Anaerobic Bacteriology

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OBJECTIVE: To determine the importance of anaerobic bacteria in clinical infections.

BACKGROUND: During the past year, this laboratory has established a capability for identifying strict anaerobic bacteria. The use of pre—reduced—anaerobically—sterilized (PRAS) media and the training of two technicians in the use and manufacture of this specialized media has enabled the recovery and identification of numerous organisms that would have otherwise been overlooked in a routine laboratory.

This media, first developed by Dr. R.E. Hungate in 1950 and subsequently modified for use in the clinical laboratory by Dr. W.E.C. Moore in 1966 enables the most fastidious anaerobic bacteria to grow. By employing these techniques in various biochemical media, identification of the organisms can be accomplished. By carefully excluding any atmospheric oxygen from contact with the media, more than twice the number of organisms can be recovered from clinical specimens than by use of standard methods utilizing the Brewer anerobic jar or fluid thioglycolate media. Once recovery of the fastidious organisms is accomplished, antibiotic sensitivity profiles can be established using prereduced agar plates and directed antibiotic therapy can be attempted by the clinician. Moore has noted that many anaerobic bacteria are resistant to certain antibiotics and has indicated the need for more efficient culture methods and accurate sensitivity data.

While our research has not yet demonstrated any of the anaerobic bacteria to be the cause of any disease process (except for tetanus), it is important to note that many lesions have been examined and discovered to harbour these fastidious organisms. We believe that specific treatment directed towards eliminating these organisms, be they the causative agent or opportunists, results in more rapid healing. McDonald has demonstrated the synergistic properties of several of the organisms that we have recovered from lesions with mixed flora. Table 1 shows that from 194 specimens examined, we recovered 237 anaerobic species of bacteria and 491 various species of aerobic bacteria. It would be unwise to assume that these anaerobic organisms were of no importance in the lesions that we cultured.

Tables 2, 3 and 4 present the identification of anaerobic bacteria identified using the techniques employed in this laboratory.

Use of PRAS techniques has enabled the rapid recovery of Clostridium tetani from numerous umbilical specimens. During such recovery, it was noted that numerous lesions were insignificant and had been ignored before clinical symptoms of tetanus had appeared. A search of the literature revealed that while some authors, like B.P. Davis, et al, in their 1967 text, Microbiology, state that spores may remain dormant in healed wounds, no one had presented any data regarding this phenomenon. This laboratory proceeded to design experiments to demonstrate the inability of the Cl. tetani spores to germinate in nontraumatic wounds.

Our experiments in working with Cl. tetani had indicated that most lesions harbouring the tetanus bacillus also contained several other species of bacteria. There was never a pure culture of Cl. tetani from the 60 specimens examined for bacteria. Tetanus spores require an environment with a low Eh (oxidation—reduction potential) for germination. In non—traumatic wounds or in wounds without a simultaneous pyogenic

infection, the normal vascularity would remain intact and maintain a normal tissue Eh. It was postulated that non—traumatic injection of a pure suspension of Cl. tetani spores would not result in germination of the spores and that clinical tetanus would not occur in these animals.

Spores of Cl. tetani were obtained by drying 2 week old broth cultures of Cl. tetani in sealed, sterile petri dishes. After 3 weeks, the dried material was rendered into a powder and checked by way of Gram stain and inoculation on appropriate media for purity. Counts were made of the spore suspension.

White mice were inoculated IM, with 0.1 ml of spore suspension using a 27 gauge hypodermic needle. Every effort was made to insure a non—traumatic inoculation. Duplicate inoculations were made into mice that had recently received tetanus antitoxin. None of the inoculation sights showed any sign of trauma nor did any mice develop clinical tetanus after 28 days of observation.

Subsequent inoculations of coagulase positive Staphylococcus aureus into the leg muscle of mice inoculated 14 days prior with Cl. tetani spores resulted in clinical tetanus and death of 2 of seven animals. Tetanus occurred in these animals 4 days after the Staph. aureus inoculation. The remaining 5 animals were sacrificed 30 days after spore injection and had no signs of tetanus. Staph. aureus inoculation of mice protected with antitoxin prior to spore inoculation produced a typical lesion but no tetanus.

These findings indicate that the spores of Cl. tetani can remain dormant in healthy tissue for as long as 28 days without germination. The presence of the spores was demonstrated by subsequent inoculation with a pyogenic bacteria that caused tissue necrosis and enabled germination of the dormant spore.

