ACUTE DENTOALVEOLAR ABSCESS

(Microbiological and clinical studies)

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THESIS

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DECLARATION

This thesis is the original work of the author.

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PREFACE

The experimental work described in this thesis was undertaken in the Department of Oral Surgery and Department of Oral Medicine and Pathology, University of Glasgow, during the period of October 1983 to September 1986. The work was supported by a Research Fellowship from Bencard.

Some of the techniques used in this thesis are modifications of previously published work and some are original techniques developed by the author with the supervisors. All the work, apart from the preparation of the histological sections and determination of acidic end products of metabolism, was carried out personally by the author.

Parts of the work reported in this thesis have been published in scientific journals or have been presented at scientific conferences as follows:

A Publications

(i) Published papers (2)

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Lewis M.A.O., McGowan D.A. & MacFarlane T.W. (1986) Short-course high-dose amoxycilin in the treatment of acute dento-alveolar abscess.

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Journal of Dental Research **63:** 509

Lewis M.A.O., MacFarlane T.W. & McGowan D.A. (1985) Antibiotic sensitivity patterns in acute dentoalveolar abscess.

Journal of Dental Research **64:** 669

Lewis M.A.O., MacFarlane T.W. & McGowan D.A. (1986) Pathogenicity of bacterial isolates from acute dentoalveolar abscess.

Journal of Dental Research **65:** 510

B Presentations at scientific meetings

Lewis M.A.O., MacFarlane T.W., MacKenzie D. & McGowan D.A. Quantification of anaerobic and facultative bacteria in acute dentoalveolar abscesses.

British Society for Dental Reseach, London, England. April, 1984.

Lewis M.A.O., MacFarlane T.W. & McGowan D.A. Antibiotic sensitivity patterns in acute dentoalveolar abscess.
British Society for Dental Reseach, Warwick, England. March, 1985.

Lewis M.A.O., MacFarlane T.W. & McGowan D.A. Pathogenicity of bacterial isolates from acute dentoalveolar abscess.
British Society for Dental Reseach, Dundee, Scotland. April, 1986.

McGowan D.A., Lewis M.A.O. & MacFarlane T.W. Short-course high-dose amoxycillin compared with conventional penicillin therapy in the treament of acute dentoalveolar abscess.
IXth International Conference on Oral and Maxillofacial Surgery Vancouver, Canada. May, 1986.

Lewis M.A.O., McGowan D.A. and MacFarlane T.W. Quantitative microbiology and antibiotic sensitivity patterns in acute dentoalveolar abscess.
IXth International Conference on Oral and Maxillofacial Surgery Vancouver, Canada. May, 1986.

Lewis M.A.O., MacFarlane T.W. & McGowan D.A. Reliability of primary antibiotic sensitivity testing of acute dentoalveolar abscess.
British Society for Dental Reseach, London, England. March, 1987.

C Presentations at invited postgraduate meetings

Microbiology of acute dentoalveolar abscesses.
Dental Research Seminar, Glasgow Dental Hospital and School, Scotland. February, 1984.

Quantitative microbiology of acute dentoalveolar abscess.
Dental Seminar, Riyadh-Al-Kharj Hospital, Kingdom of Saudi Arabia. May, 1984.

Microbiological studies of acute dentoalveolar abscess.
Dental Research Seminar, Glasgow Dental Hospital and School, Scotland. February, 1986.

Dentoalveolar abscess - Which bugs? Which drugs?
Royal Odonto-Chirurgical Society of Scotland, Royal College of Surgeons of Edinburgh, Scotland. January, 1987.

ABBREVIATIONS

ABB	Anaerobic Blood Broth
ATCC	American Type Culture Collection
cfu	colony forming units
cfu/ml	colony forming units per millilitre
CO_2	carbon dioxide
g	gram
g/l	grams per litre
IU	International units
ICS	International Collaborative Study
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
NCTC	National Collection of Type Cultures
No.	number
mg	milligram
ml	millilitre
mm	millimitre
μm	micron
nm	nanometre
p	probability
r.p.m.	revolutions per minute
SD	standard deviation
μg	microgram
μg/ml	microgram per millilitre
vol.	volume
vol/vol	volume to volume
wt/vol	weight to volume

SUMMARY

Acute dentoalveolar abscess is a common infection of man which originates at the root apex of a tooth with a necrotic pulp and spreads locally into the surrounding facial tissues. Recent studies have shown that these abscesses are usually polymicrobial with anaerobic gram-positive cocci or anaerobic gram-negative bacilli being the predominant species. Although the literature contains qualitative information about the types of microorganisms involved, little quantitative data is available on the overall microbial load and the relative proportions of individual bacterial strains. The present study was performed to characterise and quantify the microbial species present in each of 50 acute dentoalveolar abscesses using aspiration sampling and culture techniques capable of isolating strictly anaerobic bacteria. Not only did the results confirm that acute dentoalveolar abscesses usually contain a mixture of bacterial species (mean number of species per abscess, 3•3) but also revealed that organisms were present in high concentrations (mean bacterial load, $7•9 \times 10^6$ cfu/ml). Forty per cent of the abscesses studied had a purely anaerobic flora whereas 6 per cent yielded facultative bacteria only. Although a mixture of facultative and strictly anaerobic bacterial species was cultured from 27 abscesses, strict anaerobes predominated in all but 5 of these cases. A wide range of bacteria was isolated but Strep. milleri, Peptococcus species, Peptostreptococcus species, B. oralis, B. gingivalis and B. melaninogenicus were the most frequent. Not only were strict anaerobes isolated more often than facultative bacteria but they were also recovered in higher concentrations. The results of this study

confirmed the polymicrobial nature of acute dentoalveolar abscess and provided scientific data to support the current general impresssion that strict anaerobes predominate in these lesions.

A comparison of the microbiological findings and the symptoms of pain, swelling and temperature for each patient showed that pus samples yielding a strictly anaerobic flora, especially fusobacteria, tended to be recovered from patients who had experienced symptoms for a longer period of time than those who had abscesses with a facultative flora.

In polymicrobial infections involving commensal bacteria it is difficult to decide if one species is more pathogenic than another. Since little is known about the pathogenicity of the microorganisms isolated from acute dentoalveolar abscesses, an experimental model of infection in mice was developed to determine if individual strains or mixed cultures were capable of producing a subcutaneous abscess. Strains of Strep. milleri, anaerobic gram-positive cocci and anaerobic gram-negative bacilli were found to be pathogenic. Differences were noted both in the subsequent recovery of the bacteria inoculated and in the type of tissue reaction produced. B. melaninogenicus and F. nucleatum produced localised abscesses and were recovered in high concentrations significantly more frequently than other bacteria. Strep. milleri and anaerobic gram-positive cocci also produced localised abscesses but these were accompanied by less necrosis of the surrounding tissues and then bacteria were not always recovered in high concentrations. B. gingivalis was also infrequently recovered in

high concentrations, but in contrast to the other bacteria, this species on occasions produced a diffuse tissue reaction rather than a localised abscess. The variations in tissue reaction observed may partially explain the differences in the clinical presentation of acute dentoalveolar abscess seen in patients.

The next area investigated was the antibiotic sensitivity of acute dentoalveolar abscesses. This information is useful since it is sometimes necessary to prescribe antibiotics in the treatment of these acute infections and usually before microbiological results are available. Primary and secondary antibiotic sensitivity tests were performed on 50 pus samples using a comparative disc diffusion method. The antibiotics tested included amoxycillin, ampicillin, cephaloridine, clindamycin, erythromycin, penicillin, metronidazole and tetracycline. Primary and secondary antibiotic tests gave very similar results which suggests that testing of primary cultures is as reliable as the more widely used secondary tests. Although the majority of isolates were sensitive, no single antibiotic was found to be effective against all the bacterial strains. Resistance to the antibiotics other than metronidazole was demonstrated by occasional strains of Strep. milleri, B. distasonis, B. melaninogenicus and F. nucleatum. All the facultative isolates were resistant to metronidazole and all the strict anaerobes were sensitive.

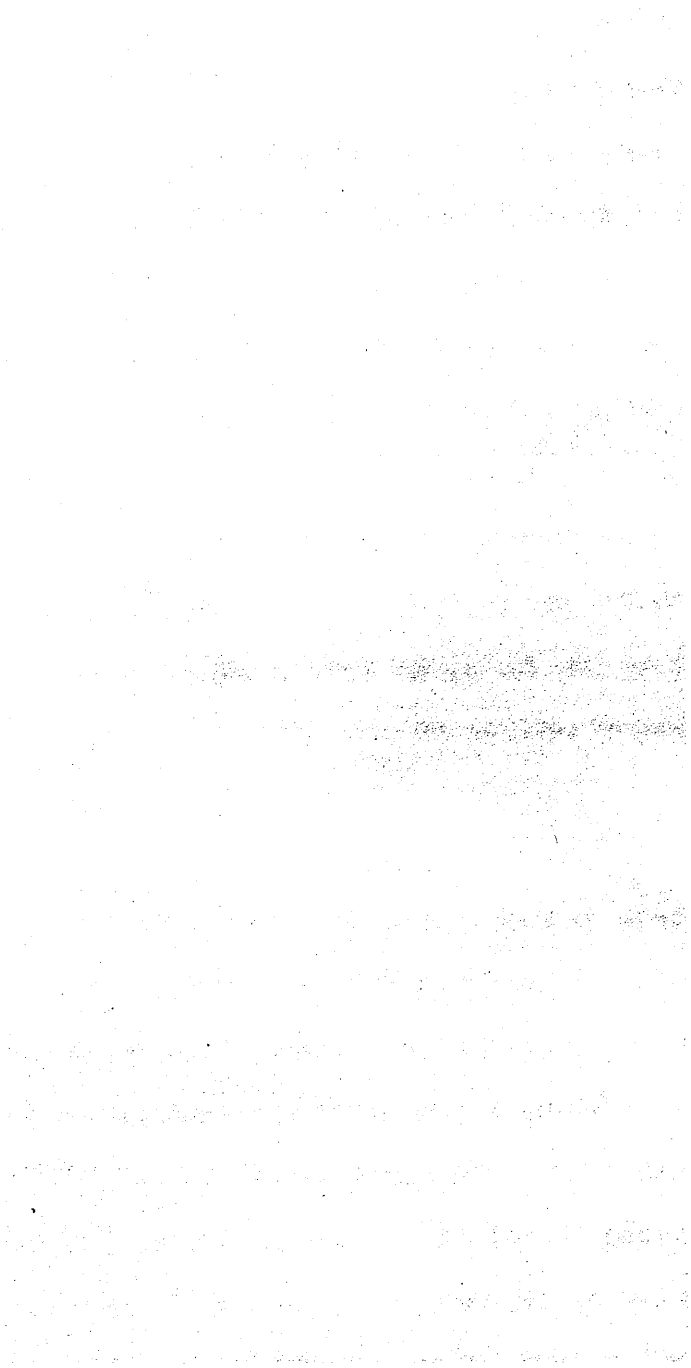
The susceptibility of a selection of bacteria classified as resistant or moderately sensitive by the disc diffusion tests was investigated in more detail using agar dilution and broth dilution methods. In general, determination of minimum inhibitory

concentration and minimum bactericidal concentration confirmed the sensitivity results of the agar diffusion disc tests. The results also indicated that the growth of a small number of isolates was not inhibited by levels of antibiotic which are normally obtained in the blood following conventional doses.

The final study was a clinical investigation to assess the effectiveness of a short-course of high-dose amoxycillin compared to a longer conventional regimen of phenoxymethylpenicillin. Subjective and objective recordings of pain, swelling, lymphadenopathy and temperature were made in 60 patients over a treatment period of 7 days. Since the two experimental groups, (30 patients each), were well matched for presenting symptoms, bacterial culture and surgical management valid comparisons could be made and any differences related to antibiotic therapy. The results indicated that a short-course of high-dose amoxycillin (two 3g sachets, second sachet taken 8 hours after the first) was as effective as a conventional course of penicillin (250 mg tablet four times daily for five days). A greater reduction in swelling during the first 24 hours of treatment was noted in the patients who received the high-dose amoxycillin regimen. This observation may be due to the greater liklihood of high-doses achieving inhibitory levels of antibiotic at the site of infection.

In conclusion the results of this thesis highlight the importance of strict anaerobes in acute dentoalveolar abscesses, provide further information on the pathogenicity and antibiotic sensitivity of the bacterial species involved and demonstrate the

efficacy of short-course high-dose antibiotic therapy in treatment of this acute dental infection.



CHAPTER 1

ACUTE DENTOALVEOLAR ABSCESS

1.1 INTRODUCTION

1.1.1 Historical introduction

Man has suffered from dental disease since earliest times (Wells, 1964) and the presence of dental caries in modern wild anthropoid apes has led to suggestions that it could have affected man's ancestors (Bothwell, 1972). Evidence of dental caries and dental abscesses is seen in human jaws discovered at archeological sites throughout the world. The earliest example is probably the 11 carious teeth present in the skull of Rhodesian man (c. 50,000 B.C.), 4 of which have abscess cavities around their roots (Bothwell, 1972). The maxilla shown in Figure 1.1 is dated from approximately 2,500 B.C. and is representative of the early cases found in the United Kingdom. It was excavated from a three-chambered cairn at Loch Calder, Caithness (Corcoran, 1964 - 1965) and has an obvious abscess cavity surrounding the root apices of an upper molar tooth.

The first written evidence of acute dental abscesses is likely to be the references to the "puncturing of gums" for the relief of toothache in Chinese medical works from 2,700 B.C. (Taylor, 1922) and the descriptions of "painful swellings of the teeth" in the Ebers' medical papyrus, an ancient Egyptian manuscript dated from approximately 1,550 B.C. (Guerini, 1909). The Ebers' papyrus lists the ingredients of a number of pastes to be used for relief of dental pain and refers to "throbbing of the bennut blister in the teeth",

Figure 1.1: Maxilla, dated c. 2,500 B.C. The bone loss around the root apices of the upper left first molar is believed to have been caused by a dentoalveolar abscess.

which is believed to describe dental abscess.

The decoration of a Phoenician burial vase found in the Crimea and dated c. 1,000 B.C. is possibly one of the earliest pictorial recordings of a person suffering from a dental abscess (Figure 1.2). The skull that was found with the vase has bony cavities around the roots of the lower molars which would support the presence of a dental abscess (Lufkin, 1948) but whether this infection was responsible for the death of the man is open to speculation. Evidence that dental abscesses were responsible for severe illness and death in these early days is provided by Hippocrates, who stated, "In the case of necrosis of the tooth the supervening of a strong fever with delirium gives reason to fear a fatal exit" (Guerini, 1909). Hippocrates also gave one of the earliest descriptions of rudimentary dental forceps that could be used for the extraction of abscessed teeth.

It is apparent from reviews of dentistry from earliest times to the beginning of this century that dental abscesses have affected man throughout the ages (Guerini, 1909; Lufkin, 1948). Their management has included the application of pastes onto painful areas of the mouth, incision of tissue swellings and extraction of affected teeth. How successful these were is unknown but death due to the spread of dental infection into the head and neck would appear to have been a well recognised complication.

Figure 1.2: Line drawing of an illustration on a Phoenician burial vase, dated 1,000 B.C., believed to be one of the earliest pictorial recordings of treatment for a dental abscess.

A major advance in the understanding of dental abscess was the appreciation by Miller (1890) of the involvement of microorganisms. The subsequent discovery of penicillin and development of a range of antibiotics resulted in dramatic improvements in the control of human infection, including dental abscesses. However, although dental abscess is no longer regarded as a life-threatening condition serious sequelae such as Ludwig's angina still occasionally occur (Gold and Sager, 1974; Bounds, 1985).

1.1.2 Terminology

A variety of terms have been used in the literature to describe dental abscesses (Table 1.1) and this has led to confusion concerning the exact lesion to which reference was made. It is important to define accurately the different types of abscess that occur in the mouth and to be able to distinguish one that originates from a dead tooth from one that may have a similar clinical presentation but have a different aetiology.

Dentoalveolar abscess would appear to be the most appropriate term as it highlights the dental origin of the lesion and includes the involvment of the surrounding alveolar bone. Grossman (1946) defined dentoalveolar abscess as " a localised collection of pus in the alveolar bone at the root apex of a tooth following the death of the pulp, with extension of infection through the apical foramen into periapical tissues". This definition is clear and therefore the term dentoalveolar abscess will be used throughout this thesis, with the prefix acute since it was this stage of the infection which was studied.

First Author	Year	Terminology
Gilmer	1914	Acute apical abscess
Head	1919	Apical abscess
Fraser	1923	Periapical abscess
Bulleid	1931	Acute alveolar abscess
Grossman	1946	Dentoalveolar abscess
Bourgoyne	1949	Periapical abscess
Alin	1954	Acute dentoalveolar abscess
Cawson	1962	Acute local periodontitis
Feldmann	1966	Submucous abscess
Sabiston	1977	Dental pyogenic infection
Chow	1978	Periapical abscess
Bartlett	1979	Perimandibular space infection
von Konow	1981	Dentoalveolar abscess
Aderhold	1981	Dentogenous pyogenic infection
Oguntebi	1982	Periapical abscess
Williams	1983	Abscess of endodontic origin
Heimdahl	1985	Oro-facial infection of odontogenic origin

Table 1.1: Terminology used to describe acute dental abscess.

1.1.3 Prevalence

Although no precise information is available concerning the prevalence of acute dentoalveolar abscess in the United Kingdom, British texts of Oral Surgery refer to it as a common clinical condition (Sowray, 1985; Howe, 1985). In recent years there has been a reduction in the incidence of dental caries in parts of the United Kingdom (Anderson et al., 1982) and since caries is generally assumed to be the most frequent cause of acute dentoalveolar abscess there is likely to be a reduction in the incidence of acute dentoalveolar abscess in the future.

1.2 AETIOLOGY

1.2.1 Development

The stages involved in the development of an acute dentoalveolar abscess are shown in Figure 1.3 and are well described in texts of Oral Pathology (Shafer et al., 1983; Soames and Southam, 1985).

The dental pulp is a highly vascular and organised collection of connective tissue contained within the pulp cavity of a tooth. The apical foramen is the route by which blood vessels, lymphatics and nerves enter the pulp chamber (Figure 1.4), although in some cases vessels may also gain access through accessory canals in the apical region. Like any other tissue the pulp will react to noxious external stimuli by producing an acute inflammatory response, described as pulpitis. Since swelling of the pulp tissue is prevented by the surrounding calcified walls of the tooth inflammatory oedema increases the pressure within the pulp chamber leading to ischaemia.

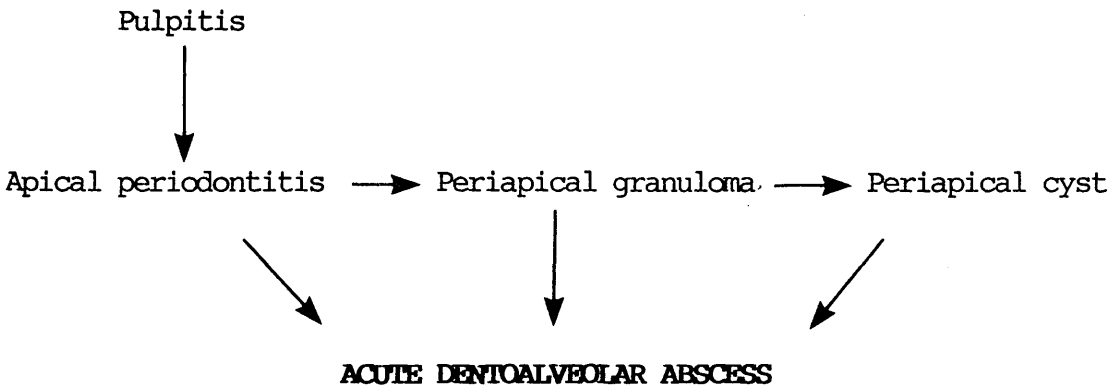


Figure 1.3: Stages of development of acute dentoalveolar abscess.

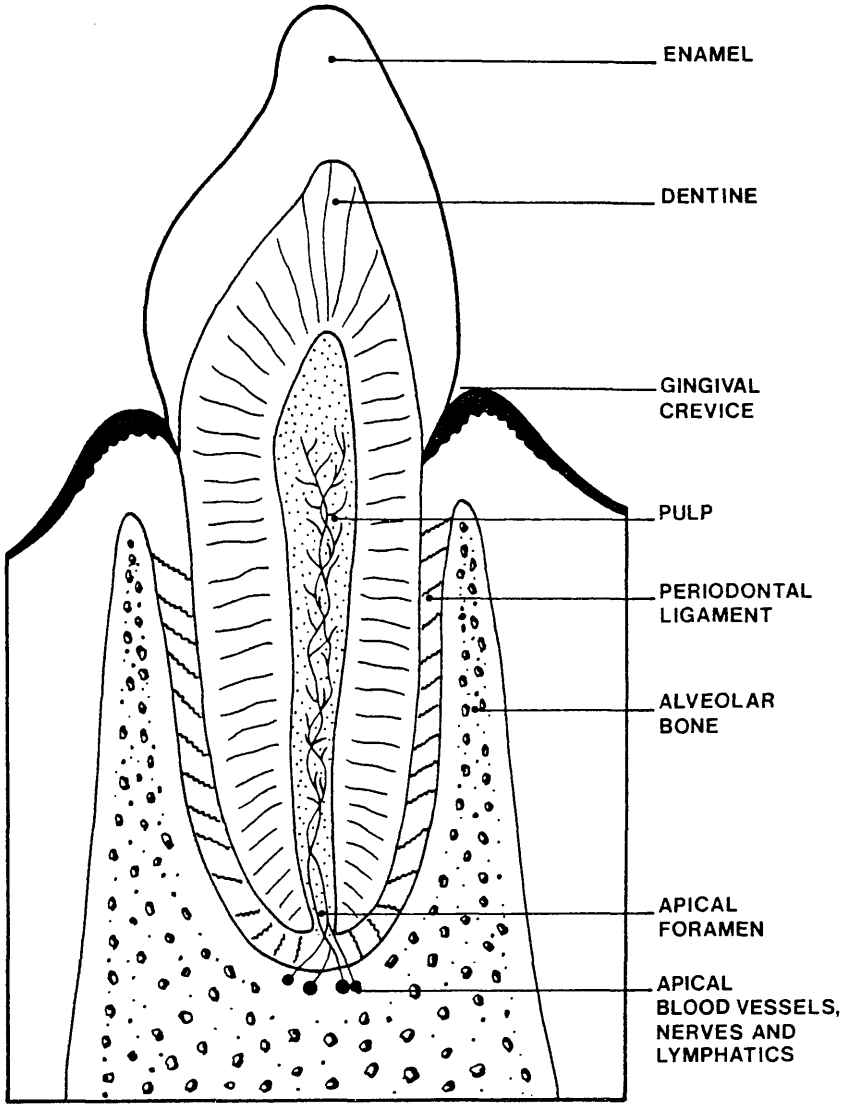


Figure 1.4: Representative structure of a single-rooted tooth and supporting tissues (saggital section).

In time localised destruction of the pulp occurs which eventually extends to the entire pulp with resultant liquefaction and necrosis.

Causes of pulpitis leading to pulp death include; caries, heat production during dental cavity preperation, chemical irritation from dental restorative materials, physical entry of microorganisms into the pulp during cavity preparation or following a fracture of the tooth crown and finally severence of the apical vessels as a result of trauma (Cawson, 1984). Death of the dental pulp, for whatever cause, is followed by an inflammatory response in the structures surrounding the root apex (Bahn, 1985), known as apical periodontitis. How this develops is uncertain but is likely to involve local diffusion of bacterial endotoxins, immune complexes and proteolytic enzymes from the pulp via the apical foramen (Naidorf, 1972; Morse, 1977; Torabinejad and Bakland, 1978; Schoenfeld et al., 1982). Apical periodontitis may progress rapidly into an acute dentoalveolar abscess but due to the vascular elements in the periapical tissues a chronic inflammatory condition, the periapical granuloma, frequently forms (Bahn, 1985; Soames and Southam, 1985).

The periapical granuloma is usually symptomless and may remain undetected for many months or years during which time approximately half undergo change to produce a periapical cyst (Lalonde and Luebke, 1968). Either of these lesions may subsequently become infected and develop into an acute dentoalveolar abscess, although the reasons for these changes and the source of the infecting microorganisms are uncertain. Three pathways of bacterial invasion

have been preposed (Figure 1.5); 1. spread from the pulp through apical foramen, 2. spread from the gingival margin via the periodontal ligament and 3. spread via the blood vessels of the circulation. The most likely route is direct spread of bacteria from a pulp infected due to destruction of the crown by dental caries, but this is not always the case since dentoalveolar abscesses can develop from teeth without caries. It has been suggested that in these circumstances bacteria reach the apical area via the general circulation or vessels in the periodontal ligament since animal experiments have shown that bacteria can spread by these routes (Robinson and Boling, 1941; Burke and Knighton, 1960; Grossman, 1967).

1.2.2 Spread of infection

Once an abscess at the apex of a tooth is established infection can spread by a number of pathways (Figure 1.6). The possible routes are dictated by the density of surrounding bone, the relationship of the root apex to the facial muscles and anatomy of the adjacent tissue planes (Birn, 1972). These structures will determine the point at which discharge of pus will occur and whether the infection presents intraorally or extraorally.

The vast majority of dental abscesses remain within the facial tissues although there is always the potential danger of infection spreading backwards into the tissue spaces of the head and neck. Fatalities have been reported following the spread of dentoalveolar abscess into the pharynx (Fein and Mohnac, 1973; Marks et al., 1974), the cavernous sinus (Haymaker, 1945) the brain (Ingham

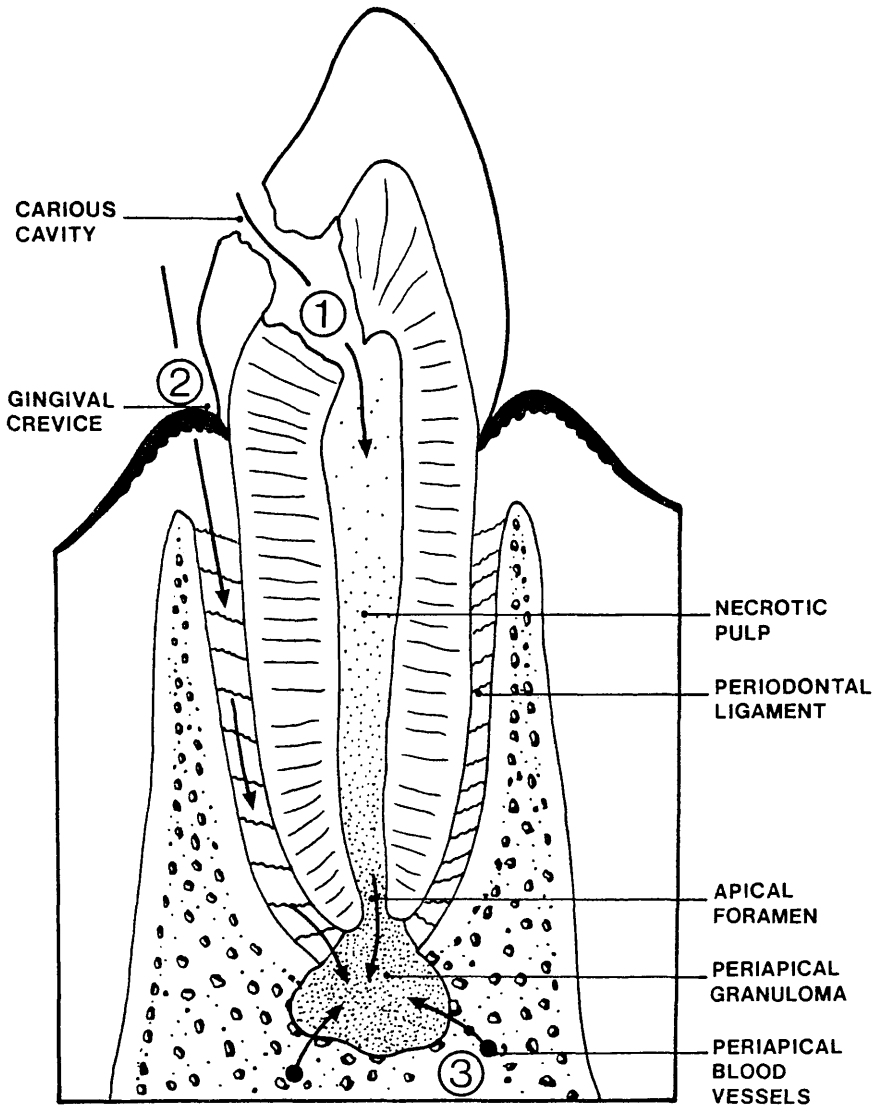


Figure 1.5: Proposed pathways of bacterial invasion of the pulp and periapical tissues. 1. via the apical foramen, 2. via the periodontal ligament and 3. via the blood stream.

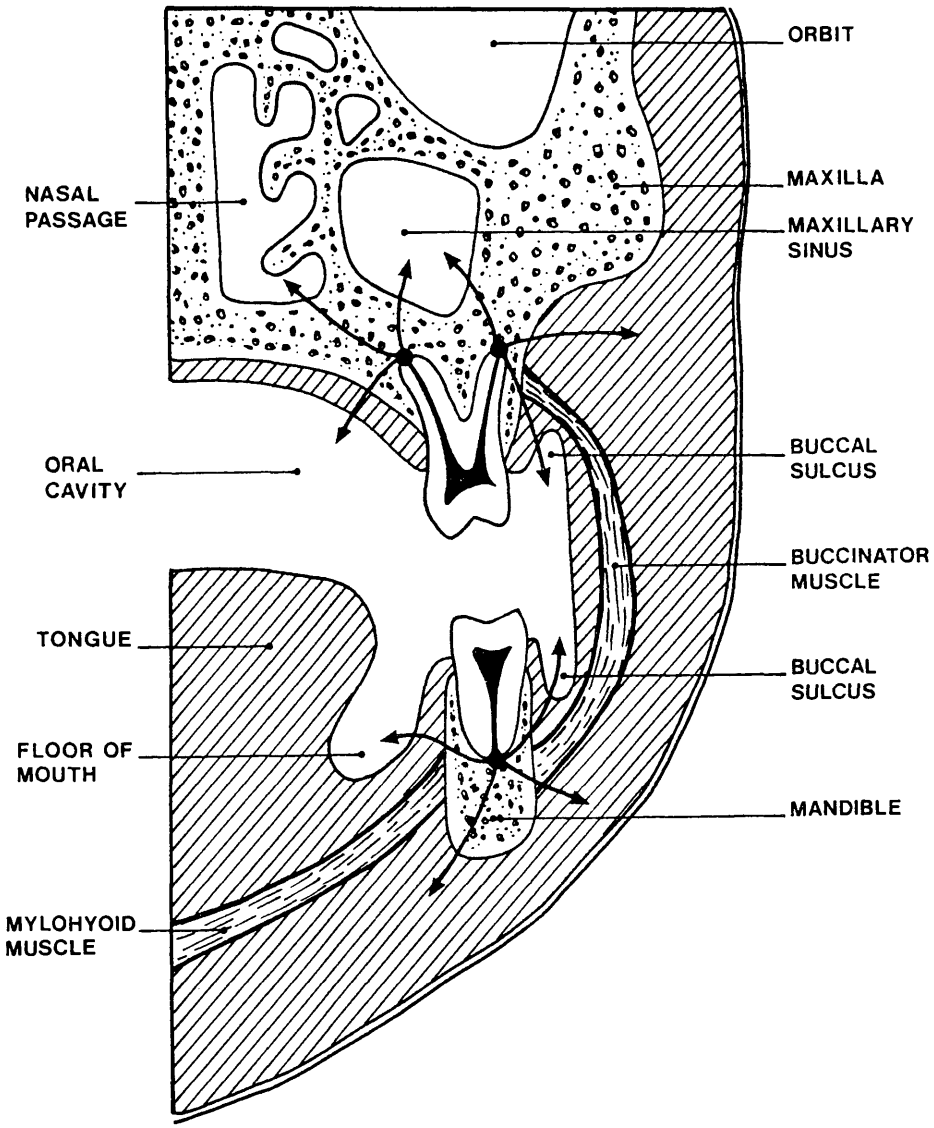


Figure 1.6: Pathways (arrows) by which pus may track from an acute dentoalveolar abscess (coronal section of left side, at plane of first molar tooth).

et al., 1978), the meninges (Hedström et al., 1980), the orbit (Gold and Sager, 1974), the myocardium (Palank et al., 1979) and the mediastinum (Moncada et al., 1978; Bounds, 1985).

1.3 CLINICAL PRESENTATION

Guralnick (1984) described a typical history of a patient suffering from an acute dentoalveolar abscess as follows; "The patient may have had violent pain and swelling 12 or 24 hours previously. He may also recall that for the past month or two his tooth had been sensitive to hot and cold foods but he had delayed visiting his dentist because the discomfort was moderate and intermittent. Last night, however, intense pain had developed and his face had begun to swell. The pain had continued to increase, he had not slept and is exhausted, and he is now seeking help." Diagnosis of acute dentoalveolar abscess can usually be made relatively easily from a clinical history such as this, although the presenting clinical symptoms described by individual patients with can vary widely depending on a number of different factors. These factors include the duration and site of infection, host defence mechanisms and the virulence of the causative microorganisms. Heimdahl et al., (1985) have suggested that the clinical syptoms of pain, swelling and fever are more severe in patients from whom anaerobic gram-negative bacilli are isolated although this aspect of acute dental abscesses has not been studied by other workers. Symptoms may range from trivial to extremely serious and in order to avoid the the potentially fatal complications described in Section 1.2.2 it is important that a rapid diagnosis is made and treatment instigated.

1.3.1 Pain

All the patients with orofacial infections studied by Schuen et al., (1974) and von Konow and Nord (1983) were in pain at the time of initial presentation. The pain associated with dentoalveolar abscess (Summary, Table 1.2) frequently starts as diffuse discomfort in one part of the mouth which later intensifies and becomes localised to one tooth. The pain is characteristically severe and throbbing, with analgesics giving little or no relief. Many patients report a sleepless night prior to presentation (von Konow and Nord, 1983) although a temporary relief of pain may be experienced when swelling appears. Release of pressure as the pus perforates the tight periostium and enters the lax soft tissues is thought to be responsible for this temporary reduction in pain (Moore, 1976).

1.3.2 Swelling

Once the infection has perforated the periosteum the inflammatory exudate can produce soft tissue oedema which may be apparent extraorally (Figure 1.7) or intraorally (Figure 1.8), depending on the factors described in Section 1.2.2. When swelling is severe, eye opening or jaw movements may be limited in addition to the overlying skin becoming erythematous and warm to the touch (Figure 1.9).

1.3.3 Appearance of affected tooth

Intraoral examination will usually reveal the causative tooth relatively easily as it is frequently extremely sensitive to touch and thus prevents the patient from eating on the affected side. Commonly the tooth involved has obvious caries (Figures 1.10 and

Character of pain	-	Dull, throbbing and severe, with sharp exacerbations when the involved tooth is bitten on or touched.
Site of pain	-	Affected tooth.
Radiates to	-	Ear (lower tooth) and cheek, eye and temple (upper tooth).
Precipitated by	-	Biting upon or touching involved tooth.
Timing	-	Continuous, worse at night and mealtimes. Prevents sleep.
Other symptoms	-	When swelling appears pain is reduced although involved tooth is still acutely tender to touch.

Table 1.2: Clinical nature of pain associated with acute dentoalveolar abscess (After Howe, 1971).

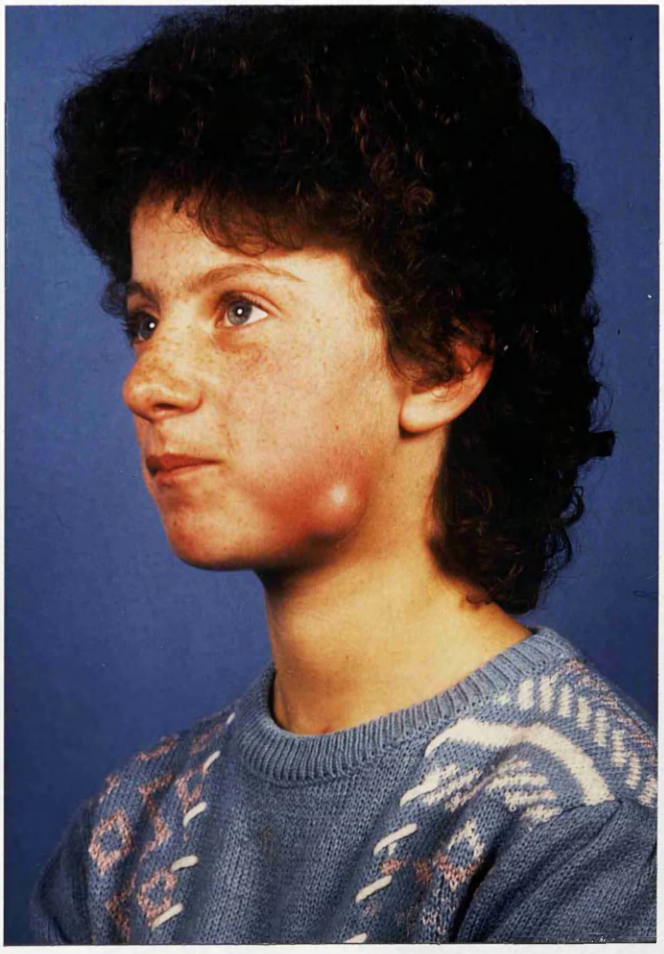


Figure 1.7: Localised swelling of left cheek due to an acute dentoalveolar abscess affecting the lower left first molar.

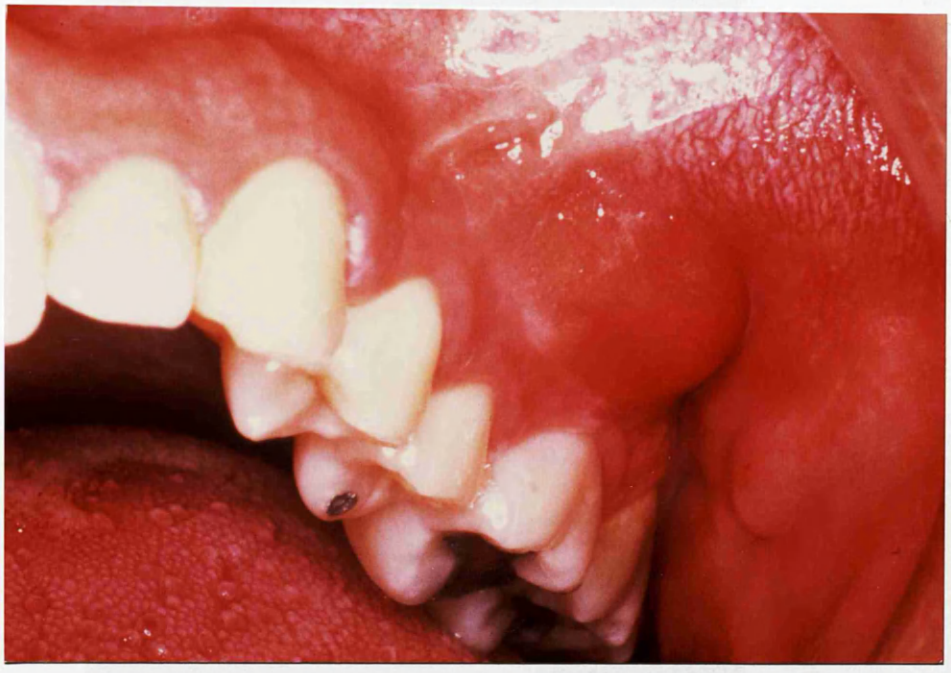


Figure 1.8: Acute dentoalveolar abscess presenting as a fluctuant swelling in the buccal sulcus, at the level of the root apex of the upper left first molar.

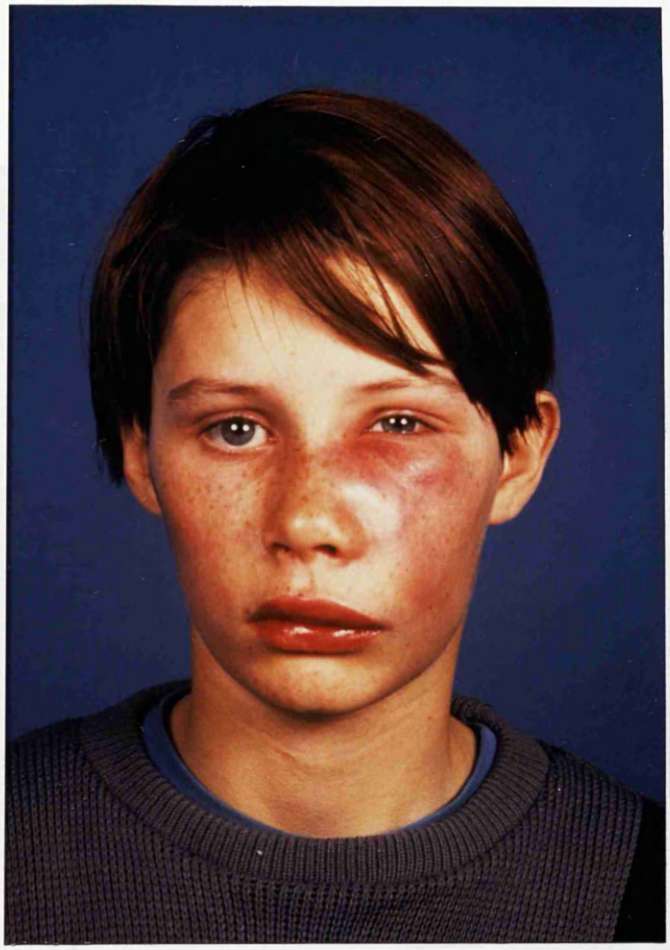


Figure 1.9: Facial appearance of a 15 year old girl with an acute dentoalveolar abscess affecting the upper left canine. Swelling has led to partial closure of the left eye and erythema of overlying skin.

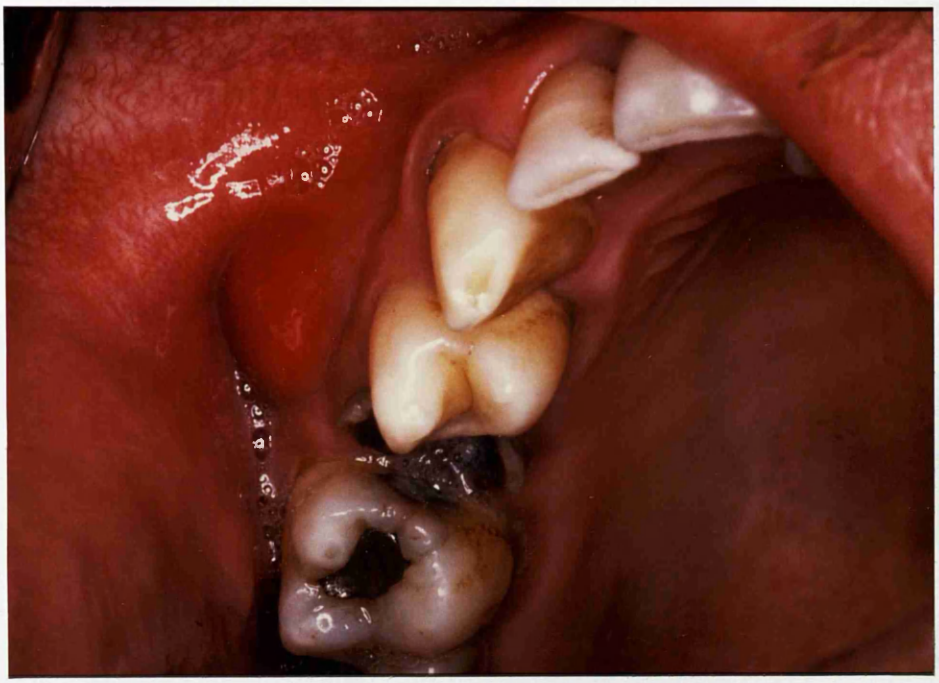


Figure 1.10: Acute dentoalveolar abscess affecting a grossly carious upper right second premolar.

