Quantitative bacteriology of acute dento-alveolar abscesses

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Summary. A qualitative and quantitative bacteriological study was performed on pus specimens obtained by needle aspiration of 50 acute dento-alveolar abscesses. Most samples contained a mixture of species (average 3.3); 20 (40%) of the abscesses contained anaerobes alone, 3 (6%) contained facultative anaerobes only and the remaining 27 (54%) contained mixtures of both types of bacteria, with anaerobes predominating. In total, 166 bacterial strains were isolated, 75% of which were strictly anaerobic; the most common species were *Peptococcus* spp, *Bacteroides oralis* and *B. melaninogenicus*. Among facultative anaerobes, *Streptococcus milleri* was particularly common. The mean concentration of bacteria in each abscess was $10^{6.9 \pm 0.2}$. The mean concentration of anaerobic bacteria was $10^{6.2 \pm 0.1}$ and of facultatively anaerobic bacteria $10^{5.7 \pm 0.2}$.

Introduction

Most acute dento-alveolar abscesses (periapical abscess, apical abscess, submucous abscess) are polymicrobial infections containing a variety of facultative and strictly anaerobic bacteria. However the identity of the bacteria isolated varies considerably. “*Streptococcus viridans*” was most commonly reported in the early studies (Alin and Aagren, 1954; Feldmann and Larje, 1966) while recent reports have described an increase in the isolation of strictly anaerobic bacteria and the occurrence of abscesses with a purely anaerobic flora (Sabiston *et al.*, 1976; Aderhold *et al.*, 1981; Oguntebi *et al.*, 1982). The differences between the early and the more recent reports are probably due to improved sampling and isolation techniques. Although it is now well recognised that strict anaerobes predominate in dento-alveolar abscesses, there is little quantitative information on the composition of the overall microbial load, and the present study was undertaken to provide such data. Quantitative information is required to further understanding of the pathogenesis of mixed infection, which has implications for treatment.

Materials and methods

Specimens

Pus samples were obtained from adult patients presenting with acute dento-alveolar abscess at the Casualty Receiving Clinic of Glasgow Dental Hospital and School.

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Patients had received no antibiotic therapy in the preceding 3 months and had no known concurrent illness. Case selection was limited to periapical abscesses that had extended through the alveolar bone to produce fluctuant intra-oral swelling. Abscesses were excluded if there was any evidence of a communication into the oral cavity through a sinus tract or the gingival margin.

Sampling

Patients rinsed their mouths with chlorhexidine 0.2% solution for 30 s. The abscesses were then isolated with sterile cotton wool rolls and the overlying mucosa was wiped with a cotton wool bud soaked in chlorhexidine 0.2% solution. Surface anaesthesia was achieved by use of a fine ethyl chloride jet. Pus was obtained by aspiration with a 21-gauge needle on a 5-ml disposable syringe and was transferred to the laboratory for immediate processing.

Culture

Each sample (0.5 ml) was diluted in 19.5 ml of Anaerobic Blood Broth (Gibco) and mixed by mechanical agitation (Whirlmixer, Fisons Scientific Ltd, Loughborough, Leicestershire) under anaerobic conditions for 30 s. Further dilutions of 1 in 800, 1 in 1600 and 1 in 16 000 were prepared in Anaerobic Blood Broth. Duplicate 50- μ l volumes of each dilution were inoculated on to Columbia Blood Agar (Oxoid) and modified Campylobacter selective medium (Skirrow, 1977) (Gibco) by use of a spiral plater (Don Whitley Scientific Ltd, Shipley, West Yorkshire). Plates were incubated at 37°C for 7–10 days in an anaerobic chamber (Forma Scientific, USA). Each dilution was similarly plated on to Columbia Blood Agar and incubated at 37°C for 7 days in an atmosphere of air with CO₂ 10%.

Quantification and identification of isolates

Plates containing well separated colonies were selected for study. Different morphological colony types were counted and representatives removed for identification. Initial characterisation of isolates was based on atmospheric requirements, reaction in Gram's stain and microscopic morphology. Facultative anaerobes were identified by standard methods (Buchanan and Gibbons, 1974; Cowan, 1974; Hardie and Bowden, 1974; Slack and Gerencser, 1975; Killian 1976; Sutter *et al.*, 1980) including the API 20 system for streptococci.

