

Molecular Characterization of Syphilis in Patients in Canada: Azithromycin Resistance and Detection of *Treponema pallidum* DNA in Whole-Blood Samples versus Ulcerative Swabs[∇]

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Although detection of *Treponema pallidum* DNA in whole-blood specimens of syphilis patients has been reported, it is uncertain at what stage of the disease such specimens are most suitable for the molecular diagnosis of syphilis. Also, few studies have directly compared the different gene targets for routine laboratory diagnostic usage in PCR assays. We examined 87 specimens from 68 patients attending two urban sexually transmitted disease clinics in Alberta, Canada. PCR was used to amplify the *T. pallidum* *tpp47*, *bmp*, and *polA* genes as well as a specific region of the 23S rRNA gene linked to macrolide antibiotic susceptibility. In primary syphilis cases, PCR was positive exclusively (75% sensitivity rate) in ulcerative swabs but not in blood specimens, while in secondary syphilis cases, 50% of the blood specimens were positive by PCR. Four out of 14 (28.6%) of our PCR-positive syphilis cases were found to be caused by an azithromycin-resistant strain(s). Our results confirmed that swabs from primary ulcers are the specimens of choice for laboratory diagnostic purposes. However, further research is required to determine what specimen(s) would be most appropriate for molecular investigation of syphilis in secondary and latent syphilis.

Syphilis, caused by the spirochete *Treponema pallidum*, is a complicated disease that can be divided into different stages (31). Other than in congenital or transfusion-acquired syphilis, *T. pallidum* gains access to the body through the mucus membranes, causing the primary lesion or chancre, which contains large numbers of the spirochete demonstrable by microscopy. It is believed that within a short time, the organism disseminates throughout the body via the bloodstream and/or lymphatics. Because *T. pallidum* cannot be grown on artificial culture media, laboratory diagnosis of syphilis is traditionally made by detection of the treponemal spirochetes in clinical specimens by using either microscopy (dark-field microscopy, silver staining, or fluorescent antibody staining), the rabbit infectivity test, or serology. Each of these approaches has its limitations. Dark-field microscopy requires the microscopist to have experience, and the method cannot reliably distinguish pathogenic *T. pallidum* from commensal spirochetes which may be present in some body sites. Fluorescent antibody staining using polyclonal antisera to *T. pallidum* has poor specificity (17). Although monoclonal antibody to the 37-kDa protein is specific (11), it is not widely available. Serology depends on the host's development of either nontreponemal reaginic antibodies or specific antibody to *T. pallidum*. In the former case, responses to the cardiolipid antigens are also induced by other infectious agents or conditions and can therefore produce

false-positive syphilis serology results (7). In the latter case, specific antibody response to treponemal antigens may be delayed, and hence, such tests may suffer from poor sensitivity during the early primary phase of infection. Furthermore, patients with low numbers of CD4⁺ lymphocytes as a result of human immunodeficiency virus infection may have an aberrant immune response and abnormal syphilis serology despite infection (10, 19). Finally, the rabbit infectivity test requires the use of live animals and live *T. pallidum* and has not been widely used in routine clinical diagnostic laboratories. Apart from these drawbacks, neither serology nor microscopy allows the microorganism to be characterized for epidemiologic study or for antibiotic susceptibility.

Modern DNA technologies have enabled most clinical laboratories to implement molecular diagnostic approaches, including PCR, restriction fragment length polymorphisms, and DNA sequencing for the molecular characterization of pathogens. A molecular typing scheme that is based on the characterization of two *T. pallidum* repeat genes, *arp* and *tpr*, has been developed by Pillay et al. (26). Since *T. pallidum* cannot be cultured on artificial media, these modern techniques are well suited to complement existing techniques for laboratory investigation of syphilis infection.

