DIAGNOSIS OF GONORRHOEA USING A GENETIC TRANSFORMATION TEST AT A VENEREAL DISEASE CLINIC IN THAILAND

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INTRODUCTION

Gram-stain examination and culture of clinical specimens are used routinely in diagnosing *Neisseria gonorrhoeae* infections, however both techniques have important limitations. Gram stain examination is relatively insensitive for the diagnosis of cervical infections even performed by experienced personnel (Rothenberg *et al.*, 1969). Selective culture media must be prepared properly, stored at 4°C, and promptly incubated in a CO_2 enriched atmosphere at 37°C after inoculation (Reyn and Bentzon, 1972). Both procedures are difficult to perform in remote medical facilities in tropical countries. Even if these difficulties are overcome *Neisseria gonorrhoeae* are difficult to transport from a small hospital or clinic where these specimens are often collected to a central laboratory for processing.

A genetic transformation test (GTT) to detect gonococcal DNA in clinical specimens has previously been described (Zubrzycki and Weinberger, 1980). In this test DNA is extracted from a clinical specimen by simple base-acid lysis. A drop of this lysate is then placed on chocolate agar which has been heavily inoculated with a mutant of *N. gonorrhoeae* which grows at 30°C, but not at 37°C. The plate is then incubated in a candle jar at 37°C for several days. If gonococcal DNA is present in the lysate it will transform the *N. gonorrhoeae* mutant allowing it to grow at 37°C.

This study was performed to determine whether the GTT could successfully identify *N. gonorrhoeae* in specimens which were 10 to 14 days old and had been mailed over a great distance.

MATERIALS AND METHODS

Collecting and processing of clinical specimens: Using sterile cotton swabs, two samples of urethral discharge were taken from each of 37 men and four samples of endocervical mucous were taken from each of 159 women at the Cholburi Venereal Disease clinic, Thailand. The first urethral and cervical specimens taken were dried and saved at room temperature for examination in the GTT. The second urethral swab was cultured at the Cholburi clinic. A duplicate set of inoculated culture plates from the same specimens were sent to the bacteriology laboratory of the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok. The plates were put into a candle extinction jar which was kept at ambient temperature (25°- 30°C) during the 2 hour trip to the AFRIMS laboratory. The third and fourth cervical swabs were used for culturing at the Cholburi clinic.

The swabs for the GTT were each placed into half of a self-sealing syringe envelope

(Chieftan, American Hospital Supply, Evanston, Ill.). The envelopes were numbered 1 through 196. The only other identification mark was the letter "U" to denote urethral, or "C" to denote cervical specimens. The specimens, which were collected between April 20 and April 24, 1981, were kept in a refrigerator in Bangkok until all of the specimens were collected. They were then sent to the United States via airmail. The specimens were not refrigerated or handled in any special manner during the shipment. They arrived at the Temple University School of Medicine in Philadelphia on May 4th (10-14 days after the specimen collection). One hundred and thirty were examined in the GTT upon arrival. The remaining 66 were left at room temperature and were examined the following day. Those examined with the GTT on either day were chosen at random.

Culture and identification methods: Swabs were inoculated onto Thayer-Martin chocolate agar plates in the form of a "Z" pattern which was then cross-streaked with a loop. Inoculated plates were put into candle extinction jars and incubated at 36°C for 48 hours at Cholburi clinic and duplicates were sent to the AFRIMS laboratory for incubation under similar conditions. Colonies typical of gonococci which were oxidase-positive and consisted of gram-negative diplococci were confirmed as being *N. gonorrhoeae* by routine sugar tests (Rothenberg *et al.*, 1976).

The genetic transformation test: The GTT of urethral and cervical specimens was conducted as previously described (Zubrzycki and Weinberger, 1980). Clinical swabs were placed in a 13 x 100 mm test tube containing 0.5 ml of GC buffer, squeezed against the side of the tube and discarded. 0.2 ml of 0.3 N NaOH containing 0.005% phenol red (Gibco, Grand Island, NY, 0.5% solution) as a pH indicator was then added, and the tube was left at room temperature for five minutes. For neutralization of the lysate 0.2-0.3 ml of 0.2 N HCl was added (a change in color indicated acid neutralization). The tube was then incubated at 68°C for ten minutes and then allowed to cool to room temperature. Several colonies of an overnight culture of the *N. gonorrhoeae* mutant, TSA-2 which grows at 30°C but not at 37°C, was then resuspended in GC buffer and evenly smeared on a chocolate agar plate in a manner similar to that used for an antibiotic disc susceptibility test. A drop of the lysate from the clinical swab was then spotted on the agar lawn of TSA-2. After incubation for 48 hours at 37°C in a candle jar, a spot of growth appeared if the lysate contained *N. gonorrhoeae* DNA. Lysates of *N. gonorrhoeae* and non-gonorrhoea were included as positive and negative controls with each plate.