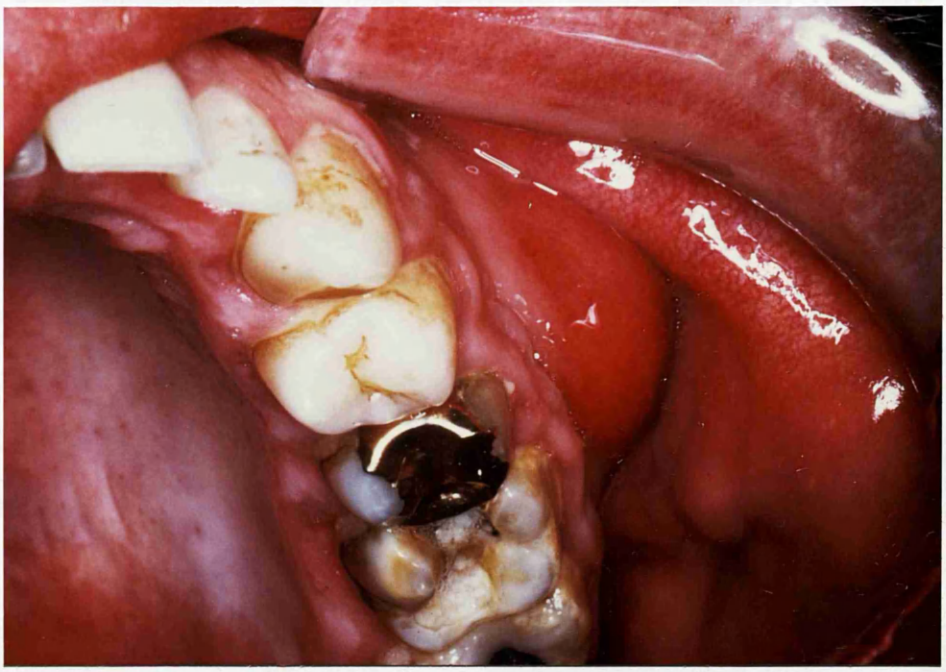


Figure 1.11: Acute dentoalveolar abscess affecting a carious upper left first molar.

1.11) or a large dental restoration. Pulp death can also lead to grey/black discolouration of a tooth and therefore such teeth should be considered as a possible source of infection even if they show no evidence of caries or are un-restored. The pulp of an abscesssed tooth will have lost its sensory nerve supply and therefore should not respond to the application of heat (melted gutta percha) or cold (frosting ethyl chloride on a pledget of cotton-wool). These clinical observations and electrical devices, which test pulp vitality, can therefore be used to assist diagnosis of pulp death (Grossman, 1981).

1.3.4. Lymphadenopathy

Acute dentoalveolar abscess can cause tender enlargement of the cevical lymph nodes (Chisholm et al., 1978) and this can be detected clinically by palpation of the submandibular region and neck.

1.3.5. Temperature

Body temperature may be elevated in patients with acute dentoalveolar abscesses and Heimdahl et al., (1985) reported a range in body temperature from 36•8°C to 39•0°C in 58 patients with orofacial infections although no details were given of how and at what site the temperature was recorded. It is possible that increased blood flow in inflammatory oral swellings produce a purely localised elevation of temperature which would result in body temperature measured by oral means being falsely high (Topazian and Goldberg, 1981).

1.3.6 Haematological investigations

Determination of white blood cell count (WBC) and erythrocyte sedimentation rate (ESR) has been found to be of little help in measuring the severity of acute orofacial infections (von Konow and Nord, 1983; Heimdahl et al., 1985).

1.3.7 Radiographic examination

An acute dentoalveolar abscess can be such a rapidly progressive lesion that there may be no radiographic evidence of its presence. However the majority of abscesses develop from a longstanding chronic apical granuloma and therefore radiolucent areas are seen at the root apices of affected teeth due to localised resorption of bone (Grossman, 1981). Examples of the varying degrees of radiolucency are shown in Figures 1.12, 1.13 and 1.14.

1.4 MICROBIOLOGY

1.4.1 Introduction

Microorganisms have long been suspected of playing an important role in pathological conditions of the pulp and periapical area. Almost a century ago Miller (1894) demonstrated by microscopy the presence of many morphological variants of bacteria in the necrotic root canals of teeth.

Although acute dentoalveolar abscess is a common clinical condition the total number of papers in the literature describing the bacterial species cultivated from this infection is small. Details of the methods and microbiological findings of the majority of investigations that have been performed are presented in three tables

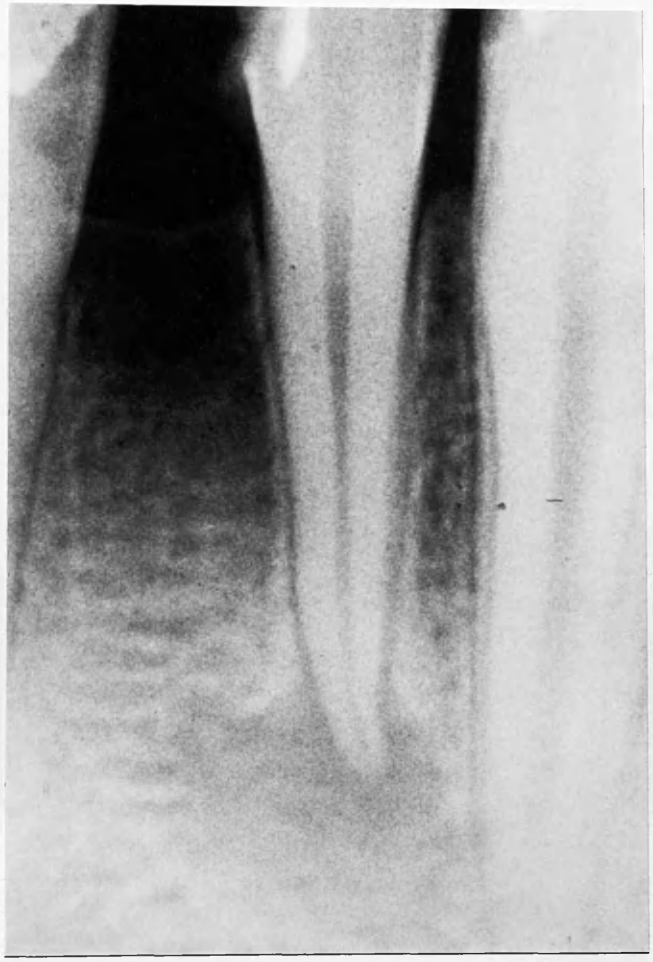


Figure 1.12: Intraoral periapical radiograph of a lower lateral incisor demonstrating an area of apical radiolucency.

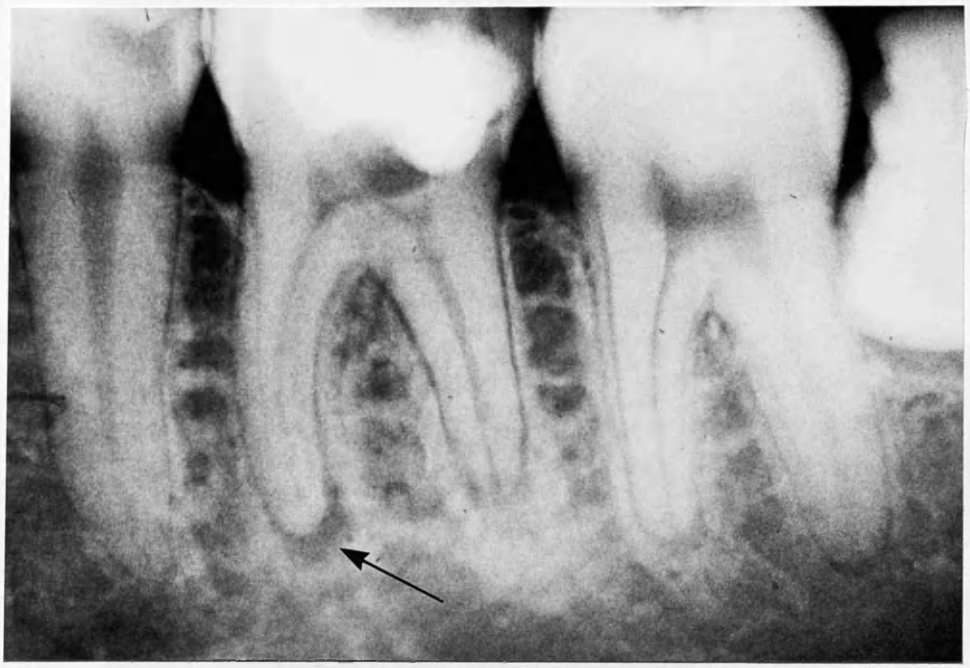


Figure 1.13: Intraoral periapical radiograph showing radiolucent area at the apex of the mesial root of a lower first molar (arrowed). There is also evidence of caries under the large amalgam restoration in the tooth.

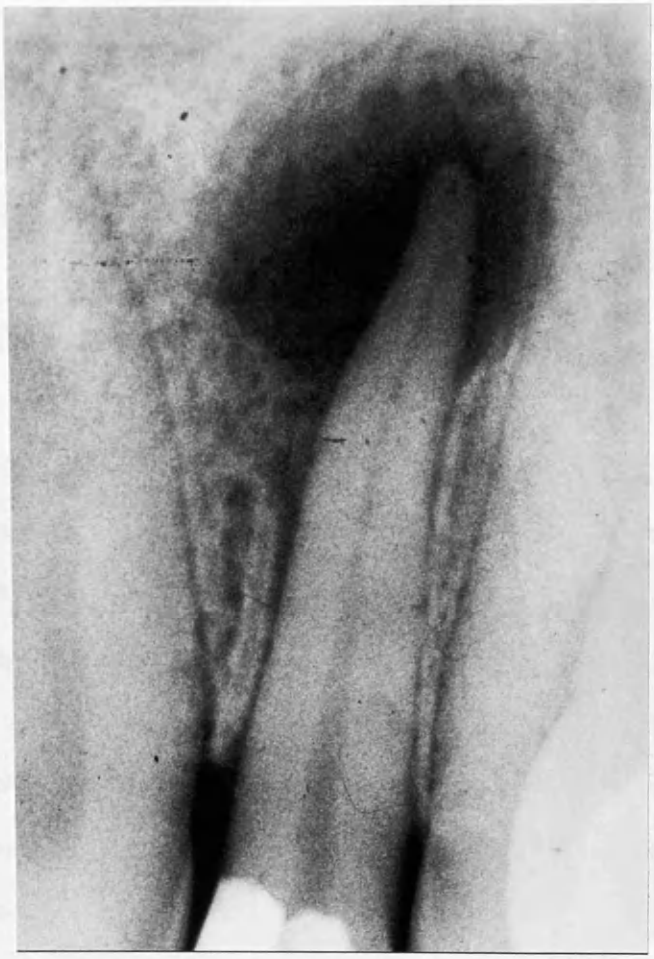


Figure 1.14: Intraoral periapical radiograph of an upper right lateral incisor showing marked area of periapical radiolucency.

in this section; prospective studies performed prior to 1974 are shown in Table 1.3 and those performed after 1974 in Table 1.4. A division was made at 1974 since it was approximately at that time that culture techniques capable of recovering strictly anaerobic bacteria became widely available. A selection of retrospective reviews reported since 1974 are presented in Table 1.5. The bacterial nomenclature used in the tables is the same as that used in the original report.

1.4.2 Early investigations (Table 1.3)

Although van Leeuwenhoek in the 17th century was the first of the early microscopists to recognise a relationship between microorganisms and oral infections (Dobell, 1958), it was not till much later that Miller (1890) produced the first detailed description of the microoganisms found in the mouth in health and disease. Miller (1890) stated that alveolar abscess was caused by the spread of bacteria from the oral cavity into the apical tissues via the pulp and stressed the seriousness of these infections since they are deep seated within bone.

The belief that a dental abscess represented a primary focus of infection responsible for serious secondary infections at other sites of the body was one of the main reasons that the earliest microbiological investigations of dental abscesses were performed. Prior to these investigations it was generally assumed that Staphylococcus albus and Staphyloccocus aureus were responsible for dental abscesses, although this was based on the clinical features rather than bacterial analysis. Gilmer and Moody (1914) carried out

one of the first mircobiological studies of acute dental abscesses and reported that although staphylococci were present in many of the samples studied the predominant microorganisms were streptococci.

Head and Roos (1919) studied 130 pus samples obtained from 100 dental abscesses and also reported a predominance of streptococci since "Streptococcus viridans" was isolated from all but 6 specimens. A wide range of other bacterial species were also isolated but, apart from streptococci, a strictly anaerobic cocco-bacillus was the only microorganisms discussed in detail. Head and Roos (1919) noted that this pigment-producing organism was cultured in combination with streptococci from abscesses which demonstrated evidence of apical necrosis on radiographs and therefore believed that these bacteria played an important role in acute dental abscesses.

In the subsequent microbiological studies by Fraser, (1923), Broderick, (1925), Bullied, (1931), Alin and Ågren, (1954) and Ludwig, (1957) the predominant isolates were "Strep. viridans" and Staphylococcus species. However the importance of obtaining uncontaminated samples from an abscess in the oral cavity started to be discussed in more detail. Specimens obtained by extraction of the abscessed tooth ran a high risk of contamination from saliva and therefore the use of aspiration and swab sampling became more widespread. In addition to the problem of contamination Feldmann and Larje (1966) stressed the need for strict anaerobe culture methods with selective enriched media and although they reported a predominance of "Strep. viridans", strict anaerobes (particulary anaerobic streptococci) were also frequently isolated.

Reference	Year	No. of cases	Sampling Method	Anaerobic Culture	Predominant Organisms	
					Facultative Anaerobes	Strict Anaerobes
Gilmer & Moody	1914	42	Pipette or Extraction	Yes	Streptococcus spp.	Fusiform bacilli Black-pigmented organisms
Head & Roos	1919	100	Extraction	Yes	Strep. viridans	Gram-negative cocco-bacillus
Fraser	1923	120	Extraction	No	Strep. viridans Staphylococcus spp.	
Broderick	1925	100	Extraction	No	Strep. viridans Staphylococcus spp.	
Bulleid	1931	16	Aspirate or Extraction	Yes	Streptococcus spp. Staphylococcus spp.	Gram-positive bacillus
Alin & Ågren	1954	27	Aspirate	Yes	Strep. viridans	
Ludwig	1957	65	Aspirate or Swab	Yes	Strep. viridans Staph. albus Non-haemolytic Streptococcus spp. Neisseria spp. Diphtheroids	
Feldmann & Larje	1966	73	Swab	Yes	Strep. viridans Diphtheroids	Streptococcus spp.

Table 1.3: Prospective microbiological studies of acute pyogenic dental infection 1914 - 1973.

1.4.3 Recent investigations (Table 1.4)

Sabiston and Gold (1974) probably performed the first study that had sufficient regard to the problems of specimen contamination in addition to using microbiological techniques capable of isolating highly oxygen-sensitive organisms. Since the number of cases studied was small the authors stressed that their findings should not be used as a basis for generalisations regarding the bacterial cause of dental abscesses. However the results, which revealed that the majority of isolates were strict anaerobes, did indicate the need for more extensive studies of dental infections using aspiration and anaerobic culture methods.

A comparison of the methods used and the findings of subsequent microbiological studies of acute dental infection reveals that those employing aspiration sampling and anaerobic culture reported a predominance of strictly anaerobic species (Papers 4, 6, 7, 9, 10, 11, 12, 13, 14, 16, 17, 18 and 20). The bacterial species most frequently isolated in these studies were strains of facultative gram-positive cocci, anaerobic gram-positive cocci and anaerobic gram-negative bacilli. Notably five of the investigations did not isolate staphylococci although strains of this species were occasionally cultured in the remainder. Cumming et al., (1984) (Paper 19) found a predominance of facultative organisms, in a study of acute dental abscesses, despite the use of aspiration sampling and anaerobic culture methods. A relatively high number of staphylococci were also isolated by Cumming et al., (1984) but the reason for these contradictory findings are not known, although they could in part be due to the small number of cases studied. Investigations which

collected samples on swabs and used limited culture techniques (Papers 2, 3, 5, 8, 15) have tended to report a predominance of facultative organisms, mainly streptococci and staphylococci.

The mean number of bacterial species isolated from pus samples range from 1•0 to 7•4 in the studies shown in Table 1.4. When strict anaerobic culture methods were not used the values ranged from 1•0 to 1•6 (mean 1•2) whereas in those studies employing anaerobic culture the figures are 3•1 to 7•4 (mean 3•7). A reasonable interpretation of these differences is that if good anaerobic techniques are not used the subsequent microbiology results will be inaccurate due to failure in isolating a range of bacterial species.

Although some studies shown in Table 1.4 were performed in in Scandinavia, United Kingdom and West Germany the majority were carried out in North America. The results obtained from different parts of the world are very similar and there does not appear to be geographical differences with regard to the types of bacterial species isolated. There would also appear to be no difference in the bacterial species isolated with respect to age of the patients. The spectrum of bacteria isolated by Brook et al., (1981) from 12 children aged between 5 and 16 years with acute dental abscesses was very similar to those obtained in studies using similar microbiological techniques on samples obtained from adult patients.

Many of the microbiological investigations of acute dental infection have not been specific and have included samples from a

Table 1.4: Prospective microbiological studies of acute pyogenic dental infection 1974 - 1986.

	Reference	Year	No. of Cases	Mucosa/Skin Prep.	Sampling Method	Time to Culture	Culture Media and Conditions (Time of Incubation)			Predominant Organisms		Mean No. of isolates per sample
							Air	CO_2	Anaerobic	Facultative anaerobes	Strict anaerobes	
1.	Sabiston and Gold	1974	8	ND	Aspirate	30 mins	Anaerobe Laboratory (10 days)		Manual*	Streptococcus spp.	F. nucleatum Bacteroides spp.	3.1
2.	Gabrielson and Stroh	1975	84+	ND	ND	ND	Yes (ND)	ND (ND)	Yes (ND)	Strep. viridans N. catarrhalis		1.6
3.	Turner et al.,	1975	66	Extraoral Betadine Intraoral Sterile gauze	Swab	ND	Yes (3 days)	ND	Yes (3 days)	Strep. viridans Staph. epidermidis		1.0
4.	Sabiston et al.,	1976	65+	Sterile gauze	Aspirate	30 mins	Anaerobe Laboratory (10 days)		Manual	Streptococcus spp.	F. nucleatum Peptostr. micros Actinomyces spp. Peptostr. anaerobius B. melaninogenicus B. ruminicola	3.8
5.	Epstein and Scopp	1977	13+	ND	Swab	ND	Yes (ND)	No	No	Strep. viridans		1.4
6.	Ingham et al.,	1977	25+	ND	Aspirate	2 hrs	MacConkey's agar Blood agar (7 days)		Blood agar Blood agar + naladixic acid (7 days)	Staphylococcus spp. Strep. viridans	G +ve bacilli G -ve bacilli G +ve bacilli	7.4

	Reference	Year	No. of Cases	Mucosa/Skin Prep.	Sampling Method	Time to Culture	Culture Media and Conditions (Time of Incubation)			Predominant Organisms		Mean No. of isolates per sample
							Air	CO_2	Anaerobic	Facultative anaerobes	Strict anaerobes	
7.	Chow et al.,	1978	31+	ND	Aspirate and tissue	ND	Anaerobe Laboratory Manual* (ND)			Streptococcus spp.	Bacteroides spp. Peptostreptococcus spp.	4.0
8.	Hunt et al.,	1978	74+	ND	Swab	ND	Blood agar (4 days)	No	Blood agar (4 days)	Strep. viridans Staphylococcus spp.		1.0
9.	Bartlett and O'Keefe	1979	21	ND	Aspirate	10 mins	MacConkey's agar (2 days)	Peptic digest of sheep blood agar 5% sheep blood agar (2 days)	Brucella - base blood agar (7 days)	α-haemolytic Streptococcus spp.	Peptococcus spp. Peptostreptococcus spp. B. melaninogenicus E. lentum F. nucleatum	5.8
10.	Kannangara et al.,	1980	61+	ND	Aspirate and tissue	ND	Anaerobe Laboratory Manual** (ND)			Staph. epidermidis α-haemolytic Streptococcus spp.	Bacteroides spp. Peptococcus spp. Peptostreptococcus spp.	3.3
11.	Aderhold et al.,	1981	50	ND	Aspirate	Immediate	Blood agar (1 day)	No	Schaedler agar + blood Schaedler KV agar + blood Tarozzi broth (7 days)	Streptococcus spp.	B. melaninogenicus Fusobacterium spp. Peptococcus spp.	3.6

Table 1.4 (Cont.)

	Reference	Year	No. of Cases	Mucosa/Skin Prep.	Sampling Method	Time to Culture	Culture Media and Conditions (Time of Incubation)			Predominant Organisms		Mean No. of isolate per sample
							Air	CO_2	Anaerobic	Facultative anaerobes	Strict anaerobes	
12.	Brock et al.,	1981	12	Betadine	Aspirate	20 mins	MacConkey's agar (2 days)	Sheep blood agar Chocolate agar (2 days)	Brucella agar Blood agar + K and V Blood agar + alcohol Thioglycolate broth (4 days)		B. melaninogenicus Peptococcus spp.	4.9
13.	von Konow et al.,	1981	57+	Extra-oral Chlorhexidine in ethanol Intra-oral Iodine	Aspirate	Immediate	Yes (10 days)	No	Yes (10 days)	Strep. milleri	Fusobacterium spp. Bacteroides spp. Strep. intermedius Peptococcus spp. B. ruminicola Eubacterium spp.	4.0
14.	Oguntebi et al.,	1982	10	Iodine	Aspirate	10 mins	Tripticase soy agar with sheep blood Mitis salivarius agar Actinomyces selective medium Fusobacterium selective medium Eikenella selective medium (7 days)	(7 days)	(7 days)	Strep. mitis	F. nucleatum Peptostr. micros Actinomyces spp. Peptostr. anaerobius B. melaninogenicus B. ruminicola	2.5
15.	Hunt and Meyer	1983	246+	ND	Aspirate and Swab	Promptly	Blood agar (4 days)	No	Blood agar (4 days)	Strep. viridans Staph. aureus Staph. epidermis	Peptostreptococcus spp.	1.1

Table 1.4 (Cont.)

	Reference	Year	No. of Cases	Mucosa/Skin Prep.	Sampling Method	Time to Culture	Culture Media and Conditions (Time of Incubation)			Predominant Organisms		Mean No. of isolates per sample
							Air	CO_2	Anaerobic	Facultative anaerobes	Strict anaerobes	
16.	Williams et al.,	1983	10	Sterile gauze	Aspirate	15 mins	No	No	Sheep blood agar (7 days)		F. nucleatum Peptostreptococcus spp.	4.5
17.	von Konow and Nord	1983	59+	Yes (ND)	Aspirate	Promptly	Anaerobe Laboratory Manual** (ND)			Strep. milleri	F. nucleatum B. melaninogenicus Bacteroides spp. B. ruminicola Peptostreptococus micros E. lentum Strep. intermedius	3.0
18.	Labriola et al.,	1983	50+	Yes (ND)	Aspirate	1 hr	Anaerobe Laboratory Manual** (21 days)			Streptococcus spp.	Bacteroides spp. B. melaninogenicus Peptococcus spp. B. oralis B. ruminicola	3.2
19.	Cumming et al.,	1984	10+	Chlorhexidine	Aspirate	30 mins	Blood agar Chocolate agar MacConkey's agar Cooked meat broth (2 days)	No	Blood agar Chocolate agar MacConkey's agar (4 days)	Strep. viridans Staph. albus		2.5

Table 1.4 (Cont.)

	Reference	Year	No. of Cases	Mucosa/Skin Prep.	Sampling Method	Time to Culture	Culture Media and Conditions (Time of Incubation)			Predominant Organisms		Mean No. of isolates per sample
							Air	CO_2	Anaerobic	Facultative anaerobes	Strict anaerobes	
20.	Heimdahl et al.,	1985	58+	Extraoral Chlorhexidine in ethanol Intraoral Iodine	Aspirate			Anaerobe Laboratory Manual** (10 days)		Strep. milleri	F. nucleatum B. ruminicola B. melaninogenicus Peptostreptococcus spp.	3.4

Anaerobe Laboratory Manual Methodology: (Holdeman and Moore, 1972)* (Holdeman, Cato and Moore, 1977)**

Air	CO_2	Anaerobic
Blood agar	Blood agar	Brain heart infusion agar roll tube Blood agar

\+ Specimens obtained from a variety of acute dental infections in addition to acute dentoalveolar abscess.

ND No details.

Table 1.4 (Cont.)

variety of conditions, such as infection secondary to fractured mandibles, infected extraction sockets and pericoronitis. In this situation where microbiological findings from a variety of infections are presented together it is difficult to deteremine if different bacterial species are responsible for different types of lesion. Only 6 of the studies which used strict anaerobic culture methods listed in Table 1.4 were limited to acute dentoalveolar abscess (Papers 1, 9, 11, 12, 14, 16) and of these only two (Papers 9 and 11) examined more than 21 cases. These two studies reported that the most frequently isolated bacteria were facultative streptococci, peptostreptococci, peptococci, bacteroides, fusobacteria and eubacteria.

1.4.4 Retrospective investigations (Table 1.5)

A number of retrospective microbiological studies of dental infections have been performed and these have the advantage of being able to collect results from large numbers of specimens over long periods of time. However Sims (1974) has stressed that the findings of this type of study require to be viewed with caution because of variations in sampling methods and limitations of the techniques used in routine microbiological situations. It is perhaps not suprising therefore to find that retrospective studies of acute dentoalveolar abscess have frequently reported "Strep. viridans" as the predominant organisms.

Reference	Year	No. of cases	Sampling Method	Anaerobic Culture	Predominant Organisms	
					Facultative Anaerobes	Strict Anaerobes
Sims	1974	1000	Various	Yes	Strep. viridans Neisseria spp. Staph. epidermidis	
Woods	1975	80	Paper point	No	Strep. viridans Pyogenic streptococci	
von Schuppan et al.,	1978	919	No details	Yes	Microaerophilic Streptococcus spp.	B. melaninogenicus Fusobacterium spp.
Woods	1981	129	Paper point or Platinum wire	No	Strep. viridans Strep. pyogenes	
Morey et al.,	1984	173	Paper point or Swab	Yes	Strep. viridans Gram-positive organisms	

Table 1.5: Retrospective microbiological studies of acute pyogenic dental infection 1974 - 1986.

1.4.5 Summary

Acute dentoalveolar abscess is a relatively common dental infection but the knowledge of the microbiology involved appears to be incomplete. The difficulty of avoiding contamination of samples during collection in addition to the stringent demands on sampling and culture methods are probably the main reasons for the confusion in the results reported. Improved sampling and cultivation techniques used in recent investigations have resulted in a decrease in the isolation of facultative organisms and an increase in the culture of strict anaerobes. It would appear that acute dentoalveolar abscess contains a complex mixture of facultative and strictly anaerobic microorganisms that are also regarded as members of the normal oral flora.

Although it has become increasingly apparent that many of the bacteria present in acute dentoalveolar abscesses are strict anaerobes there is very little quantitative information on the relative proportions of individual isolates in the overall microbial load. Data provided by Williams et al., (1983) for 10 dentoalveolar abscesses has indicated that high concentrations of viable bacteria are present in these infections and that strict anaerobes predominate. However, there would appear to be a need for quantitative information from a larger number of abscesses.

1.5 PATHOGENICITY OF BACTERIAL ISOLATES

1.5.1 Introduction

Experimental animals have been used to investigate the pathogenicity of bacteria isolated from dental tissues since the earliest days of microbiology. Miller (1890) described the formation

of subcutaneous abscesses following the inoculation of particles of necrotic dental pulps under the skin of mice but also noted that the severity of these abscesses was variable, leading to the death of some animals whilst others had minimal symptoms. Since these early observations further experiments have been performed in an attempt to establish differences in the pathogeic potential of bacteria isolated from mixed dental infections. Head and Roos (1919) inoculated isolates from acute dental abscess by the intra-peritoneal route into mice and quinea pigs. Facultative streptococci were found to have low pathogenicity since only high doses were lethal. However an anaerobic gram-negative cocco-bacillus, which could only be successfully grown in combination with streptococci, was reported to have considerable pathogenicity. Intra-peritoneal inoculation of a mixture of these two organisms usually resulted in death of an adult mouse within 36 hours.

There is little additional information in the literature concerning the pathogenicity of bacterial species specifically isolated from acute dentoalveolar abscesses (van Steenbergen et al., 1984a) but it is probable that useful data can be gained from the studies of bacterial species isolated from other dental diseases, such as periodontits or pulpitis, since many of the species encountered are the same.

1.5.2 Studies of bacterial strains isolated from periodontal disease or necrotic dental pulps

A variety of animals have been employed in the pathogenicity testing of bacteria associated with necrotic pulps or periodontal

disease. The male mouse was used by Van Steenbergen et al., (1982), Brook et al., (1983), Brook and Walker, (1984) and Roenterink et al., (1984) whereas other workers have selected quinea pigs (Sundqvist et al., 1979) or rats (Mayrand and Mcbride, 1980). Animal experiments have involved either intra-peritoneal or subcutaneous inoculation. Extraoral inoculation sites have included the groin, back or foot (Brook and Walker, 1984, van Steenbergen et al., 1982; Roenterink and de Graaff, (1985). The intraoral sites used include the cheek (Takazoe and Nakomara, 1971) and the hard palate (Roeterink et al., 1984). In an attempt to create experimental conditions similar to those within human teeth some workers have introduced bacteria into the pulp chambers of rats (Kakehashi et al., 1965) or monkeys (Dahlen et al., 1982; Möller et al., 1981; Fabricus et al., 1982a, 1982b) whilst Sundqvist et al., (1984) and Moorer et al., (1984) implanted teflon or polythene tissue cages, containing known bacterial suspensions, subcutaneously in guinea pigs and rabbits.

A large part of the work investigating the pathogenicity of oral bacteria has involved black-pigmented bacteroides (Hite et al., 1949; MacDonald et al., 1963; Socransky and Gibbons, 1965; Takazoe and Nakamura, 1971). In these studies small laboratory animals, such as guinea pigs, were inoculated with combinations of bacteria which had originally been obtained from periodontal and endodontal sites. When black-pigmented bacteroides were present typical and transmissable infections were produced. However when they were absent from the inoculum the remaining organisms did not appear to possess pathogenic properties (MacDonald et al., 1963; Socransky and Gibbons, 1965). These experiments indicated that the presence of

black-pigmented bacteroides was essential for experimental infections and highlighted the fact that these bacteria are important in the pathogenicity of mixed bacterial populations. Although black-pigmented bacteroides have usually been found to be non-infective in pure culture exceptions have been reported (MacDonald et al., 1963; Takazoe et al., 1971; Kastelein et al., 1981). More recently it has been demonstrated that major differences in pathogenicity exist between various black-pigmented bacteroides (van Steenbergen et al., 1982; Roenterink et al., 1984; Roenterink et al., 1985) with B. gingivalis and B. melaninogenicus emerging as the most pathogenic species.

The reasons for the importance of bacteroides in abscesses is not clear but a number of virulence factors which may enable them to resist host defence systems have been suggested. It has been shown that strains possessing capsular material are more pathogenic than strains of the same species which do not (Takazoe et al., 1971; Okuda and Takazoe, 1973; Brook et al., 1983; Brook, 1986, 1987) and it has been suggested that the presence of capsular material may prevent the phagocytosis of these bacteria (Okuda and Takazoe, 1973; Simon et al., 1982). In addition to the presence of capsular material black-pigmented bacteroides can also produce a number of toxins, some of which directly inhibit polymorphonuclear leucocyte chemotaxis (Botta et al., 1985; Rotstein, 1985). (For reviews of virulence factors associated with anaerobic gram-negative bacilli see, Hofstad, (1984) and Slots and Genco, (1984).

Although most work has concentrated on bacteroides, Brook and Walker (1984, 1986) have stressed the need for investigation of the pathogenicity of fusobacteria and gram-positive cocci. The work described in the previous section of this chapter indicates that these bacterial species are frequently isolated from acute dentoalveolar abscesses and therefore may well play an important role in the development of these infections.

1.5.3 Summary

There have been few studies performed investigating the pathogenicity of bacteria isolated from acute dental abscesses but the findings of studies using bacterial strains isolated from necrotic pulps and periodontal disease are probably applicable to acute dentoalveolar abscess. Differences in the bacterial strains, experimental methods and animals used probably explain the variable findings in the literature. In general however it would appear that Bacteroides species are the most likely pathogens but the exact mechanisms of their pathogenicity are still unknown.

1.6 ANTIBIOTIC SENSITIVITY

1.6.1 Introduction

When the use of antibiotic therapy is indicated as part of the treatment of acute dental abscess it is often necessary to prescribe a drug before antibiotic susceptibility has been determined. It is therefore important that information is available which provides not only the identity of the likely causative bacteria but also describes their sensitivity to a range of antibiotics.

1.6.2 Antibiotic sensitivity tests

Approximately half of the microbiological studies listed in Table 1.4 contain details of antibiotic sensitivity testing (Papers 1, 2, 3, 5, 6, 8, 10, 11, 13, 15, and 17) and provide *in vitro* sensitivity patterns to a wide range of antibiotics.

It would appear that the vast majority of bacterial species isolated from dentoalveolar abscess are sensitive to a range of antibiotics, such as penicillin, erythromycin and clindamycin. Aderhold *et al.*, (1981) performed one of the most extensive of these studies and reported that less than 10 per cent of the facultative and strictly anaerobic isolates were resistant to a range of antibiotics (Table 1.6).

Phenoxymethylpenicillin and benzylpenicillin have been found to be two of the most effective antibiotics since the incidence of penicillin-resistant isolates has been low in the majority of studies. Those studies which cultured staphylococci found that many strains were resistant to penicillin (Gabrielson and Stroh, 1975; Epstein and Scopp, 1977) but, as was discussed in Section 1.4, the true significance of staphylococci in dental infection is doubtful. However clinical failure of penicillin has been reported (Heimdahl *et al.*, 1980; Bahn *et al.*, 1981) and one explanation was the presence of beta-lactamase producing bacteroides. Since it is likely that bacteroides play a major role in the development of dental abscesses the apparent recent emergence of penicillin-resistant strains may well have clinical implications.

	Aerobes		Anaerobes	
Antibiotic	No. of strains	Percent. resistant	No. of strains	Percent. resistant
Penicillin	22	4.6	56	0
Ampicillin	49	0	130	1.5
Oxacillin	36	2.8	103	1.0
Ticarcillin	37	0	104	1.0
Azlocillin	38	0	118	0.9
Mezlocillin	38	0	118	0.9
Cephalothin	49	4.1	114	0.9
Cefazolin	37	2.7	104	1.0
Cefamandole	39	0	122	0.8
Cefuroxime	37	0	122	1.6
Cefoxitin	42	4.8	119	0.8
Tetracycline	49	4.1	130	0.8
Chloramphenicol	48	0	130	0
Gentamicin	49	40.8	129	100.0
Sisomicin	43	39.5	105	100.0
Tobramycin	42	40.5	105	100.0
Amikacin	39	41.0	104	99.0
Erythromycin	49	6.1	129	0.8
Clindamycin	49	8.2	129	0
Sulphonamide	49	49.0	129	34.9

Table 1.6: Antibiotic sensitivity of aerobic and anaerobic bacteria isolated from acute dentogenous pyogenic infections. (After Aderhold *et al.*, 1981).

Sensitivity to ampicillin has not been investigated as often as penicillin but the information available indicates that it produces similar sensitivity patterns (Epstein and Scopp, 1977; Hunt et al., 1978; Sabiston and Gold 1974; Turner et al., 1975; Aderhold et al., 1981). Amoxycillin has a very similar antibacterial spectrum to ampicillin (Neu, 1974) and although the sensitivity of isolates from dental abscesses to this antibiotic has rarely been studied many strains are likely to be sensitive.

Other antibiotics which have been used to treat acute dental infections include erythromycin, clindamycin, cephalosporins and metronidazole. Although Heimdahl et al., (1981) reported a relatively high incidence of resistance to erythromycin the majority of studies have described it as an effective antibiotic. The vast majority of bacterial strains isolated from acute dental infections have been found to be sensitive to clindamycin (Aderhold et al., 1981; Gabrielson and Stroh, 1975). Aderhold et al., (1981) reported that 95 per cent of bacterial strains isolated from acute dentoalveolar abscesses were sensitive to 5 different cephalosporins (cephalothin, cefazolin, cefamandole, cefuroximine and cefoxitin). In the studies by Turner et al., (1975), Epstein and Scopp (1977) and Hunt et al., (1978) all the isolates were sensitive to cephalothin.

The increasing awareness that strict anerobes are frequently isolated from acute dental infections has resulted in investigations of their sensitivity to nitroimidazoles such as metronidazole (Ingham et al., 1977), ornidazole (von Konow and Nord, 1983) and tinidazole (von Konow et al., 1981). Although the vast majority of strict

anaerobes have been found to be sensitive, all the facultative isolates tested in these studies were resistant to these agents.

1.6.3 Summary

A comparison of the results of in vitro susceptibility tests performed on bacterial strains isolated from acute dental infections reveals that although the majority are sensitive to a range of antibiotics rarely has a single drug been found to be 100 per cent effective. Variations in the reported incidence of antibiotic resistance are probably due to differences in the spectrum of microorganisms studied and the method of testing. Production of beta-lactamase among oral bacteria is rare but has been implicated in the clinical failure of penicillin in the treatment of a small number of acute oro-facial infections (Heimdahl et al., 1980).

The antibiotic sensitivity reported in studies of acute dentoalveolar abscesses were performed on individual isolates but it has been suggested that testing of primary cultures may also be useful (Waterworth and del Piano, 1976). There is however no information on the reliability of this type of testing for acute dentoalveolar abscesses.

1.7 TREATMENT

1.7.1 Introduction

The treatment of an acute dentoalveolar abscess varies among individuals depending on the extent of the lesion, presence of trismus and the general health of the patient. Therapy can be divided

into local and general measures with the relative importance of each being different in each individual case. The first principle of treatment is to achieve drainage of any pus present and to ensure the removal of the source of infection (Piecuch, 1983). The management of acute dentoalveolar abscess is described in many texts of Oral Surgery (Howe, 1985; Sowray, 1985) and therefore will only be outlined in this chapter.

1.7.2 Local measures

Whenever a fluctuant swelling containing pus is present within the tissues surgical drainage must be established and maintained. This may be achieved by incision of the soft tissue swelling via an intraoral or extraoral approach, or both, depending on the location of the pus. Knowledge of the anatomy of the head and neck is essential when attempting to establish surgical drainage as facial tissue spaces must be opened without causing damage to the major nerves and blood vessels passing through them. Incisions through mucosa or skin should be of adequate length and be placed at a point that allows maximum drainage by gravity, which may be maintained by the placement of a sterile plastic or rubber drain.

If the affected tooth is to be retained then an opening should be made through the crown into the necrotic pulp chamber to allow drainage of any pus present within the tooth. Alternatively if the tooth is unsuitable for restoration then it should be extracted. In the past it was a popular belief that acute infection had to be resolved before extraction could be performed but it has been found that extraction of teeth at the time of initial presentation is not

associated with an increase in the incidence of post-extraction complications (Krogh, 1951; Martis and Karakasis, 1975), although general anaesthesia may be required since local anaesthesia cannot always be achieved in areas of acute inflammation (Brown, 1981).

1.7.3 General measures

Bed rest and adequate hydration are advisable for patients showing signs of poor health, such as raised temperature and cervical lymphadenopathy. Analgesics, such as aspirin, paracetamol, or codeine phosphate are usually required for the relief of pain.

It has been stated that antibiotics are prescribed too frequently in the treatment of acute dentoalveolar abscess (Zallen and Black, 1976) since local measures are often sufficient for resolution to occur (Piecuch, 1982). It is generally agreed however that antibiotic therapy is indicated in the case of a poorly localised abscess or when the patient is in poor health (Topazian and Goldberg, 1981). Penicillin has traditionally been regarded as the drug of choice in the treatment of acute dentoalveolar abscess (Topazian and Goldberg, 1981; Guralnick, 1984; Trieger, 1984) and it is common place to prescribe a course of 250 mg tablets for 7 days. However traditional regimens such as this have been challenged (Anonymous, 1979) and shorter courses of antibiotics at higher doses have been found to be as effective as conventional regimens for acute otitis media (Bain et al., 1985) and for urinary tract infection (Bailey and Abbott, 1977).

Penicillin can be given by mouth, intramuscularly or intravenously and causes minimal disturbance of the normal oral flora (Heimdahl et al., 1982). In addition to penicillin, related derivatives, such as cloxacillin and amoxycillin, have also been recommended for treatment of dentoalveolar abscess (Hooley, 1969; Morey et al., 1984). Erythromycin is the drug of choice for patients known to be hypersensitive to penicillin (Heimdahl and Nord, 1983; Guralnick, 1984) and can also be given by mouth, although it may cause marked disturbance of the oral microflora (Heimdahl and Nord, 1982). It has also been suggested that clindamycin should be regarded as an alternative to penicillin for penicillin-sensitive patients (de Viries and Francis, 1984) but Mehrhof (1976) has stated that because of the well recognised association between clindamycin therapy and antibiotic-associated colitis clindamycin should only be used when microbiological results indicate the presence of B. fragilis.

Cumming et al., (1984) have suggested that the cephalosporin, cefadroxil, be added to the list of drugs used in the treatment of acute oral infections and the in vitro sensitivity tests performed by Aderhold et al., (1981) would support the use of cephalosporins.

Metronidazole is highly active against obligate anaerobes and has been found clinically to be as effective as penicillin in the treatment of acute oro-facial infections (Ingham et al., 1977). A related nitroimidazole, ornidazole, has also been recommended as an alternative to penicillin for patients who are allergic to penicillin or when beta-lactamase producing anaerobes are present (Heimdahl et

al., 1980; von Konow and Nord, 1983).

1.7.4 Summary

Acute dentoalveolar abscess can often be managed by local measures alone but occasions do arise when general measures, including the use of antibiotics may be necessary. Although a number of different antibiotics are available penicillin is still probably the most widely used. Emphasis on the likely importance of strict anaerobes in acute dentoalveolar abscess has resulted in the recommendation that nitroimidazoles, such as metronidazole and ornidazole, should be used more frequently.

In some fields of medicine there has recently been discussion concerning the successful use of short-course high-dose antibiotic therapy as an alternative to conventional regimens but this aspect of chemotherapy has not been applied to acute dental infections.

1.8 AIMS OF THE PRESENT STUDY

The review of the literature presented in the previous sections of this chapter has revealed that there is paucity of scientific information on a number of aspects of the microbiology of acute dentoalveolar abscess.

Little quantitative data is available to support the general impression that strictly anaerobic bacteria predominate numerically in the flora of acute dentoalveolar abscesses. There have also been few investigations concerning the relationship between individual

bacterial species and the severity of clinical symptoms. In additional, although it is generally accepted that the flora of acute dentoalveolar abscess is usually polymicrobial few experiments have been performed to assess the pathogenicity of bacteria isolated. It is therefore uncertain if all the species present are equally pathogenic or if there are differences between them.

Penicillin has traditionally been regarded as the antibiotic of choice in the treatment of acute dental abscess but clinical failure of this antibiotic has resulted in discussion concerning whether or not alternative agents should be used routinely. Although the antibiotic sensitivity of pus samples obtained from acute dental abscesses has been investigated the majority of studies have only reported results for facultative bacteria and therefore little data is available on the susceptibility of strict anaerobes. Sensitivity testing is traditionally performed on individual isolates however it has been suggested that testing of primary cultures of pus is also useful although the reliability of performing this type of test on pus samples obtained from acute dentoalveolar abscesses has not investigated.

Lastly high-dose short-course antibiotic regimens have recently been found to produce similar clinical resolution as traditional regimens for a variety of acute infections but there is no information on the effectiveness of this type of regimen in the teatment of acute dentoalveolar abscesses.

The experiments described in this thesis were undertaken in order to obtain further information on the microbiology of acute dentoalveolar abscesses and the effectiveness of antibiotics in their management .

The initial study (Chapter 2) was designed to determine the identity and concentration of the bacterial species present in individual acute dentoalveolar abscesses and to relate the severity of the clinical symptoms of pain, swelling, lymphadenopathy and fever to the microorganisms present in pus samples.

Animal experiments (Chapter 3) were used to investigate the pathogenicity of pure and mixed cultures of the bacterial species most frequently isolated from the acute dentoalveolar abscess described in Chapter2.

Chapter 4 describes primary and secondary antibiotic sensitivity tests (disc diffusion method) carried out on pus samples obtained from acute dentoalveolar abscesses. Both types of tests were performed so as to provide not only further information on the sensitivity of the pus samples to a range of antibiotics but also to enable a comparison to be made between the results obtained by primary and secondary testing.

Since disc diffusion tests provide only an approximate estimate of antibiotic sensitivity, the minimum inhibitory concentration and minimum bactericidal concentration of bacterial strains classified as moderately sensitive or resistant in Chapter 4

was determined to provide a more detailed assessment of antibiotic susceptibility (Chapter 5).

On the basis of the sensitivity results obtained in Chapters 4 and 5 a clinical trial was performed to assess the effectiveness of high-dose amoxycillin compared to penicillin in the treatment of acute dentoalveolar abscess (Chapter 6).