Over the past 2 decades, a number of investigators have described PCR procedures for the diagnosis of syphilis based on the detection of different *T. pallidum* gene targets (6, 8, 9, 14, 23, 33). Most of the procedures used swab specimens obtained from ulcers or cerebrospinal fluid (CSF). Although detection of *T. pallidum* DNA in whole blood has been reported (18, 30), it is uncertain at what stage of the disease such specimens are most suitable for PCR diagnosis of syphilis. To

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TABLE 1. PCR primer sequences used in this study to amplify the different target genes

Gene target	Forward primer	Reverse primer	Amplicon size (bp)
tpp47	1F 5' GACAATGCTCACTGAGGATAGT 3'	1R 5' ACGCACAGAACCGAATTCCTTG 3'	658
	2F 5' TTGTGGTAGACACGGTGGGTAC 3'	2R 5' TGATCGCTGACAAGCTTAGGCT 3'	496
bmp	F1 5' CTCAGCACTGCTGAGCGTAG 3'	R1 5' AACGCCTCCATCGTCAGACC 3'	976
	F2 5' CAGGTAACGGATGCTGAAGT 3'	R2 5' CGTGGCAGTAACCGCAGTCT 3'	506
polA	5' TGC GCG TGT GCG AAT GGT GTG GTC 3'	5' CAC AGT GCT CAA AAA CGC CTG CAC G 3'	377
23S rRNA gene	5' GTA CCG CAA ACC GAC ACA G 3'	5' AGT CAA ACC GCC CAC CTA C 3'	628
β-Globin gene	5' CAAGGTGAACGTGGATGAAG 3'	5' CCTGAAGTTCTCAGGATCCACG 3'	395

date, there have not been any studies to directly compare the different target genes for routine laboratory diagnostic usage in PCR assays.

In Canada, PCR is not commonly used for laboratory investigation of syphilis. Therefore, we report our experience with using PCR to diagnose syphilis in various specimens, using PCR primers that target three different *T. pallidum* genes (*bmp*, *tpp47*, and *polA*) as well as a specific region of the 23S rRNA gene that has been linked to macrolide antibiotic resistance (15). Our objective is to study the suitability of different clinical specimens as well as the different *T. pallidum* gene targets for molecular diagnosis and characterization of syphilis in patients in Alberta, Canada. Alberta has been experiencing an outbreak of predominantly heterosexual infectious syphilis since 2001, with recent rises among men who have sex with men (MSM) (27).

MATERIALS AND METHODS

Syphilis notification and diagnosis in Alberta. Alberta is a western Canadian province with a population of 3.4 million. Laboratories and health care providers are legally required to report all cases of syphilis to the provincial Ministry of Health, Alberta Health and Wellness, using a standard sexually transmitted infection notification form. The sexually transmitted infection notification documents the patient's demographic information and clinical (including recent antibiotic usage) and laboratory findings. Staging of cases is based on national sexually transmitted disease (STD) guidelines (28). Patients with syphilis are staged on a combination of clinical and epidemiologic considerations, as well as laboratory investigations, like the direct examination of specimens (either dark-field microscopy or fluorescent antibody to *T. pallidum*) and syphilis serological tests. All cases are staged by one of three STD medical consultants. The following case definitions for the different stages of syphilis are based on provincial and national documents (1, 2).

Primary syphilis is defined by (i) the identification of *T. pallidum* by dark-field microscopy, fluorescent antibody, or equivalent examination of material from a chancre or a regional lymph node or (ii) the presence of one or more typical lesions (chancres) and reactive treponemal serology, regardless of nontreponemal test reactivity, in individuals with no previous history of syphilis or (iii) the presence of one or more typical lesions (chancres) and at least a fourfold increase in the titer over that of the last known nontreponemal test in individuals with a past history of syphilis treatment.

Secondary syphilis is defined by (i) the identification of *T. pallidum* by microscopy, as in primary syphilis, or equivalent examination of mucocutaneous lesions, condylomata lata, and reactive serology (nontreponemal and treponemal) or (ii) the presence of typical mucocutaneous lesions, alopecia, loss of eyelashes and the lateral third of eyebrows, iritis, generalized lymphadenopathy, fever, malaise or splenomegaly, and either a reactive serology (nontreponemal and treponemal) or at least a fourfold increase in titer over that of the last known nontreponemal test.

Early latent syphilis is said to occur in an asymptomatic patient with reactive serology (nontreponemal and treponemal) who within the past 12 months had one of the following: nonreactive serology or symptoms suggestive of primary or secondary syphilis or exposure to a sexual partner with primary, secondary, or early latent syphilis.

Late latent syphilis is said to occur in an asymptomatic patient with persistently reactive treponemal serology (regardless of nontreponemal serology reactivity)

who does not meet the criteria for early latent disease and who has not been previously treated for syphilis.

