Interference of Neisseria gonorrhoeae Growth by Other Bacterial Species

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Growth of Neisseria gonorrhoeae from clinical specimens has been enhanced by the use of selective media that inhibit the simultaneous growth of other microorganisms. One explanation for this enhancement could be that certain other bacteria inhibit gonococcal growth. This hypothesis was examined by testing 167 bacterial isolates for in vitro gonococcal inhibition; 34.1% of the isolates failed to inhibit the gonococcus, but 12.0% produced weak inhibition and 53.9% strongly inhibited N. gonorrhoeae. The pattern of in vitro gonococcal inhibition was consistently the same for all the individual isolates within some species, but individual isolates within other bacterial species varied in their ability to inhibit the gonococcus. Consistently strong in vitro N. gonorrhoeae inhibitors were Citrobacter diversus, Enterobacter cloacae, Serratia marcescens, and Pseudomonas. The in vivo significance of gonococcal interference was demonstrated in the subcutaneous chamber model of N. gonorrhoeae infection.

The isolation of Neisseria gonorrhoeae from individuals with gonorrhea can be difficult when the clinical specimen is heavily contaminated with other microorganisms. However, the use of selective media that inhibit these other microorganisms has facilitated the recovery of the gonococcus. The detection of N. gonorrhoeae in vaginal specimens was increased from 54.2 to 96.6% by the use of selective media (20).

The mechanism(s) responsible for the failure to isolate gonococci from heavily contaminated specimens is unknown, but it could be overgrowth of the slow-growing gonococcus by the other more rapidly growing microorganisms. Interference with gonococcal growth by other microorganisms may also be involved. This possibility is supported by the frequent clinical laboratory observation that there is no gonococcal growth in the vicinity of another colony but good growth on other areas of the culture medium. In vitro gonococcal growth inhibition has also recently been described on areas of media where other microorganisms were previously grown. This occurred with two Staphylococcus epidermidis isolates, one Neisseria meningitidis isolate, and Candida albicans isolates (10, 14, 16).

In this study we examined a variety of bacteria frequently identified in clinical laboratories to determine their ability to inhibit the gonococcus. Our purpose was to establish the frequency of N. gonorrhoeae inhibition by other bacteria and to identify which bacteria account for this interference. In addition, in vivo gonococcal inhibition was examined in guinea pigs. It was not the purpose of this report to define the biochemical mechanisms of gonococcal inhibition or to examine its clinical significance in the prevention of gonococcal infection.

MATERIALS AND METHODS

Bacteria. Two N. gonorrhoeae isolates were used in this study: isolate 2686 and isolate B. Isolate 2686 has been passed in vitro more than 500 times and has been characterized in several publications (5, 16, 17, 34). Isolate B was recently isolated from a patient with gonococcal urethritis. Virulent colony types (13) of these two isolates have been maintained by selective in vitro passage. N. meningitidis isolates were obtained from the bacteria bank in the Venereal Disease Research Branch of the Center for Disease Control.

One hundred and sixty-seven consecutive aerobic bacterial isolates identified by the Special Bacteriology Unit of the Center for Disease Control were used in this study.

In vitro assay of bacteria interference. Our original technique (14, 16) was a modification of the bacteriocin assay described by Counts (6). Briefly, the potential inhibitor bacterium was inoculated in an approximately 2-cm-wide linear streak on 20 ml of GC agar base supplemented with IsoVitalex (BBL) (GCB medium) in a 15- by 100-mm petri dish. The inoculate was incubated for 24 h in a candle extinction jar at 36.5°C. If the growth was not confluent, the colonies were cross-streaked within the 2-cm-wide area and the dish was reincubated for another 24 h. The quantity of inoculum was standardized to the point of confluent growth, since bacterial species vary in their rate of growth on GCB...
medium. When this bacterial growth was confluent, it was carefully removed with cotton-tipped applicators and the residual bacteria were killed by exposure to chloroform vapors. The potentially inhibited bacteria were then cross-streaked over the entire medium, and the plate was reincubated in a candle extinction jar at 36.5°C for 20 h. Evidence of bacterial interference was manifested by failure of the second bacteria to grow in the area where the initial bacteria had grown.

This technique was slightly modified in the current studies with respect to the cross-streaking of the second or potentially inhibited bacteria. Instead of cross-streaking the entire plate in an identical manner, half was lightly cross-streaked by using a single 24-h-old colony, and the other half received a heavy inoculum—the growth contained in four 3-mm bacterial loops.