The concluding chapter (7) inter-relates the various findings of the previous chapters to summarise the microbiological information which could assist the clinical management of acute dentoalveolar abscess and to highlight areas which require further study.

CHAPTER 2

QUANTITATIVE MICROBIOLOGY OF ACUTE DENTOALVEOLAR ABSCESS

2.1 INTRODUCTION

Recent microbiological studies of acute dental infection have found that pus samples from acute dentoalveolar abscesses are usually polymicrobial and frequently contain strict anaerobes (Labriola et al., 1983; von Konow and Nord, 1983; Heimdahl et al., 1985). There is however little quantitative information in the literature related to either the concentration of the overall microbial load or the relative proportions of each of the bacterial species present.

Sabiston et al., (1976) stressed the need for quantitative study of acute dental abscess and discussed the likely predominance of strict anaerobes but concluded that the methods they had employed in a study of 65 oro-facial infections were not sufficiently accurate to allow them to report quantitative results. A small amount of quantitative data was provided by Williams et al., (1983) who cultured high concentrations of viable bacteria from 10 acute dentoalveolar abscesses and reported that strict anaerobes accounted for the major part of the flora.

Increasing intrest in the microbiology of dental disease has led to the investigation of possible correlations between certain bacteria and the severity of clinical symptoms. Bacteroides melaninogenicus has been associated with symptomatic infected dental

pulps (Sundqvist, 1976; Griffee et al., 1980) and the presence of anaerobic gram-negative bacilli correlated with the occurence of severe symptoms of pain, swelling and fever in patients with acute dental abscess (Heimdahl et al., 1985).

Further information on the occurence of strict anaerobes in polymicrobial dental infections is required as it may well have implications for treatment. Therefore the aims of this chapter were to quantify the individual viable bacterial species present in acute dentoalveolar abscesses and to investigate any relationship between the microbial findings and the clinical symptoms experienced by the patients.

2.2 MATERIALS AND METHODS

2.2.1 Selection of patients

Material for this study was obtained from adult out-patients who presented with acute dentoalveolar abscess at the Casualty Receiving Clinic of Glasgow Dental Hospital and School. Patients were excluded from the study if they had any concurrent illness or had received antibiotics in the preceding three months. Case selection was limited to acute dentoalveolar abscesses which had progressed to produce fluctuant swelling in the soft tissues surrounding the affected tooth. Patients were also excluded if there was evidence of a communication between the abscess and the oral cavity, either through a sinus opening onto the oral mucosa or via the gingival margin.

2.2.2 Clinical details

The sex and age of each patient were noted and the identity of the tooth involved was recorded according to Palmer's dental notation (Palmer, 1891), the standard system used in the United Kingdom.

Subjective and objective assessments were made of the clinical symptoms of pain, swelling, lymphadenopathy and temperature.

Pain

Severity of pain was measured using a 10 cm visual analogue scale (Revill et al., 1976). One extremity of the line (0 cm) was labelled "no pain" and the other (10 cm) "unbearable pain" (Figure 2.1). Patients were asked to place a mark on the scale at the point which corresponded to the amount of pain they were experiencing. A numerical value of pain was obtained by measuring the distance (cm) from zero to the mark. The number of days that the patient had experienced pain prior to presentation was also recorded.

Swelling

The number of days the patient had been aware of any swelling were noted.

Lymphadenopathy

Lymphadenopathy was considered to be present if one or more cervical lymph nodes were palpable and tender.

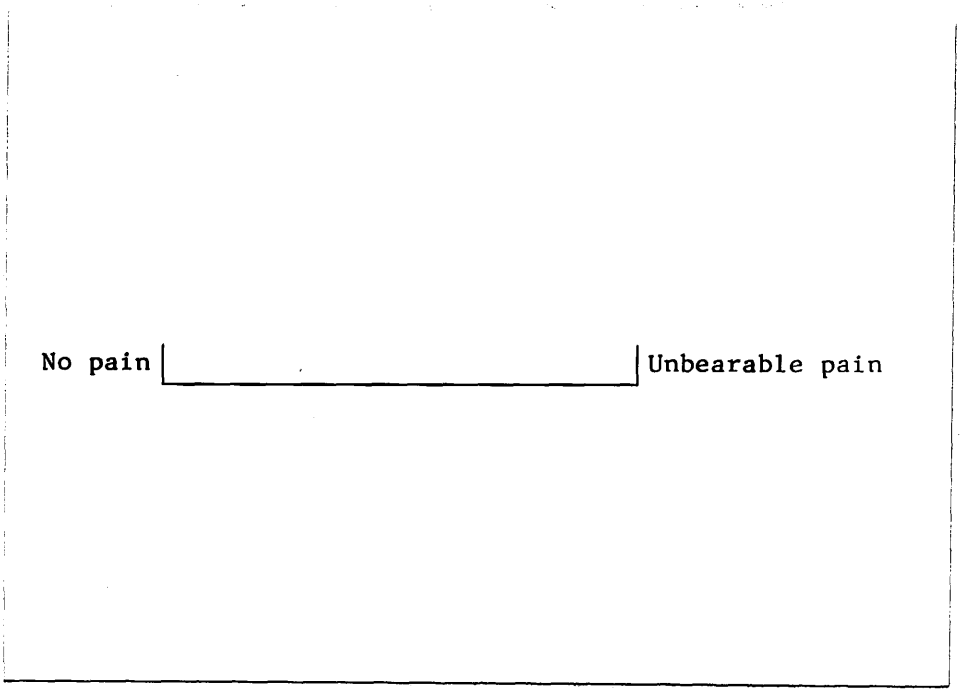


Figure 2.1: Design of a 10 cm linear analogue scale which was used to measure severity of pain.

Temperature

Sub-lingual temperature and axillary temperature was measured using a clinical mercury thermometer held in position for 90 seconds.

2.2.3 Sampling procedure

Patients rinsed their mouths for 30 seconds with a solution of 0•2 per cent chlorhexidine gluconate (Central Sterile Fluids Laboratory, Knightswood Hospital, Glasgow). The abscess was then isolated intraorally with sterile cotton-wool rolls and the surface mucosa wiped with a cotton-wool bud soaked in 0•2 per cent chlorhexidine gluconate solution. Anaesthesia of the mucosa was achieved by the application of a fine ethyl chloride spray (Bengue and Co. Ltd., Maidenhead) for 5 seconds. A 5 ml disposable syringe fitted with a 21 gauge needle (Becton Dickinson UK Ltd., Oxford) was used to aspirate pus (Figure 2.2). Samples of less than 0•5 ml or containing visible blood were not included in the study. Specimens were transferred immediately to the laboratory for microbiological processing.

2.2.4 Microbiological preparation

A smear of each sample was prepared on a glass microscope slide and stained by Gram's method (Baker and Breach, 1980).

A 0•5 ml volume of each sample was added to 19•5 ml Anaerobic Blood Broth (ABB) (Gibco Europe, Paisley) and subjected to vortex mixing for 30 seconds using a whirlmixer (Fisons Scientific, Loughborough) under anaerobic conditions. Three further dilutions

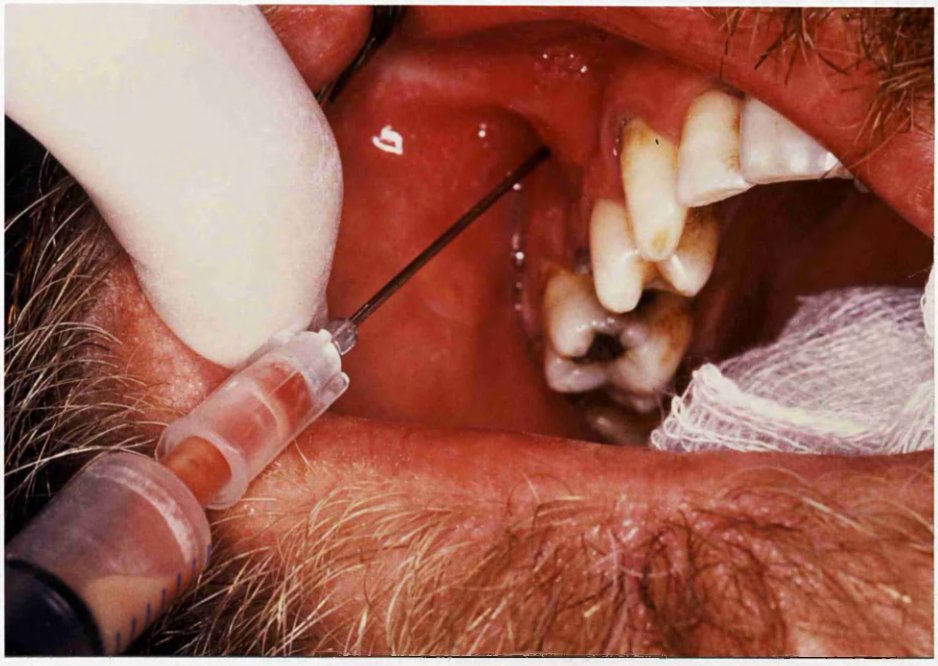


Figure 2.2: Needle aspiration of pus from an acute dentoalveolar abscess affecting a grossly carious upper right second premolar.

were made in ABB (Gibco Europe, Paisley) to achieve concentrations of 1 in 800, 1 in 1,600 and 1 in 16,000. Duplicate 50 μl volumes of each dilution were inoculated onto Columbia Blood Agar (Oxoid Ltd., Basingstoke) and modified Campylobacter medium, containing 0•1 ml campylobacter antibiotic mix per 100 ml agar (Gibco Europe, Paisley) as described by Skirrow (1977), using a spiral plater (Don Whitely Scientific, Shipley). In the spiral system an agar plate, which is rotating on a turntable, is inoculated with 50 μl of fluid sample by a stylus moving in a straight line from the centre of the plate to the periphery (Figure 2.3). The combined movement of plate and stylus produces a spiral pattern of inoculation which results in an increasing dilution of the sample on the agar surface towards the periphery of the plate.

2.2.5 Incubation

Inoculated plates of each dilution were incubated at 37°C for 7 to 10 days in an anaerobic chamber (Appendix I) (Figure 2.4). Plates of Columbia Blood Agar (Oxoid Ltd., Basingstoke) inoculated with each dilution were also incubated at 37°C for 7 days in an atmosphere of air with carbon dioxide 10 per cent (Laboratory Thermal Equipment, Oldham).

2.2.6 Quantification of growth

The Columbia Blood Agar culture plates (Oxoid Ltd., Basingstoke) of each abscess were examined and one containing between 10^3 and 10^4 well separated colonies was selected for study (Figure 2.5). Using a calibrated metal stamp the surface of the culture plate

Figure 2.3: Spiral plate system used to inoculate solid media with a 50 μl volume of fluid specimen in a spiral pattern.

Figure 2.4: Anaerobic chamber (Don Whitely Scientific Ltd., Shipley) containing a gas mixture of 85 per cent nitrogen, 10 per cent hydrogen and 5 per cent carbon dioxide, maintained at 37°C.

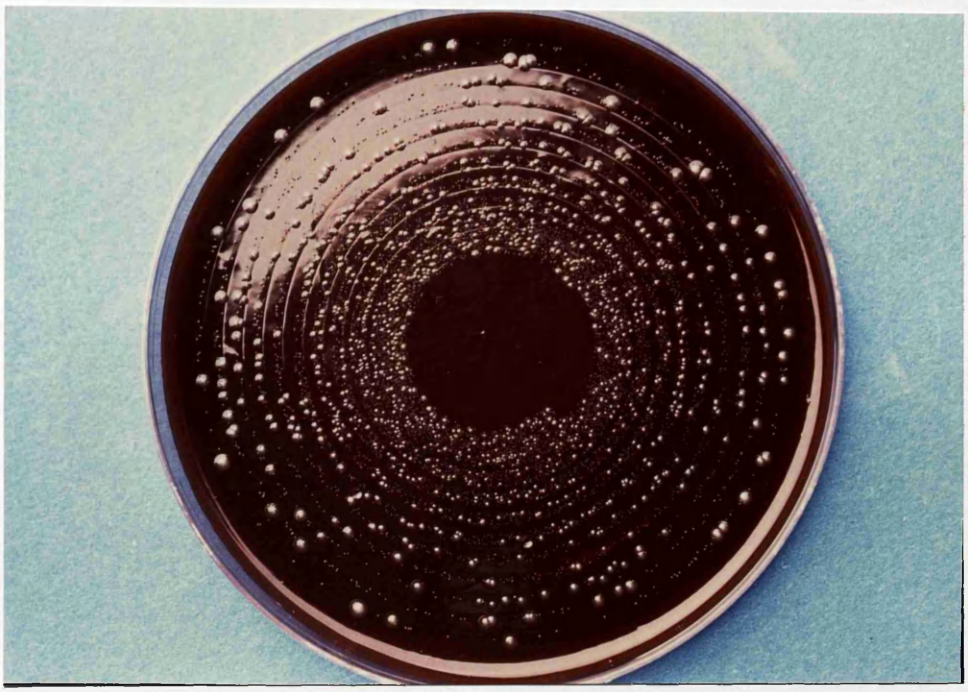


Figure 2.5: Incubated Columbia Blood Agar plate inoculated, using the spiral system, with a pus sample obtained from an acute dentoalveolar abscess.

was split into a number of areas, each of which had received a known volume of inoculum (Figure 2.6). An area of the plate, containing a minimum of 100 colonies, was chosen and the numbers of each different morphological colony type counted (Figure 2.7). Identical colonies in the corresponding area on the opposite side of the plate were also counted and the two values added. The number of colony forming units per millilitre (cfu/ml) of each isolate in the pus sample was calculated using the equation;

$$\text{cfu/ml} = \frac{n}{v} \times d \times 1000$$

where n = number of colonies in area counted

v = volume of sample inoculated onto the area counted (μl)

d = dilution of sample

2.2.7 Identification of isolates

Four single colonies of each morphological type present on the Columbia Blood Agar (Oxoid Ltd., Basingstoke) or modified Campylobacter selective medium (Gibco Europe, Paisley) were individually subcultured onto a fresh Columbia Blood Agar (Oxoid Ltd., Basingstoke) to obtain pure cultures. The plates of modified Campylobacter selective medium (Gibco Europe, Paisley) were not used for quantification but were included in the study to assist the isolation of bacteroides. Initial characterisation of isolates was based on Gram stain reaction (Baker and Breach, 1980), microscopic morphology and on their ability to grow in the following conditions; air, air with carbon dioxide 10 per cent and an atmosphere of 85 per cent nitrogen, 10 per cent hydrogen and 5 per cent carbon dioxide.

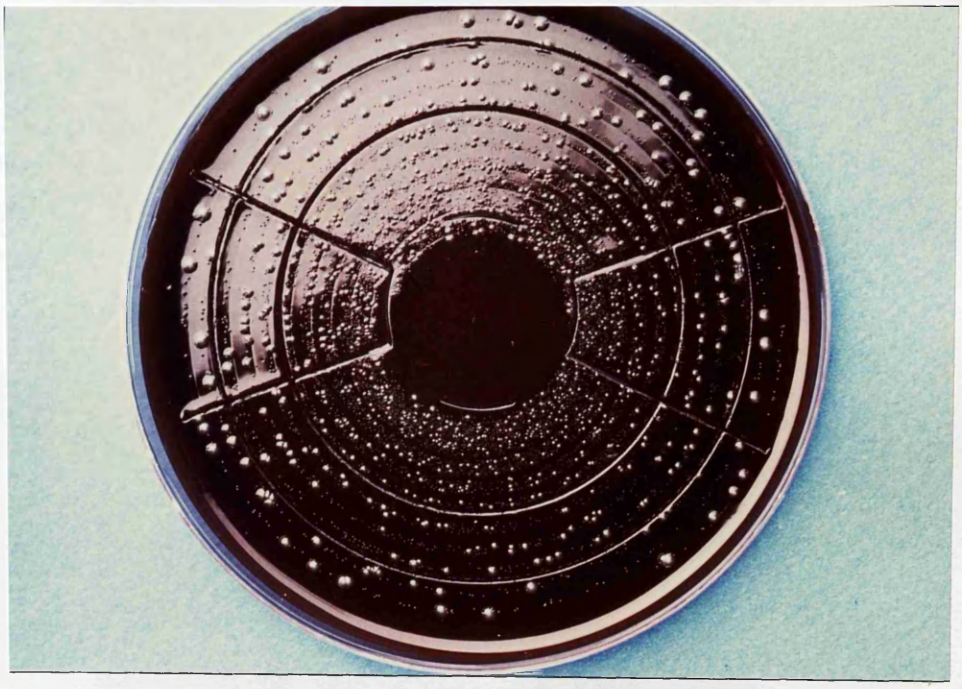


Figure 2.6: Incubated anaerobic culture plate which has been divided into areas which received a known volume of inoculum.

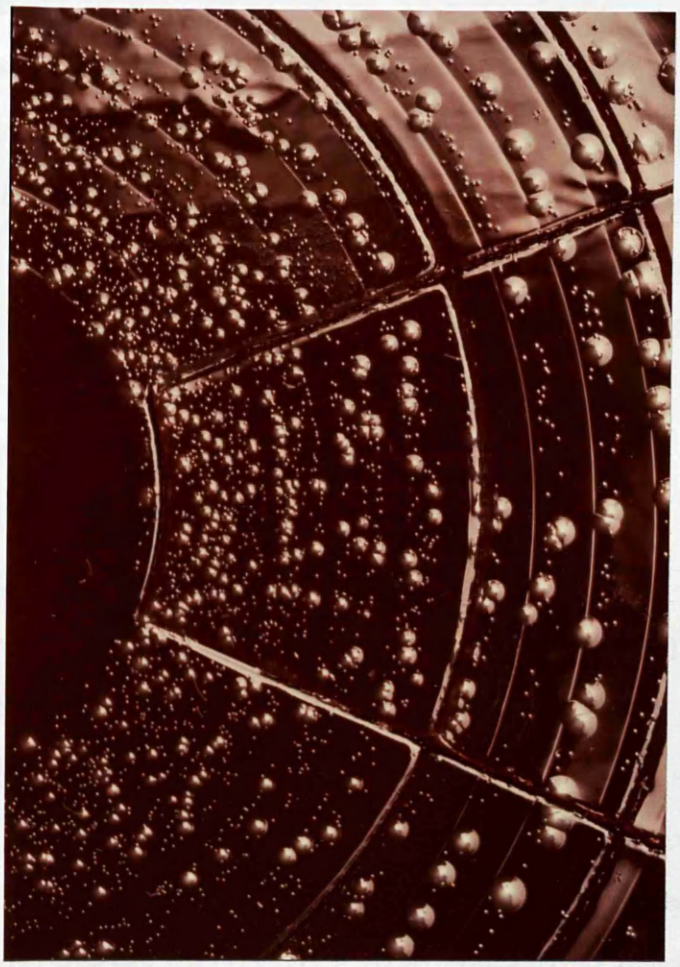


Figure 2.7: Incubated anaerobic culture plate demonstrating growth of 2 different morphological colony types. The small colonies were subsequently identified as Peptococcus species and the large colonies as Streptococcus milleri.

Facultative gram-positive cocci were identified to species level using the API 20 system for streptococci (API Laboratory Products, Basingstoke). Other facultative organisms were identified according to a combination of standard methods (Buchanan and Gibbons, 1974; Cowan and Steel, 1974; Hardie and Bowden, 1974; Sutter et al., 1980). Identity of Actinomyces species was according to Slack and Gerencser (1975) and identity of Haemophilus species according to Killian (1976). Detection of acidic end products of metabolism using a tachophor (Model LKB 2127, LKB Instruments, Bromma, Sweden) was performed, as described by Shah et al., (1985), to assist the identification of Arachnia propionica.

Strict anaerobes were characterised to species level using the Minitek system (Becton Dickinson UK Ltd., Oxford). However strictly anaerobic gram-positive cocci, other than Strep. intermedius and Strep. constellatus, could not be reliably identified with the Minitek system (Becton Dickinson UK Ltd., Oxford) and were therefore differentiated into Peptococcus species or Peptostreptococcus species depending on their susceptibility to a disc containing novobiocin 5 μg (Wren et al., 1977).

2.2.8 Storage of isolates

In order to preserve fresh cultures of all the bacterial isolates each strain was stored within three subcultures of initial isolation using a freeze dryer (Edwards High Vacuum, Crawley).

2.3 RESULTS

2.3.1 Patients

Pus was obtained from 50 acute dentoalveolar abscesses in patients who were otherwise healthy and had not received antibiotics in the preceding 3 months. There were 38 males and 12 females with an age range of 14 to 65 years and a mean of 31 years.

2.3.2 Tooth affected

None of the abscesses investigated were associated with deciduous teeth. Thirty four of the abscesses originated from a permanent upper tooth and 16 from a permanent lower tooth. The distribution of the abscessed teeth is shown in Figure 2.8 and the most commonly affected were the upper lateral incisors and the upper molars.

2.3.3 Appearance of pus smears stained by Gram's method

All the pus samples demonstrated the presence of polymorphonuclear leukocytes and microorganisms. In 25 of the smears a mixture of large numbers of gram-positive cocci and gram-negative cocco-bacilli was apparent (Figure 2.9). A combination of gram-positive cocci and gram-positive bacilli was present 6 cases and in 19 specimens gram-positive cocci alone were detected.

2.3.4 Bacterial species isolated

Bacteria were cultured from all the pus samples examined and details of the microorganisms isolated from each abscess is shown in Table 2.1. A total of 166 bacterial strains were cultured, of which 123 (74 per cent) were strict anaerobes and 43 (26 per cent)

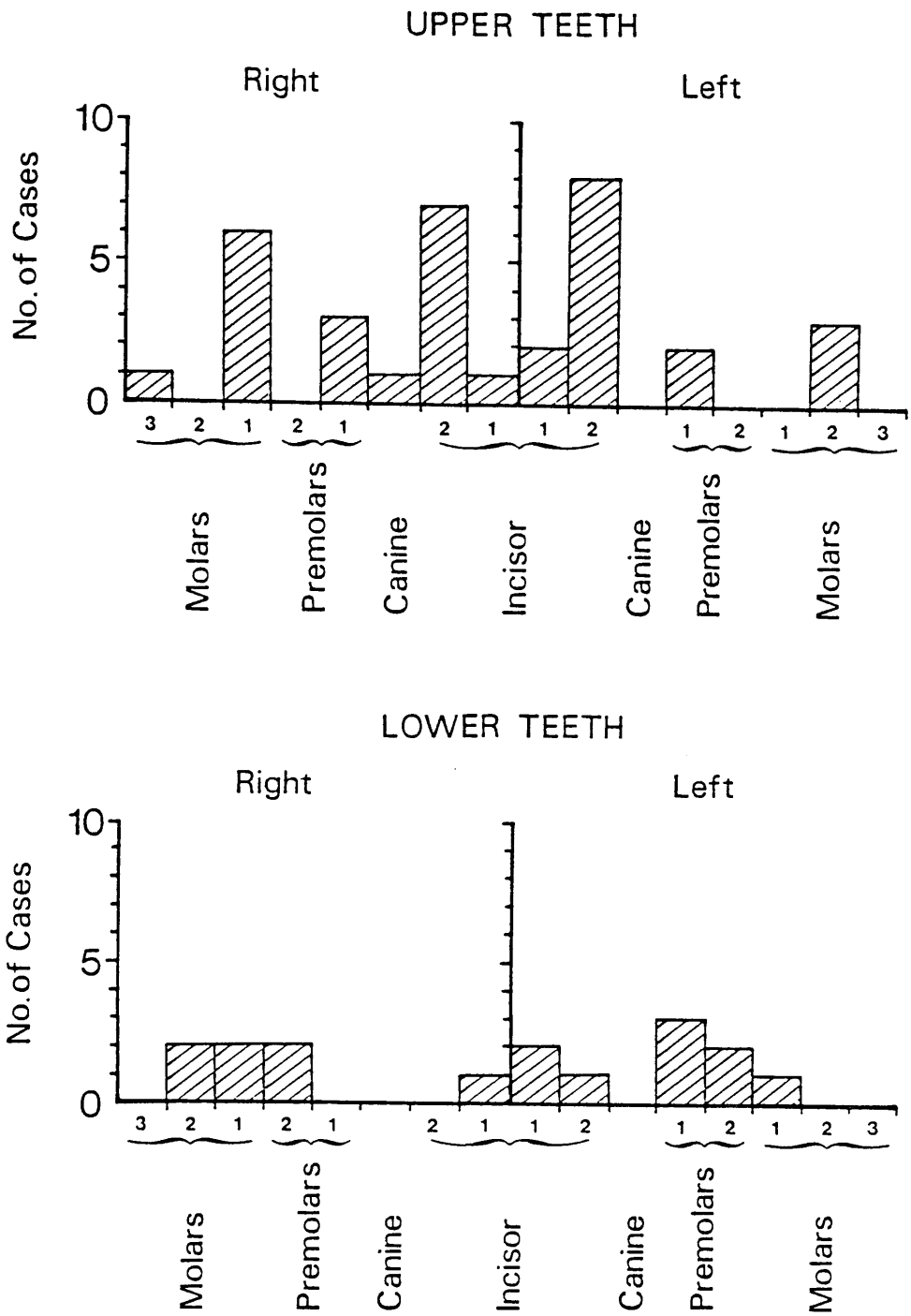


Figure 2.8: Distribition of permanent teeth involved in 50 acute dentoalveolar abscesses.

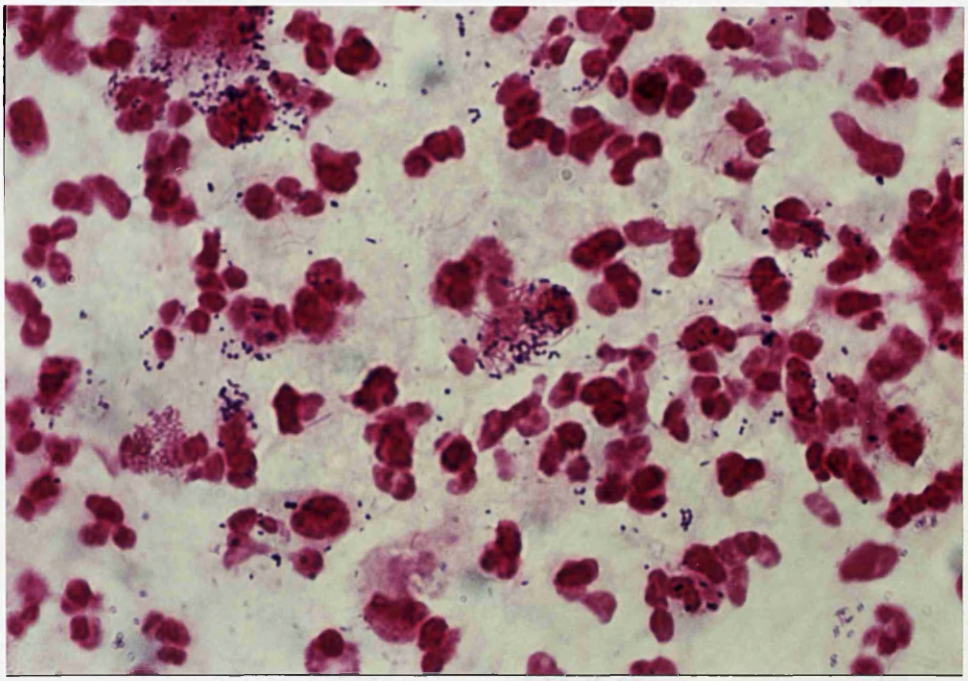


Figure 2.9: Smear of pus obtained from an acute dentoalveolar abscess and stained by Gram's method.

Table 2.1: Identity and concentration of bacterial strains isolated from 50 acute dentoalveolar abscesses.

Patient No.	Bacterial Species Isolated	cfu/ml	Percentage strict anaerobic content
1	Peptococcus species	2.4×10^5	100
2	Peptococcus species	3.5×10^4	100
	Bacteroides gingivalis	1.5×10^4	
3	Streptococcus milleri	7.9×10^4	0
4	Streptococcus milleri	2.1×10^4	
	Bacteroides intermedius	4.0×10^4	
	Peptococcus species	1.0×10^2	67
	Fusobacterium nucleatum	1.0×10^2	
	Actinomyces naeslundi	1.0×10^2	
5	Streptococcus milleri	1.1×10^7	0
	Streptococcus mitis	1.1×10^7	
6	Streptococcus milleri	3.3×10^6	
	Bacteroides gingivalis	7.4×10^6	
	Bacteroides intermedius	9.4×10^6	86
	Bacteroides oralis	1.0×10^6	
	Bacteroides oralis	2.6×10^6	
7	Streptococcus intermedius	7.4×10^5	100
	Peptococcus species	3.9×10^5	
8	Bacteroides ruminicola	7.2×10^7	
	Peptococcus species	4.2×10^7	100
	Peptostreptococcus species	6.9×10^5	
	Veillonella parvula	1.2×10^5	
9	Streptococcus milleri	8.5×10^4	
	Peptococcus species	5.9×10^4	46
	Bacteroides oralis	1.3×10^4	
10	Streptococcus mitis	3.3×10^7	
	Bacteroides distasonis	3.6×10^5	6
	Bacteroides ureolyticus	2.3×10^5	
	Bacteroides intermedius	1.7×10^6	

Patient No.	Bacterial Species Isolated	cfu/ml	Percentage strict anaerobic content
11	Streptococcus intermedius	1.1×10^6	
	Peptococcus species	4.8×10^5	100
	Bacteroides melaninogenicus	8.8×10^4	
12	Streptococcus intermedius	4.5×10^8	100
	Fusobacterium nucleatum	8.6×10^5	
13	Streptococcus milleri	6.4×10^8	
	Peptostreptococcus species	1.7×10^8	21
	Propionibacterium acnes	5.4×10^6	
14	Streptococcus milleri	2.4×10^4	
	Capnocytophaga ochracea	1.2×10^5	25
	Streptococcus milleri	6.1×10^4	
	Peptostreptococcus species	7.1×10^4	
15	Bacteroides oralis	1.4×10^7	
	Streptococcus milleri	3.0×10^6	
	Haemophilus parainfluenzae	6.0×10^6	97
	Bacteroides ruminicola	4.8×10^5	
	Peptostreptococcus species	2.4×10^8	
16	Lactobacillus fermentum	2.4×10^5	
	Streptococcus sanquis	8.9×10^4	9
	Actinomyces odontolyticus	2.4×10^5	
	Bacteroides melaninogenicus	5.2×10^4	
17	Streptococcus mitis	8.8×10^4	
	Streptococcus milleri	6.4×10^4	79
	Fusobacterium mortiferum	2.8×10^5	
	Bacteroides gingivalis	3.0×10^5	
18	Streptococcus milleri	1.4×10^7	
	Bacteroides oralis	3.5×10^4	99
	Bacteroides oralis	2.5×10^9	
19	Streptococcus milleri	2.2×10^5	
	Peptococcus species	2.6×10^8	99
	Bacteroides oralis	2.1×10^7	
20	Actinomyces meyeri	5.9×10^4	98
	Peptostreptococcus species	4.2×10^6	

Table 2.1 (Cont.)

Patient No.	Bacterial Species Isolated	cfu/ml	Percentage strict anaerobic content
21	Peptococcus species	7.1×10^4	100
22	Bacteroides gingivalis	4.6×10^8	100
	Peptostreptococcus species	1.4×10^8	
23	Streptococcus mutans	2.6×10^4	
	Bacteroides oralis	3.9×10^5	96
	Peptococcus species	2.7×10^5	
24	Peptococcus species	4.0×10^6	
	Fusobacterium nucleatum	2.0×10^6	100
	Bacteroides oralis	2.4×10^5	
25	Streptococcus milleri	3.7×10^5	
	Peptococcus species	3.8×10^5	91
	Fusobacterium nucleatum	3.5×10^6	
	Bacteroides gingivalis	1.0×10^2	
26	Bacteroides gingivalis	3.4×10^4	
	Bacteroides melaninogenicus	3.5×10^5	100
	Peptostreptococcus	1.2×10^4	
	Veillonella parvula	1.6×10^5	
27	Streptococcus sanquis	4.1×10^6	
	Bacteroides gingivalis	6.5×10^5	64
	Peptococcus species	6.7×10^6	
28	Streptococcus milleri	2.7×10^7	
	Bacteroides oralis	2.3×10^6	
	Lactobacillus salivarius	2.6×10^4	87
	Bacteroides ruminicola	2.0×10^7	
	Bacteroides oralis	2.0×10^7	
	Peptococcus species	1.5×10^8	
29	Eikenella corrodens	8.2×10^4	
	Bacteroides uniformis	4.9×10^5	90
	Peptococcus species	1.3×10^6	
	Arachnia propionica	5.9×10^4	
30	Peptococcus species	7.6×10^7	100
	Bacteroides gingivalis	3.1×10^5	

Table 2.1 (Cont.)

Patient No.	Bacterial Species Isolated	cfu/ml	Percentage strict anaerobic content
31	Peptococcus species	1.3×10^7	100
	Bacteroides oralis	1.1×10^8	
32	Peptococcus species	2.0×10^5	100
	Peptostreptococcus species	8.9×10^3	
33	Peptococcus species	2.1×10^5	
	Eubacterium lentum	4.1×10^4	100
	Bacteroides oralis	8.9×10^3	
	Bacteroides gingivalis	2.4×10^4	
34	Peptococcus species	7.2×10^4	
	Bacteroides gingivalis	4.1×10^4	100
	Bacteroides ruminicola	1.6×10^3	
	Bacteroides melaninogenicus	2.3×10^5	
35	Peptococcus species	1.2×10^9	
	Bacteroides melaninogenicus	1.9×10^7	100
	Bacteroides intermedius	7.2×10^7	
	Bacteroides intermedius	1.8×10^8	
36	Streptococcus milleri	1.4×10^8	
	Bacteroides melaninogenicus	1.4×10^6	57
	Peptococcus species	1.9×10^8	
37	Streptococcus milleri	6.4×10^5	
	Peptococcus species	5.1×10^7	
	Bacteroides gingivalis	4.8×10^6	97
	Streptococcus milleri	1.0×10^6	
	Bacteroides oralis	6.4×10^6	
38	Streptococcus milleri	1.2×10^5	
	Bacteroides melaninogenicus	1.2×10^6	
	Peptococcus species	9.0×10^7	99
	Bacteroides oralis	1.0×10^6	
	Peptostreptococcus species	8.0×10^5	
39	Streptococcus milleri	1.6×10^7	
	Peptococcus species	3.0×10^8	
	Streptococcus milleri	1.3×10^7	91
	Streptococcus constellatus	8.7×10^5	
	Bacteroides ruminicola	5.4×10^5	
40	Streptococcus milleri	1.3×10^6	0

Table 2.1 (Cont.)

Patient No.	Bacterial Species Isolated	cfu/ml	Percentage strict anaerobic content
41	Bacteroides melaninogenicus	2.2×10^5	
	Peptococcus species	4.7×10^6	
	Peptostreptococcus species	2.9×10^6	100
	Bacteroides oralis	4.4×10^6	
	Bacteroides gingivalis	1.4×10^7	
42	Lactobacillus fermentum	1.6×10^5	
	Peptococcus species	5.6×10^4	57
	Peptostreptococcus species	1.6×10^5	
43	Streptococcus milleri	4.0×10^7	
	Peptococcus species	7.4×10^8	
	Peptostreptococcus species	1.4×10^8	
	Bacteroides oralis	7.9×10^5	96
	Bacteroides oralis	6.4×10^5	
	Bacteroides melaninogenicus	4.8×10^6	
	Veillonella parvula	2.1×10^7	
	Fusobacterium nucleatum	1.8×10^7	
44	Streptococcus milleri	1.6×10^8	
	Bacteroides melaninogenicus	1.1×10^8	45
	Fusobacterium nucleatum	2.4×10^7	
45	Streptococcus milleri	6.8×10^4	50
	Peptococcus species	7.0×10^4	
46	Peptococcus species	3.5×10^7	
	Bacteroides ruminicola	1.1×10^7	100
	Bacteroides melaninogenicus	6.4×10^5	
47	Bacteroides capillosus	4.6×10^7	
	Bacteroides gingivalis	6.0×10^5	100
	Peptostreptococcus species	1.3×10^7	
48	Streptococcus sanquis	6.0×10^5	
	Haemophilus parainfluenzae	4.0×10^5	98
	Peptococcus species	2.2×10^7	
	Bacteroides oralis	2.9×10^7	
49	Streptococcus milleri	3.0×10^5	
	Peptococcus species	5.2×10^5	24
	Bacteroides melaninogenicus	4.3×10^5	
50	Bacteroides oralis	3.0×10^8	
	Bacteroides gingivalis	7.3×10^7	100
	Peptostreptococcus species	1.1×10^8	

Table 2.1 (Cont.)

facultative anaerobes (Table 2.2). The majority of the organisms were either facultative gram-positive cocci, anaerobic gram-positive cocci or anaerobic gram-negative bacilli. The bacterial species most frequently isolated were B. oralis, B. gingivalis, B. melaninogenicus, Peptococcus species, Peptostreptococcus species and Strep. milleri.

2.3.5 Number of species and bacterial combinations occurring in individual abscesses

There was a mean of 3·3 bacterial species per abscess, with a range of 1 to 8 species. The distribution of the abscesses according to the number of bacterial species cultured from each case is shown in Figure 2.10. A single species was cultured from 4 of the abscesses, 2 cases involving Strep. milleri and 2 cases involving Peptococcus species. From the remaining 46 abscesses more than one species was cultured.

The occurrence of combinations of different bacterial groups in the abscesses with a mixed flora is shown in Table 2.3. The most frequently encountered combinations were; 1. anaerobic gram-negative bacilli with anaerobic gram-positive cocci (62 per cent of abscesses), 2. anaerobic gram-negative bacilli with facultative gram-positive cocci (42 per cent of abscesses) and 3. facultative gram-positive cocci with anaerobic gram-positive cocci (38 per cent of abscesses).

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

Table 2.2: Identity of 166 bacterial strains isolated from 50 acute dentoalveolar abscesses.

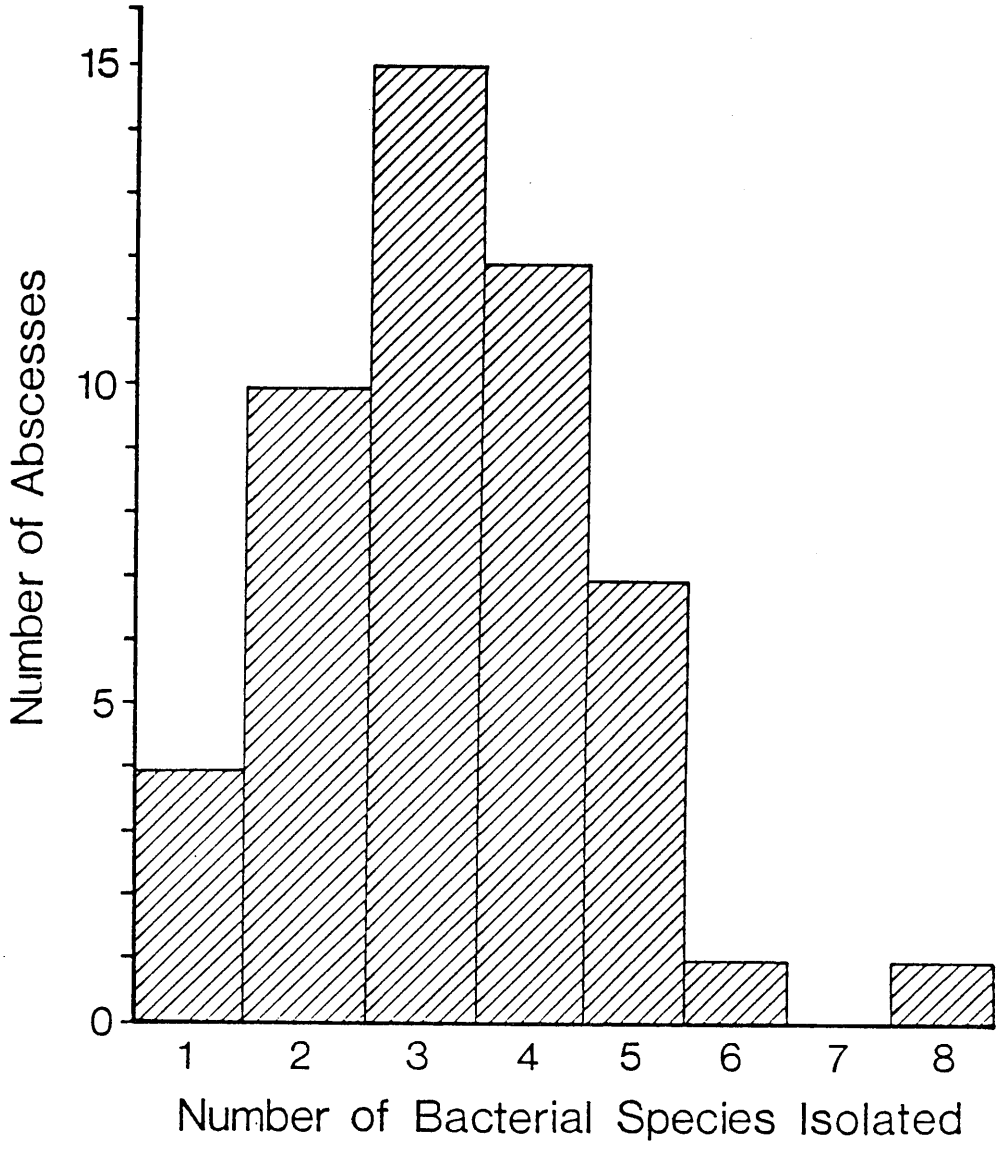


Figure 2.10: Distribution of 50 acute denotoalveolar abscesses according to the number of bacterial species cultured.

Index group of bacteria	Percentage of abscesses containing, in addition to index group, Facultative anaerobes: Gram-positive cocci	Facultative anaerobes: Gram-positive bacilli	Facultative anaerobes: Gram-negative bacilli	Strict anaerobes: Gram-positive cocci	Strict anaerobes: Gram-positive bacilli	Strict anaerobes: Gram-negative cocci	Strict anaerobes: 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	0	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	..

Table 2.3: Bacterial combinations encountered in 50 acute dentoalveolar abscesses.

Combinations of the most frequently isolated bacterial species are shown in Table 2.4. The most frequent pairings were Peptococcus species with Strep. milleri (12 cases), Peptococcus species with B. oralis (11 cases), Peptococcus species with B. gingivalis (11 cases) and B. oralis with Strep. milleri (9 cases).

2.3.6 Viable bacterial counts

The highest viable colony count for a single organism was $1{\cdot}2 \times 10^9$ cfu/ml and the lowest $1{\cdot}0 \times 10^2$ cfu/ml. The mean concentration, expressed as $\log_{10}$, for each group of isolates is shown in Table 2.5. The mean viable count ($\log_{10}$) for facultative organisms was 5·7 and for strict anaerobes 6·2.

The largest viable microbial load for an abscess was $2{\cdot}5 \times 10^9$ and the lowest $5{\cdot}0 \times 10^4$. The mean load, expressed as $\log_{10}$ cfu/ml, for the 50 abscesses was 6·9.

2.3.7 Percentage strictly anaerobic content

Each abscess was classified into one of five groups according to the percentage of the flora that consisted of strictly anaerobic bacteria.

Group 1	-	0 per cent
Group 2	-	1 to 33 per cent
Group 3	-	34 to 65 per cent
Group 4	-	66 to 99 per cent
Group 5	-	100 per cent

Index bacterial species	Number of abscesses containing, in addition to index species								
	Strep. milleri	Pepto. species	Peptostrep. species	B. gingivalis	B. oralis	B. melanin-ogenicus	B. intermedius	B. ruminicola	F. nucleatum
Strep. milleri	..	12	4.	4	9	4	3	3	5
Pepto. species	12	..	6	9	11	6	3	5	4
Peptostrep. spp.	4	6	..	3	4	3	1	2	2
B. gingivalis	4	9	3	..	5	3	2	1	2
B. oralis	9	11	4	5	..	3	1	2	2
B. melaninogenicus	4	6	3	3	3	..	0	2	2
B. intermedius	3	3	1	2	1	0	..	0	0
B. ruminicola	3	5	2	1	2	2	0	..	0
F. nucleatum	5	4	2	2	2	2	0	0	..

Table 2.4: Most frequent combinations of bacterial species encountered in 50 acute dentoalveolar abscesses.