Neurosyphilis is defined by reactive treponemal serology (regardless of nontreponemal serology reactivity) and one of the following: reactive VDRL test result in nonbloody CSF or clinical evidence of neurosyphilis and CSF pleocytosis (particularly lymphocytes) in the absence of other known causes or clinical evidence of neurosyphilis and elevated CSF protein in the absence of other known causes.

Tertiary syphilis other than neurosyphilis is defined by reactive treponemal serology (regardless of nontreponemal test reactivity) together with characteristic abnormalities of the cardiovascular system, bone, skin, or other structures, in the absence of other known causes of these abnormalities, and no clinical or laboratory evidence of neurosyphilis.

Congenital syphilis is defined by the identification of *T. pallidum*, by microscopy as in primary syphilis, in material from nasal discharges, skin lesions, placenta, umbilical cord or autopsy material of a neonate (up to 4 weeks of age), or reactive serology (nontreponemal and treponemal) from venous blood (not cord blood) of an infant or child with clinical, laboratory, or radiographic evidence of congenital syphilis, whose mother is without documented evidence of adequate treatment.

Clinical specimens. EDTA whole-blood, serum, CSF, and swab specimens from ulcers or skin lesions were obtained from patients attending STD clinics in the province of Alberta, with the exception of one penile swab which was taken from a patient in the Northwest Territories, but the specimen was sent to the Alberta Provincial Public Health Laboratory for analysis. All patients included in this study had a history and/or symptoms suggestive of syphilis, with the exception of one of the nonsyphilis cases (see Table 2), who was an elderly woman with a urinary tract infection for whom a swab for syphilis PCR investigation was included by error. Laboratory personnel performing the PCR assays for *T. pallidum* were not aware of the results of the clinical and other laboratory investigations for making a diagnosis of syphilis or indicating that a patient does not have syphilis.

Ulcer or skin lesion specimens from patients suspected of having primary or secondary syphilis were collected by first gently removing necrotic material or crusts from the lesions with sterile gauze and gently expressing clear exudates from the lesion. The exudates expressed from the lesion were absorbed with Dacron swabs and sent to the laboratory in 3 ml of transport medium supplied with the Roche Amplicor kit (Roche Diagnostic Systems, Inc., Mississauga, Ontario, Canada) or universal transport medium (Copan International, Murrieta, CA). From some suspected syphilis cases, 3 to 5 ml EDTA whole-blood specimens were collected. Specimens were sent on cool packs (4°C) to the Alberta Provincial Laboratory for Public Health within 1 day. When there was a delay in specimen transport to the National Microbiology Laboratory, specimens were frozen and shipped on dry ice.

DNA extraction. Extraction of DNA from 200 µl of whole blood, serum, and plasma was accomplished using the QIAamp DNA mini kit (Qiagen, Mississauga, Ontario, Canada) according to the manufacturer's instructions. DNAzol (Invitrogen, Burlington, Ontario, Canada) was used to extract DNA from CSF specimens according to the manufacturer's instructions. Extraction of DNA from clinical specimens was carried out in a clean room separated from all other DNA work to minimize potential contamination of clinical specimens with exogenous *T. pallidum* DNA. As a control for DNA extraction/specimen quality and detection of PCR inhibitory substances in the clinical specimens, PCR amplification of the human β-globin gene was performed on all clinical samples.

PCR methods. The PCR assay for *T. pallidum* was based on the gene targets *tpp47*, *bmp*, and *polA* according to methods described in the literature (6, 14, 25). The primers used, their sequences, and their amplicon sizes are described in Table 1. All PCRs were performed in an ABI9700 GeneAmp PCR system (Applied Biosystems, Foster City, CA).

PCR assays were performed in a volume of 50 μ l containing 5 μ l of DNA extracted from clinical specimens or controls as a template. Each run contained a positive control (extracts from rabbit testis infected with the Nichols strain of *T. pallidum*) as well as a no-template (water) negative control. Each reaction mixture contained 1 μ l of 10 mM concentrations of deoxynucleoside triphosphates (dATP, dGTP, dCTP, dTTP) (Invitrogen), 5 μ l of 10 \times PCR HotStar buffer (Qiagen), 2 μ l of 25 mM MgCl₂ (Qiagen), 1.25 units of HotStar Taq (Qiagen), 1.2 μ l of each primer (100 ng/ μ l), and distilled H₂O. The PCR mix for the β -globin gene PCR also contained 10 μ l of Q solution (Qiagen). Amplification of the *bmp* and *tpp47* genes were performed by nested PCR, and 1 μ l of the first-round PCR product was used as the template for the second-step PCR.

