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Rapid Presumptive Identification of Anaerobes in Blood Cultures by Gas-Liquid Chromatography

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Production of volatile and nonvolatile metabolic acids in blood culture broths by aerobic, facultative anaerobic, and obligate anaerobic bacteria was analyzed by gas-liquid chromatography. Anaerobic blood culture isolates were presumptively identified by the qualitative analysis of volatile fatty acids. Isolates, with a characteristic Gram stain reaction and cellular morphology, were identified by the following acid patterns: *Bacteroides fragilis* group with acetic and propionic acids; *Fusobacterium* with acetic, butyric, and usually propionic acids; *Veillonella* with acetic and propionic acids; gram-positive cocci with acetic and butyric acids; and *Clostridium* with acetic and butyric acids.

Analysis by gas-liquid chromatography (GLC) of volatile and nonvolatile metabolic acids was initially used by Holdeman and associates for the taxonomic classification of obligate anaerobes (3) and is commonly used now in many clinical laboratories to supplement biochemical tests for anaerobic identification. Gorbach and co-workers (2) and others (1, 5) used direct GLC analysis to presumptively identify the presence of anaerobes in clinical specimens. Wüst (6) reported the presumptive identification of anaerobic bacteremias by the quantitative GLC analysis of positive blood culture broths. Although Wüst analyzed the volatile and nonvolatile metabolic acids with a chromatograph equipped with flame ionization detectors, most microbiology laboratories use chromatographs with the less sensitive and less expensive thermal conductivity detectors.

In the present study, positive blood culture broths were qualitatively analyzed by GLC for the presence of metabolic acids. Most anaerobic blood culture isolates could be accurately identified by the presence of characteristic volatile fatty acids detected with a chromatograph equipped with a thermal conductivity detector.

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MATERIALS AND METHODS

Blood cultures, collected from patients in Barnes Hospital, were analyzed during a 6-month period from November 1977 through April 1978. Blood was inoculated (10%, vol/vol) into one bottle each of 100 ml of tryptic soy broth and 100 ml of thiol broth (Difco Laboratories, Detroit, Mich.), under vacuum with CO_2 and with 0.025% sodium polyanetholsulfonate. The routine processing of these cultures has been described previously (4). Additional simulated positive blood cultures were prepared by injecting stock bacterial cultures into 10-day-old negative blood culture broths.

On the day of initial detection, all positive broths were analyzed by GLC for metabolic by-products. Approximately 3 ml of broth was removed and centrifuged at 3,000 × *g* for 10 min. Methylated derivatives of the nonvolatile acids and ether extracts of the volatile acids were prepared from the supernatant fluid according to the methods described by Holdeman and co-workers (3).

A Gow-Mac (model 69-550) gas chromatograph equipped with a thermal conductivity detector and dual column oven was used. The stainless-steel columns were packed with 15% SP-1220/1% H_3PO_4 on 100/200-mesh acid-washed Chromosorb W (Supelco Inc., Bellefonte, Pa.). Operating conditions included: column temperature, 150°C; injection port temperature, 175°C; detection block temperature, 165°C; bridge current, 175 mA; and helium gas flow, 80 to 100 ml/min. A total of 15 μl of each sample was injected into the chromatograph.

Volatile and nonvolatile acid standards (Capco, Sunnyvale, Calif.) were run daily. In addition, each lot of the tryptic soy and thiol broths, with and without added blood, was analyzed for the presence of the acids.

Although the amounts of metabolic acids produced by the isolates in either tryptic soy or thiol broth differed quantitatively, there were no consistent qualitative differences. The results presented herein are applicable to both broths unless otherwise stated.

RESULTS

Cultures. A total of 128 positive blood cultures were analyzed in this study, including 30 cultures inoculated wth stock laboratory isolates. An additional nine polymicrobial cultures, from which 24 organisms were recovered, were also processed.

Analysis of negative blood culture

broths. Small amounts of acetic, lactic, and succinic acids were consistently detected in uninoculated tryptic soy and thiol broths, as well as significant quantities of oxaloacetic acid in 8 of 12 lots of thiol broth. The quantities of lactic, succinic, and oxaloacetic acids, but not acetic acid, were increased in broths inoculated with blood.

