CULTURE SITES IN THE DIAGNOSIS OF GONORRHEA IN WOMEN[1]

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Asymptomatic women present an important problem for the control of gonorrhea. Diagnosis has been accomplished by the culturing of endocervical and rectal specimens. The addition of a third site, the urethra, is suggested, and the idea of a single culture plate is advanced with a view to saving costs.

Introduction

Successful control of gonorrhea has been hampered in large measure by the asymptomatic occurrence of *Neisseria gonorrhoeae* in infected women (*1,2*). This hidden reservoir contributes greatly to the perpetuation of gonorrhea in the population. If the disease's spread is to be effectively controlled, both the symptomatic patient and the asymptomatic carrier must be found and properly treated.

Laboratory Techniques

A number of methods have been used to detect the presence of *N. gonorrhoeae* infections in women, including: direct smear examination either by Gram stain or by direct fluorescent antibody staining (*3*), detection of gonococcal antibody (*4*), and cultural isolation of the bacterial agent (*5*). At present this last is the most efficient and widely preferred approach (*6,7*). The recommended culture technique, described by Thayer and Martin (*5*), utilizes a chocolate agar base medium with yeast supplement and the antibiotics vancomycin, colistimethate, and nystatin to inhibit the growth of saprophytic bacteria.

Sites Cultured

Although the culture medium and conditions of incubation of inoculated cultures are extremely important for isolation of the agent, a culture's overall efficiency (defined as maximum recovery of *N. gonorrhoeae*) is still directly related to the adequacy of the specimen that the physician obtains (*8*).

Thus, although the diagnosis of gonorrhea infection in the female depends ultimately on cultural isolation of *N. gonorrhoeae,* selection of the sites from which to take the specimens is a key step in the process. Several authors have indicated the value of using more than one culture site for the diagnosis of gonorrhea in females (*1,8*). It has been claimed that the endocervix and rectum are the two most efficient sites to use in combination (*6*), and indeed they are the two that have been advocated

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by the U.S. Center for Disease Control (*9*). The urethral culture was considered impractical, and also, cost considerations mitigated against the addition of a third site.

The authors recently conducted a study to determine which combination of specimens taken from female endocervical, urethral, and/or rectal sites yields the greatest number of positive cases of gonorrhea. The current recommended procedure for diagnostic screening in women—that is, the separate culturing of specimens from the endocervix and from the rectum—was also reexamined.

Materials and Methods

A total of 556 women were seen at the Venereal Disease Clinic, Alameda County Health Care Services Agency, Oakland, California, U.S.A., from February to June 1975. Patients who presented themselves at the clinic during this period either with symptoms or with histories as gonorrhea contacts were admitted to the study. Each patient was interviewed and then given a pelvic examination, at which time culture specimens were taken from the endocervix, urethra, and rectum using a sterile cotton-tipped applicator. Immediately after collection, the specimens were placed in a tube of 1% Mueller-Hinton holding medium. The tubes were then transported to the laboratory and the contents inoculated onto Thayer-Martin medium within three hours after collection. The Mueller-Hinton holding medium and the Thayer-Martin medium were prepared in the Public Health Service Laboratory. All cultures were incubated at 36°C ± 0.5° in a 5% CO_2 environment for 24 to 48 hours. Typical colonies that were oxidase-positive and contained Gram-negative diplococci were reported as presumptively positive for *N. gonorrhoeae*. Since *N. meningitidis* may account for 5 per cent of the *Neisseria* specimens found in anogenital cultures, biochemical confirmations were performed on rectal isolates when only the rectal site gave a positive culture (*10*). These confirmations were performed in cystine trypticase agar medium as described by Kellogg (*11*).

Results

Of the 556 patients in the study, 154, or 27.7 per cent, were found to have either one or more cultures positive for *N. gonorrhoeae*. The distribution of the recovery results is shown in Figure 1 and Table 1. As it had been expected, the endocervix was the most efficient site for isolation of the agent, being positive for 138 of the total of 154 culture-positive patients (89.6 per cent). The urethra produced the second greatest number of positives—104, or 67.5 per cent. The rectum yielded 61, or 39.6 per cent. Of the total of 154 positive cases, 44 of them (28.6 per cent) were positive at all three sites; 53 (34.4 per cent) were positive only in the endocervix and the urethra; and 8 (5.2 per cent) were positive only in the endocervix and the rectum. Among those identified at only one site, 33 (21.4 per cent of the 154 diagnosed) were positive in the

Figure 1. Specimens positive for *Neisseria gonorrhoeae* by site cultured, Alameda County, California, U.S.A. (February-June 1975).

