An unclassified *Eubacterium* taxon in acute dento-alveolar abscess

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Summary. The microflora of pus samples aspirated from 50 acute dento-alveolar abscesses was examined. A total of 143 bacterial strains was isolated, consisting predominantly of *Prevotella* spp., α -haemolytic *Streptococcus* spp., *Peptostreptococcus* spp. and *Eubacterium* spp. An unclassified asaccharolytic *Eubacterium* taxon was encountered in 17 (34%) of the abscesses. This taxon was found to have a positive association with *Fusobacterium* spp. and a negative association with α -haemolytic *Streptococcus* spp.

Introduction

The microbial flora of acute dento-alveolar abscess was traditionally thought to consist of facultative streptococci and staphylococci, either alone or in combination.^{1,2} However, aspiration sampling of abscesses rather than the use of swabs revealed that the organisms previously reported were likely to represent contaminants rather than to be a true reflection of the abscess microflora.³ More recently, the use of improved methods of anaerobic incubation and identification techniques for slow growing, non-reactive strict anaerobes has revealed the frequent presence of *Peptostreptococcus* spp., *Prevotella* spp. and *Fusobacterium* spp. in acute dento-alveolar abscesses.^{4,5}

Asaccharolytic *Eubacterium* spp. are strictly anaerobic gram-positive bacilli which, in the past, have been difficult to culture and identify. *Eubacterium* spp. are being implicated increasingly in chronic periodontal disease^{6–8} and have been found to account for up to 50% of the anaerobic flora in subgingival plaque.⁹ These organisms have been isolated also from carious dentine¹⁰ and necrotic root canals^{11,12} but little information is available concerning their presence in suppurative periapical infection. The uncertainty of the role of these organisms in dental disease may be due in part to difficulties with the taxonomy of the group since a variety of unnamed taxa have been described.^{7,9,13,14}

The aim of the present study was to determine the prevalence of asaccharolytic *Eubacterium* spp. in acute suppurative dento-alveolar infection and to detect any microbial associations between these organisms and other members of the abscess microflora.

Materials and methods

Patients with acute dento-alveolar abscess attending the Examination and Emergency Clinic of Cardiff Dental Hospital and School were included in the study. Pus specimens were obtained by needle aspiration and transferred promptly to the laboratory for processing. Pus was inoculated on to blood agar plates (Blood Agar Base No. 2, Lab M, Bury, supplemented with sheep blood, TCS Biological Ltd, Buckingham, 5%), chocolate agar and Fastidious Anaerobe Agar (Lab M) supplemented with sheep blood 5% which were incubated in air, in air plus CO₂ 5% and in an anaerobic cabinet (N₂ 80%, CO₂ 10%, H₂ 10%), respectively. Aerobic growth was assessed after 24 and 48 h and plates incubated anaerobically were examined daily for 7 days in all. Representatives of morphologically different colony types were sub-cultured for pure growth and identified to genus level by conventional methods.¹⁵ Strains of asaccharolytic *Eubacterium* spp. were identified according to enzyme and protein profiles combined with analysis of metabolic end-products.¹³

The association between asaccharolytic *Eubacterium* spp. and other bacterial isolates was assessed by calculation of odds ratio as described by Socransky *et al.*¹⁶

Results

Pus samples were obtained from 50 patients, of whom 23 were being treated with an antibiotic at the time of sampling. A total of 142 bacterial strains was isolated with a mean of 2.9 species/abscess. The predominant isolates were *Prevotella* spp. (31 strains), α -haemolytic *Streptococcus* spp. (30 strains), *Pepto-*

Table I. Bacterial species isolated from 50 acute dento-alveolar abscesses

Bacterial species	Number of isolates	Number of isolates from patients on	
		no antibiotic (n = 27)	antibiotic (n = 23)
α -Haemolytic <i>Streptococcus</i> spp.	30	16	14
Non-haemolytic <i>Streptococcus</i> spp.	3	2	1
<i>S. anginosus</i>	1	0	1
<i>Actinomyces</i> spp.	4	0	4
<i>Peptostreptococcus</i> spp.	29	14	15
<i>Eubacterium</i> C ₂	17	7	10
<i>E. brachy</i>	1	1	0
<i>E. timidum</i>	1	1	0
Saccharolytic <i>Eubacterium</i> spp.	9	8	1
Unidentified gram-positive bacillus	1	1	0
<i>Veillonella</i> spp.	7	4	3
<i>Prevotella</i> spp.	31	21	10
<i>Fusobacterium</i> spp.	8	6	2
Total	142	81	61

