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Airborne non-sporeforming anaerobic bacteria

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INTRODUCTION

A large proportion of postoperative infections after clean surgery are thought to be exogenous. For aerobic bacteria different routes of transmission have been thoroughly studied. Airborne infection has been considered very important in infections after total hip replacement (Charnley, 1972). Anaerobic non-sporing bacteria have been found in deep late infections after total hip replacement (Kamme *et al.* 1974; Schwan *et al.* 1977; Petrini, Nord & Welin-Berger, 1978). However, infections caused by anaerobic bacteria have been considered endogenous, and little is known about the routes of transmission for these bacteria.

The aim of this investigation has been to study the survival of anaerobic non-sporeforming bacteria in the air and environment to make it possible to study their routes of transmission in the operating room later.

MATERIAL AND METHODS

Bacteriological methods

Anaerobic air samples and all floor samples were cultured on fresh brain heart infusion blood agar with addition of yeast extract, vitamin K and haemin. The contact plates for the floor samples also contained 0·5% Tween 80. Aerobic samples from air were cultured on blood agar. All plates were incubated at 37 °C. The Gas Pak system was used for anaerobic incubation.

Bacteria growing anaerobically were subcultured and incubated aerobically. Those which grew aerobically were grouped as facultative anaerobes, all others as strict anaerobes so that the term strict anaerobes here therefore also includes microaerophilic bacteria. Anaerobes were further identified by gram reaction, cell morphology and gas chromatography (Holdeman, Cato & Moore, 1977). All anaerobic plates were incubated for 4 days except the membrane filter samples that were incubated for 6 days. Aerobic samples were examined after 2 days.

Air samplers

A Casella slit sampler MK II, capacity 30, 175, and 700 l/min and a Sartorius filter Sampler MD2, maximum capacity 45 l/min were used. Gelatin filters type SM 12652, pore size 3 μm and 50 mm diam., or cellulose-membrane filter type SM 11306, pore size 0·45 μm and 47 mm diam., from Sartorius Membranfilter were used in the filter sampler. An Andersen sampler Model 10-800, with six impactor stages, flow rate 28·3 l/min was used for size grading bacteria-carrying

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particles. The volume sampled by each sampler was checked and the flow rates found to be correct. Student's *t*-test and paired sample *t*-test were used in statistical analysis.

EXPERIMENTS AND RESULTS

Recovery of airborne anaerobic bacteria with the Casella slit sampler

The influence of sampling time. With a sampling volume of 30 l/min alternating samples were taken for 2 and 5 min in one series of experiments and for 5 and 10 min in another series. As shown in Table 1*a*, a reduction from 36 c.f.u./m^3 to 31 c.f.u./m^3 was noted in the yield of strict anaerobic bacteria when the sampling time was lengthened from 2 to 5 min, and from 27 to 23 c.f.u./m^3 when lengthened from 5 to 10 min. These reductions were not statistically significant.

The influence of air volumes sampled. In the same room 2 min samples were taken. In one series of experiments alternating samples were taken with air flow rates of 30 and 700 l/min. In another the rates compared were 30 and 175 l/min.

The yield of strict anaerobes was 65 and 60 c.f.u./m^3 when the sampling rates were 30 and 700 l/min; that difference was not statistically significant. When the sampling rates were 30 and 175 l/min the yield was the same, 20 c.f.u./m^3 (Table 1*b*). There was a great difference in the yield of bacteria and the proportion of strict anaerobes between the two series of experiments, presumably due to the different number of people present during the sampling.

Recovery of airborne anaerobic bacteria with a filter sampler

The influence of sampling time. Half-min samples were made in an occupied room. Afterwards sterile air was sampled through every other filter in a laminar flow bench for a further 9·5 min. Series of samples were made with both gelatin and cellulose membrane filters. Each filter pair, the one exposed to sterile air and the other not exposed, was put onto agar plates and incubated at the same time.

When gelatin filters were used exposure to air for a further 9·5 min reduced the yield of strict anaerobic bacteria from 1146 to 810 c.f.u./m^3. This difference was statistically significant (Table 2).

