APPLIED MICROBIOLOGY, May 1974, p. 927-932

Vol. 27, No. 5
Printed in U.S.A.

Bacteremia After Tooth Extractions Studied with the Aid of Prereduced Anaerobically Sterilized Culture Media

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Received for publication 29 October 1973

Both prereduced molten agar and broth and aerobic molten agar and broth were inoculated with blood samples collected from patients with periodontitis, but in otherwise good health, both before and after extraction of two or more teeth. Postoperative blood samples from 23 of 25 patients sampled yielded anaerobic and facultative species. Colony counts from nine samples yielded from less than 1 to over 100 colonies per ml of blood. Organisms detected were species belonging to the genera *Bacteroides*, *Fusobacterium*, *Peptostreptococcus*, *Leptotrichia*, *Propionibacterium*, *Peptococcus*, *Veillonella*, plus *Streptococcus mitis*, *S. salivarius*, vibrio forms, and strains resembling *S. mutans*. The data indicate that prereduced anaerobically sterilized culture medium with polyanethol sulfonate is effective for detecting anaerobic species in bacteremia and that anaerobic species can be prevalent in bacteremias immediately after tooth extraction in patients with periodontitis.

Anaerobic bacteria have been detected in human blood under a variety of conditions including (i) bacteremia after oral surgery, (ii) bacterial endocarditis (3), and (iii) septicemia associated with other types of systemic infections (10).

Approximately 60 studies of bacteremia after various oral treatments have been reported. Most reports describe use of only aerobic culture media, or thioglycolate medium. Only five reports emphasized and described use of anaerobic culture conditions (5, 11, 12, 15, 16) such as the Brewer jar. None of these employed the prereduced medium technology (4, 9). Moreover, little attention has been given to quantitative determination of aerobic and anaerobic bacteria in the bloodstream.

A modification of Hungate prereduced anaerobically sterilized medium for cultivation of anaerobes has been used at the Virginia Polytechnic Institute Anaerobe Laboratory to develop a comprehensive classification of anaerobic bacteria (4) and has been found sensitive and applicable to cultivation of anaerobes in clinical laboratories (9).

Prereduced anaerobically sterilized medium should also provide a sensitive means for detection and study of anaerobes in conditions of bacteremia and septicemia.

This initial report is limited to a description of findings derived from the application of aerobic and prereduced culture media to the study of bacteremia after tooth extractions. A prereduced anaerobically sterilized agar culture bottle, which served as the equivalent of an anaerobic agar pour plate, was used to obtain colony counts of bacteria in the blood samples. (This work was presented at the Annual Meeting of the International Association for Dental Research, Chicago, Ill., March 1971.)

MATERIALS AND METHODS

Moore E medium (4) was selected for use in this study because of our prior use of that medium in an investigation which demonstrated the applicability of prereduced anaerobically sterilized media to detect anaerobes in clinical samples in a clinical diagnostic laboratory (9). This does not imply any superiority of E medium over other formulations of prereduced anaerobically sterilized media for blood culturing. In a pilot comparison, prereduced E medium was compared with commercial prereduced brain heart infusion, vitamin K, heme medium (Robbin Laboratory, Carrboro, N.C.) prepared under anaerobic conditions. Colony counts were made from serial dilutions of 10 anaerobic clinical isolates cultivated in roll tubes prepared with the two different kinds of media.

Moore E medium was prepared with rumen fluid (Robbin Laboratory, Carrboro, N.C.) with the addition of 1.5% (wt/vol) agar and 0.05% (wt/vol) sodium polyanethol sulfonate (SPS) (Hoffman-LaRoche),

