

## NOTES

### Molecular Typing of *Treponema pallidum* in South Africa: Cross-Sectional Studies

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**We evaluated a molecular subtyping system for *Treponema pallidum* for its ability to differentiate between strains obtained from male patients with primary syphilis in South Africa. Of 201 *T. pallidum*-positive specimens, 161 were typeable, revealing 35 subtypes. The unique subtypes identified in Durban, Cape Town, and Carletonville and the total number of subtypes suggested that the strain population was very diverse and varied geographically.**

Syphilis is endemic in South Africa. A recent national survey of antenatal clinic attendees in South Africa revealed a syphilis seroprevalence of 4.9% (1). Although the rates in this group of women have been declining since 1998, the prevalence of the disease in the country's general population remains unacceptably high by world standards.

In this cross-sectional study, we sought to determine the diversity of *T. pallidum* strains circulating in a country where syphilis is common and to obtain an indication of the discriminatory ability of a PCR-based typing system for *T. pallidum* (4) by using genital ulcer specimens. By use of a multiplex PCR assay (3) or a *poLA* PCR assay (2), *T. pallidum* DNA was detected in 201 of 1,954 consecutive male patients with genital ulcers and attending STD clinics in Durban, Kwa-Zulu-Natal; Cape Town, Western Province; Johannesburg, Gauteng; Carletonville, Gauteng; and Welkom, Orange Free State (Table 1).

DNA was extracted from a 40- to 200- $\mu$ l aliquot of the specimen by using a QIAamp blood/tissue kit (Qiagen, Santa Clarita, Calif.) with the following modification: 3  $\mu$ l of a 1:10 dilution of calf thymus DNA (10.5  $\mu$ g/ $\mu$ l; Sigma Chemical Co., St. Louis, Mo.) was added to the Eppendorf centrifuge tube before ethanol precipitation to increase the yield of *T. pallidum* DNA. The repeat region of the *arp* gene and three members of the *tpr* gene family were amplified by using the following PCR primer pairs: ARP1A (5'-CAA GTC AGG ACG GAC TGT CC CTT GC-3') and ARP2A (5'-GGT ATC ACC TGG GGA TGC GCA CG-3') and B1 (5'-ACT GGC TCT GCC ACA CTT GA-3') and A2 (5'-CTA CCA GGA GAG GGT GAC

GC-3'). All other experimental conditions were as previously described (4, 5).

Of the 201 *T. pallidum*-positive specimens, 161 (80.1%) contained sufficient DNA for typing. Of the 40 specimens (19.9%) that could not be subtyped, 26 were found negative by both *tpr* and *arp* gene PCR assays, while 14 were typeable by the *tpr* gene PCR method only (data not shown). This result could have been due to the fact that the *tpr* gene PCR assay is a nested PCR and is therefore more sensitive than the *arp* gene PCR assay. Other possible reasons for our inability to type *T. pallidum* from these specimens include (i) insufficient DNA in the specimen, (ii) degradation of DNA during storage, and (iii) false-positive diagnostic PCR results.

TABLE 1. Clinical specimen collection by site in South Africa ( $n = 1,954$ )

City or town	Collection date	No. of specimens	No. (%) of specimens positive for <i>T. pallidum</i> <sup>a</sup>
Durban	June–August 1996	199	29 (15)
	June–August 1997	158	15 (9.4)
	January 1998	72	6 (8.3)
	July–October 1998	400	33 (8.3)
Cape Town	April–August 1995	180	58 (32.2)
Johannesburg	May–October 1996	159	19 (12)
Carletonville	July–November 1998	115	18 (16)
	August 1998–May 2000	398	21 (5.3)
Welkom	January–November 1996	273	12 (4.4)

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<sup>a</sup> As determined by a multiplex PCR assay or a *poLA* PCR assay. Typing experiments were not performed on 10 samples because of inadequate or no specimen.

