Possible role of the anaerobe in tonsillitis

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SUMMARY Anaerobic bacteria were isolated from all tonsils removed from children at routine tonsillectomy; 75.6% of specimens yielded moderate to heavy growth and 80% of tonsils contained more than one anaerobic species. This recovery rate fell to 56% after a 10-day course of metronidazole before tonsillectomy—in only 14.6% of cases were anaerobes isolated in significant numbers. Surface swabbing of the tonsils permitted recovery of a similar spectrum of anaerobic bacteria but resulted in an overall loss of both aerobic and anaerobic pathogens. A comparison was made between the flora of acutely inflamed tonsils and “healthy” tonsils: over 90% of both groups yielded anaerobic bacteria, but they were present in significant numbers in 56.2% of swabs taken from acutely inflamed tonsils compared with 24% of swabs from “healthy” children. The isolation rate for aerobic pathogens was 37.5% and 16% respectively.

In many cases of acute and recurrent acute tonsillitis, a microbiological diagnosis is not made. One possible explanation is that the bacteria sampled by the surface swabbing technique are not an accurate reflection of the flora of the tonsillar tissue.¹ Alternatively laboratory isolation techniques may be incriminated. It has been suggested that hitherto unrecognised penicillin-sensitive bacteria cause non-streptococcal tonsillitis.² The aetiological role of anaerobic bacteria has received little attention. The aim of this study was to establish the detailed anaerobic bacteriology of children's tonsils at various pathological stages, in an attempt to delineate a pattern of causal relationship.

Patients and methods

PATIENTS

Whole tonsils removed at routine tonsillectomy by two of us (AGB and PT) were submitted for bacteriological examination. A total of 41 specimens was examined, all from children with a past history of uncomplicated recurrent tonsillitis. At the time of removal the tonsils were not clinically inflamed. With their parents' consent, 41 more children were given oral metronidazole (7.5 mg/kg three times a day) for 10 days before tonsillectomy, after which the excised tonsils were cultured in an identical manner. A comparable group of 37 children aged 3-12 years, who had been referred to the ear, nose,

and throat department with histories of recurrent acute tonsillitis, was selected for tonsillar swabbing. With the aid of the light of a head-lamp, sterile cotton wool swabs were rubbed over the tonsillar surfaces, care being taken to avoid oral contamination. The swabs were transported to the laboratory in Stuart's transport medium.

Tonsillar swabs were similarly taken by AGB and PT from 25 children, aged 2-17 years, who were referred to the ENT department for problems unrelated to their tonsils (controls), and from 16 children, aged 2-18 years, with a clinical diagnosis of acute tonsillitis.

BACTERIOLOGICAL CULTURE MEDIA

Prepared horse blood agar and chocolate agar plates (Lab M, Salford, Lancs) were chosen as the aerobic culture media. A selective medium for *Corynebacterium diphtheriae* was not used. The basal medium for the anaerobic blood agar plates comprised Columbia agar base plus yeast extract 0.5% wt/vol to which were added the following: haemin (BDH Ltd) 0.005 g/l; menadione (Sigma Ltd) 0.0005 g/l; L-cysteine (BDH Ltd) 0.3 g/l; horse blood (Oxoid Ltd) 5% v/v. The selective medium initially chosen included vancomycin 0.0075 g/l and kanamycin 0.075 g/l. Neomycin 0.075 g/l was later substituted for kanamycin after the results of preliminary experiments which indicated a low recovery rate of *Fusobacterium* spp with the vancomycin-kanamycin combination. The plates for anaerobic culture were freshly poured as required.

sealed in plastic bags, and their surfaces dried before use.

VIRUS CULTURE

It was not possible to perform virus culture on the majority of specimens. However, 20 of the tonsils from children with recurrent tonsillitis were inoculated on to three cell lines: monkey kidney, HEP2, and MRC5.

PREPARATION OF SPECIMENS

The tonsils were transported in sterile universal containers with the minimum of delay from theatre to the laboratory. Transport medium was not used. Each tonsil was sectioned with a sterile scalpel and impression smears of the cut surface were stained by the methods of Gram and Leishman. The tissue was then chopped with scissors to a very fine consistency and a standard loopful inoculated on to each of the four bacteriological media. Preliminary investigations had shown that washing the tonsils in sterile saline was an unnecessary procedure. Smears were likewise prepared from the tonsillar swabs, which were then inoculated on to the plates in the routine manner.

