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Short Communication

Evaluation of the Diagnostic Efficacy of PCR for *Ureaplasma urealyticum* Infection in Indian Adults with Symptoms of Genital Discharge

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SUMMARY: *Ureaplasma urealyticum* genital infection may lead to severe clinical implications if left undiagnosed and untreated. The present study was conducted to evaluate the diagnostic efficiency of a polymerase chain reaction (PCR) assay and to determine the prevalence of *U. urealyticum* in Indian adults with symptoms of genital discharge. Cervical swabs, vaginal swabs and male urethral swabs from 100 patients attending an sexually transmitted disease clinic at a tertiary care hospital in Delhi were screened prospectively for infection with *U. urealyticum*. The prevalence of *U. urealyticum* was found to be 32% by culture and 45% by PCR. *U. urealyticum* was recovered from 8 (47%) and 37 (45%) symptomatic men and women, respectively. The agreement between PCR and culture was 93.75%. PCR improved the test sensitivity by 13% compared to culture. The results confirm the need to use a sensitive and reliable molecular method to prevent the underdiagnosis of ureaplasma infection and to facilitate better clinical management of this infection in India.

Neisseria gonorrhoeae and *Treponema pallidum*, the "classical" sexually transmitted pathogens continue to produce serious illnesses. In addition to these pathogens, recent studies have also demonstrated a strong association between abnormal urogenital findings and the detection of *Ureaplasma urealyticum* (1). However, the prevalence and complete pathogenic potential of this organism is still to be elucidated in the Indian adult population.

Genital mycoplasma infections are commonly diagnosed by culture, which is time-consuming, costly, and requires expertise. Recently, polymerase chain reaction (PCR) has been reported to offer a better diagnostic performance than culture (2). However, financial constraints limit its use in developing countries like India. The present study was conducted to evaluate the diagnostic efficiency of a PCR assay and to determine the prevalence of *U. urealyticum* in Indian adults with symptoms of genital discharge. The study was approved by the Ethical Committee of our institute.

A total of 100 symptomatic patients (83 women and 17 males) attending the sexually transmitted disease (STD) outpatient clinic of a tertiary care hospital in India from June 2003 to April 2005 were enrolled in the study. The specimens included 29 cervical swabs, 54 vaginal swabs and 17 male urethral swabs. Samples were transported in 2 ml pleuropneumonia-like organisms medium (PPLO broth) and were stored at 4°C before processing. Samples were inoculated into PPLO broth supplemented with urea and incubated at 37°C in 5% CO_2. The remaining material was frozen at −70°C for PCR testing. The broths were inspected twice daily and subsequently subcultured onto PPLO agar plates containing urea. The plates were examined periodically for 2 weeks for the characteristic colonies of ureaplasma. Each sample was thawed and centrifuged at 14,000 × g for 30 min. Pellets were treated with 500 μl of lysis buffer (10mM Tris-HCl, pH 8.0, 0.45% Triton X-100, and 0.45% Tween 20) and proteinase K (100 μg/ml) at 55ºC for 1 h. DNA was extracted with phenol-chloroform-isoamyl alcohol and was ethanol precipitated. The oligonucleotides used to amplify a 429-bp region of the *U. urealyticum* were chosen from the published nucleotide sequence of the urease structural gene (3). The primers used were 5′- TCT GCT CGT GAA GTA TTA C-3′ (sense) and 5′- ACG ACG TCC ATA AGC AAC T-3′ (antisense). The 25 μl amplification reaction mixture containing 2.5 μl of 10 × PCR buffer (1 × PCR buffer is 10 mM Tris-HCl [pH 8.8 at 25ºC], 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1% Triton X-100), 200 μM (each) deoxynucleoside triphosphate mixture, 10 pmol of each primer, 1 unit of Taq polymerase and 5 μl of sample DNA. A thermal controller (MJ Research, Waltham, Mass., USA) was used to process the samples through 35 cycles with initial denaturation at 95ºC for 5 min; cyclic denaturation at 94ºC for 1 min, annealing at 56ºC for 1 min, extension at 72ºC for 1 min, and final extension at 72ºC for 5 min. The PCR product was analyzed by electrophoresis on 2.0% agarose gels.

The prevalence of *U. urealyticum* as determined by culture was 32% and PCR was 45%. *U. urealyticum* was recovered from 8 (47%) and 37 (45%) symptomatic men and women, respectively. The agreement between the PCR and culture was 93.75%. Of the 100 patients' results analyzed, 30 specimens were both culture- and PCR-positive. An additional 13 specimens were PCR-positive but culture-negative (Table 1). Upon analysis of the test results according to specimen type it was observed that in cervical specimens, PCR detected

Table 1. Number of specimens positive for *Ureaplasma urealyticum*

Specimen type	Tested	No. of specimens	
		Culture positive (%)	PCR positive (%)
Cervical swab	29	10 (34)	13 (45)
Vaginal swab	54	18 (33)	24 (44)
Urethral swab	17	4 (24)	8 (47)
Total	100	32 (32)	45 (45)

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U. urealyticum in 13 (44.8%) of 29 specimens, whereas culture detected it in 10 (34.4%) of 29 specimens. In vaginal specimens, PCR detected ureaplasma in 24 (44.4%) of 54 specimens (only 18 of which were culture-positive). Culture detected ureaplasma in 4 (23.5%) of 17 urethral specimens, whereas PCR was positive in 8 (47.05%) specimens. The only specimens that were culture-positive and PCR-negative were 2 vaginal specimens. The sensitivity, specificity, and positive and negative predictive values of PCR were 94, 81, 70 and 96%, respectively.

The role of *U. urealyticum* in many human diseases has been established, but the difficulty of culturing it may have led to an underestimation of its role in clinical entities. Many studies have evaluated PCR for the detection of *U. urealyticum* in clinical specimens and have shown it to be highly sensitive and specific (4). However, hindering factors like cost and specialized infrastructure have precluded the use of PCR for diagnostic purposes in developing countries like India. To our knowledge, the present study is the first to evaluate the diagnostic efficiency of PCR in Indian adults with symptoms of genital discharge. The overall sensitivity of culture in our study was 32% (24% in males and 34% in females). In clinical specimens, PCR enhanced the detection rate of *U. urealyticum* by 13%. The enhanced sensitivity of genital mycoplasma detection with PCR is consistent with the findings reposted in the literature (5). The two PCR- negative but culture-positive vaginal specimens suggest that there is an inhibitory component in this specimen type. It is important to consider that failure to identify infection can lead to sequelae like pelvic inflammatory disease, infertility, ectopic pregnancy, etc. It is therefore emphasized that efforts to diagnose and treat ureaplasma infection should be an essential component of every STD control program in India. Although financial constraints may impede the routine use of molecular diagnostic methods in our country, it should be kept in mind that the cost of treating the clinical sequelae of undiagnosed and untreated ureaplasma infections may far exceed the cost of these detection assays.

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