Bacterial Interference by Group B Streptococci with Aerobic and Anaerobic Genital Tract Streptococci and Nonstreptococcal Aerobic Bacteria of the Female Genital Tract

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Abstract : *Target groups composed of 10 isolates of each of the following: S. viridans, non-hemolytic streptococci not group B or D, group A streptococci, group B streptococci, peptostreptococci, coagulase-negative staphylococci, S. aureus, G. vaginalis, E. coli, 9 enterococci, 9 group C or G streptococci; 7 lactobacilli and 7 diphtheroids were tested for inhibition by a test panel of a group of 10 or 41 group B streptococci (GBS). The GBS did not inhibit the growth of E. coli, coagulase negative staphylococci or S. aureus. They uniformly inhibited the groups A, B, C and G streptococci, lactobacilli and G. vaginalis. One isolate of diphtheroids was inhibited by 37 of 41 GBS; the other six isolates were uniformly inhibited. Variable inhibition was observed with the viridans streptococci, non-group B or D streptococci and enterococci; however, inhibition or noninhibition was uniform for a given isolate against the entire group B streptococcal test panel. The 23 isolates of the group B streptococci from neonates or adults with septicemia, did not differ from isolates from patients with only local disease in their ability to inhibit other species tested. The group B streptococci uniformly inhibited the aerobic lactobacilli, diphtheroids and G. vaginalis but had no effect on the coagulase-negative staphylococci, S. aureus or E. coli. These studies suggest that group B streptococci may be significant regulators of the female genital tract bacterial flora. (Thai J Obstet Gynaecol 1991;3:.45-52.)*

Key words : bacterial interference, group B streptococci, female genital tract

When quantitative and qualitative bacteriological studies are performed on normal cervical and vaginal vault bacterial flora, the dominant aerobic groups of bacteria are the lactobacilli, diphtheroids, staphylococci and streptococci[1-6]. The dominant anaerobic groups are composed of the

Gram positive bacilli (which include lactobacilli, eubacteria and clostridia), peptostreptococci and the Bacteroidaceae. Group B streptococci (GBS) constitute a potentially important subgroup within the streptococci. Not only is it a frequent constituent of normal genital tract bacterial flora, but it is also the most frequent monomicrobial cause of disease in both parturitional gravida and neonate[7-10].

This paper analyzes the ability of 23 isolates of GBS obtained from septicemic patients and 18 isolates of GBS from alternate sites to inhibit other constituents of the female genital tract bacterial flora.

Materials and Methods

Strains

Initially, an inhibitor test panel of 41 isolates of group B streptococci was examined. These inhibitor isolates were tested against target cultures of other streptococci and aerobic bacteria common in the vaginal flora. Twenty-three of the 41 group B streptococci were obtained from blood cultures. Of the 23 isolates from septicemic patients, 15 were from infants with early onset and late onset group B streptococcal disease. The non-septicemic isolates were isolates from random genital tract or wound cultures. Because of the uniformity of inhibition observed within the entire 41 isolates of group B streptococci, the inhibitor test panel was reduced to 10 isolates of group B streptococci for certain target strains. Of these 10 isolates, 5 isolates were derived from cases of early onset neonatal septicemia, and 5 were incidental isolates from genital tract cultures. Internal controls were run with each test.

The target cultures included 10 viridans streptococci, 10 group B streptococci, 10 non-hemolytic streptococci not group B or D, 10 group A streptococci, 9 group C or G streptococci, 10 peptostreptococci, 9 enterococci, 10 coagulase-negative staphylococci, 10 S. aureus, 10 E. coli, 7 lactobacilli, 7 diphtheroids, and 10 G. vaginalis.

Media

Tripticase Soy Agar (TSA, Baltimore Biological Laboratories, Baltimore, Maryland) was used for both layers in the overlay procedure. Organisms were maintained on Tripticase Soy Agar supplemented with 5% sheep blood (BAP, Scott Laboratories, Fiskeville, Rhode Island).

Overlay assay

Overlays performed were a modification of the technique described by Fredericq[11] and modified by Crowe, Sanders and Longley[12] and Murray and Rosenblatt[13]. Each strain of group B streptococcus was inoculated onto a one square centimeter area of a 15 ml TSA plate. The assays were performed in duplicate. Four strains per plate were tested. Each plate was rerun in a separate test under code.

The organisms were incubated for 18-24 hours in 10% carbon dioxide at 35° C. They were overlaid with 7.5 ml molten TSA which was allowed to solidify. The target strain was then inoculated onto the top of the fresh TSA in the following manner.