and was prepared by published methods (4). This concentration of SPS was not inhibitory to pure and mixed cultures of oral anaerobes in preliminary diffusion plate studies. SPS was found later in quantitative studies to inhibit growth of a *Bacteroides melaninogenicus*, *Peptococcus anaerobius*, and a *Peptostreptococcus magnus* strain even in the presence of blood as indicated by Holdeman and Moore (4). Therefore, concentrations of SPS above should be limited to 0.025% as used by others (17). That concentration did not inhibit *B. melaninogenicus*, *P. magnus*, or *Veillonella alcalescens*, but still showed distinct inhibition of the *P. anaerobius* in our hands. Quantities (30 ml) of agar medium at 50 C (pH 7.4 to 7.6) were dispensed with an automatic dispensing pipette into 8-oz (0.24 liter) clear-glass prescription bottles ("Duraglas," Owens-Illinois, Toledo, Ohio), flushed with oxygen-free 10% CO_2 in nitrogen, and closed with moistened tight-fitting #1 black rubber stoppers (4). Stoppers were held in place with bakelite screw caps (1). The bottles used have a wide flattened side and withstood autoclaving better than other inexpensive bottles tested. Lowering the autoclave pressure by slow cooling was essential to avoid breakage. In the autoclave, bottles were separated with suitable partitions to prevent breakage of other bottles if one bottle did break. Before use, the agar medium was melted in an autoclave and cooled to 45 C.

A patient's arm was cleansed with soap and disinfected with 1% tincture of iodine followed by 70% isopropyl alcohol, each applied twice, using a separate sterile gauze sponge for each application. A sterility test sample from each patient's arm consisted of placing about 0.1 ml of prereduced broth on the prepared site of a patient's arm, moving it around firmly on the skin with the sterile needle tip, and aspirating it back into a sterile syringe. This was then cultured in prereduced and aerobic culture media. A 10-ml amount of venous blood was taken from the antecubital fossa, both before teeth were extracted and immediately after the last extraction was completed.

From the first nine patients, 3 ml of blood was injected through the rubber stopper into the bottle containing 30 ml of molten E medium agar with the 20-gauge collection needle. To avoid air leakage, the needle hub had to be grasped firmly to be withdrawn from the stopper if the needle was not locked securely to the syringe. A 1-ml amount of blood was injected into a rubber-stoppered, screw-cap tube containing 9 ml of prereduced E medium broth prepared by the same formulation; 5 ml was also cultured aerobically in 100 ml of Trypticase soy broth (BBL) and 1 ml was cultured in a 14-ml Trypticase soy agar pour plate. From the remainder of the patients, 3 ml of blood was inoculated into 30-ml volumes of the respective aerobic and prereduced agar media, and 2 ml of blood was inoculated into 30-ml volumes of the respective aerobic and prereduced broth media for consistency.

After gentle mixing, the prereduced E medium agar culture bottle was placed on its flat side to harden. Bottles were incubated horizontally with the agar side up to avoid confluent growth caused by moisture condensation. Examining the thick blood agar layer was aided by use of a high-intensity, illumination candling device (Fig. 1).

A dissecting microscope aided inspection and colony detection. Bottles were incubated for 3 weeks before discarding. Most colonies were detectable in 4 to 5 days, although some required 10 days for growth.

Samples were taken from 25 healthy adult patients with gingival disease, after extraction of two or more teeth.

Growth was subcultured to roll tubes, and pure isolates were classified according to the V.P.I. criteria (4) by means of Gram reaction, morphology, fermentation, and biochemical tests. A low-cost gas chromatograph (model 69-500, Gow-Mac Instrument Co., Madison, N.J.) was used to determine fermentation products of anaerobes and to aid identification according to published criteria (4).

RESULTS

In a pilot comparison of prereduced E agar with prereduced brain heart infusion (BHI) agar, colony counts of 10 strains from clinical sources were performed in roll tubes of the two media. Three *Fusobacterium nucleatum* strains produced approximately 10% higher counts in BHI than in E agar. Three strains of *B. melaninogenicus* species and one *Leptotrichia* strain, two strains of *Veillonella*, and one *Propionibacterium* (not speciated)