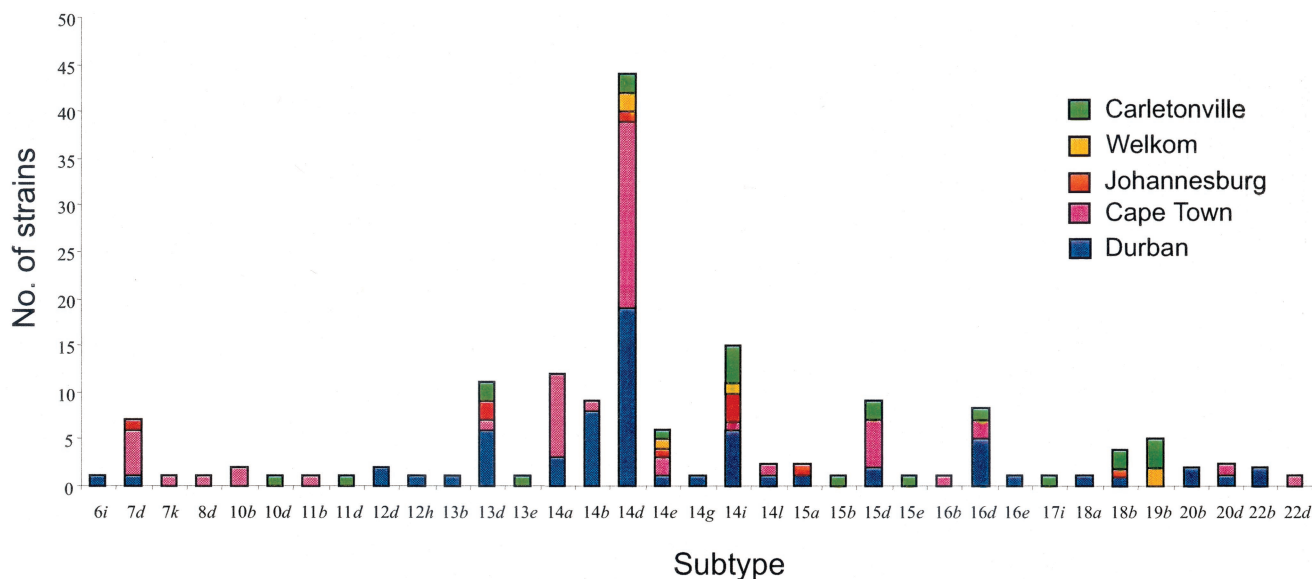


FIG. 1. Distribution of *T. pallidum* subtypes in South Africa (n = 161).

A total of 15 *arp* repeat sizes (6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 22 repeats) and 10 *MseI* restriction fragment length polymorphism patterns (*a, b, d, e, g, h, i, j, k, and l*) were identified in this study. By combining the *arp* repeat sizes and *MseI* restriction fragment length polymorphism patterns (4, 5), we observed 35 subtypes (Fig. 1): 22 subtypes among the 67 specimens from Durban, 17 subtypes among the 55 specimens from Cape Town, 14 subtypes among the 23 specimens from Carletonville, 7 subtypes among the 10 specimens from Johannesburg, and 4 subtypes among the 6 specimens from Welkom. The majority of strains (44 of 161, 27.3%) belonged to subtype 14*d*: 19 of 67 (28.3%) from Durban, 20 of 55 (36.3%) from Cape Town, 2 of 6 (33.3%) from Welkom, 1 of 10 (10%) from Johannesburg, and 2 of 23 (8.7%) from Carletonville. The second most common subtype was 14*i*, present in 15 of 161 specimens (9.3%), followed by 14*a* and 13*d*, found in 12 of 161 specimens (7.4%) and 11 of 161 specimens (6.8%), respectively. Of the remaining specimens, 9 of 161 (5.5%) were subtype 14*b*, 8 of 161 (4.9%) were subtype 16*d*, 7 of 161 (4.3%) were subtype 7*d*, 5 of 161 (3.1%) were subtype 19*b*, and 4 of 161 (2.4%) were subtype 18*b*; 2 strains

(1.2%) each belonged to subtypes 10*b*, 12*d*, 15*a*, 20*b*, 20*d*, and 22*b*; and the remaining 17 strains had unique subtypes.

Subtypes 14*d*, 14*e*, and 14*i* were identified in specimens from all five sites studied, while subtype 13*d* was found in four sites and subtypes 7*d*, 15*d*, 16*d*, and 18*b* were each found in three sites. Subtype 14*a*, which included the third highest number of strains, was identified only in specimens from Durban and Cape Town. A number of subtypes were unique to specimens from Durban, Cape Town, and Carletonville. No unique subtypes were found in specimens from either Johannesburg or Welkom.

The first 20 typeable specimens obtained from genital ulcer disease patients in Durban beginning in June 1996 were compared with the first 20 typeable specimens collected from patients in July 1998 (Fig. 2). Thirteen subtypes were identified among the 20 specimens collected during 1996, while 10 subtypes were identified among the 20 specimens from 1998. Four subtypes were identified in specimens from both 1996 and 1998; four specimens from 1996 had the 14*d* subtype, compared with seven in 1998. There were nine unique subtypes

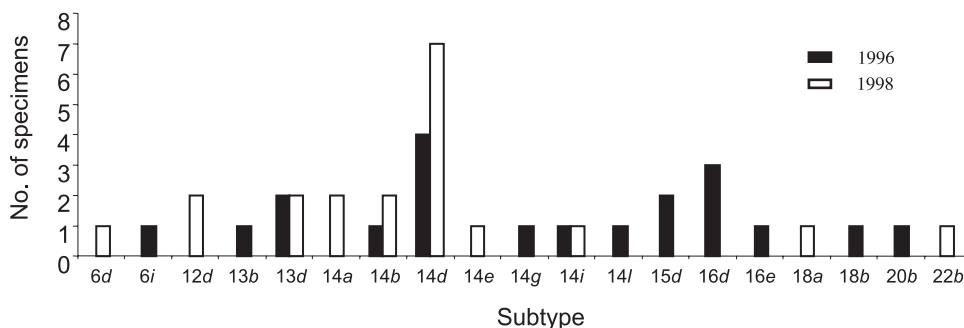


FIG. 2. Comparison of *T. pallidum* strain patterns in Durban in 1996 (n = 20) versus 1998 (n = 20).

