A Study of Bacterial Flora of Landfall

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Abstract

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. 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. 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 constituent's present.

Keywords: *Bacterial Flora, Landfall, Microbiological Flora, Bacterial Composition*

1. INTRODUCTION

Anaerobic bacteria had been identified previously, it was not until publication of the work of Gorbach et al. and, soon afterward, that of Ohm and Galask, Galask et al. and Hill, that the role of anaerobic bacteria both in maintaining health and in causing disease became more clearly defined. Gorbach et al. 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.

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. 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. 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.

2. REVIEW OF LITERATURE

P. D. Klite, (2010) Intestinal bacterial flora and transit time of three neotropical bat species. J. Bacteriol. 90:375-379. 1965.-Quantitative studies on the intestinal bacterial flora of three neotropical bat species revealed the following average bacterial populations: Molossus major, 104.8 bacteria per intestinal contents; Carollia perspicillata, 103.3; Chilonycteris rubiginosa, 103.9. In comparison, laboratory mice had an average of 109.7 bacteria per intestinal contents. Of 236 bacterial isolates obtained from 60 bats, bacteria of the Klebsiella-Aerobacter-Serratia group were found most frequently, followed by enterococci and Proteus spp. Bacteria of eight other groups were less frequently recovered. A large intestine, cecum, or appendix was absent in all three bat species, and the intestinal length was one-third to one-fifth of that in a mouse of comparable weight. The transit time through the short bat intestine was 15 min. The possible relationship of these unusual anatomical and physiological phenomena to the ability of Histoplasma capsulatum to survive in bat feces is discussed.

Iran J Microbiol, (2010) Despite improvements in modern diagnosis and therapies, hospital acquired infections remain a leading problem of global health systems. Healthcare workers mobile phones is a reservoir for potential pathogens. Despite the high possibility of being contaminated, mobile phones are rarely clean and are often touched during or after examination of patients and handling of specimens without proper hand washing. The main objective of the present study was to isolate, identify different types of bacteria and their antibiotic sensitivity from mobile phones of healthcare workers and non-health-care workers. Samples were collected aseptically by rolling over the exposed surfaces of the mobile phones inoculated on the agar plates and incubated aerobically. After incubation, plates were examined for growth. Bacteria were identified and antibiotic sensitivity was tested as per standard microbiological procedures. In this study a total of 175 samples were examined, out of which 125 samples were from healthcare workers (HCWs), 50 samples were from non-healthcare workers (non-HCWs). Among the mobile phones of HCW‟s from ICUs, Acinetobacter baumannii (36.84%) was the predominant organism isolated followed by methicillin resistant Staphylococcus aureus (MRSA) (21.05%). Predominant organism isolated from HCW‟s in operation theater was MRSA (46.66%). Out of 50 worker‟s non-HCWs mobile phones samples cultured, 23 (46.00%) samples yielded growth of six different types of bacteria.

L El Oufir, (2005) Eight healthy volunteers (four methane excretors and four non-methane excretors) were studied for three, three week periods during which they received a controlled diet alone (control period), and then the same diet with cisapride or loperamide. At the end of each period, mean transit time (MTT) was estimated, an H2 lactulose breath test was performed, and stools were analysed. In the control period, transit time was inversely related to faecal weight, sulphate reducing bacteria counts, concentrations of total short chain fatty acids (SCFAs), propionic and butyric acids, and H2 excreted in breath after lactulose ingestion. Conversely, transit time was positively related to faecal pH and tended to be related to methanogen counts. Methanogenic bacteria counts were inversely related to those of sulphate reducing bacteria and methane excretors had slower MTT and lower sulphate reducing bacteria counts than non-methane excretors. Compared with the control period, MTT was significantly shortened ($p < 0.05$) by cisapride and prolonged ($p < 0.05$) by loperamide (73 (11) hours, 47 (5) hours and 147 (12) hours for control, cisapride, and loperamide, respectively, mean (SD)). Cisapride reduced transit time was associated with (a) a significant rise in faecal weight, sulphate reducing bacteria, concentrations of total SCFAs, and propionic and butyric acids and breath H2 as well as (b) a significant fall in faecal pH and breath CH4 excretion, and (c) a non-significant decrease in the counts of methanogenic bacteria. Reverse relations were roughly the same during the loperamide period including a significant rise in the counts of methanogenic bacteria and a significant fall in those of sulphate reducing bacteria.

3. 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. It has 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.

4. 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 to 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 clearly 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". 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.

