JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1997, p. 2899–2903 Vol. 35, No. 11
0095-1137/97/$04.00+0

Vaginal Carriage of Enterotoxigenic *Bacteroides fragilis* in Pregnant Women

PIOTR LESZCZYNSKI,[1] ALEX VAN BELKUM,[2]* HANNA PITUCH,[1] HENRI VERBRUGH,[2] AND FELICJA MEISEL-MIKOLAJCZYK[1]

Department of Clinical Bacteriology, Warsaw Medical Academy, 02-004 Warsaw, Poland,[1] and Department of Medical Microbiology & Infectious Diseases, University Hospital Rotterdam, 3015 GD Rotterdam, The Netherlands[2]

Received 21 February 1997/Returned for modification 13 June 1997/Accepted 5 August 1997

***Bacteroides fragilis* is an anaerobic bacterial species that is involved in gynecological infections and pathology. The incidence of vaginal carriage is largely unknown, and in order to study this, 120 pregnant women attending a general hospital for delivery were examined. Cultures were positive for eight of these women (6.6%). Interestingly, potential clonal relatedness could be demonstrated among several of the nonenterotoxigenic *B. fragilis* strains. Among the strains, only one produced metalloprotease enterotoxin. The presence of the gene for the metalloprotease, giving rise to the pathogenic effect on cultured eukaryotic HT29/C1 cells, was confirmed by a newly designed specific PCR assay. The enterotoxigenic *B. fragilis* (ETBF) strain was analyzed with the help of arbitrarily primed PCR (AP-PCR) and PCR-mediated ribotyping. The ETBF strain was shown to be genetically different compared to several other strains obtained from diverse sources. Our data indicate a relatively high vaginal *B. fragilis* carriage rate among pregnant women in Warsaw, Poland. Although neither ETBF nor *B. fragilis* colonization presented a clinical problem, the possible genetic relatedness among the colonizing *B. fragilis* strains indicates the need for additional research in the field of ETBF transmission and molecular epidemiology.**

Bacteroides fragilis is an important and frequently isolated non-spore-forming anaerobic etiological agent of infections, and research on its pathogenicity in the human genital tract is still in progress (40). Representatives of the *B. fragilis* group may sometimes be isolated from the vagina, uterine cervix, fornix, urethra, and external genitals of women (14, 26). However, *B. fragilis* does not belong to the normal flora of the vagina (10, 11, 36). It is known that bacteria from the *B. fragilis* group are agents of infection of the abdominal cavity organs, pelvis, and the genitourinary system (6, 10, 14, 36). *B. fragilis* is causally related to pelvic inflammatory disease (18, 37) and may be an etiological agent of ovary, Fallopian tube, and Bartholin's gland abscesses (2, 4). It may participate in local and multiorgan infections after various forms of gynecological and obstetrical surgery (13, 33). Furthermore, *B. fragilis* is associated with cases of septic abortion (35), intrauterine infection (2, 5, 42), postpartum endometritis (22), and sepsis (33).

There are also reports on severe neonatal infections, such as sepsis (5) and meningitis (45, 47). These latter papers present data from 1969 to 1989, and it was discovered that 3% of all cases of neonatal meningitis have a *B. fragilis* etiology. Krohn et al. (22) have observed a correlation between the occurrence of deliveries before 34 weeks of gestation and the concentration of *B. fragilis* rods in vaginal discharge. Germain et al. (15) correlated the presence of *B. fragilis* strains with the low birth weight of neonates born preterm. This suggests that the intrauterine development of the fetus is influenced by this microorganism. As a matter of fact, in studies with animal models it appeared that administration of *B. fragilis* endotoxin resulted in newborns with low birth weights (1).

Recently, a new virulence factor of *B. fragilis* was described. A protein with an estimated molecular mass of 20 kDa displaying enterotoxigenic properties was identified (32). At first, the pathogenicity of so-called enterotoxigenic *B. fragilis* (ETBF) was connected with the gastrointestinal tract only (34, 43). More recently, isolates were also reported to be recovered from clinical specimens other than the gastrointestinal tract (19, 28–31). It seems that strains possessing the novel enterotoxigenic property may play a significant role in sepsis (19), although this is still controversial (33). The complete 1,191-nucleotide-long coding sequence of the metalloprotease enterotoxin was recently determined (21).

An epidemiological aspect of *B. fragilis* infection is that it can be acquired in the hospital (16, 29), although infections caused by this microorganism are often defined as endogenous. Elhag and Senthilselvan (12) claim that *B. fragilis* may live freely in the hospital environment and infect patients. Myers et al. (34) suggest that tap water contaminated with municipal sewage is a possible source of infection. Also, Domingues et al. (9) claim that *B. fragilis* infections may have an external source. However, little is still known about the human carriage of ETBF and its clinical relevance.