When using cellulose-membrane filters the prolonged exposure to air reduced the yield of strict anaerobic bacteria from 2375 to 2339 c.f.u./m^3, a not statistically significant reduction (Table 2).

Comparison between sampling methods

Comparison between sampling with gelatin and cellulose-membrane filters. Half, 2 and 10 min samples were collected with both types of filters. When 0·5 and 2 min samples were taken exposure of one filter type was followed by exposure of the other. Ten-min samples were taken in pairs with two identical samplers.

In all experiments the recovery of strict anaerobes with cellulose-membrane filters was lower than with gelatin filters (Table 3). The number found on cellulose membrane filters never exceeded 76% of that found on gelatin filters. In the

Table 1. *Recovery of anaerobic bacteria with a Casella slit sampler*

(*a*) The influence of time: air flow rate 30 l/min

Sampling time (min)	Total no.* c.f.u./m³ mean value	Strict anaerobes c.f.u./m³ mean value	Strict anaerobes % of total	Number of samples
2	95	36	38	40
5	102	31	30	20
5	91	27	30	18
10	82	23	28	18

(*b*) The influence of volume: time 2 min

Sampling volume/ min (l)	Total no.* c.f.u./m³ mean value	Strict anaerobes c.f.u./m³ mean value	Strict anaerobes % of total	Number of samples
30	273	65	24	20
700	230	60	26	18
30	33	20	61	20
175	33	20	61	20

* Total no: The total yield of bacteria when incubated anaerobically and therefore including both facultative and strict anaerobic bacteria.

Table 2. *The influence of sampling time on recovery of airborne anaerobic bacteria with a filter sampler*

Filter type	Sampling time (min)	Total no.* c.f.u./m³ mean value	Strict anaerobes c.f.u./m³ mean value	Strict anaerobes % of total	Number of samples
Gelatin	0·5	2490	1146	46	25
	9·5+0·5	1923	810	42	24
Cellulose	0·5	5267	2375	45	30
	9·5+0·5	5322	2339	44	30

* Total no: The total yield of bacteria when incubated anaerobically and therefore including both facultative and strict anaerobic bacteria.

10 min samples the yield of facultative anaerobes was 46 and 47 c.f.u./m³ on gelatin and cellulose membrane filters respectively, while the yield of strict anaerobes was 61 c.f.u./m³ on gelatin and 44 c.f.u./m³ on cellulose-membrane filters. This difference is statistically highly significant.

Comparison between Casella slit sampler and gelatin filter sampler. In three series of experiments 2, 5 and 10 min parallel samples were made.

The results are presented in Table 4. There was a striking difference in the yield of strict anaerobic bacteria between the two methods, the gelatin filter being much more effective. In the 10 min samples, the yield of strict anaerobes on gelatin filters was 58 c.f.u./m³, and only 26 c.f.u./m³ when using the slit sampler. The difference in the yield of facultative anaerobic bacteria between the two methods was not as large. In the 2 and 5 min samples it was not significant.

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Table 3. *Comparison between sampling with gelatin and cellulose membrane filters*

Filter type	Sampling time (min)	Facultative anaerobes c.f.u./m³ mean value	Strict anaerobes c.f.u./m³ mean value	No. of samples	Statistical significance: Fac. an.	Statistical significance: Str. an.
Gelatin	0·5	504	687	25	$P < 0·05$	N.S.*
Cellulose	0·5	364	523	25		
Gelatin	2	90	154	20	N.S.*	$P < 0·05$
Cellulose	2	71	104	20		
Gelatin	10	46	61	15	N.S.*	$P < 0·001$
Cellulose	10	47	44	15		

* Not significant.

Table 4. *Comparison between gelatin filter sampler and Casella slit sampler*

Method	Sampling time (min)	Facultative anaerobes c.f.u./m³ mean value	Strict anaerobes c.f.u./m³ mean value	No. of samples	Statistical significance: Fac. an.	Statistical significance: Str. an.
Gelatin filter	2	25	83	16	N.S.*	$P < 0·001$
Slit sampler	2	23	35	16		
Gelatin filter	5	34	71	22	N.S.*	$P < 0·001$
Slit sampler	5	29	29	22		
Gelatin filter	10	31	58	13	$P < 0·05$	$P < 0·001$
Slit sampler	10	22	26	13		

* Not significant.