INCUBATION OF CULTURES

The aerobic blood agar plates were examined after overnight incubation at 37°C, as were the chocolate plates which were incubated in candle jars. All anaerobic plates were incubated in 10-litre anaerobic jars (Don Whitley Scientific) fitted with freshly activated palladium catalyst, which were evacuated to an internal pressure of 440 mm Hg and the vacuum replaced with a gas mixture of 10% CO₂, 90% H₂. Growth was first seen after 48 hours' incubation and was inspected daily for a total of six days.

IDENTIFICATION OF BACTERIA

Aerobic bacteria were identified according to the methods of Cowan (1974).³ All anaerobic isolates were identified to species level as far as possible using the biochemical criteria and gas-liquid chromatographic patterns of Holdeman *et al.*⁴

MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATIONS

In view of the fact that the majority of strains of *Bacteroides melaninogenicus* recovered from the children's tonsils were resistant on disc testing to 1 unit of benzylpenicillin, the MICs of penicillin and ampicillin were determined for 38 of the isolates. A suspension of each organism was prepared in nutrient broth to a concentration of 2×10^4 cells/ml, from which 1 μ l was applied to the surface of well-dried, freshly prepared blood agar plates con-

taining antibiotic concentrations ranging from 0.003-32.0 μ g/ml. The lowest concentration of penicillin and ampicillin preventing colony formation after incubation in an anaerobic jar at 37°C for 48 hours was taken as the MIC for that strain. A strain of *Staphylococcus aureus* of known MIC was included in each set of tests.

The production of penicillinase by a few of these strains was assessed using Intralactam strips (Mast Laboratories Ltd, Bootle, Merseyside). No attempt was made to induce penicillinase by growing the strains of *B. melaninogenicus* in the presence of penicillin before testing them by the strip method.

Results

STAINED FILMS OF SPECIMENS

In general, the Gram and Leishman stained smears were unhelpful: stainable material bore little correlation to subsequent bacteriological findings. A notable exception was a case of Vincent's stomatitis, presenting as a painful oropharynx, in which tonsillar swabs stained with carbol fuchsin showed an abundance of fusobacteria and spirochaetal forms.

CULTURE OF WHOLE TONSILS

Aerobic pathogens

A semiquantitative assessment of bacterial growth was made in terms of +++ (heavy) to $\pm$ (scanty). β -haemolytic streptococci of Lancefield's groups A, C, or G were isolated in significant numbers from 15 out of 41 tonsils (36.6%), and a heavy to moderate growth of *Haemophilus influenzae* was recovered from 14 out of 41 tonsils (34.1%). There were no isolates of *Streptococcus pneumoniae*. *Staph. aureus* was isolated in large numbers from seven specimens (17%).

Anaerobic isolates (Table 1)

Eighty per cent of the tonsils contained more than one anaerobic species. By using a 5 μ g metronidazole disc on the non-selective anaerobic plate, the heavy growth of anaerobes was readily discernible from the large zone of inhibition. Simultaneous inspection of the neomycin-vancomycin plates facilitated the recovery of individual anaerobic species. After six days' incubation, anaerobes were isolated from 100% of the tonsils. There was moderate to heavy anaerobic growth in 75.6% of specimens.

B. melaninogenicus was the most frequently isolated anaerobic bacterium, being present in 95% of the tonsils examined. In 46% of these specimens, more than one colonial type of *B. melaninogenicus* was detectable. Full identification of some isolates was impossible, but the majority belonged to the two

Table 1 A summary of the anaerobic bacteria isolated from the five main groups of specimens

Anaerobe	No. of specimens				
	Tonsils (41)	Tonsils after 10 days metronidazole (41)	Tonsil swabs (recurrent tonsillitis) (37)	Tonsil swabs (acute tonsillitis) (16)	Tonsil swabs (controls) (25)
<i>Bacteroides melaninogenicus</i> ss <i>melaninogenicus</i> and ss <i>intermedius</i>	50	18	34	19	16
<i>Bacteroides melaninogenicus</i> ss unidentified	12	10	10	4	10
<i>Bacteroides</i> spp (unidentified)	28	7	19	7	7
<i>B. ruminicola</i> ss <i>ruminicola</i>	3	0	0	0	0
<i>Fusobacterium</i> spp	22	6	10	5	16
<i>Leptotrichia buccalis</i>	2	0	0	0	0
<i>Veillonella parvula</i>	0	0	2	2	0
<i>Peptococcus/peptostreptococcus</i> spp	7	0	1	0	0
<i>Eubacterium</i> spp	5	0	9	2	6
<i>Clostridium</i> spp	0	0	0	0	0
<i>Bifidobacterium</i> spp	2	0	0	0	0
Total	131	41	85	39	55
Anaerobic bacteria isolated per specimen	3.2	1.0	2.3	2.4	2.2