Table II. Average odds ratios of association between *Eubacterium* C₂ and other species isolated from dento-alveolar abscesses

Organism	Odds ratio
α -Haemolytic <i>Streptococcus</i> spp.	0.4*
Non-haemolytic <i>Streptococcus</i> spp.	0.92
<i>S. anginosus</i>	0
<i>Actinomyces</i> spp.	0
<i>Peptostreptococcus</i> spp.	0
<i>Eubacterium brachy</i>	0
<i>E. timidum</i>	0
Saccharolytic <i>Eubacterium</i> spp.	0
Unidentified gram-positive bacillus	0
<i>Veillonella</i> spp.	0
<i>Prevotella</i> spp.	1.09
<i>Fusobacterium</i> spp.	12.5†

* Negative association < 0.5.

† Positive association > 2.0.

streptococcus spp. (29 strains) and *Eubacterium* spp. (28 strains) (table I). The spectrum of bacteria isolated from patients with no history of antibiotics in the preceding 3 months was similar to that found in patients who had received antibiotics during the preceding 3 months or were taking an antibiotic at the time of sampling.

The *Eubacterium* spp. isolated were slow growing, usually only appearing after incubation for 4 days but then often becoming the most numerous organism by 7 days. Of the 28 strains of *Eubacterium* spp., 17 belonged to the putative *Eubacterium* spp. Cluster 2 (C₂) described by Wade *et al.*¹³

Analysis of odds ratios revealed that *Eubacterium* C₂ had a positive correlation with *Fusobacterium* spp. and a negative correlation with α -haemolytic *Streptococcus* spp. (table II).

Discussion

It is now generally accepted that the microflora of acute dento-alveolar abscesses usually contains a

combination of facultative streptococci and strictly anaerobic bacteria. In recent years, improved methods of incubation and identification of strict anaerobes have revealed that *Peptostreptococcus* spp. and *Prevotella* spp. are frequently encountered in suppurative dental infections. The results of the present study suggest that the newly described taxon *Eubacterium* C₂ is also frequently present in pus aspirated from acute dento-alveolar abscess. It is possible that previous studies of acute suppurative dental infections failed to isolate these organisms either because of short periods of incubation or problems associated with identifying non-reactive anaerobic gram-positive bacilli. *Eubacterium* C₂ resembles *E. lentum* which is also generally non-reactive in biochemical tests. Isolates described as *E. lentum* have frequently been cultured from the root canals of teeth.^{11,12} The two species may be distinguished by culture on FAA, as this medium contains arginine which stimulates the growth of *E. lentum*, giving rise to relatively large colonies, whereas colonies of *Eubacterium* C₂ remain < 1 mm in diameter even after prolonged incubation.⁸

Analysis of the microbiological findings of the present study revealed a positive association between *Eubacterium* C₂ and *Fusobacterium* spp. Therefore, it is possible that these two species may interact to give enhanced pathogenic potential to the abscess flora similar to the interactions shown experimentally for other combinations of gram-positive and gram-negative strict anaerobes isolated from acute dento-alveolar abscesses.¹⁷ The negative association with α -haemolytic streptococci is interesting and could be interpreted as supporting the proposal that the mixed flora of acute dento-alveolar abscess is initially predominantly facultative and becomes progressively strictly anaerobic with time.

In conclusion, the use of prolonged anaerobic incubation and techniques capable of identifying strictly anaerobic gram-positive bacilli have revealed

that *Eubacterium* C₂ taxon is frequently present in the flora of acute dento-alveolar abscesses. Further studies

are required to provide information on the role of this group and other *Eubacterium* spp. in dental disease.

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