

Bacterial Isolates From Patients With Preterm Labor With and Without Preterm Rupture of the Fetal Membranes

H. Mikamo,* Y. Sato, Y. Hayasaki, K. Kawazoe, Y.X. Hua, and T. Tamaya

Department of Obstetrics and Gynecology, School of Medicine, Gifu University, Gifu, Japan

ABSTRACT

Objective: The aim of this study is to describe the bacterial flora of women in preterm labor with or without premature rupture of membranes.

Methods: Retrospective studies of 239 patients with preterm labor were performed.

Results: One hundred and twenty-three of 239 patients with preterm labor (51.5%) had bacterial vaginosis. Seventy of the 239 patients with preterm labor (29.3 %) developed premature rupture of the membranes (preterm PROM). Of the 70 patients with preterm PROM, 51 (72.9%) had bacterial vaginosis. Therefore, 51 of the 123 patients with bacterial vaginosis (41.5%) developed preterm PROM. An increased number of organisms detected from the vaginal discharge in patients with preterm labor was associated with preterm PROM by Cochran-Armitage test. An increased number of organisms detected from the vaginal discharge in patients with preterm labor complicated with bacterial vaginosis was significantly associated with preterm PROM by Cochran-Armitage test.

Conclusions: In preterm labor, the number of different species detected in the vagina provide sensitive and specific prediction of preterm PROM in patients with preterm labor. *Infect. Dis. Obstet. Gynecol.* 7:190–194, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS

preterm labor; preterm PROM; prediction; bacterial vaginosis

Preterm birth is the leading perinatal complication.^{1–3} Since intrauterine infection from organisms found in the genital tract has been implicated in both the etiology and adverse sequelae of preterm premature rupture of the membranes (preterm PROM),^{3–6} culturing the vagina has not been an essential part of clinical management. Although there are many studies that evaluate such cultures in the prediction of intrauterine pregnancies, they have been focused on the identification of bacteria.^{4–6} The aim of this study is to investigate the significance of bacterial culture in predicting preterm PROM in patients with preterm labor.

MATERIALS AND METHODS

Subjects

From 1992 to 1996, we sampled the vaginal discharge of 239 patients with preterm labor that were referred to the hospital of the School of Medicine of Gifu University for further assessment. The patients were Japanese married women of middle-class socioeconomic status who had had no sexual intercourse in the past 10 days, had no history of sexual transmitted diseases, and had taken no antibiotic therapy during pregnancy. Patients with chlamydia, trichomoniasis, or candidiasis infection were excluded from the study. Consent to the

*Correspondence to: Hiroshige Mikamo, MD, PhD, Department of Obstetrics and Gynecology, School of Medicine, Gifu University, 40, Tsukasa-machi, Gifu-city, Gifu 500-8705, Japan. E-mail: mikamo@cc.gifu-u.ac.jp

study was obtained from the patients and the committee of the institution.

Diagnosis of Bacterial Vaginosis

Bacterial vaginosis was diagnosed with intact membranes in 239 patients by the presence of the following four criteria:⁷⁻¹⁰ thin and homogenous vaginal discharge, vaginal pH above 4.5, positive amine "whiff" test, and the presence of clue cells in wet-mount preparations of vaginal discharges.

Sampling Method

Vaginal discharge was collected from the posterior vaginal fornix with a sterilized cotton wool swab, which gathers 0.05 mL of sample per swab.

Culture Methods

Immediately after collection, the swab specimen (0.05 mL) was suspended in 5 mL of the anaerobic diluent for culture.⁸⁻¹¹ The composition of the anaerobic buffer was as follows: 4 g of KH_2PO_4 , 6 g of Na_2HPO_4 , 1 g of L-cysteine-HCl-H₂O, 1 g of Tween 80 (Sigma, St. Louis, MO), and agar, and 1,000 mL of distilled water with a pH of 7.2. All the components were mixed in a solution and heated at 80°C for 30 minutes. All tubes were sterilized in an autoclave at 115°C for 20 minutes. A 9-mL aliquot of the buffer was transferred to each test tube, air in the tube was immediately replaced with CO₂, and the tube was sealed with a butyl rubber stopper.

After samples were suspended in the buffer, the tubes were resealed under a continuous stream of CO₂ gas of commercial grade to drive air out. Exposure of samples to atmospheric oxygen was restricted to 5 minutes or less. Incubation commenced immediately after a sample was suspended in a solution. A small aliquot for the subsequent culture was aspirated with a syringe via a butyl rubber stopper.

For quantitative bacterial determination, serial dilutions were made with the anaerobic buffer. The time lag from the specimen collection to the quantitative determination was within 1 hour.