subspecies *melaninogenicus* and *intermedius*. Of considerable interest was the observation that many of the isolates were resistant to 1 unit penicillin discs. Subsequent MIC determinations on 18 of these strains showed that 14 (77.8%) had an MIC for penicillin ≥ 4.0 $\mu\text{g/ml}$. Penicillinase production could not be detected by the strip method.

There were 31 isolates of other *Bacteroides* spp. Species identification was inconclusive in most cases, but there were three isolates of *B. ruminicola* ss *ruminicola*. *Fusobacterium nucleatum* accounted for 50% of the 22 fusobacteria isolated. Most of the tonsils which grew fusobacteria contained a heavy growth of more than one species. *F. necrophorum* and *F. naviforme* were isolated from three specimens, the remainder were unidentified. There were two isolates of *Leptotrichia buccalis*. There was a low isolation rate of anaerobic cocci (17.1%). This could be attributed to the nature of the selective medium which was chosen deliberately to suppress the growth of the facultative, Gram-positive flora of the oropharynx. *Peptococcus magnus* was the commonest isolate. Five strains of *Eubacterium* spp were cultured from the tonsils, but no clostridia were isolated. Bifidobacteria were recovered from two specimens.

Virus culture

No viruses were isolated from the 20 specimens examined.

TONSILLAR FLORA AFTER 10-DAY COURSE OF METRONIDAZOLE

Aerobic pathogens (Table 2)

β -haemolytic streptococci of Lancefield's groups A, C, or G were recovered from eight out of 41 tonsils (19.5%), *H. influenzae* was isolated from 34.1%, and

Table 2 Effect of metronidazole on the aerobic flora of the tonsils

	No. of specimens		
	Untreated tonsils (%)	Tonsils after metronidazole (%)	Total (%)
Aerobic pathogens	25 (61)	29 (70.7)	54 (65.9)
No significant aerobic pathogens	16 (39)	12 (29.3)	28 (34.1)

No statistically significant difference.

a heavy growth of *Staph aureus* was grown from 24.4%. No pneumococci were isolated.

Anaerobic isolates (Table 3)

Anaerobic bacteria were recovered from only 56% of children's tonsils after a prior course of metronidazole, and they were present in scanty amounts. There was a significant growth (+ + + + +) of anaerobes from only six tonsils, accounting for 14.6% of the specimens. *B. melaninogenicus* was recovered from 91.3% of those tonsils with any anaerobic flora. Penicillin resistance was again

Table 3 Effect of metronidazole on the anaerobic flora of the tonsils

	No. of specimens		
	Untreated tonsils (%)	Tonsils after metronidazole (%)	Total (%)
Anaerobes	41 (100)	23 (56.1)	64 (78.1)
	31* (75.6)	6* (14.6)	
No anaerobes	0	18 (43.9)	18 (21.9)

*Good growth (+ + + + +).

$\chi^2 = 20.57$, $p < 0.001$.

Considering good* anaerobic growth only: $\chi^2 = 31.24$, $p < 0.001$.

observed and the MICs of six strains tested ranged from 4.0-16.0 µg/ml for penicillin and 4.0- > 32.0 µg/ml for ampicillin.

Seven strains of other *Bacteroides* spp were isolated but only three were in large numbers. There were six isolates of *Fusobacterium* spp; two were identified as *F gonidiaformans*. No other anaerobic species was recovered.

FLORA OF TONSILLAR SWABS FROM CHILDREN WITH HISTORIES OF RECURRENT TONSILLITIS

Table 4 indicates the impaired recovery of both aerobes and anaerobes from tonsillar swabs compared with the bacterial flora of whole tonsils.