Bacterial group	Number of isolates	Mean concentration (log_{10} cfu/ml) ± SD
Facultative anaerobes	43	5•7 ± 0•2
Gram-positive cocci	32	6•1 ± 0•2
Gram-positive bacilli	7	4•6 ± 0•4
Gram-negative bacilli	4	5•6 ± 0•4
Strict anaerobes	123	6•2 ± 0•1
Gram-positive cocci	50	6•3 ± 0•2
Gram-positive bacilli	3	6•3 ± 0•8
Gram-negative cocci	2	5•9 ± 0•7
Gram-negative bacilli	68	6•1 ± 0•2

Table 2.5: Number of isolates and mean concentration of each bacterial group obtained from 50 acute dentoalveolar abscesses.

A purely facultative flora was cultured from 3 cases, a purely strictly anaerobic flora from 20 cases and a mixture of both types of organism was isolated from 27 abscesses. In 36 (72 per cent) of the abscesses strictly anaerobic bacteria accounted for between 66 per cent and 100 per cent of the cultivable microbial load (Figure 2.11).

2.3.8 Clinical Symptoms

Details of the clinical symptoms of pain, swelling, presence of tender cervical lymph nodes and temperature recorded for each patient are shown in Table 2.6 (Note the patient identification number used in Table 2.6 corresponds to the patient number used in Table 2.1). The mean results of the clinical symptoms are shown in Table 2.7. Forty-six patients were in pain at the time of presentation and the severity score ranged from 0•7 to 9•9, with a mean of 4•9. The number of days that a patient had been in pain prior to presentation ranged from 1 to 6, with a mean of 1•8 days.

All the patients presented with swelling, which had been present for between 1 and 14 days. The majority of patients (82 per cent) had had swelling for less than 3 days and the mean for the group was 2 days.

Tender enlarged cervical lymph nodes were detected in only 4 of the 50 patients studied.

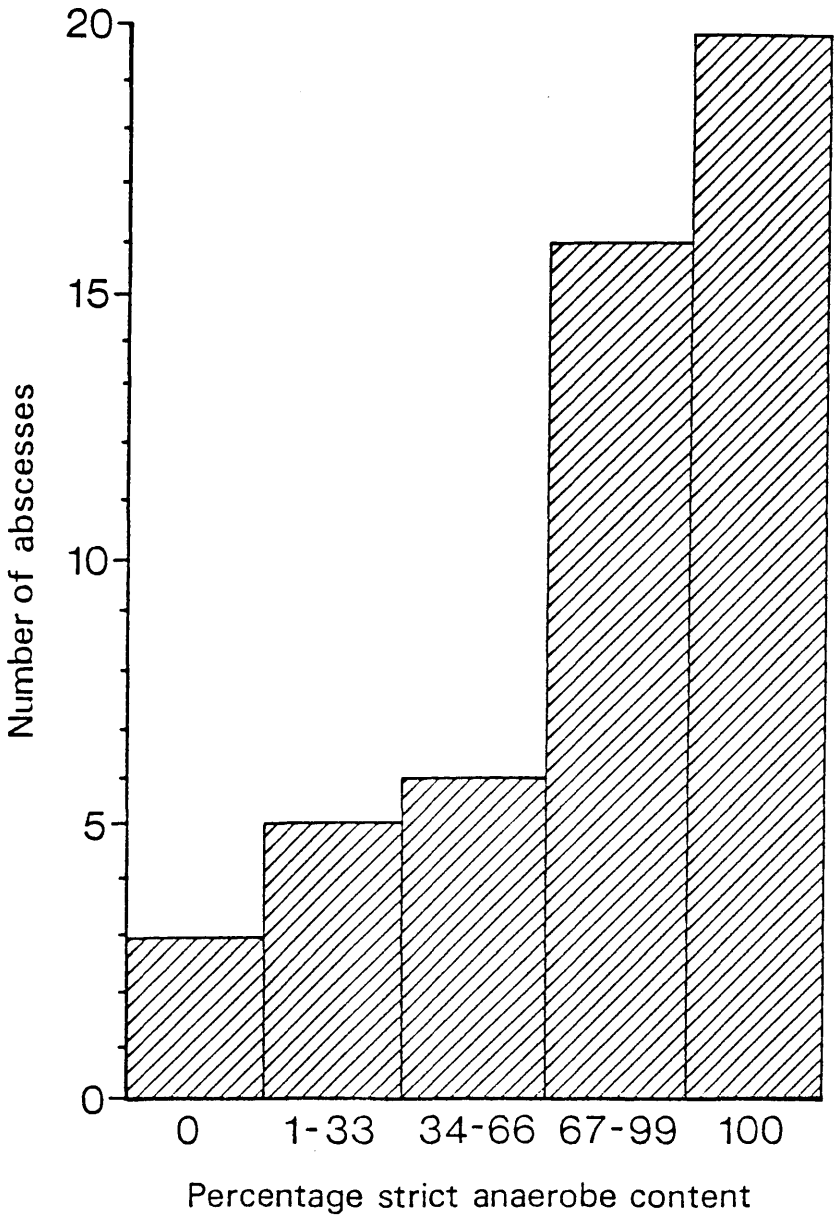


Figure 2.11: Distribution of 50 abscesses according to the percentage of the microbial count that was strictly anaerobic.

Table 2.6: Clinical symptoms for 50 patients with acute dentoalveolar abscess at the time a pus sample was obtained.

Patient Number	Pain	No. of days of pain	Severity of pain	Swelling	No. of days of swelling	Oral temp. (°C)	Axilla temp. (°C)	Tender nodes	Abscess group
1	Yes	3	9.9	Yes	1	37.4	37.1	No	5
2	Yes	2	9.0	Yes	2	37.3	36.9	No	5
3	No	0	0.0	Yes	1	37.4	37.0	No	1
4	Yes	2	2.0	Yes	1	37.9	37.4	No	4
5	No	0	0.0	Yes	2	36.9	36.6	No	1
6	Yes	3	1.2	Yes	3	37.8	37.0	No	4
7	Yes	1	0.7	Yes	1	36.8	36.8	No	5
8	Yes	2	4.0	Yes	7	37.8	37.4	No	5
9	Yes	1	7.5	Yes	1	37.3	36.8	No	3
10	Yes	2	2.8	Yes	1	37.2	36.8	No	2
11	Yes	4	4.5	Yes	4	36.8	36.4	No	5
12	Yes	1	5.0	Yes	1	37.0	36.7	No	5

Patient Number	Pain	No. of days of pain	Severity of pain	Swelling	No. of days of swelling	Oral temp. (^{O}C)	Axilla temp. (^{O}C)	Tender nodes	Abscess group
13	Yes	6	5.6	Yes	2	37.4	36.9	No	2
14	Yes	2	3.2	Yes	2	37.2	37.0	Yes	2
15	Yes	2	7.5	Yes	1	37.4	36.8	No	4
16	Yes	2	7.2	Yes	1	36.9	36.4	No	2
17	Yes	1	8.2	Yes	1	36.8	36.6	Yes	4
18	Yes	2	3.3	Yes	1	37.4	36.8	Yes	4
19	No	0	0.0	Yes	2	36.8	36.4	No	4
20	Yes	1	0.4	Yes	1	37.0	36.5	No	4
21	No	0	0.0	Yes	4	36.8	36.8	No	5
22	Yes	4	4.5	Yes	4	37.2	36.8	No	5
23	Yes	1	3.7	Yes	1	37.0	36.4	No	4
24	Yes	5	2.6	Yes	5	37.7	37.0	No	5
25	Yes	4	7.5	Yes	14	27.2	37.0	No	4

Table 2.6 (Cont.)

Patient Number	Pain	No. of days of pain	Severity of pain	Swelling	No. of days of swelling	Oral temp. (°C)	Axilla temp. (°C)	Tender nodes	Abscess group
26	Yes	2	4.8	Yes	1	37.4	37.0	No	5
27	Yes	3	8.0	Yes	3	36.8	36.4	No	3
28	Yes	1	9.0	Yes	1	37.2	37.0	No	4
29	Yes	1	1.7	Yes	1	36.8	36.4	No	4
30	Yes	1	2.5	Yes	2	37.4	36.8	No	5
31	Yes	3	5.8	Yes	3	37.0	36.8	No	5
32	Yes	3	5.8	Yes	1	38.0	37.3	No	5
33	Yes	1	3.8	Yes	1	36.8	36.5	No	5
34	Yes	2	3.5	Yes	2	37.4	37.0	No	5
35	Yes	1	2.8	Yes	1	37.4	37.0	No	5
36	Yes	2	3.7	Yes	2	36.8	36.5	No	3
37	Yes	1	4.7	Yes	1	36.8	36.4	No	4

Table 2.6 (Cont.)

Patient Number	Pain	No. of days of pain	Severity of pain	Swelling	No. of days of swelling	Oral temp. (°C)	Axilla temp. (°C)	Tender nodes	Abscess group
38	Yes	2	9.8	Yes	1	36.8	36.4	No	4
39	Yes	2	5.2	Yes	2	37.3	36.8	No	4
40	Yes	1	4.6	Yes	1	36.9	36.5	No	1
41	Yes	2	2.1	Yes	2	36.8	36.4	No	5
42	Yes	2	8.2	Yes	1	37.5	37.3	No	3
43	Yes	2	6.7	Yes	2	37.5	37.0	Yes	4
44	Yes	1	9.5	Yes	1	37.5	37.0	No	3
45	Yes	1	2.9	Yes	1	36.8	36.4	No	3
46	Yes	2	8.2	Yes	2	36.8	36.4	No	5
47	Yes	2	7.9	Yes	2	36.8	36.4	No	5
48	Yes	1	8.0	Yes	1	36.8	36.4	No	4
49	Yes	1	7.2	Yes	1	36.8	36.4	No	2
50	Yes	1	9.7	Yes	1	37.4	37.0	No	5

Table 2.6 (Cont.)

Clinical Symptom		
Pain present	Yes - 46	No - 4
Mean number of days	1•8 ± 1•2	
Mean severity of pain	4•9 ± 2•9	
Swelling present	Yes - 50	No - 0
Mean number of days of swelling	2•0 ± 2•1	
Cervical lymph nodes tender to palpation	Yes - 4	No - 46
Mean oral temperature (°C)		37•1 ± 0•3
Mean axillary temperature (°C)		36•7 ± 0•3

Table 2.7: Summary of clinical symptoms at time of presentation of 50 patients with acute dentoalveolar abscess.

The mean oral temperature was 37•1°C (range 36•8°C to 37•4°C) and 26 patients had an oral temperature of 37•2°C or higher, which was regarded as being raised from the normal value of 36•6°C. The mean axillary temperature was 36•7°C (range 36•4°C to 37•4°C) and 17 patients had an axillary temperature of 37•0°C or higher, which was regarded as being raised from the normal value of 36•5°C.

2.3.9 Comparison of clinical symptoms and microbiological findings

The 50 patients were split, as described in section 2.3.7, into 5 groups depending on the proportion of the cultivated flora which consisted of strict anaerobes. The mean values of pain and swelling are shown in Table 2.8 and mean values of lymphadenopathy and temperature in Table 2.9. A comparison, using Student's t test, between the mean value of each clinical symptom in each goup did not reveal any statistically significant difference.

The details of clinical symptoms were examined to determine if there was any relationship between individual bacterial species and severity of symptoms. The only correlation that could be demonstrated in this way was for fusobacteria. The mean values of pain, swelling and temperature for the patients depending on whether fusobacteria were isolated from the pus or not are shown in Table 2.10. A comparison of these values revealed that each of the clinical symptoms was more severe in the patients from whom fusobacteria were isolated, although this difference was only significant (Student's t test) for the values of mean axillary temperature.

Clinical symptom	Strict anaerobe percentage of abscess flora				
	0	1 - 33	34 - 66	67 - 99	100
PAIN					
Present	1	5	6	15	19
Not present	2	0	0	1	1
Mean No. of days ± SD	0·3 ± 0·6	2·6 ± 1·9	1·6 ± 0·8	1·6 ± 0·9	2·1 ± 1·2
Mean severity ± SD	1·5 ± 2·6	5·2 ± 2·1	6·6 ± 2·6	4·9 ± 3·2	4·8 ± 2·8
SWELLING					
Present	3	5	6	16	20
Not present	0	0	0	0	0
Mean No. of days ± SD	1·3 ± 0·6	1·4 ± 0·5	1·5 ± 0·8	1·5 ± 1·0	2·3 ± 1·6

Table 2.8: Comparison of the clinical symptoms of pain and swelling present at time of sampling and strict anaerobe percentage of flora subsequently cultured.

Clinical symptom	Strict anaerobe percentage of abscess flora				
	0	1 - 33	34 - 66	67 - 99	100
TENDER NODES					
Present	0	1	0	3	0
Not present	3	4	6	13	20
TEMPERATURE					
Mean oral temperature (°C) ± SD	37•0 ± 0•3	37•1 ± 0•3	37•1 ± 0•3	37•1 ± 0•3	37•2 ± 0•3
Mean axillary temperature (°C) ± SD	36•7 ± 0•3	36•7 ± 0•3	36•7 ± 0•4	36•7 ± 0•3	36•8 ± 0•3

Table 2.9: Comparison of the clinical symptoms of lymphadenopathy and temperature present at time of sampling and strict anaerobe percentage of flora subsequently cultured.

Clinical feature	Fusobacteria not isolated in specimen	Fusobacteria isolated in specimen	Significance*
Number of patients	43	7	
Mean number of days of pain	1·7 ± 1·1	2·2 ± 1·6	NS+
Mean Severity of pain	4·7 ± 3·0	5·9 ± 2·8	NS
Mean number of days of swelling	1·7 ± 1·2	2·3 ± 1·9	NS
Mean oral temperature (°C)	37·1 ± 0·3	37·4 ± 0·4	NS
Mean axillary temperature (°C)	36·7 ± 0·3	37·0 ± 0·2	$p < 0·05$

+ NS = Not significant

* Student's t test

Table 2.10: Mean clinical symptoms for 50 patients presenting with acute dentoalveolar abscess depending on whether fusobacteria were isolated from the pus sample obtained.

2.4 DISCUSSION

2.4.1 Methodology

Many different methods of collecting and processing pus samples from acute dentoalveolar abscesses have been described and this is probably the main reason for the confusing microbiological findings.

Sampling method

Firstly it is essential to obtain a pus specimen that is free from salivary or plaque contamination and is therefore a true sample of the infection. Moore and Russell (1972) reported that cultures of pus samples from acute dentoalveolar abscesses collected on swabs yielded a greater number of bacterial species than aspirates of the same lesion and suggested that this increase was due to the introduction of salivary contaminants. It could be concluded therefore that swabbing is not a reliable method of sampling pus from acute dental abscesses. In addition to problems of contamination of swabs, Collee et al., (1974) have shown that recovery of strictly anaerobic bacteria from this type of sample is poor due to retention and inactivation of organisms on the swab. Aderhold and Frenkel (1985) further emphasised the need to collect aspirates of pus from acute dentoalveolar abscess and also recommended chemical disinfection of the overlying mucosa or skin to reduce the risk of sample contamination. For the above reasons all samples in the present study were obtained by aspiration following preparation of the oral mucosa with chlorhexidine solution.

Exposure of a pus sample to air and delay before processing could potentially result in loss of oxygen sensitive bacteria. Bartlett et al., (1976) reported minimal loss of anaerobic bacteria from purulent specimens of at least 2 ml after 24 hours exposure to air, although strains of fusobacteria and peptostreptococci were not recovered from samples processed after 4 hours exposure. Carlsson et al., (1976) investigated the oxygen tolerance of anaerobic bacteria isolated from necrotic dental pulps and found that all the strains tested could withstand 2 hours exposure to air on solid media supplemented with blood. In order to minimise the risk of loss of viable bacteria from the samples in the present study, processing was completed within 30 minutes of collection.

Bacterial quantification

Methods of quantifying bacterial populations are designed to evaluate either the total number of bacteria present in a given volume of specimen (total count) or the number of living bacteria (viable count). The basic methods available for determining the viable bacterial count of specimens are described in standard microbiological texts (Postgate, 1969). In the pour plate or roll tube method a known amount of sample is added to molten nutrient agar, held at 40°C, before being poured into petri dishes or glass tubes and incubated. The number of colonies visible within the agar after incubation are counted and related to the dilution of the sample to permit calculation of the viable count. In the drop plate method, developed by Miles and Misra (1938), the sample is diluted and then a standard volume inoculated onto the surface of pre-prepared solid medium. After incubation, the colonies growing on the agar surface are

counted and viable count determined. Various modifications of these basic techniques have been devised in order to reduce the amount of materials used and shorten the time taken to process the specimens. These have included microdroplet methods (Sharpe and Kilsby, 1971; Kelly, 1978) and techniques of mechanically inoculating plates (Trotman, 1971).

Gilchrist et al., (1973) developed a mechanical spread plate technique, known as the Spiral System, that inoculates a known volume of fluid sample in a spiral pattern onto the surface of solid medium. Although the initial cost for the equipment is high there are long-term economic savings since less materials and technical time are involved. The system has been used successfully for examination of bacterial contamination of food products (Gilchrist et al., 1977; Kramer et al., 1979) and when compared with pour plate or other spread plate methods for the enumeration of candida the spiral plater produced statistically similar results (Walsh et al., 1985). The Spiral System has also been used to determine the proportions of Strep. mutans, Strep. sanquis and lactobacilli in dental plaque (Loesche and Straffon, 1979) and to monitor changes in the microbial flora of periodontal disease (Loesche et al., 1984, 1985). The spiral plater was used in the present study because of the advantages mentioned above and since it has been employed successfully by previous workers to quantify mixtures of oral bacteria. The experience gained by its use would indicate it is a convenient method for the quantification of viable bacteria in pus samples obtained from acute dentoalveolar abscess but requires the preparation of

sample dilutions to ensure that a culture plate with suitable growth is obtained. The density of growth should include a large number of colonies to ensure accurate quantification but should also allow reliable subculture of individual morphological colony types for identification. In the present study four representatives of each colony type were removed for identification. On no occasion was there conflicting identification of these isolates and therefore it was assumed that all the colonies of one morphological type were the same species. Ideally all the colonies in the area of the plate counted should be identified but this would have involved a great amount of time, expense and materials. The observation that the majority of samples produced 3 or 4 distinct colony types and identification of representative colonies demonstrated each was a different species further supports the use of the Spiral System for analysis of microbial samples containing mixtures of oral bacteria.

Anaerobic techniques

Increased interest in oxygen-sensitive organisms has resulted in the development of a number of techniques for anaerobic incubation. The three most widely used methods are the anaerobic glove box (incubator), the GASPAK jar system and the rollstreak tube system. In a comparison of these methods Kilgore et al., (1973) reported a similar recovery of bacteria with each method. Although the anaerobic chamber is expensive and requires more laboratory space than other methods it have the advantage of allowing the handling of cultures without exposure to oxygen.

Culture media and incubation

In any microbiological investigation of acute infection it is essential that the culture media used are capable of isolating all the bacterial species which are present in the sample obtained. Enriched blood agar has been recommended, both by the Anaerobe Laboratory Manual (Holdeman et al., 1977) and the Wadsworth Anaerobic Laboratory Manual (Sutter et al., 1980), for routine use. Although blood agar is the most widely used medium the majority of studies of acute dental infections have also included some selective media (See Table 1.4). In the present study samples were inoculated onto modified Campylobacter selective medium in addition to blood agar to assist the isolation of anaerobic gram-negative bacilli.

The Anaerobe Laboratory Manual (Holdeman et al., 1977) and the Wadsworth Anaerobic Laboratory Manual (Sutter et al., 1980) both recommend that culture plates for anaerobic growth should be incubated for 6 to 7 days before a final examination is made. In some studies anaerobic plates were incubated for only 3 or 4 days (Turner et al., 1975; Hunt et al., 1978; Brook et al., 1981; Hunt and Meyer, 1983; Cumming et al., 1984) and therefore it is possible that the presence of strains of the more slow growing strict anaerobes was not detected. In the present study plates were examined daily and colony counts performed after 7 days incubation. Plates were re-incubated for a further 3 days prior to being discarded to ensure that colonies of any slow growing bacteria would be recorded.

Identification

Identification of facultative anaerobes, which in the present study were predominantly gram-positive cocci, was performed using well established techniques and little difficulty was experienced. The accuracy of the API 20 system for streptococci (API Laboratories, Basingstoke), which was used in the present study, has been investigated and has been found to produce reliable results for species of "Strep. viridans", such as Strep. milleri and Strep. sanguis (Appelbaum et al., 1984; Colman and Ball, 1984).

Classifications of strictly anaerobic bacteria have been proposed by Bergey's Manual (Buchannan and Gibbons, 1974) and the Virginia Polytechnic Insitute Manual (Holdeman et al., 1977). However as a result of new information updated classification systems are constantly being suggested which has led to multiple names for the same species in the literature (Finegold, 1979).

Commercially produced microsystems, which test a series of enzyme and carbohydrate reactions, are now available and provide a convenient method for identifying anaerobic bacteria. Minitek (BBL Microbiology Systems, Cockeysville, MD, USA) and API 20A (API Systems, Montalieu Vercieu, France) are two such systems that have gained acceptance since both have been found to produce reliable profiles for many species of strict anaerobes (Appelbaum et al., 1983; Karachewski et al., 1985). However, these identification systems were developed on data bases for bacterial species encountered in general medical microbiology and would appear to have limitations when employed for the identification of anaerobic bacteria that are found

predominantly in the mouth. In the present study the Minitek system was used and difficulty was encountered in the identification of 2 groups of bacteria, namely anaerobic gram-positive cocci and black-pigmented bacteroides.

Of the anaerobic gram-positve cocci species cultured strains of Strep. intermedius and Strep. constellatus could be identified but remaining isolates produced tests reactions that were difficult to interpret as either positive or negative. At the present time classification of anaerobic gram-positive cocci by conventional tests is unsatisfactory (Smith et al., 1986a). Although alternative systems of identification based on cell surface components have recently been investigated (Smith et al., 1986a: 1986b) until this group of bacteria can be characterised more reliably it has been suggested that simple identification schemes are used (Watt et al., 1984). Therefore, in the present study it was decided to limit the identification of the majority of the anaerobic gram-positive cocci isolated to Peptococcus species or Peptostreptococcus species according to their sensitivity to novobiocin (Wren et al., 1977).

Black-pigmented bacteroides is one group of strictly anaerobic bacteria that has been the subject of reclassification in recent years. Originally B. melaninogenicus was used to describe a group of anaerobic gram-negative bacilli which produced a black pigment when cultured on blood agar. The species was subsequently divided into 3 subspecies; 1. melaninogenicus, 2. intermedius and 3. asaccharolyticus (Holdeman and Moore, 1973), which were later each

given species status (Finegold and Barnes, 1977). More recently B. asaccharolyticus has been divided into two sperate species, B. asaccharolyticus (non-oral isolates) and B. gingivalis (oral isolates) (Coykendall et al., 1980). The heterogeneity of these two species is now well recognised (Slots, 1981) but identification using the Minitek system does not permit differentiation further than B. asaccharolyticus. Since the isolates in the present study had been cultured from oral specimens all those that were identified by Minitek as B. asaccharolyticus were classified as B. gingivalis.

Since the beginning of the present study a further pigment-producing Bacteroides species, Bacteroides endodontalis, has been proposed (van Steenbergen et al., 1984). The proposed species is very similar to B. gingivalis and B. assachrolyticus and therefore it is possible that some of the strains of B. gingivalis or other asaccharolytic Bacteroides species isolated in the present study may conform to the definition of B. endodontalis. The haemaglutination, enzyme and growth tests necessary for the identification of B. endodontalis have been described by (van Winkelhoff et al., 1985) and these could be applied to the bacterial strains cultured in the present study in the future since fresh cultures of each isolate have been stored in freeze dried ampoules.

In conclusion, great care was taken during the present study to avoid contamination of specimens during sampling and to ensure that the microbiological techniques used were capable of isolating, quantifying and identifying, as accurately as possible, all the facultative or strictly anaerobic bacteria that were present in each

specimen.

2.4.2 Microbiological findings: Bacterial species

Viable bacteria were isolated from all the abscesses examined in the present study, which is a similar finding to the majority of other microbiological investigations of acute dentoalveolar abscess. However, failure to culture bacteria from a small number of specimens was reported by Sabiston et al., (1976), von Konow et al., (1981) and von Konow and Nord (1983) during studies that included a similar number of abscesses and used similar culture methods to the one reported here.

A single bacterial species was cultured from 4 abscesses whilst the remainder were polymicrobial with a mean of 3•3 bacterial species per pus sample. A comparison of this value and those reported by other studies which were limited to acute dentoalveolar abscess and used aspiration sampling, with strict anaerobic culture methods, may be made from the information presented in Table 2.11. The mean value obtained in the present study is similar to the findings of these studies, especially those of Aderhold et al., (1981) who also examined 50 abscesses.

Of the 166 bacterial strains isolated in the present study 123 (74 per cent) were strict anaerobes which is almost identical to the 73 per cent reported for the 50 acute abscesses studied by Aderhold et al., (1981). The proportion of strictly anaerobic isolates found in other studies of acute dentoalveolar abscesses

Authors	No. of cases studied	Mean No. of species isolated per abscess	Percent. of total isolates strict anaerobes
Sabiston and Gold (1974)	8	3•1	58
Bartlett and O'Keefe (1979)	21	5•8	68
Aderhold et al., (1981)	50	3•6	73
Brook et al., (1981)	12	4•9	89
Oguntebi et al., (1982)	10	2•5	48
Williams et al., (1983)	10	4•5	70
Present study	50	3•3	74

Table 2.11: Mean number of bacterial species per abscess and percentage of isolates which were strictly anaerobic in studies of acute dentoalveolar abscess which used aspiration sampling and strict anaerobic culture methods.

(Table 2.11) has been variable, although the small numbers of abscesses involved in some investigations may be partly responsible for the differences.

Facultative gram-positive cocci

In early studies "Strep. viridans" accounted for at least 90 per cent of the isolates (Head and Roos, 1919; Fraser, 1923; Bulleid, 1931; Alin and Ågren, 1954; Feldmann and Larje, 1966) but in the present study only 19 per cent of the isolates belonged to the "Strep. viridans" group. Staphylococcus species were also frequently isolated in these early studies but were not encountered in the present study. These findings support the opinion of Aderhold et al., (1981) that the high incidence of "Strep. viridans" and Staphylococcus species in early studies was probably due to contamination.

In the present study the most frequently isolated facultative species was CO_2-dependent Strep. milleri, which is in agreement with the findings of von Konow et al., (1981), von Konow and Nord, (1983) and Heimdahl et al., (1985). Strains of microaerophilic streptococci were also frequently encountered by other workers (Hunt et al., 1978; Aderhold et al., 1981; Brook et al., 1981; Hunt and Meyer, 1983) but were not identified to species level. However it is possible that many of these isolates were in fact CO_2-dependent Strep. milleri.

In general American taxonomists do not formally recognise Strep. milleri and it is not included in their approved list of bacterial names (Sherman et al., 1980). Strep. intermedius and

Strep. constellatus generally conform to the definition of Strep. milleri (Facklam, 1984) and it is possible that some strains identified as Strep. constellatus or Strep. intermedius in studies using American nomenclature would be classified as Strep. milleri by microbiologists using alternative nomenclature. Therefore it is not possible to determine the occurence of Strep. milleri, as defined in the present study, in American studies but it is likely that they were encountered but classified under alternative names.

Strep. milleri may well be more pathogenic than previously appreciated as it has been isolated from brain abscess, pleural empyema, lung abscess, maxillary sinusitis and intra-abdominal abscess (van der Auwera, 1985). In addition to this finding the spread of Strep. milleri from dental abscesses has been implicated in cases of fatal endocarditis (Denning et al., 1984) and brain abscesses (Ingham et al., 1978). In the present study Strep. milleri was the organism isolated from 2 of the 4 abscesses which contained only one bacterial species, a finding which was also reported for one of the 10 dental abscesses studied by Williams et al., (1983). It seems likely therefore that the importance of Strep. milleri in acute dentoalveolar abscess has been underestimated in the past.

Facultative gram-positive bacilli

Facultative gram-positive bacilli have not been frequently isolated from dentoalveolar abscess and only 3 strains of Actinomyces species were cultured in the present study, one strain of A. meyeri, one of A. naeslundi and one of A. odontolyticus. Actinomyces species

are common inhabitants of the oral cavity (Howell et al., 1962) and strains have been frequently isolated from root canals and periapical granulomas (Sundqvist and Reutering, 1980). Occasional strains of the Actinomyces species encountered in the present study were also isolated in other studies (Sabiston and Gold 1974; Turner et al., 1975; Sabiston et al., 1976; Bartlett and O'Keefe 1979; Brook et al., 1981; von Konow et al., 1981).

Although no strains of Actinomyces israeli were isolated from any of the abscesses in the present study or those included in the studies listed in Table 1.4 the presence of this species in periapical tissues has been demonstrated by histological methods (Sundqvist and Reuterving, 1980). Since the culture media and prolonged incubation periods used in the present study were similar to those used successfully by the Diagnostic Oral Microbiology Unit of Glasgow Dental Hospital and School to isolate A. israeli it is probable that this organisms is rarely involved in acute dentoalveolar abscess.

Arachnia propionica was cultured from one pus sample in the present study and from one acute dentoalveolar abscess studied by Bartlett and O'Keefe, (1979). However, this would appear to be only encountered occasionally since it was not isolated in any of the other studies included in Table 1.4.

In the present study strains of Lactobacillus species were isolated from 3 abscesses, a low incidence of recovery which is in agreement with the studies in Table 1.4. It is generally accepted that lactobacilli are involved in caries of dentine and since caries

is believed to be one of the main causes of acute dentoalveolar abscess, it is perhaps surprising to find how rarely this genus is cultured from pus from these lesions.

Facultative gram-negative cocci

No strains of facultative gram-negative cocci were isolated in the present study which is a similar finding to the majority of investigations. Sabiston et al., (1976) however isolated 5 unidentified strains in a study of 65 abscesses and Labriola et al., (1983) cultured Neisseria from one pus sample in a study of 50 abscesses. Therefore it can be concluded that facultative gram-negative cocci are not frequently present in acute dentoalveolar abscesses.

Facultative gram-negative bacilli

Strains of facultative gram-negative bacilli were isolated from only 4 abscesses. The majority of studies have failed to isolate facultative gram-negative bacilli although Aderhold et al., (1981) and von Konow et al., (1981) reported the occurence of small numbers of Haemophilus species. This group of bacteria appear to be infrequently involved in acute dentoalveolar abscess.

Anaerobic gram-positive cocci

As a group anaerobic gram-positive cocci were frequently isolated from the pus samples in the present study. Approximately twice as many strains of Peptococcus species were isolated than strains of Peptostreptococcus species. Some studies, which have also

isolated large numbers of anaerobic gram-positive cocci, have described similar proportions to those reported here (Kannagara et al., 1980; Aderhold et al., 1980; von Konow et al., 1981) whilst others have described either a predominance of Peptostreptococcus species (Sabiston et al., 1976; von Konow and Nord, 1983; Williams et al., 1983; Heimdahl et al., 1985) or approximately equal numbers of both types (Bartlett and O'Keefe, 1979; Labriola et al., 1983). The present confusion concerning the identification of anaerobic gram-positive cocci is probably in part responsible for variations in the reported occurence of different species types. However it would appear that as a group anaerobic gram-positive cocci are frequently involoved in acute dentoalveolar abscess.

Anaerobic gram-positive bacilli

In the present study only two strains of gram-positive bacilli were isolated; one strain each of Propionibacterium acnes and Eubacterium lentum. Although the majority of other studies have reported a low incidence of the isolation of these bacteria, Bartlett and O'Keefe (1979) isolated 8 strains of E. lentum from 21 abscesses, von Konow et al., (1981) 10 strains from 57 abscesses and von Konow and Nord (1983) 13 strains from 55 abscesses. The reason for the relatively frequent isolation of anaerobic gram-positive bacilli in these studies when compared to that found in the present study is uncertain. However since at least one strain was cultured it would seem likely that if others had been present in the samples then they should have also been detected. It is therefore probable that the differences in the reported incidence of eubacteria are due to real variations in the bacterial flora encountered.

Anaerobic gram-negative cocci

Although most investigations of acute dentoalveolar abscess have failed to isolate anaerobic gram-negative cocci, 3 strains of Veillonella species were cultured in the present study. The isolation of a small number of strains of this species was also reported by Bartlett and O'Keefe, (1979), Aderhold et al., (1981) and Brook et al., (1981). As a group therefore, anaerobic gram-negative cocci would not appear to be frequently involved in acute dentoalveolar abscess.

Anaerobic gram-negative bacilli

In the present study anaerobic gram-negative bacilli formed the largest bacterial group and this finding supports the results of Aderhold et al., (1981), Brook et al., (1981), Williams et al., (1983), and Heimdahl et al., (1985). The most frequently isolated species was B. oralis which is in agreement with the findings of Labriola et al., (1983). Strains of B. gingivalis, B. melaninogenicus and B. intermedius were also frequent isolates in the present study and this compares well with the findings of Bartlett and O'Keefe, (1979), Aderhold et al., (1981), and Brook et al., (1981). In addition to these species, B. ruminicola was isolated from six abscesses in the present study which is a similar occurence to that reported by Oguntebi et al., (1982) and Labriola et al., (1983). In two studies B. ruminicola was the most frequently isolated Bacteroides species (von Konow et al. 1981; Heimdahl et al., 1985).

The original strains of the recently proposed Bacteroides species, B. endodontalis, were isolated from root canals of teeth but the species has subsequently been isolated from periapical abscesses (van Winkelhoff et al., 1985). As discussed previously some of the isolates that were identified as B. gingivalis in the present study may conform to the definition of B. endodontalis and therefore this species may well have been encountered in the present study if the criteria defined by van Steenbergen et al., (1984b) had been used.

B. fragilis is a recognised pathogen in medical microbiology but was not isolated from any of the abscesses in the present study or from any of those reviewed in Section 1.4 that were limited to acute dentoalveolar abscesses. However, Kannagara et al., (1980) and Chow et al., (1978) reported the isolation of B. fragilis from pus samples obtained from a range of acute pyogenic dental infections, including mandibular osteomyelitis and infected fractured mandibles. It is possible therefore that although B. fragilis does not appear to be involved in acute dentoalveolar abscess it may well be encountered in other acute dental infections.

The most frequently isolated anaerobic gram-negative bacilli in studies by Sabiston et al., (1976), Aderhold et al., (1981) and Oguntebi et al., (1983) were fusobacteria rather than bacteroides. Fusobacteria were isolated from only seven cases in the present study, which is a similar incidence to that reported by Bartlett and O'Keefe, (1979), Brook et al., (1981) and Williams et al., (1983). Since the methods used in all these studies and the present one were capable of isolating fusobacteria, any differences noted are probably due to

variations in the microflora of individual abscesses included in each study.

Thus from the above comparisons it would appear that the spectrum of bacterial species isolated in the present study is in agreement with the findings of other microbiological studies of acute dentoalveolar abscesses that used aspiration sampling and anaerobic culture methods. The majority of isolates belong to one of three bacterial groups, namely facultative gram-positive cocci (Strep. milleri), anaerobic gram-positive cocci (Peptococcus species and Peptostreptococcus species) and anaerobic gram-negative bacilli (B. oralis, B. melaninogenicus, B. gingivalis, B. ruminicola and F. nucleatum).

2.4.3 Microbiological findings: Quantitative results

The microbial load of acute dentoalveolar abscesses has rarely been quantified. The abscesses in the present study were found to contain between $10^{4.7}$ to $10^{9.4}$ cfu/ml pus, which is a similar range of viable counts to that reported for the 6 abscesses studied by Williams et al., (1983). The mean viable count for strict anaerobes was greater than the mean of the facultative isolates, thus not only were facultative organisms less frequently isolated than strict anaerobes but when they were present they were also less numerous.

Twenty (40 per cent) of the abscesses had a flora consisting purely of strict anaerobes, 3 (6 per cent) had a purely facultative flora and the remaining 27 (54 per cent) contained a mixture of

bacterial types. The findings of the microbiological studies of acute dentoalveolar abscess which used aspiration sampling and anaerobic culture methods are shown in Table 2.12. The figures reported are very variable but this may partly be due to the small number of cases involved in some investigations.

There are virtually no data in the literature concerning the relative proportions of facultative and strictly anaerobic bacteria in the overall microbial load of mixed cultures obtained from dental abscesses. The results of the present study have shown that the flora of the majority of mixed cases consists predominantly of strict anaerobes which confirms the suspicion of Sabiston and Gold (1974) and Sabiston et al., (1976) and supports the results reported for the small number of abscesses by Williams et al., (1983).

2.4.4 Relationship between clinical symptoms and microbiogical findings

Sundqvist (1976) and Griffee et al., (1980) have discussed a relationship between the presence of B. melaninogenicus in necrotic dental pulps and symptoms of pain, swelling and exudate. In addition Heimdahl et al., (1985) have reported that anaerobic gram-negative bacilli, in particular F. nucleatum, are isolated more frequently from pus samples obtained from acute dentoalveolar abscesses in patients with severe clinical symptoms than from patients with mild symptoms. In the present study a similar relationship between the presence of fusobacteria and the severity of the patient's clinical symptoms was demonstrated although this was only significant in relation to the axillary temperature.

Authors	No. of cases studied	Type of flora		
		FO	Mixed	AO
Bartlett and O'Keefe, (1979)	21	0	100	0
Aderhold et al., (1981)	50	4	68	28
Brook et al., (1981)	12	0	67	33
Oguntebi et al., (1982)	10	20	70	10
Williams et al., (1983)	10	10	30	60
Present study	50	6	54	40

Table 2.12: Percentage of cases containing facultative anaerobes only (FO), strict anaerobes only (AO) or both types of organism (mixed) in studies of acute dentoalveolar abscess which used aspiration sampling and strict anaerobic culture methods.

2.4.5 Source of bacteria

The source of the bacterial species isolated from dentoalveolar abscesses is likely to be the oral flora but the route by which bacteria spread into the periapical region of teeth and initiate abscess formation is still not clear. The three pathways that have been proposed were discussed in Section 1.3 and include; the necrotic pulp, the vessels of the periodontal ligament and the general circulation.

The pus samples in the present study contained many of the same bacterial species and in the same concentrations as found in the necrotic pulps of teeth by Zavistoski et al., (1980) and this similarity would support the concept that bacteria spread into the apical region directly from the dental pulp through the apical foramen. It is probable that the bacteria originally gained access to the pulp via a carious cavity although other pathways must be possible as the spectrum of organisms isolated from the abscesses in the present study was also similar to that found in necrotic pulps of teeth with intact crowns (Bergenholtz, 1974; Wittgow and Sabiston, 1975).

The similarity of the spectrum of microorganisms isolated in the present study and that found at the gingival crevice (Socransky, 1977; Newman and Sims, 1979; Slots, 1977) supports the proposal that the source of bacteria in acute dentoalveolar abscess can be from the gingivae. This observation may help explain acute dentoalveolar abscesses which occur in relation to non-carious, apparently intact teeth.

Seeding of bacteria from one body site to another via the blood stream is a well recognised phenomenon. Brook (1987) has recently shown, using experimental animals, that following the intravenous inoculation of bacteroides and anaerobic gram-positive cocci there was a higher subsequent recovery of bacteria from body sites, such as the liver and kidney, when capsulate strains were used rather than non-capsulate strains. Although the presence of capsule was not determined in the present study the finding that bacteroides and anaerobic gram-positive cocci were frequently isolated from acute dentoalveolar abscesses suggests that these bacteria, particulary capsulate strains, may have gained access to the periapical area from other body sites via the blood stream.

2.5 CONCLUSIONS

(i) Acute dentoalveolar abscesses contain high numbers of viable bacteria and are usually polymicrobial infections.

(ii) A wide range of bacterial species are encountered but Peptococcus species, Strep. milleri, B. oralis, Peptostreptococcus species, B. gingivalis, and B. melaninogenicus are the species most frequently isolated.

(iii) The flora of an acute dentoalveolar abscess is usually either purely or predominantly anaerobic, although occasionally facultative bacteria are cultured alone.

(iv) In the present study there was an apparent relationship between the presence of fusobacteria and an increase in the severity of clinical symptoms.

CHAPTER 3

PATHOGENICITY OF BACTERIAL STRAINS ISOLATED FROM ACUTE DENTOALVEOLAR ABSCESS

3.1 INTRODUCTION

The results of the microbiological investigation described in Chapter 2 have demonstrated that acute dentoalveolar abscesses are usually polymicrobial and involve a wide range of bacterial species. The bacterial species encountered however can be regarded as commensals since they are members of the healthy oral microflora, their main habitats being the gingival crevice (Socransky, 1977) and the tongue (van der Velden et al., 1986). Therefore there is uncertainty if there are variations in pathogenicity between the different organisms present. The need for the study of the pathogenicity of bacteria isolated from mouth has been stressed (Chow et al., 1978) but in a review of mixed infections of the oral cavity van Steenbergen et al., (1984a) were unable to find any studies which described the pathogenic properties of the microorganisms encountered in acute oral abscesses. However, it is likely that useful information may be obtained from reports describing the bacteria associated with periodontal disease or necrotic dental pulps since many of the species cultured from these conditions are the same as those prevalent in acute dentoalveolar abscess.

Because of the belief that black-pigmented bacteroides play an important role in periodontal disease, pathogenicity experiments involving oral bacteria have tended to concentrate on this group of

organisms. Initially it was reported that although infections could be produced by mixtures of bacteria obtained from periodontal sites, pure cultures of individual isolates were almost invariably non-pathogenic. However, when B. melaninogenicus was deleted from these mixtures the residual inocula did not produce infections (MacDonald et al., 1963; Socransky and Gibbons, 1965). This observation led to the opinion that B. melaninogenicus was a major pathogen in periodontal disease.

More recently pure cultures of other species of oral bacteroides, such as B. gingivalis, B. intermedius, B. oralis and B. ruminicola, have been found to be capable of producing experimental infections in mice and rats (Kastelein et al., 1981; van Steenbergen et al., 1982; Roeterink et al., 1985; Pancholi et al., 1985). In addition, it has been reported that recent clinical isolates of fusobacteria and anaerobic gram-positive cocci (both traditionally thought to be non-pathogenic in pure culture) contribute to experimental infections produced by mixed cultures (Brook and Walker, 1984, 1986). Therefore there is increasing evidence that certain bacterial species presently regarded as members of the commensal oral flora are likely to be more pathogenic than previously appreciated.

The aim of the present study was to investigate the pathogenicity of pure cultures and combinations of bacterial species isolated from pus samples collected from patients with acute dentoalveolar abscess.

3.2 MATERIALS AND METHODS

3.2.1 Bacterial species

The bacterial strains used in this study were isolated from the acute dentoalveolar abscesses described in Chapter 2 and had been stored in freeze dried ampoules since initial culture. The identity of the 20 bacterial strains used in the experiments using pure cultures is shown in Table 3.1 and the identity of the 10 bacterial strains used in experiments where combinations of two bacterial species were investigated is shown in Table 3.2.

3.2.2 Preparation of inoculum

Freeze dried cultures of each bacterial species were reconstituted in Anaerobic Blood Broth (ABB) (Gibco Europe, Paisley) and streaked onto Columbia Blood Agar (Oxoid Ltd., Basingstoke), which was subsequently incubated at 37°C in an anaerobic chamber (Appendix I) for 5 days. Single colonies were subcultured on to fresh Columbia Blood Agar plates (Oxoid Ltd., Basingstoke) and incubated anaerobically (Appendix I) at 37°C for 5 days. The identity of the test bacteria was confirmed using the methods of identification described in section 2.2.7.

Using a sterile cotton-wool swab approximately 40 to 50 bacterial colonies were inoculated into 20 ml ABB (Gibco Europe, Paisley) and placed in an anaerobic chamber (Appendix I). Facultative organisms were incubated at 37°C for 24 hours and strictly anaerobic organisms for 48 hours. After incubation the concentration of the resultant broth was adjusted to approximately 2×10^7 cfu/ml using a spectrophotometer (Pye Unicam, Cambridge) and previously determined

Organism Group	Bacterial Species	Number of Strains	Strain Code No.
CO_2-dependent gram-positive cocci	Streptococcus milleri	2	116, 031
Anaerobic gram-positive cocci	Peptococcus species	3	008, 058, 090
	Peptostreptococcus species	1	080
Anaerobic gram-negative bacilli	Bacteroides gingivalis	2	221, 222
	Bacteroides intermedius	2	005, 023
	Bacteroides melaninogenicus	4	092, 114, 117, 734
	Bacteroides oralis	3	109, 075, 083
	Bacteroides ruminicola	1	024
	Fusobacterium nucleatum	2	110, 112

Table 3.1: Identity of the 20 test organisms used in pure culture experiments.

