Fluorescent Antibody Method of Detecting Gonorrhea in Asymptomatic Females

AD HARRIS, W. E. DEACON, Ph.D., JOHN TIEDEMANN, M.D., and WILLIAM L. PEACOCK, Jr., B.A.

GONORRHEA has not been as responsive to the wide usage of penicillin and other therapeutic antibiotics during the past several years as has syphilis in this country (*1*, *2*).

Symptoms of *Neisseria gonorrhoeae* infection cause more infected males than females to seek adequate treatment and cure (*3*). It is therefore probable that the untreated foci of this infection are most frequently in the female. The *N. gonorrhoeae* has been shown to be present in varying percentages of females named as sexual contacts to male patients with gonorrhea (*4–7*).

A fluorescent antibody technique has been found to be an effective and rapid method for the detection of *N. gonorrhoeae* in the female (*8*). This study was designed to explore the usefulness of the fluorescent antibody method for detecting *N. gonorrhoeae* in females who had no signs or symptoms indicating gonorrheal infection and who had not been named as sexual contacts of male gonorrhea patients.

Methods

Two hundred thirteen routine-admission female jail inmates, 162 Negro and 51 white, were the patients for this study. These inmates received a careful speculum examination at, or shortly after, admission to jail, and only those found to be free of any signs or symptoms of gonococcal infection were selected for study.

Individual sterile cotton-tipped applicators were used for obtaining specimens from cervical, urethral, and vaginal sites of each patient. Each swab was immediately rotated on the surface of an enriched chocolate agar slant and then allowed to remain in the culture tube. Tubes were placed in a candle jar within 30 minutes, and the jar was placed in a 35° C. incubator approximately 2 hours later. After approximately 20 hours incubation, the growth on the slant was removed with the original swab, and moderately heavy smears were prepared. After fixation in 3 percent formalin, smears were stained with fluorescein-labeled *N. gonorrhoeae* antiserum and examined on a darkfield microscope with ultraviolet light. This entire technique has been described in detail by Deacon and co-workers (*8*), who refer to it as the delayed FA method for detection of *N. gonorrhoeae*.

Results

More Negro than white inmates were examined (table 1), and the number and percentage of *N. gonorrhoeae* identifications were slightly higher in the Negro group (20.9 percent) than in the white group (19.6 percent). However, in the Negro group, 10.3 percent of the sites examined were positive compared with 13.7 percent of the sites examined in the white group. This indicates that a slightly greater number of sites per person were positive in the white group (0.41) than in the Negro group (0.31).

Mr. Harris, Dr. Deacon, and Mr. Peacock are with the Venereal Disease Research Laboratory, Communicable Disease Center, Public Health Service, Chamblee, Ga. Mr. Harris is director, Dr. Deacon is a medical microbiologist, and Mr. Peacock is a bacteriologist. Dr. Tiedemann is medical director of the diagnostic and treatment center, Fulton County Health Department, Atlanta, Ga.

The distribution of sites from which *N. gonorrhoeae* was identified in both Negro and white patients is shown in table 2. In the Negro patients the 50 positive site findings included 11 vaginal, 20 cervical, and 19 urethral. Four-fifths of these positive findings were from the cervical and urethral sites combined. The white patients had 21 positive site findings: 9 from the vagina, 5 from the cervix, and 7 from urethral areas. Twelve of the 21 positive site findings from this group (57 percent) were from the urethra or cervix. This difference between the findings in the Negro and white patients is further emphasized by the positive results from cervical sites only, with 12 occurring in the Negro group and none in the white group.

The number of positive findings at two or more sites was also dissimilar. *N. gonorrhoeae* was found in 13 of 34 patients in the Negro group and in 7 of 10 patients in the white group. These dissimilarities are, in part, associated with a greater frequency of positive findings at vaginal sites in the white group (9 of 10 positive patients) compared with the Negro group (10 of 34 positive patients).

Table 1. Fluorescent antibody identification of Neisseria gonorrhoeae from 213 asymptomatic female jail inmates

Race	Patients examined			Sites examined [1]		
	Total	Number positive	Percent positive	Total	Number positive	Percent positive
Negro	162	34	20.9	486	50	10.3
White	51	10	19.6	153	21	13.7
Total	213	44	20.6	639	71	11.1

[1] Vagina, cervix, and urethra.

Table 2. Number of sites [1] from which Neisseria gonorrhoeae was identified by fluorescent antibody method, by race

Sites positive	Negro	White
All 3 [2]	3	4
Any 2	10	3
1 or more	50	21
Vagina	11	9
Vagina only	0	2
Cervix	20	5
Cervix only	12	0
Urethra	19	7
Urethra only	9	1

[1] 639 sites examined.
[2] Vagina, cervix, and urethra.

Table 3. Results of reexamination [1] of 74 female jail inmates by fluorescent antibody method for detection of Neisseria gonorrhoeae without intervening therapy.

Number of patients	Initial examination	Reexamination
51	Negative	Negative.
13	Positive [2]	Positive.[2]
6	Positive [2]	Negative.
4	Negative	Positive.[2]

[1] 7 days after initial examination.
[2] One or more sites (vagina, cervix, or urethra).

Table 4. Results of reexamination of 30 female jail inmates by the fluorescent antibody method for detection of Neisseria gonorrhoeae after penicillin therapy

Number of patients	Pretherapy examination	Post-therapy examinations		
		First [1]	Second [2]	Third [3]
17	Positive	Negative		
8	Positive	Negative	1 positive [4] 7 negative	
5	Positive	Negative	Negative	Negative.

[1] 5 days post-therapy.
[2] 12 days post-therapy.
[3] 19 days post-therapy.
[4] At urethral sites only.