Table 4 Comparison of aerobic and anaerobic bacterial isolation rates from tonsil tissue and surface swabbing. (Children in both groups had histories of chronic recurrent tonsillitis)

	No. of specimens		
	Whole tonsils (%)	Tonsil swabs (%)	Total (%)
Aerobic pathogens	25 (61)	9 (24.3)	34 (31)
Anaerobic bacteria	41 (100)	35 (94.6)	76 (69)
	31* (75.6)	20* (54)	

*Good growth (+ +/+ + +).
No statistically significant difference.

Aerobic pathogens

Six out of 37 specimens yielded β-haemolytic streptococci of Lancefield's groups A, C, or G (16.2%), there were only three isolates of *H influenzae* (8.1%) and *Staph aureus* was not cultured in large amounts from any of the specimens. A significant growth of *Strep pneumoniae* was obtained from one tonsil.

Anaerobic isolates

Anaerobic bacteria were isolated from 35 out of 37 tonsillar swabs (94.6%), 54% of the specimens yielded more than one anaerobic species, and 81% contained *B melaninogenicus*. Good growth (+ +/+ + +) of *B melaninogenicus ss melaninogenicus* and *ss intermedius* accounted for 34% of these strains. The penicillin MICs of those strains that were resistant on disc testing to 1 unit of penicillin ranged from 0.5- > 32.0 µg/ml.

There were 19 isolates of other species of bacteroides and 10 *Fusobacterium* spp. *Veillonella parvula* was recovered from two of the swabs and there was a single isolate of a peptostreptococcus. Nine strains of *Eubacterium* spp were isolated, some of which were identified as *E aerofaciens*.

FLORA IN ACUTE TONSILLITIS

Aerobic pathogens

A heavy growth of β-haemolytic streptococci of Lancefield's groups A, C, or G was obtained from 31.2% of the specimens, *H influenzae* was isolated from two out of 16 samples (12.5%), and there was one isolate of a significant growth of *Strep pneumoniae* (6.2%).

Anaerobic isolates

Anaerobic bacteria were isolated from 93.7% of these swabs, of which 66.7% contained more than one anaerobic species. Once again, *B melaninogenicus* was the most prevalent anaerobe, present in 100% of specimens yielding an anaerobic flora and 60% of the isolates were in large numbers.

Other *Bacteroides* spp were recovered from seven out of 16 specimens, and a single *Fusobacterium* sp was recovered from five swabs. Vincent's organisms were seen in a carbol fuchsin-stained smear of material from an adolescent, but no fusobacteria were cultured. There were two isolates each of *Veillonella parvula* and *Eubacterium* spp.

FLORA OF "HEALTHY" TONSILS

Table 5 compares the bacteriology of "healthy" tonsils with that of the acutely inflamed state.

Table 5 Comparison of aerobic and anaerobic bacterial isolation rates from tonsil swabs in acute tonsillitis and from "healthy" children

	No. of specimens		
	Acute tonsillitis (%)	Controls (%)	Total (%)
Aerobic pathogens	6 (37.5)	4 (16)	10 (21)
Anaerobic bacteria	15 (93.7)	23 (92)	38 (79)
	9* (56.2)	6* (24)	

*Good growth (+ +/+ + +).
No statistically significant difference.

Aerobic pathogens

Pyogenic streptococci were recovered from only two of the 25 specimens (8%); there was an identical recovery rate for *H influenzae*. No *Strep pneumoniae* was isolated. A heavy growth of *Staph aureus* was present in one specimen.

Anaerobic isolates

It was possible to grow anaerobic bacteria from 92% of the swabs, but they were present in large numbers in only six specimens (24%). More than one anaerobic species was recovered from 64% of the swabs. As before, *B melaninogenicus* was the

commonest anaerobic isolate, occurring in 95.6% of those specimens with an anaerobic flora, but only 27.3% of the isolates were in large numbers.

There were seven isolates of other *Bacteroides* spp and 16 isolates of *Fusobacterium* spp. Six specimens yielded *Eubacterium* spp.

MICS

Table 6 shows the sensitivities of 38 strains of *B melaninogenicus* selected from those isolated from the children's tonsils. Overall, 52.6% of the strains were inhibited by 4 µg penicillin/ml and 50% were inhibited by 4 µg ampicillin/ml. However, 63.1% of these tonsillar isolates were not inhibited by ≥ 4 µg penicillin/ml and 57.9% possessed MICs ≥ 4 µg ampicillin/ml.

