Understanding the Bacterial Flora of the Female Genital Tract

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The microbiological flora of the lower female genital tract provides a dynamic, complex example of microbial colonization, the regulation of which is not fully understood. When an exogenous bacterial species, with its array of virulence factors, is introduced into the host, disease does not always occur. Conversely, under selected conditions, commensal endogenous bacteria—for example, *Gardnerella vaginalis* and group B streptococci—can participate in disease processes. Disease caused by both exogenous and endogenous bacteria correlates positively with a markedly increased level of bacterial replication. The key question is what determines the quantity of a given bacterium at any given time. For disease to occur, exogenous or endogenous bacteria that possess pathogenic prerequisites must attain replicative dominance. Their ability to do so is potentially governed by inhibitory or synergistic interrelationships with other microbes.

QUALITATIVE MICROBIOLOGY

The microbiological flora of the lower female genital tract is a dynamic, complex example of microbial colonization, the regulation of which is not fully understood. Much of what we know about the bacterial composition of the female genital tract is derived from qualitative, descriptive studies [1–10]. The fund of information that such studies have provided with regard to the microbial flora of the lower female genital tract is derived from qualitative, descriptive studies [1–10]. The fund of information that such studies have provided with regard to the microbial flora of the lower female genital tract is weakened by the intrinsic technical limitations that are inherent in the studies. Often, even the usefulness of qualitative data is negatively affected by inappropriate or suboptimal methods of data collection, failure to use appropriate transport systems or enriched media, or a lack of stringent anaerobic technique in the processing and culture of specimens.

The importance of using specialized media is illustrated in a study of *Clostridium difficile* by Bramley et al. [11]. These investigators evaluated cultures of vaginal specimens obtained from 522 women who made a total of 902 visits to a family planning clinic, and they found this organism in only 1 patient. However, when a specialized medium that contained 0.2% para cresol was used, a higher rate of isolation (11%) was obtained.

One can only speculate as to how many more microbial species would have been recovered if truly optimal media and methods had been used for all studies reported in the literature prior to the 1970s. Isolation techniques used prior to the 1970s resulted in a gross underestimation of the importance of anaerobic bacteria as major constituents of the normal flora of the female genital tract. Failure to use appropriate transport systems as well as failure to use optimal media and anaerobic culture techniques have compromised the results of many studies with regard to the delineation of the bacterial constituents present.

Although anaerobic bacteria had been identified previously, it was not until publication of the work of Gorbach et al. [2] and, soon afterward, that of Ohm and Galask [3], Galask et al. [5], and Hill [7], that the role of anaerobic bacteria both in maintaining health...
and in causing disease became more clearly defined. Gorbach et al. [2] demonstrated that, in women of reproductive age, anaerobic bacteria outnumbered aerobic bacteria in a ratio of approximately 10:1. This ratio clearly reflects a dynamic colonization process. For example, although adolescent subjects appeared to have a greater prevalence of anaerobic bacteria, aerobic bacteria appeared to become more abundant with advancing age, onset of sexual activity, and parity. A study of postmenopausal women who were either receiving or not receiving estrogen replacement therapy found that such therapy had no effect on facultative organisms; however, anaerobic isolates tended to be less prevalent among women who received such therapy. A notable exception, however, were anaerobic lactobacilli, which appeared to be more prevalent in the tissue of women receiving estrogen therapy [12].

**QUANTITATIVE MICROBIOLOGY**

Combined qualitative and quantitative studies require a quantum increase in technical effort and, as a consequence, tend to be limited in scope despite yielding richer information [13–18]. Recent studies have begun to focus more on the fact that the density of microbial colonization appears to be relevant not only to the condition of asymptomatic individuals but, also, to the initiation of disease states, in which it is a critical factor [13, 18, 19]. The microbial load for a given organism appears to influence the relative risk of symptomatic infection; however, in the absence of quantitative data, data that have been extrapolated from qualitative studies (e.g., the prevalence rates of individual species) are used as a surrogate for quantitative data. The concept exploited is that organisms of which there are a great number are readily found in cultures, whereas those species that are fewer in number may not be noticed during primary isolation.