PCR conditions for *bmp*, *tpp47*, and *polA* genes were as follows: 95°C for 15 min, followed by 40 cycles at 95°C for 40 s, 58°C for 1 min (*bmp* gene) or 65°C for 1 min (*tpp47* and *polA* genes), and 72°C for 1 min, followed by 72°C for 5 min. Conditions for amplification of the β -globin gene were as follows: 95°C for 15 min, followed by 40 cycles at 95°C for 30 s, 56°C for 45 s, and 72°C for 1 min, followed by 72°C for 7 min. PCR products were analyzed on a 1.5% agarose gel, visualized by staining with ethidium bromide, and compared to a molecular size marker of a 100-bp ladder (New England BioLabs, Pickering, Ontario, Canada).

Detection of azithromycin resistance. PCR amplification of the 23S rRNA gene, and subsequent restriction enzyme digestion analysis by MboII were carried out as described by Lukehart et al. (15).

The azithromycin resistance genotype was confirmed by DNA sequencing of the PCR products after purification with a QIAquick PCR purification kit (Qiagen, Mississauga, Ontario, Canada). The DNA sequences of both strands obtained by the DNA analyzer 3730xl (Applied Biosystems, Foster City, CA) were edited, assembled, and aligned with published sequences obtained from both azithromycin-sensitive and -resistant strains by using software from DNASTar, Inc. (Madison, WI).

RESULTS

From February 2007 to January 2008, 86 specimens were submitted from two urban STD clinics in Alberta for PCR testing for syphilis. One penile swab specimen originated from a patient in the Northwest Territories (therefore, not a resident of Alberta), but the specimen was routed to the province of Alberta for analysis. These 87 specimens were obtained from 68 subjects presenting with lesions suggestive of primary (genital or oral ulcers), secondary (rash, lymphadenopathy, condyloma lata), or congenital syphilis meeting current surveillance criteria, with the exception of one swab specimen from a nonsyphilis patient, which was included by error. Of these 68 patients, 47 (69.1%) were male with ages ranging from 0 (newborn) to 67.5 years, with a median age of 43.0 years. The 21 female patients ranged in age from 0 (newborn) to 97.3 years, with a median age of 27.9 years.

Among the 68 patients, 41 were diagnosed with syphilis, 3 were previously treated past syphilis cases, and 24 did not have syphilis, based on laboratory findings and clinical evaluation (including one patient giving a biological false-positive syphilis serology result and three babies with positive syphilis serology tests due to the presence of maternal antibody in their specimens). Among the 41 syphilis patients, 19 were diagnosed with primary syphilis, 9 with secondary syphilis, 10 with latent syphilis, and 3 with congenital syphilis. From the 41 syphilis cases, the following specimens were obtained: 19 ulcer swabs, 2 tissue, 2 CSF, and 30 blood samples. Multiple specimens were obtained from eight patients, as follows: five patients with paired swab and blood specimens; one with three swab specimens from different sites; one with a blood specimen and a CSF specimen; one with two blood, two tissue, and one ulcer swab specimens. Single specimens were obtained from 33 patients (22 blood samples, 10 swab samples, and one CSF sam-

ple). Table 2 describes the PCR results from these 53 clinical specimens.

The three treponemal gene PCR assays (*tpp47*, *bmp*, and *polA*) gave concordant results in all specimens collected from syphilis patients, regardless of the specimen types (blood or swab) or the stages of disease (primary or secondary). Nineteen (36%) of the 53 specimens were positive by PCR. Overall, 12 (63%) of the 19 swabs, versus 5 (17%) of the 30 blood specimens, collected from patients with syphilis were positive for treponemal DNA by PCR, but only 14 of the 41 syphilis cases were diagnosed by PCR, with an overall positive rate of 34%. However, when PCR results were analyzed according to the stage of the disease and the specimen types, a different picture emerged. There were 17 swabs and 9 blood specimens collected from 19 primary syphilis cases, with 10 cases providing swab specimens only, 4 giving blood specimens only, and 5 providing both blood and swab specimens. Although only 9 (47%) of the 19 primary syphilis cases were positive by the PCR test and none were positive by PCR using blood specimens, 9 of the 15 primary syphilis cases were positive for treponemal DNA by PCR examinations of the swab specimens collected, which translated into a positive rate of 60%. Moreover, of these 15 primary syphilis cases, three gave swab specimens that were found to be negative for the β -globin gene and, hence, may be regarded as inadequate specimens. If these three specimens were discarded, the positivity rate increased to 75% (9 positive out of 12 cases).