We have set out to determine the *B. fragilis* carriage rate in a large group of pregnant women attending a gynecology department in the Medical Academy Hospital in Warsaw, Poland. In order to assess the medical relevance of enterotoxin production among the strains, all isolates of *B. fragilis* were assayed by monitoring cythopathologic effects on cell cultures and by a newly developed PCR test for detection of the metalloprotease toxin gene. Another aim of the present study was to genetically compare strains of *B. fragilis* isolated from the women's genitourinary tracts.

MATERIALS AND METHODS

Study group. A total of 120 women (age range, 18 to 40 years) were admitted for delivery (at term) to the Second Clinic of Obstetrics and Gynecology (Medical Academy Hospital, Warsaw) and were examined for *B. fragilis* carriage in the vagina. Samples were taken during the first stage of labor. The sampling lasted from January to May 1991 (23). All women were in good health and remained in

* Corresponding author. Mailing address: Department of Medical Microbiology & Infectious Diseases, University Hospital Rotterdam, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. Phone: 31-10-4635813. Fax: 31-10-4633875. E-mail: vanbelkum@bacl.azr.nl.

the hospital for 4 to 5 days. All children were born without infectious complications.

Clinical material and reference strains. Vaginal swabs were taken with commercially available cotton tips, and the material was stored in the accompanying transport medium (Biomed, Cracow, Poland). Cultivation of the swabs for *B. fragilis* was performed on brucella bile esculin selective medium containing gentamicin within a period of 12 h after clinical sampling. The cultures were incubated under anaerobic conditions by means of the Gasbox system (bioMerieux, Marcy l'Etoile, France) at 37°C. The following *B. fragilis* isolates were included in the study as reference strains: strains from the genitourinary systems of females with miscellaneous pathologies isolated in our laboratory (strains 22, 44, 48, 49, 54, and 60) (see Table 1 and Fig. 1), one strain isolated in 1977 from a patient suffering from peritonitis (strain 210), international reference strains (strains IPLE 323 and NCTC 11295), and two strains (strains BE17 and BE61) from the private collection of the Free University Amsterdam (Amsterdam, The Netherlands). Two additional strains (strains 101 and BRA1), isolated from the vaginas of pregnant women, were included in the study. The biochemical identification of these latter two strains was aberrant but, although doubtful, indicative of *B. fragilis.*

Strain identification. Isolates were identified to the species level on the basis of the biochemical tests available in the Rapid 32A assay (bioMerieux). Susceptibility to metronidazole was determined by the agar dilution method according to the recommendations of the National Committee for Clinical Laboratory Standards. Production of the enterotoxin was determined phenotypically on an HT29/C1 cell line by the method described elsewhere (48). PCR-mediated detection of the toxin gene is described separately below.

DNA isolation. The *B. fragilis* strains were cultured for 24 h in liquid brain heart infusion medium. The culture was centrifuged at 4,000 × *g* for 20 min, and bacterial pellets were processed for DNA extraction and purification with guanidine isothiocyanate and Celite affinity chromatography (3).

AP-PCR and PCR ribotyping. PCR-mediated typing of the strains was performed essentially as described previously (27). Primers ERIC1 and ERIC2 (enterobacterial repetitive intergenic consensus) were used separately and in combination (46). One additional primer, primer AP7, was used to confirm the findings obtained with the ERIC primers. The sequence of AP7 primer was 5′-GTGGATGCGA-3′. Variations in the lengths of the ribosomal intergenic spacer regions were analyzed by use of the SP1 primer (specific for the 16S rRNA gene) and the SP2 primer (specific for the 23S rRNA gene) by protocols described previously (7, 27). Forty-cycle PCR programs were applied. For arbitrarily primed PCR (AP-PCR) the following PCR program was used: 1 min at 94°C, 2 min at 25°C, and 2 min at 74°C. For the ribotyping the PCR machines were programmed as follows: 1 min at 94°C, 1 min at 55°C, and 1 min 74°C, with SP1 and SP2 primers. After 1% agarose gel electrophoresis in TBE (Tris-borate-EDTA) buffer, the banding patterns were visualized on a UV transilluminator. Any different banding pattern, even those differing by a single DNA fragment, was marked with a separate capital letter.