Quantitative studies of upper and lower female genital tract disease due to exogenous bacterial species (e.g., *Neisseria gonorrhoeae*) and endogenous bacterial species (e.g., *Gardnerella vaginalis*) have demonstrated one common finding: increased numbers of bacteria are found during the course of disease. The studies that have been published to date, although technically imperfect, do provide some information regarding the dynamics of the bacterial flora of the female genital tract.

**BACTERIOLOGICAL STUDIES OF THE NORMAL FLORA**

Studies of the normal bacterial flora of the female genital tract are primarily limited to characterization of the types of bacteria present in women who do not have identifiable disease. Studies by Bartlett et al. [5], Larsen and Galask [8], and Gopperud et al. [12] (see tables 1 and 2) have effectively delineated the principal bacteria that reside in the female genital tract, although they have not delineated their quantitative interrelationship. In terms of planning empirical therapy, it may be just as important to know which organisms are not isolated with high frequency as it is to know which organisms are commonly isolated.

**PATHOGENS AND COMMENSAL ORGANISMS**

Within colonized tissues, such as those of the female genital tract, what constitutes a pathogen is dependent not only on the type of offending microorganism and its intrinsic virulence but, also, on the species complexity of the flora—that is, the relative dominance, in numbers, of the various bacteria that can be recovered—in individual asymptomatic patients. According to traditional thinking, a pathogen was a microbe that was genetically endowed with a factor that, when expressed, caused disease. This postulate became central to the concept of the monomicrobial etiology of infectious diseases, which was derived from correlation of the disease back to the etiological agent. Examples that fit this concept well are diseases caused by *N. gonorrhoeae* or *Treponema pallidum*.

However, the mere presence of an unknown, exogenous, potentially pathogenic species does not necessarily constitute disease when disease is defined in terms of symptoms. Understanding how specific bacteria produce disease has been tied

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**Table 1. Prevalence of aerobic (facultative) isolates reported in vaginal flora studies published in the literature.**

<table>
<thead>
<tr>
<th>Aerobic isolate</th>
<th>Prevalence in vaginal flora, %</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Gram-positive rods</td>
<td></td>
</tr>
<tr>
<td>Diphtheroids</td>
<td>3</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>18</td>
</tr>
<tr>
<td>Gram-positive cocci</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>5</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td></td>
</tr>
<tr>
<td>α-Hemolytic</td>
<td>8</td>
</tr>
<tr>
<td>β-Hemolytic</td>
<td>3</td>
</tr>
<tr>
<td>Nonhemolytic</td>
<td>0</td>
</tr>
<tr>
<td>Group D</td>
<td>2</td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Klebsiella</em> and <em>Enterobacter</em> species</td>
<td>0</td>
</tr>
<tr>
<td><em>Proteus</em> species</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas</em> species</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** Design of table is based on [83].
normal bacterial constituents of the female genital tract are clearly not disease.* Once the knowledge of virulence properties, which allow the bacteria to function as monoetiological agents. Such microorganisms as Neisseria gonorrhoeae, Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae, Listeria monocytogenes, and Trichomonas vaginalis are not ordinarily part of the flora of the female genital tract. They bring the potential for disease to the vaginal/endocervical area by virtue of their inherent biological properties, although the presence of these properties does not guarantee that disease will occur. Once the normal bacterial constituents of the female genital tract are defined, one is confronted with having to explain why apparently commensal bacteria (e.g., G. vaginalis, group B streptococci and Escherichia coli) can cause disease.

More than a century after Pasteur introduced the idea of monoetiological disease (the idea that 1 pathogen causes 1 disease), we still struggle with the definition of the term “pathogen” [24]. In the middle of the 19th century, Pasteur provided evidence that the presence of an organism, such as the anthrax bacillus, in a host is associated with disease; however, there frequently has been a tendency to think that the mere presence of certain organisms is synonymous with disease. It was not until the early part of the 20th century that Theobald Smith introduced the idea that disease was the result of the interplay between microbial virulence, dominance of the organism in terms of numbers, and the innate defenses of the host [25].