Discussion

It is well recognised that anaerobic bacteria are abundant among the indigenous flora of the oropharynx.^{5,6} Their pathogenic potential is realised in a variety of localised clinical disorders: alveolar abscesses,⁷ peritonsillar abscesses,⁸ cervical adenitis,⁹ otitis media and mastoiditis.^{10,11} The oropharynx may thence act as the portal of entry for an ensuing anaerobic bacteraemia and distant sepsis.^{12,13,14} Nevertheless, despite the recognised association between anaerobic bacteria and oropharyngeal sepsis, there is a paucity of microbiological work detailing the aerobic and anaerobic flora of the tonsils in health and disease. Our study was done to assess the importance of anaerobes in tonsillar pathology.

Two major factors facilitated the recovery of a wide range of anaerobic bacteria: the use of neomycin-vancomycin as a selective medium to avoid overgrowth by facultative streptococci, and prolonged anaerobic incubation.¹⁵ It is appreciated that a similar spectrum of anaerobic bacteria may be cultured from saliva (personal observation), hence at all times tonsillar swabbing was performed with good illumination to reduce contamination of specimens with the microflora of the oropharynx. Our results confirm the observation of previous workers^{1,16} that the conventional technique of sampling the surface flora of tonsils is associated with an impaired recovery rate of both aerobic and

anaerobic pathogens. Nevertheless, in view of the fact that the only two bacterial species isolated solely from the macerated tonsils were *Leptotrichia buccalis* and *Bifidobacterium* spp, both of doubtful pathogenic potential, it was considered that the bacteriology of a well-taken tonsillar swab provided a satisfactory indication of the deep flora.

Sprinkle and Veltri¹⁷ showed that the tonsils of children suffering from recurrent adenotonsillitis had an abnormal oro- and nasopharyngeal flora, which reverted to a non-pathogenic microflora after adenotonsillectomy. In the absence of surgical intervention, a complexity of factors must be responsible for the perpetuation of this abnormal bacteriology. In our study all the tonsils excised from children with past histories of recurrent tonsillitis contained anaerobic bacteria. They were recovered in much lower numbers from healthy children. There may be two explanations for this difference: that once tonsillar tissue has been subjected to repeated infections (possibly viral), a suitably anaerobic microenvironment is created which favours the proliferation of endogenous oral flora; or alternatively that anaerobic bacteria are the prime pathogens. Administration of metronidazole, a bactericidal antibiotic with specific activity against obligate anaerobes and capable of good tissue penetration, led to a statistically highly significant reduction in the anaerobic flora of the tonsils. This raises the interesting possibility of an alternative treatment for tonsillitis—if the anaerobic bacteria are abolished, subsequent tonsillar pathology may be averted.

Pre-eminent among the anaerobic isolates was *B melaninogenicus*, the prevalence of which in the tonsillar habitat was recognised as early as 1921 by Oliver and Wherry.¹⁸ The isolation rate of this species from the gingival crevice seems to be related to age: figures vary from around 20% for children up to 12 or 13 years¹⁹ to 40% for 5 to 7 year olds.²⁰ However, in our work, *B melaninogenicus* was isolated in moderate numbers (++) growth) from the tonsils of children as young as 2 years. Research into the antecedent drug histories of many of these children showed an alarming frequency of prescriptions for various penicillin derivatives; this probably explains the high percentage of strains resistant to penicillin. Other workers have remarked upon the diminishing sensitivity of *B melaninogenicus* to

Table 6 Sensitivity of *B melaninogenicus* to penicillin and ampicillin

Antibiotic	No. of strains	Cumulative % strains inhibited by (µg/ml)												
		0-007	0-015	0-03	0-06	0-125	0-25	0-5	1	2	4	8	16	32
Penicillin	38	2.6	10.5	18.4	21	—	—	23.6	31.5	36.8	52.6	73.6	97.3	100
Ampicillin	38	—	7.8	13.1	21	—	26.3	31.5	36.8	42.1	50	57.8	68.4	100

penicillin;^{21 22 23} if penicillin continues to be prescribed for tonsillitis, the tonsils may become a naedus of penicillin-resistant bacteria. Further studies are in progress to test the hypothesis that anaerobic bacteria play a significant role in the aetiology of acute and recurrent tonsillitis.

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