In certain experiments, after exposure to chloroform vapors but before cross-streaking with the second bacteria, 0.2 ml of broth was overlaid on the medium and allowed to penetrate. This broth was prepared by dissolving 15 g of protease peptone no. 3 and the lyophilized contents of a 10-ml vial of IsoVitaleX in 10 ml of distilled water. Each 0.2 ml of the broth contained 0.3 g of protease peptone no. 3 and 0.2 ml of IsoVitaleX—the quantity of the two nutrients in 20 ml of GCB medium.

In vivo assay of bacterial interference. A variation of the guinea pig model of gonococcal infection was used (2). Garra's subcutaneous chamber technique (7) was modified in that 2.5-cm polyethylene tubes with open ends were implanted into the subcutaneous areas of adult Hartley strain guinea pigs (26). Two to 4 weeks later, the chambers were challenged by the percutaneous injection of bacteria. Each in vivo study involving various numbers of N. gonorrhoeae and another bacterium was done in a animal having multiple chambers. If the in vivo inhibition varied from the in vitro results, both assays were repeated.

These bacteria for challenge were suspended in Eagle minimal essential medium and adjusted to an optical density of 0.3 at 530 nm (approximately 10⁶ colony-forming units [CFU]/ml). Log dilutions in Eagle minimal essential medium were made, and 0.1-ml samples were plated on GCB medium. The bacteria were incubated overnight, and then the colonies on the plate with the greatest number of discrete colonies were counted. The number usually ranged from 30 to 300 colonies/plate. Portions (0.10 ml) of the various bacterial suspensions were percutaneously injected into the subcutaneous chambers. If two different bacterial species were simultaneously injected, each was introduced separately in a 0.1-ml sample. The in vivo interaction of N. gonorrhoeae and each of the other bacteria was evaluated by adding the following bacteria or mixtures of bacteria to individual subcutaneous chambers: 10⁶ CFU of N. gonorrhoeae and 10⁷ CFU of the other bacterium; 10⁶ CFU of N. gonorrhoeae and 10⁵ CFU of the other bacterium; 10⁵ CFU of N. gonorrhoeae and 10⁴ CFU of the other bacterium; 10⁴ CFU of N. gonorrhoeae alone; and 10⁵ CFU of the other bacterium alone.

At various intervals after the guinea pig challenge, 0.05 ml of chamber fluid was cultured on GCB medium and on an identical medium containing vancomycin-colistin-nystatin inhibitor (35). The latter inhibited the growth of bacteria other than the gonococcus. This inhibition was essential if the chambers contained N. gonorrhoeae and a gonococcal inhibitor, since the latter organism sometimes inhibited the in vitro growth of the former. The GCB medium was effective for growing the eight bacterial species evaluated in the in vivo assay.

In vitro growth of bacteria contained in the chamber fluid was approximated as 4+ for confluent growth, 3+ for individual colonies too numerous to count, 2+ for greater than 50 CFU, and 1+ for fewer than 50 CFU.

RESULTS

Several patterns of gonococcal interference were observed in the in vitro assay (Fig. 1). No inhibition in areas of either light or heavy N. gonorrhoeae growth was designated type A or no interference. Type B interference was defined as no inhibition in the area of heavy N. gonorrhoeae growth but inhibition in the area of light N. gonorrhoeae growth confined to the area of potential inhibitor growth. Type C interference was similar to type B except that the zone of inhibition in the area of light N. gonorrhoeae growth extended beyond the area of potential inhibitor growth. Inhibition in the areas of both light and heavy N. gonorrhoeae growth was designated type D. The in vitro assay was only semiquantitative, and additional patterns might have been found if a greater range of gonococcal inocula had been tested.

When the 167 bacterial isolates were tested for possible N. gonorrhoeae inhibition, 110 or 65.9% demonstrated some degree of inhibition. Occasionally, the inhibition of gonococcal isolate 2686 differed from that of isolate B, but the difference was no more than one type of inhibition pattern; A ⇌ B, B ⇌ C, and C ⇌ D were occasionally seen, but A ⇌ C, A ⇌ D, and B ⇌ D variances were not observed. If this variability was encountered, the strongest inhibitory pattern was arbitrarily taken as the pattern for the inhibitor bacterial isolate. The most frequently observed pattern was type C (Table 1). Among the various bacterial species tested, some were consistent in their pattern of inhibition as either being noninhibitors (type A) or strong inhibitors (types C or D) (Table 2). Other species were variable in that some strains within the species produced no inhibition (type A), others produced weak inhibition (type B), and others produced strong inhibition (Table 2). A bacterial species was included in Table 2 only if three or more different isolates were tested for gonococcal interference.