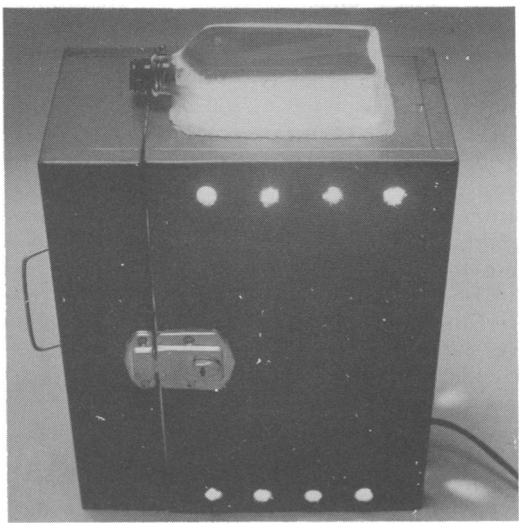

FIG. 1. *Dense blood agar in the anaerobic culture bottles was examined with a high-intensity illuminator. A 150-W bulb and porcelain socket were attached to the bottom of a metal box beneath a 6- by 12-cm rectangular opening 12 cm above the bulb. The portable metal file box used (approximately 25 by 14 by 32 cm) was ventilated as shown and lined with asbestos. A green-tinted glass plate can be inserted between the bulb and bottle as a heat barrier, to permit prolonged examinations.*

showed essentially identical counts in both media.

Cultures taken from the surface skin of patients' arms before disinfection revealed mainly white colonies of coagulase- and mannitol-negative, gram-positive cocci classified as *Staphylococcus epidermidis*. Samples taken after disinfection produced no growth aerobically or anaerobically. Preoperative control blood samples also produced no growth.

Table 1 lists bacterial species cultivated from 25 patients immediately after extraction of two to seven teeth without use of antibiotic premedication. The only facultative strains detected were species of *Streptococcus* and *Neisseria*. Members of 11 different anaerobic genera or groups were detected.

Table 2 lists the frequency of detection of bacterial species in the blood cultures.

Table 3 shows colony counts of bacteria detected in aerobic and anaerobic media from the first nine patients' postextraction blood samples. Since smaller amounts of blood (1 ml) were used in the aerobic agar cultures than were used in the anaerobic agar cultures (3 ml), the values are not directly comparable. Different volumes were used in order to simulate aerobic culture procedures used in a hospital laboratory. Thus, a total of 6 ml of blood, including that added to the broth medium, was cultured aerobically, whereas a total of only 4 ml was cultured anaerobically. Despite this inequality, three times more anaerobic strains were detected than aerobic strains in the first nine samples. Colony counts were not tabulated on all of the remaining samples, but no significant change was observed in the ratio of 2.7:1 of anaerobic to facultative strains detected.

DISCUSSION

Anaerobes were detected in the blood samples from 23 out of 25 patients who yielded positive blood cultures immediately after tooth extractions. That the species detected were derived from the patients' blood rather than the skin was demonstrated by the negative skin cultures of the collection site on the patients' arms, and by the negative preoperative blood samples.

In other studies the incidence of bacteremia after extractions has ranged from 15 to 88% (5). The higher incidences were generally obtained with the aid of the more common types of anaerobic culture procedures. The highest percentage of anaerobic bacterial species recovered after tooth extractions in other studies was 57%. The number of anaerobic bacteria detected in this study per person was over twice that reported in other studies of bacteremia after dental extractions.

Although it is well established that strict anaerobes, as defined by Loesche (7), such as *Treponema* species, occur at the margins of diseased gingival tissues in adults (6, 8), no such anaerobes were recovered from these patients' blood. However, *Treponema* species usually require nutrients that were not added to the media. Although such fastidious species are not always believed to be of much significance as systemic pathogens (13), it is of interest that fastidious spirochetes are found in some pulmonary abscesses (4).

Anaerobic methods other than the use of prereduced media may also be applicable to the study of blood samples and other clinical specimens if carefully used. Washington and Martin have obtained favorable results with several kinds of liquid anaerobic media (17).

McMinn and Crawford (9) reported the superiority of prereduced media over the GasPak anaerobic jar system when the latter was used in the manner employed in the past by many hospitals. Other investigators who employed several ways of improving the use of the GasPak system appear to have found it equal to the use of prereduced medium for cultivating intermediate anaerobes (2, 13). These methods of improvement included careful rejuvenation of the catalyst between uses by heating to 160 C for several hours (2, 13) and use of blood plates preincubated in an anaerobic jar (2), plus use of an anaerobic or prereduced transport medium to preserve the anaerobic status of their clinical samples until media were inoculated for incubation in the GasPak system (2, 13).