Organism Group	Bacterial Species	Number of Strains	Strain Code No.
CO_2-dependent gram-positive cocci	Streptococcus milleri	2	116, 031
Anaerobic gram-positive cocci	Peptococcus species	2	058, 090
	Peptostreptococcus species	1	080
Anaerobic gram-negative bacilli	Bacteroides intermedius	2	023, 005
	Bacteroides oralis	1	109
	Bacteroides melaninogenicus	1	114
	Fusobacterium nucleatum	1	112

Table 3.2: Identity of the 10 test organisms used in bacterial combination experiments.

growth absorbance curves (Appendix II). In order to achieve a concentration of approximately 2 x 10^8 cfu/ml the broths were concentrated by centrifuging at 3,000 r.p.m. for 15 minutes. The volume of the supernatant removed was measured and the residue re-suspended in 1/10th the volume of ABB (Gibco Europe, Paisley). Suspensions containing two bacterial species were prepared by mixing equal volumes of pure culture broths at this stage of inoculum preparation.

Each suspension was then centrifuged at 3,000 r.p.m. for 15 minutes and the volume of each supernatant measured before it was discarded. The residue pellet was re-suspended in an identical volume of ABB containing 2 per cent agar (Gibco Europe, Paisley), that had been boiled and allowed to cool to 45°C. To ensure thorough mixing, the newly constituted suspension was immediately subjected to vortex-mixing for 30 seconds using a whirlmixer (Fisons Scientific, Loughborough). A 0•5 ml sample was removed and added to 19•5 ml of ABB (Gibco Europe, Paisley) to allow subsequent determination of viable bacterial content using the methods described in sections 2.2.4 to 2.2.6. The remaining suspension was allowed to cool to room temperature and was inoculated into mice within 1 hour.

Sterile ABB (Gibco Europe, Paisley) containing 2 per cent agar (Gibco Europe, Paisley) was used as the control inoculum.

3.2.3 Experimental animals

Animals were obtained from an inbred colony of male albino white mice (BALB-C) maintained under conventional laboratory conditions at the Department of Bacteriology and Immunology, Western Infirmmary, Glasgow. Adult mice, in a weight range of 22 g to 26 g, were removed from the stock colony as required and caged in groups of 3 or 4 with food and water ad libitum.

3.2.4 Inoculation of animals

Inoculation of all bacterial suspensions and control media was made using a 1 ml disposable syringe (Gillette Surgical, Islesworth) fitted with a Nr. 18 microlance needle (Becton Dickinson UK Ltd., Oxford). Each mouse was lightly anaesthetised using ether in an anaesthetic jar. On removal from the jar, 0•2 ml of inoculum was introduced subcutaneously into the right groin of each animal, held by hand in the supine position on a cork board (Figure 3.1). Animals rapidly regained consiousness after inoculation.

The general health of the mice was assessed daily and the groin examined for evidence of developing lesions. On the eighth day the animals were sacrificed by cervical dislocation.

3.2.5 Microbiological assessment

A 25 μl microsyringe (Burke Electronics Ltd., Glasgow) was used to aspirate a sample of fluid from the site of inoculation. If 25 μl was obtained the specimen was added to 2 ml ABB (Gibco Europe, Paisley). If less than 25 μl was obtained the specimen was discarded. The syringe was washed out between animals, firstly with 2 per cent

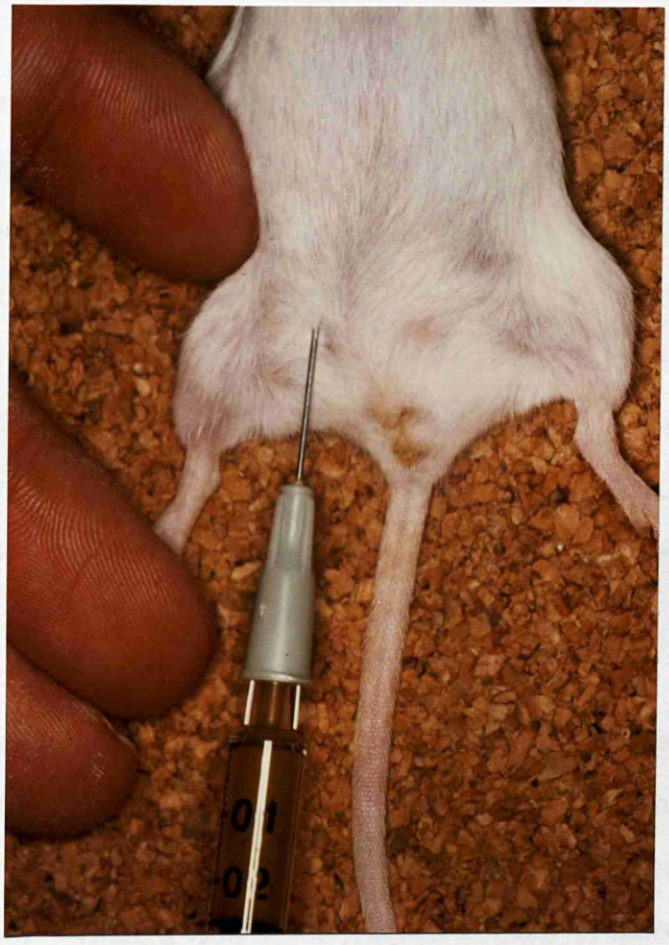


Figure 3.1: Subcutaneous inoculation of 0·2 ml of bacterial suspension into right groin of lightly anaesthetised male mouse.

phenolic disinfectant (Stericol, Sterling Industrial, Sheffield) and secondly with distilled water. Samples were transported to the laboratory within 1 hour and their viable bacterial content determined using the methods described in section 2.2.4 to 2.2.6.

3.2.6 Histological assessment

Following microbial sampling the right hind leg was removed from the animal by gross dissection using scissors and tissue forceps. The necropsy was immediately pinned to a cork block and placed in 10 per cent phosphate buffered formalin at pH 7 for 72 hours. If a gross abscess was evident the specimen was sectioned and trimmed to produce two tissue blocks as shown in Figure 3.3. If there was no sign of abscess formation then the material was prepared as shown in Figure 3.4 to produce 3 tissue blocks.

Histological processing was carried out in an automatic tissue processer (Shandon Scientific Company Ltd., London) and tissue enbedded in paraffin wax (Table 3.3). Using a microtome (E. Leitz Instruments Ltd., Luton) 5 μm thick sections were prepared from each block and stained with haematoxylin and eosin (Culling et al., 1985). All sections were code numbered without details of the bacterial inoculum involved being known and examined randomly.

3.2.7 Statistical analysis

Fisher's exact test was used to determine the significance of any differences in the results obtained.

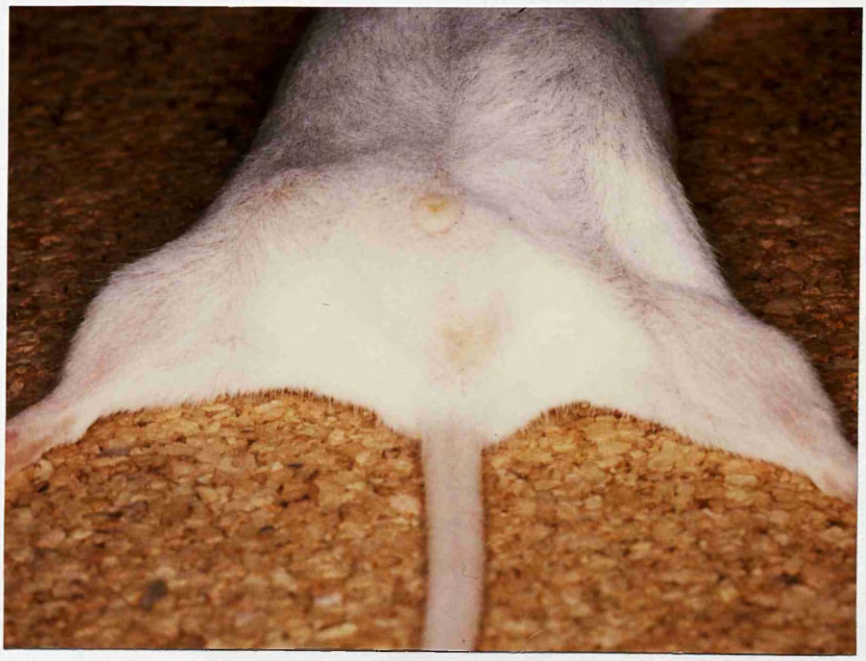


Figure 3.2: Localised abscess, visible as a swelling, in right groin region of a male mouse 8 days after inoculation.

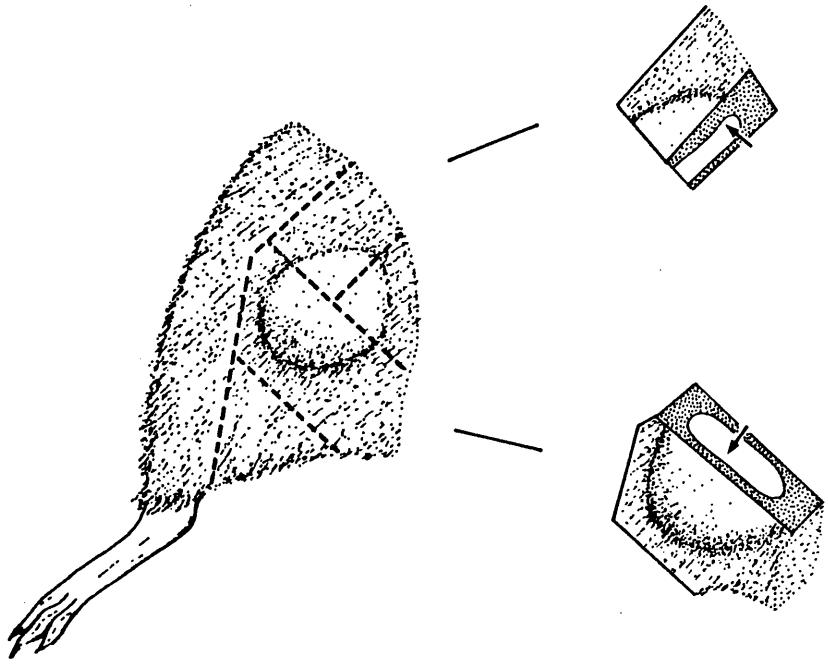


Figure 3.3: Preparation of tissue blocks from animals which had gross swelling at the site of inoculation.

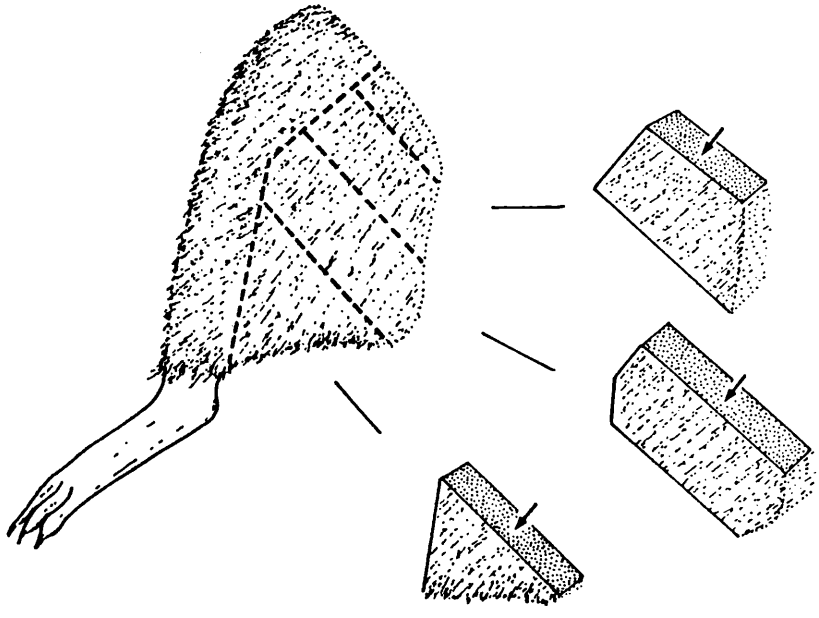


Figure 3.4: Preparation of tissue from animals which did not have gross swelling at the site of inoculation.

1	50% spirit	0•5 hours
2	70% spirit	1 hour
3	90% spirit	2 hours
4	methylated spirit	1 hour
5	methylated spirit	1 hour
6	absolute alcohol	3 hours
7	absolute alcohol	3 hours
8	absolute alcohol chloroform	1 hour
9	chloroform	2•5 hours
10	chloroform	4 hours
11	paraffin wax	2 hours
12	paraffin wax	2•5 hours

Table 3.3: Processing cycle used to prepare necrospy material obtained from experimental infections in mice.

3.3 RESULTS

The pathogenicity of 9 bacterial species consisting of 20 different bacterial strains was investigated in 81 mice and the pathogenicity of 11 combinations of 2 bacterial strains in 55 mice. Although 1 mouse died 3 days after inoculation the remaining 135 mice survived till the day of sacrifice without showing signs of distress or decline in general health. The results are presented in two sections; firstly, for experiments involving the inoculation of pure cultures and secondly, for experiments involving the inoculation of a combination of two bacterial species.

3.3.1 Microbiological findings of pure culture experiments

The viable bacterial content of the inoculum introduced into each mouse and the concentration of viable bacteria in pus samples recovered at the time of sacrifice are shown in Table 3.4 (summary Table 3.5). All the animals were challenged with the same volume of inoculum at a concentration of approximately 10^8 cfu/ml (range $1 \cdot 1 \times 10^8$ cfu/ml to $8 \cdot 2 \times 10^8$ cfu/ml) and a sample of fluid aspirated from the site of inoculation after 8 days. An aspirate of at least 25 μl was not obtained from the experimental site of 8 mice (10 per cent of the total inoculated) and details for these animals are combined in the results with those of the 15 animals from which samples were negative on culture. On 19 occasions the concentration of colonies cultured from the sample was less than 10^3 cfu/ml and these were classified as low viable growth (LVG). In 38 of the samples the bacterial strain inoculated was recovered at concentrations between 10^7 cfu/ml and 10^9 cfu/ml.

Table 3.4: The concentration of viable organisms in the inoculum and aspirate of experimental absccesses together with the tissue reaction produced by 9 bacterial strains inoculated into 80 mice.

Bacterial species (Strain number)	Animal number	Conc. of inoculum ($x10^8$ cfu/ml)	Conc. of aspirate ($x10^8$ cfu/ml)	Tissue reaction
Strep. milleri (116)	0865	8.0	0.4	B
	0875	8.0	0.4	B
	0885	8.0	0.7	B
	0895	8.0	LVG+	B
Strep. milleri (031)	0825	3.6	—x	A
	0835	3.6	LVG	B
	0845	3.6	LVG	B
	0855	3.6	LVG	B
Strep. milleri (031)	3004	3.9	LVG	B*
	3014	3.9	—	A
	3024	3.9	—	A
Peptococcus sp. (090)	3514	3.1	—	A*
	3524	3.1	LVG	B*
	3534	3.1	—	A
Peptococcus sp. (008)	0705	2.4	LVG	B
	0715	2.4	LVG	B
	0725	2.4	—	A
	0735	2.4	—	A
Peptococcus sp. (058)	0745	5.6	—	A*
	0755	5.6	—	A
	0765	5.6	LVG	B
	0775	5.6	LVG	B
Peptostrep. sp. (080)	0785	2.6	0.2	B
	0795	2.6	0.2	B
	0805	2.6	0.1	C
	0815	2.6	LVG	B
B. intermedius (023)	1545	3.2	0.4	C
	1555	3.2	0.3	C
	1565	3.2	0.3	C
	1575	3.2	LVG	C
B. intermedius (005)	0665	5.1	0.6	C
	0675	5.1	0.3	C
	0685	5.1	0.4	C
	0695	5.1	—	A

Bacterial species (Strain number)	Animal Number	Conc. of inoculum ($x10^8$ cfu/ml)	Conc. of aspirate ($x10^8$ cfu/ml)	Tissue reaction
B. intermedius (005)	3034	2.3	4.1	C
	3044	2.3	3.1	C
	3054	2.3	3.3	C
B. gingivalis (221)	2015	5.3	0.5	C
	2025	5.3	0.6	C
	2035	5.3	LVG	B
	2045	5.3	LVG	B
B. gingivalis (222)	2055	4.8	—	A
	2065	4.8	0.4	C*
	2075	4.8	—	A*
	2085	4.8	—	A
B. oralis (109)	3574	7.7	—	A
	3584	7.7	0.3	C
	3594	7.7	7.7	C
B. oralis (075)	1615	1.7	—	B*
	1625	1.7	—	A*
	1635	1.7	—	A*
	1645	1.7	—	B
B. oralis (083)	1465	2.1	LVG	B
	1475	2.1	0.4	C
	1485	2.1	0.6	B
	1495	2.1	0.3	C
B. melaninogenicus (114)	3364	1.1	5.0	C
	3374	1.1	16.9	C
	3384	1.1	10.4	C
B. melaninogenicus (734)	1585	2.6	LVG	C
	1595	2.6	LVG	C
	1605	2.6	LVG	C
	1615	2.6		
B. melaninogenicus (092)	1545	3.1	0.3	C
	1555	3.1	0.3	C
	1565	3.1	0.2	B
	1575	3.1	LVG	C
B. melaninogenicus (117)	3394	1.2	—	B
	3404	1.2	—	A
	3414	1.2	—	B

Table 3.4 (Cont.)

Bacterial species (Strain number)	Animal number	Conc. of inoculum (x10^8 cfu/ml)	Conc. of aspirate (x10^8cfu/ml)	Tissue reaction
B. ruminicola (024)	1505	3.0	0.6	C
	1515	3.0	—	A*
	1525	3.0	0.3	C
	1535	3.0	—	A
F. nucleatum (112)	0625	8.2	0.8	C
	0635	8.2	0.4	C
	0645	8.2	0.6	C
	0655	8.2	0.2	C
F. nucleatum (110)	3544	2.6	0.7	C
	3554	2.6	1.2	C
	3564	2.6	0.6	C

$^+$LVG Low Viable Growth (less than 10^3 cfu/ml)

x— No Growth

⊛ Animal died on Day 3

* Aspirate of at least 25 μl not obtained

Table 3.4 (Cont.)

Bacterial Species	No. of Strains	No. of animals	No. of animals with pus containing (cfu/ml)		
			NG+	LVG*	$10^7 - 10^9$
Strep. milleri	2	11	3	5	3
Peptococcus spp.	3	11	6	5	0
Peptostrep. spp.	1	4	0	1	3
B. oralis	3	11	5	1	5
B. melaninogenicus	4	14**	3	4	6
B. intermedius	2	11	1	1	9
B. gingivalis	2	8	3	2	3
B. ruminicola	1	4	2	0	2
F. nucleatum	2	7	0	0	7
Total			23	19	38

+NG = No growth *LVG = Low viable growth (less than 10^3 cfu/ml)

** One animal died on Day 3 and therefore is not included in the results.

Table 3.5: Distribution of experimental animals according to the viable bacterial content of pus samples recovered from 80 experimental abscesses using pure cultures.

Examination of the results in Table 3.5 reveals that although each bacterial strain tested was recovered from at least one mouse, F. nucleatum and Peptostreptococcus species were the only bacteria species recovered on every occasion they were used.

3.3.2 A comparison of the recovery of different bacterial species

The concentrations at which bacterial species belonging to each of the 3 bacterial groups were recovered is shown in Table 3.6. A comparison of these results revealed that gram-negative bacilli were recovered at high concentrations significantly more often than either CO_2-dependent gram-positive cocci ($p < 0{\cdot}05$) or anaerobic gram-positive cocci ($p < 0{\cdot}001$). There was no significant difference between the recovery of CO_2-dependent gram-positive cocci and that of anaerobic gram-positive cocci.

A comparison was made between the frequency with which different bacterial species were recovered at high concentrations (10^7 to 10^9 cfu/ml) rather than low concentrations (less than 10^3 cfu/ml) or not at all (Table 3.7). Some bacterial species were recovered significantly more often at high concentrations than others and these were;

(a) all test species, with the exception of Strep. milleri, more often than Peptococcus species,

(b) F. nucleatum more often than Strep. milleri, Peptococcus species, B. gingivalis, B. melaninogenicus and B. oralis.

(c) B. melaninogenicus more often than Strep. milleri

Organism Group	No. of Animals	Viable bacteria recovered cfu/ml		
		NG+	LVG*	10^7 - 10^9
CO_2-dependent				
gram-positive cocci	11	3	5	3
Anaerobic				
gram-positive cocci	15	5	7	3
Anaerobic				
gram-negative bacilli	54	14	8	32

+NG = No growth

*LVG = Low viable growth

Table 3.6: Concentration of viable bacteria recovered from 80 experimental abscesses inoculated with approximately 10^8 cfu.

	COMPARATIVE SPECIES								
INDEX SPECIES	Strep. milleri	Peptococcus sp.	Peptostrep. sp.	B. gingivalis	B. intermedius	B. melaninogenicus	B. oralis	B. ruminicola	Fusobacterium
Strep. milleri	..	..	..	..	..	..	..	..	..
Peptococcus spp.	..	..	..	..	..	..	..	..	..
Peptostrep. spp.	..	<0.001	..	..	..	..	..	..	..
B. gingivalis	..	<0.001	..	..	..	..	..	..	..
B. intermedius	..	<0.001	..	..	..	..	..	..	..
B. melaninogenicus	<0.05	<0.05	..	..	..	..	..	..	..
B. oralis	..	<0.05	..	..	..	..	..	..	..
B. ruminicola	..	<0.001	..	..	..	..	..	..	..
Fusobacterium	<0.001	<0.001	..	<0.05	..	<0.05	<0.05	..	..

p values indicate the significance with which the index species was recovered more frequently at $10^7 - 10^9$ cfu/ml than comparative species.

.. Not significant.

Table 3.7: A comparison of the recovery of test species at a concentration of $10^7 - 10^9$ cfu/ml against low or no growth.

3.3.3 Histopathological findings of inoculation of sterile semi-solid agar

Inoculation of sterile medium into 10 control animals failed to produce any gross swelling at the site of inoculation. Histological examination of tissue from all the control animals demonstrated traces of the medium in the subcutaneous tissues surrounded by macrophages but there was minimal evidence of an acute inflammatory infiltrate (Figures 3.5).

3.3.4 Histopathological findings in experiments using single bacterial species

Histological examination of tissue from the sites inoculated with viable bacteria revealed 3 different tissue reactions and the histopathological features of each are described in Table 3.8. Examples of each tissue reaction are shown in Figures 3.6 to 3.10. Histological material from each animal (2 sections from those animals with obvious swelling at the time of sacrifice and 3 sections from those with no swelling) was examined and classified into one of the 3 tissue reactions. The results are shown in Table 3.2 and a summary of the reactions produced by each bacterial species in Table 3.9.

F. nucleatum was the only species to produce the same tissue reaction (Type C) on every occasion it was tested whereas the remaining organisms produced more than one type of reaction. Strep. milleri and Peptococcus species produced either Type A or Type B reaction and Peptostreptococcus species Type B or Type C. B. intermedius and B. rumincola produced only Type A or Type C. The remaining Bacteroides species produced at least one of each of the 3

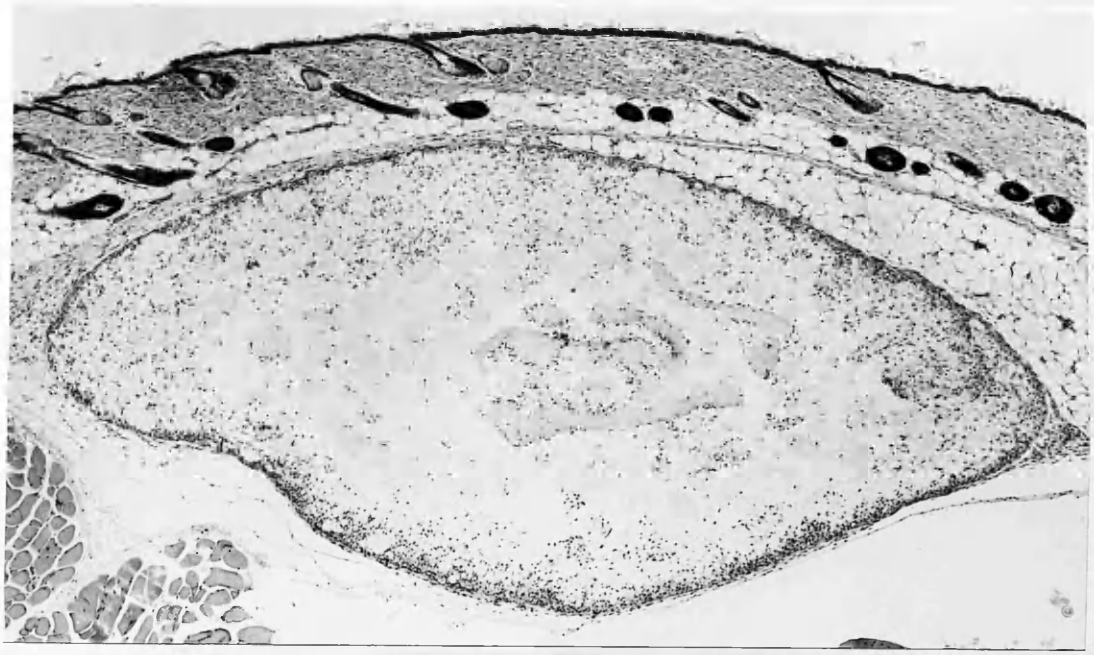


Figure 3.5: Low-power photomicrograph of tissue reaction produced 8 days after subcutaneous inoculation of sterile semi-solid agar into the groin of an adult male mouse. A discrete collection of inoculum, surrounded and diffusely infiltrated by macrophages, is seen in the subcutaneous tissues which show minimal evidence of an acute inflammatory reaction (x 35).

Tissue response	Histological features
A	Diffuse subcutaneous inflammatory infiltrate consisting predominantly of polymorphonuclear leucocytes with smaller numbers of macrophages (Figure 3.6).
B	Discrete area of necrosis surrounded by polymorphonuclear leucocytes and macrophages (Figures 3.7 and 3.8).
C	Discrete area of necrosis and prominent polymorphonuclear leukocytes infiltrate. Separate encirculating bands of necrosis and granulation tissue are also present (Figures 3.9 and 3.10).

Table 3.8: Classification of the 3 types of tissue response produced by subcutaneous inoculation of bacterial suspension into the groin of mice.

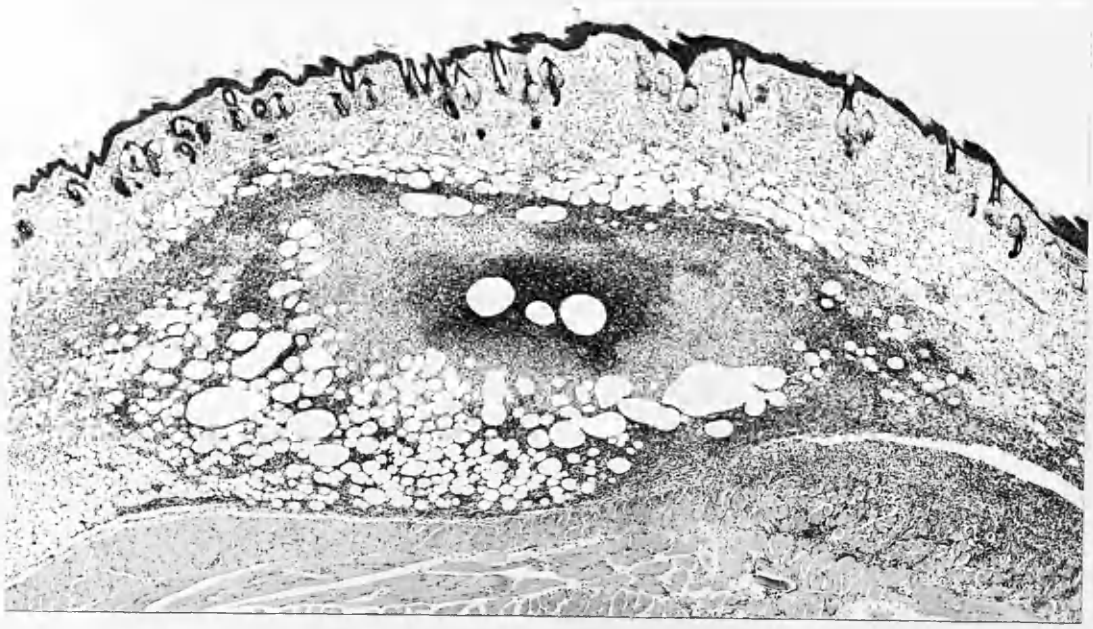


Figure 3.6: Low-power photomicrograph of Type A tissue reaction produced 8 days after inoculation of a suspension of B. gingivalis into the groin of an adult male mouse. A diffuse inflammatory infiltrate, predominately polymorphonuclear leukocytes, is present in the subcutaneous connective tissue. The overlying epithelium and underlying muscle show minimal acute inflammatory reaction (x 35).

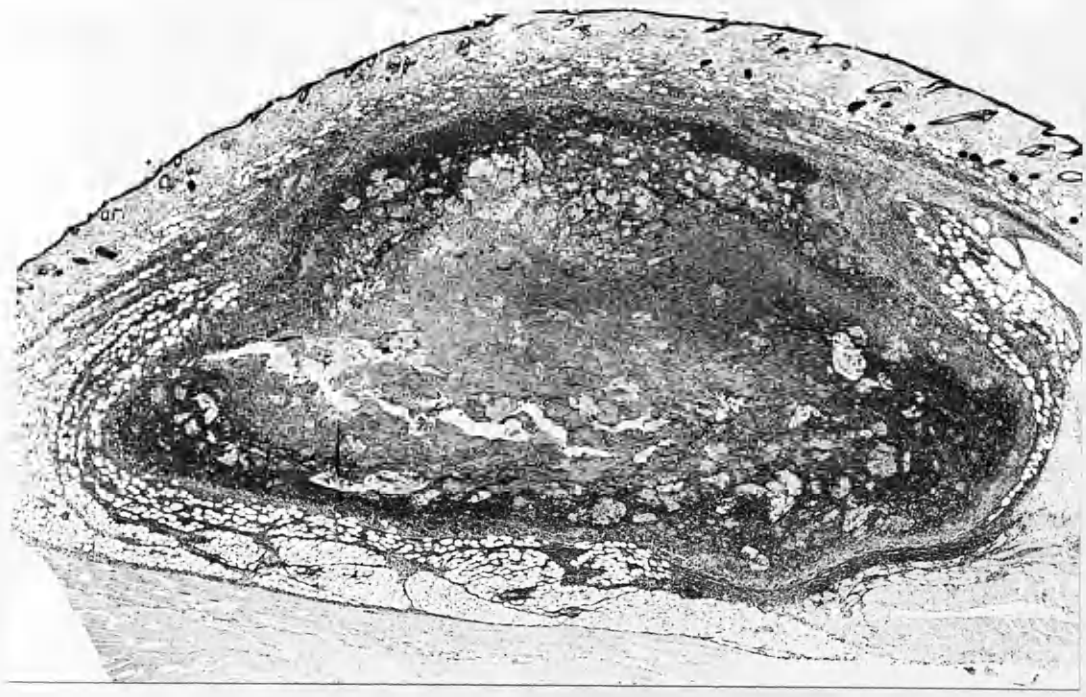


Figure 3.7: Low-power photomicrograph of Type B tissue reaction produced 8 days after subcutaneous inoculation of a suspension of Strep. milleri into the groin of an adult male mouse. An abscess has developed with a central area of necrosis surrounded by a large number of acute inflammatory cells (x 25).

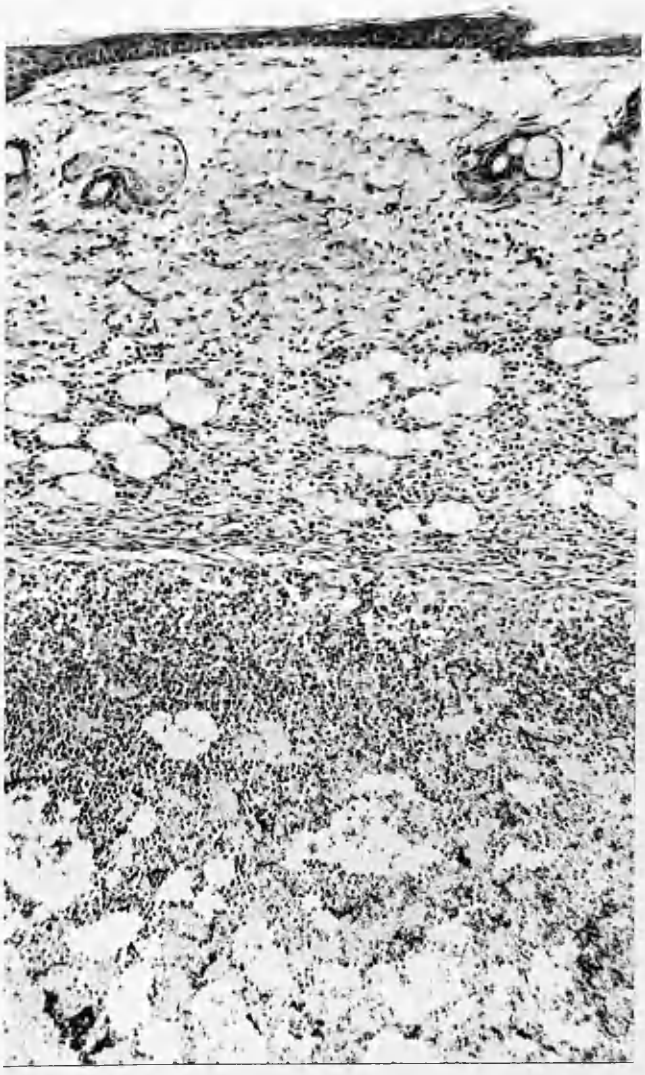


Figure 3.8: High-power photomicrograph of the Type B tissue reaction shown in Figure 3.7. The inflammatory cell infiltrate consists predominantly of polymorphonuclear leukocytes with smaller numbers of macrophages (x 150).

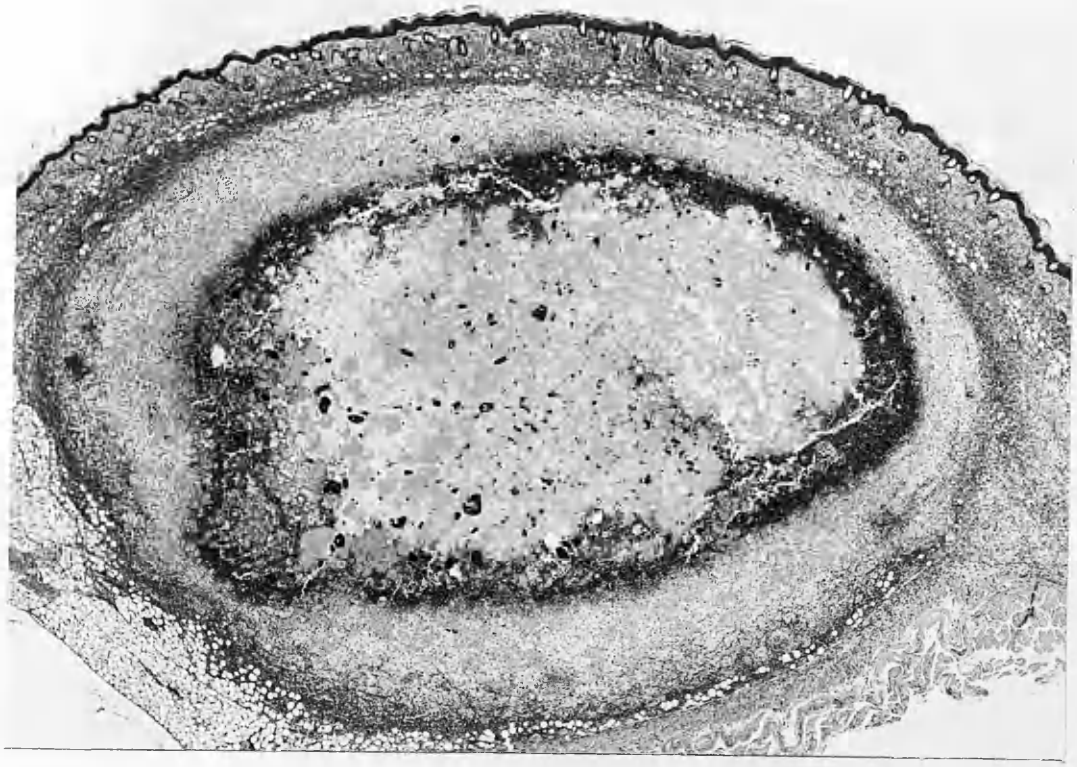


Figure 3.9: Low-power photomicrograph of Type C tissue reaction produced 8 days after subcutaneous inoculation of a suspension of F. nucleatum into the groin of an adult male mouse. An abscess has developed with an area of central necrosis surrounded by 3 distinct bands of inflammatory reaction (x 25).

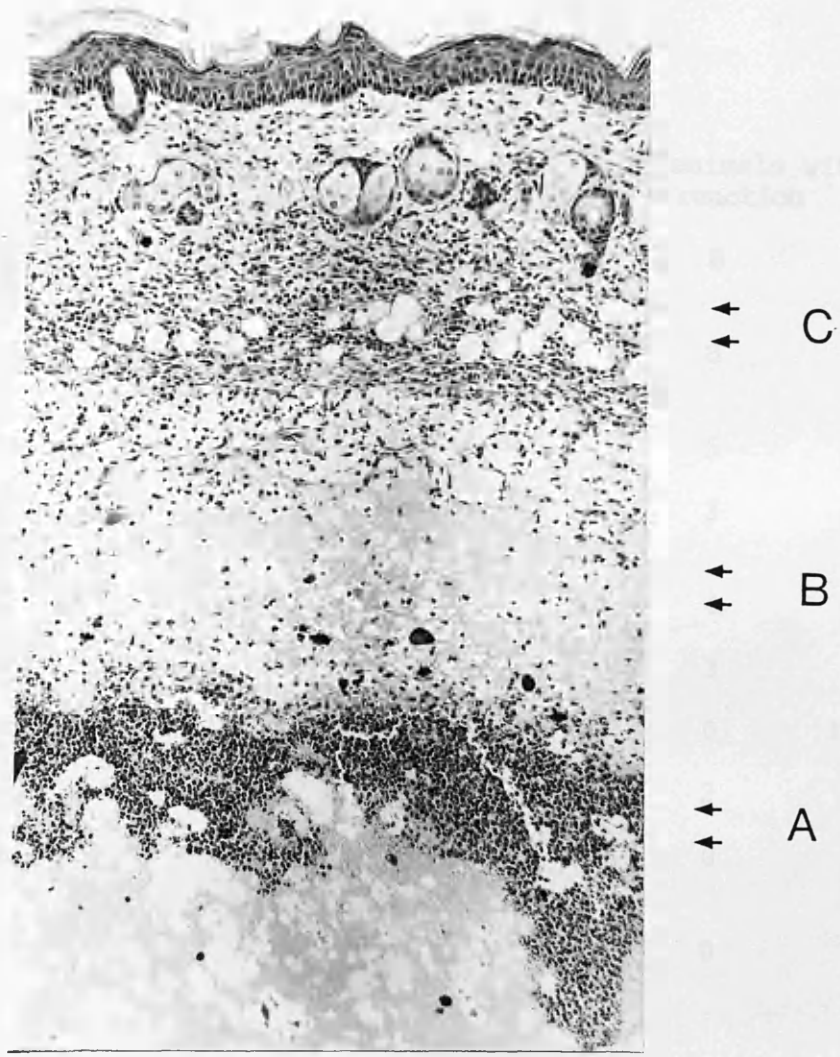


Figure 3.10: High-power photomicrograph of the Type C tissue reaction shown in Figure 3.9. The peripheral layers of inflammatory reaction are seen to consist of; (A) polymorphonuclear leukocytes, (B) necrotic polymorphonuclear leukocytes and macrophages and (C) granulation tissue (x 150).

Bacterial Species	No. of Strains	No. of animals	No. of animals with tissue reaction		
			A	B	C
Strep. milleri	2	11	3	8	0
Peptococcus spp.	3	11	6	5	0
Peptostrep. spp.	1	4	0	3	1
B. oralis	3	11	3	4	4
B. melaninogenicus	4	14*	1	3	9
B. intermedius	2	11	1	0	10
B. gingivalis	2	8	3	2	3
B. ruminicola	1	4	2	0	2
F. nucleatum	2	7	0	0	7

* One animal died on Day 3 and therefore is not included in the results.

Table 3.9: Distribution of experimental animals according to type of tissue reaction produced by 9 bacterial strains in 80 mice.

tissue reactions.

3.3.5 A comparison of the type of tissue reaction produced by each bacterial species

A comparison of the frequency with which test organisms produced a Type A reaction rather than a Type B or Type C revealed that the only significant differences were for Peptococcus species. This organism produced a Type A reaction more frequently than B. intermedius ($p < 0{\cdot}05$), B. melaninogenicus ($p < 0{\cdot}05$) and F. nucleatum ($p < 0{\cdot}05$).

A comparison of the frequency with which each species produced a Type C reaction rather than Type A or Type B is shown in Table 3.10. Both F. nucleatum and B. intermedius produced a Type C reaction significantly more frequently than Strep. milleri, Peptococcus species, Peptostreptococcus species, B. gingivalis and B. oralis. A Type C reaction was produced by B. oralis and B. melaninogenicus more frequentley than Strep. milleri and Peptococcus species. There were no other significant differences between the test species.

When results from bacterial strains were compiled to give results for bacterial groups no significant difference was found between the 3 groups for the ability to produce Type A reaction rather than Type B or Type C. However anaerobic gram-negative bacilli did produce a Type C reaction statistically more often than Type A ($p < 0{\cdot}001$) or Type B ($p < 0{\cdot}001$). There was no statistical difference between the type of tissue reaction produced by anaerobic gram-

	COMPARATIVE SPECIES								
INDEX SPECIES	Strep. milleri	Peptococcus sp.	Peptostrep. sp.	B. gingivalis	B. intermedius	B. melaninogenicus	B. oralis	B. ruminicola	Fusobacterium
Strep. milleri		..	..	..	..	..	..	..	..
Peptococcus spp.	..		..	..	..	..	..	..	..
Peptostrep. spp.	..	..		..	..	..	..	..	..
B. gingivalis	..	..	..		..	..	..	..	..
B. intermedius	<0.001	<0.001	<0.05	<0.05		..	<0.05	..	..
B. melaninogenicus	<0.001	<0.001	..	..	..		..	..	..
B. oralis	<0.05	<0.05	..	..	..	..		..	..
B. ruminicola	..	..	..	..	..	..	..		..
Fusobacterium	<0.001	<0.001	<0.05	<0.05	..	..	<0.05	..	

p values indicate the significance with which the index species produced Type C reaction more often than the comparative species.

.. Not significant.

Table 3.10: A comparison of the test species which produced a Type C reaction rather than a Type A or Type B.

positive cocci or CO_2-dependent gram-positive cocci.

3.3.6 A comparison of the concentration of viable bacteria recovered from each type of tissue reaction

The concentration of bacteria recovered from the 3 types of tissue reaction are shown in Table 3.11. Viable bacteria were not recovered from any of the Type A reactions but they were recovered from 21 of the 25 Type B reactions and all of the 36 Type C reactions. Viable bacteria were recovered at high concentrations from Type C reactions statistically more frequently than from Type B reactions ($p < 0{\cdot}001$).

3.3.7 Microbiological findings of experiments using a combination of 2 bacterial species

The viable bacterial content of the inoculum introduced into each mouse and the concentration of each bacterial strain recovered at sacrifice on Day 8 are shown in Table 3.12 (summary Table 3.13). All the animals were challenged with the same volume of inoculum at a concentration of approximately 10^8 cfu/ml (range $0{\cdot}7 \times 10^8$ to $7{\cdot}3 \times 10^8$ cfu/ml) and a sample of fluid aspirated from the experimental site after 8 days. An aspirate of 25 μl/ml was not obtained from the experimental site of 2 animals (4 per cent of the total inoculated) and these are combined, in the results, with the details of the 2 animals from which samples were negative on culture. The samples yielding no growth on culture were obtained from 2 animals that had been inoculated with a combination of either a Strep. milleri and Peptostreptococcus species. From 7 mice only one of the two bacterial strains inoculated was recovered. The bacterial species lost from the

Tissue reaction	No. of mice with tissue reaction	No. of samples recovered at each concentration		
		NG+	LVG*	$10^7 - 10^9$ cfu/ml
Type A	19	19	0	0
Type B	25	4	14	7
Type C	36	0	5	31

+NG = No growth *LVG = Low viable growth

Table 3.11: Distribution of 80 experimental abscesses according to tissue reaction type and concentration of viable bacteria recovered.

Table 3.12: Concentration of bacterial inoculum, concentration of viable organisms recovered and tissue reaction produced by 11 bacterial combinations inoculated into 55 mice.