Two possible reasons for N. gonorrhoeae in-
hition variability within a bacterial species were investigated. To determine if the site of isolation of a gonococcal inhibitor influenced its degree of gonococcal inhibition, we analyzed the interference patterns of the three variable inhibitors that had been studied most frequently as a function of their site of isolation. Table 3 presents the ratio of strong inhibitors to the number tested as a function of the anatomical site of isolation. To determine if in vitro passage of a bacterium influences its ability to inhibit the gonococcus, a fresh clinical isolate of N. meningitidis was passed in vitro 55 times. This organism was a weak (type B) inhibitor, so either an increase or decrease in N. gonorrhoeae inhibition could be observed. The inhibition of each of the two gonococcal isolates by this group B meningococcus did not change substantially after in vitro passage.

Twenty randomly selected C or D gonococcal inhibitors were further studied to determine if they could be inhibited by gonococci in the in vitro assay. Gonococcal isolates 2686 and B were each initially grown as the center streak, and the GCB medium plates were subsequently cross-streaked with each of the 20 strong inhibitors. No bacterial interference was observed in this aspect of the study.

The same 20 C or D gonococcal inhibitors were used in the medium replenishment experiments. A preliminary study had shown that the nutrients of GCB medium (protease pep-
The interaction of N. gonorrhoeae and S. faecalis, and it also shows the incidental finding that fewer gonococci than S. faecalis established an infection in a subcutaneous chamber, i.e., 10^6 CFU of S. faecalis and 10^5 CFU of N. gonorrhoeae.

Contrasting with the relationship of N. gonorrhoeae to S. faecalis and Listeria, the other two in vitro A inhibitors (Propionibacterium acnes and an Enterococcus) predominated over the gonococcus in the in vivo system. This variability between in vitro and in vivo infection was confirmed when each assay was repeated. Although the P. acnes was cultured with the gonococcus after 4-day infections, by 10 days the gonococcus could only be recovered from the control chamber where it alone had been inoculated. After the chambers had only a single bacterial isolate (N. gonorrhoeae or P. acnes), challenge of a P. acnes-infected chamber with up to 10^6 CFU of N. gonorrhoeae failed to infect. Introduction of 10^6 CFU of P. acnes into an N. gonorrhoeae infection failed to infect, but 10^6 CFU of P. acnes did establish a co-infection. A somewhat analogous situation was seen with the Enterococcus. After a brief co-infection seen after 4 days in a single chamber, the N. gonorrhoeae was eliminated from all co-challenged chambers by 10 days. Challenge of the Enterococcus with and IsoVitaleX could be added to petri dishes already containing the agar, salts, and buffers. After the nutrients were evenly distributed over the surface and allowed to penetrate the medium, an inoculum of one N. gonorrhoeae was added, and the nutrients were evenly distributed over the surface and allowed to penetrate the medium, an inoculum of one N. gonorrhoeae colony resulted in a quantity of growth comparable to that on GCB medium prepared in the usual manner. When the GCB nutrients were added to the medium after the chloroform treatment but before cross-streaking, the pattern of gonococcal inhibition by the 20 C or D inhibitors was unchanged.

The same 20 C or D gonococcal inhibitors were again used in the in vitro assay to evaluate possible inhibition of meningococci. A variety of responses was observed, ranging from that of Providencia stuartii, which failed to inhibit any of the eight meningococci, to that of Pseudomonas aeruginosa, which strongly inhibited (D type) not only the gonococcus but also all eight meningococci.

The in vivo interactions of N. gonorrhoeae and two type A gonococcal inhibitors (Streptococcus faecalis and a Listeria) were similar. Coinfections persisted for the duration of the experiment, which was 21 days for N. gonorrhoeae and the Listeria and 61 days for N. gonorrhoeae and S. faecalis. Table 4 illustrates the interaction of N. gonorrhoeae and S. faecalis, and it also shows the incidental finding that fewer gonococci than S. faecalis established an infection in a subcutaneous chamber, i.e., 10^6 CFU of S. faecalis and 10^5 CFU of N. gonorrhoeae.