Because Koch’s postulates stated that monoetiological disease could be demonstrated through production of infections in animals, many studies were done in which animals could be successfully infected with microbial pathogens. Often, however, these infections eventuated only when a change in the microenvironment was created as part of the experimental model of infection. For example, peritonitis might be more efficiently induced when blood is added to the inoculum. Gangrene has been known to develop when calcium chloride is implanted into muscle along with the Clostridium species. In rodents, vaginal infection with Candida albicans requires estrogen replacement therapy for the animal host. Bacteria that are normal constituents of the vaginal flora of the host have the potential to cause symptoms of disease, but they apparently require some alteration in the microenvironment to do so. C. albicans, group B Streptococcus (GBS), G. vaginalis, and Escherichia coli, which are organisms that are commonly isolated from the lower female genital tract, can, under select circumstances, cause disease.

Although they are not indigenous to the microflora of the genital tract, organisms that are commonly termed “pathogens,” such as H. influenzae, S. pneumoniae, S. pyogenes, and T. vaginalis, may also be present without causing symptoms, much in the way that organisms that are part of the normal flora of the genital tract are typically present. These seeming inconsistencies focus on a critical question regarding the pathogenesis of infectious diseases: what enables a given organism to produce disease?

For endogenous bacteria of the female genital tract, the microbiological environment may supersede selected inherent virulence factors in terms of importance; at the very least, the microbiological environment may affect the bacterial expression of virulence factors [26]. Theoretically, if a virulence factor is constitutive, the number of organisms present will determine the amount of the virulence factor available to promote infec-

### Table 2. Prevalence of anaerobic microorganisms present in cultures of cervical and vaginal specimens obtained from asymptomatic women (according to the results of selected reports)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Percentage of isolates, according to reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides species</td>
<td></td>
</tr>
<tr>
<td>B. bivius&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>B. fragilis</td>
<td>4 40 12 16</td>
</tr>
<tr>
<td>B. melaninogenicus&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>18 46</td>
</tr>
<tr>
<td>Bifidobacterium species</td>
<td></td>
</tr>
<tr>
<td>C. perfringens</td>
<td>3 4</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
</tr>
<tr>
<td>Any</td>
<td>4 0</td>
</tr>
<tr>
<td>Eubacterium species</td>
<td></td>
</tr>
<tr>
<td>Fusobacterium species</td>
<td></td>
</tr>
<tr>
<td>Gaffkya species&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus species</td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcus species</td>
<td></td>
</tr>
<tr>
<td>P. asaccharolyticus</td>
<td></td>
</tr>
<tr>
<td>P. magnus</td>
<td>11 17</td>
</tr>
<tr>
<td>P. prevotii</td>
<td>17 21</td>
</tr>
<tr>
<td>Any</td>
<td>11 33</td>
</tr>
<tr>
<td>Peptococcus species</td>
<td></td>
</tr>
<tr>
<td>P. aerobius</td>
<td></td>
</tr>
<tr>
<td>P. intermedius</td>
<td>34 15</td>
</tr>
<tr>
<td>P. micros</td>
<td>5 10</td>
</tr>
<tr>
<td>P. productus</td>
<td>7 8</td>
</tr>
<tr>
<td>Any</td>
<td>64 65 8</td>
</tr>
<tr>
<td>Propionibacterium species</td>
<td></td>
</tr>
<tr>
<td>Veillonella species</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Dashes denote that no specific information is available.

<sup>a</sup> Prevotella bivia.
<sup>b</sup> Prevotella melaninogenic.
<sup>c</sup> Aerococcus species.
<sup>d</sup> Peptostreptococcus species.
The number of organisms may be controlled by means of the antagonistic or synergetic interaction between the different microbial species present. Three relatively common conditions that involve the female genital tract—namely, vaginal candidiasis, bacterial vaginosis, and infection with GBS—show evidence of regulation.

