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RESEARCH ARTICLE

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Vaginal microbiota in healthy pregnant women and prenatal screening of group B streptococci (GBS)

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Abstract The microbiota of the lower female genital tract was evaluated from vaginal swabs obtained from 623 healthy pregnant women at gestation periods of 35–40 weeks. Isolated and identified microorganisms were expressed as percentages of total samples. As expected, lactobacilli made up the dominant vaginal microbiota (70%). Enterobacteriaceae, mainly *Escherichia coli*, *Klebsiella* spp. and *Proteus*, were present in 38% of the samples, which might reflect the possible contamination of vaginal tract with rectal microorganisms. *Candida albicans* was present in 10% of healthy pregnant woman assayed. Streptoccocci (*Streptococcus* sp. and *Enterococcus faecalis* with 3% and 4%, respectively) and other gram-positive cocci (*Staphylococcus* sp., 5%), along with other microorgansisms such as *Gardnerella vaginalis* (5%) and *Pseudomonas aeruginosa* (2%) may represent a potential infection risk. *Streptococcus agalactiae* (group B streptococci β-hemolytic, GBS) was detected in 7% of the samples. GBS infection is a leading cause of neonatal morbidity and mortality in the developed world. Furthermore, GBS was often co-isolated with *C. albicans* (54.5%) in the samples. A complete and detailed evaluation of the vaginal biota swab, with particular attention to the presence of potential pathogens such as GBS, is a preventive strategy that can provide useful information to obstetricians and gynecologist in managing the last days of pregnancy and delivery.

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Introduction

The microbiota 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. Such studies are weakened by intrinsic technical limitations. 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 techniques in the processing and culture of specimens. Isolation techniques used before the 1970s resulted in an underestimation of the importance of anaerobic bacteria as major constituents of the normal microbiota of the female genital tract [12].

When a very simple microbiota exists, as it does in young adolescents, lactobacilli are usually dominant, and when only a single isolate is recovered, it is usually a *Lactobacillus* species. 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 one species may be present in an individual. Sexual activity, tampon use, childbirth, and various other occurrences in the reproductive life of women are associated with an increasing complexity of the microbiota. *Streptococcus agalactiae* (group B streptococci β-hemolytic, GBS) are commonly found among the microbiota of the pharynx, gastrointestinal tract, and vagina. Historically, GBS were considered a cause of bovine mastitis (in 1887). Although known in veterinary medicine, GBS were virtually ignored as human pathogen until the late 1930s. In 1935, Lancefield and Hare identified GBS in vaginal cultures from asymptomatic postpartum

women. In 1938, three cases of fatal puerperal sepsis were described; this was the first report of GBS as a human pathogen. In the 1960s, some reports noted the role of GBS in perinatal infections [1]. Subsequently, an increasing number of studies in the obstetric and pediatric literature documented a growing concern with neonatal sepsis and/or meningitis due to GBS. GBS remain the predominant cause of invasive bacterial disease in the neonatal period. Studies in the USA demonstrated a risk of 1–2 cases per 1,000 live births. Incidence rates for different European countries vary between 0.24 and 1.26 per 1,000 live births [19]. Due to modern neonatal intensive care, fatality rates have declined during the last few decades but still range from 4 to 6% [17]. A considerable number of children, especially among meningitis survivors, suffer long-term sequelae. Prematurity is one of the risk factors for GBS disease, and morbidity and mortality rates are higher among these patients.

Therefore, with the overall improvements in survival rates, the clinical community has turned its attention to research on disease prevention [4]. The consensus guidelines have urged institutions and caregivers to adopt a prevention strategy for GBS disease and recommended the following approaches: (1) a screening-based strategy, and (2) a risk-based strategy, as discussed by Schuchat [18] (Table 1). Preventive strategies based only on maternal risk factors would prevent less than half the cases of neonatal diseases [8]. Therefore main goal of this study was to assess the benefits of a complete and detailed evaluation of the vaginal biota swab, with particular attention to the presence of potential pathogens such as GBS.

Materials and methods

A total of 623 vaginal swabs from 623 healthy pregnant woman were collected from January 1998 to December 2000. Samples were taken by gynecologists and obstetricians in their consulting room. Vaginal swabs were transported to the laboratory on Amies medium (IASA, Rubí, Spain). Patient ages ranged from 18 to 45 years (mean = 29 years), and with a gestation period of 35–40 weeks (mean = 38 weeks). Microbial colonies were obtained from 603 samples (96.8%); 20 samples (3.2%) yielded no growth.

Samples were inoculated on tryptone soy agar (Merck, Darmstadt, Germany), blood agar (Merck, Darmstadt, Germany) and Todd-Hewitt broth with 25 mg gentamicin/l and 25 mg nalidixic acid/l (Oxoid, Hampshire, England). Cultures were incubated at 37 °C for 4 days. Axenic cultures obtained were identified following standard methods [2,16]. Serological typing of streptococci was carried out using an agglutination test (Menarini Diagnostics).