In all instances, rapid placement of clinical samples in a reduced atmosphere by whatever means appeared to be of major importance. In that light and in light of differences of procedure employed, there appears to be no discrepancy between those reports (2, 13) and that of McMinn and Crawford (9), who endeavored only to test the GasPak system as they found it used in clinical laboratories.

It is not unlikely that aerobic bacteria obscure the importance of anaerobes in blood cultures. Of interest was an abundance of *Staphylococcus* species detected on patients' arms in this study before careful disinfection of their skin. If the skin is not carefully disinfected, it appears that such residential staphylococci could easily contaminate blood cultures, even on a repeated basis. That was considered to be a problem in some bacteremia studies by at least one other investigator (5). This may be

TABLE 1. *Microorganisms isolated from the blood of patients after tooth extraction*

Patient no.	Microorganisms cultivated
1	*Bacteroides melaninogenicus*
	Streptococcus mitis
	Vibrio[a] forms
	Actinomyces naeslundii
	Streptococcus hemolyticus
	Probable *Leptotrichia* species
2	*Bacteroides melaninogenicus*
	Streptococcus mitis
	Vibrio[a] forms
	Bacteroides oralis
	Peptococcus magnus
	Peptostreptococcus intermedius
	Fusobacterium nucleatum
	Probable *Leptotrichia* species
3	*Bacteroides melaninogenicus*
	Streptococcus mitis
	Streptococcus sp. (resembling *S. mutans*)
	Vibrio[a] forms
4	*Bacteroides melaninogenicus*
	Streptococcus salivarius
	Streptococcus mitis
	Peptostreptococcus intermedius
5	*Bacteroides melaninogenicus*
	Veillonella parvula
	Streptococcus mitis
	Streptococcus sp. (resembling *S. mutans*)
	Fusobacterium nucleatum
	Bacteroides oralis
	Bacteroides fragilis
	Branching filaments (lost to subculture)
	Probable *Leptotrichia* species
6	*Peptococcus magnus*
7	*Bacteroides melaninogenicus*
	Streptococcus mitis
	Streptococcus sp. (resembling *S. mutans*)
	Vibrio[a] forms
	Fusobacterium nucleatum
	Veillonella parvula
	Probable *Leptotrichia* species
	Branching filaments
8	*Bacteroides melaninogenicus*
	Vibrio[a] forms
	Peptococcus asaccharolyticus
9	*Bacteroides melaninogenicus*
	Bacteroides oralis
	Streptococcus mitis
10	*Streptococcus mitis*
	Streptococcus salivarius
	Bacteroides melaninogenicus
11	*Streptococcus mitis*
	Streptococcus salivarius
12	*Bacteroides* sp.
	Fusobacterium nucleatum
	Fusobacterium sp.
	Bacteroides melaninogenicus
13	0
14	*Streptococcus* sp. (resembling *S. mutans*)
	Streptococcus mitis
15	*Peptostreptococcus intermedius*
	Arachnia propionica
	Propionibacterium acnes
	Fusobacterium nucleatum
16	*Veillonella parvula*
	Streptococcus mitis
17	*Actinomyces naeslundii*
18	*Propionibacterium* sp.
	Bacteroides melaninogenicus
	Veillonella parvula
19	0
20	*Fusobacterium nucleatum*
	Actinomyces naeslundii
	Streptococcus mitis
	Bacteroides corrodens
	Neisseria catarrhalis
	Streptococcus salivarius
21	*Fusobacterium nucleatum*
	Streptococcus mitis
	Peptococcus asaccharolyticus
	Veillonella alcalescens
	Peptococcus magnus
	Vibrio[a] forms
	Bacteroides melaninogenicus
22	*Streptococcus* sp. (resembling *S. mutans*)
	Streptococcus mitis
	Actinomyces naeslundii
	Fusobacterium nucleatum
	Peptostreptococcus sp. lost to subculture
	Veillonella parvula
	Streptococcus sp. (resembling *S. mutans*)
	Bacteroides melaninogenicus
	Vibrio[a] forms
23	*Fusobacterium nucleatum*
	Peptostreptococcus sp.
	Peptococcus magnus
	Streptococcus mitis
24	*Bacteroides melaninogenicus*
	Bacteroides oralis
	Actinomyces viscosus
	Streptococcus mitis
	Fusobacterium nucleatum
	Vibrio[a] forms
	Bacterionema matruchotii
	Propionibacterium sp.
25	*Actinomyces naeslundii*
	Bacterionema matruchotii
	Streptococcus mitis
	Peptostreptococcus anaerobius
	Bacteroides oralis