**C. albicans: vaginal candidiasis.** During the past several decades, the many published surveys of vaginal flora specimens obtained from asymptomatic women have clearly shown that *C. albicans* may be present without the typical symptoms of yeast vaginitis. In a study by Glover and Larsen [27], the results of successive cultures of vaginal flora specimens obtained from women who were followed throughout pregnancy indicated that *Candida* species may be present in stable association with the genital epithelium. Moreover, the majority of women who have vaginal yeast also carry the organism in the gut. The typical rate of yeast carriage varies among populations and increases both after puberty and during pregnancy, which suggests an important role for host physiology in cases of vaginal candidiasis.

A relationship between estrogen levels and bacterial colonization has been recognized almost since the inception of studies of normal vaginal flora; this relationship holds true for *Candida* species as well. For example, rats are resistant to colonization by *Candida* species, unless the animals have an amount of estrogen sufficient to cause vaginal cornification [28]. Growth of bacteria in the flora of the genital tract is stimulated by estrogen [28, 29]. Prevalence of *Lactobacillus* species and prevalence of yeast in different populations tend to show that the times when prevalence of *Lactobacillus* species is highest (during the reproductive years and, especially, during pregnancy) are also the times when the prevalence of *Candida* species is highest. Hydrogen peroxide–producing *Lactobacillus* species may co-colonize with *Candida* species [28]. Although *Candida* species are less susceptible to the microbicidal effects of hydrogen peroxide than are non–catalase producers, such as *N. gonorrhoeae* and *Streptococcus agalactiae*, *Candida* species could be inhibited by hydrogen peroxide [30, 31]. This is presumably due to the fact that hydrogen peroxide damages cellular membranes unimpeded by the intracellular catalase [32]. Classically, vulvovaginal candidiasis occurs in association with a significant increase in the number of colony-forming units of *Candida* species that are present in the tissue-invasive form. Any microbiological influence that allows the yeast concentration to increase may result in the development of symptoms, and any microbiological effect that suppresses the number of yeast could ensure that it remains as a commensal organism [33, 34].

Both studies of animal models and observation of humans suggest that there is an inverse relationship between bacterial and yeast floras with respect to prevalence and numerical abundance. Savage [35], in a classic study of the gastric flora of mice, found that 2 distinct tissue areas exist, and these are normally colonized by a nearly pure culture of *Lactobacillus* species and a nearly pure culture of yeast, respectively. When *Lactobacillus* species were eliminated by means of antibiotic treatment, the yeast took over the microbiological void. This observation suggested control of yeast through an antagonistic effect of the *Lactobacillus* species. Conversely, *Candida* species may exert their own antagonistic effects on populations of bacteria.

Monif and Carson [36] compared the patterns of isolates from women with and without co-isolation of *C. albicans*. In the presence of *C. albicans*, selected aerobic and anaerobic gram-positive isolates were diminished in a statistically lower frequency. Hipp et al. [37] had previously demonstrated that *Candida* species can produce a substance that suppresses the growth of *N. gonorrhoeae*. Later, Shah and Larsen [38, 39] showed that *Candida* species can produce gliotoxin, which, along with other inhibitory compounds in appropriate concentrations, is antagonistic toward various bacteria.

**G. vaginalis: bacterial vaginosis (BV).** The relationship of *G. vaginalis* to disease is not simply a phenomenon of cause and effect. *G. vaginalis* can be a common constituent of the vaginal flora of women [40, 41], yet only a relatively small percentage of these women have symptoms or have a clinically significant vaginal discharge. McCormack et al. [40] identified the presence of *G. vaginalis* in the vaginal samples obtained from 150 of 446 women who visited a student health center and who were free of clinically overt disease. Again, the difference between colonization and disease appears to be partially a function of the magnitude of replication of bacteria. Quantitative bacteriological studies have shown that symptoms that involve *G. vaginalis* are associated with >10⁵ cfu per gram of vaginal fluid [18]. If reintroduction occurs after therapy as a result of sexual contact with an untreated partner, the patient is usually asymptomatic; however, in such patients, the quantitative counts are <10⁵ cfu per gram of vaginal fluid. For disease to occur, not only must there be an environment that will sustain *G. vaginalis* as a constituent of the microbiological flora, but something must happen to free the bacteria from the inhibitory restraints that govern the magnitude of its replication.

