Anaerobic organisms in postoperative wounds

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SUMMARY A survey of the bacteria found in postoperative wounds was undertaken during a 14-month period. The yields of aerobic and anaerobic bacteria in 65 appendicectomy wounds were compared; 42 wounds yielded aerobes and 51 anaerobes. Seventy-eight other operation wounds yielded anaerobes, and, overall, 33 wounds yielded anaerobes only. *Bacteroides* sp were the most common anaerobic organisms isolated from all operation sites except the lung.

The examination of infected postoperative wounds for bacteria permits rational chemotherapy. Previous reports of bacteria found in postoperative wounds have shown a predominance of aerobes over anaerobes. In two large national surveys (National Academy of Sciences—National Research Council, 1964; Public Health Laboratory Service, 1960) anaerobes were isolated from only 2.3% of sampled wounds in one, and were not mentioned in the other. More recent surveys (Hoffmann and Gierhake, 1969; Gupta *et al.*, 1972; Peach and Hayek, 1974) of the bacterial flora found in wounds have yielded an increased incidence of anaerobes. Leigh *et al.* (1974) reported the recovery of *Bacteroides* species, but not other anaerobes, in 28 of 30 post appendicectomy wounds. Willis and Study Group (1976) stated that 'most infections that develop after intestinal surgery' are caused by anaerobic bacteria.

In this report the yields of aerobic and anaerobic bacteria are compared in 65 post appendicectomy wounds, and the recovery of anaerobic bacteria from other wounds is described.

Methods

SPECIMENS

All swabs or samples of pus received in the laboratory from postoperative wounds between 1 December 1974 and 31 January 1976 were included in the survey. The swabs were serum-coated and, after sampling a wound, were driven to a depth of about 2 inches (5 cm) in Cary-Blair (1964) transport

medium. They were sent to the laboratory via the hospital portering system. The survey was limited to the first specimen taken from each wound.

MICROSCOPIC EXAMINATION

A Gram film was made from each specimen, and the morphology and number of bacteria present were noted.

MEDIA AND CULTURE

The media and conditions of culture have been described in full previously (Wren *et al.*, 1976). Swabs and purulent material were plated directly on to Columbia blood agar and MacConkey agar for aerobic culture; and on to Columbia blood agar and selective Brucella agar containing 5% horse blood, 0.5 µg/ml menadione, and 75 µg/ml kanamycin for anaerobic culture. Swabs and samples of pus were then inserted into Robertson's cooked meat medium and incubated for 48 hours before subculture on to Columbia blood agar and MacConkey agar aerobically and kanamycin anaerobically.

Direct plate cultures were examined after 24 and 48 hours' incubation. Anaerobic plate cultures were re-examined after a total of five days' incubation. Plate cultures from Robertson's cooked meat medium were examined after three days' incubation.

Anaerobic jars were prepared according to Watt and Collee (1974) using a gas mixture of 90% H₂ and 10% CO₂; 8.5 g catalyst in a modified sachet (Baldwin, 1975) was used in each jar. The catalyst was freshened each week by heating to 160°C for one hour.

IDENTIFICATION OF BACTERIAL ISOLATES

All bacteria isolated were identified for genus and the majority for species. Aerobic bacteria were identified

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according to conventional means using methods described by Cowan (1974).

Anaerobic bacteria were identified by morphology, biochemical tests, and antibiograms, as described by Sutter *et al.* (1975). Nomenclature followed that of Holdeman and Moore (1972).

Results

APPENDICECTOMY WOUNDS

A total of 65 consecutive specimens from appendicectomy wounds with a purulent discharge were examined during the study period (Table 1). Fifty-one wounds yielded anaerobic bacteria and 42 yielded aerobic bacteria. Six wounds yielded aerobes without anaerobes; 15 yielded anaerobes only and, of these, 12 gave a pure growth of a single anaerobic strain.

The different species of bacteria obtained from appendicectomy wounds are listed in Table 2. Eighty-two strains of aerobes and 97 strains of anaerobes were recovered from the 65 wounds examined. *Escherichia coli* composed more than half the aerobic strains, and *Bacteroides* species formed about three-quarters of the anaerobic strains. Ninety-

Table 1 Aerobic and anaerobic bacteria recovered from 65 purulent appendicectomy wounds

Appendicectomy wounds yielding;	Total	%
Aerobes and anaerobes	36	56
Aerobes only	6	9
Anaerobes only	15	23
No growth	8	12
Total	65	100