In the 10 min samples the gelatin filter gave 31 c.f.u./m³ of facultative anaerobes and the slit sampler 22 c.f.u./m³, this difference was significant.

Comparison between Andersen sampler and gelatin filter sampler. Parallel series of samples at 10 min were made. The gelatin filter was found to be much more effective in sampling strict anaerobes, the yield was 166 c.f.u./m³ when the gelatin filter was used but only 54 c.f.u./m³ when the Andersen sampler was used. On the other hand, there was no significant difference between these methods in sampling facultative anaerobes (Table 5).

Composition of bacteria in occupied rooms

In an occupied room 10 min samples were taken continuously for 1·5 h with gelatin filter. Every other filter was incubated anaerobically and every other aerobically. This was done twice in the same room, but different people were

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Table 5. *Comparison between gelatin filter sampler and Andersen sampler*

(The time was 10 min)

Method	Facultative anaerobes c.f.u./m³ mean value	Strict anaerobes c.f.u./m³ mean value	No. of samples	Statistical significance: Fac. an.	Statistical significance: Str. an.
Gelatin filter	83	166	9	N.S.*	$P < 0{\cdot}05$
Andersen sampler	89	54	9		

* Not significant.

Table 6. *The proportion of anaerobes in occupied rooms found on 2 different occasions*

Facultative anaerobes	Strict anaerobes: Gram-positive rods: Prop. spp.	Strict anaerobes: Gram-positive rods: Other	Strict anaerobes: Gram-positive cocci
77·8	185·8	27	1·6
65·8	30·6	1·8	0·9

The total yield of bacteria found on aerobic incubation is set to 100.

present. The results are presented in Table 6, and show that anaerobic non-sporing bacteria were present in large numbers in the air of occupied rooms. Their numbers compared with those of the aerobes vary. When the total yield of bacteria when incubated aerobically is set to 100 on each occasion, the strict anaerobes are 214·4 one day (Table 6) and 33·3 the other (Table 6). In both cases, anaerobic propionibacteria account for the great majority of the strict anaerobic bacteria. Other strict anaerobes found were gram-positive rods (not *Propionibacterium* spp.) often *Actinomyces* species, and gram-positive cocci, mostly *Peptococcus* and *Peptostreptococcus* species.

Die-away rate of anaerobic non-sporeforming bacteria in the air

To achieve a high count of airborne bacteria, 5 persons walked in a small ($\approx$ 18 m³) room without ventilation for 10 min. After that 4 persons left the room and consecutive half min samples were taken with gelatin filters. In Fig. 1 (*a*) and (*b*) the results from 3 such experiments are represented. The removal of bacteria from the air corresponded to a die-away rate, K, (Bourdillon, Lidwell & Lovelock, 1948) of 4·5, 4·1 and 5·5 for aerobic bacteria and 4·8, 5·7 and 3·1 for anaerobic propionibacterium sp.

Size distribution of bacteria-carrying particles in occupied rooms

Fourteen 10 min samples were taken with an Andersen sampler in the laboratory and incubated anaerobically. The results are presented in Table 7.