PCR-mediated detection of the enterotoxin gene. Several potential PCR primers were designed on the basis of the nucleotide sequence described previously (32). The sequences of these primers and the corresponding positions in the partial metalloprotease gene sequence (32) are as follows: forward 1 primer (5′-GAGCCGAAGACGGTGTATGTGATTTGT-3′), positions 1 to 27; forward 2 primer (5′-GAGAGAGAATTGGAAGTACTA-3′), positions 30 to 50; reverse 1 primer (5′-TGCTCAGCGCCCAGTATATGACCTAGT-3′), positions 401 to 429; reverse 2 primer (5′-GGGATAAGTATCCAGTAAATGTAGCAT-3′), positions 455 to 481. None of the primers showed significant overlap with sequences available through the GenBank database, and the order of the nucleotides corresponded to those obtained from data for the full sequence (21). The forward and reverse primers were applied in all possible pairwise combinations in order to establish the sensitivities of the tests. By using serially diluted DNA from ETBF NCTC 11295, it was shown that the forward 1 primer plus the reverse 1 primer provided the most sensitive combination. Use of these primers allows the forward 2 primer to be used as a confirmatory DNA probe. The combined PCR-probe assay was studied for species specificity (see Table 2 for a survey of all bacterial species and strains used). The assays thus developed were performed in incubation volumes of 25 μl containing approximately 50 ng of DNA and 0.08 U of *Taq* polymerase. The PCR program consisted of the following combinations of steps: 4 min at 94°C plus 40 cycles of 1 min at 94°C, 1 min at 52°C, and 1 min at 74°C. Amplicons were detected by straightforward electrophoresis, the nature of the amplicons was once again confirmed by probe hybridization.

RESULTS

A total of 120 swabs taken from the female genital tract were examined, and eight strains of *B. fragilis* were isolated (incidence, 6.6%). Only one of these vaginal isolates (isolate 131) produced enterotoxin in the cytotoxicity assay with the HT29/C1 cell line. Furthermore, a positive cytotoxicity result was obtained with the reference strains (ATCC 43858, ATCC 43859, 86-5443-2-2, 3-101-5, and NCTC 11295) and with five gastrointestinal isolates from our laboratory (isolates W1, W2, 32, 154, and 76D). The data provided by the newly developed PCR test were completely concordant with the observed cytotoxicity (Table 1). Furthermore, the DNA in the combined PCR probe test did not cross-react with DNA isolated from any of the species mentioned in Table 2. In the exceptional case that an amplicon of a different size was synthesized, probing confirmed the aberrant nature of these probably aspecifically amplified products. All strains exhibited susceptibility to metronidazole (MICs, less than 2 g/liter). Only strain NCTC 11295 exhibited growth in the presence of metronidazole at a concentration of 32 g/liter.

All strains of *B. fragilis* isolated from the female genital tract were compared by genetic methods. Two strains from the *B. fragilis* group (strains BRA1 and 101) presented biochemical characteristics which were similar to those of genuine *B. fragilis* species. These two aberrant strains were also included in the genetic study. Random primers ERIC1 and ERIC2 were used for typing by AP-PCR, but amplification with these primers separately did not allow for the differentiation of any of the *B. fragilis* strains; identical banding patterns were obtained upon electrophoresis. Only when a combination of the primers ERIC1-ERIC2 was used could the strains be differentiated, although most strains could still not be separated on a genetic basis (Table 1). Apparently, PCR with ERIC primers is quite unrevealing for *B. fragilis*. No major differences between DNA banding patterns were observed; only limited diversity could be visualized (types A1 through A4; Fig. 1 and Table 1). Strains 101 and BRA1, the ones not belonging to the *B. fragilis* species according to their biochemical tests, had very distinct banding patterns and were classified patterns B and C, respectively. AP-PCR with primer AP7 more clearly resolved the *B. fragilis* strains. Among the gynecological isolates, five distinct genotypes could be defined. Ribotyping by PCR did not provide additional resolution, but it did confirm the classification based on AP7-mediated AP-PCR. The 14 strains from gynecological sites were subdivided into six different overall PCR types (by AP-PCR plus PCR ribotyping; Table 1).

The *B. fragilis* strains could be divided into seven different overall DNA types. A total of 5 of 14 strains isolated from female genital tract were classified as overall type I. Four of these strains were isolated from the vaginas of pregnant women, and one was isolated from the vagina of a nonpregnant woman with clinical symptoms of vaginitis. Four strains were classified as type V, which was also established for reference strain IPLE 323. Two strains were isolated from pregnant women, and the other two were isolated from nonpregnant women, one of whom had vaginitis and the other one of whom present with an abscess of the uterine adnexa. One strain isolated from a pregnant woman belonged to type IV and was identical to our earlier isolate 210. Two other strains isolated from pus (from a patient with an abscess of the uterine adnexa and a patient with vaginitis) were classified as type VII. Strains belonging to type VII were not isolated from pregnant women. One strain was enterotoxigenic and presented unique DNA fingerprints (Table 1).