The complexity of microbial interrelationships is further suggested by the finding that effective clinical and microbiological cures can be achieved by use of metronidazole in only 75%–80% of patients [41, 42]. For metronidazole to function effectively, an organism must have a functional nitroreductase system. Only 15%–22% of *Gardnerella* isolates have this enzyme system. This observation has led investigators to speculate that the mechanism by which metronidazole has its effect involves its impact on the concomitantly flourishing anaerobic bacteria...
population, which sustains dominance in conjunction with Gardnerella species [43].

The complexity of interspecies interactions has not been completely unraveled with regard to BV. Among the prevailing conditions in the patient who has BV are an elevated pH level (range, 5.5–5.5) and the presence of various primary amines and polyamines, which can be detected on the basis of the presence of the characteristic fishy smell that they emit after KOH has been added [44, 45]. It is not clear what precipitates the condition, and several causes could be suggested. A decrease in the number of lactobacilli could result in decreased production of hydrogen peroxide and acid, thereby allowing for unrestricted growth of other constituents of the flora. A shift in the redox potential favoring anaerobic bacterial may in turn contribute to proteolysis, thereby leading to a more alkaline environment [46]. The proteins and amino acids that are released may be metabolized by anaerobic bacteria as a result of the production of amines, which contribute to both the alkalinization of the vaginal environment and the odor problem, and which may also contribute to vaginal irritation [47]. Some anaerobic bacteria produce succinic acid, which is known to diminish the efficacy of neutrophilic phagocyte activity; this may allow for some species to proliferate [48]. Increased growth of Gardnerella species can augment production of hemolysin, which may further blunt any phagocytic protection that might otherwise occur in the vaginal milieu [49].

**GBS: perinatal group B streptococcal disease.** Because of the relative abundance of studies that deal with GBS, there are sufficient fragments of information that one can use to infer some aspects of intergens bacterial regulation. Certain clues lie in the demographics of the diseases caused by GBS. Although GBS is a leading cause of perinatal and maternal postpartum septicemia, the incidence of disease is grossly disproportional to that of colonization [50, 51]. Depending on the use of special media and the number of anatomical sites sampled, 14%–25% of women have GBS as a constituent of their vaginal flora. The best statistics are those that correlate the incidence of perinatal septicemia with material factors, including maternal antibody. Prior to the implementation of protocols for avoidance of GBS disease, the overall incidence of GBS perinatal septicemia was 1.2–3 cases per 1000 live births. The greater the quantity of GBS present (i.e., the greater the density of colonization), the greater the probability of disease [52, 53]. Maternal fever during parturition is the factor associated with the highest incidence of disease, followed by the presence of asymptomatic GBS bacteriuria in a gravida [54, 55]. A study of newborns with GBS septicemia has demonstrated that isolates recovered from these subjects have a greater ability to attach to epithelial cells than do isolates from newborns without septicemia [56]. Although some of the genetic requisites are known, the need for high multiplicity of GBS has also been recognized. These observations emphasize the importance of discovering what regulates the number of colony-forming units per gram of vaginal fluid.

Chaisilwattana and Monif [57] have published the most extensive study that explores the ability of GBS to inhibit gram-positive and gram-variable constituents of the bacterial flora of the female genital tract. By use of an agar overlay assay technique, test strains of GBS were first inoculated and then were allowed to reach a level of heavy growth. The plate was then overlaid with new media. The target strain was then inoculated onto the fresh agar and was incubated to achieve heavy growth.