In contrast to the primary syphilis cases where positive PCR results were found exclusively using swab specimens, all PCR-positive cases among the secondary syphilis cases were identified by tests on their blood specimens, with the exception of one case in which treponemal DNA was also detected in tissue and ulcerative swab specimens (Table 2).

Only one of the three congenital syphilis cases was positive by PCR using blood specimens. None of the latent syphilis cases (including eight early latent, one late latent, and one with central nervous system involvement) gave positive PCR results from the nine blood specimens, one swab specimen, and one CSF specimen tested.

Besides the three swab specimens from primary syphilis cases that were deemed to be inadequate due to a negative PCR result for the β -globin gene, two CSF specimens (one from a secondary syphilis case and one from a latent case with central nervous system involvement) were also negative, suggesting a lack of inflammatory cells and disease activity. The specificity of the PCR assays appeared to be excellent (100%), with all 27 nonsyphilis cases being negative, and the specificity did not appear to be affected by the specimen types (Table 2).

The 23S rRNA gene segment containing the macrolide active site was also detectable by PCR from the 19 positive specimens. Restriction fragment length polymorphism analysis of the PCR amplicons showed that in 12 specimens (from 10 cases), the *T. pallidum* organisms were sensitive to azithromycin, while the other 7 specimens (from four cases) were resistant. The wild-type stains were from females (two cases), heterosexual males (four cases), and MSM (four cases). Azithromycin-resistant organisms were identified in MSM (three cases, including one bisexual male) and in one baby with

TABLE 2. Syphilis PCR results by patient diagnosis, number of patients ($n = 68$), and number of specimens ($n = 87$)

Patient diagnosis	No. of patients	Case no.	Specimen type (no. of specimens)	
			Positive specimens ($n = 19$)	Negative specimens ($n = 68$)
Primary syphilis	19	1		Blood (1)
		2	Swab (1)	
		3		Swab (1) (β -globin negative)
		4		Swab (1) (β -globin negative)
		5		Blood (1)
		6	Swab (1)	Blood (1)
		7	Swab (1)	
		8	Swab (1)	Blood (1)
		9	Swab (1)	Blood (1)
		10		Blood (1)
		11		Blood (1)
		12		Swab (1) (β -globin negative)
		13	Swab (1)	
		14	Swab (1)	Blood (1)
		15		Blood (1), swab (1)
		16	Swab (1)	
		17		Swab (1)
		18	Swab, cervix (1), lip (1), vaginal (1)	
		19		Swab (1)
Secondary syphilis	9	1	Blood (1), tissue (2), swab (1)	Blood (1)
		2	Blood (1)	
		3	Blood (1)	
		4		CSF (1) (β -globin negative)
		5	Blood (1)	
		6		Blood (1)
		7		Blood (1)
		8		Blood (1)
		9		Blood (1)
Congenital syphilis	3	1	Blood (1)	
		2, 3		Blood (2)
Early latent syphilis	8		(0)	Blood (8)
Infectious syphilis with neurologic involvement	1		(0)	Blood (1)
Late latent syphilis	1		(0)	CSF (1) (1 β -globin negative)
Old treated syphilis	3	1	(0)	Swab (1)
		2		Swab (1)
		3		Blood (2)
Noncases ^a	24		(0)	Blood/serum (12)
				CSF (1)
				Umbilical fluid (1)
				Tissue (1) (skin scraping, inguinal)
			Swabs (16) (3 β -globin negative)	

^a Twenty-four cases include 20 cases with no syphilis diagnosis, one biological false-positive reaction, and three babies with positive serologic tests for syphilis due to maternal antibody transfer.

congenital syphilis, whose father reported sexual contact in China.

DISCUSSION

The use of PCR for the study of syphilis has been explored for several purposes, including diagnosis of syphilis, typing of strains for understanding of the molecular epidemiology of the disease, and assessing antimicrobial resistance. The use of PCR as a diagnostic tool for syphilis has not been popular, and most reports have employed the test on ulcer specimens from primary lesions. This study examines the detection of treponemal DNA in blood or ulcer specimens taken from patients at different stages of disease.