DISCUSSION

To our knowledge only a few studies have determined the incidence of vaginal *B. fragilis* carriage in prepartal and nonpregnant women (24, 26). In the study by Levinson et al. (25) no *B. fragilis* was detected, whereas Lindner et al. (26) encountered unspecified *Bacteroides* spp. in 4% of healthy women. The latter microbiologists defined similar carriage rates in pregnant women, but in cases of cervicitis the frequency of *Bacteroides* spp. detection rose to 27 to 28%. However, the

TABLE 1. Genetic and phenotypic analysis of *B. fragilis* strains of different origins

Strain group and no.	Type by the following test:			Overall DNA type[b]	Metronidazole susceptibility[c]	Presence of enterotoxin	
	ERIC1-ERIC2 PCR[a]	AP7 PCR	Ribosomal spacer			In tissue culture	By PCR
Strains isolated from vaginas of pregnant women							
99	A	A	A	I	S	−	−
107	A	A	A	I	S	−	−
131	A	C	A	II	S	+	+
227	A	A	A	I	S	−	−
229	A	D	C	IV	S	−	−
241	A1	E	C	V	S	−	−
297	A	A	A	I	S	−	−
301	A2	E	C	V	S	−	−
Strains involved in diverse gynecological pathologies[d]							
22	A	A	C	VI	S	−	−
44	A	A	C	VI	S	−	−
48	A	E	C	V	S	−	−
49	A3	G	A	VII	S	−	−
54	A	E	C	V	S	−	−
60	A	A	A	I	S	−	−
Other strains							
210	A3	D	ND[e]	IV	S	−	−
IPLE323	A	E	C	V	S	−	−
B17	A	A	A	I	S	−	−
B61	A4	G	C	VII	S	−	−
NCTC11295	A	H	D	VIII	R	+	+
Strains from *B. fragilis* group							
BRA1	C	F	ND	IX	S	−	−
101	B	B	B	III	S	−	−

[a] The subtypes obtained by ERIC1-ERIC2 AP-PCR indicated by a numeral; patterns A1 through A4 differ by a single band of low-intensity staining.
[b] The overall DNA type is a summary of data obtained by AP-PCR and ribotyping by PCR in which the individual typing characteristics have been combined into a single digit.
[c] S, susceptible; R, resistant.
[d] Pathologies varied from colpitis and Bartholin's glands abscess to wounds to vaginitis.
[e] ND, not determined.

epidemiological aspects of *B. fragilis* transmission are still largely unknown. *B. fragilis* strains isolated from the genital tracts of healthy pregnant women and strains isolated from patients with various gynecological and obstetrical pathologies were examined in the present study. Many investigators have paid attention to phenotypic differentiation among strains (12). Our intention was to compare strains isolated from pregnant women attending a large medical center by the use of AP-PCR and ribotyping by PCR.

Studies describing the molecular subtyping of *B. fragilis* are sparse: the most recent review on the subject, dating back to 1992, proclaims that besides serological assays, exploration of the newer molecular biology-based techniques is mandatory (44). The recent availability of various monoclonal antibodies recognizing capsular antigens shows interisolate degrees of variability too extensive to be helpful in typing studies (38). More recently, the first DNA typing studies were presented. However, on the basis of those studies, in which intergenic ribosomal spacer analysis (7, 27), ribotyping combined with insertion element mapping (41), or AP-PCR (8, 27, 41) was used, no guidelines for molecular typing could be deduced. Moreover, the AP-PCR assays used by other investigators were performed with primers different from those that we used. For these reasons we suggest that at present the application of at least two different typing methods in combination generates epidemiologically valid molecular data, as confirmed by an earlier study from our laboratories (27). On the basis of banding patterns, we have divided all strains into 10 types. The use of the combination of the ERIC1 and ERIC2 primers has allowed us to confirm that strains 101 and BRA1 do not belong to the *B. fragilis* species. This is based on the fact that the DNA banding patterns were different to a very large extent. The largest group having homologous banding patterns was group I, which consisted of five strains. These strains were similar enough to be recognized as a single clonal type. Two strains (strains 241 and 301), isolated from healthy pregnant women, belong to type V. Strains of this type were also isolated from a patient with an adnexal abscess and a patient with vaginitis. Particularly interesting was the isolation of ETBF strains from the female genital tract. To our knowledge the present report highlights the first examples of colonization of the female genital tract by ETBF. One of 14 genital tract isolates presented with enterotoxigenic activity against the HT29/C1 cell line. A clear genetic difference was seen after application of the various AP-PCR primers.