Bacterial species (Strain number)	Animal number	Cons. of inoculum ($x10^8$ cfu/ml)	Conc. of aspirate ($x10^8$ cfu/ml)	Tissue reaction
Strep. milleri (116) + Peptococcus sp. (058)	2795	3.2	0.2	B
		2.1	0.1	
	2805	3.2	0.5	B
		2.1	—[x]	
	2815	3.2	LVG[⊛]	B
		2.1	—	
	2825	3.2	LVG	B
		2.1	LVG	
Strep. milleri (031) + Peptococcus sp. (058)	2715	3.0	—	A
		3.0	—	
	2725	3.0	0.4	B
		3.0	0.1	
	2735	3.0	0.2	B
		3.0	0.2	
	2745	3.0	—	A
		3.0	—	
Strep. milleri (116) + Peptostrep. sp. (080)	2835	3.2	LVG	B
		1.6	—	
	2845	3.2	LVG	B
		1.6	—	
	2855	3.2	LVG	B
		1.6	—	

Bacterial species (Strain number)	Animal number	Conc. of inoculum ($x10^8$ cfu/ml)	Conc. of aspirate ($x10^8$ cfu/ml)	Tissue reaction
	2865	3.2	0.4	C
		1.6	0.1	
Strep. milleri (031) + Peptostrep. sp. (080)	2755	3.0	—	A*
		2.6	—	
	2765	3.0	0.3	B
		2.6	—	
	2775	3.0	—	A*
		2.6	—	
	2785	3.0	0.3	B
		2.6	—	
Strep. milleri (031) + B. intermedius (005)	3064	2.3	0.1	C
		3.9	0.1	
	3074	2.3	0.2	C
		3.9	0.1	
	3084	2.3	0.1	C
		3.9	0.1	
Strep. milleri (031) + B. intermedius (023)	1855	4.8	0.6	C
		2.8	0.4	
	1865	4.8	0.9	C
		2.8	0.7	
	1875	4.8	0.7	C
		2.8	0.6	
	1885	4.8	LVG	A
		2.8	LVG	

Table 3.12 (Cont.)

Bacterial species (Strain number)	Animal number	Conc. of inoculum ($x10^8$ cfu/ml)	Conc. of aspirate ($x10^8$ cfu/ml)	Tissue reaction
Strep. milleri (116) + B. intermedius (005)	3094	2.9	0.1	C
		3.9	0.2	
	3104	2.9	0.9	C
		3.9	0.3	
	3114	2.9	1.3	C
		3.9	1.1	
Strep. milleri (116) + B. melaninogenicus (114)	3334	2.9	4.5	C
		1.2	1.5	
	3344	2.9	0.8	C
		1.2	1.6	
	3354	2.9	2.2	C
		1.2	2.4	
Strep. milleri (031) + F. nucleatum (112)	1975	7.3	0.2	C
		5.2	0.2	
	1985	7.3	0.8	C
		5.2	0.6	
	1995	7.3	0.6	C
		5.2	0.5	
	2005	7.3	0.6	C
		5.2	0.6	
Peptococcus sp. (058) + B. intermedius (023)	1815	3.0	LVG	B
		2.8	LVG	
	1825	3.0	0.2	C
		2.8	0.2	

Table 3.12 (Cont.)

Bacterial species (Strain number)	Animal number	Conc. of inoculum ($x10^8$ cfu/ml)	Conc. of aspirate ($x10^8$ cfu/ml)	Tissue reaction
	1835	3.0	0.2	B
		2.8	0.2	
	1845	3.0	LVG	C
		2.8	LVG	
Peptococcus (090)	3424	0.6	19.2	C
+				
B. melaninogenicus (114)		1.1	3.5	
	3434	0.6	18.8	C
		1.1	4.2	
	3444	0.6	13.7	C
		1.1	5.9	
Peptococcus sp. (090)	3604	0.7	0.5	C
+				
B. oralis (109)		3.5	0.3	
	3614	0.7	LVG	C
		3.5	LVG	
	3624	0.7	LVG	B
		3.5	LVG	
Peptococcus (058)	1935	3.0	0.6	C
+				
F. nucleatum (112)		3.3	0.4	
	1945	3.0	0.5	C
		3.3	0.7	
	1955	3.0	0.9	C
		3.3	1.8	
	1965	3.0	0.6	C
		3.3	2.0	

Table 3.12 (Cont.)

Bacterial species (Strain number)	Animal number	Conc. of inoculum ($x10^8$ cfu/ml)	Conc. of aspirate ($x10^8$ cfu/ml)	Tissue reaction
Peptostrep. sp. (080) + B. intermedius (023)	1775	1.4	0.8	C
		2.8	0.9	
	1785	1.4	0.2	C
		2.8	0.2	
	1795	1.4	0.4	C
		2.8	0.1	
	1805	1.4	0.1	C
		2.8	0.4	
Peptostrep. sp. (080) + F. nucleatum (112)	1895	1.4	0.8	C
		5.3	0.8	
	1905	1.4	0.4	C
		5.3	0.2	
	1915	1.4	0.3	C
		5.3	0.7	
	1925	1.4	0.8	C
		5.3	0.3	

⊛ LVG — Low Viable Growth (less than 10^3 cfu/ml)

x ___ — No Growth

* — Aspirate of at least 25 μl not obtained

Table 3.12 (Cont.)

original inoculum mixture were Peptococcus species (2 cases) and Peptostreptococcus species (5 cases) when combined with Strep. milleri. From 38 animals both organisms were recovered at high concentrations (10^7 to 10^9 cfu/ml).

3.3.8 A comparison of the recovery of the bacterial strains from experiments using a combination of 2 bacterial species

The number of abscesses from which both bacterial species were recovered at high concentrations was compared to the number from which one or both species were recovered either at low concentrations or not at all (Table 3.13). Both bacterial strains were recovered at high concentrations statistically more often in combinations containing gram-negative bacilli than those which did not ($p < 0{\cdot}001$).

3.3.9 Histopathological findings in experiments using a combination of 2 bacterial species

The tissue reactions produced by combinations of bacteria were similar to those produced by pure cultures. Representative sections were examined from each animal and classified as Type A, Type B or Type C according to the histological parameters described in Table 3.8.

The type of tissue reaction produced in each animal is shown in Table 3.12, with a summary in Table 3.13. Combinations containing B. melaninogenicus or F. nucleatum produced the same type of tissue reaction (Type C) on every occasion. The remaining bacterial combinations produced more than one type of tissue reaction.

Table 3.13: Recovery of viable bacteria and tissue reaction produced in 55 mice inoculated with bacterial combinations at a concentration of approximately 10^8 cfu/ml.

Bacterial Combination	No. of animals	No. of aspirates containing both bacterial species at concentrations		No. of animals with tissue reaction type		
		NG[+] or LVG[*]	$10^7 - 10^9$ cfu/ml	A	B	C
Strep. milleri + Peptococcus	8	5	3	2	6	0
Strep. milleri + Peptostrep. spp.	8	7	1	2	5	1
Strep. milleri + B. melaninogenicus	3	0	3	0	0	3
Strep. milleri + B. intermedius	10	1	9	1	0	9
Strep. milleri + F. nucleatum	4	0	4	0	0	4

[+] NG = No growth

[*] LVG = Low viable growth

Bacterial Combination	No. of animals	No. of animals containing both bacterial species at concentrations		No. of animals with tissue reaction type		
		NG or LVG	10^7 - 10^9 cfu/ml	A	B	C
Peptococcus + B. intermedius	4	2	2	0	2	2
Peptococcus + B. melaninogenicus	3	0	3	0	0	3
Peptococcus + B. oralis	3	2	1	0	1	2
Peptococcus + F. nucleatum	4	0	4	0	0	4
Peptostrep. spp. + B. intermedius	4	0	4	0	0	4
Peptostrep. spp. + F. nucleatum	4	0	4	0	0	4
Total	55	17	38	5	14	36

Table 3.13 (Cont.)

3.3.10 A comparison of the type of tissue reaction produced by each bacterial combination

A comparison of the frequency with which bacterial combinations produced a Type A reaction rather than Type B or Type C revealed that there were no statistically significant differences.

A comparison of the frequency with which combinations produced a Type C reaction rather than Type A or Type B (Table 3.14) revealed that 7 of the 9 combinations containing anaerobic gram-negative bacilli produced a Type C reaction significantly more often than combinations of CO_2-dependent and strictly anaerobic gram-positive cocci. The 2 exceptions were combinations of B. intermedius with Peptococcus species and B. oralis with Peptococcus species. No other significant differences were found.

The results obtained for individual combinations were compiled to produce information for bacterial group combinations (Table 3.15) and a comparison made between the type of tissue reaction produced. A combination of CO_2-dependent gram-positive cocci and anaerobic gram-positive cocci produced a Type A reaction rather than a Type B or Type C significantly more often than the combinations containing anaerobic gram-negative bacilli ($p < 0{\cdot}05$). Bacterial combinations containing anaerobic gram-negative bacilli produced a Type C reaction rather than Type A or Type B statistically more frequently than combinations of CO_2-dependent gram-positive cocci and anaerobic gram-positive cocci ($p < 0{\cdot}001$).

INDEX COMBINATION	COMPARATIVE COMBINATION	
	Strep. milleri Peptococcus sp.	Strep. milleri Peptostrep. sp.
Strep. milleri Peptococcus sp.		..
Strep. milleri Peptostrep. sp.	..	
Strep. milleri B. intermedius	<0.001	<0.05
Strep. milleri B. melaninogenicus	<0.001	<0.001
Strep. milleri F. nucleatum	<0.01	<0.01
Peptococcus sp. B. intermedius	..	..
Peptococcus sp. B. melaninogenicus	<0.001	<0.05
Peptococcus sp. B. oralis	..	..
Peptococcus sp. F. nucleatum	<0.001	<0.001
Peptostrep. sp. B. intermedius	<0.001	<0.001
Peptostrep. sp. F. nucleatum	<0.001	<0.001

p values indicate significance with which the index combination produced a Type C rather than a Type A or B (Fisher's exact test).

Table 3. 14: A comparison of the ability of the test bacterial combinations to produce a Type C tissue reaction rather than Type A or B.

Bacterial Combination	No. of aniamls	No. of animals with tissue reaction			No. of animals containing both bacterial species at concentrations	
		A	B	C	LVG	$10^7 - 10^9$ cfu/ml
CO_2-dependent gram-positive cocci and Anaerobic gram-positive cocci	16	4	11	1	12	4
CO_2-dependent gram-positive cocci and Anaerobic gram-negative bacilli	17	1	0	16	1	16
Anaerobic gram-positive cocci and Anaerobic gram-negative bacilli	22	0	3	19	3	19

Table 3.15: Distribution of tissue reactions produced by bacterial combinations and the number of abscesses in each combination that contained both bacterial species at similar concentrations.

3.3.11 A comparison of the concentration of viable bacteria recovered from eact type of tissue reaction

The number of pus samples obtained from each type of tissue reaction that contained both isolates at high concentrations is shown in Table 13.16. A comparison of these results demonstrated that Type C reactions contained both species at high concentrations significantly more often than either Type A ($p < 0{\cdot}001$) or Type B ($p < 0{\cdot}001$).

3.4 DISCUSSION

3.4.1 Methodology

A number of methods have been used previously to study the pathogenicity of bacterial strains isolated from the oral cavity and this may in part explain the differences in the reported results. Small laboratory animals, such as mice, rats, guinea pigs and rabbits, have been used with the groin, back, hind foot, hard palate or cheek pouch as the site of experimental infection. In recent years Brook and Walker (1983, 1984, 1986) have established subcutaneous inoculation into the groin of a mouse as a simple model for determination of bacterial pathogenicity. This model was employed in the present study because mice were readily available and are easily maintained. In addition, subcutaneous inoculation into the groin is easy to perform, does not appear to cause distress to the animal and allows the development of an abscess to be observed without the need for sacrifice.

Tissue Reaction	No. of animals	No. of abscesses from which one or both strains were recovered at low concentration or not at all	No. of abscesses from which both strains were recovered at a concentration of between 10^7 - 10^9 cfu/ml
Type A	5	5	0
Type B	14	10	4
Type C	36	2	34

Table 3.16: Distribution of abscesses containing both bacterial species in high concentrations according to tissue reaction.

In early bacterial pathogenicity studies, attempts to establish pure culture infections in experimental animals met with limited success. It was found subsequently that the addition of substances such as caecal contents or hog gastric mucin to the inoculum had an adjuvant effect and greater numbers of abscesses were produced (Hill et al., 1974). However concern regarding variations in the composition of these suspending media resulted in the development by Walker and Wilkins (1976) of a semi-solid medium containing 0·25 per cent agar (wt/vol agar) that also assisted abscess development. Although the precise role of semi-solid agar is unknown it may protect bacteria from phagocytosis and other immune responses of the host. Other suspending media which have been used include, P-Y glucose broth, (Sundqvist et al., 1979), BM medium (Kastelein et al., 1981; van Steenbergen et al., 1982; Roenterink et al., 1984) and normal saline (Brook and Walker, 1983, 1984, 1986). A semi-solid medium, consisting of Anaerobic Blood Broth and agar, was used in the present study since it supported the viability of all the test bacterial strains used and each inoculum could be prepared rapidly and relatively easily from a 24 hour or 48 hour culture. The observation that inoculation of a sterile semi-solid agar based on an anaerobic blood broth did not produce an obvious acute inflammatory response would indicate that the different tissue reactions recorded when bacteria were included in the inoculum were due to the bacterial strains used rather than to the suspending medium.

The recent observation that increased numbers of capsulate anaerobic bacteria were present in pus obtained from oro-facial abscesses, compared with organisms present in the normal oral flora, has provided

evidence of a possible pathogenic role for capsular material (Brook, 1986). Since the production of capsular material may be lost under laboratory conditions (Ward and Berkely, 1980) bacterial strains which are repeatedly subcultured over extended periods may lose pathogenic potential which was present at the time of initial isolation. Repeated subculture or use of type strains may therefore be partly responsible for the low pathogenicity previously reported for some oral bacterial species. In the present study, although the presence of capsular material was not investigated, all the bacterial strains used had been stored in freeze dried ampoules soon after initial isolation and were subcultured on 3 occasions only prior to inoculation into the experimental animals.

The methods used to quantify the pathogenicity of bacteria in experimental animals have also varied and although these have usually included both microbiological and histopathological examinations (Sundqvist et al., 1979; Brook et al., 1983) some studies have employed only one method of quantitation. For example, in the studies of oral strains of black-pigmented bacteroides by van Steenbergen et al., (1982) and Roenterink et al., 1984, 1985) no attempt was made to recover the test bacteria from the inoculation site and pathogenicity was graded purely on histopathological changes. In the present study pus was collected on Day 8 and viable counts were performed both to ensure that the organisms present were the same as those in the original inoculum and to detect any quantitative changes which had occurred since the day of inoculation. It was originally intended to a use the method of histological assessment described by

Joiner et al., (1980) to enable quantification of abscess volume, however when it became apparent that localised abscesses did not always develop it was decided to record histopathological features rather than determine lesion volume.

Care was taken throughout the present study to ensure that fresh cultures of bacterial strains were tested and that the methods used were capable of characterising the infections produced both microbiologically and histopathologically.

3.4.2 Findings of experiments involving inoculation of pure cultures of bacterial strains

Little information is available concerning the pathogenicity of bacterial species isolated from acute dental infections. Investigations of oral bacteria have tended to involve strains of Bacteroides species isolated from periodontal disease or necrotic dental pulps since early studies demonstrated that, apart from B. melaninogenicus, oral bacteria were non-pathogenic in pure culture (MacDonald et al., 1963; Socransky and Gibbons, 1965). In contrast to these early findings, the present study has revealed that in addition to B. melaninogenicus infections could be produced by pure cultures of all the bacterial strains tested.

Quantitative microbiological results demonstrated that the concentration of the viable bacteria subsequently recovered from the experimental infections was variable. Although viable bacteria were not cultured from all the pus samples obtained, the majority of samples yielded bacterial loads which fell into one of two groups; (a)

concentrations of 10^3 cfu/ml or less or (b) concentrations between 10^6 and 10^9 cfu/ml. Studies which have used quantitative microbiological techniques to examine pus specimens obtained from experimental infections induced by Bacteroides species have reported recovery at concentrations of approximately 10^9 to 10^{10} cfu/ml (Sundqvist et al., 1979; Pancholi et al., 1985). These concentrations are higher than those recorded for the strains of Bacteroides species in the present study but may partly be due to the different methods of quantification used. Other than the use of serial 10-fold dilutions no details were provided by Sundqvist et al., (1979) or Pancholi et al., (1985) concerning how viable concentration of samples was determined. The mean recovery concentration of F. nucleatum in the study by Brook and Walker (1986) was of the order of 10^7 cfu/ml, a figure similar to that obtained for F. nucleatum in the present investigation. There does not appear to be information in the literature concerning the recovery of facultative or stictly anaerobic gram-positive cocci from experimental infections and therefore no quantitative data is available with which to compare the findings of the present study.

The differences in viable concentration noted in the present study suggest that certain bacterial species are more able to resist the mouse defence systems than others. Species of anaerobic gram-negative bacilli, such as F. nucleatum and B. melaninogenicus, were recovered more frequently at high concentrations than species of anaerobic gram-positive cocci or Strep. milleri and therefore appear to be more resistant to the host defences. Bacterial factors which enable organisms to resist killing by the host must play a major role

in the pathogenesis of acute dentoalveolar abscess. Slots and Genco (1984) reviewed the microbial pathogenicity of black-pigmented Bacteroides species, Capnocytophaga species and Actinobacillus actinomycetemcomitans in human periodontal disease and listed a number of factors which are of probable importance in evasion of host defence system. Those believed to be produced by B. gingivalis and B. intermedius, two of the bacterial species included in the present study, are shown in Table 13.17. In addition to this range of factors, the production of capsular material has been reported to enable Bacteroides species to evade phagocytosis (Okuda and Takazoe, 1973).

It is probable therefore that strains of Bacteroides species present in acute dentoalveolar abscess possess a number of potential properties which assist bacterial survival in the host tissues. The observation in the present study that facultative and strictly anaerobic gram-positive cocci can survive in host tissues suggests that they are also capable of evading the host defence systems, probably by using similar mechanisms to those described for bacteroides.

The bacterial strains tested in the present study produced either a spreading infection or a localised abscess in the subcutaneous tissues. The observation that pure cultures of a number of different Bacteroides species produced infections contrasts with the earlier findings of MacDonald et al., (1963) and Socransky and Gibbons (1965) but compares well with the more recent investigations that have described pathogenic strains of B. oralis, B. ruminicola and

Item	B. gingivalis	B. intermedius
Inhibition of polymorpho-nuclear leukocytes		
Leukotoxin	-	-
Chemotaxis-inhibitors	+	+
Decreased phagocytosis and intracellular killing	+	+
Resistance to complement mediated killing	+	+
Lymphocyte alterations	?	?
Endotoxicity	weak	weak
Immunoglobulin proteases		
IgA	+	+
IgG	+	+
Fibrinolysin	strong	weak
Superoxide dismutase	+	+
Catalase	-	-

Table 3.17: Bacterial factors of importance in the evasion of host defence systems (After Slots and Genco, 1984).

B. gingivalis in addition to B. melaninogenicus (Kastelein et al., 1981; van Steenbergen et al., 1982; Brook et al., 1983). These studies have also found that infections produced by pure cultures of black-pigmented bacteroides are usually of one of two types, either a purulent abscess localised more or less at the site of inoculation or a rapidly spreading gangrenous infection. Pure cultures of asaccharolytic strains, such as B. gingivalis, have tended to produce a spreading type of infection (MacDonald et al., 1963; Kastelein et al., 1981), whereas saccharolytic strains have usually caused a more localised abscess (Takazoe et al., 1971; Sundqvist et al., 1979). The observations in the pesent study that B. melaninogenicus and B. intermedius produced localised abscess whilst B. gingivalis, on occasions, produced a spreading infection rather than a localised abscess support these findings and those of van Steenbergen et al., (1982).

Bacteria may produce tissue destruction either by direct action or indirectly by inducing inflammatory or immune reactions that are in part harmful to the host. Slots and Genco (1984) reviewed the factors produced by B. intermedius and B. gingivalis which can potentially destroy gingival tissue and the periodontal ligament (Table 3.18). The production of some or all of these factors by strains of Bacteroides species used in the present study may well partly explain the different tissue reactions observed subsequently in histological sections.

Apart from details of the possible pathogenicity factors produced by certain Bacteroides species there is little similar

Item	B. gingivalis	B. intermedius
Enzymes		
Collagenase	+	+
Trypsin-like	+	-
Gelatinase	strong	moderate
Aminopeptidases	+/-	-
Phospholipase A	+	+
Alkaline phosphotase	+	+
Acid phosphotase	+	+
Toxic factors		
Epitheliotoxin	+	+
Fibroblast growth inhibitors	+	+
Endotoxicity	weak	weak
Lipopoly saccharide-induced bone resorption	+	+
Other bone-resorbing factors	?	?
Volatile sulfur compounds	+	+
Butyric and propionic acids	+	-
Indole	+	+
Ammonia	+	+
Polyclonal B-cell activators	+	+

Table 3.18: Bacterial factors with potential to destroy gingival tissue and periodontal attachment (After, Slots and Genco, 1984).

information available for bacteria such as fusobacteria, Strep. milleri or anaerobic gram-positive cocci which are also frequently cultured from acute dentoalveolar abscess.

Attempts to produce experimental infections in small animals by inoculation of fusobacteria have been performed, but apart from intra-hepatic abscesses in rabbits (Abe et al., 1976) and mice (Hill et al., 1974) these have met with limited success. More recently Brook and Walker (1986) used subcutaneous inoculation into mice as a method of assessing the pathogenicity of fusobacteria and found that pure cultures of 7 of the 8 strains of F. nucleatum, freshly isolated from a variety of body sites, produced abscesses in 90 per cent of the mice inoculated. The results of the present study would appear to support the finding that pure cultures of F. nucleatum are pathogenic. In an earlier study Brook and walker (1984) investigated the pathogenicity of facultative gram-positive cocci (Strep. constellatus and Strep. intermedius) and anaerobic gram-positive cocci (Peptococcus species and Peptostreptococcus species) in pure culture and in combination with other organisms such as B. assacharolyticus, Staph. aureus and E. coli. Pure cultures of facultative and anaerobic gram-positive cocci were found to produce abscesses in 364 of the 400 mice involved although, the lesions were smaller than those produced by the other bacteria tested and 90 per cent of the lesions resolved spontaneously.

The findings of the present study provide further evidence that pure cultures of facultative gram-positive cocci and anaerobic gram-positive cocci are pathogenic. It is therefore likely that these

organisms produce some of the pathogenic factors described for Bacteroides species by Slots and Genco (1984). In addition Brook (1986) has demonstrated that strains of anaerobic gram-positive cocci isolated from acute dentoalveolar abscess possess capsular material and this may also assist pathogenicity.

3.4.3 Findings of experiments involving the inoculation of a combination of two bacterial species

When a combination of two bacterial species was inoculated into the mice the same 3 types of tissue reaction to those produced by pure cultures developed. However both test bacterial strains were subsequently recovered more frequently in high concentrations from experimental sites which had been inoculated with a bacterial combination. In addition it was noted that bacterial strains which had tended previously to be recovered in low concentrations from pure culture experiments were more frequently recovered in high concentrations from mixed infections involving anaerobic gram-negative bacilli. There is little information in the literature which provides quantitative microbiological results for experimental infections involving mixtures of bacteria. Brook and Walker (1986) reported that F. nucleatum and Staph. aureus were recovered in higher concentrations from infections induced by a combination of these organisms than from infections caused by pure cultures of each species. These findings and those of the present study suggest that anaerobic gram-negative bacilli in some way either protect other bacteria or provide a suitable environment for the proliferation of other organisms. It has been proposed that one of the ways by which this effect is produced is by interference with opsinisation or processes of intracellular

killing (Jones and Gemmell, 1986).

Brook and Walker (1986) reported that not only were organisms recovered in high concentrations from infections involving F. nucleatum but the abscesses produced by combinations of bacteria were larger than those produced by pure cultures of bacteria. In an earlier study Brook and Walker (1984) noted that the presence of facultative gram-positive cocci or anaerobic gram-positive cocci enhanced the abscesses produced by other aerobic and anaerobic bacteria. Both these observations are in agreement with the general findings of the present study however the exact role and relative importance of individual organisms in acute dentoalveolar abscess is still not fully understood. It would appear however that pathogenic synergistic relationships probably exist between many of the bacterial species present in the microflora of acute dentoalveolar abscess.

3.4.4 Summary

It was noted in early studies of the pathogenicity of oral bacteria that the inclusion of B. melaninogenicus in a mixed inoculum was essential to produce infection in animals. This observation led to the opinion that B. melaninogenicus was closer to an overt pathogen than any of the other bacterial species normally residing in the mouth. The results of the present study confirm the pathogenicity of B. melaninogenicus but also support the findings of other more recent investigations that have shown that strains of other Bacteroides species are also capable of causing infections when inoculated subcutaneously into mice. In addition the present results show that strains of Strep. milleri, Peptoccus species, Peptostreptococcus

species and F. nucleatum are also capable of producing experimental infections in pure culture. The observation that pure cultures of anaerobic gram-negative bacilli; (i) tended to produce abscesses with peripheral necrosis, (ii) were recovered in high concentrations significantly more often than other the bacterial species tested and (iii) appear to protect other bacterial strains present in mixed infections, suggests that these micoorganisms play a major role in the aetiology of acute dentoalveolar abscess.

3.5 CONCLUSIONS

(i) Subcutaneous inoculation of bacterial strains into the groin of mice appears to be a satisfactory method of investigating the pathogenicity of bacterial strains isolated from acute dentoalveolar abscesses.

(ii) The bacterial species tested varied in their pathogenicity with respect to the tissue reactions produced and the concentration of viable organisms recovered from the experimental infections.

(iii) Fusobacteria and bacteroides tended to produce abscesses with obvious peripheral necrosis and were recovered in high concentrations.

(iv) Strep. milleri and anaerobic gram-positive cocci tended to produce abscesses without peripheral necrosis and were recovered in low concentrations.

(v) The differences in pathogenicity of the bacterial species tested in this study may help explain the variations seen in the clinical presentation of acute dentoalveolar abscesses in man.

CHAPTER 4

PRIMARY AND SECONDARY ANTIBIOTIC SENSITIVITY TESTING OF PUS FROM ACUTE DENTOALVEOLAR ABSCESS

4.1 INTRODUCTION

Antibiotic therapy is often prescribed empirically in the treatment of acute dentoalveolar abscess and penicillin has traditionally been regarded as the drug of choice. However Williams et al., (1983) have questioned this practice since clinical failure of penicillin, believed to be due to the presence of beta-lactamase producing bacteroides, has been reported for these abscesses (Heimdahl et al., 1980). There would appear therefore to be a need for information on the sensitivity of the microbial flora of acute dentoalveolar abscesses to a range of antibiotics.

Antibiotic susceptibility studies of dental infections have provided data on the sensitivity of facultative anaerobes (Gabrielson and Stroh, 1975; Epstein and Scopp, 1977; Woods, 1981) but few have given details for strictly anaerobic bacteria (Sabiston and Grigsby, 1977; Aderhold et al., 1981). The isolation of penicillin-resistant bacteroides and the recent emphasis on the importance of strict anaerobes in acute dentoalveolar abscess has highlighted the need for further information on the antibiotic susceptibility of these bacteria (Allard et al., 1978; von Konow and Nord, 1983).

Antibiotic sensitivity tests are usually performed on pure cultures of individual isolates however susceptibility testing of primary cultures of pus has also been recommended (Stokes and Ridgway, 1980). Primary testing of pus samples obtained from general hospital casualty departments and wards has been found to be useful (Waterworth and del Piano, 1976) but there is no information on the reliability of similar tests performed on the mixed flora obtained from acute dentoalveolar abscesses.

The aim of the present study was to determine the antibiotic sensitivity patterns of pus samples obtained from acute dentoalveolar abscesses using both primary and secondary antibiotic sensitivity tests.

4.2 MATERIALS AND METHOD

4.2.1 Pus specimens and bacterial isolates

The pus samples and individual bacterial strains used in this study were obtained from the acute dentoalveolar abscesses described in Chapter 2.

4.2.2 Control organisms

Staphylococcus aureus (NCTC 6571) was used as the control organism both in the primary testing of pus samples and the secondary testing of facultative organisms. The control strain for the secondary testing of strict anaerobes was Clostridium perfringens (NCTC 8237). Stock cultures of both these organisms were maintained throughout the study by monthly subculture on Columbia Blood Agar (Oxoid Ltd., Basingstoke) and stored, after 24 hours incubation at

37°C, under anaerobic conditions at room temperature (Appendix I).

4.2.3 Antibiotic sensitivity testing

Primary and secondary antibiotic tests were performed on Columbia Blood Agar (Oxoid Ltd., Basingstoke) using method B described by Stokes and Waterworth (1972), referred to subsequently in this thesis as Stokes method. A 0•1 ml volume of pus was placed in a sterile plastic 5 ml bijou (Sterilin Ltd., Teddington) and a sterile wire loop (external diameter, 4mm) (Medical Wire and Equipment Company Ltd., Corsham) used to transfer a standard amount of pus onto the centre of each of two agar plates. A sterile cotton-wool swab was then used to spread the pus evenly over the equator of these plates. A suspension of Staph. aureus was prepared by adding 3 or 4 colonies to 2 ml of sterile distilled water and a small loopfull spread evenly over over the poles of the plate using a sterile swab. Secondary antibiotic plates were similary inoculated with the appropriate control and tests organisms. Antibiotics were tested in the form of impregnated paper discs (Mast, Bootle) placed on each plate as shown in Figure 4.1. The identity and content of the 8 antibiotics included in the present study is shown in Table 4.1. All plates were incubated for 4 days at 37°C in an anaerobic chamber (Appendix I).

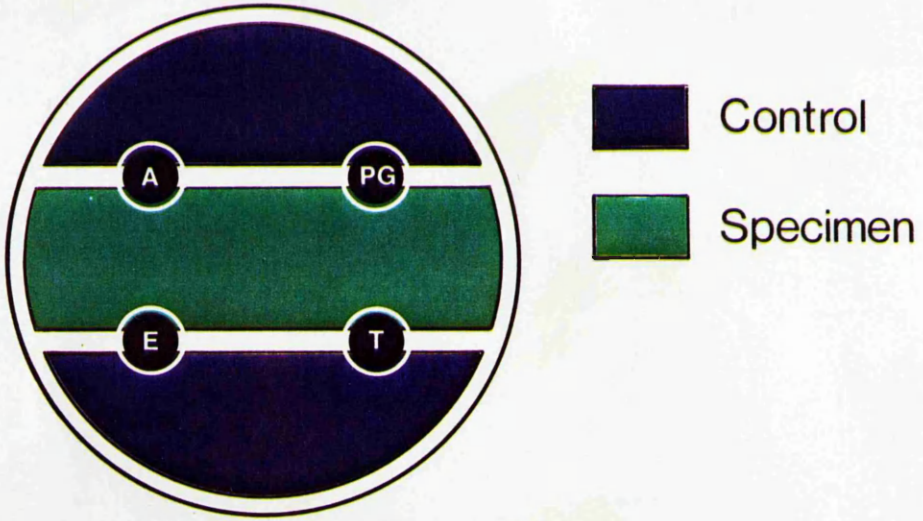


Figure 4.1: Inoculation pattern for antibiotic susceptibility testing (Method B as described by Stokes and Waterworth, 1972). Antibiotics, in disc form, are placed in one of four positions; amoxycillin (A), penicillin (PG), erythromycin (E) and tetracycline (T).

Antibiotic	Disc content (μg)
Amoxycillin	10
Ampicillin	10
Cephaloridine	5
Clindamycin	2
Erythromycin	15
Metronidazole	5
Penicillin	2 units
Tetracycline	10

Table 4.1: Concentration of antibiotic discs used in sensitivity testing of primary cultures and bacterial isolates from acute dentoalveolar abscess.

4.2.4 Interpretation of plates

Plates were examined after 2 days and 4 days incubation and zones of inhibition in the test area compared to those of the control areas. Results were recorded as;

Sensitive: - zone radius equal but not more than 3 mm smaller than control.

Moderately sensitive: - zone more than 3 mm radius but smaller than the control by 3 mm.

Resistant: - zone of 3 mm radius or less.

Using these criteria each pus sample and individual isolate was classified as sensitive, moderately sensitive or resistant to each of the antibiotics. An example of an incubated sensitivity test of a primary culture is shown in Figure 4.2 and a representative secondary sensitivity plate in Figure 4.3.

4.2.5 Secondary antibiotic sensitivity

Due to the polymicrobial nature of most (94 per cent) of the abscesses the following criteria were used in recording the overall secondary antibiotic sensitivity of each abscess. If one or more of the isolates was resistant to a particular antibiotic then the abscess was recorded as resistant to that antibiotic. If no organism was resistant but at least one isolate was found to be moderately sensitive, the secondary sensistivity was recorded as moderately sensitive. If all isolates were sensitive, the abscess was recorded sensitive.

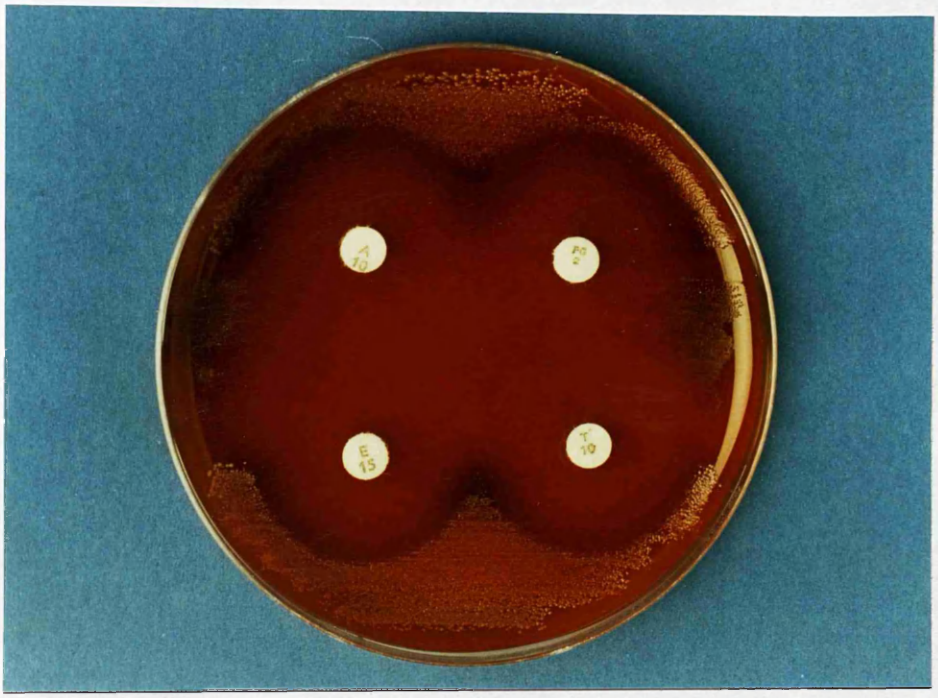


Figure 4.2: Incubated primary antibiotic sensitivity plate (Stokes method) demonstrating sensitivity to erythromycin (E) and moderate sensitivity to amoxycillin (A), tetracycline (T) and penicillin (PG). The control organism used was Staph. aureus (NCTC 6571).

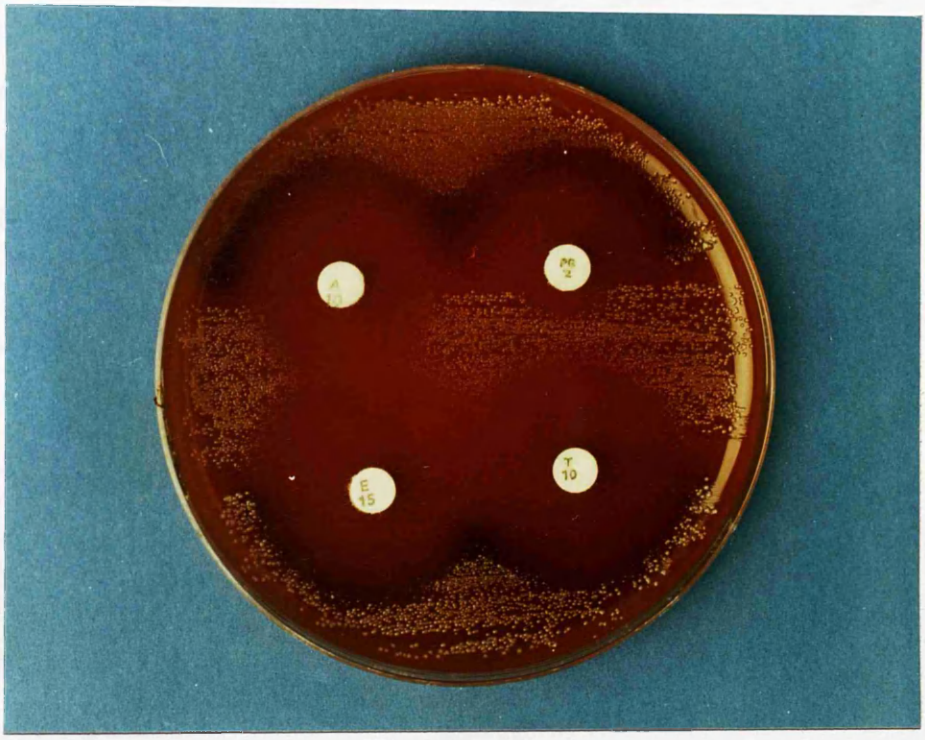


Figure 4.3: Incubated secondary antibiotic sensitivity plate (Stokes method) inoculated with a pure culture of Strep. milleri demonstrating sensitivity to erythromycin (E) and moderate sensitivity to amoxycillin (A), tetracycline (T) and penicillin (PG). The control organism used was Staph. aureus (NCTC 6571).

4.3 RESULTS

4.3.1 Antibiotic sensitivity testing of primary cultures

Since none of the sensitivity plates inoculated with pus yielded confluent growth it was possible to obtain a primary sensitivity result for all the samples. Most samples produced optimal colony density but growth was limited to the edges of the test area for approximately half of the specimens which yielded strict anaerobes only. In these circumstances, the zones of inhibition were larger than those of the control and the samples were recorded as sensitive.

The results of tests performed on primary culture of the 50 pus samples are shown in Table 4.2 and Figure 4.4. Although clindamycin was the most effective antibiotic, the majority (88 per cent) of pus samples were also sensitive or moderately sensitive to the other antibiotics with the exception of metronidazole. The 30 samples containing at least one facultative anaerobe were all resistant to metronidazole.

4.3.2 Antibiotic testing of bacterial isolates

The identity of the 166 bacterial strains isolated from the pus samples is shown in Table 4.3 and the results of sensitivity testing of pure cultures in Table 4.4 and Figure 4.5. The vast majority of the isolates were either sensitive or moderately sensitive to the antibiotics used and less than 4 per cent were resistant to antibiotics other than metronidazole. All the facultative anaerobes (26 per cent of the total number of isolates tested) were resistant to metronidazole and all the strict anaerobes sensitive.

Antibiotic	Number of Pus Samples		
	Sensitive	Moderately Sensitive	Resistant
Clindamycin	48	1	1
Amoxycillin	44	5	1
Erythromycin	43	6	1
Ampicillin	43	5	2
Cephaloridine	39	6	5
Penicillin	31	13	6
Tetracycline	38	8	4
Metronidazole	20	0	30

Table 4.2: Antibiotic susceptibility of primary cultures of pus samples obtained from 50 acute dentoalveolar abscesses.

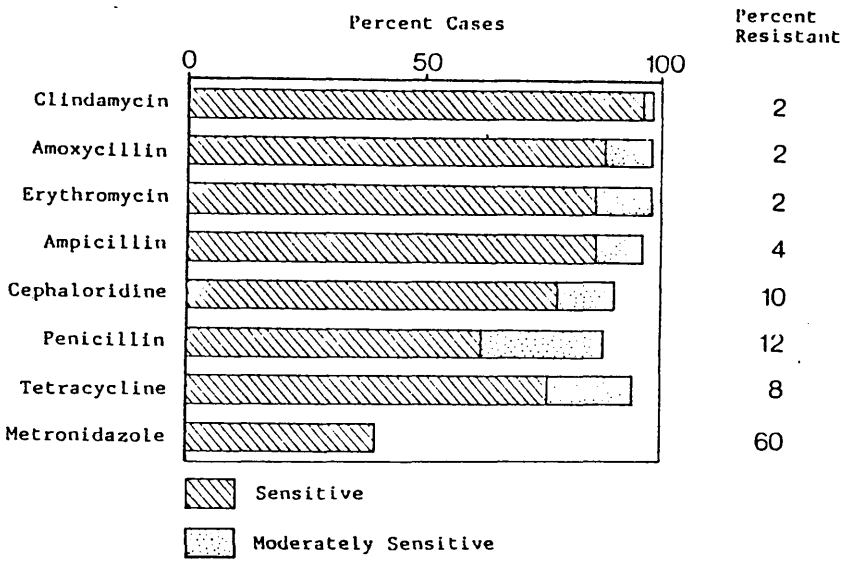


Figure 4.4: Effectiveness of 8 antibiotics against primary culture of pus samples obtained from 50 acute dentoalveolar abscesses.

Group 1	Facultative gram-positive cocci		32
		Streptococcus milleri	25
		Streptococcus mitior	3
		Streptococcus sanguis	3
		Streptococcus mutans	1
Group 2	Anaerobic gram-positive cocci		50
		Peptostreptococcus spp.	14
		Peptococcus spp.	32
		Streptococcus intermedius	3
		Streptococcus constellatus	1
Group 3	Anaerobic gram-negative bacilli		68
		Bacteroides oralis	20
		Bacteroides gingivalis	14
		Bacteroides melaninogenicus	12
		Bacteroides intermedius	5
		Bacteroides ruminicola	6
		Bacteroides distasonis	1
		Bacteroides ureolyticus	1
		Bacteroides capillosus	1
		Bacteroides uniformis	1
		Fusobacterium nucleatum	6
		Fusobacterium mortiferum	1
Group 4	"Miscellaneous" Isolates		16
		Lactobacillus fermentum	2
		Lactobacillus salivarius	1
		Actinomyces odontolyticus	1
		Actinomyces naeslundi	1
		Actinomyces meyeri	1
		Arachnia propionica	1
		Haemophilus parainfluenzae	2
		Capnocytophaga ochracea	1
		Eikenella corrodens	1
		Propionibacterium acnes	1
		Eubacterium lentum	1
		Veillonella spp.	3

Table 4.3: Identity and grouping of 166 bacterial strains isolated from 50 acute dentoalveolar abscesses.

Antibiotic	Number of strains		
	Sensitive	Moderately Sensitive	Resistant
Clindamycin	156	8	2
Amoxycillin	153	12	1
Erythromycin	155	10	1
Ampicillin	152	11	3
Cephaloridine	146	14	6
Penicillin	139	22	5
Tetracycline	141	21	4
Metronidazole	123	0	43

Table 4.4: Antibiotic susceptibility of 166 bacterial strains isolated from 50 acute dentoalveolar abscesses.

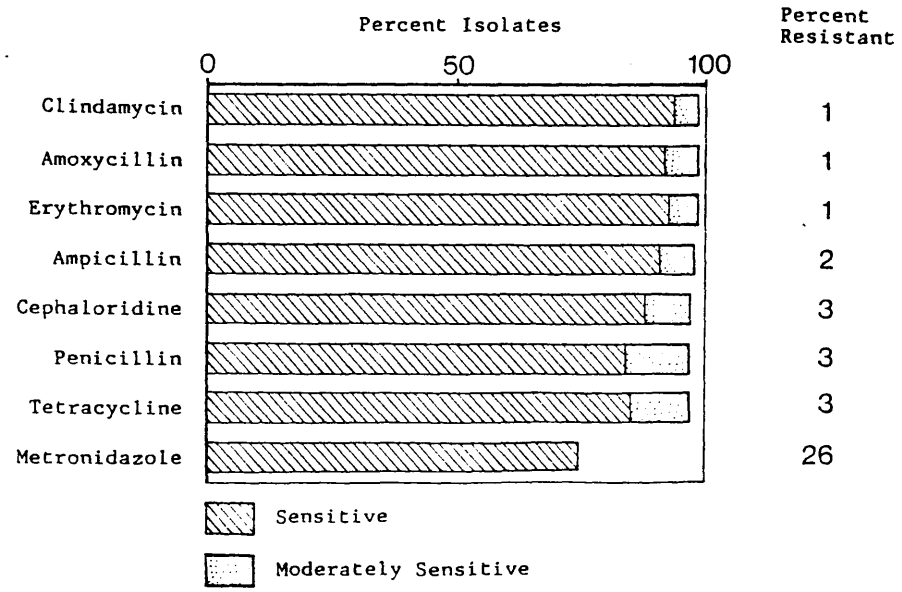


Figure 4.5: Effectiveness of 8 antibiotics against 166 bacterial strains isolated from 50 acute dentoalveolar abscesses.