Detection of methylated nonvolatile acids. Detectable quantities of only succinic acid were produced by the *Bacteroides fragilis* group (7 of 13 isolates), *Escherichia coli* (8 of 11 isolates), and *Proteus* species (4 of 6 isolates). Lactic acid was produced by three isolates of *Clostridium*, one anaerobic nonsporeforming bacillus (a *Lactobacillus* sp.), two anaerobic cocci, and five aerobic cocci. Other nonvolatile acids were not detected.

Detection of volatile fatty acids. Summarized in Tables 1 and 2 are the major volatile fatty acids which were produced by the aerobic or facultative anaerobic and obligate anaerobic bacteria, respectively. Summarized in Table 3 are the volatile fatty acid patterns which were used to identify the most common obligate anaerobic blood culture isolates. A total of 12 of 13 isolates of *B. fragilis* group, and one isolate of *Proteus morganii*, produced significant quantities of acetic and propionic, but not butyric, acids. All five isolates of *Fusobacterium* produced acetic and butyric acids, and four produced propionic acid. The two isolates of *Veillonella* produced both acetic and propionic acids, whereas the two aerobic gram-negative cocci which were tested, *Neisseria gonorrhoeae* and *N. meningitidis*, did not produce any volatile acids. Five of the 10 obligate anaerobic gram-positive cocci produced both acetic and butyric acids and were differentiated from the aerobic cocci, which produced only acetic acid. Eight of nine *Clostridium* isolates, including all three isolates of *Clostridium perfringens*, produced

TABLE 1. *Detection of volatile fatty acids produced by aerobic or facultative anaerobic bacteria*

Organism	No.	Major volatile fatty acids detected[a]
Escherichia coli (11)[b]	9	A
	2	A, (P)
Klebsiella pneumoniae (7)	3	A
	2	A, (IV)
	1	A, (P), (B)
	1	(A)
Proteus (6)	4	A
	1	A, P
	1	A, (C)
Pseudomonas aeruginosa (6)	6	(A)
Acinetobacter calcoaceticus (3)	3	(A)
Staphylococcus aureus (6)	4	A
	2	(A)
Staphylococcus epidermidis (5)	4	A
	1	(A)
Streptococcus (9)	5	A
	3	(A)
	1	None
Neisseria (2)	2	None
Listeria (2)	2	A
Corynebacterium (8)	4	A
	1	A, P
	3	None
Bacillus (6)	1	A, (P)
	1	A, B, (IB), (IV)
	4	(A)

[a] A, Acetic; P, propionic; IB, isobutyric; B, butyric; IV, isovaleric; V, valeric; C, caproic. Trace amounts of acids are indicated by parentheses.

[b] Number in parentheses is total number of isolates.

TABLE 2. *Detection of volatile fatty acids produced by obligate anaerobic bacteria*

Organism	No.	Major volatile fatty acids detected[a]
Bacteroides fragilis group (13)[b]	7	A, P
	3	A, P, IV
	1	A, P, IB, B
	1	A, P, IB, (B), IV
	1	A, P, (B)
Bacteroides spp. (5) (not *B. fragilis*)	2	A, (P)
	1	A
	2	None
Fusobacterium (5)	4	A, P, B
	1	A, B
Veillonella (2)	2	A, P
Peptococcus (8)	2	A, B
	2	A, P
	2	A, (P), B
	1	A
	1	None
Peptostreptococcus (2)	1	A, (P), B
	1	None
Clostridium perfringens (3)	2	A, P, B
	1	A, B
Clostridium spp. (6) (not *C. perfringens*)	5	A, B
	1	A
Propionibacterium (7)	3	A, P
	2	A, P, IV
	1	A, P, IV, V
	1	(A)
Eubacterium (3)	1	A
	2	A, (P), (B)
Bifidobacterium (2)	2	(A)
Lactobacillus (1)	1	A

[a] See Table 1, footnote *a*.

[b] See Table 1, footnote *b*.