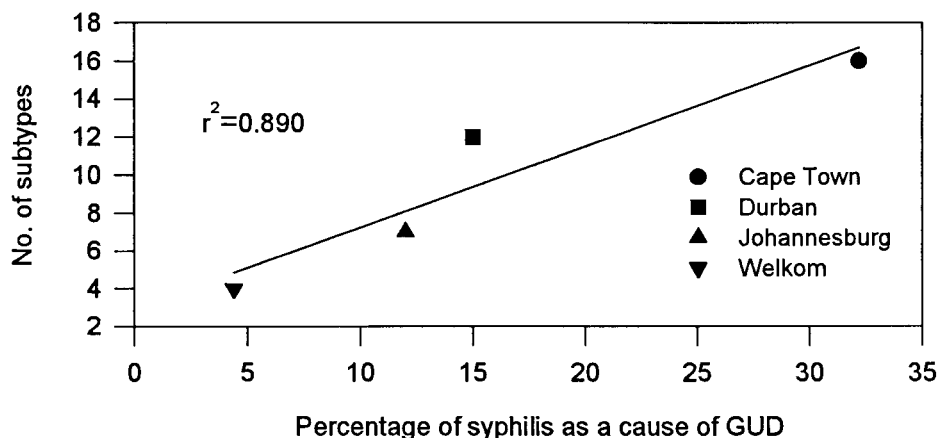


FIG. 3. Number of subtypes versus percentage of syphilis infections as a cause of genital ulcer disease (GUD) by city in South Africa.

among the specimens from 1996 and six unique subtypes among the 1998 specimens.

The presence of many different *T. pallidum* subtypes among persons in Durban and Cape Town may reflect the large numbers of *T. pallidum*-positive specimens from these areas. However, when the total number of strains per total number of subtypes was determined for each site (data not shown), the strain diversity in descending order of sites was Johannesburg, Welkom, Carletonville, Durban, and Cape Town. However, the high strain diversity that was observed in Johannesburg, Welkom, and Carletonville may not be an accurate representation because disproportionately smaller numbers of *T. pallidum*-positive strains were available for analysis in these areas compared to Cape Town and Durban. Furthermore, if specimens were collected from epidemiologically linked cases, the degree of genetic diversity would appear falsely low.

The high strain diversity observed here may reflect long-term endemicity of the disease in South Africa, with many untreated individuals with infectious forms of syphilis, allowing transmission to susceptible contacts. In addition to the genetic diversity of endemic strains of *T. pallidum*, the introduction of new strains by travelers to and from other neighboring countries may have contributed to the large number of different circulating strains. We examined whether there was a correlation between the number of subtypes and the prevalence of syphilis and observed that the number of subtypes was directly proportional to the relative prevalence of syphilis (the Pearson  $r^2$  value was 0.890) in South Africa (Fig. 3). While the correlation was of borderline statistical significance ( $P = 0.057$ ), it

may indicate that the diversity of *T. pallidum* subtypes is higher when the prevalence of syphilis is high.

The observation of predominant subtypes and the demonstration of strain diversity in this study suggest that the typing system used here may be useful for other countries where syphilis is endemic. However, to validate its usefulness in contributing to an understanding of the epidemiology of syphilis, it will be necessary to demonstrate the same *T. pallidum* subtype within individual sexual networks.

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#### REFERENCES

1. Department of Health. 2001. Eleventh national HIV and syphilis seroprevalence survey of women attending public antenatal clinics in South Africa, October 2000. Department of Health, Pretoria, South Africa.
2. Liu, H., B. Rodes, C. Y. Chen, and B. Steiner. 2001. New tests for syphilis: rational design of a PCR method for detection of *Treponema pallidum* in clinical specimens using unique regions of the DNA polymerase I gene. *J. Clin. Microbiol.* **39**:1941-1946.
3. Orle, K. A., C. A. Gates, D. H. Martin, B. A. Body, and J. B. Weiss. 1996. Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. *J. Clin. Microbiol.* **34**:49-54.
4. Pillay, A., H. Liu, C. Y. Chen, B. Holloway, A. W. Sturm, B. Steiner, and S. A. Morse. 1998. Molecular subtyping of *Treponema pallidum* subspecies *pallidum*. *Sex. Transm. Dis.* **25**:408-414.
5. Sutton, M. Y., H. Liu, B. Steiner, A. Pillay, T. Mickey, L. Finelli, S. Morse, L. E. Markowitz, and M. E. St. Louis. 2001. Molecular subtyping of *Treponema pallidum* in an Arizona County with increasing syphilis morbidity: use of specimens from ulcers and blood. *J. Infect. Dis.* **183**:1601-1606.