The GBS test panels uniformly inhibited group A, B, C, and G streptococci, lactobacilli, G. vaginalis, and most diphtheroid strains. Variable inhibition by GBS was observed with viridans streptococci, nonhemolytic (neither group B nor group D) streptococci, peptostreptococci, and enterococci. The GBS test panels did not inhibit the growth of either coagulase-negative staphylococci or S. aureus. The 23 GBS isolates from neonates or adults with septicemia did not differ from the 18 isolates from subjects without septicemia, with regard to their ability to inhibit the challenge bacteria. When converse testing was done, the growth of GBS isolates was uniformly inhibited by coagulase-negative staphylococci and by the majority of the enterococci, but it was not inhibited by S. aureus.

The stab/chloroform technique was used for confirmation of inhibition. In this technique, the GBS isolate is embedded into the agar, which is then incubated for 24 h. The GBS colonies are then killed as a result of exposure to chloroform, and the plate is then covered with the challenge bacteria. In addition to confirming the results of the overlay agar assay, the stab/chloroform technique demonstrated that, regardless of the cause of the inhibition, the presence of live GBS was not a prerequisite and that the inhibitor had the ability to diffuse through the agar. Both in vitro techniques require that the density of GBS reach maximal levels of growth.

Qualitative studies of cultures of vaginal/cervical bacteriological specimens obtained from asymptomatic women challenge the interpretation of in vitro observations. GBS are not infrequently isolated along with lactobacilli, G. vaginalis, coagulase-negative staphylococci, and enterococci. Deeper analysis does reveal data that are compatible with those of in vitro studies. In a large qualitative study, Carson et al. [58] demonstrated that whenever GBS was isolated, no other β-hemolytic Streptococcus species was present. Conversely, when another β-hemolytic Streptococcus species was present, GBS was not concomitantly present.

The results of in vitro studies of bacterial inhibition may be difficult to relate to the results of qualitative studies of vaginal flora. The ability of GBS to inhibit bacterial growth in vitro is based on it being present in a concentration of $>10^5$ cfu/mL, and such high-density populations of GBS may not often be seen in vivo. In a review of a number of clinical studies that...
dealt with bacterial vulvovaginitis, Monif [19] identified 4 cases of GBS vulvovaginitis for which both qualitative assessment and some form of quantitative assessment were available, and for which appropriate culture techniques had been employed. In each case, GBS was present at a high multiplicity. In 3 of the 4 cases, single co-isolates were identified: *Escherichia coli* in 2 cases and *S. aureus* in 1. GBS does not inhibit the growth of either of these bacteria in vitro. This report indicates that when the issue of bacterial density is effectively addressed, in vivo observations tend to parallel those derived from in vitro experiments.

The studies cited above suggest that a critical level of bacterial replication must be achieved to support the disease-producing capability of either an exogenous or an endogenous bacterial strain within the female genital tract. Whether a bacterial strain within the female genital tract attains the requisite population level appears to be governed, to a significant degree, by the microbes that are concomitantly present.

**REGULATION OF BACTERIAL FLORA**

“Bacterial interference” is the term applied to in vivo situations in which indigenous microbial species regulate colonization by pioneering exogenous microorganisms. Bacterial interference can occur for a variety of reasons. These reasons may include the production of antimicrobial substances by the interfering organism, the efficient use of some substrate in the local environment, preemptive attachment to tissue sites, or a more rapid rate of growth than that of competing organisms [59–63]. Quantitative relationships among bacterial species appear to be a key regulator of bacterial interference. The magnitude of the inhibitory effect may be the result of the potency of the inhibitory substance and the number of producing organisms.