Development of a PCR assay for the detection of the enterotoxin gene was straightforward; the primer probe combination deduced from the primary sequence of the metalloprotease gene appeared to be highly specific. In order to more specifically define the reliability of our new PCR test, a number of additional ETBF strains were assayed (data not shown). Reference strains ATCC 43858, ATCC 43859, 86-5443-2-2,

TABLE 2. Survey of bacterial species and isolates used for specificity analysis of the PCR probe test for the enterotoxin gene

Species or isolate		
Acinetobacter anitratus	*Acinetobacter baumannii*	*Acinetobacter Iwoffi*
Bacillus circulans	*Bacteroides merdae*	*Bacteroides ovatus*
Bacteroides thetaiotomicron	*Bacteroides uniformis*	*Bacteroides ureolyticus*
Bacteroides vulgatus	*Burkholderia cepacia*	*Citrobacter diversus*
Citrobacter freundii	*Clostridium difficile* toxin positive	*Clostridium difficile* toxin negative
Clostridium perfringens	*Clostridium sporogenes*	*Enterobacter cloacae*
Enterococcus casselliflavus	*Enterococcus durans*	*Enterococcus faecalis*
Enterococcus faecium	*Enterococcus gallinarum*	*Escherichia coli*
Flavimonas oryzihabitans	*Fusobacterium mortiferum*	*Fusobacterium necrophorum*
Fusobacterium nucleatum	*Fusobacterium varium*	*Haemophilus influenzae*
Haemophilus parainfluenzae	*Hafnia alvei*	*Klebsiella oxytoca*
Klebsiella pneumoniae	*Lactobacillus* spp.	*Listeria monocytogenes*
Moraxella catarrhalis	*Morganella morganii*	*Peptococcus asaccharolyticus*
Peptostreptococcus anaerobius	*Prevotella bivia*	*Prevotella buccae*
Prevotella intermedia	*Proteus mirabilis*	*Proteus vulgaris*
Providencia rettgeri	*Pseudomonas aeruginosa*	*Pseudomonas fluorescens*
Pseudomonas putrefaciens	*Rhodococcus equi*	*Salmonella enteritidis*
Salmonella typhimurium	*Serratia liquefaciens*	*Serratia marcescens*
Staphylococcus aureus	*Staphylococcus epidermidis*	*Staphylococcus haemolyticus*
Staphylococcus hominis	*Staphylococcus saprophyticus*	*Staphylococcus simulans*
Staphylococcus warneri	*Stenotrophomonas maltophilia*	*Streptococcus bovis*
Streptococcus spp. group A (β-hemolytic)	*Streptococcus* spp. group B (β-hemolytic)	*Streptococcus* spp. group C (β-hemolytic)
Streptococcus spp. group F (β-hemolytic)	*Streptococcus* spp. group G (β-hemolytic)	*Streptococcus pneumoniae*
Veillonella parvula	Viridans group streptocci	

W1, W2, 32, 154, and 76D, all of which were sensitive to metronidazole, clearly tested positive by the cytotoxicity test and PCR. Interestingly, a similar PCR was recently described by Japanese researchers (20). They used an upstream primer that was similar to ours but chose a downstream primer from a different location. Although those investigators did not report the results of specificity testing, they suggested that the presence of the enterotoxin gene predisposes a strain to successful infection of blood. Apparently, the enterotoxin functions as a virulence factor that contributes to penetration into vessels or prolonged survival in the circulation, an issue that deserves additional study. In light of recent reports on the high carriage rates of ETBF among Italian adults and children (39) and our current data, the role of the enterotoxin in various other disease processes may be a relevant issue for further study. Genetic studies of the metalloprotease locus provided intriguing additional data. Preliminary analysis of neighboring open reading frames revealed the hidden presence of another gene which potentially encodes a cobra cytotoxin-type protein molecule (21). The activity of this protein is still unidentified, but it might be interesting and potentially clinically relevant to study the expression of the gene and the toxic activity of its product as well.

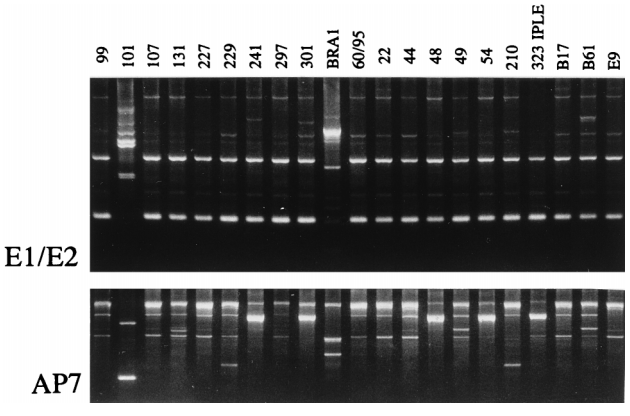

FIG. 1. AP-PCR of ETBF strains from pregnant women and women suffering from diverse gynecological syndromes. The strain names are indicated above the lanes and correspond to those given in Table 1. Only in the case of strain NCTC 11295 was an aberrant identification given (laboratory number E9). The results obtained with the ERIC1-ERIC2 combination (E1/E2) or the AP7 primer are presented.