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<table>
<thead>
<tr>
<th>Type of inhibition</th>
<th>Bacteria</th>
<th>No. of isolates tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (A type)</td>
<td>K. pneumoniae</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>S. faecalis</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Enterococcus</td>
<td>7</td>
</tr>
<tr>
<td>Variable (A, B, C, and D types)</td>
<td>E. coli</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>P. stuartii</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Alpha Streptococcus</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>E. agglomerans</td>
<td>4</td>
</tr>
<tr>
<td>Strong (C and D types)</td>
<td>C. diversus</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>E. cloacae</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: Enterococci further identified by additional biochemical tests.
coccus-infected chambers with up to 10^6 CFU of N. gonorrhoeae did not establish a gonococcal infection, but 10^5 CFU of the Enterococcus introduced into an established N. gonorrhoeae infection of 23 days' duration eliminated the gonococcus within 3 days.

The experiments involving three of the D inhibitors (Staphylococcus aureus, E. coli, and Pseudomonas cepacia) resulted in similar findings. When injected into the animals simultaneously with N. gonorrhoeae, each of these D inhibitors eliminated the gonococcus within 3 days (E. coli and P. cepacia) to 10 days (S. aureus). When 10^3 CFU of the same three inhibitors were introduced into established gonococcal infections, the N. gonorrhoeae were eliminated within 2 days (E. coli and P. cepacia) to 8 days (S. aureus). Introduction of up to 10^6 CFU of N. gonorrhoeae into established S. aureus, E. coli, and P. cepacia infections failed to either eliminate the latter or even establish coinfections. Table 5 illustrates the interaction of N. gonorrhoeae and E. coli.

Findings were somewhat different when the fourth D inhibitor (Staphylococcus epidermidis) was tested in the in vivo system. In those chambers challenged simultaneously with N. gonorrhoeae and S. epidermidis, the latter eventually was the sole organism recovered, but this took up to 60 days. Challenge with up to 10^6 CFU of N. gonorrhoeae into an established S. epidermidis infection did not establish a coinfection.

**DISCUSSION**

This study has extended prior studies by showing that a variety of bacterial species are capable of inhibiting gonococcal growth. It also demonstrated that inhibition of N. gonorrhoeae growth by another bacterium can occur in an in vivo model of gonococcal infection.

**Table 5. In vivo interaction of N. gonorrhoeae and E. coli**

<table>
<thead>
<tr>
<th>Day</th>
<th>Procedure</th>
<th>Animal chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>Challenge</td>
<td>10^9/10^6</td>
</tr>
<tr>
<td>4</td>
<td>Culture</td>
<td>0/4</td>
</tr>
<tr>
<td>11</td>
<td>Culture</td>
<td>0/3</td>
</tr>
<tr>
<td>21</td>
<td>Culture</td>
<td>0/4</td>
</tr>
<tr>
<td>31</td>
<td>Culture</td>
<td>0/4</td>
</tr>
<tr>
<td>34</td>
<td>Rechallenge</td>
<td>10^9</td>
</tr>
<tr>
<td>36</td>
<td>Culture</td>
<td>0/4</td>
</tr>
</tbody>
</table>

a The procedure was either challenge of a subcutaneous chamber with a bacterial inoculum or the culture of a chamber to determine the status of the bacterial infection.

b Results for challenge procedure: N. gonorrhoeae inoculum/E. coli inoculum (colony-forming units).

c Results for culture procedure: N. gonorrhoeae growth/ E. coli growth (0 to 4+).

Most bacteria tested in the in vitro assay inhibited N. gonorrhoeae to some degree. Various patterns of gonococcal inhibition were observed; they ranged from the inhibition of only a relatively light gonococcal inoculum to inhibition of a relatively heavy inoculum. Variability in the degree of in vitro bacterial interference has been described in other bacterial systems, with somewhat different techniques being used (12).

Some bacterial species were consistent in either their ability to inhibit or their failure to inhibit the gonococcus. In other species individual strains varied in their ability to inhibit the gonococcus. The number of isolates examined among those bacteria consistent in their pattern of inhibition was relatively smaller than the number tested among the variable inhibitors. If more isolates had been examined, additional species might have demonstrated variability.