A 0.4 OD at 450 nm of the target strain was made in physiological saline. A 1:10 dilution was made in saline, and a 2 ml quantity of this was inoculated onto the freshly overlaid plate. The excess was siphoned off, and the plates were incubated for 24 hours at 35° C in 10% carbon dioxide. After incubation, the assays were examined for inhibition or no inhibition of growth of the target strain.

Results

Streptococcus viridans

Seven isolates were inhibited by all group B streptococci examined in 101 tests (Table 1). Three isolates were not inhibited by group B streptococcal test panel. When inhibition was observed, the phenomena was uniform for the entire 10 or 41 group B streptococcal panel.

Non-hemolytic streptococci, not group B or D

Of the 10 target isolates of the non-hemolytic streptococci, not group B or D, 9 isolates were inhibited while 1 isolate was not. Comparable inhibition was produced by all of group B streptococci tested.

Enterococci

Of 9 isolates of enterococci, only 1 isolate was inhibited by group B streptococci while the remaining 8 were not (Talbe 1). The results were uniform for both inhibition and noninhibition for the entire group B streptococcal panel, however, the degree of inhibition did vary from isolate to isolate. When 5 strains of the enterococci were used as the inhibitor strain, all 10 isolates of group B streptococci isolates tested in 50 challenge experiments were inhibited.

Group A streptococci

For the 10 target isolates of group A streptococci, all were inhibited by group B streptococci in the 193 challenge experiments (Table 1).

Group B streptococci

For the 10 target isolates of group B streptococci, inhibition was total in 193 challenge experiments (Table 1).

Group C or G streptococci

For the 9 challenge isolates of group C (7) or group G (2) streptococci, inhibition was total in the 183 challenge experiments (Table 1).

Peptostreptococci

Of the 10 peptostreptococci, 7 challenge isolates were inhibited completely. Three of the 10 were uninhibi-

Table 1 Inhibition of target bacteria by group B streptococci isolates

Taret bacteria	Number of strains tested	Number of observations	Number of strains / Number of observations	
			Inhibited	Non-inhibited
Viridans streptococci	10	193	7/101	3/92
Non-hemolytic streptococci-not group B or D	10	193	9/183	1/10
Enterococci group A	9	276	1/41	8/235
Streptococci group B	10	193	10/193	0
Streptococci group C (7) or G (2)	10	193	10/193	0
Streptococci Pepto-streptococci	9	183	10/183	0
Coagulase-negative staphylococci	10	193	7/132	3/61
Staphylococcus aureus	10	193	0	10/193
Escherichia coli	10	193	0	10/193
Lactobacilli	7	163	7/163	0
Diphtheroids	7	194	7/190*	0/4
Gardnerella vaginalis	10	19	10/193	0

*4 of group B streptococci in the panel of one isolate were not inhibitory.

ted. The target isolates exhibited a uniform pattern of inhibition or noninhibition by group B streptococci. The presence or absence of inhibition for the individual species of the peptostreptococci is listed in Table 2.

Coagulase-negative staphylococci

None of the 10 target isolates tested in 193 individual challenge experiments was inhibited by group B streptococci (Table 1). When five

strains of coagulase-negative staphylococci were used as the inhibitors, all 10 group B streptococcal isolates tested in 50 challenge experiments were inhibited.

Staphylococcus aureus

None of the 10 target isolates tested in 193 individual challenge experiments was inhibited (Table 1). When 5 strains of S. aureus were used as the inhibitor cultures, none of 10 group B streptococci isolates tested in 50 challenge experiments was inhibited.

Escherichia coli

None of the 10 isolates tested in 255 individual challenge experiments was inhibited by group B streptococci (Table 1).

Lactobacilli

All 7 target isolates tested in 163 individual challenge experiments were inhibited (Table 1).

Diphtheroids

All 7 target isolates tested individually were inhibited. One isolate had a variable pattern of inhibition such that of the 194 individual experiments, 190 showed inhibition (Table 1).

Gardnerella vaginalis

All 10 target isolates tested in 193 individual challenge experiments were inhibited (Table 1).

Table 2 In vitro bacterial interference by the group B stereptococci on strains of peptostreptococci

Strain of peptostreptococci	Number of test strains of group B streptococci	Percentage of inhibition
P. tetradius	10	100
P. tetradius	10	0
P. anaerobius	41	100
P. anaerobius	10	100
P. anaerobius	10	100
P. micros	41	100
P. micros	10	0
P. asaccharolyticus	41	0
p. asaccharolyticus	10	100
P. asaccharolyticus	10	100

Discussion

The initial concept of bacterial interference eminated from the observations of Pasteur and Joubert[14]. They noted that some urine cultures of B. anthracis would die if they became contaminated with other bacteria.