Because reference strain NCTC 11295 is known to be resistant to metronidazole (17) and is also an enterotoxin producer (42a), we have examined all strains in order to obtain an answer to the question of whether there is a general relationship between enterotoxin production and resistance to metronidazole. We were not able to demonstrate such a relationship. All Polish *B. fragilis* strains including the single ETBF strain were susceptible to 2 g of metronidazole per liter.

The present results lead us to state that strains of ETBF isolated from the female genital tract are apparently polyclonal, although the number of strains tested is too limited to allow us to draw general conclusions. Among the nonenterotoxigenic strains, however, clonal relationships can be observed; overall, types I and V occur relatively frequently. More definite characterization of genetic variation among strains of *B. fragilis* must be made available. This can be performed by studying well-defined strains of *B. fragilis* by both molecular and phenotypic typing protocols. Comparison of epidemiological and typing data may ultimately define an optimal strategy for the molecular typing of this particular anaerobic bacterial species. Finally, determination of the clinical relevance of both *B. fragilis* and ETBF carriage in the vagina requires additional studies.

ACKNOWLEDGMENTS

These studies were performed as part of the Polish-Dutch Research and Development Agreement and were supported in part by grant C/13035/96.

The control strain IPLE 323 was obtained from the Institut Pasteur (Lille, France), whereas strains E2, BE17, and BE61 were kindly provided by F. Namavar (Free University Amsterdam, Amsterdam, The Netherlands). Several *Clostridium difficile* control strains were pro-

vided by S. W. Rothman (Walter Reed Institute, Washington, D.C.). M. Sebald (Institut Pasteus, Paris, France) is gratefully acknowledged for providing *Prevotella bivia*, *Clostridium sporogenes*, and several ETBF strains (ATCC 43858, ATCC 43859, 86-5443-2-2, and 3-101-5).