In our study, although the percentages of specimens (36%) and patients (34%) with syphilis that were PCR positive were low compared to those of other studies, the data offered some insights. When patients were separated into those with primary

versus secondary syphilis, the percentages of patients giving positive PCR results were found to be roughly equal—47% versus 44%, respectively. In the primary syphilis patients, treponemal DNA was detected only in ulcerative swab specimens and not in their blood specimens. Of the nine secondary cases, only two cases provided specimen types other than blood (CSF in one case and ulcerative swab and tissues in the other). Therefore, it was not possible to know if specimen types other than blood would yield higher positive rates among this group of patients.

Since treponemal DNA could be detected only from the ulcerative lesions in primary syphilis cases, this is the most suitable type of specimen for this group of patients. Therefore, when the only patients (15 cases listed in Table 2) that provided swab specimens were examined, nine of them were PCR positive, and the positivity rate increased from 34% to 60%. Among the 15 cases that provided swab specimens, specimens from 3 appeared to be inadequate due to the inability to detect

the β -globin gene in them, which may suggest the lack of host pus and/or epithelial cells in the specimens. The presence of PCR inhibitory substances in these three specimens was excluded by spiking them with a positive control (500 copies of target genes) and repeating the PCR assay that now yielded positive results. If these three inadequate specimens are removed from the calculation, the percentage of cases testing positive by PCR increased to 75% (9 cases out of a total of 12 cases providing adequate specimens). This compares favorably with an 80% sensitivity rate, compared with serological testing, of a real-time PCR assay that detects the *polA* gene in ulcerative swab specimens (13).

Our data demonstrated the detection of treponemal DNA in blood specimens, exclusively in secondary syphilis cases. Although our positive detection rate of 44% in the secondary syphilis patients is almost identical to the 46% reported in another study that measured the *polA* gene in blood specimens of persons with syphilis (18), in that study, the 13 PCR-positive syphilis cases included three with incubating syphilis, one each of primary and secondary syphilis, and eight with latent syphilis.

While others had reported a much higher PCR-positive rate in ulcerative swab specimens, such as 93% in the study of Sutton et al. (30), it might be related to the stringent criteria for obtaining swab specimens for PCR. For example, in the study of Sutton et al., only dark-field-positive genital lesions were obtained for PCR, and when there was no lesion and rapid plasma reagin was reactive, blood was drawn for PCR, which gave a positive rate of 27%.

Despite reports of detecting treponemal DNA in blood specimens of patients with latent syphilis (12, 18, 30), none of the 10 latent syphilis cases examined in this study were positive by PCR. This is in contrast to the 47% to 75% (depending on if only adequately obtained ulcerative swab specimens were included in the analysis) and 44% positive rates of our PCR assays for primary and secondary syphilis cases, respectively. Therefore, it is unlikely that our failure to get positive PCR results in the group of patients with latent syphilis is related to the procedures that we use in our PCR protocol but more likely to be due to low treponemal levels in blood in latent syphilis patients. Although many publications have appeared over the past several years on the use of PCR for syphilis diagnosis, the only consistent finding is the high success rate of detecting treponemal DNA in swab specimens obtained from primary syphilis cases (24, 30). PCR detection of treponemal DNA from non-primary-syphilis cases is still uncommon, and the sensitivity rates reported are not as high or consistent as those reported for primary syphilis cases using specimens taken from ulcerative lesions (18, 21, 30). Therefore, further investigations into specimen types and the timing of specimen collection are required before PCR can be recommended routinely for the investigation of syphilis beyond the primary stage of the disease.