TABLE 3. *Summary of characteristic volatile fatty acid patterns associated with common anaerobic isolates*

Organism	Volatile fatty acids			No. of isolates	No. correctly identified
	Acetic	Propionic	Butyric		
Bacteroides fragilis group	+	+	−	13	12
Fusobacterium	+	+/−	+	5	5
Veillonella	+	+	−	2	2
Gram-positive cocci	+	−	+	10	5
Clostridium	+	−/+	+	9	8

acetic and butyric acids, whereas only one other gram-positive bacillus, a *Bacillus* species, produced these volatile acids.

Production of volatile fatty acids in polymicrobial bacteremias. Nine polymicrobial bacteremias occurred in this study. Five polymicrobial cultures were initially detected by Gram staining the blood culture broth. In two of the other four cultures, isolates of the *B. fragilis* group were detected by the presence of pleomorphic gram-negative bacilli associated with the production of acetic and propionic, but not butyric, acids. However, in two mixtures of *B. fragilis* and *Fusobacterium*, the production of acetic, propionic, and butyric acids was characteristic of *Fusobacterium* strains, and *B. fragilis* was not initially detected.

DISCUSSION

In the present study, anaerobic blood culture isolates were presumptively identified by the qualitative analysis of the volatile metabolic acids with a chromatograph equipped with thermal conductivity detectors. Analysis of the nonvolatile acids was not necessary. Wüst (6) reported that succinic acid was a characteristic metabolic product for *Bacteroides* spp., although he noted that it was also produced by facultative anaerobic and aerobic bacteria. Gorbach and co-workers (2) also reported that succinic acid was produced by *E. coli*. In our experience the amounts of succinic acid that were produced by *Bacteroides* and *Enterobacteriaceae* isolates were indistinguishable. Both aerobic and anaerobic gram-positive cocci produced similar quantities of lactic acid. Although lactic acid was produced by *Clostridium* (three of nine isolates) and *Lactobacillus*, and was not associated with the aerobic gram-positive bacilli analyzed herein, we believe the limited information obtained with this analysis does not justify the additional extraction.

In the present study, an accurate presumptive identification of most obligate anaerobic blood culture isolates was possible with the volatile acid patterns alone (Table 3). Although Wüst (6) found butyric and isovaleric acids associated with *B. fragilis*, these acids were not commonly detected in the present study (Table 2). Media composition, particularly the ratio of carbohydrates and peptones, influences production of metabolic acids (G. L. Lombard, F. S. Thompson, and A. Y. Armfield, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C29, p. 282). However, because tryptic soy broth was used in both our study and Wüst's, the decreased detection of butyric and isovaleric acids is likely due to the decreased sensitivity of the thermal conductivity detector used in this study compared with the flame ionization detector used in Wüst's study. In addition, Wüst did not observe quantities of propionic acid greater than that detected in uninoculated broth. We cannot readily explain this difference from our study, although Holdeman and co-workers (3) also reported that propionic acid was consistently associated with the *B. fragilis* group. One isolate of the *B. fragilis* group, which produced acetic, propionic, isobutyric, and butyric acids, was misidentified as a *Fusobacterium* sp. In addition, one facultative anaerobic gram-negative bacillius, *P. morganii*, produced acetic and propionic acids and was misidentified as *B. fragilis* group. Only 5 of 10 anaerobic gram-positive cocci were differentiated from the aerobic gram-positive cocci by the volatile acid patterns. Eight of nine isolates of *Clostridium* produced both acetic and butyric acids, a pattern which was observed with only 1 of 16 aerobic gram-positive bacilli. Some isolates of *Eubacterium*, including two of the three isolates in the present study, produce acetic acid and variable amounts of butyric acid (3). Although the volatile acid pattern does not differentiate these two genera, *Eubacterium* is an uncommon isolate in blood cultures, and *Clostridium* strains commonly produce hemolysis and gas in the cultures.

At the present time in this laboratory we analyze blood culture broths by GLC for volatile metabolic acids if pleomorphic or fusiform gram-negative bacilli, or gram-positive bacilli, associated with hemolysis or gas production, are observed in the broths. By this procedure, we can accurately identify isolates of *B. fragilis* group, *Fusobacterium*, and *Clostridium*. Although identification of other anaerobic organisms can

be made in most instances, the analysis is not routinely performed because the organisms are not frequently recovered in blood cultures. Identification of all isolates must still be confirmed by appropriate biochemical tests and standardized analysis by GLC.

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