To determine the susceptibility patterns of different bacterial groups the results were divided into the 4 groups as shown in Table 4.3.

The antibiotic sensitivity of facultative gram-positive cocci (Group 1) is shown in Table 4.5 and Figure 4.6. The majority of bacterial strains in this group were sensitive or moderately sensitive to the all the antibiotics tested except metronidazole. Three strains of Strep. milleri gave the following antibiotic sensitivity patterns; one strain was resistant to penicillin, ampicillin, cephaloridine and erythromycin, one strain resistant to penicillin and cephaloridine, and a third strain resistant to tetracycline. None of the isolates in Group 1 were resistant to clindamycin or amoxycillin but all were resistant to metronidazole.

The 50 strains of anaerobic gram-positive cocci (Group 2) were very susceptible to all the antibiotics tested (Table 4.6 and Figure 4.7). All strains were sensitive to clindamycin, amoxycillin and metronidazole but a minority (less than 6 per cent) were moderately sensitive to erythromycin, ampicillin, cephaloridine, penicillin and tetracycline. The only isolate in Group 2 that demonstrated resistance was a strain of Strep. intermedius, growth of which was not inhibited by tetracycline.

The 68 strains of anaerobic gram-negative bacilli (Group 3) were also very susceptible to the antibiotics tested (Table 4.7 and Figure 4.8). All were sensitive to metronidazole but a small number (less than 7 per cent) were moderately sensitive to the other

Antibiotic	Number of Strains		
	Sensitive	Moderately Sensitive	Resistant
Clindamycin	29	3	0
Amoxycillin	24	8	0
Erythromycin	27	4	1
Ampicillin	25	6	1
Cephaloridine	23	7	2
Penicillin	15	15	2
Tetracycline	16	13	3
Metronidazole	0	0	32

Table 4.5: Antibiotic susceptibility of 32 strains of facultative gram-positive cocci (Group 1) isolated from 50 acute dentoalveolar abscesses.

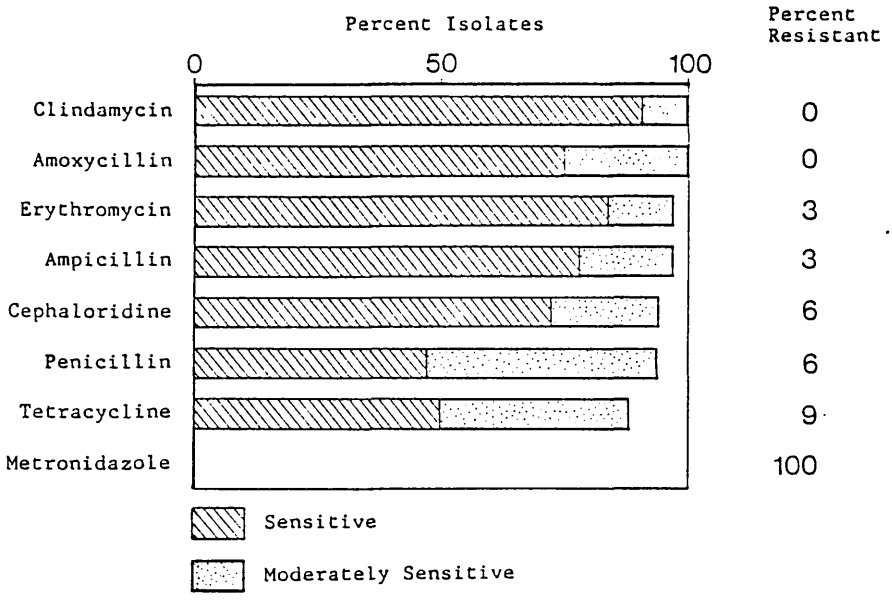


Figure 4.6: Effectiveness of 8 antibiotics against 32 strains of facultative gram-positive cocci (Group 1) isolated from 50 acute dentoalveolar abscesses.

Antibiotic	Number of Strains		
	Sensitive	Moderately Sensitive	Resistant
Clindamycin	50	0	0
Amoxycillin	50	0	0
Erythromycin	49	1	0
Ampicillin	49	1	0
Cephaloridine	48	2	0
Penicillin	49	1	0
Tetracycline	46	3	1
Metronidazole	50	0	0

Table 4.6: Antibiotic susceptibility of 50 strains of strictly anaerobic gram-positive cocci (Group 2) isolated from 50 acute dento-alveolar abscesses.

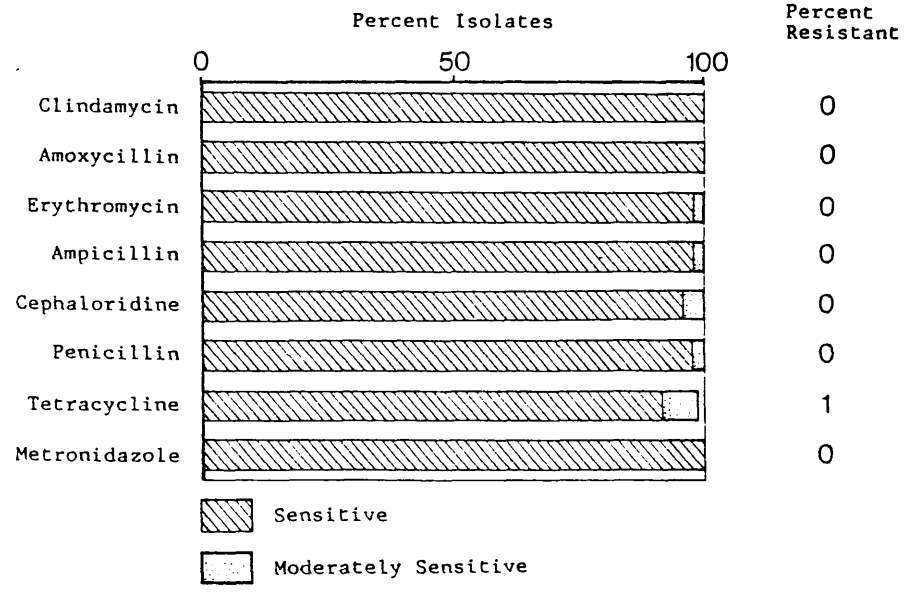


Figure 4.7: Effectiveness of 8 antibiotics against 50 strains of anaerobic gram-positive cocci (Group 2) isolated from 50 acute dento-alveolar abscesses.

Antibiotic	Number of Strains		
	Sensitive	Moderately Sensitive	Resistant
Clindamycin	64	4	0
Amoxycillin	66	1	1
Erythromycin	65	3	0
Ampicillin	65	1	2
Cephaloridine	64	2	2
Penicillin	63	2	3
Tetracycline	63	5	0
Metronidazole	68	0	0

Table 4.7: Antibiotic susceptibility of 68 strains of strictly anaerobic gram-negative bacilli (Group 3) isolated from 50 acute dento-alveolar abscesses.

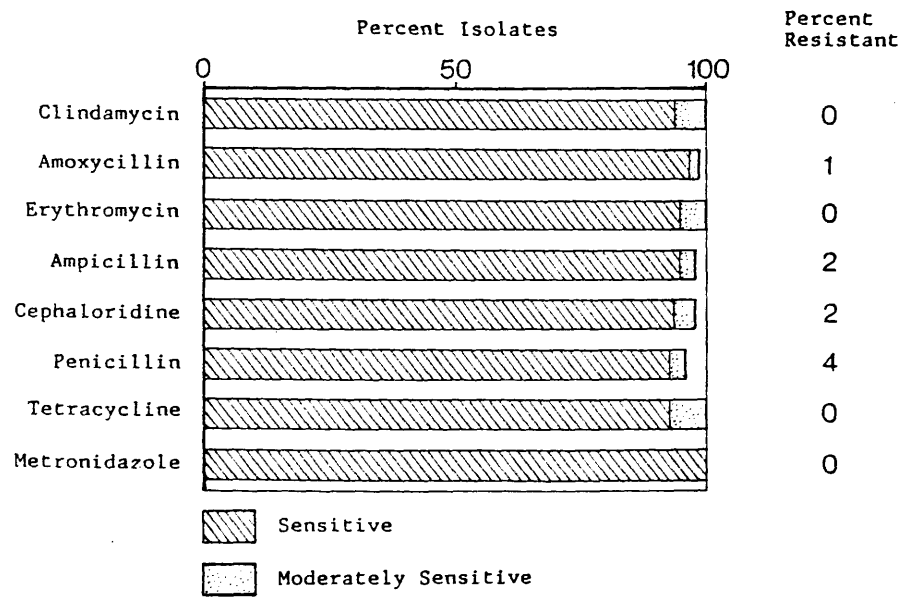


Figure 4.8: Effectiveness of 8 antibiotics against 68 strains of anaerobic gram-negative bacilli (Group 3) isolated from 50 acute dento-alveolar abscesses.

antibiotics. The single strain of B. distasonis isolated was resistant to amoxycillin, ampicillin, penicillin and cephaloridine. One strain of B. melaninogenicus was resistant to penicillin and ampicillin and one strain of F. nucleatum resistant to penicillin and cephaloridine.

The results for the 7 strains of facultative gram-positive bacilli, 4 strains of facultative gram-negative cocci, 2 strains of anaerobic gram-positive bacilli and 3 strains of anaerobic gram-negative cocci (Group 4), are shown in Table 4.8 and Figure 4.9. Tetracycline was the only antibiotic to which all these isolates were sensitive. The strict anaerobes were sensitive to metronidazole but the facultatives were resistant. None of the isolates were resistant to amoxycillin, ampicillin, penicillin and erythromycin however a minority (less than 4 per cent) were moderately sensitive to antibiotics other than metronidazole. The strains of Actinomyces naeslundi and Eikenella corrodens were resistant to clindamycin and cephaloridine.

4.3.3 Comparison of primary and secondary antibiotic tests

A comparison, using χ^2 test and Fisher's exact test, between the results obtained by primary testing and the results of secondary tests (Table 4.9) revealed no statistically significant differences. The majority of differences which occurred were minor and involved a change from sensitive to moderately sensitive or vice versa. The incidence of resistance to amoxycillin, ampicillin, erythromycin cephaloridine, tetracycline and metronidazole was the same by either method. Although only one sample was found to be resistant to

Antibiotic	Number of Strains		
	Sensitive	Moderately Sensitive	Resistant
Clindamycin	13	1	2
Amoxycillin	13	3	0
Erythromycin	14	2	0
Ampicillin	13	3	0
Cephaloridine	11	3	2
Penicillin	12	4	0
Tetracycline	16	0	0
Metronidazole	5	0	11

Table 4.8: Antibiotic susceptibility of 16 bacterial strains (Group) 4 isolated from 50 acute dentoalveolar abscesses.

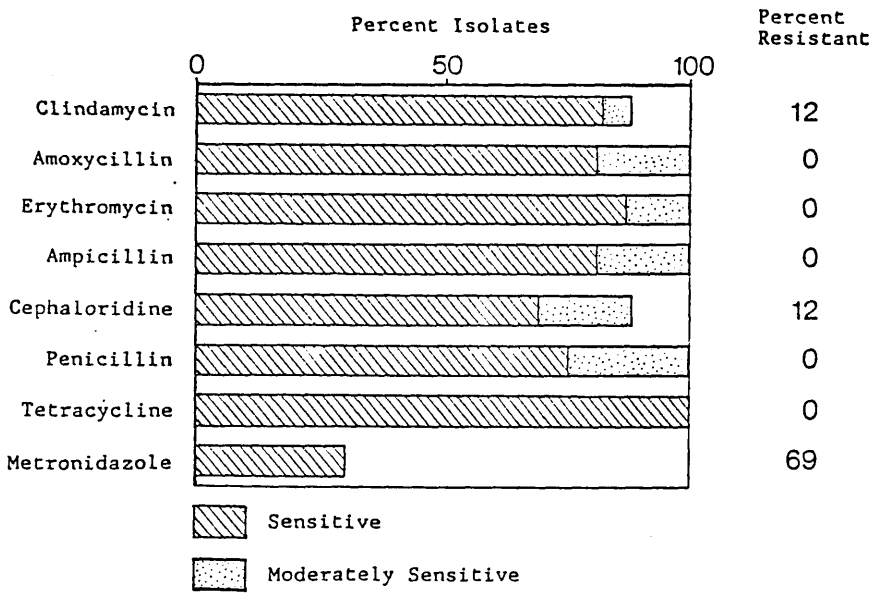


Figure 4.9: Effectiveness of 8 antibiotics against 16 bacterial strains (Group 4) isolated from 50 acute dentoalveolar abscesses.

Antibiotic	Test	Number of Cases		
		Sensitive	Moderately sensitive	Resistant
Clindamycin	Primary	48	1	1
	Secondary	43	5	2
Amoxycillin	Primary	44	5	1
	Secondary	38	11	1
Erythromycin	Primary	43	6	1
	Secondary	40	9	1
Ampicillin	Primary	43	5	2
	Secondary	37	11	2
Cephaloridine	Primary	39	6	5
	Secondary	35	10	5
Penicillin	Primary	31	13	6
	Secondary	31	15	4
Tetracycline	Primary	38	8	4
	Secondary	31	15	4
Metronidazole	Primary	20	0	30
	Secondary	20	0	30

Table 4.9: Comparison of primary and secondary antibiotic sensitivity testing of 8 antibiotics on samples from 50 acute dentoalveolar abscesses.

clindamycin during primary tests, secondary testing revealed that two abscesses contained a clindamycin-resistant strain. The additional resistant isolate was identified as a strain of Eik. corrodens. Six pus samples were classified as resistant to penicillin by primary tests whereas only 4 samples in secondary tests yielded penicillin-resistant strains.

4.4 DISCUSSION

4.4.1 Methodology

Many different techniques are available for the determination of antibiotic sensitivity of bacteria but disc diffusion methods are probably the most widely used, especially in routine diagnostic laboratories (Garrod et al., 1981). In an attempt to standardise methods worldwide an International Collabrative Study (ICS) examined different techniques over a period of 10 years. Although the ICS was unable to recommend a particular test for routine use, it did propose the acceptance of a modification of the method described by Ericsson (1960) (Ericsson and Sherris, 1971). This modified test has been officially adopted by Sweden while laboratories in the United States tend to use the Kirby-Bauer method (Bauer et al., 1966). The World Health Organisation (1977) subsequently advised that the choice of test should be left to individual laboratories but stressed the need for quality control.

Although test methods are now well established for facultative bacteria, they cannot be simply applied to strict anaerobes. The activity of some antibiotics, such as erythromycin, is affected by anaerobic conditions (Rosenblatt and Schoenknecht, 1972;

Rosenblatt, 1981) which would alter the size of inhibition zone. Since the standard zones for the Kirby-Bauer method (Bauer et al., 1966) were determined using facultative organisms they should not be applied to susceptibility testing of strict anaerobes (Rosenblatt, 1981; Wilkins, 1977). For this reason many microbiological laboratories use tests based on either an agar or broth dilution technique. Agar dilution tests are suitable for the testing a large number of individual isolates at one time whereas broth dilution methods become unwieldy in large studies due to the amount of laboratory time involved in their preparation.

Brown and Kothari (1978a, 1978b) used the Comparative and Stokes method, both as described by the Association of Clinical Pathologists (Stokes and Waterworth, 1972), the ICS method (Ericsson and Sherris, 1971) and the Kirby-Bauer method (Bauer et al., 1966) to test the susceptibility of 41 strains of rapidly growing pathogenic bacteria. It was noted that major disagreements between the results obtained by each of these four methods occured in less than 10 per cent of the tests. Stokes method (Stokes and Waterworth, 1972) was used in the present study since it is easily performed, would appear to be reliable and can be used to test strict anaerobes if Cl. perfringens is employed as a control (Stokes and Ridgway, 1980).

4.4.2 Analysis of experimental findings

While the antibiotic susceptibility of individual bacterial strains isolated from acute dentoalveolar abscess has been reported previously no information is available for the testing of primary

cultures of pus. Therefore in this discussion it was decided to firstly compare the secondary antibiotic sensitivity patterns of the present study with previous investigations and then relate them to the results of primary testing.

Turner et al., (1975) determined the sensitivities of 42 strains of "Strep. viridans", which had been isolated from acute dental abscesses, by the Kirby-Bauer (Bauer et al., 1966) method and found all were sensitive to ampicillin, penicillin, cephaloridine and erythromycin but 8 (19 per cent) were resistant to tetracycline. These findings are very similar to those obtained for the facultative gram-positive cocci, which were predominately Strep. milleri, in the present study. The minor differences may be due to variations in the identity of the strains tested although this is not certain since isolates studied by Turner et al., (1975) were simply characterised as "Strep. viridans" and not identified to species level. It is probable however that a number of different species of oral streptococci were involved since Turner et al., (1975) obtained specimens by a swab method and were therefore likely to contain a variety of contaminants from saliva.

Kannagara et al., (1980) used the agar dilution method described by Wilkins (1977) to determine the sensitivity of strict anaerobes isolated from a variety of oro-facial infections, including acute abscesses. All the strains studied were found to be sensitive to clindamycin but a small number of anaerobic gram-negative bacilli were resistant to penicillin, a similar finding to the present study. B. fragilis accounted for the majority of penicillin-resistant

anaerobes isolated by Kannagara et al., (1980) but most had been obtained from cases of mandibular osteomyelitis, infected extraction sockets or fractured mandibles rather than dentoalveolar abscesses. In the present study B. fragilis was not isolated from any of the abscesses.

Using an unspecified agar diffusion test Aderhold et al., (1981) investigated the effectiveness of 20 antibiotics against isolates from 50 acute dental abscesses (Reviewed in Section 1.6.2 and Table 1.6). The results can be summarised as follows; all facultative anaerobes were sensitive to ampicillin, approximately 5 per cent were resistant to penicillin, 4 per cent to tetracycline, 6 per cent to erythromycin and 8 per cent to clindamycin. None of the strict anaerobes were resistant to penicillin or clindamycin and less than 2 per cent were resistant to ampicillin, tetracycline and erythromycin. The number and identity of the facultative and strict anaerobes tested by Aderhold et al., (1981) was very similar to that of the present study and the susceptibility findings are almost identical.

Von Konow et al., (1981) tested the susceptibilities of 25 facultative bacteria, (predominately streptococci), and 178 strict anaerobes, (predominately gram-positive cocci and gram-negative bacilli), isolated from acute dental abscesses using an agar dilution method. All the facultative bacteria were sensitive to penicillin but 16 per cent were resistant to erythromycin, clindamycin, and doxycycline. The effect of metronidazole was not investigated but all facultative organisms were resistant to tinidazole, a drug closely

related to metronidazole. Of the strict anaerobes tested only 4 strains, one each of B. distasonis, and B. ruminicola and 2 unidentified bacteroides, were resistant to penicillin. In the present study the strain of B. distasonis tested was also found to be resistant to penicillin but the 6 strains of B. ruminicola were sensitive. In general the results reported by von Konow et al., (1981) are similar to those of the present study although there was a relatively high incidence of resistance to clindamycin (6 per cent of isolates) and to erythromycin (20 per cent of isolates). Unfortunately the identity of the resistant strains involved was not given so it is difficult to speculate on reasons for the disagreements. Tinidazole was reported to be effective against only 70 per cent of the strict anaerobes since some strains of anaerobic streptococci, actinomyces, lactobacilli, and propionibacteria were recorded as resistant. However strains of these bacterial species have been known to grow aerobically or in air with carbon dioxide after subculture (Watt and Jack, 1977; Rogosa and Sharpe, 1959; Wilson, 1983) which questions the accuracy of the classification by von Konow et al., (1981) of these strains as strict anaerobes.

Hunt and Meyer (1983) tested the sensitivity of 279 bacterial isolates from acute dental infections using the Kirby-Bauer method (Bauer et al., 1966). Four of the 95 strains of "Strep. viridans" were resistant to ampicillin, 15 resistant to penicillin and 12 resistant to erythromycin, a higher incidence than found in the present study. The reason for this difference may be the fact that some of the specimens studied by Hunt and Meyer (1983) had been obtained on swabs and therefore were likely to contain strains of

"Strep. viridans" introduced from saliva. Although the 34 strains of peptostreptococci were sensitive to erythromycin approximately 15 per cent were resistant to penicillin and ampicillin. In the present study none of the anaerobic gram-positive cocci were resistant to these antibiotics. A possible explanation for this difference may be the employment by Hunt and Meyer (1983) of the Kirby-Bauer method (Bauer et al., 1966), an inappropriate susceptibility test for strict anaerobes.

Labriola et al., (1983) determined the susceptibility of 62 facultative and 115 strict anaerobes isolated from oro-facial abscesses to penicillin, using the method described by Wilkins (1977). All 45 strains of facultative streptococci and the majority of strict anaerobes were sensitive but 1 strain of Peptostreptococcus species, 2 strains of fusobacteria and 20 strains of bacteroides, predominately B. melaninogenicus, B. oralis and B. ruminicola, were resistant. A small percentage of the pus samples studied by Labriola et al., (1983) were obtained from patients who were taking antibiotics at the time of sampling and this may partially explain the relatively high incidence of resistance to penicillin when compared with the results of the present study. Heimdahl et al., (1981) studied the incidence of penicillin-resistant bacteroides in saliva and found that specimens obtained from patients who had received penicillin prior to the study contained significantly more penicillin-resistant bacteroides than specimens obtained from patients who had not taken penicillin. None of the samples in the present study were obtained from patients who had received antibiotic therapy during the 3 months prior to the investigation.

The effectiveness of five antibiotics against 196 bacterial strains isolated from dental abscesses was investigated by Heimdahl et al., (1985). The 22 facultative organisms tested were streptococci and all were found to be sensitive to penicillin, erythromycin, doxycycline and clindamycin but resistant to tinidazole. The strict anaerobes tested were reported as sensitive to clindamycin but a small number of strains were resistant to other antibiotics. The isolates involved were 2 strains of Veillonella species, 1 strain B. pneumosintes, 2 strains B. oralis and 1 strain of E. lentum which were resistant to penicillin, and 7 strains of Veillonella species, 1 strain of anaerobic Streptococcus species , 1 strain of Peptostreptococcus species and 7 strains of F. nucleatum which were resistant to erythromycin. These findings are similar to those of the present study apart from the relatively large number of strains of Veillonella species and F. nucleatum which were resistant to erythromycin. Heimdahl et al., (1981) had previously emphasised the effect of antibiotic therapy prior to sampling and therefore factors other than previous erythromycin therapy were likely to be responsible for this finding.

In summary the results of the present study would appear to be in general agreement with those of other recent investigations of acute dental infections. The vast majority of bacteria isolated from acute dentoalveolar abscesses are sensitive to a number of antibiotics including amoxycillin, ampicillin, penicillin, erythromycin, cephaloridine and tetracycline. Strict anaerobes are sensitive to the nitroimidazoles, such as metronidazole, tinidazole and ornidazole, although there is still confusion in some studies regarding the

classification of certain isolates as true strict anaerobes. Resistance to the antibiotics presently used to treat acute dentoalveolar abscess would appear to be a relatively rare phenomenon.

4.4.3 Reliability of tests performed on primary cultures

A comparison of the two types of sensitivity test performed in the present study revealed that each produced similar results. Differences in interpretation of sensitive and moderately sensitive were relatively frequent but may well be due to the difficulty of recording zones of inhibition of some of the slower growing strict anaerobes. However differences between sensitive and moderately sensitive are probably of little clinical significance. More important are disagreements involving a difference in classification between sensitive and resistant but these only occured in the results for 3 (6 per cent) abscesses.

A criticism of primary testing is that the inoculum can not be standardised but Stokes and Ridgway, (1981) and Garrod et al., (1981) have stated that a suitable inoculum can often be achieved by inoculating swabs of pus samples directly onto agar plates. The results of the present study would support these claims, although some samples containing strict anaerobes alone gave growth which was difficult to see.

Waterworth and del Piano, (1976) were able to give sensitivity reports from primary culture tests for 63 per cent of pus samples obtained from casualty departments. Lower percentages were

reported for samples obtained from hospital wards and body sites which have an indigenous flora but it was felt that the reliabilty of primary cultures would be greater if the specimens from patients who were taking an antibiotic prior to sampling had been excluded from the study. In the present study none of the samples used were obtained from patients who had received antibiotics during the 3 months prior to sampling and great care was taken to obtain uncontaminated specimens. Major disagreements in interpretation of sensitivity occured in only 6 per cent of the abscesses a finding which supports the opinion of Waterworth and del Piano (1976) that primary testing of pus samples obtained from acute infections is a useful practice.

Primary testing assists the detection of small numbers of resistant organisms in a predominantly sensitive population and can provide the clinician with sensitivity information more rapidly than secondary testing, especially when slow growing strict anaerobes are present. The results of present study would support the use of susceptibility testing of primary cultures of pus obtained from acute dentoalveolar abscesses.

4.5 CONCLUSIONS

(i) The vast majority of bacterial species isolated from acute dentoalveolar abscesses are sensitive or moderately sensitive to the antibiotics presently used to treat acute dental infections.

(ii) Resistance to each of the antibiotics tested was demonstrated by a minority of strains.

(iii) All facultative and CO_2-dependent isolates were resistant to metronidazole but all strict anaerobes were sensitive.

(iv) Testing of primary cultures of pus is a reliable method of determining antibiotic sensitivity of acute dentoalveolar abscess.

CHAPTER 5

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION AND THE MINIMUM BACTERICIDAL CONCENTRATION OF ANTIBIOTICS FOR BACTERIAL STRAINS ISOLATED FROM ACUTE DENTOALVEOLAR ABSCESS

5.1 INTRODUCTION

Comparative disc diffusion tests, such as Stokes method (Stokes and Waterworth, 1972), are widely employed in routine diagnostic microbiology to assess antibiotic sensitivity because they are easily performed and give reproducible results. Although these tests only provide an approximate guide to antibiotic sensitivity this is sufficient for the majority of clinical situations. Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) provides a more accurate assessment of bacterial sensitivity (Stokes and Ridgway, 1980) but is frequently considered to be too time consuming for general use. In the past therefore the determination of MIC and MBC has often been reserved for the study of important slow-growing bacteria, such as Mycobacterium tuberculosis, or for bacterial strains isolated from the blood of patients with endocarditis or septicaemia where bactericidal activity is important.

Although in recent years there has been an increase in the performance of MIC determination, it is unlikely to become standard practice in United Kingdom because of the amount of technical time involved in performing these tests (Garrod et al., 1981). Therefore it has been suggested that centres capable of performing MIC tests on

a survey basis do so periodically to monitor any changing patterns of sensitivity to commonly used antibiotics and to determine the effect of new agents (Sutter and Finegold, 1976). Such surveys have provided MIC values for a variety of antibiotics and bacterial strains (Sutter and Finegold, 1976; Appelbaum and Chatterton, 1978; Hanson and Martin, 1980) but little information is available for bacterial strains isolated from acute dental abscesses (Allard et al., 1978).

The need to determine the MIC of antibiotics for bacteria isolated from the mouth has been stressed since it is unclear if their susceptibilities differ to those of non-oral strains of the same species (Baker et al., 1983). However, few studies have involved oral isolates and those that have, were performed in North America or Scandinavia. Therefore not only is there little information concerning oral bacteria generally but it is also possible that published values are not applicable to strains isolated in the United Kingdom due to geographical variations in antibiotic sensitivity.

The aims of the present study were firstly, to determine in detail the in vitro susceptibilities of bacterial strains isolated from acute dentoalveolar abscesses to antibiotics which are commonly used to treat acute dental infections and secondly to compare the results obtained with the sensitivity classification obtained by Stokes method in Chapter 4.

5.2 MATERIALS AND METHOD

5.2.1 Bacterial strains

All the test strains were originally isolated during the study described in Chapter 2 and had been stored in freeze dried ampoules since initial isolation.

The identity of the isolates chosen for study is shown in Table 5.1 and included all those strains of Strep. milleri, anaerobic gram-positive cocci and anaerobic gram-negative bacilli which had been classified, during the study described in Chapter 4, as resistant or moderately sensitive to either amoxycillin, ampicillin, clindamycin, penicillin, erythromycin or metronidazole. A number of sensitive strains were also selected for investigation to enable comparisons to be made between MIC results and the 3 classifications of susceptibility obtained by Stokes method (Stokes and Waterworth, 1972).

5.2.2 Control organisms

Bacteroides fragilis (NCTC 9343, ATCC 25285) and Clostridium perfringens (NCTC 8237, ATCC 13124) were used as control strains as recommended by Sutter et al., (1980). Stock cultures of both these organisms were maintained throughout the study by monthly subculture on Columbia Blood Agar (Oxoid Ltd., Basingstoke), stored at room temperature in anaerobic conditions after 24 hours incubation at 37°C in an anaerobic chamber (Appendix I).

Bacteria	No. of strains	Strain codes	Antibiotic sensitivity					
			A	PG	AP	E	CD	MZ
Strep. milleri	15	004, 016, 031	+	+	+	+	+	-
		050, 034, 071	+	(+)	+	+	+	-
		036, 038, 047, 113	(+)	(+)	(+)	+	+	-
		046, 112, 121	(+)	(+)	(+)	(+)	+	-
		001	+	-	+	+	+	-
		021	(+)	-	-	-	+	-
Peptococcus species	12	003, 006, 015, 017	+	+	+	+	+	+
		022, 026, 033, 079	+	+	+	+	+	+
		082, 081	+	+	+	+	+	+
		098	+	+	+	(+)	+	+
		097	+	(+)	(+)	+	+	+
Peptostrep. species	3	058, 080, 135	+	+	+	+	+	+
B. intermedius	3	005, 011	+	+	+	+	+	+
		023	+	+	+	+	(+)	+
B. melaninogenicus	3	053, 037	+	+	+	+	+	+
		117	(+)	-	-	+	+	+
B. oralis	3	075	+	+	+	+	+	+
		083	+	+	+	(+)	+	+
		096	+	(+)	+	+	+	+
B. gingivalis	2	013, 087	+	+	+	+	+	+
B. ruminicola	2	019	+	(+)	+	+	(+)	+
		024	+	+	+	+	+	+
B. distasonis	1	020	-	-	-	+	+	+
F. nucleatum	2	032	+	+	(+)	(+)	+	+
		112	+	+	+	+	+	+

A Amoxycillin; PG Penicillin G; AP Ampicillin;

E Erythromycin; CD Clindamycin; MZ Metronidazole

Table 5.1: Identity and secondary antibiotic sensitivity of 46 bacterial strains for which minimum inhibitory concentration and minimum bactericidal concentration were determined using broth and agar dilution methods. + sensitive, (+) moderately sensitive, - resistant

5.2.3 Preparation of inoculum

The bacterial suspensions used in the agar dilution and broth dilution tests were prepared by the same method. Test organisms were streaked onto Columbia Blood Agar (Oxoid Ltd., Basingstoke) and incubated at 37°C in the anaerobic chamber (Appendix I) for 3 days. For each test strain, approximately 50 colonies from a plate of pure growth were inoculated into 20 ml of Anaerobic Blood Broth (ABB) (Gibco Europe, Paisley) and placed in an anaerobic chamber (Appendix I). Facultative organisms were incubated at 37°C for 24 hours and strict anaerobes for 48 hours. Following this the concentration of the resultant broth was adjusted to approximately 2 x 10^7 cfu/ml using spectrophotometry and growth curves (Appendix II).

5.2.4 Antibiotic tested

Dispersable tablets of amoxycillin, ampicillin, clindamycin, erythromycin, penicillin and metronidazole were obtained commercially (Mast, Bootle) and stored at 4°C. Stock solutions of each antibiotic were freshly prepared prior to each experiment by dissolving the appropriate number of tablets in Wilkins-Chalgren broth (Gibco Europe, Paisley) supplemented with heat inactivated horse serum (Gibco Europe, Paisley) at 1 per cent vol/vol. The antibiotic content of the tablets and the concentration of each antibiotic stock solution is shown in Table 5.2.

Wilkins-Chalgren broth (Gibco Europe, Paisley) supplemented with heat inactivated horse serum (Gibco Europe, Paisley) at 1 per cent vol/vol was used to prepare dilutions of the stock solution to produce plates with the antibiotic concentrations shown in Table 5.3.

Antibiotic	Tablet content (mg)	Broth dilution method		Agar dilution method	
		No. of tablets and vol. of broth	Concentration (μg/ml)	No. of tablets and vol. of broth	Concentration (μg/ml)
Penicillin G	0.8	1 in 50ml	16	1 in 10ml	80
Amoxycillin	3.2	1 in 200ml	16	1 in 20ml	160
Ampicillin	3.2	1 in 200ml	16	1 in 20ml	160
Erythromycin	0.2	4 in 50ml	16	2 in 10ml	40
Clindamycin	0.1	4 in 50ml	8	2 in 10ml	20
Metronidazole	0.4	2 in 50ml	16	1 in 10ml	40

Table 5.2: Preparation and concentration of stock solutions of test antibiotics used in MIC and MBC determination.

Antibiotic	Test Method	Concentration of Antibiotic (μg/ml)									
		0·03	0·06	0·12	0·25	0·5	1·0	2·0	4·0	8·0	16·0
Penicillin	Agar	x	x	x	x	x	x	x	x	x	ND
	Broth	x	x	x	x	x	x	x	x	x	x
Amoxycillin	Agar	x	x	x	x	x	x	x	x	x	x
	Broth	x	x	x	x	x	x	x	x	x	x
Ampicillin	Agar	x	x	x	x	x	x	x	x	x	x
	Broth	x	x	x	x	x	x	x	x	x	x
Erythromycin	Agar	x	x	x	x	x	x	x	x	ND	ND
	Broth	x	x	x	x	x	x	x	x	x	x
Clindamycin	Agar	x	x	x	x	x	x	x	ND	ND	ND
	Broth	x	x	x	x	x	x	x	x	x	ND
Metronidazole	Agar	x	x	x	x	x	x	x	x	ND	ND
	Broth	x	x	x	x	x	x	x	x	x	x

x = indicates concentration included in test

ND = Not done

Table 5.3: Range of antibiotic concentrations used in agar and broth dilution MIC tests.

5.2.5 Agar medium

Wilkins-Chalgren agar (Gibco Europe, Paisley), prepared according to manufacturers instructions (Appendix I), was used as the agar base. Antibiotic sensitivity plates were prepared by adding 2 ml of each dilution of antibiotic to 18 ml molten of agar base at 50°C (Appendix III). Immediately after pouring, the plates were gently swirled to ensure even mixing of antibiotic and after cooling were stored at 4°C for use the following day.

5.2.6 Inoculation and incubation of agar plates

A multipoint inoculator (Mast, Bootle) was used to inoculate 2 μl of the test bacterial suspensions, containing approximately 2 x 10^4 cfu, onto each plate (Figure 5.1). The 9 test organisms and 2 control strains were inoculated onto each plate at the sites shown in Figure 5.2. Each test strain was inoculated onto two areas of each plate. A plate of modified Wilkins-Chalgren agar (Gibco Europe, Paisley) containing no antibiotic was inoculated at the beginning and end of each series of test plates to act as a growth control. Plates were allowed to dry for 15 minutes before being incubated at 37°C for 4 days in an anaerobic chamber (Appendix I). A control plate was also incubated at 37°C in air to detect any contamination by facultative organisms.

5.2.7 Interpretation of plates

Plates were examined after 2 and 4 days incubation and the MIC recorded as the lowest antibiotic concentration that prevented visible growth (Figure 5.3).

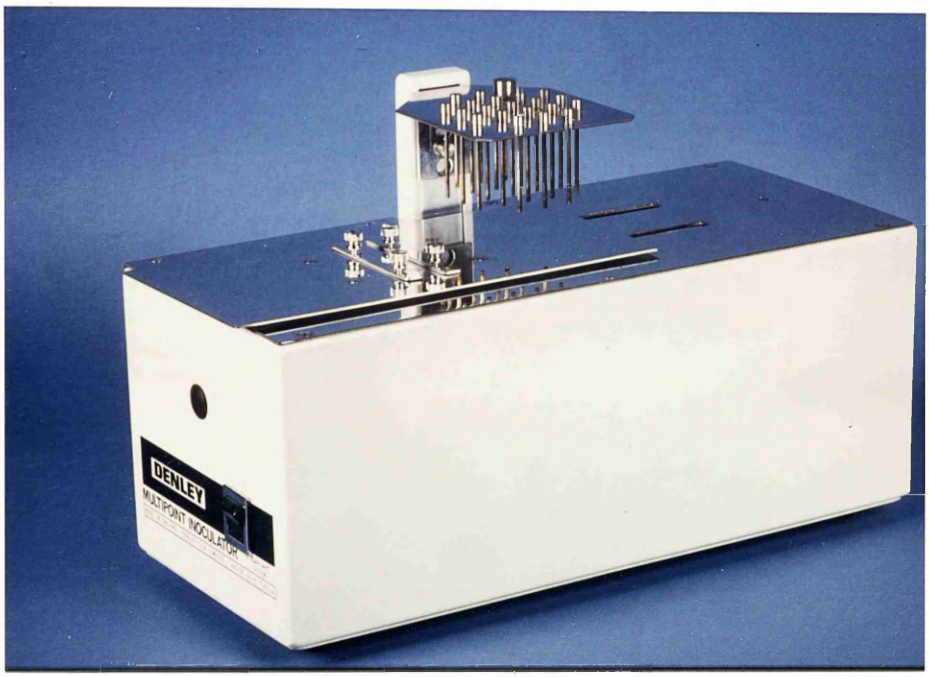


Figure 5.1: Multipoint inoculator used to inoculate 9 tests strains (in duplicate) and two control organisms onto antibiotic sensitivity agar plates.

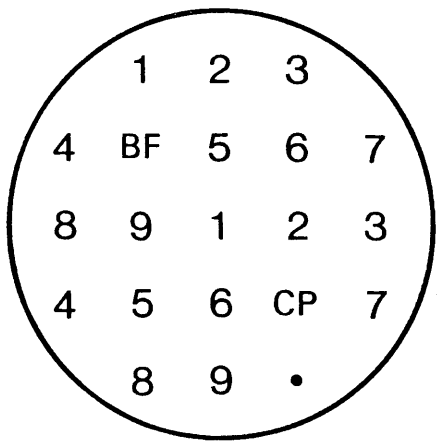
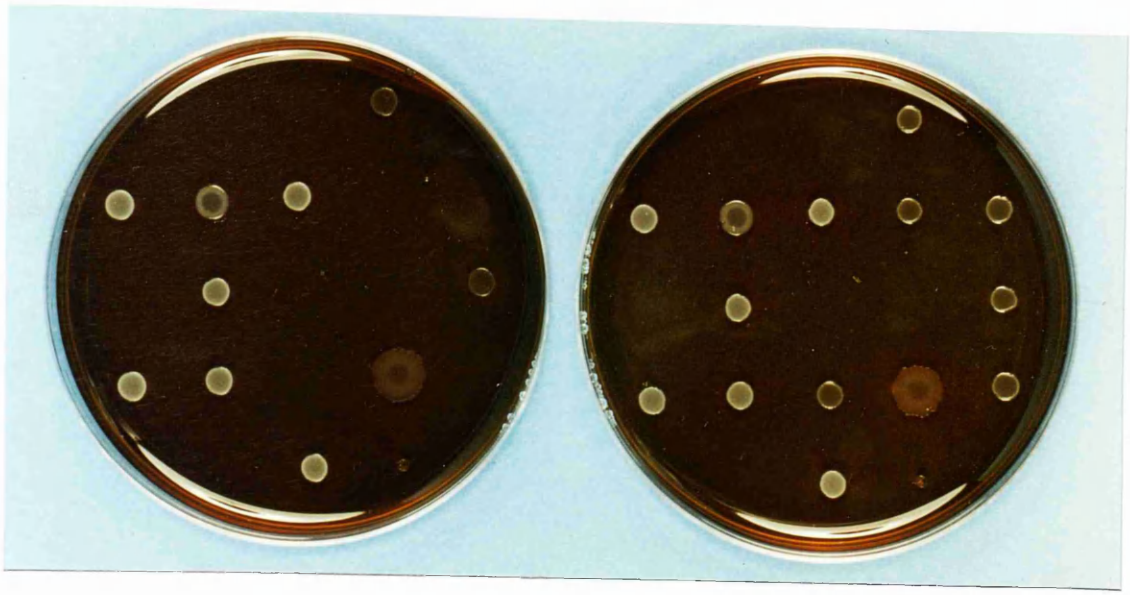
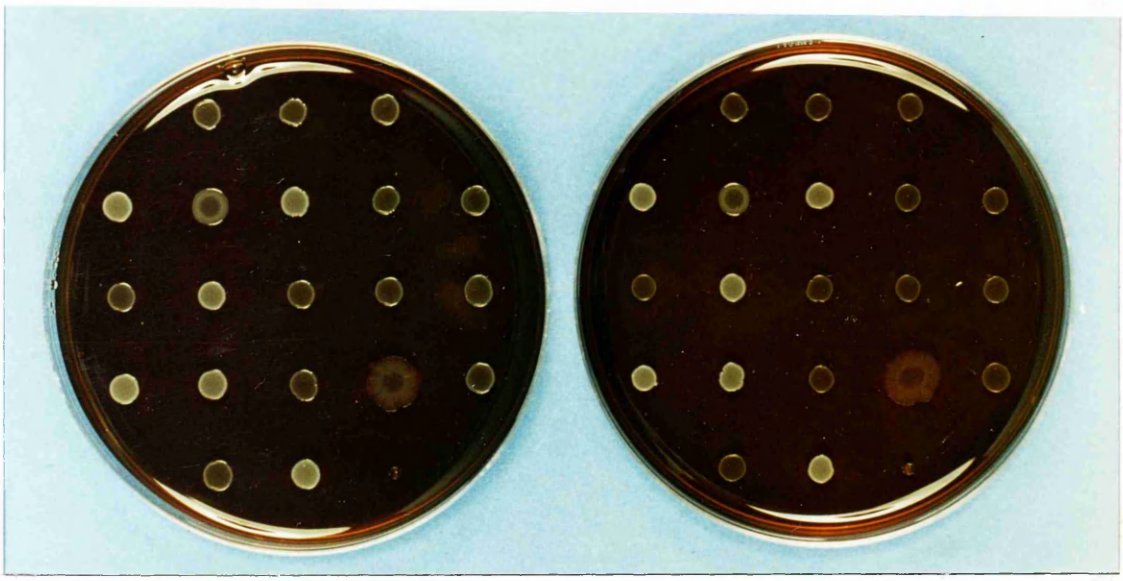


Figure 5.2: Pattern of inoculation of nine test strains (1 to 9) and two control organisms, C. perfringens (CP) and B. fragilis (BF). A reference mark is placed in bottom right area of the plate.

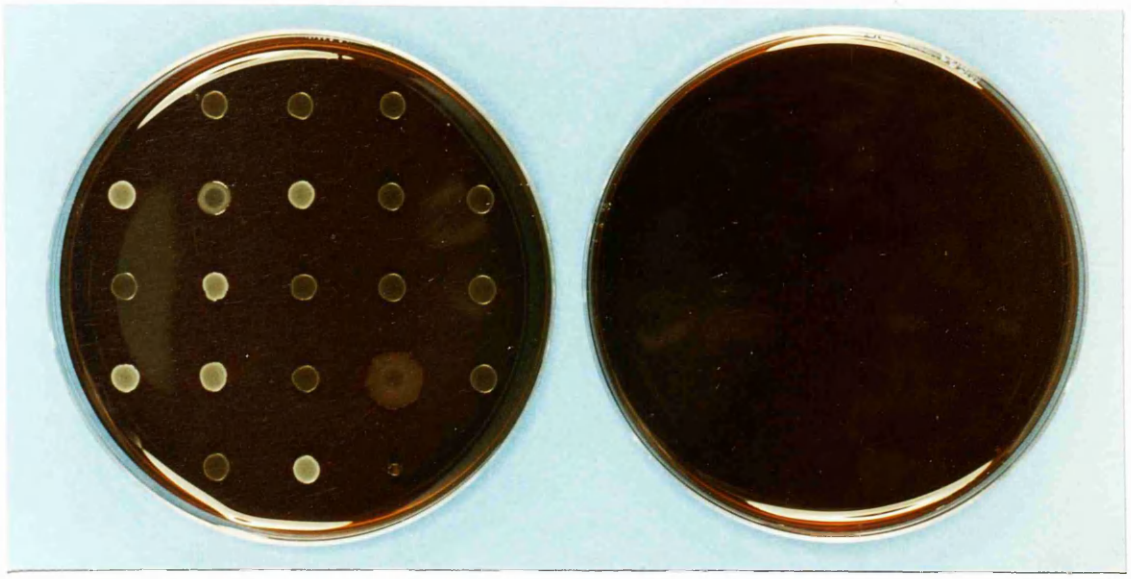


0•25 0•15



0•06 0•03

Figure 5.3: Antibiotic sensitivity plates (modified Wilkins-Chalgren agar), containing doubling-dilutions (0•03 μg/ml to 2 μg/ml) of erythromycin, inoculated with 9 strains of Strep. milleri and 2 control organisms.