One potential cause of the negative finding in our latent syphilis cases and the low sensitivity rates in primary and secondary syphilis cases may be prior antibiotic treatment. Experimental animal studies have shown that DNA from killed *T. pallidum* was removed from the body at a much higher rate than were DNA from live organisms (32). However, none of the patients reported recent use of antibiotics. Another poten-

tial reason is the sensitivity of our assay. Our PCR assay to detect *bmp* and *tp47* genes has a sensitivity of 20 cells per PCR, which translates to about 4,000 cells per ml of specimen. Although our PCR protocol used only 40 cycles of amplifications instead of the 45 amplification cycles used in two other studies (14, 18) for the detection of the *polA* gene in clinical specimens (including blood), this difference in the number of amplification cycles did not appear to contribute to the lower sensitivity of our assay. This was because we repeated our PCR assay by using 45 cycles of amplification on 15 PCR-negative samples (three swab and nine blood specimens from primary syphilis cases; two swab specimens and one blood specimen from secondary syphilis cases), and the results were still negative. However, more sensitive real-time PCR techniques that use fluorescence dyes for detection may increase the sensitivity and diagnostic yield of this method when applied to blood specimens. A third possible reason is the choice of specimen or timing of collection. Although the use of peripheral blood mononuclear cell fractions has been described to offer better detection than whole blood (12), this finding was not supported by observations in the rabbit infection model (29). Therefore, further studies with clinical specimens are required to clarify this issue. Some investigators have used biopsy specimens from skin lesions with various rates of success for the diagnosis of syphilis by PCR (75% of 12 specimens in one study and 39% of 36 specimens in another) (3, 5). The use of skin biopsy specimens for diagnostic purposes is limited in most clinical settings. Finally, the PCR assay described in this study is solely for the detection of *T. pallidum* in suspected syphilis patients. Therefore, its application and utility performance may be different from those of multiplex PCR assays for the detection and identification of multiple infectious agents in genital ulcer diseases in general (4, 16).

In light of the recent reports of the failure of azithromycin treatment of early syphilis (15) and the rapid development of azithromycin resistance in *T. pallidum* (20) as well as reports of resistance in syphilis cases from the neighboring province of British Columbia (22), an expert working group in Canada recently removed azithromycin from the treatment regimen of syphilis (28). Approximately 29% of the PCR-positive cases in this Canadian province showed in vitro evidence of resistance to macrolide antibiotics, including azithromycin. Infections caused by a resistant strain(s) were found in MSM (three cases, including one bisexual male) and in one baby with congenital syphilis, whose father likely acquired his infection in China. We recently examined nine primary syphilis cases in Shanghai, China, and all nine cases were demonstrated to be caused by macrolide-resistant *T. pallidum* (I. E. Martin, W. Gu, Y. Yang, and R. S. W. Tsang, unpublished data). Macrolide-resistant *T. pallidum* was reported in China in five congenital syphilis cases born to mothers treated with azithromycin during pregnancy (34). The observation of both azithromycin-sensitive and azithromycin-resistant syphilis cases in Alberta involving individuals with different sexual orientations raises the possibilities that different outbreaks are occurring in the heterosexual population compared with those occurring in MSM and that bisexual males could serve as the "bridge" to heterosexual persons. Very limited Canadian data on the prevalence of azithromycin resistance in *T. pallidum* are available, with 1 of 47 specimens collected between 2000 and 2003 compared with

4 of 9 specimens from MSM in 2004 to 2005 in Vancouver demonstrating resistance (22). The Canadian expert working group recommended that azithromycin not be routinely used as a treatment option for early or incubating syphilis unless adequate and close follow-up can be ensured, and it should be used only in jurisdictions where few or no azithromycin-resistant genotypes have been demonstrated (28).

Based on our data, it may be reasonable to treat selected Alberta cases with infectious syphilis with azithromycin, e.g., patients who are not MSM or bisexual males, those with no history of sexual contact or travel outside of Alberta, those with severe allergy to penicillin, and those with a high likelihood of noncompliance with a 14-day course of doxycycline. However, use of this treatment agent should be accompanied by every attempt to follow these individuals both clinically and serologically. The rapid development of resistance in other settings emphasizes the ongoing need of surveillance for azithromycin resistance in syphilis if this agent is to be used in selected situations.

Our data support the use of PCR testing of ulcerative swab specimens for the diagnosis of syphilis. PCR is likely to be of particular benefit in the primary stage of syphilis before serologic conversion has occurred. Its use in the diagnosis of secondary and latent syphilis is likely to be limited by the relatively low positivity rate from cases in these stages, but it may serve as an epidemiologic tool for positive cases. Ongoing surveillance for azithromycin resistance in syphilis continues to be important in guiding treatment recommendations for syphilis. Molecular typing analysis of our PCR-positive specimens as well as specimens from other Canadian provinces will help to delineate the molecular epidemiology of syphilis in Canada.

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