We did not find the reason for this variability among strains of some species, but we did consider two possible reasons. We studied the possibility that in vitro passage of a fresh isolate (a N. meningitidis isolate) could alter the inhibition. During 55 in vitro passages, its ability to inhibit N. gonorrhoeae growth did not change significantly. The second possibility examined was that the anatomical site of isolation might influence inhibition. We found that the percentages of strong inhibitors among S. aureus, S. epidermidis, and E. coli were similar for different anatomical sites of isolation.

A variety of mechanisms has been shown to account for in vitro bacterial interference. The inhibition of S. aureus by Serratia marcescens and Pseudomonas sp. is related to nutrient competition (37). Bacillus cereus, Proteus vulgaris, E. coli, and Enterobacter aerogenes inhibit S. aureus via an antibiotic substance (37). A heat-labile inhibitor of nicotinamide metabolism is the mechanism by which coagulase-negative staphylococci inhibit coagulase-positive staphylococci (22). The inhibitory substance by which pneumococci are inhibited by viridans streptococci has a molecular weight of approximately 12 million (12), whereas low-molecular-weight polypeptide inhibitors are produced by the oculair bacterial flora (9).

The mechanism(s) involved in in vitro gonococcal inhibition cannot be determined from our data. Media depletion by the inhibitor bacteria does not appear to be involved for several reasons. If such depletion were involved, the gonococcus might be expected to inhibit the 20 strong gonococci inhibitors when it is the initial bacterium grown on the test medium. This phenomenon was not observed. The fact that
good *N. gonorrhoeae* growth occurred with a heavy gonococcal challenge and *N. gonorrhoeae* growth was inhibited in an area of light gonococcal challenge (as in the B and C types of interference) might also suggest that medium changes are not involved, since they should not necessarily favor growth of a heavy inoculum over that of a light inoculum. Alternatively, inhibition of a light challenge overcome by a greater challenge is the pattern seen when inhibitors are added to a medium (36). A third factor suggesting that medium depletion is not the mechanism of in vitro inhibition is that replenishment of medium nutrients did not alter the patterns of inhibition.

The inhibitory mechanisms may vary with each bacterial species. The inhibition may also involve the simultaneous action of more than one mechanism (19). The inhibition could be accompanied by factors that potentiate bacterial growth, since certain bacteria are known to enhance the growth of other bacteria (8; L. F. Judge, Ph.D. thesis, Univ. of Maryland, College Park, 1958).

Gonococcal inhibition by other bacteria in the guinea pig model is evidence that antgonococcal bacterial interference is not solely an in vitro phenomenon, but may also have in vivo significance. The *Listeria* and *S. faecalis* that did not inhibit *N. gonorrhoeae* in the in vitro assay did not inhibit it in the in vivo model. These two bacteria coexisted with *N. gonorrhoeae* for the duration of their coinfection, 20 and 60 days, respectively. This relationship between in vitro type A interference and in vivo bacterial coexistence was not always seen. Two other in vitro type A inhibitors, an *Enterococcus* and *P. acnes*, coexisted with *N. gonorrhoeae* 4 days after inoculation, but by 10 days only the *Enterococcus* and *P. acnes* were present. When up to 10⁸ gonococci were introduced into an established pure *P. acnes* or *Enterococcus* infection, gonococci could not be recovered when first cultured 3 days later. Conversely, 10⁷ enterococci introduced into an established *N. gonorrhoeae* infection eliminated the gonococcus within 3 days. When 10⁸ *P. acnes* were added to an established pure gonococcal infection, no *P. acnes* could later be recovered, but when 10⁷ *P. acnes* were added to an *N. gonorrhoeae* infection, a coinfection was present when the chamber was first cultured 3 days later.

The reason the in vivo gonococcal inhibition by the *Enterococcus* and *P. acnes* was not detected by the in vitro assay cannot be determined from our data. The in vivo assay could simply be a more sensitive test of gonococcal inhibition. It could also be that in vitro the gonococcal inhibition is neutralized by medium components such as starch, which may neutralize certain toxic materials (18, 38). Another possibility is that certain in vivo interference mechanisms are not present in the in vitro assay. This could include the blood bactericidal activity, which has been described in the bacterial interference produced by staphylococci (36).