The mechanisms by which a bacterial species maintains its ecological niche are varied. Inhibitory bacterial products include a wide range of substances: low molecular weight antibiotics, metabolic products, hydrogen peroxide, lytic agents, enzymes, bacteriocins and bacteriophages[15,16].

The ultimate question for group B streptococci is how does a normal constituent of the bacterial flora of the female genital tract become the causative agent of septicemia in mother and neonate[7-9]. As demonstrated in these studies, group B streptococci has the ability *in vitro* to effect bacterial interference. If these mechanisms function *in vivo,* group B streptococci would possess the capability of defending their microbiological niche not only against other group B streptococci but also against other aerobic beta-hemolytic strains (group A, C and G streptococci). This ability appears to be uniform. The uniformity of this effect may be the result of the genetic interrelationship between hemolytic activity and bacterial interference. Brock et al[17] found that, by categorizing strains of S. zymogenes in terms of their hemolytic character, they could demonstrate uniform bacterial interference which was mediated by bacteriocins. There was no variation in the ability to inhibit bacterial replication between septicemic and nonsepticemic isolates of group B streptococci. Group B streptococcal potential for *in vitro* governance over the non-hemolytic streptococci not group B or D gamma hemolysis is significant. The majority of the isolates (95%) exhibit complete inhibition; their impact on the viridans streptococci and peptostreptococci is significantly less. Group B streptococci inhibited other common non-streptococcal Gram-positive aerobic bacteria and G.vaginalis but had no impact on the staphylococci and E. coli.

The importance of the lactobacilli may be more as regulators of the enterococci than major regulators of the female genital tract bacterial florad (FGTBF). De Klerk and Coetzec[18] studied bacterial inhibition by the lactobacilli. Using the supernatants concentrated by ammonium sulfate precipitators, they were able to demonstrate an antibacterial activity which was primarily restricted to certain members of the family Lactobacteriaceae. A significant number of enterococci were inhibited. The antibiotic-like supernatants did not impact on the Enterobacteriaceae or staphylococci. Holmberg and Hallander[19] documented the ability of S. sanguis to inhibit L. acidophilus, L. fermentum and L. casei. Pohonch[20], among others, has similarly demonstrated the ability of the streptococci to inhibit the vaginal lactobacilli.

Statistically, coagulase-negative staphylococci are more frequently present in the FGTBF than S. aureus[1-4,23]. Both coagulase-negative and -positive staphylococci have the ability to inhibit bacterial replication of other bacteria *in vitro*. Prior work by Dajani et al[21] demonstrated the ability of S. aureus to elaborate a bactericidal substance which inhibited groups A, D and G streptococci. Observations derived from clinical disease in which both staphylococci and beta-hemolytic streptococci can be concomitantly isolated from skin lesions have questioned whether the staphylococci invade sites previously infected with a beta-hemolytic organism or whether there is a significant coupling between the 2 groups of Gram-positive bacteria[22-24]. The ability of a given strain of the Enterobacteriaceae to inhibit other members of the family has been well documented[16]. The predominance of a strain of E. coli as the principal Enterobacteriaceae in FGTBF may be selected by the Bacteroidaceae. Murray and Rosenblatt[13] demonstrated that B. melaninogenicus, B. fragilis and B. oralis, while possessing significant ability to inhibit E. cloacae, E. aerogenes, Klebsiella species and S. marcescens, were ineffective against E. coli and M. morganii. The Bacteroidaceae did have moderate inhibitor activity for coagulase-negtive staphylococci but almost no activity against S. aureus. In their paper, Fusobacteria and L. fermentum had little inhibitory effect on either Gram-negative or Gram-positive bacteria. Interspecies governance among the Enterobacteriaceae is probably mediated by bacteriocins, however, the predominace of E. coli and P. mirabilis may be a direct function of their resistance to bacterial inhibition by the Bacteroidaceae and selected peptostreptococci.

Demostration of the *in vitro* ability of group B streptococci to inhibit all group A, B, C and G streptococci, lactobacilli, diphtheroids and G. vaginalis as well as most non-hemolytic streptococci not group B or D and viridans streptococci, infers that this organism may be a significant regulator of the female genital tract bacterial flora should those phenomena function *in vivo*.

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