Results obtained by reexamination of 74 patients 7 days after the initial examination are recorded in table 3. Techniques employed in reexamination were identical with those of the first examination. None of these 74 patients received antibiotic therapy between examinations. The *N. gonorrhoeae* was identified at one or more sites in 19 patients at the first examination and in only 17 patients during the second examination. However, the second examination produced positive findings in 4 of the 51 patients who had had negative findings on the first examination. Six patients with positive findings on the first examination were negative on second examination. The two examina-

tions combined produced positive findings in 23 (31 percent), compared with 19 (25.7 percent) for the first examination only and 17 (23 percent) for the second examination only.

Thirty patients were reexamined one or more times after therapy with 1,200,000 units of procaine penicillin in aluminum stearate (PAM). Results of the examinations of these patients are listed in table 4. Only one positive finding occurred on reexamination. This finding was from the urethral site only on the second reexamination (12 days after therapy) after negative findings had been obtained at the first post-treatment examination made 5 days after therapy.

Discussion

Most of the jail inmates used for this study had short sentences, and followup periods for reexamination were necessarily limited. All findings reported were obtained from female inmates at the jail, and no attempt was made to examine them after their release. These inmates were of similar economic status, so the findings obtained when divided into Negro and white classifications may not be reflective of the races as they exist at dissimilar economic levels or under other conditions. Neither could the assumption be made that results similar to those reported here would occur in segments of either race that had been otherwise selected. This study does, however, indicate that relatively large numbers of females may harbor *N. gonorrhoeae* without signs or symptoms of infection and that the fluorescent antibody method is a rapid and effective means of confirming this.

The effectiveness of any laboratory method for the detection of *N. gonorrhoeae* is dependent primarily on the efficiency of the examining physician in procuring adequate specimens from the proper site or sites in the female. Careless or casual examination by the physician could greatly reduce the number of positive findings and largely negate the value of the fluorescent antibody method, or any other laboratory procedure, for the detection of *N. gonorrhoeae.* In this study, great care was exercised in properly preparing the swab from each site that was examined. The fact that the *N. gonorrhoeae* was detected in approximately one out of each five of the asymptomatic females examined indicates that the medical examination and swab preparations were efficiently performed.

The listings in table 2 indicate the importance of an adequate examination and the taking of specimens for examination from cervical, urethral, and vaginal sites. No single site would have produced half of the positive findings obtained when all three sites were examined. Although the cervical site in the Negro patients and the vaginal site in the white patients were most productive of positive findings, many positive findings would have been missed if only these sites had been examined. These findings are reflective of the small number of *N. gonorrhoeae* organisms present in asymptomatic infection of females and therefore would probably not be similar to results obtained when examining patients with evident gonorrheal infection.

Reexamination, without intervening therapy, was made of 74 randomly selected patients in order to ascertain the value of repeated examinations. Four patients who had been negative at first examination were positive when reexamined. This finding indicates that a single examination of all three sites in the asymptomatic female, no matter how efficient, may fail to obtain some positive findings that could occur in succeeding examinations. This fact is probably also reflective of the small number of organisms present in asymptomatic infection of the female and the consequent probability of missing them by any single examination. This point is also indicated by the six patients with positive findings on first examination who were all negative on later examinations. These findings indicate the continuing value of multiple examinations, even by fluorescent antibody methods, when diagnosis or cure of the patient is the problem at hand. A single examination by the fluorescent antibody method appears to have a relatively high detection quotient, however. Therefore, this could be the approach of choice in a gonorrhea control program since many individuals not institutionally confined would not be readily available for repeat examinations and since the additional cost of multiple examinations would need to be considered.

A smaller series of 30 patients harboring *N. gonorrhoeae* were examined one or more times after a single injection of 1.2 megaunits of PAM. This part of the study was not an attempt to evaluate the efficiency of this form of therapy but rather to ascertain the influence of this amount of penicillin treatment on the fluorescent antibody method for detection of *N. gonorrhoeae*. All 30 patients were reexamined 5 days after penicillin was administered. Thirteen of these were again examined 7 days later, and only five were present for the third post-treatment examination, 7 additional days later. During this relatively short reexamination period after penicillin therapy, the *N. gonorrhoeae* was detected at the urethral site in only one patient, at the second post-treatment examination, 12 days after penicillin therapy. At the time of this positive finding this patient had no signs or symptoms of *N. gonorrhoeae* infection.

The amounts of residual pencillin at any of the sites during any of these post-treatment examinations were not determined. In order that any residual penicillin that may have been carried from the site by the swab might be neutralized, penicillinase was incorporated in media slants at the rate of 10,000 units per milliliter. This was considered to be a necessary precaution for post-penicillin-therapy examinations. The single positive finding in this group after therapy indicates that the *N. gonorrhoeae* had been killed or reduced to numbers below the detection level of the technique employed, with the single exception. Reexaminations 12 to 14 days after therapy may detect treatment failures that would not have been noted by any examination at an earlier post-therapy date.

Conclusions

The delayed fluorescent antibody method is a rapid and efficient laboratory procedure for the detection of *N. gonorrhoeae* in asymptomatic females.

Jails may be a fruitful source of asymptomatic females harboring the *N. gonorrhoeae*. Asymptomatic females may be a large untreated reservoir that continues to serve as a focus for *N. gonorrhoeae* infections.

Summary

Using the delayed fluorescent antibody method, *Neisseria gonorrhoeae* was detected in 44 (20.6 percent) of 213 female jail inmates who had no signs or symptoms of *N. gonorrhoeae* infection.

Urethral, cervical, and vaginal sites of these 213 patients were examined and *N. gonorrhoeae* was found at one or more of these sites in 44 patients. Examination of all three sites produced more positive findings than did examination of any one site or any combination of two sites.

Repeat examinations of 74 of these patients showed that additional positive findings could be obtained by a second examination.

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