Anaerobes were identified by the Minitek System (Becton-Dickinson UK Ltd, Oxford) combined with acid end-product analysis by isotachophoresis (Shah *et al.*, 1985). Anaerobic gram-positive cocci were initially differentiated on the basis of susceptibility to a disk containing novobiocin 5 µg (Wren *et al.*, 1977).

Results

A total of 166 isolates was obtained from 50 dento-alveolar abscesses; 123 (74%) of the strains were strict anaerobes and 43 (26%) were facultative anaerobes. The mean number of bacterial species was 3.3 per abscess (range 1–8), and the mean viable bacterial load was $10^{6.9 \pm 0.2}$ (range $10^{4.7}$ – $10^{9.4}$). The identity of the 166 isolates is shown in table I and the

mean concentration values for each group of organisms in table II.

Strict anaerobes were present in 47 (94%) of the abscesses; the anaerobic species most commonly isolated were *Peptococcus* spp., *Bacteroides oralis*, *B. gingivalis* and *B. melaninogenicus*. Facultative anaerobes were present in 30 abscesses, the commonest isolate being *Streptococcus milleri*.

Anaerobic gram-negative bacilli occurred

Table II. Mean concentration of bacterial groups isolated from 50 acute dento-alveolar abscesses

Bacterial group	Mean concentration (log ₁₀ cfu/ml)
Facultative anaerobes	5.7 ± 0.2
Gram-positive cocci	6.1 ± 0.2
Gram-positive bacilli	4.6 ± 0.4
Gram-negative bacilli	5.6 ± 0.4
Strict anaerobes	6.2 ± 0.1
Gram-positive cocci	6.3 ± 0.2
Gram-positive bacilli	5.7 ± 0.8
Gram-negative cocci	5.9 ± 0.7
Gram-negative bacilli	6.1 ± 0.2

Table I. Identity of 166 bacterial strains isolated from 50 acute dento-alveolar abscesses

Facultative anaerobes	Number of isolates	Strict anaerobes	Number of isolates
<i>Streptococcus milleri</i>	25	<i>Peptostreptococcus</i> spp.	14
<i>Streptococcus mitior</i>	3	<i>Peptococcus</i> spp.	32
<i>Streptococcus sanguis</i>	3	<i>Streptococcus intermedius</i>	3
<i>Streptococcus mutans</i>	1	<i>Streptococcus constellatus</i>	1
<i>Lactobacillus fermentum</i>	2	<i>Propionibacterium acnes</i>	1
<i>Lactobacillus salivarius</i>	1	<i>Eubacterium lentum</i>	1
<i>Actinomyces odontolyticus</i>	1		
<i>Actinomyces naeslundii</i>	1		
<i>Actinomyces meyeri</i>	1		
<i>Arachnia propionica</i>	1		
		<i>Veillonella parvula</i>	3
<i>Haemophilus parainfluenzae</i>	2	<i>Bacteroides oralis</i>	20
<i>Capnocytophaga ochracea</i>	1	<i>Bacteroides gingivalis</i>	14
<i>Eikenella corrodens</i>	1	<i>Bacteroides melaninogenicus</i>	12
		<i>Bacteroides intermedius</i>	5
		<i>Bacteroides ruminicola</i>	6
		<i>Bacteroides distasonis</i>	1
		<i>Bacteroides ureolyticus</i>	1
		<i>Bacteroides capillosus</i>	1
		<i>Bacteroides uniformis</i>	1
		<i>Fusobacterium nucleatum</i>	6
		<i>Fusobacterium mortiferum</i>	1
TOTAL	43	TOTAL	123

Table III. Bacterial combinations encountered in 50 acute dento-alveolar abscesses