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, 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 enough to cause vaginal cornification. Growth of bacteria in the flora of the genital tract is stimulated by estrogen. 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. 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. This is presumably due to the fact that hydrogen peroxide damages cellular membranes unimpeded by the intracellular catalase. 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.

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, yet only a relatively small percentage of these women have symptoms or have a clinically significant vaginal discharge. McCormack et al. identified the presence of G. vaginalisin 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 >107 cfu per gram of vaginal fluid. 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. 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.

5. GBS: PRENATAL 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 intergenus bacterial regulation. Certain clues lie in the demographics of the diseases caused by GBS. Although GBS is a leading cause of prenatal and maternal postpartum septicemia, the incidence of disease is grossly disproportional to that of colonization. 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 prenatal septicemia with material factors, including maternal antibody. Prior to the implementation of protocols for avoidance of GBS disease, the overall incidence of GBS prenatal 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. 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. 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. 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 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.

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. 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. 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 would 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 post pubertal asymptomatic female patients. Quantitative studies have reported that vaginal washings contain ~107 lactobacilli per gram of secretion. The most common Lactobacilllus 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. A longitudinal study has shown variability in terms of species or combinations of species over time.

In an in vitro study, Skarin and Sylwan demonstrated the ability of Lactobacillus species to inhibit the growth of several bacterial species, including G. vaginalis, Mobiluncus species, Peptostreptococcus species, and Bacteroidesspecies. They attributed this inhibition primarily to production of a low pH. Reid et al. suggested an alternate mechanism of control of the bacterial flora by the lactobacilli. They found that cell wall fragments of Lactobacillusspecies 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 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.

Special focus has been placed on the idea that hydrogen peroxide production is a mechanism of bacterial antagonism of the Lactobacillus species. Eschenbach et al. 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. 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 Lactobacillusthat 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. Holmberg and Hallander documented the ability of Streptococcus sanguinus to inhibit the growth of L. acidophilus,

Lactobacillus fermentum, and Lactobacillus casei. Phonck and Hillier et al. reported that streptococci may inhibit vaginal lactobacilli. Skarin and Sylwan 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. curtisii, Peptosteptococcus assacharolyticus, Peptostreptococcus anaerobius, Bacteroides fragilis, and Peptococcus species (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 Sylwan 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. 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 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.

6. CONCLUSION

Bacteria are the smallest and most hardy microbe in the soil and can survive under harsh or changing soil conditions. Bacteria are only 20-30% efficient at recycling carbon, have a high N content (10 to 30% N, 3-10 C:N ratio), a lower C content, and a short life span. There are basically four functional soil bacteria groups including decomposers, mutalists, pathogens and lithotrophs. Decomposer bacteria consume simple sugars and simple carbon compounds, while mutualistic bacteria form partnerships with plants including the nitrogen-fixing bacteria (Rhizobia). Bacteria can also become pathogens to plants and lithotrophic bacteria convert nitrogen, sulfur, or other nutrients for energy and are important in nitrogen cycling and pollution degradation. Actinomycetes are classified as bacteria but are very similar to fungus and decompose recalcitrant (hard to decompose) organic compounds. Bacteria have the ability to adapt to many different soil microenvironments (wet vs. dry, well oxygenated vs. low oxygen). They also have the ability to alter the soil environment to benefit certain plant communities as soil conditions change. Here, we demonstrate that while perturbations impact the whole community, distinct and eco-physiologically informative changes appear on and below the genus level, illustrating the importance of fine-scale

taxonomic identification in assessing the impacts of disturbance on microbial population. A close examination of the preferred ecological niche of pure cultures closely related to clones detected in the Tar River strictly post-disturbance illuminated possible roles for these organisms. Specifically, the post-disturbance detection of clones related to beta-proteo-bacterial methyl-trophs and verrucomicrobial taxa involved in polysaccharide degradation, in tandem with the observed sharp increase in DOC concentrations after the hurricane, suggested enhanced carbon cycling by specific microbial responders in the lower portions of the Tar River. While the geochemical regime post-disturbance (increased DOC, decreased dissolved oxygen) would be consistent with cyanobacterial bloom die-off, we hypothesize that the large freshwater pulse minimized the impacts of preceding bloom events by flushing the river system-consistent with the disappearance of cyanobacterial sequences from the clone libraries of samples collected 2 days after Hurricane Irene-and that allochthonous carbon input from floodplains and tributaries altered riverine conditions and microbial communities.

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