Staphylococcus-selective agar (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) and MacConkey agar (Becton Dickinson and Co., Cockeysville, MD) were used for aerobic culture. Sheep blood agar (Becton Dickinson) and chocolate agar (Becton Dickinson) were used for CO₂ culture.

As for *Gardnerella vaginalis*, human blood-bilayer-Tween (HBT) medium, developed by Totten et al.,¹² was used for CO₂ culture. It consists of a bottom layer of Columbia colistin-nalidixic acid agar (Becton Dickinson) supplemented with 1% Proteose Peptone no. 3 (Difco Laboratories, Detroit, MI), amphotericin B (2.0 µg/mL), and 0.0075% Tween 80 (Becton Dickinson) and a top layer of the same composition with 5% human blood added.

For anaerobic culture, *Brucella* HK (hemin, vitamin K1) RS (rabbit, sheep) blood agar (Kyokuto Pharmaceutical Co. Ltd., Tokyo, Japan) was used as a nonselective medium; -phenylethylalcohol (PEA) *Brucella* HK blood agar (Kyokuto), paromomycin/vancomycin (PV) *Brucella* HK blood agar (Kyokuto) and *Bacteroides* bile esculin (BBE) agar (Kyokuto) were used as selective media. Sabouraud dextrose agar (Becton Dickinson) was used for fungal culture.

Aerobic culture was performed at 37°C for 2 days, CO₂ culture in 5% CO₂ in air at 37°C for 3 days, anaerobic culture in a GasPak Pouch at 37°C for 7 days, and fungal culture at 37°C for 7 days.

Bacterial Identification

Each colony with an individual appearance on an agar plate was subcultured three times on a nonselective medium to obtain a pure culture for bacterial identification.⁸⁻¹¹ Among aerobes, gram-positive and catalase-positive cocci were identified by Api STAPH identification system (bioMerieux SA, Marcy l'Etoile, France). Gram-positive and catalase-negative cocci were identified by Api STREP identification system (bioMerieux SA), and gram-negative rods were identified by Enterotube II (Becton Dickinson) or Oxi/Ferm Tube II (Becton Dickinson). *Haemophilus* species and *G. vaginalis* were identified by Rap ID NH (Innovative Diagnostic System, Inc., Atlanta, GA).

Anaerobic bacteria were identified by Rap ID ANA System II (Innovative Diagnostic System). For identification of *Mobiluncus* spp., colonial characteristics were observed and a Gram stain was performed. Gram-negative or gram-variable curved rods were identified by Rap ID ANA System II. Nitrate reduction and hippuric acid hydrolysis tests were used for identification at the subspecies level. Fungi were identified by Api AUXANOGRAM (bioMerieux SA).

TABLE 1. The number of different species detected in all patients with preterm labor and those who developed preterm PROM

Number of organisms detected	Preterm labor (%)	Preterm PROM (%)
0	0 (0)	0 (0)
1	4 (1.7)	0 (0)
2	16 (6.7)	0 (0)
3	34 (14.2)	3 (4.3)
4	92 (38.5)	26 (37.1)
5	58 (24.3)	22 (31.4)
6	24 (10.0)	11 (15.7)
7	9 (3.8)	6 (8.6)
8	2 (0.8)	2 (2.9)
Total	239	70

Statistical Analysis

All results were analyzed using the Cochran-Armitage test.

RESULTS

One hundred and twenty-three of 239 patients with preterm labor (51.5%) had bacterial vaginosis. Seventy in 239 patients with preterm labor (29.3%) developed preterm PROM. Of the 70 patients with preterm PROM, 51 (72.9%) had bacterial vaginosis. Therefore, 51 in 123 patients with preterm labor complicated with bacterial vaginosis (41.5%) reduced preterm PROM. The cultures from the lower genital tract in patients with preterm labor were positive in all specimens.

The number of different species detected from the vaginal discharge in all 239 patients with preterm labor and the 70 patients with preterm PROM following preterm labor are shown in Table 1. An increase in the number of organisms detected from the vaginal discharge in patients with preterm labor was significantly associated with preterm PROM by Cochran-Armitage test ($P = 2.6 \times 10^{-8}$).

Table 2 shows the number of different species detected from the vaginal discharge in the 123 patients with bacterial vaginosis and the 51 patients with both preterm PROM following preterm labor and bacterial vaginosis. An increase in the number of organisms detected in the vaginal discharge of patients with preterm labor complicated with bacterial vaginosis was significantly associated with preterm PROM by Cochran-Armitage test ($P = 0.0030$).