A good correlation was observed between the in vitro and in vivo gonococcal inhibition by the four strong inhibitors. When each of these strong (type D) in vitro inhibitors was simultaneously introduced with *N. gonorrhoeae* into the guinea pig model, the gonococcus was eliminated whereas the strong inhibitor persisted in the site of infection. The time during which this occurred varied from less than 3 days with *E. coli* to as long as 2 months with *S. epidermidis*. When up to 10⁸ gonococci were introduced into an established infection of each of the four type D inhibitors, gonococci could not be recovered when first cultured 2 to 4 days later. Conversely, when 10⁵ of each of three of the type D inhibitors were introduced into established gonococcal infections, the gonococci were eliminated within 2 days by *E. coli* and *P. cepacia* and within 8 days by *S. aureus*.

An incidental finding in the in vivo experiments was the ease with which the guinea pig subcutaneous chambers could be infected with the eight bacteria studied in the in vivo experiments. As with the gonococcus, no foreign substances, such as mucin (21), and no immunosuppressants are needed to establish the infection. This suggests that the subcutaneous chamber technique may be a model for the study of other bacterial infections and other instances of bacterial interference. The need for an animal model to be used in bacterial interference studies has been stated (25). So far the only available model has been the chicken embryo (1, 8). Disadvantages of the chicken embryo model include the fact that the bacterial interference is usually measured by the all-or-none phenomenon of embryo survival. The guinea pig subcutaneous chamber technique allows the two bacteria to be counted as they interact over a period of days to weeks. In the chicken embryo only a small number of phagocytic leukocytes are present before hatching (24), and the serum beta globulins that contain complement components are not fully developed (27). These tools of the immune system are present in the adult guinea pig and they may be important in some instances of bacterial interference (22).

As with in vitro gonococcal bacterial inhibition, the mechanism(s) of in vivo gonococcal
inhibition is unknown and cannot be determined from our data. Each of the eight in vitro inhibition studies involving variable numbers of gonococci and other bacteria was done in a single guinea pig with five subcutaneous chambers. Two of the chambers were control chambers and were challenged with only the gonococcus or the potential inhibitor. In six animals the gonococcus was eliminated from all infected chambers, and in five of these animals the gonococcus in the control chamber persisted well beyond the elimination of the gonococcus from the other chambers. This seems to indicate that the mechanism of gonococcal elimination is confined to the locale of the inhibitor bacteria, and if a diffusible substance is involved, as suggested by the type C and D patterns of in vitro inhibition, it does not reach a systemic level capable of eliminating the gonococcus from an adjacent chamber.

Although this study did not involve patients, bacterial interference is known to have clinical significance (32, 33). Both artificial and natural staphylococcal colonization of adults' and infants' nasal mucosa and infants' umbilical cord interfered with the subsequent colonization of these sites by other staphylococcal strains. This phenomenon was successfully used to terminate S. aureus infections in a hospital nursery (4, 28–31).

The possibility of antagononcoccal bacterial interference playing some role in clinical resistance to gonorrhea has been the subject of several reports (14, 16). In these studies urethral bacteria were isolated from men who resisted multiple exposures to gonorrhea. These bacteria were evaluated in the in vitro antagononcoccal assay, and some were found to inhibit the gonococcus. This current report shows that such a qualitative approach, which monitors a single in vitro variable, may not represent the entire clinical situation. Both the in vitro and in vivo assays suggest that gonococcal inhibition by other bacteria is a quantitative phenomenon related to multiple variables. Slight alteration of the previously described in vitro inhibition assay showed that interference of a gonococcal isolate is not an all-or-none phenomenon but ranges from the inhibition of only a few gonococci to inhibition of many. The pattern of gonococcal inhibition is therefore related to the inhibitory capability of the interfering bacteria and the size of the gonococcal challenge. The in vivo assay extends this to show that gonococcal inhibition can also be a function of the number of inhibiting bacteria. In the in vivo assay also shows that the duration of interaction is yet another variable that can influence the rate at which other bacteria eliminate the gonococcus.

If the in vitro assay and the in vivo animal data have any relevance to gonococcal infection in humans, the success of antagononcoccal bacterial interference will be a function of (i) the antagononcoccal inhibitory capability of the bacteria involved, (ii) the number of inhibitors present, (iii) the size of the gonococcal challenge, and (iv) the duration of contact of the two bacteria. An adequate evaluation of the clinical significance of antagononcoccal bacterial interference should include monitoring each of these variables.

LITERATURE CITED