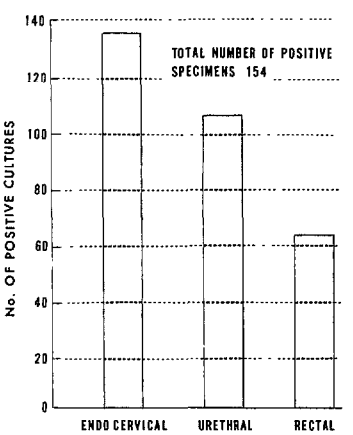

Table 1. Results of cultures for *Neisseria gonorrhoeae* by site(s) of positive specimen, Alameda County, California, U.S.A. (February-June 1975).

	Number of patients tested	Percentage
Negative	*402*	*72.3*
Positive	*154*	*27.7*
Endocervical only	33	5.9
Urethral only	7	1.3
Rectal only	9	1.6
Endocervical and urethral	53	9.5
Endocervical and rectal	8	1.4
Urethral and rectal	–	0
Endocervical, urethral, and rectal	44	7.9
Total	*556*	*100.0*

endocervix, 9 (5.8 per cent) in the rectum, and 7 (4.5 per cent) in the urethra.

Bacterial overgrowth occurred in specimens from 42 (7.6 per cent) of the 556 women tested. Of the patients with overgrown cultures, 14 had all three cultures involved. The rectal specimens were the ones most commonly overgrown, corresponding to all but four of the patients with such cultures. Overgrowth of either the endocervical or urethral culture occurred only in one patient; the endocervical and urethral cultures were both reported overgrown in two patients.

Discussion

The diagnosis of gonorrhea infection in the female is dependent on cultural isolation of *N. gonorrhoeae*. Several authors have indicated the value of using more than one culture site (*1, 8*). It has previously been shown that combination of the endocervical and rectal sites is efficient, and this is the combination that has been advocated by the U. S. Center for Disease Control (*9*).

In the present study the numbers of new positive cases found only through the rectal or urethral specimens—nine and seven, respectively—were statistically too small to determine whether the rectum would provide more new positive cases than the urethra. However, in the interest of maximum efficiency for isolation of *N. gonorrhoeae,* it would appear that the optimum recommendation would be to culture specimens from all three sites—the endocervix, the rectum, and the urethra—in each female patient examined. Of course, the consideration of cost of the additional culture, in light of the small number of positives confirmed, would at first appear to make this recommendation impractical as a routine procedure.

Prior to CDC's recommendation of the endocervical and rectal sites, the Alameda Clinic had been using specimens from the endocervix and the urethra. There was a period of time when, as an economy measure, both the endocervical and urethral specimens from the same patient were cultured on a single Thayer-Martin plate.

The need for obtaining the maximum possible yield of *N. gonorrhoeae* at reasonable cost would suggest that the practice of culturing endocervical and urethral specimens on the same agar plate might be both practical and efficient. Thus, the question of how to increase the yield of positive cases might be approached *not by identifying which are the best sites to culture individually but rather by finding ways to combine specimens on the same culture plate so that more sites can be examined without additional cost.*

The combination of culture sites on one agar plate could conceivably increase "overgrowth." In the study, 14 patients had cultures that were completely overgrown when all three sites were plated separately. If the two sites yielding the most positives are considered, 22 patients had cultures that were overgrown to some extent, including two who were positive for *N. gonorrhoeae.* For all three specimens on one culture, the number overgrown was 42; nine of these 42 patients were positive for *N. gonorrhoeae.*

Had the two most efficient sites been cultured together, the proportion of overgrowth in relation to all the women tested would have been 4 per cent; if all three sites had been combined on a single plate, it would have been 7.6 per cent.

One could assume from these figures that it might be possible to combine all three specimens on a single culture plate. However, further study is indicated to evaluate the efficacy of this recommendation,[5] primarily with respect to the problem of overgrowth.

SUMMARY

To test for the presence of *Neisseria gonorrhoeae* infection, culture specimens were taken from the endocervical, urethral, and rectal sites in 556 women seen at the Alameda County Venereal Disease Clinic, Oakland, California, U.S.A. There was no significant difference between the number of new cases found with the rectal specimens alone (nine out of a total of 154 cases diagnosed) and those revealed by the urethral site alone (seven). It is suggested, however, that the urethral specimen has importance, since it does yield cases not otherwise identified.

The possibility of culturing endocervical, rectal, and urethral specimens from a given patient together on a single Thayer-Martin plate is discussed. Such a method would afford increased possibilities of isolating *N. gonorrhoeae* at a markedly reduced cost. It is recommended that studies be undertaken in this connection.

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[5] Technique suggested for the evaluation: direct inoculation of culture plates containing modified Thayer-Martin medium, incubation of plates in 5% CO_2 within 30 minutes after inoculation of the specimen, and incubation at 36°C ± 0.5 ° for 48 hours.