In a study of consecutive cultures of vaginal flora specimens, Carson et al. [58] introduced the term “compatibility profiling” to describe the hypothesis that dominant regulatory bacteria could be identified by virtue of their ability to emerge as the sole isolate in samples in which numerical complexity did not ordinarily be observed. When this hypothesis was applied to 781 isolates, the only bacteria that achieved single-isolate status were *Lactobacillus* species and *G. vaginalis*. Once these bacteria were identified as “sole isolates,” analysis was extended to identify the co-isolate when only 2 bacteria were recovered. The most prevalent of these bacteria were added to the initial key bacteria isolated, and the process was repeated for cases in which only 3 species of bacteria were recovered. The process was again repeated with use of cultures when 4 species of bacteria were present. This iterative process of additive grouping of bacteria established that bacteria such as coagulase-negative staphylococci and the enterococci were compatible with both *Lactobacillus* species and *G. vaginalis*. By inference, those bacteria that were not present were presumed to be susceptible to bacterial interference by the target bacteria or its subsequent isolates.

Certainly, confirmation of this hypothesis will require additional in vitro evaluation. *Lactobacillus* species appeared to be the major regulator of both *G. vaginalis* and selected anaerobic bacteria. *Lactobacillus* species were identified in 131 cultures of vaginal specimens. When *Lactobacillus* species were present, *G. vaginalis* was a co-isolate in 7 cultures. In only 1 of these 7 cases were fewer than 5 isolates observed, including the anaerobic bacteria present. In this study, inhibitory organisms appeared to include coagulase-negative staphylococci, which appeared to suppress *S. aureus* and the group B streptococci, and other β-hemolytic streptococci.

**THE ROLE OF THE LACTOBACILLI**

*Lactobacillus* species are isolates that are commonly recovered from cultures of vaginal specimens obtained from postpubertal asymptomatic female patients. Quantitative studies have reported that vaginal washings contain ~107 lactobacilli per gram of secretions. The most common *Lactobacillus* species include *L. acidophilus* and *L. fermentum*; less common are *L. plantarum*, *L. brevis*, *L. jensenii*, *L. casei*, *L. delbrueckii*, and *L. salivarius*. More than 1 species may be present in an individual [64]. A longitudinal study has shown variability in terms of species or combinations of species over time [8].

In an in vitro study, Skarin and Sylwan [65] demonstrated the ability of *Lactobacillus* species to inhibit the growth of several bacterial species, including *G. vaginalis*, *Mobiluncus* species, *Peptostreptococcus* species, and *Bacteroides* species. They attributed this inhibition primarily to production of a low pH. Reid et al. [66] suggested an alternate mechanism of control of the bacterial flora by the lactobacilli. They found that cell wall fragments of *Lactobacillus* species could block attachment of bacterial uropathogens to uroepithelial cells. It is not clear whether this observation might also apply to vaginal epithelial cells or whether adherence of vaginal microorganisms to the epithelium might be blocked by this mechanism. Colonization of the introitus with *Enterobacteriaceae* species is a predisposing factor for urinary tract infection in women. Raz and Stamm [67] showed that estrogen therapy helped alleviate recurrences of urinary tract infection in a cohort of women. Several lines of evidence support a role for estrogen in increasing the density of vaginal colonization by normal flora organisms [68, 69].

Special focus has been placed on the idea that hydrogen peroxide production is a mechanism of bacterial antagonism of the *Lactobacillus* species [70–75]. Eschenbach et al. [72] advanced the concept that hydrogen peroxide, rather than pH, is a prime regulatory feature of the lactobacilli. They detected
Lactobacillus species in only 35% of women with BV; of those women who were co-colonized with G. vaginalis and Lactobacillus species, only 11% had hydrogen peroxide–producing strains.

Hillier et al. [75] demonstrated a significant correlation between the absence of hydrogen peroxide–producing lactobacilli and vaginal colonization by G. vaginalis, Bacteroides species, Peptostreptococcus species, and Mycoplasma hominis. There were no significant differences between strains of Lactobacillus that produced hydrogen peroxide and those that did not, with regard to concomitant isolation of enterococci, GBS or α-hemolytic streptococci, and catalase-positive bacteria, such as diphtheroids, coagulase-negative staphylococci, and Enterobacteriaceae species. The prevalence of M. hominis or Ureaplasma urealyticum was unaffected when cultures contained hydrogen peroxide–negative Lactobacillus species or when no lactobacilli were isolated from these cultures. When a very simple flora exists, as it does in young adolescents, the lactobacilli are usually dominant in number, and when only a single isolate is recovered, it is usually a Lactobacillus species. This is notable in view of the close physical proximity of the vaginal introitus to the perineum, with its abundant and complex flora.