Index group of bacteria	Percentage of abscesses containing, in addition to index group,						
	Facultative anaerobes			Strict anaerobes			
	Gram-positive cocci	Gram-positive bacilli	Gram-negative bacilli	Gram-positive cocci	Gram-positive bacilli	Gram-negative cocci	Gram-negative bacilli
Facultative anaerobes							
Gram-positive cocci	...	6	6	38	2	2	42
Gram-positive bacilli	6	...	2	8	0	0	8
Gram-negative bacilli	6	2	...	8	0	6	6
Strict anaerobes							
Gram-positive cocci	38	8	8	...	6	4	62
Gram-positive bacilli	2	0	0	6	...	0	6
Gram-negative cocci	2	0	0	4	0	...	2
Gram-negative bacilli	42	8	6	62	6	2	...

together with anaerobic gram-positive cocci in 62% of cases and with facultatively anaerobic gram-positive cocci in 42% of cases (table III). The species most commonly involved were *B. oralis* with *Peptococcus* spp. (22% of cases) and *B. oralis* with *S. milleri* (18% of cases). Facultatively and strictly anaerobic gram-positive cocci, most commonly *Peptococcus* spp. and *S. milleri* (24% of cases), were isolated from the same abscess on 19 occasions (38% of cases).

Facultatively anaerobic gram-positive cocci, strictly anaerobic gram-positive cocci and gram-negative bacilli were isolated in the same sample from 15 abscesses (30% of cases).

Twenty abscesses (40%) contained strict anaerobes only and 3 (6%) contained facultative anaerobes alone. The remainder contained both types of organism and, in most of these cases, the flora was predominantly anaerobic (figure). In 72% of the abscesses, strict anaerobes accounted for more than two-thirds of the total flora.

Discussion

Most microbial investigations of dento-alveolar abscesses have reported a mixed flora containing facultatively and strictly anaerobic organisms. This study is no exception and the spectrum of bacterial species isolated was very similar to that described by other recent workers (Alderhold *et al.*, 1981; von Konow *et al.*, 1981; Labriola *et al.*, 1983; Williams *et al.*, 1983). The average number of bacterial species present in each sample, 3.3, compares well with the results of Alderhold *et al.* (1981) and Sabiston *et al.* (1976), who found an average of 3.6 and 3.8 species, respectively.

The different bacterial species present in dental abscesses have rarely been quantified. Sabiston *et al.* (1976) stated that the number of colonies on plates incubated anaerobically appeared higher than those seen on plates incubated aerobically or in CO₂, but precise data were not presented. Williams

Figure. Distribution of abscesses according to percentage of content that was strictly anaerobic.

et al. (1983) assessed the relative proportions of facultatively and strictly anaerobic organisms present in 10 dental abscesses of endodontic origin. Their comparative data indicated the predominance of anaerobes, and in 6 abscesses for which quantitative information was given, the number of bacteria was in the range (3.3×10^6)–(1.2×10^9) cfu/ml of pus, figures similar to ours. Interestingly, the mean concentration of $10^{6.9}$ bacteria/ml of pus found in our study is lower than that found by Zavistoski *et al.* (1980) in the root canals of non-vital teeth ($10^{7.7}$ /g). This difference could be due to the sampling methods used.

Unexpectedly, *S. milleri* was found in 20 (40%) of the abscesses that we examined, twice as the sole organism. Von Konow and Nord (1983) similarly found *S. milleri* to be the commonest facultative anaerobe in their study of orofacial infections. Several other workers have described the isolation of micro-aerophilic α -haemolytic streptococci from dento-alveolar abscesses without identifying the isolates to species level, and it seems likely that the importance of *S. milleri* has been underestimated in the past. Sisson *et al.*, (1978) found CO₂-dependent streptococci, subsequently identified as *S. milleri*, in

various healthy and diseased sites in man, and there is need for more information on the pathogenic role of this organism in infection.

Aderhold *et al.* (1981) suggested that the early phase of abscess formation involves streptococci which prepare the environment for subsequent anaerobic invasion. Our results support this concept because on the two occasions that *S. milleri* was isolated in pure culture, the samples were obtained from the patients on the first day of clinical symptoms. Samples containing a predominantly or purely anaerobic flora tended to come from patients who had had symptoms for 2–3 days.