The organisms isolated from the vaginal discharge in patients with preterm labor are listed in

TABLE 2. The number of different species detected in all patients with preterm labor and bacterial vaginosis (BV) and those with preterm PROM

Number of organisms detected	Preterm labor and BV (%)	Preterm PROM and BV (%)
0	0 (0)	0 (0)
1	0 (0)	0 (0)
2	1 (0.8)	0 (0)
3	4 (3.3)	1 (2.0)
4	48 (39.0)	16 (31.4)
5	40 (32.5)	17 (33.3)
6	20 (16.3)	9 (17.6)
7	8 (6.5)	6 (11.2)
8	2 (1.6)	2 (4.0)
Total	123	51

Table 3. The dominant organisms detected were *Staphylococcus haemolyticus*, *Streptococcus agalactiae*, *Escherichia coli*, *Fusobacterium nucleatum*, and *Prevotella bivia*. However, the particular organisms for preterm labor were not specified.

The organisms isolated from the vaginal discharge in patients with preterm PROM following preterm labor are listed in Table 4. The dominant organisms detected were *S. haemolyticus*, *S. agalactiae*, *E. coli*, *F. nucleatum*, and *P. bivia*. However, the particular organisms for preterm PROM were not specified.

DISCUSSION

Recent studies have reported the relation between preterm PROM and bacterial vaginosis;^{3-5,11} this condition is characterized by depletion of vaginal *Lactobacilli* with increased *G. vaginalis* in association with such organisms as *Bacteroides* species, *Prevotella* species, *Peptostreptococcus* species, and *Mycoplasma* species.⁷⁻¹¹ This study suggests that the increased number of organisms detected from the vaginal discharge might be predictive of the prevalence of preterm labor and preterm PROM. This means that the number of organisms detected in the vagina provides sensitive and specific prediction of intrauterine infection with aerobic or anaerobic organisms in pregnancies with preterm PROM. Most studies associated with vaginal flora in pregnancies have found that it is the relative quantity of organisms.^{3-5,11} However, great efforts are needed in the quantitative assays. Therefore, we gave attention to the numbers of different species detected.

In most cases with positive amniotic fluid cul-

TABLE 3. Organisms detected from the vaginal discharge of patients with preterm labor

Bacteria	Number of strains	% of total (1019)
Aerobe	558	54.8
Gram-positive cocci	290	28.5
<i>Staphylococcus aureus</i>	16	1.6
<i>Staphylococcus epidermidis</i>	49	4.8
<i>Staphylococcus haemolyticus</i>	21	2.1
<i>Staphylococcus hominis</i>	3	0.3
<i>Staphylococcus simulans</i>	5	0.5
<i>Staphylococcus xylosus</i>	2	0.2
<i>Staphylococcus</i> spp.	5	0.5
<i>Streptococcus agalactiae</i>	70	6.9
<i>Streptococcus bovis</i>	3	0.3
<i>Streptococcus oralis</i>	7	0.7
<i>Streptococcus pyogenes</i>	8	0.8
<i>Streptococcus sanguis</i>	5	0.5
<i>Streptococcus</i> spp.	5	0.5
<i>Enterococcus avium</i>	4	0.4
<i>Enterococcus faecalis</i>	54	5.3
<i>Enterococcus faecium</i>	20	2
<i>Gemella morbillorum</i>	5	0.5
Gram-positive cocci	8	0.8
Gram-positive bacilli	107	10.5
<i>Gardnerella vaginalis</i>	100	9.8
Gram-positive bacilli	7	0.7
Gram-negative bacilli	161	15.8
<i>Burkholderia cepacia</i>	8	0.8
<i>Enterobacter cloacae</i>	15	1.5
<i>Escherichia coli</i>	88	8.6
<i>Klebsiella pneumoniae</i>	24	2.4
<i>Proteus mirabilis</i>	7	0.7
<i>Proteus vulgaris</i>	7	0.7
<i>Pseudomonas aeruginosa</i>	9	0.9
Gram-negative bacilli	3	0.3
Anaerobe	461	45.2
Gram-positive cocci	133	13.1
<i>Peptostreptococcus anaerobius</i>	46	4.5
<i>Peptostreptococcus asaccharolyticus</i>	5	0.5
<i>Peptostreptococcus magnus</i>	42	4.1
<i>Peptostreptococcus micros</i>	27	2.6
<i>Peptostreptococcus prevotii</i>	5	0.5
<i>Peptostreptococcus tetradius</i>	3	0.3
<i>Peptostreptococcus</i> spp.	5	0.5
Gram-positive bacilli	129	12.7
<i>Mobiluncus curtisii</i> subsp. <i>curtisii</i>	48	4.7
<i>Mobiluncus curtisii</i> subsp. <i>holmesii</i>	36	3.5
<i>Mobiluncus mulieris</i>	45	4.4
Gram-negative bacilli	199	19.5
<i>Bacteroides distasonis</i>	5	0.5
<i>Bacteroides fragilis</i>	31	3
<i>Bacteroides thetaiotaomicron</i>	15	1.5
<i>Bacteroides uniformis</i>	2	0.2
<i>Bacteroides</i> spp.	5	0.5
<i>Fusobacterium nucleatum</i>	31	3
<i>Prevotella bivia</i>	61	6
<i>Prevotella buccae</i>	2	0.2
<i>Prevotella disiens</i>	25	2.5
<i>Prevotella intermedia</i>	5	0.5
<i>Prevotella melaninogenica</i>	5	0.5
<i>Prevotella oralis</i>	5	0.5
<i>Prevotella</i> spp.	4	0.4
Gram-negative bacilli	4	0.4
Total	1019	