REFERENCES

1. **Beckman, I., F. Meisel-Mikolajczyk, P. Leszczynski, M. Brooijmans, and H. C. S. Wallenburg.** 1993. Endotoxin induced fetal growth retardation in the pregnant guinea pig. Am. J. Obstet. Gynecol. **168:**714–718.
2. **Bergan, T.** 1983. Anaerobic bacteria as cause of infections in female genital organs. Scand. J. Gastroenterol. **85:**37–47.
3. **Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
4. **Brook, I.** 1989. Aerobic and anaerobic microbiology of Bartholin's abscess. Surg. Gynecol. Obstet. **169:**32–34.
5. **Brook, I.** 1990. Bacteremia due to anaerobic bacteria in newborns. J. Perinatol. **10:**351–356.
6. **Brook, I., and M. L. Myhal.** 1991. Adherence of *Bacteroides fragilis* group species. Infect. Immun. **59:**742–744.
7. **Cartwright, C. P., F. Stock, S. E. Beekmann, E. C. Williams, and V. J. Gill.** 1995. PCR amplification of rRNA intergenic spacer regions as a method for epidemiologic typing of *Clostridium difficile*. J. Clin. Microbiol. **33:**184–187.
8. **Claros, M. C., D. M. Citron, S. H. Gerardo, and E. J. C. Goldstein.** 1996. Comparison of German and US *B. fragilis* and *Bacteroides thetaiotaomicron* clinical isolates using PCR fingerprinting, p. 230. *In* Abstracts of the Anaerobe Society of the Americas Congress on Anaerobic Bacteria and Anaerobic Infections.
9. **Domingues, R. M., M. de Oliveira, R. Hirata, Jr., A. F. de Andrade, and M. C. Ferriera.** 1995. *B. fragilis*: an exogenous pathogen. Int. J. Med. Microbiol. Virol. Parasitol. Infect. Dis. **282:**296–302.
10. **Duerden, B. I., and B. S. Drasar.** 1991. Anaerobes in human disease. Edward Arnold Division of Hodder & Stoughton, London, United Kingdom.
11. **Easmon, C. S. F.** 1992. The bacterial flora of the female genital tract. J. Obstet. Gynecol. **12:**S2–S4.
12. **Elhag, K. M., and A. Senthilselvan.** 1988. A serogrouping scheme for the study of the epidemiology of *B. fragilis*. J. Med. Microbiol. **27:**199–205.
13. **Failla, D. M., and J. R. Dalovisio.** 1995. *B. fragilis* arthritis following cesarean section. Clin. Infect. Dis. **21:**1341–1342.
14. **Finegold, S. M.** 1995. Anaerobic infections in humans: an overview. Anaerobes **1:**3–9.
15. **Germain, M., M. Krohn, S. Hillier, and D. Eschenbach.** 1994. Genital flora in pregnancy and its association with intrauterine growth retardation. J. Clin. Microbiol. **32:**2162–2168.
16. **Gorbach, S. L., and J. G. Bartlett.** 1974. Anaerobic infections. N. Engl. J. Med. **290:**1177–1184.
17. **Ingham, H. R., S. Eaton, C. W. Venables, and P. C. Adams.** 1978. *B. fragilis* resistant to metronidazole after long-term therapy. Lancet **8057:**214.
18. **Kasper, D. L., D. A. Eschenbach, M. E. Hayes, and K. K. Holmes.** 1978. Quantitative determination of the serum antibody response to the capsular polysaccharide of *B. fragilis* subspecies fragilis in women with pelvic inflammatory disease. J. Infect. Dis. **138:**74–80.
19. **Kato, N., R. Jotwani, H. Kato, K. Tanaka-Bandoh, K. Watanabe, and K. Ueno.** 1995. Isolation of enterotoxigenic *B. fragilis* from extraintestinal sites by cell culture assay. Clin. Infect. Dis. **20:**S141.
20. **Kato, N., H. Kato, K. Watanabe, and K. Ueno.** 1996. Association of enterotoxigenic *Bacteroides fragilis* with bacteremia. Clin. Infect. Dis. **23:**S83–S86.
21. **Kling, J. J., R. L. Wright, J. S. Moncrief, and T. D. Wilkins.** 1997. Cloning and characterization of the gene for the metalloprotease enterotoxin of *Bacteroides fragilis*. FEMS Microbiol. Lett. **146:**279–284.
22. **Krohn, M. A., S. L. Hillier, M. L. Lee, L. K. Rabe, D. A. Eschenbach.** 1991. Vaginal *Bacteroides* species are associated with an increased rate of preterm delivery among women in preterm labor. J. Infect. Dis. **164:**88–93.
23. **Lang, N., and H. Werner.** 1980. Diagnosis of pure and mixed anaerobic infections in obstetrics and gynaecology. Infection **8:**S201–S202.
24. **Leszczynski, P., F. Meisel-Mikolajczyk, M. Dworczynska, L. Cwyl-Zembrzuska, and L. Marianowski.** 1995. Occurrence of *B. fragilis* strains in full term and post term pregnancies. Ginekol. Pol. **66:**324–329. (In Polish.)
25. **Levinson, M., L. Corman, E. Carrington, and D. Kaye.** 1977. Quantitative microflora of the vagina. Am. J. Obstet. Gynecol. **127:**80–85.
26. **Lindner, J. G., F. H. Plantema, and J. A. Hoogkamp-Korstanje.** 1978. Quantitative studies of the vaginal flora of healthy women and of obstetric and gynaecological patients. J. Med. Microbiol. **11:**233–241.
27. **Martirosian, G., A. Van Belkum, W. Van Leeuwen, F. Meisel-Mikolajczyk, and H. Verbrugh.** 1996. PCR ribotyping and AP PCR for the comparison of enterotoxigenic *B. fragilis* strains from two Polish university hospitals. Clin. Microbiol. Infect. **3:**102–108.
28. **Meisel-Mikolajczyk, F.** 1995. *B. fragilis* strains producing enterotoxin, new factor of pathogenicity. Post. Microbiol. **37:**197–209. (In Polish.)
29. **Meisel-Mikolajczyk, F., and A. Dworczynski.** 1973. Chemical composition of endotoxins of *Eggerthella convexa* (*B. fragilis*) strains. Bull. Acad. Pol. Sci. Biol. **21:**193–197.
30. **Meisel-Mikolajczyk, F., G. S. Rouyan, C. Henry, and M. Sebald.** 1995. Enterotoxigenic *B. fragilis* strains from France and Poland, abstr. 3.16, p. 62. *In* Abstracts of the World Congress on Anaerobic Bacteria and Infections.
31. **Meisel-Mikolajczyk, F., E. Zawidzka, K. Solarz, P. Leszczynski, A. Rokosz, W. Klos, G. Martirosian, A. van Belkum, and G. Rouyan.** 1996. A comparative study of enterotoxin producing *B. fragilis* strains isolated in Poland, p. 220. *In* Abstracts of Anaerobe Society of the Americas Congress on Anaerobic Bacteria and Anaerobic Infections.
32. **Moncrief, J. S., R. Obiso, Jr., L. A. Barroso, J. J. Kling, R. L. Wright, R. L. van Tassel, D. M. Lyerly, and T. D. Wilkins.** 1995. The enterotoxin of *Bacteroides fragilis* is a metalloprotease. Infect. Immun. **63:**175–181.
33. **Mundy, L., and C. Sears.** 1996. Detection of toxin production by *B. fragilis*: assay development and screening of extraintestinal clinical isolates. Clin. Infect. Dis. **23:**269–276.
34. **Myers, L. L., D. S. Shoop, L. L. Stackhouse, F. S. Newman, R. J. Flaherty, G. W. Letson, and R. B. Sack.** 1987. Isolation of enterotoxigenic *Bacteroides fragilis* from humans with diarrhoea. J. Clin. Microbiol. **25:**2330–2333.
35. **Nastro, L. J., and S. M. Finegold.** 1973. Endocarditis due to anaerobic gram-negative bacilli. Am. J. Med. **54:**482–496.
36. **Oyston, P. C., and P. S. Handley.** 1991. Surface components of *B. fragilis* involved in adhesion and haemagglutination. J. Med. Microbiol. **34:**51–55.
37. **Paavonen, J., V. V. Valtonen, D. L. Kasper, M. Malkamaki, and P. H. Makela.** 1981. Serological evidence for the role of *B. fragilis* and Enterobacteriaceae in the pathogenesis of acute pelvic inflammatory disease. Lancet **8215:**293–295.
38. **Pantosti, A., R. Colangeli, A. O. Trzianabos, and D. L. Kasper.** 1995. Monoclonal antibodies to detect capsular diversity among *Bacteroides fragilis* isolates. J. Clin. Microbiol. **33:**2647–2652.
39. **Pantosti, A., M. Grazia, A. Frate, A. Sanfilippo, F. D'Ambrosio, and M. Malpeli.** 1997. Detection of enterotoxigenic *Bacteroides fragilis* and its toxin in stool samples from adults and children in Italy. Clin. Infect. Dis. **24:**12–16.
40. **Patrick, S., J. P. McKenna, S. O'Hagan, and E. Dermott.** 1996. A comparison of the haemagglutination and enzymatic activities of *B. fragilis* whole cells and outer membrane vesicles. Microb. Pathog. **20:**191–202.
41. **Podglajen, I., J. Breuil, I. Casin, and E. Collatz.** 1995. Genotypic identification of two groups within the species *B. fragilis* by ribotyping and by analysis of PCR-generated fragment patterns and insertion sequence content. J. Bacteriol. **177:**5270–5275.
42. **Romero, R., M. Mazor, R. Morrotti, C. Avila, E. Oyarzun, A. Insunza, M. Parra, E. Behnke, F. Montiel, and G. H. Cassell.** 1992. Infection and labour. VII. Microbial invasion of the amniotic cavity in spontaneous rupture of membranes at term. Am. J. Obstet. Gynecol. **166:**129–133.