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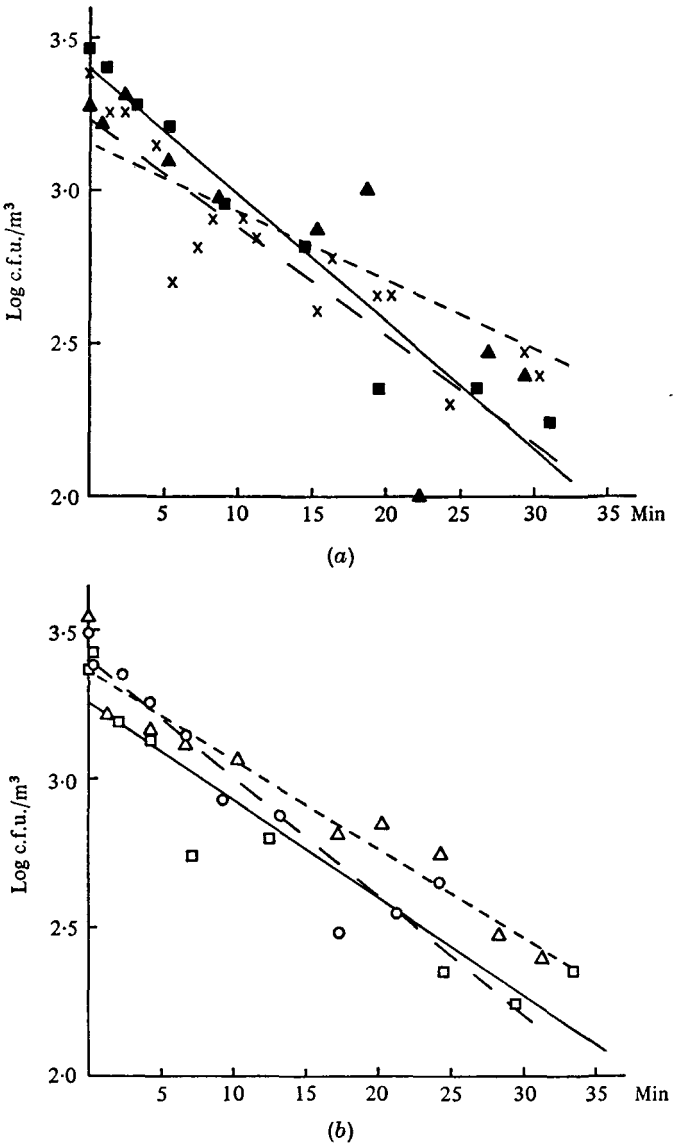

Fig. 1. Die-away rate in air of bacteria generated by humans. (a) Anaerobic bacteria (Propionibacterium sp): – – – ×, Expt. 1, K = 4·8; —— ■, Expt. 2, K = 5·7; - - - - - ▲, Expt. 3, K = 3·1. (*b*) Aerobic bacteria: —— □, Expt. 2, K = 4·5; - - - - - △, Expt. 3, K = 4·1; – – – ○, Expt. 4, K = 5·5.

There was no significant difference between the size of particles carrying facultative and strict anaerobic bacteria, the majority of bacteria falling on the first three stages.

Survival of anaerobic bacteria on floors

In two series of experiments a person walked in an airtight box (3·2 m³) for 5 min. The box was then left closed and samples collected from the floor, in the

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Table 7. *Size distribution of bacteria-carrying particles in occupied rooms*

	Percentage with equivalent diameter larger than				
	7·0 μm	4·7 μm	3·3 μm	2·1 μm	1·1 μm
Facultative anaerobes	35·7	59·4	77·3	90	97·9
Strict anaerobes	24·8	56·6	81·6	87·9	95·3

Table 8. *Survival of anaerobic bacteria on floors*

(*a*)

	Samples collected after			
	72 h	72 h	96 h	96 h
Total no. when inc. aerobically (C.f.u./plate, mean value)	21	46·4	18·1	15·4
Total no. when inc. anaerobically (C.f.u./plate, mean value)	32·9	52·8	23·1	13·3
Strict anaerobes (C.f.u./plate, mean value)	17	19·5	10·1	3·8
% strict anaerobes of total no. when inc. anaerobically	52	37	44	29

(*b*)

	Samples collected after					
	1 h	5 h	24 h	48 h	72 h	96 h
Total no. when inc. aerobically (C.f.u./plate, mean value)	32	35·5	17·5	9·3	8·3	6·8
Total no. when inc. anaerobically (C.f.u./plate, mean value)	27·3	23·5	24·8	6·3	7·8	5·5
Strict anaerobes (C.f.u./plate, mean value)	8·5	5	12·5	1·5	2	2
% strict anaerobes of total no. when inc. anaerobically	31	21	50	24	26	36

first series after 72 h and in the second after 96 h. For sampling 8 Rodac plates were used for aerobic and 8 for anaerobic incubation.