TABLE 4. Organisms detected from the patients with preterm PROM

Bacteria	Number of strains	% of total (801)
Aerobe	448	55.9
Gram-positive cocci	232	29
<i>Staphylococcus aureus</i>	12	1.5
<i>Staphylococcus epidermidis</i>	39	4.9
<i>Staphylococcus haemolyticus</i>	17	2.1
<i>Staphylococcus hominis</i>	2	0.2
<i>Staphylococcus simulans</i>	4	0.5
<i>Staphylococcus xylosus</i>	2	0.2
<i>Staphylococcus</i> spp.	4	0.5
<i>Streptococcus agalactiae</i>	58	7.2
<i>Streptococcus bovis</i>	3	0.3
<i>Streptococcus oralis</i>	5	0.6
<i>Streptococcus pyogenes</i>	7	0.9
<i>Streptococcus sanguis</i>	3	0.4
<i>Streptococcus</i> spp.	3	0.4
<i>Enterococcus avium</i>	3	0.4
<i>Enterococcus faecalis</i>	44	5.5
<i>Enterococcus faecium</i>	16	2.0
<i>Gemella morbillorum</i>	4	0.5
Gram-positive cocci	6	0.7
Gram-positive bacilli	85	10.6
<i>Gardnerella vaginalis</i>	80	10.0
Gram-positive bacilli	5	0.6
Gram-negative bacilli	131	16.4
<i>Burkholderia cepacia</i>	6	0.7
<i>Enterobacter cloacae</i>	13	1.6
<i>Escherichia coli</i>	70	8.7
<i>Klebsiella pneumoniae</i>	21	2.6
<i>Proteus mirabilis</i>	5	0.6
<i>Proteus vulgaris</i>	6	0.7
<i>Pseudomonas aeruginosa</i>	8	1.0
Gram-negative bacilli	2	0.2
Anaerobe	107	13.4
Gram-positive cocci	107	13.4
<i>Peptostreptococcus anaerobius</i>	37	4.6
<i>Peptostreptococcus asaccharolyticus</i>	4	0.5
<i>Peptostreptococcus magnus</i>	34	4.2
<i>Peptostreptococcus micros</i>	22	2.7
<i>Peptostreptococcus prevotii</i>	4	0.5
<i>Peptostreptococcus tetradius</i>	3	0.4
<i>Peptostreptococcus</i> spp.	3	0.4
Gram-positive bacilli	106	13.2
<i>Mobiluncus curtisii</i> subsp. <i>curtisii</i>	42	5.2
<i>Mobiluncus curtisii</i> subsp. <i>holmesii</i>	27	3.4
<i>Mobiluncus mulieris</i>	37	4.6
Gram-negative bacilli	140	17.5
<i>Bacteroides distasonis</i>	4	0.5
<i>Bacteroides fragilis</i>	27	3.4
<i>Bacteroides thetaiotaomicron</i>	12	1.5
<i>Bacteroides uniformis</i>	2	0.2
<i>Bacteroides</i> spp.	4	0.5
<i>Fusobacterium nucleatum</i>	1	0.1
<i>Prevotella bivia</i>	51	6.4
<i>Prevotella buccae</i>	2	0.2
<i>Prevotella disiens</i>	19	2.4
<i>Prevotella intermedia</i>	4	0.5
<i>Prevotella melaninogenica</i>	4	0.5
<i>Prevotella oralis</i>	4	0.5
<i>Prevotella</i> spp.	3	0.4
Gram-negative bacilli	3	0.4
Total	801	100

tures, the same organisms were recovered from vaginal swabs.¹¹ This finding is compatible with the hypothesis that the lower genital tract is the source of the offending organisms in intrauterine infection associated with preterm labor.