Sexual activity, tampon use, childbirth, and various other occurrences in the reproductive life of women are associated with an increasing complexity of the flora, but one must ask how Lactobacillus organisms are able to retain their dominant status for long periods of time. Of equal importance, one must ask how Lactobacillus species occasionally cease to be the dominant type of organism. Do other microorganisms have the ability to emerge with the same dominance as lactobacilli in some women? If so, what circumstances allow for the development of a flora that is not dominated by Lactobacillus species?

Inhibitory proteins have been isolated from strains of Lactobacillus acidophilus [76]. Holmberg and Hallander [62] documented the ability of Streptococcus sanguinus to inhibit the growth of L. acidophilus, Lactobacillus fermentum, and Lactobacillus casei. Phonck [77] and Hillier et al. [75] reported that streptococci may inhibit vaginal lactobacilli. Skarin and Sylvan [65] used L. acidophilus and L. lactus to analyze bacterial inhibition on the predominant organisms cultured from women with BV. Organisms such as G. vaginalis, Mobiluncus mulieris, M. curtisi, Peptostreptococcus assacharolyticus, Peptostreptococcus anaerobius, Bacteroides fragilis, and Peptococcus (now classified as “Peptostreptococcus species”) were inhibited by lactobacilli. In this study, the ability to acidify the medium was better correlated with inhibition than was production of hydrogen peroxide. Skarin and Sylvan [65] found that L. acidophilus produced wider zones of inhibition on plate assays and more lactic acid than did Lactobacillus lactis.

A number of reports have emphasized that production of hydrogen peroxide is the key feature in the antimicrobial action of lactobacilli. Zheng et al. [31] demonstrated that, although hydrogen peroxide had little effect on the quantity of viable N. gonorrhoeae in culture at neutral pH, the peroxide became more effective at acidic pH. Although this result was obtained with a catalase-negative organism, Larsen and White [30] showed a similar result with the catalase-producing C. albicans. Perhaps it is appropriate to conclude that probiosis in vivo is likely to be multifactorial and that synergy between several factors exists.

THE CONCEPT OF PROBIOTICS

Recognition that one microbial species can inhibit a different species of microbe has generated an interest in the exploitation of this phenomenon for the benefit of the well-being of humans or animals [78]. A term related to probiotics is “prebiotics,” which refers to the feeding of substrates that promote the development of a benign microflora. For example, it is known that consumption of fructo-oligosaccharides selects for the development of an intestinal flora dominated by Bifidobacterium species [79].

Bifidobacterium is only one of several microbial genera that have probiotic potential. Most proposed probiotics are gram-positive bacteria, including enterococci, various Lactobacillus species, Clostridium butyricum, and Bacillus species, in addition to Bifidobacterium bifidus [80, 81]. In addition to these gram-positive bacteria, the yeast Saccharomyces cerevisiae has been used as a probiotic. Although promising probiotic approaches to vaginal colonization are under consideration, the advice of Gorbach [82] is as relevant to the vagina as it is to the gut, “The purported benefits for any probiotic must pass the highest standards of scientific scrutiny before the claims can be accepted.”

CONCLUSIONS

Studies have shown that the microbiological environment may supersede the selected virulence of a given bacterial species in the production of disease. The observations that suggest the importance of environmental factors are intertwined with the issue of microbial replication. For disease to occur, exogenous or endogenous bacteria that possess pathogenic prerequisites must attain replicative dominance. Their ability to do so is potentially governed by inhibitory or synergistic interrelationships with other microbes. Although the lactobacilli are key regulators when they are dominant in number, their ability to maintain governance is influenced by other bacterial species within the microflora of the genital tract.

References

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Flora of the Female Genital Tract • CID 2001:32 (15 February) • e77