Results and discussion

A list of the vaginal aerobic (and/or anaerobic facultative) microbiota in 623 healthy pregnant women is shown in Table 2 as the frequency of positive results with respect to the total number of samples. The results are compared with those obtained previously [11]. The vagina of the adult female generally is weakly acidic and contains significant amounts of *L. acidophilus* and

Table 1. Strategies for the prevention of early onset group B streptococcal (GBS) diseases [18]

Approach	Prenatal component	Intrapartum component
Screening-based strategy	Obtain vaginal and rectal swab specimens at 35-37 weeks gestation and inoculate in selective broth media; assess history of previous infant(s) with GBS disease and presence of GBS bacteriuria during current pregnancy	Offer intrapartum penicillin (ampicillin, clindamycin or erythromycin) to women with GBS identified on prenatal cultures, previous infant(s) with GBS disease, GBS bacteriuria during this pregnancy
Risk-based strategy	Determine history of previous infant(s) with GBS disease and presence of GBS bacteriuria during current pregnancy	Offer intrapartum penicillin (ampicillin, clindamycin or erythromycin) to women with previous infant(s) with GBS disease, GBS bacteriuria during this pregnancy

Table 2. A comparison of the prevalence of the aerobic (and/or facultative anaerobic) isolates in vaginal microbiota studies. Percentage indicates the frequency of positive results with respect to the total number (603) of positive samples

Aerobic (and/or anaerobic facultative) isolates	Number of isolates[a]	Prevalence in vaginal biota (%)[a]	Prevalence in vaginal biota (%)[b]
Lactobacilli	436	70	60
Diphteroids	60	10	40
Staphylococcus aureus or sp.	31	5	2
Staphylococcus epidermidis	44	7	50
Streptococcus agalactiae	44	7	15
Sreptococcus sp. (nonhemolytic)	19	3	20
Enterococcus faecalis	25	4	28
Escherichia coli	168	27	18
Klebsiella spp./*Enterobacter* spp.	6	1	10
Proteus spp.	63	10	5
Pseudomonas sp.	12	2	0.1
Gardnerella vaginalis	30	5	–
Candida albicans	63	10	–

[a]This work
[b]Results of [11]

L. fermentus. Lactobacilli seem to control the bacterial microbiota in this microhabitat, primarily by the production of acids that decrease pH, which inhibits the growth of microorganisms such as *G. vaginalis*, *Peptostreptococcus* sp. and *Bacteroides* sp. It has been suggested that lactobacilli form a continuous biofilm which blocks the attachment of other bacteria, including pathogenic ones [14]. Special attention has been given to the idea that hydrogen peroxide production is a mechanism of bacterial antagonism of *Lactobacillus* species. Hillier et al. [9] showed a significant correlation between the absence of hydrogen-peroxide-producing lactobacilli and vaginal colonization by *G. vaginalis, Bacteroides* sp., *Peptostreptococcus* sp. and *Mycoplasma hominis*.

Within colonized tissues, 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 microbiota– that is, the relative dominance, in numbers, in individual asymptomatic patients. The mere presence of a potentially pathogenic species does not necessarily cause disease as defined in terms of symptoms. For example, *Candida albicans* may be present without the typical symptoms of yeast vaginitis (see Table 2; *C. albicans* is present in 10% of healthy pregnant woman included in the assays). Yeast carriage varies among populations and increases both after puberty and during pregnancy, which suggests an important role of the host physiology [12]. Estrogen promotes the growth of bacteria in the genital tract [7]. The prevalence of both *Lactobacillus* species and yeast in different populations indicates that the times of the highest prevalence of *Lactobacillus* species (during the reproductive years and, especially, during pregnancy) coincide with those of the highest prevalence of *Candida* species. Enterobacteriaceae, mainly *Escherichia coli*, *Klebsiella* spp. and *Proteus* spp., were present in 38% of the samples; this result may reflect possible contamination of the vaginal tract by rectal microorganisms. The colonization with Enterobacteriaceae species is also a predisposing factor for urinary tract infection in women [15]. Anaerobic microbiota were also detected (*Bacteroides* spp. was present in 12% of the samples). Streptococci, other gram-positive cocci, and others potential pathogens, such as *G. vaginalis*, *P. aeruginosa* and *C. albicans*, indicate a potential risk of infection to the mother.

Anywhere from 5% to 35% of women are asymptomatic carriers of GBS in the genital tract [3, 10, 20]. In our study, *Streptococcus agalactiae* (GBS) was detected in 7% of the samples (see Table 2). Note that there are significant geographic variation in the percentage of women colonized with GBS [20]. Up to 60% of the colonized women will carry the organism intermittently. Indeed, colonization of the vagina may actually reflect also a contamination from the rectum, with the gastrointestinal tract being the principal reservoir of this organism [6]. At birth, one in two infants who are born to colonized mothers will themselves be colonized on the skin or mucosal surfaces. Approximately 98% of colonized newborns have no symptoms, but 1–2% develop early-onset disease, in which sepsis, pneumonia, or meningitis occur during the first week of life. The vast majority of early-onset infections are evident within hours of birth [18]. GBS are frequently co-isolated with *C. albicans*; 54.5% of samples positive for GBS were also positive to *C. albicans*. This results is in accordance with those of other studies [5,13].

In 223 of 623 women examined (36%), potentially pathogenic microorganisms were isolated that could disrupt the normal progression of the last weeks of pregnancy or even delivery. Thus, we conclude that, during the last weeks of pregnancy, the identification of microbial populations present in vaginal swabs can provide detailed information about the vaginal microbiota at this time. This may help to reduce, at low cost, any possible infectious disease problems at the neonatal, delivery, and postpartum stages.

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