Most bacteria responsible for ascending infections are derived from the indigenous genital flora.¹¹ Anaerobic bacteria are of major importance.¹¹ Many of these anaerobes are considered potentially pathogenic, as they have been frequently isolated in patients with genital infections.^{5-6,11} Genera such as the anaerobic cocci, *Bacteroides* spp., *Prevotella* spp., and *Fusobacterium* spp. have been implicated in maternal or neonatal infections accompanying premature labor or PROM.^{5-6,11}

In a matched case-control study of 54 consecutive women in preterm labor, Gravett et al.¹³ found a significant association between bacterial vaginosis and preterm labor (43% of study patients versus 14% of control patients, $P = 0.02$, relative risk ratio of 3.80). Martius et al.¹⁴ performed a similar study at the same center using Gram stain to diagnose bacterial vaginosis and had similar findings. Bacterial vaginosis was significantly associated with preterm labor (odds ratio of 2.3), as was the presence of *Chlamydia trachomatis* and the absence of *Lactobacilli* species. In this study, 70% of women with preterm labor had PROM.

The results of our study add to the existing evidence that bacterial vaginosis is an independent risk factor for preterm birth and suggest that the timing of this infection in gestation significantly affects this risk. It remains to be shown whether this association is causative or whether bacterial vaginosis is associated with some as yet unidentified factor that initiates preterm labor and birth. Although bacterial vaginosis is commonly found in pregnant women, preterm birth usually does not occur in these pregnancies. In addition, it remains unclear whether treatment of pregnant women with bacterial vaginosis decreases their risk of preterm birth. A large, well-controlled, randomized trial of treatment for bacterial vaginosis in pregnancy is needed to answer this question.

REFERENCES

1. Rush RW, Kierse MJ, Howat P. The contribution of preterm labour to perinatal mortality. *BMJ* 1976;2:965-968.
2. Morrison J. Preterm birth: a puzzle worth solving. *Obstet Gynecol* 1990;76:5-12.
3. James D. Preterm prelabour rupture of membranes. *Arch Dis Child* 1991;66:812-815.
4. Romero R, Mazor M, Wu YK, et al. Infection in the pathogenesis of preterm labor. *Semin Perinatol* 1988;12:262-279.
5. Kurki T, Sivonen A, Renkonen O, Savia E, Ylikorkala O. Bacterial vaginosis in early pregnancy and pregnancy outcome. *Obstet Gynecol* 1992;80:173-177.
6. Hay PE, Lamont RF, Taylor-Robinson D, Morgan DJ, Ison C, Pearson J. Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. *BMJ* 1994;308:295-298.
7. Spiegel CA, Davick P, Totten PA, et al. *Gardnerella vaginalis* and anaerobic bacteria in the etiology of bacterial (nonspecific) vaginosis. *Scand J Infect Dis Suppl* 1983;40:41-46.
8. Mikamo H, Izumi K, Ito K, et al. Study on treatment of bacterial vaginosis with oral administration of metronidazole or cefdinir. *Chemotherapy* 1994;40:362-368.
9. Mikamo H, Kawazoe K, Izumi K, et al. Bacteriological epidemiology and treatment of bacterial vaginosis. *Chemotherapy* 1996;42:78-84.
10. Mikamo H, Kawazoe K, Izumi K, Watanabe K, Ueno K, Tamaya T. Comparative study on vaginal or oral treatment of bacterial vaginosis. *Chemotherapy* 1997;43:60-68.
11. Mikamo H, Kawazoe K, Sato Y, Izumi K, Tamaya T. Studies on the clinical implications of anaerobes, especially *Prevotella bivia*, in obstetrics and gynecology. *J Infect Chemother* 1998;4:177-187.
12. Totten PA, Amsel R, Hale J, Piot P, Holmes KK. Selective differential human blood bilayer media for isolation of *Gardnerella (Haemophilus) vaginalis*. *J Clin Microbiol* 1982;15:141-147.
13. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm labor associated with subclinical amniotic fluid infection with bacterial vaginosis. *Obstet Gynecol* 1986;67:229-237.
14. Martius J, Krohn MA, Hillier SL, Stamm WE, Holmes KK, Eschenbach DA. Relationship of vaginal *Lactobacillus* species, cervical *Chlamydia trachomatis*, and bacterial vaginosis to preterm birth. *Obstet Gynecol* 1988;76:89-95.