42a. **Sebald, M. (Institut Pasteur, Paris, France).** Personal communication.

43. **Shoop, D. S., L. L. Myers, and J. B. LeFever.** 1990. Enumeration of enterotoxigenic *B. fragilis* in municipal sewage. Appl. Environ. Microbiol. **56:**2243–2244.
44. **Tabaqchali, S., M. Wilks.** 1992. Epidemiological aspects of infections caused by *Bacteroides fragilis* and *Clostridium difficile*. Eur. J. Clin. Microbiol. Infect. Dis. **11:**1049–1057.
45. **Unhanand, M., M. M. Mustafa, G. H. McCracken, Jr., and J. D. Nelson.** 1993. Gram-negative enteric bacillary meningitis: a twenty-one-year experience. J. Pediatr. **122:**15–21.
46. **Versalovic, J., T. Koeuth, and J. R. Lupski.** 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res. **19:**6823–6831.
47. **Webber, S. A., P. Tuohy.** 1988. *B. fragilis* meningitis in a premature infant successfully treated with metronidazole. Pediatr. Infect. Dis. J. **7:**886–887.
48. **Weikel, C. S., F. D. Grieco, J. Reuben, L. L. Myers, and R. B. Sack.** 1992. Human colonic epithelial cells, HT29/C1, treated with crude *Bacteroides fragilis* enterotoxin dramatically alter their morphology. Infect. Immun. **60:** 321–327.