Table 1.

Anaerobic Specimens (April 1971 – March 1972)

Hospitals	Source (lesion)	No. of specimens	Anaerobic Organism Isolated				Aerobic org.
			Clostridium	Bacteroides	Cocci	Gram–pos.–rod (non–spore former)	
Children's Hosp.	Umbilical swab	60	68	25	45	1	260
	Miscellaneous*	23	18	13	11	1	85
Chulalongkorn Hosp.	Corneal ulcer	29	–	–	–	4	–
	Umbilical swab	15	6	2	3	–	42
	Wounds	34	6	8	3	–	58
	C.S.F.	7	–	–	–	–	–
	Miscellaneous*	23	2	5	6	2	34
Other (Army Hosp., Rama Hosp.)	Miscellaneous*	3	2	3	2	–	4
	Total	194	102	56	70	9	491

* Note Table 4

Table 2.

Children's Hospital

Source	Anaerobic Organism Isolated							
	Clostridium		Bacteroides		Cocci		Gram—pos.—rod (non—spore former)	
Umbilical (60)	C. tetani	18	B. fragilis	18	Peptococcus magnus	17	Eubacterium lentum	1
	C. perfringens	14	B. terebrans	3	P. prevotii	16		
	C. sordellii	8	B. melaninogenicus	1	P. asaccharolyticus	6		
	C. bifermentans	10	F. fusiforme	2	Peptostreptococcus			
	C. sporogenes	4			— intermedius	1		
	C. sphenoides	2			— anaerobius	2		
	C. tertium	2			Veillonella			
	C. subterminale	2			— alcalescens	1		
	C. novyi A	2			— parvula	2		
	C. histolyticum	1						
	C. paraputrificum	1						
	C. innocuum	1						
	C. limosum	1						
Miscellaneous (23)*	C. tetani	4	B. fragilis	4	Peptococcus magnus	2	Propionibacterium — anaerobium	1
	C. perfringens	6	B. corodens	3	P. prevotii	2		
	C. bifermentans	2	B. oralis	1	P. asaccharolyticus	2		
	C. cadaveris	2	F. necrophorus	3	Peptostreptococcus			
	C. sphenoides	1	F. fusiforme		— intermedium	1		
	C. sporogenes	1			Veillonella			
	C. noyvi A	1			— alcalescens	1		
	C. capitovale	1			— parvula	3		

* note Table 4

Table 3.

Chulalongkorn Hospital

Source	Anaerobic Organism Isolated			
	Clostridium	Bacteroides	Cocci	Gram—pos.—rod (non—spore formed)
Corneal ulcer (29)	—	—	—	P. propionicum 2 P. granulosum 2
Umbilical	C. tetani 2 C. perfringens 1 C. bifermentans 1 C. sphenoides 1 C. capitovale 1	B. fragilis 1 B. ferebrans 1	Peptococcus intermedius 1 P. asaccharolyticus 1 Peptostreptococcus anaerobius 1	
Wound (34)	C. perfringens 1 C. sphenoides 2 C. novyi A 1 C. bifermentans 1 C. limosum 1	B. fragilis 6 F. necrophorus 1 F. fusiforme 1	Peptococcus magnus 2 P. asaccharolyticus 1	Eubacterium lentum 1
Miscellaneous (23)*	C. sordelli 1 C. perfringens 1	B. fragilis 2 B. corodens 1 B. melaninogenicus 1 F. necrophorus 1	Peptococcus magnus 3 P. asaccharolyticus 2 P. prevotii 1	Bifidobacterium spp. 1 Eubacterium lentum 1

* Note Table 4

Table 4.

Other Hospitals

Source	Anaerobic Organism Isolated						
	Clostridium		Bacteroides		Cocci		Gram—pos.—rod (non—spore farmer)
Miscellaneous (3)*	C. perfringens	2	B. fragilis	2	Peptococcus prevotii	1	—
			B. terebrans	1	P. asaccharolyticus	1	

* Identification of miscellaneous specimens

Miscellaneous (Chulalongkorn Hosp.):—

Pleural effusion
Cervical swab
lesion of mouth
Burns
Hemoculture
Peritoneal fluid

Miscellaneous (Children's Hosp.):—

Hemoculture
Pleural fluid
Swab from ear
Swab from teeth
Wounds

Miscellaneous (Other):—

Hemoculture
Wound
Stool