Table 2 Bacteria recovered from 65 appendicectomy wounds

Aerobes	No.	Anaerobes	No.
<i>Escherichia coli</i>	47 (2)	<i>Bacteroides fragilis</i>	58 (10)
Beta haemolytic streptococci	9	<i>Bacteroides melaninogenicus</i>	7
<i>Proteus mirabilis</i>	6	<i>Bacteroides species</i>	7 (1)
Microaerophilic streptococci*	3	<i>Fusobacterium nucleatum</i>	1
<i>Streptococcus faecalis</i>	3	<i>Peptococcus constellatus</i>	1
<i>Staphylococcus aureus</i>	2 (1)	<i>Peptococcus prevotii</i>	1
<i>Streptococcus viridans</i>	2	<i>Peptococcus species</i>	3
<i>Klebsiella aerogenes</i>	2	<i>Peptostreptococcus micros</i>	1
<i>Klebsiella oxytoca</i>	2	<i>Peptostreptococcus intermedius</i>	1
<i>Pseudomonas aeruginosa</i>	2	<i>Peptostreptococcus species</i>	4
<i>Staphylococcus epidermidis</i>	1	<i>Clostridium perfringens</i>	5 (1)
<i>Proteus vulgaris</i>	1	<i>Clostridium sordellii</i>	1
<i>Haemophilus influenzae</i>	1	<i>Clostridium species</i>	1
<i>Micrococcus species</i>	1	NSGPB†	6
Total	82	Total	97

Figures in parentheses indicate pure cultures of one strain.

*Grown anaerobically initially but aerobically on prolonged culture.

†Non-sporing Gram-positive bacillus.

Table 3 Operation wounds yielding anaerobic bacteria during period of survey (excepting appendicectomy wounds)

Site of operation	No. of wounds yielding	
	Aerobes + anaerobes	Anaerobes only
Stomach and small intestine	10	2
Large intestine	13	3
Abdominal wall		
Laparotomy*	6	3
Hernia repair	3	2
Female genital tract†	15	6
Prostate‡	3	2
Lung§	5	0
Miscellaneous	5	0
Total	60	18

*Intestine not incised.

†Caesarean section (10), abdominal hysterectomy (9), vulvectomy (1), oophorectomy (1).

‡Prostatectomy (5).

§Pneumectomy (5).

||Splenectomy (1), insertion of nail and plate (1), nephrectomy (1), pyelolithotomy (2).

one per cent of the aerobic strains and 89% of the anaerobic strains belonged to species frequently found in the intestinal tract.

OPERATION WOUNDS OTHER THAN APPENDICECTOMY

During the survey anaerobic organisms were recovered from 78 septic wounds of operations other than appendicectomy (Table 3). Eighteen wounds yielded anaerobic organisms only, and, of these, nine yielded more than one strain of an anaerobe.

One hundred and thirty-six strains of anaerobes and 100 strains of aerobes were recovered from wounds that yielded both types of organisms. This result indicates a predominance of anaerobic strains in those wounds in which aerobic organisms were also present.

Overall, 143 postoperative wounds yielded anaerobic bacteria during the survey. One hundred and twenty-eight wounds resulted from operations in which the peritoneal cavity had been opened, but in 15 of these operations neither the intestinal nor the female genital tracts were incised. The remaining 15 wounds were of operations outside the abdominal cavity; five involved the prostate and five were pneumonectomies (Table 4).

Bacteroides species accounted for 70% of the anaerobic strains recovered from wounds after abdominal surgery and for 44% of strains obtained from wounds outside the abdominal region (Table 4). A total of 165 strains of *Bacteroides* species were recovered during the survey; 113 were *B. fragilis*, 31 were *B. melaninogenicus*, 4 were *B. corrodens*, and 17 were not assigned to species. Fifty-three strains of *Peptococcus* and *Peptostreptococcus* species were recorded; 20 of these were found in wounds after

Table 4 Anaerobic bacteria recovered from 78 operation wounds (excepting appendicectomy wounds)

Anaerobic bacteria	Operation site					Total (78)
	Stomach and small intestine (12)	Large intestine (16)	Laparotomy* or hernia repair (14)	Female genital tract (21)	Prostate, lung, and miscellaneous† (15)	
<i>Bacteroides</i> spp	15	24	20	21	13	93
<i>Fusobacterium</i> spp	1	1	1	1	2	6
<i>Peptococcus</i> spp	3	4	1	15	6	29
<i>Peptostreptococcus</i> spp	4	2		5	2	13
<i>Clostridium</i> spp	3	4		2	6	15
Non-sporing Gram-positive bacillus	2	1	1	3	1	8
<i>Veillonella</i> spp		1			1	2
<i>Megasphaera</i> sp		1				1
Total	28	38	23	47	31	167

Figures in parentheses indicate number of operations.

*Intestine not incised.

†Operations as in Table 2.

operations on the female genital tract. Twenty-two strains of *Clostridium* species were cultured, 14 from wounds after abdominal surgery.