[a] Morphological identification.

TABLE 2. *Microorganisms detected in 23 of 25 postextraction blood samples*

Microorganism	Incidence in 25 samples	
	No.	%
Streptococcus mitis	17	68
Bacteroides melaninogenicus	14	56
Fusobacterium nucleatum	10	40
Vibrio forms	8	32
Streptococcus species (resembling *S. mutans*)	6	24
Bacteroides oralis	5	20
Veillonella parvula	5	20
Actinomyces naeslundii	5	20
Streptomyces salivarius	4	18
Peptococcus magnus	4	18
Leptotrichia species (probable)	4	18
Peptostreptococcus intermedius	3	12
Peptostreptococcus species	2	8
Peptococcus assachrolyticus	2	8
Peptococcus species	2	8
Propionibacterium species	2	8
Branching filament (anaerobic)	2	8
Neisseria catarrhalis	1	4
Streptococcus hemolyticus	1	4
Bacteroides fragilis	1	4
Bacteroides corrodens	1	4
Bacteroides species	1	4
Fusobacterium species	1	4
Peptostreptococcus anaerobius	1	4
Propionibacterium acnes	1	4
Actinomyces viscosus	1	4
Arachnia propionica	1	4
Bacterionema matruchotii	1	4

TABLE 3. *Viable organisms detected (per ml of blood) from nine patients*

Patient no.	Viable organisms detected (per ml of blood)	
	Anaerobically prepared bottles (3 ml of blood)	Aerobic pour plates (1 ml of blood)
31	29	3
2	14	3
33	8	1
12	9	1
35	28	13
36	1	0
37	131	82
38	4	0
25	1	0

important when considering the involvement of *S. epidermidis* as compared with other species in cardiac infections of patients with heart valve prostheses. It is also possible that "viridans streptococci" so often reported in bacteremias associated with endocarditis may not always exist alone in such conditions. Detection of the alpha hemolytic (viridans) streptococci may well be overaccentuated, and anaerobes may be missed by the aerobic culture methods commonly used.

The importance of anaerobes in clinical bacteremias has been documented by other researchers. Felner and Dowell reviewed 30 cases of anaerobic endocarditis (3) and one-third were believed to be of oral origin. McVay and Sprunt (10) documented several cases of fatal septicemia caused by the anaerobic *Bacteroides* species. Wilson et al. (18) recently reported that members of the family *Bacteroidaceae* were etiological agents in 78% of hospital patients with clinical bacteremias. Such documentation supports the need for wider application of culture methods than can effectively detect anaerobes in blood cultures. The significance of the more fastidious anaerobic species as agents of systemic infections remains to be determined.

The concept that oral bacteria pose an important source of systemic anaerobic infections is reinforced by the fact that the bloodstream must frequently be exposed to various anaerobic oral species which predominate in plaque masses on the teeth and impinge upon diseased gingival tissues of most adults (6, 9, 19). Another study in our laboratory has shown that even tooth brushing of diseased gingival tissues can cause plaque organisms to enter the circulating blood (14).

On the basis of these initial data, use of prereduced, anaerobically sterilized culture medium containing polyanethol sulfonate appears to provide a sensitive method for detection of anaerobic species in blood samples.

The quantitative use of prereduced medium described here should also be helpful to further investigate bacteremias that may follow various kinds of oral treatments. These methods should also be applicable to determine the effectiveness of measures to reduce or control bacteremias in patients with a high risk of heart valve infections.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant RR-05333 and a grant from United Health Services of North Carolina.

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