)	19 (90.5)	111 (88.8)	5 (100.0)	1 (50.0)
100% condom use	4 (1.5)	0 (0)	2 (2.6)	0 (0)	2 (1.6)	0 (0)	0 (0)
HIV infection,	242 (90.6)	32 (84.2)	68 (89.5)	16 (76.2)	120 (96.0)	4 (80.0)	2 (100)
CD4, mean (SD), cells/mm^3	520 (255)	429 (181)	498 (240)	464 (225)	570 (269)	613 (331)	90 (104)
Log_{10} HIV RNA, mean (SD), copies/ml	2.21 (1.39)	2.38 (1.47)	2.35 (1.43)	3.20 (1.52)	1.88 (1.19)	3.02 (1.58)	5.18 (0.72)
On cART	197 (81.4))	26 (81.3)	52(76.5)	9 (56.2)	107 (89.2)	3 (75.0)	0 (0)
RPR titer, median (IQR)	64 (32, 128)	64 (32, 256)	128 (64, 256)	128 (64, 256)	64 (16, 128)	32 (8, 32)	NA
Log_2 RPR titer ≥5	212 (79.4)	32 (84.2)	69 (90.8)	20 (95.2)	86 (68.8)	3 (60.0)	2 (100)

Table 2. Univariate and multivariate analysis of the factors associated with positive polymerase-chain-reaction results for *Treponema pallidum* in patients without oral ulcers

	Univariate analysis			Multivariate analysis		
	Oral swab with PCR positive (n=98)	Oral swab with PCR negative (n=147)	*P*-value	Adjusted OR	95% CI	*P*-value
Age, mean (SD), years	31.2 (6.6)	34.3 (7.4)	0.11	0.96	0.92-1.00	0.08
Syphilis stage, n (%)			<0.001			
Primary syphilis	4 (4.1)	17 (11.6)		1		
Secondary	46 (46.9)	27 (18.4)		6.79	1.97-23.38	0.002
Other stages	48 (49.0)	103 (70.0)		1.83	0.56-5.97	0.32
Oral sex, n (%)	91 (92.9)	129 (87.8)	0.65	2.87	0.21-39.56	0.43
Condom use on 100% occasions	3 (3.1)	1 (0.7)		0.16	0.01-1.97	0.15
CD4>350	60 (61.2)	110 (81.5)	0.05	0.77	0.19-3.14	0.72
PVL <20 copies/ml, n (%)	49 (50.0)	72 (53.3)	0.58	1.57	0.82-2.99	0.17
RPR titer ≥1:32	87 (88.8)	103 (70.0)	0.001	2.23	1.02-4.89	0.045

Conclusions

1. Detection of treponemal DNA in the oral cavity using PCR assays is not uncommon in MSM who practice unprotected oral sex, even in the syphilitic individuals with apparently intact oral mucosa.
2. Barrier protection to prevent transmission of STDs at all points of sexual contact, including oral-genital or oral-anal contacts can not be overemphasized.