In one experiment samples were taken 1, 5, 24, 48, 72 and 96 h after the walk in the box. Each sampling was made with four Rodac plates for aerobic and four for anaerobic incubation. It was possible to remain outside the box when sampling and long gloves covering hands and arms were used to avoid dispersal of bacteria during sampling. Two sedimentation plates were exposed from one sampling to the next to estimate airborne contamination during the experiment. The number of bacteria found on these was less than 1 c.f.u. per plate.

As shown in Table 8*a* a high number of strict anaerobes could be found on the floor 72 and 96 h after dispersal. In both series of experiments the strict anaerobes

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formed 30–50 % of the total number of bacteria found. The majority of the anaerobes were *Propionibacterium* spp.

Table 8*b* summarizes the last experiment. The total number of aerobic bacteria was reduced from 32 c.f.u./plate to 6·8 c.f.u./plate between 1 and 96 h. Strict anaerobes were reduced from 8·5 to 2 c.f.u./plate during the same time period. The greatest loss seems to have been between 24 and 48 h. The total number of bacteria found was lower in this experiment than in the other owing to the fact that a different person was used as a disperser.

DISCUSSION

The main source of airborne aerobic bacteria is the human skin (Noble *et al.* 1976). In spite of the fact that a large proportion of skin bacteria are anaerobic the presence of anaerobic non-sporeforming bacteria in the air has been very little studied. The present investigation shows that anaerobic non-sporeforming bacteria can be found in the air in large amounts. The majority of the bacteria found are *Propionibacterium* spp.

In a comparison between three air samplers, a Sartorius membrane filter sampler with gelatin or cellulose filter, a Casella slit sampler and an Andersen sampler, it was found that the membrane filter sampler with gelatin filter gave the highest yield of anaerobic bacteria. As shown in Table 3 the yield of anaerobic bacteria when cellulose filters were used was 72 % of that when gelatin filters were used. When the Casella or Andersen sampler was used the yield was 45 and 33 %, respectively, of that when the membrane filter sampler was used with gelatin filters.

The reason for the bad results with the Casella and Andersen samplers is not understood. It is not due to a general difference in sampling efficiency as the yield of facultative anaerobes was about the same with all sampling methods. Drying or exposure to air of the medium may be of some importance. The main difference between the filter sampler and the two others is that air is drawn direct onto the medium when sampling with the Casella or Andersen sampler, whereas the filter is placed on fresh anaerobic medium after the sampling period. The effect of the air, however, must be very rapid as neither minimizing the sampling volume nor shortening the sampling time could be shown to have any effect when sampling with the Casella sampler.

The Sartorius membrane filter sampler is very convenient for taking samples in operating rooms as the filter holders can be sterilized and placed in the immediate vicinity of the operation wound. A drawback, however, is that the air volume sampled is rather small, about 45 l/min, which gives few bacteria in highly ventilated rooms, It would therefore be an advantage if sampling could be made for a longer time than a few minutes. In a series of experiments contaminated air was therefore sampled with the filter sampler for half a min and after that sterile air was sampled for another 9·5 min. This reduced the recovery of anaerobes from the gelatin filter by about 30 % but had no effect when sampling with cellulose filters.

In one series of experiments the die-away rate of anaerobic bacteria in the air was studied. It was found to be the same as for aerobic bacteria. Consistent with this were the findings with the Andersen sampler where the size distribution of particles carrying facultative and strict anaerobes was the same. The se results indicate that anaerobic bacteria are carried on the same type of material as aerobic bacteria, i.e. skin scales. It also shows that airborne transmission is as plausible a route of infection in the operating room for these bacteria as for aerobic bacteria. Although peptococci and peptostreptococci were found in the air their number was too small to make it possible to draw any valid conclusions concerning their survival in the air. The survival of *Propionibacterium* spp. on the floor was as good as the survival of aerobic bacteria at least up to 96 h. This indicates the possibility of indirect transfer of these bacteria as well. It is thus still an open question whether postoperative anaerobic infections after hip surgery are endogenous or exogenous. An investigation on transfer of anaerobes in the operating room has therefore now started.

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