Viable bacteria were present in large numbers in dentoalveolar abscesses that had progressed to produce fluctuant oral swellings and the bacterial load was predominantly anaerobic. Certain combinations of anaerobic gram-positive cocci, anaerobic gram-negative bacilli and facultatively anaerobic streptococci occurred frequently. The relative importance of each of these bacterial species in mixed infections requires further investigation.

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REFERENCES

- Aderhold L, Knothe H, Frenkel G 1981 The bacteriology of dentogenous pyogenic infections. *Oral Surgery, Oral Medicine, Oral Pathology* **52**:583–587.
- Alin K, Aagren E 1954 The bacterial flora of odontogenic infections and its sensitivity to antibiotics. *Acta Odontologica Scandinavica* **12**:85–98.
- Buchanan R E, Gibbons N E (eds) 1974 *Bergey's Manual of determinative bacteriology*, 8th edn. The Williams and Wilkins Company, Baltimore, MD.
- Cowan S T 1974 Cowan and Steel's Manual for the identification of medical bacteria, 2nd edn. Cambridge University Press, Cambridge.
- Feldmann G, Larje O 1966 The bacterial flora of submucous abscesses originating from chronic exacerbating osteitis. *Acta Odontologica Scandinavica* **24**:129–145.
- Hardie J M, Bowden G H 1974 The normal microbial flora of the mouth. In Skinner FA, Carr JG (eds). *The normal microbial flora of man* (Society for Applied Bacteriology Symposium Series No. 3). Academic Press, London, pp 47–83.
- Kilian M 1976 A taxonomic study of genus *Haemophilus*, with the proposal of a new species. *Journal of General Microbiology* **93**:9–62.
- Labriola J D, Mascaro J, Alpert B 1983 The microbiologic flora of orofacial abscesses. *Journal of Oral and Maxillofacial Surgery* **41**:711–714.
- Oguntibi B, Slec A M, Tanzer J M, Langeland K 1982 Predominant microflora associated with human dental periapical abscesses. *Journal of Clinical Microbiology* **15**:964–966.
- Sabiston C B, Grigsby W R, Segerstrom N 1976 Bacteriology of pyogenic infections of dental origin. *Oral Surgery, Oral Medicine, Oral Pathology* **41**:430–435.
- Sisson P R, Ingham H R, Selkon J B 1978 A study of carbon dioxide-dependent strains of *Streptococcus milleri*. *Journal of Medical Microbiology* **11**:111–116.
- Shah H N, Nash R A, Hardie J M, Weetman D A, Geddes D A, MacFarlane T W 1985 Detection of acid end products of metabolism by anaerobic gram-negative bacteria. In: Goodfellow M, Minnikin D E (eds) *Chemical methods in bacterial systematics*. Academic Press, Orlando, FA, pp 317–340.
- Skirrow M B 1977 *Campylobacter enteritis*: a "new" disease. *British Medical Journal* **2**:9–11.
- Slack J M, Gerencser M A 1975 *Actinomyces, filamentous bacteria: biology and pathogenicity*. Burgess Publishing Company, Minneapolis, MN.
- Sutter V L, Citron D M, Finegold S M 1980 *Wadsworth anaerobic bacteriology manual*, 3rd edn. C. V. Mosby Company, St Louis, MO.
- von Konow L, Nord C E, Nordenram A 1981 Anaerobic bacteria in dentoalveolar infections. *International Journal of Oral Surgery* **10**:313–322.
- von Konow L, Nord C E 1983 Ornidazole compared to phenoxymethylpenicillin in the treatment of orofacial infections. *Journal of Antimicrobial Chemotherapy* **11**:207–215.
- Williams B L, McCann G F, Shoenknecht F D 1983 Bacteriology of dental abscesses of endodontic origin. *Journal of Clinical Microbiology* **18**:770–774.
- Wren M W D, Eldon C P, Dakin G H 1977 Novobiocin and the differentiation of peptococci and peptostreptococci. *Journal of Clinical Pathology* **30**:620–622.
- Zavistoski J, Dzink J, Onderdonk A, Bartlett J 1980 Quantitative bacteriology of endodontic infections. *Oral Surgery, Oral Medicine, Oral Pathology* **49**:171–174.