Neisseria gonorrhoeae: Colonial Morphology of Rectal Isolates

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Received for publication 22 November 1971

Four principal colony types of gonococci have been previously described, and it has been shown that primary isolates from the urethra and cervix are primarily of colony types 1 and 2. In the present work, gonococcal isolates from the rectum were also shown to be predominantly colony types 1 and 2. Visualization and typing of gonococcal colonies in primary rectal isolates were facilitated by the use of medium containing vancomycin, colistin, nystatin, and, in some cases, trimethoprim lactate. Control experiments showed that these agents sometimes caused minor alterations in colonial morphology; but with knowledge of these alterations satisfactory colonial typing could be made.

Kellogg et al. (1, 2) described four morphological types of gonococcal colonies and showed that colony types 1 and 2 were associated with virulence. They also found that type 1 colonies predominated in isolates from males with typical gonococcal urethritis. Sparling and Yobs (3) found a predominance of colony types 1 and 2 in isolates from the male urethra and female endocervix. In the present study, the colony types of gonococci present in rectal isolates are described.

MATERIALS AND METHODS

Consecutive female patients presenting for venereal disease evaluation at a local clinic were studied without regard to symptoms, signs, or history. The females included known sexual contacts of males with gonorrhea and those with symptomatic complaints. Also included were females seen for routine examination on admission to a local institution for delinquent adolescents. Specimens were obtained by inserting a moistened sterile cotton swab into the rectum and rotating the swab slightly. Upon removal, the swab was brushed in a "2" pattern onto the culture material and then cross-streaked in a diffuse pattern.

Studies of the colonial morphology of gonococci must take into account the instability of the small gonococcal colonies; unsuitable in vitro passage results in a progression of small to large colonies (2). For this reason, examinations of the colony morphology of clinical isolates are best done with the least number of in vitro passages. The incorporation of selective antibiotics into the isolation media allows colony typing of the primary isolate, even though the inocula may be contaminated by undesirable bacteria. The clinical specimens used in this study were inoculated onto two selective antibiotic media. Both media contained GC medium base (BBL) without hemoglobin, plus 1% defined supplement (Iso Vitalex-BBL). One medium, "VCN" medium, contained VCN inhibitor combination (5) (vancomycin, 3 μg/ml; colistin, 7.5 μg/ml; nystatin, 12.5 units/ml); the other, "VCNT" medium, contained VCN inhibitor combination plus 5 μg of trimethoprim lactate per ml to suppress possible proteus swarming and overgrowth. The transparent nature of both media facilitated colony typing.

All cultures were incubated in a candle extinction jar for 20 hr at 36 C. Colonies were identified as Neisseria gonorrhoeae on the basis of colony morphology, oxidas reaction, Gram stain, and sugar fermentation. For each positive culture, 100 colonies were counted, if possible, and typed according to the morphological criteria of Kellogg (2).

RESULTS

Preliminary control studies were carried out to determine whether the selective antibiotics contained in the media had any influence on colony morphology. Known colony types established by selective passage in the laboratory were transferred from antibiotic-free media to media with antibiotics, and slight alterations were noted (Fig. 1). For example, the dark, sharp borders characteristic of type 2 were occasionally seen with type 1 colonies grown on antibiotic media. Crenated borders are also typical of type 2, but the antibiotics could induce a similar picture with the other types, and surface granularity, normally a characteristic of type 3, occasionally was seen with types 1, 2, and 4. The degree to which the antibiotics induced crenated borders or a granular surface was least evident with type 4. Anti-
Fig. 1. Neisseria gonorrhoeae colony types 1, 2, 3, and 4. Colonies grown on media with (a) and without selective antibiotics; 2a contains both type 1 and type 2 colonies (125×).
Fig. 1—continued
biotic-induced changes in colony morphology were similar with VCN and VCNT media. Alterations in colony morphology showed considerable strain variability as some specimens did not develop significant crenation or granularity when grown on the VCN or VCNT media.

Thirty of the 216 females examined by rectal culture were positive for *N. gonorrhoeae*. Proteus overgrowth on VCN medium occurred in four instances and precluded detection of gonococci. In these four cases, colony typing was possible from the VCNT medium because the proteus had been suppressed by the trimethoprim. Type 1 colonies predominated in 60% of the isolates, and type 2 colonies were most prominent in another 20%. The remaining 20% showed equal prevalence of types 1 and 2. Some cultures had an occasional type 3 or 4, but these never exceeded 10% of the colonies.

The data presented here are in accord with those of Kellogg (2) for urethral isolates and of Sparling and Yobs (3) for endocervical specimens. Our finding that the gonococcal colony types associated with virulence (2) are found in the rectum also fits well with the clinical and epidemiological evidence that gonorrhea can be transmitted by rectal intercourse.

There has recently been an intriguing report (4) that type 4 colonies predominated in the rectal isolate of a patient with gonococcal arthritis. While we found no instance of such a situation in our patients, it should be kept in mind that none of our patients appeared to have systemic involvement. Sparling and Yobs (3) found that 1 of 114 males and none of 72 females had predominantly type 4 colonies in their urethral or endocervical isolates. Thus, had our study included a larger number of patients, it is conceivable that an occasional isolate of predominantly type 3 or 4 would have been found.

**ACKNOWLEDGMENTS**

We thank T. O. Vinson, H. Palmer, and R. Havird for providing access to patients.

**LITERATURE CITED**