RESULTS

The urethral specimens from 37 men and the cervical specimens from 159 women were studied. The results of the culture method and of the GTT for determining gonorrhoea infections are shown in Table 1.

There was 100% concordance with the urethral specimens between the GTT and the combined culture results from the Cholburi Venereal Disease and the AFRIMS laboratories. Two specimens which were transported to Bangkok from Cholburi were negative for *N. gonorrhoeae* in the AFRIMS laboratory, but were positive in the GTT and contained *N. gonorrheoae* when specimens were incubated on selective media in a candle jar at 37°C within one hour of collection at the Cholburi Venereal Disease clinic laboratory. The GTT identified 31 of 41 (76%) cervical swabs that were positive by culture. In ten cervical specimens from which *N. gonorrhoeae* were isolated the GTT was negative, and two failed to grow the organism, but the GTT was positive. Overall the GTT

identified 62 of the 72 (86%) clinical specimens from which *N. gonorrhoeae* were isolated.

DISCUSSION

The comparison between examining urethral specimens in the GTT and the standard culture method in this study and a previous study in the Atlanta and Philadelphia study were similar (Jaffe *et al.*, 1982). The GTT on mailed urethral specimens was as sensitive as culturing specimen directly.

The sensitivity of the culture method for isolating gonococci from cervices is reported to be between 90 and 95% (Schnale *et al.*, 1969; Windall *et al.*, 1980). A factor affecting the sensitivity of the standard culturing techniques used in this study, was the fungal overgrowth of culture media in the Thai laboratories, due to the excessive heat and humidity of the climate, and the transportation of the specimens. To increase the sensitivity of the culture method for gonococci, multiple cervical samples were taken and cultured as described. Eight of the 41 (20%) cervical specimens which contained *N. gonorrhoeae* would have been missed due to overgrowth by fungi, if only one cervical swab had been cultured.

The GTT identified 76% of cervical specimens from which *N. gonorrhoeae* were isolated by standard bacteriological methods. Two cultures may have contained vancomycin sensitive organisms (Cross *et al.*, 1971; Reyn and Bentzon, 1972; Brorson *et al.*, 1973), which may account for the two specimens which were positive in the GTT but from which *N. gonorrhoeae* was not isolated (Table 1). Another explanation stems from the indiscriminate use of antibiotics by the patients. Partial antibiotic therapy could result in the shedding of predominantly dead gonococci which could not be detected by culture but whose DNA could be detected by the GTT.

Table 1

Comparison of the standard methods and the genetic transformation test (GTT) in identifying *N. gonorrhoeae*.

	Standard bacteriological methods			
	Urethral specimens		Cervical specimens	
GTT	Pos.	Neg.	Pos.	Neg.
Positive	31	0	31	2
Negative	0	6	10	116

The 76% sensitivity of the GTT on cervical specimens in this study was unexpectedly low when compared with the 94% sensitivity in a previous study (Caldwell *et al.*, 1971). There are no good explanations for the specimens from which *N. gonorrhoeae* were isolated in Thailand, but in which the GTT were negative. Perhaps the mixed flora in the cervical specimens flourished in the hot and humid climate and thus affected the GTT, possibly by destroying the gonococcal DNA. This possibility should be investigated in the future. Nevertheless the GTT appears to be a promising approach to the diagnosis of gonorrhoea in areas where a diagnostic bacteriology laboratory is not available.

SUMMARY

A genetic transformation test (GTT), a technique in which gonococcal DNA is detected in clinical specimens, was used to search for *Neisseria gonorrhoeae* infections in 37 men and 159 women at the Venereal Disease clinic in Cholburi, Thailand. Swabs were collected in duplicate from cervical specimens from 159 women and from urethral specimens from 37 men. One of each specimen was cultured on Thayer-

Martin media while the other was mailed to the United States at room temperature for the GTT which involved a delay of 10 to 14 days. With the urethral specimens *N. gonorrhoeae* was identified in 84% (31/37) of specimens and there was 100% concordance between the results of the GTT and culturing specimens directly on Thayer-Martin media. With cervical specimens *N. gonorrhoeae* was isolated from 26% (41/159) by the standard culture technique and 19% (13/159) by the GTT. Seventy-six percent of the culture positive specimens were positive with the GTT and two specimens from which *N.* gonorrhoeae were not isolated were positive in the GTT. The GTT technique enables physicians to send swab collected from patients with suspected gonorrhoea without any special transport media to a central laboratory for laboratory diagnosis of gonorrhoeal infections. This technique which uses reagents which are available in most bacteriology laboratories, should facilitate surveillance of gonorrhoea especially when specimens are collected in clinics where bacteriology laboratory facilities are not available.

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