Discussion

The association of anaerobic bacteria with post-operative wound infection is emphasised by the results of this survey. Anaerobes were recovered from 79% of all septic appendicectomy wounds and from 78 other purulent wounds during the survey. A larger number of strains and of pure cultures of anaerobes than of aerobes were recovered from septic appendicectomy wounds. These results, and those of certain previous studies (Hoffmann and Gierhake, 1969; Leigh *et al.*, 1974), demonstrate the presence of anaerobic bacteria in the majority of septic post-operative appendicectomy wounds.

Bacteroides sp formed the largest proportion of anaerobic strains recovered, being particularly predominant in wounds after abdominal surgery where 70% of the strains belonged to this species compared to 44% of the strains recovered from other wounds. The distribution of the species of aerobic and anaerobic organisms was comparable to that of previous studies (Thadepalli *et al.*, 1972; Leigh *et al.*, 1974; Gilmore and Sanderson, 1975). The majority of both aerobic and anaerobic species recovered from abdominal wounds belonged to those of the bowel flora (Moore *et al.*, 1969), providing indirect evidence that the bowel is the main source of organisms in infected abdominal wounds. The higher incidence of *Peptococcus* and *Peptostreptococcus* species from wounds of the respiratory and female genital tracts reflects the normal flora of these regions (Smith, 1975).

Most studies of the bacteriology of septic post-appendicectomy wounds have yielded more strains of aerobes than of anaerobes, and this report is one of the few to reverse their proportions. There may be several explanations for the predominance of an-

aerobes in this survey, including the method of transport of specimens, the laboratory techniques adopted for isolation of anaerobes, and the antibiotic treatment of the patients. In this study specimens were sent to the laboratory in the depths of a column of Cary-Blair medium, while in many previous studies dry swabs were transported to the laboratory unprotected from oxygen or desiccation.

The laboratory bench techniques adopted for the recovery of anaerobes have been described previously (Wren *et al.*, 1976). They were subsequently shown (Wren, 1976) to be as successful for the recovery of anaerobes from clinical specimens as the use of an anaerobic chamber. We feel that plating swabs on to enriched and selective media, with careful prevention of exposure of both specimens and cultures to atmospheric oxygen, and the incubation of inoculated plates in an anaerobic atmosphere containing 10% CO₂ with fresh catalyst for up to five days help to reveal the true incidence of anaerobic infection. These techniques are not necessarily all used in bacteriology laboratories, and previous surveys of postoperative wound infection must be viewed in the light of the techniques practised by the laboratory.

A defect of the present study, as of others, is the absence of information of the antibiotics received by the patients in the survey. It may be argued that antibiotics commonly used by surgeons after operations are often inactive against anaerobic organisms. This was demonstrated in a survey of 112 patients by Kelly and Warren (1978), in which 24 of their patients received antibiotics but only six of them received a drug active against anaerobes. If antibiotics had been used extensively in our patients the bacteriological results would probably be biased in favour of anaerobes. However, in those wounds (both appendicectomy and others) in which aerobic as well as anaerobic organisms were recovered, the total number of anaerobic strains was larger than the total number of aerobic strains. The recovery of aerobes in

these patients tends to indicate that they had not received antibiotics at the time of wound sampling and that the influence of antibiotics, in this subgroup at least, may be discounted.

The pathogenic role of anaerobes in postoperative wound sepsis is supported by the clinical evidence of this study. Fifteen of 65 appendicectomy wounds and 19 other operation wounds yielded anaerobes only. In each of these wounds a purulent discharge was present, and many showed tissue destruction and foul odour.

Most wounds, however, including 36 of the 65 septic post-appendicectomy wounds, yielded both aerobes and anaerobes. The relative importance of the two types of organism in the initiation of sepsis in an operation wound is not known (Willis, 1975). The clinical impression that purulent wounds often heal after treatment with antibiotics inactive against *Bacteroides* species and other anaerobic organisms may indicate that sepsis is enhanced by the presence of both aerobes and anaerobes. Kelly and Warren (1978) observed that the incidence of postoperative wound infection varied with the type of organism grown from a wound swab taken at operation. Where both aerobes and anaerobes were recovered from the wound at operation 71% of wounds later became infected; when anaerobes or aerobes alone were recovered only 13% and 22% respectively became infected.

A possible mechanism for bacterial synergy in the causation of postoperation sepsis has been described by Ingham *et al.* (1977). These authors showed that phagocytosis of certain aerobic organisms was inhibited by *Bacteroides melaninogenicus* and other anaerobes, and this finding may indicate an important pathogenic role for anaerobes. It is not known what effect aerobes may have on the phagocytosis of anaerobes, but there are many other potential opportunities for synergy between aerobes and anaerobes in pathogenesis.

This survey demonstrates the predominance of anaerobic bacteria in septic appendicectomy wounds. Anaerobes were found frequently in other wounds of the abdomen, female genital tract, and chest. Indeed, it is wise to assume the presence of anaerobes in purulent surgical wounds when treatment has to be undertaken before the results of bacteriology are available.

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