Control 2•0

1•0 0•5

Figure 5.3: (Cont.) All test organisms were inhibited by 0•5 μg/ml and the control strains by 2 μg/ml after 4 days anaerobic incubation at 37°C.

5.2.8 Broth medium

Wilkins-Chalgren broth (Gibco Europe, Paisley) supplemented with heat inactivated horse serum (Gibco Europe, Paisley) was used to prepare broth with the antibiotic concentrations shown in Table 5.3. A 2 ml volume of each antibiotic concentration was placed in separate 5 ml sterile bijous (Sterilin Ltd., Teddington) using a fresh sterile pipette.

5.2.9 Inoculation and incubation of broths

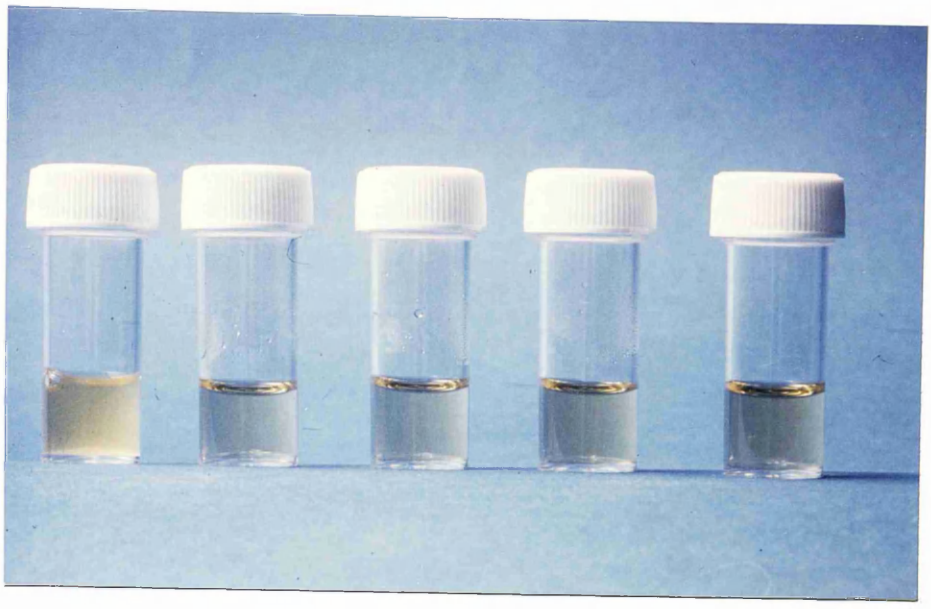
Using a sterile pipette each bijou was inoculated with 50 μl of bacterial suspension to achieve a final concentration of approximately 5×10^5 cfu/ml. A bijou containing Wilkins-Chalgren broth (Gibco Europe, Paisley) supplemented with heat inactivated horse serum (Gibco Europe, Paisley) but without antibiotic was similarly inoculated at the end of each test to act as a control. The bijous were capped with perforated lids and incubated at 37°C for 4 days in an anaerobic chamber (Appendix I).

5.2.10 Interpretation of broths

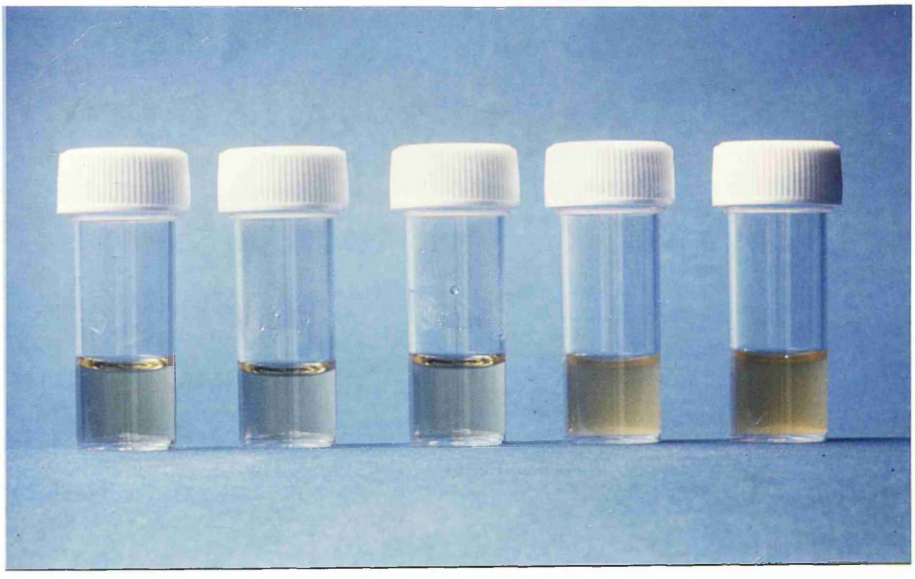
All the bijous were examined after 2 and 4 days incubation and the MIC recorded as the lowest concentration of antibiotic that prevented visible growth (Figure 5.4).

5.2.11 Determination of minimum bactericidal concentration (MBC)

On the fourth day 10 μl was removed from each broth showing no growth and inoculated onto a quarter plate of Columbia Blood Agar (Oxoid Ltd., Basingstoke) using a standardised sterile wire loop (Medical Wire and Equipment Company Ltd., Corsham). Plates were



Control 8•0 4•0 2•0 1•0



0•5 0•25 0•12 0•06 0•03

Figure 5.4: Bijous containing modified Wilkins-Chalgren broth inoculated with Strep. milleri and showing inhibition of growth at 0•12 μg/ml of clindamycin after 4 days anaerobic incubation at 37°C.

incubated at 37°C in the anaerobic chamber (Appendix I) for 4 days and the MBC recorded as the broth with lowest antibiotic concentration that gave no growth after subculture.

5.3 RESULTS

5.3.1 MIC of antibiotics for the control strains

During the period of study the MIC of each antibiotic for B. fragilis NCTC 9343 and C. perfrigens NCTC 8237 was determined on 6 occasions by each method and used as the control values. The values obtained of each antibiotic for these two strains was never more than 2 double dilutions away from the reference MIC or outside the reference MIC ranges shown in Table 5.4.

5.3.2 MIC and MBC of antibiotics for test bacterial species

The MIC values obtained by the agar dilution method and the MIC/MBC values obtained by the broth dilution method for each antibiotic are presented in Tables 5.5 to 5.10.

Penicillin (Table 5.5)

Penicillin inhibited the growth of the majority of the test strains at a concentration of 1 μg/ml but was ineffective against 1 strain of Strep. milleri, 2 strains of peptococci, 1 strain of B. melaninogenicus and 1 strain of B. distasonis at this level. Growth of 14 of the 15 strains of Strep. milleri was inhibited by 0·12 μg/ml but the MIC for Strep. milleri 021 was 2 μg/ml. Although all the peptococci were inhibited by a concentration of 0·5 μg/ml by the agar method 2 μg/ml was required to prevent growth of 2 strains by

Control strain	Antibiotic	Reference MIC (μg/ml)	Number of agar tests, Same as reference	Number of agar tests, In range or ± 2 double dilutions from reference	Number of broth tests, Same as reference	Number of broth tests, In range or ± 2 double dilutions from reference
B. fragilis NCTC 9343	Amoxycillin+	16	6	0	4	2
	Ampicillin+	16	5	1	4	2
	Clindamycin*	1	0	6	0	6
	Erythromycin+	1-4		6		6
	Metronidazole++	0·2-3·1		4**		4**
	Penicillin G*	32	0	6	0	6
C. perfringens NCTC 13124	Amoxycillin+	0·25	0	6	0	6
	Ampicillin+	0·25	5	1	4	2
	Clindamycin*	0·06	0	6	0	6
	Erythromycin+	2	2	4	5	1
	Metronidazole++	0·2-1·6		4**		4**
	Penicillin*	0·12	5	1	4	2

Reference source: * Sutter et al., 1979; + Garrod, 1981; ++ Wüst, 1977.

** MIC of metronidazole determined on 4 occasions only.

Table 5.4: Accepted MIC values and distribution of values obtained during MIC determination (6 tests) for 46 bacterial strains isolated from acute dentoalveolar abscesses.

Bacteria	No. of strains tested		Cumulative percentages susceptible to concentration (ug/ml)										
			0.03	0.06	0.12	0.25	0.50	1.00	2.00	4.00	8.00	16.0	>16.0
Strep. milleri	15	MIC (a)	27	93	..	..	..	..	100				
		MIC (b)	..	60	93	..	..	..	100				
		MBC	..	46	93	..	..	..	..	100			
Peptococcus spp.	12	MIC (a)	8	33	42	66	100						
		MIC (b)	..	..	17	42	83	92	100				
		MBC	..	..	8	42	..	..	100				
Peptostreptococcus spp.	3	MIC (a)	66	100									
		MIC (b)	..	33	..	100							
		MBC	..	..	33	66	100						
B. intermedius	3	MIC (a)	33	66	..	..	100						
		MIC (b)	..	33	..	100							
		MBC	..	..	..	33	100						
B. melaninogenicus	3	MIC (a)	..	..	..	33	66	..	..	..	100		
		MIC (b)	..	33	66	..	..	..	..	..	..	..	100
		MBC	..	33	..	66	..	..	..	..	..	..	100
B. oralis	3	MIC (a)	..	..	33	66	100						
		MIC (b)	..	33	..	..	66	100					
		MBC	..	33	..	..	66	100					
B. gingivalis	2	MIC (a)	50	..	..	100							
		MIC (b)	..	100									
		MBC	..	50	100								
B. ruminicola	2	MIC (a)	50	100									
		MIC (b)	..	100									
		MBC	..	100									
B. distasonis	1	MIC (a)	..	..	..	..	..	..	..	..	100		
		MIC (b)	..	..	..	..	..	..	..	..	100		
		MBC	..	..	..	..	..	..	..	..	100		
F. nucleatum	2	MIC (a)	..	100									
		MIC (b)	..	50	100								
		MBC	..	..	..	..	..	..	100				

Table 5.5: Activity of penicillin G against 46 bacterial strains using agar (a) and broth (b) dilution methods.

the broth method. Using the agar dilution method B. melaninogenicus 117 was inhibited by 8 μg/ml penicillin but when the broth method was employed this strain was resistant to the highest concentration of penicillin tested (16 μg/ml). Therefore for this strain the MIC and MBC were recorded as greater than 16 μg/ml using the broth technique. Only 2 other strains, one each of Strep. milleri and B. distasonis, were not killed by 2 μg/ml of penicillin. The MBC for these bacteria was 4 μg/ml and 8 μg/ml of penicillin respectively.

Ampicillin (Table 5.6) and Amoxycillin (Table 5.7)

The activity of ampicillin and amoxycillin was very similar and a concentration of 0·5 μg/ml inhibited the growth of the majority of isolates. Only 2 strains, B. distasonis 020 and B. melaninogenicus 117, were not inhibited by a concentration of 2 μg/ml of either antibiotic. The MIC of ampicillin and amoxycillin was 4 μg/ml for B. distasonis 020 and was either 4 μg/ml or greater than 16 μg/ml for B. melaninogenicus 117 depending on the test method used.

The MBC of ampicillin for B. distasonis 020 was 8 μg/ml and for amoxycillin was 4 μg/ml but B. melaninogenicus 117 was not killed by the highest concentration tested (16 μg/ml) of either antibiotic.

Erythromycin (Table 5.8)

A level of 1 μg/ml erythromycin inhibited the growth of the test bacteria other than 2 strains of Peptococcus species, 1 strain of B. oralis, 1 strain of B. ruminicola and 1 strain of F. nucleatum. These bacteria were inhibited and killed at levels of between 2 μg/ml and 4 μg/ml.

Bacteria	No. of strains tested		Cumulative percentages susceptible to concentration (ug/ml)										
			0.03	0.06	0.12	0.25	0.50	1.00	2.00	4.00	8.00	16.0	>16.0
Strep. milleri	15	MIC (a)	..	27	33	100							
		MIC (b)	..	..	53	100							
		MBC (b)	..	..	20	80	100						
Peptococcus spp.	12	MIC (a)	8	..	..	58	83	100					
		MIC (b)	..	..	33	66	75	83	100				
		MBC (b)	..	..	17	..	58	69	100				
Peptostreptococcus spp.	3	MIC (a)	..	33	100								
		MIC (b)	..	..	33	66	..	100					
		MBC (b)	..	..	33	66	..	..	100				
B. intermedius	3	MIC (a)	..	66	..	..	100						
		MIC (b)	..	66	..	100							
		MBC (b)	..	33	..	..	..	..	100				
B. melaninogenicus	3	MIC (a)	..	33	..	66	..	..	..	..	100		
		MIC (b)	..	33	66	..	..	..	..	..	..	..	100
		MBC (b)	..	..	66	..	..	..	..	..	..	..	100
B. oralis	3	MIC (a)	..	33	66	100							
		MIC (b)	..	33	..	66	..	100					
		MBC (b)	..	..	..	66	..	100					
B. gingivalis	2	MIC (a)	50	..	100								
		MIC (b)	..	50	100								
		MBC (b)	..	..	100								
B. ruminicola	2	MIC (a)	..	..	..	100							
		MIC (b)	..	50	..	100							
		MBC (b)	..	50	..	100							
B. distasonis	1	MIC (a)	..	..	..	..	..	..	..	100			
		MIC (b)	..	..	..	..	..	..	..	100			
		MBC (b)	..	..	..	..	..	..	..	..	100		
F. nucleatum	2	MIC (a)	..	..	50	100							
		MIC (b)	..	50	100								
		MBC	..	..	..	100							

Table 5.6: Activity of ampicillin against 46 bacterial strains using agar (a) and broth (b) dilution methods.

Bacteria	No. of strains tested		Cumulative percentages susceptible to concentration (ug/ml)										
			0.03	0.06	0.12	0.25	0.50	1.00	2.00	4.00	8.00	16.0	>16.0
Strep. milleri	15	MIC (a)	..	33	80	100							
		MIC (b)	..	..	87	100							
		MBC	..	..	47	87	100						
Peptococcus spp.	12	MIC (a)	..	25	42	75	100						
		MIC (b)	..	8	33	..	58	83	100				
		MBC	..	..	17	34	..	50	100				
Peptostreptococcus spp.	3	MIC (a)	..	66	100								
		MIC (b)	..	..	33	66	100						
		MBC	..	..	33	..	66	100					
B. intermedius	3	MIC (a)	..	50	..	..	100						
		MIC (b)	..	33	..	..	..	100					
		MBC	..	..	..	33	..	..	100				
B. melaninogenicus	3	MIC (a)	..	..	..	33	66	..	..	100			
		MIC (b)	..	..	33	66	..	..	..	..	100		
		MBC	..	..	33	66	..	..	..	..	..	..	100
B. oralis	3	MIC (a)	..	33	..	100							
		MIC (b)	..	33	66	100							
		MBC (b)	..	33	66	100							
B. gingivalis	2	MIC (a)	50	100									
		MIC (b)	..	..	100								
		MBC (b)	..	..	100								
B. ruminicola	2	MIC (a)	..	100									
		MIC (b)	..	100									
		MBC (b)	..	100									
B. distasonis	1	MIC (a)	..	..	..	..	..	..	..	100			
		MIC (b)	..	..	..	..	..	..	..	100			
		MBC (b)	..	..	..	..	..	..	..	100			
F. nucleatum	2	MIC (a)	..	100									
		MIC (b)	..	50	100								
		MBC (b)	..	..	100								

Table 5.7: Activity of amoxycillin against 46 bacterial strains using agar (a) and broth (b) dilution methods.

Bacteria	No. of strains tested		Cumulative percentages susceptible to concentration (ug/ml)										
			0.03	0.06	0.12	0.25	0.50	1.00	2.00	4.00	8.00	16.0	>16.0
Strep. milleri	15	MIC (a)	..	60	87	93	100						
		MIC (b)	..	13	47	100							
		MBC (b)	..	..	33	47	80	100					
Peptococcus spp.	12	MIC (a)	..	17	..	..	50	92	100				
		MIC (b)	8	..	..	..	33	85	100				
		MBC (b)	..	8	..	..	17	42	83	100			
Peptostreptococcus spp.	3	MIC (a)	..	100									
		MIC (b)	50	100									
		MBC (b)	..	66	100								
B. intermedius	3	MIC (a)	..	..	33	66	100						
		MIC (b)	..	..	..	66	100						
		MBC (b)	..	..	..	..	33	66	100				
B. melaninogenicus	3	MIC (a)	..	33	..	66	..	100					
		MIC (b)	..	66	..	100							
		MBC (b)	..	33	..	66	100						
B. oralis	3	MIC (a)	..	..	33	..	..	66	100				
		MIC (b)	..	..	33	..	..	100					
		MBC (b)	..	..	..	33	..	66	100				
B. gingivalis	2	MIC (a)	..	..	..	100							
		MIC (b)	..	50	100								
		MBC (b)	..	..	..	100							
B. ruminicola	2	MIC (a)	..	..	..	..	50	..	100				
		MIC (b)	..	..	..	..	..	..	100				
		MBC (b)	..	..	..	..	..	..	100				
B. distasonis	1	MIC (a)	..	..	..	100							
		MIC (b)	..	..	..	100							
		MBC (b)	..	..	..	..	..	100					
F. nucleatum	2	MIC (a)	..	..	..	..	50	..	..	100			
		MIC (b)	..	..	..	..	50	..	..	100			
		MBC (b)	..	..	..	..	..	..	50	100			

Table 5.8: Activity of erythromycin against 46 bacterial strains using agar (a) and broth (b) dilution methods.

Clindamycin (Table 5.9)

The majority of bacterial strains tested were sensitive to clindamycin since Peptococcus 097 and Peptostreptococcus 080 were the only strains not killed by a concentration of 0·5 μg/ml. The MBC of clindamycin for these two anaerobic gram-positive cocci was 1 μg/ml.

Metronidazole (Table 5.10)

The MIC and MBC of metronidazole was only determined for strictly anaerobic bacteria. Only 2 strict anaerobes, Peptococcus species 081 and 097, were not inhibited by 1 μg/ml of metronidazole. Although these strains had an MIC of 0·25 μg/ml by the agar dilution method their MIC and MBC by the broth test was 4 μg/ml. The MBC for F. nucleatum 032 and B. ruminicola 019 was 2 μg/ml and these strains were the only other bacteria not killed by 1 μg/ml of metronidazole.

5.3.3 A comparison of MIC results obtained by broth dilution and agar dilution methods

In general the MIC results obtained by the two methods of testing were in agreement. The broth dilution method however tended to give a higher MIC value than the agar dilution method but only on 3 occasions was the difference more than 2 double dilutions.

5.3.4 A comparison of MIC and MBC results by the broth dilution method

For the majority of strains the MBC was either the same or 1 double dilution higher than the MIC (Table 5.11). The largest difference between MIC and MBC was 4 double dilutions. This occured twice and involved penicillin for 2 strains of F. nucleatum.

Bacteria	No. of strains tested		Cumulative percentages susceptible to concentration (ug/ml)									
			0.03	0.06	0.12	0.25	0.50	1.00	2.00	4.00	8.00	16.0	>16.0
Strep. milleri	15	MIC (a)	..	40	100								
		MIC (b)	6	46	87	100							
		MBC	..	33	46	..	100						
Peptococcus spp.	12	MIC (a)	50	75	92	..	100						
		MIC (b)	33	66	83	92	100						
		MBC	33	..	66	..	..	100					
Peptostreptococcus spp.	3	MIC (a)	..	33	66	100							
		MIC (b)	33	..	66	..	100						
		MBC	..	..	66	..	..	100					
B. intermedius	3	MIC (a)	66	..	..	100							
		MIC (b)	66	..	..	100							
		MBC	..	66	..	100							
B. melaninogenicus	3	MIC (a)	..	..	66	100							
		MIC (b)	..	..	33	100							
		MBC	..	..	..	100							
B. oralis	3	MIC (a)	66	..	100								
		MIC (b)	33	66	100								
		MBC	..	66	100								
B. gingivalis	2	MIC (a)	100										
		MIC (b)	50	100									
		MBC	50	..	100								
B. ruminicola	2	MIC (a)	..	..	50	100							
		MIC (b)	..	..	50	100							
		MBC	..	..	..	100							
B. distasonis	1	MIC (a)	100										
		MIC (b)	100										
		MBC	100										
F. nucleatum	2	MIC (a)	..	..	100								
		MIC (b)	..	..	100								
		MBC (b)	..	..	100								

Table 5.9: Activity of clindamycin against 46 bacterial strains using agar (a) and broth (b) dilution methods.

Bacteria	No. of strains tested		Cumulative percentages susceptible to concentration (ug/ml)									
			0.03	0.06	0.12	0.25	0.50	1.00	2.00	4.00	8.00	16.0	>16.0
Peptococcus spp.	12	MIC (a)	..	..	33	100							
		MIC (b)	..	33	42	..	75	83	..	100			
		MBC	..	..	..	50	..	83	..	100			
Peptostreptococcus spp.	3	MIC (a)	..	..	66	100							
		MIC (b)	..	33	..	..	66	100					
		MBC	..	..	..	33	66	100					
B. intermedius	3	MIC (a)	..	..	66	100							
		MIC (b)	..	..	33	66	100						
		MBC	..	..	..	..	100						
B. melaninogenicus	3	MIC (a)	..	..	33	66	100						
		MIC (b)	33	..	..	66	100						
		MBC	..	..	..	..	100						
B. oralis	3	MIC (a)	..	..	33	100							
		MIC (b)	..	33	66	..	100						
		MBC	..	..	66	..	100						
B. gingivalis	2	MIC (a)	..	..	..	100							
		MIC (b)	50	..	..	100							
		MBC	..	..	50	100							
B. ruminicola	2	MIC (a)	..	..	100								
		MIC (b)	..	..	..	..	50	100					
		MBC	..	..	..	..	..	50	100				
B. distasonis	1	MIC (a)	..	100									
		MIC (b)	..	..	100								
		MBC	..	..	100								
F. nucleatum	2	MIC (a)	..	..	100								
		MIC (b)	..	..	..	50	100						
		MBC	..	..	..	..	50	..	100				

Table 5.10: Activity of metronidazole against 31 bacterial strains using agar (a) and broth (b) dilution methods.

	Number of doubling dilutions that MBC was higher than MIC.				
Antibiotic	**0**	**1**	**2**	**3**	**4**
Penicillin	31	12	1	0	2
Amoxycillin	21	20	4	1	0
Ampicillin	25	17	4	0	0
Erythromycin	15	21	7	3	0
Clindamycin	22	15	9	0	0
Metronidazole*	14	11	5	1	0

* Only 31 strains tested against metronidazole.

Table 5.11: Relationship between MBC and MIC of 6 antibiotics for 46 bacterial strains isolated from acute dento-alveolar abscesses.

5.3.5 A comparison between MIC and results of secondary antibiotic sensitivity tests

The serum concentration of each antibiotic obtained following the recommended oral dosage is shown in Table 5.12 (Wise, 1978). In this comparison a bacterial strain with an MIC greater than the normally expected serum concentration was regarded as demonstating resistance.

"Sensitive strains"

All the strains tested in the present study which were classified as sensitive according to Stokes method (Stokes and Waterworth, 1972) in Chapter 4 were also recorded as sensitive by the MIC determination reported in this chapter.

"Moderately sensitive strains"

The MIC of the antibiotics for the bacterial strains tested which were classified as moderately sensitive to at least one antibiotic by Stokes method (Stokes and Waterworth, 1972) in Chapter 4 are shown in Table 5.13.

Penicillin

A level of 0•12 μg/ml of penicillin inhibited the growth of all but 1 of the 13 strains classified as moderately sensitive to this antibiotic. Since the expected serum level following a routine oral dose of penicillin is 1 μg/ml to 3 μg/ml it follows that the 12 of these strains can be regarded as sensitive. However a concentration of 2 μg/ml was required to inhibit Peptococcus species 097 which suggests that this strain had a reduced sensitivity to

Antibiotic	Concentration (μg/ml)
Penicillin	1-3
Ampicillin	2-3
Amoxycillin	4-6
Erythromycin	1-2
Clindamycin	2-4
Metronidazole	4

Table 5.12: Expected serum concentrations of 6 antibiotics achieved in healthy adults following recommended oral doses (Wise, 1978).

Table 5.13: MIC values for the bacterial strains that were classified in Chapter 4 as moderately sensitive to at least one antibiotic.

		Agar MIC (μg/ml)	Broth MIC (μg/ml)
Penicillin			
Strep. milleri	034	0·06	0·06
	038	0·06	0·06
	046	0·06	0·12
	047	0·06	0·06
	050	0·06	0·06
	071	0·06	0·06
	111	0·03	0·12
	113	0·03	0·12
	121	0·03	0·12
Peptococcus spp.	097	0·5	2·0
B. oralis	096	0·12	0·06
B. ruminicola	019	0·06	0·06
Amoxycillin			
Strep. milleri	021	0·06	0·12
	036	0·12	0·12
	038	0·12	0·12
	046	0·25	0·25
	047	0·25	0·12
	111	0·06	0·12
	113	0·06	0·12
	121	0·06	0·12
B. melaninogenicus	117	4·0	8·0

		Agar MIC (μg/ml)	Broth MIC (μg/ml)
Ampicillin			
Strep. milleri	036	0•25	0•12
	038	0•25	0•25
	046	0•25	0•25
	047	0•25	0•12
	111	0•06	0•12
	113	0•06	0•12
	121	0•06	0•12
Peptococcus spp.	097	0•25	2•0
F. nucleatum	032	0•25	0•06
Erythromycin			
Strep. milleri	046	0•12	0•25
	111	0•06	0•06
	121	0•06	0•25
Peptococcus spp.	098	0•06	0•5
B. oralis	083	2•0	2•0
F. nucleatum	032	4•0	4•0
Clindamycin			
B. intermedius	023	0•03	0•03
B. ruminicola	019	0•25	0•25

Table 5.13: (Contd)

penicillin.

Amoxycillin

Growth of the 8 strains of Strep. milleri classified as moderately sensitive was inhibited by 0·25 μg/ml amoxycillin and this would confirm that they were sensitive to this antibiotic. Although B. melaninogenicus 117 had been classified as moderately sensitive to amoxycillin the agar dilution test gave an MIC of 4 μg/ml and the broth dilution test an MIC of 8 μg/ml. These figures would indicate that this strain was resistant to amoxycillin since the expected serum level is between 4 μg/ml and 6 μg/ml.

Ampicillin

The MIC of ampicillin was determined for 9 strains that had been recorded as moderately sensitive to this antibiotic in Chapter 4. Growth of F. nucleatum 032 and 7 strains of Strep. milleri was inhibited by 0·25 μg/ml ampicillin which would indicate they were sensitive to this antibiotic. The MIC for Peptococcus species 097 was 2 μg/ml a level which would suggest this strain had reduced sensitivity to ampicillin since the expected serum level is between 2 μg/ml and 3 μg/ml.

Erythromycin

The MIC of erythromycin for B. oralis 083 and F. nucleatum 032, both classified as moderately sensitive in Chapter 4, was 2 μg/ml and 4 μg/ml respectively. Since the expected serum level is between 1 μg/ml and 2 μg/ml it is likely that both these strains were resistant to erythromycin. A concentration of 1, μg/ml of erythromycin

was required to inhibit the growth of Peptococcus species 098 and 3 strains of Strep. milleri therefore these strains should probably be regarded as moderately sensitivite to this antibiotic.

Clindamycin

The MIC of clindamycin for B. intermedius 023 was 0•03 μg/ml and for B. ruminicola 019 was 0•25 μg/ml. Although both these strains were classified as moderately sensitive to clindamycin in Chapter 4 their MIC values would indicate that they were sensitive to this antibiotic since the expected serum level is between 2 μg/ml and 4 μg/ml.

"Resistant strains"

The MIC of the antibiotics for the bacterial strains tested which were classified as resistant by Stokes method (Stokes and Waterworth, 1972) in Chapter 4 are shown in Table 5.14

Penicillin

Growth of 3 of the 5 strains recorded as resistant to penicillin in Chapter 4 was not inhibited by 2 μg/ml which supports resistance to this antibiotic. The MIC of penicillin for Strep. milleri 001 and F. nucleatum 032 was 0•12 μg/ml which is a concentration that suggests that these strains were sensitive to this antibiotic rather than resistant.

	Strain code	Agar MIC (μg/ml)	Broth MIC (μg/ml)
Penicillin			
Strep. milleri	001	0•12	0•12
Strep. milleri	021	2•0	2•0
B. melaninogenicus	117	8•0	>16•0
B. distasonis	020	8•0	8•0
F. nucleatum	032	0•06	0•12
Amoxycillin			
B. distasonis	020	4•0	4•0
Ampicillin			
Strep. milleri	021	0•25	0•25
B. melaninogenicus	117	8•0	>16•0
B. distasonis	020	4•0	4•0
Erythromycin			
Strep. milleri	0•1	0•5	0•25

Table 5.14: MIC values for the 5 bacterial strains that were classified in Chapter 4 as resistant to at least one antibiotic.

Amoxycillin

The MIC of amoxycillin for B. distasonis 020, the only organism recorded as resistant to this antibiotic in Chapter 4, was 4 μg/ml. This finding would confirm the reduced sensitivity obtained by Stokes method (Stokes and Waterworth, 1972).

Ampicillin

The MIC of ampicillin for B. melaninogenicus 117 and for B. distasonis 020 was greater than 4 μg/ml which would support their resistant classification. Growth of Strep. milleri 021 was inhibited at a level of 0•25 μg/ml and this suggests that it should be regarded as sensitive rather than resistant.

Erythromycin

Only 1 bacterial strain, Strep. milleri 021, was recorded as resistant to erythromycin in Chapter 4 and growth of this organism was inhibited by 0•5 μg/ml by agar dilution and 0•25 μg/ml by broth dilution. These MIC values suggest that Strep. milleri 021 was sensitive to erythromycin rather than resistant.

In summary this comparison of MIC value and classification of antibiotic sensitivity according to Stokes method (Stokes and Waterworth, 1972) has revealed that;

(i) Growth of all the bacterial strains which were had been recorded as sensitive by Stokes method (Stokes and Waterworth, 1972) was inhibited by a concentration below that expected in serum following routine doses. This finding would therefore support their

classification as sensitive.

(ii) All but one of the strains which were recorded as moderately sensitive by Stokes method (Stokes and Waterworth, 1972) had a MIC below the expected serum level which would indicate that the majority of these strains were fully sensitive. The single strain which had an MIC above these levels should be regarded as resistant.

(iii) The MIC for 6 of the 10 antibiotic - bacterial strain combinations which were classified as resistant by Stokes method (Stokes and Waterworth, 1972) was above the expected serum levels which would confirm resistance. The MIC for the remaining 4 combinations was below the expected serum levels and this indictes that the strains involved should be considered as sensitive.

5.4 DISCUSSION

5.4.1 Methodology

The different methods available for determination of MIC were outlined by Brown and Blowers (1978) and are based either on antibiotic diffusion through agar or antibiotic dilution in agar or broth.

In agar diffusion tests the zone of inhibition around an antibiotic disc, placed on standardised agar medium, is measured and the MIC calculated from standard regression lines which relate zone size to MIC. In North America the Kirby-Bauer method (Bauer et al., 1966) is the accepted agar diffusion test for aerobes and facultative bacteria whereas in Sweden the ICS method (Ericsson and Sherris, 1971)

is used. Since both these tests depend on facultative organisms as the reference standards they should not be employed in testing strict anaerobes (Lorian, 1980). Although alternative methods of testing strict anaerobes by an agar diffusion have been preposed (Kwok et al., 1975) tests based on dilution methods are the type now most widely used.

In dilution methods a suspension of the test organism is inoculated onto a solid medium or into a liquid medium containing a known concentration of antibiotic. Agar dilution methods have the advantage of allowing a large number of bacterial strains to be tested at one time but do not permit determination of MBC. In contrast broth dilution methods allow the determination of MBC but involve a great deal of laboratory time if large numbers of strains are to be tested. In the present study it was decided to use both a broth dilution method and an agar dilution method since this would enable a comparison to be made between the MIC values obtained by each type of test.

It is essential to standardise the inoculum used during MIC determination and in the majority of studies this has been achieved by comparing the optical density of the inoculum with that of a freshly prepared barium sulphate solution (Lennette, 1980). The optical density of this preparation is often referred to as a 0•5 McFarland standard and is generally assumed to contain approximately 10^8 organisms per ml. In the present study it was felt that a particular McFarland standard would not indicate a constant viable count due to

the wide variety of bacteria to be tested. Therefore cultures of each test organism were adjusted to a concentration of approximately 2×10^7 cfu/ml using spectrophotometry and growth curves (Appendix II). A concentration of 2×10^7 cfu/ml was chosen since it produced the inoculum (10^4 cfu in a 5 to 8 mm diameter spot) recommended by Ericsson and Sherris (1971) when using the multipoint inoculator for the agar test. In addition by adding 50 μl of this suspension to 2 ml of broth the bacterial concentration (10^5 to 10^6 cfu/ml) recommended by Ericsson and Sherris (1971) in the broth test was conveniently achieved.

The growth medium used during MIC and MBC determination must be able to provide adequate growth of all the bacteria tested. Mueller-Hinton agar has been used for susceptibility testing of facultative bacteria for many years but will not support the growth of the majority of strict anaerobes (Sutter et al., 1973). Brucella agar supplemented with 5 per cent sheep blood was suggested as a standard test medium for anaerobes but Wilkins and Chalgren (1976) were concerned about the possible variations that the inclusion of blood may introduce. A new medium, Wilkins-Chalgren agar, was therefore proposed and in North America it has become the recommended agar for determining the antibiotic susceptibility of strictly anaerobic bacteria (Sutter et al., 1980).

Walker et al., (1979) developed a new agar, medium V, for the susceptibility testing of anaerobic bacteria isolated from periodontal disease. Medium V was claimed to produce similar results to Wilkins-Chalgren agar but had the advantage of supporting the

growth of strains of Actinobacillus actinomycetemcommitans, anaerobic vibrios, corroding bacteroides and campylobacter which had been found to grow poorly on Wilkins-Chalgren agar. These bacterial species were not encountered in the abscesses studied in Chapter 2 and therefore it was decided to use Wilkins-Chalgren agar and broth, as recommended by Sutter et al., (1980).

During the present study however some strains of peptostreptococci, peptococci and B. gingivalis were found to grow poorly both on Wilkins-Chalgren agar and in the equivalent broth. It was found that growth of these species was greatly improved by supplementing the agar with lysed horse blood and the broth with heat inactivated horse serum. The need to add blood to Wilkins-Chalgren medium to support the growth of strains of anaerobic gram-positive cocci and bacteroides was also reported Murray and Niles (1983) and Citron (1983). This practice would appear to be permissible as long as the MIC of the control organisms remains within an accepted range.

The majority of studies determining the MIC of bacteria isolated from the oral cavity have read tests after 2 days incubation (Allard et al., 1978; von Konow et al., 1981; Sutter et al., 1983). However in the present study some test organisms, even after the addition of blood to the media, required 4 days of incubation to obtain good growth on control plates and in control broths. Similar findings were reported by Baker et al., (1983) who therefore recommended that incubation should be continued for 4 days, with results being read after 2 and 4 days. A 4 day incubation period was

therefore used in the present study.

5.4.2 Experimental findings

Bacterial strains isolated from acute dental abscess

Allard et al., (1978) used an agar dilution test to determine MIC values of nine antibiotics to 96 strains of facultative alpha-haemolytic streptococci and 81 strict anaerobes isolated from acute oral infections. The facultative streptococci, which included 12 strains of Strep. milleri, were all found to be susceptible to penicillin (MIC range 0•004 μg/ml to 1•0 μg/ml) and erythromycin (MIC range 0•016 μg/ml to 0•5 μg/ml). The majority of facultative streptococci were also sensitive to clindamycin although a concentration of between 4 μg/ml and 16 μg/ml was required to inhibit the growth of 10 of the strains investigated. Apart from a relatively high incidence of resistance to clindamycin these results are similar to those found for Strep. milleri in the present study. With regard to strict anaerobes, Allard et al., (1978) reported that the only isolates not inhibited by low levels of penicillin were 3 strains of B. fragilis, for which the MIC was between 8 μg/ml and 16 μg/ml. The MIC of erythromycin for approximately 20 per cent of the anaerobes was 1 μg/ml or greater and a concentration of 1 μg/ml of clindamycin inhibited the growth 97 per cent of the strains of strict anaerobes. Apart from the relatively large percentage of isolates that were resistant to erythromycin these results are in agreement with those of the present study. It is difficult to discuss the reasons for these differences since the identity of strictly anaerobic strains was not provided by Allard et al., (1978).

Von Konow and Nord (1983) used the agar dilution method described by Ericsson and Sherris (1971) to determine the MIC of penicillin and ornidazole for 20 facultative strains and 157 strict anaerobes isolated from acute oral infections. Results for individual strains were not given but strains which were not inhibited by 4 μg/ml of the antibiotics tested were discussed. Growth of all the facultative isolates, which included 16 strains of Strep. milleri, was inhibited by 0•125 μg/ml of penicillin but a number of anaerobic strains were found to be resistant to 4 μg/ml penicillin. The identity of the resistant organisms being; 2 strains of B. oralis, 2 strains of B. pneumonistes, 2 strains of Veillonella parvula, and 2 strains Eubacterium lentum. The 15 strains of B. melaninogenicus and 2 strains of B. distasonis tested were inhibited by 4 μg/ml. These results are similar to those of the present study since it was found that the growth of all but 1 of the 15 strains of Strep. milleri tested was inhibited by 0•125 μg/ml penicillin and only two isolates, B. melaninogenicus 117 and B. distasonis 020, were not inhibited by 4 μg/ml of penicillin.

Heimdahl et al., (1985) used the agar dilution method described by Ericsson and Sherris (1971) to determine the MIC of penicillin, erythromycin, doxycycline, clindamycin and tinidazole for 22 facultative bacteria and 174 strict anaerobes isolated from acute oral infections. Like the present study Heimdahl et al., (1985) found that facultative bacteria were sensitive to antibiotics other than a nitroimidazole such as tinidazole or metronidazole. All 22 the facultative strains, which included 18 strains of Strep. milleri, were inhibited by 4 μg/ml of each antibiotic. However although all 15

strains of B. melaninogenicus and the single strain of B. distasonis tested were inhibited by 4 μg/ml penicillin, 2 strains of Veillonella parvula, 2 strains of B. oralis, 1 strain of B. pneumosintes and 1 strain of E. lentum were not. In the present study all the test organisms apart from two strict anaerobes, (one strain each of B. melaninogenicus and B. distasonis), were sensitive to 4 μg/ml penicillin, erythromycin or clindamycin. Therefore it would appear that resistance to these 3 antibiotics is a rare phenomenon for bacterial species isolated from acute dentoalveolar abscess.

This comparison has revealed that the MIC values obtained in the present study appear to be similar to those reported in other studies of isolates obtained from acute dental abscess. Growth of the majority of bacteria cultured from these lesions is inhibited by concentrations of antibiotics that are achieved in the serum following standard oral doses of the agents used to treat dental infections. The minor differences in the reported sensitivity patterns are probably due to expected variations in the identity of individual strains encountered.

There is little information available for bacteria isolated from acute dentoalveolar abscessses however useful information may be gained by performing a comparison of the results of the present study with the MIC values reported for organisms isolated from periodontal disease since the same bacterial species are frequently encountered in both infections.

Bacterial strains isolated from periodontal disease

Newman et al., (1979) used broth and disc diffusion tests to determine the MIC of 7 different antibiotics (ampicillin, chloramphenicol, clindamycin, erythromycin, metronidazole, penicillin, and tetracycline) for 38 bacterial strains isolated from a variety of periodontal diseases. The results included MIC values of each antibiotic for 2 strains of B. melaninogenicus and 4 strains of F. nucleatum. The values obtained for B. melaninogenicus were 0·08 μg/ml ampicillin, 0·31 μg/ml clindamycin, 0·06 μg/ml erythromycin, 0·10 μg/ml metronidazole and 0·09 μg/ml penicillin. The values for F. nucleatum were 0·04 μg/ml ampicillin, 0·01 μg/ml clindamycin, 1·98 μg/ml erythromycin, 0·04 μg/ml metronidazole and 0·02 μg/ml penicillin. Because of the small number of strains involved it is difficult to make close comparisons with these figures and the results of the present study. However the observation that the MIC values of penicillin and ampicillin obtained for B. melaninogenicus 117 were higher than those reported for the two strains of B. melaninogenicus studied by Newman et al., (1979) would support the conclusion that B. melaninogenicus 117 was representative of the occasional strain of this species that has reduced sensitivity to the penicillin group of antibiotics.

Walker et al., (1985) reported the in vitro susceptibilities of a large number (between 369 and 966) of bacterial strains isolated from periodontal diseases to a range of antibiotics. The study provided information on the inhibitory concentrations of penicillin, ampicillin, amoxycillin, erythromycin and metronidazole for strains of bacteroides, fusobacteria, peptostreptococci and facultative

streptococci. The majority of isolates were susceptible to benzylpenicillin (MIC less than 2 μg/ml), ampicillin (MIC less than 3 μg/ml) and amoxycillin (MIC less than 4 μg/ml). Growth of approximately 10 per cent of strains of bacteroides and peptostreptococci was not inhibited by 2 μg/ml penicillin or 3 μg/ml ampicillin. Although a concentration of 2 μg/ml clindamycin inhibited the growth of practically all the bacteroides, fusobacteria and gram-positive cocci a level of 8 μg/ml was required for some strains of peptostreptococci. All the facultative streptococci were resistant to metronidazole (MIC greater than 32 μg/ml). Although the majority of fusobacteria and bacteroides were sensitive to low levels of this agent, growth of 10 per cent of the strains of peptostreptococci was not inhibited by a level of 32 μg/ml. Therefore no single antibiotic emerged as being inhibitory for all organisms encountered but Walker et al., (1985) concluded that several different antibiotics had good to excellent activity against bacteria associated with periodontal disease. In general these results are similar to those found in the present study. Minor differences may be due to differences in the bacterial strains which predominate at the gingival crevice and those present in acute dentoalveolar abscesses.

It would appear therefore that the MIC values reported for strains of bacterial species isolated from periodontal disease are comparable to those obtained for similar species isolated from acute dentoalveolar abscess. Bacterial species present in both infections would appear to be sensitive to a range of antibiotics.

Bacterial strains isolated from non-oral sites

One of the reasons for the determination of the MIC of antibiotics for bacterial strains isolated from oral infections was the uncertainty whether they differed in susceptibility from strains of the same species isolated from non-oral sites (Baker et al., 1983). Therefore it would seem appropriate in this discussion to make a comparison between the findings of the present study and the MIC values reported for similar bacterial species isolated from body sites other than the mouth.

Sutter and Finegold (1976) investigated the susceptibility of 492 anaerobic bacteria, the majority of which were recent isolates from a variety of non-oral sites. The activity of 23 antibacterial agents was assessed using an agar dilution technique and the strains tested included a number of bacteroides, peptococci and peptostreptococci. In general the results reported for ampicillin, amoxycillin, and erythromycin were very similar to those recorded for the strains isolated from acute dentoalveolar abcesses in the present study. A notable difference however was a relatively high incidence (24 per cent) of resistance to metronidazole (MIC greater than 1 μg/ml) demonstrated by strains of peptostreptococci. In the present study all the strains of anaerobic gram-positive cocci tested were inhibited by a concentration of 1 μg/ml. Since only 2 strains of peptostreptococci were included in the present study it is not possible to determine if there are significant differences in sensitivity of oral and and non-oral isolates of this anaerobic gram-positive species.

Appelbaum and Chatterton (1978) used an agar dilution test to determine the susceptibility patterns of 10 antibacterial agents to 265 anaerobic bacteria isolated from a variety of clinical sites. The results revealed that the growth of all but a small number of the test strains was inhibited by 2 μg/ml of penicillin or 2 μg/ml ampicillin, a similar finding to the present study. Applebaum and Chatterton (1978) also found that the vast majority of anaerobic gram-positive cocci were susceptible to low concentrations of penicillin and only occasional strains of peptococci or peptostreptococci were resistant to 2 μg/ml of ampicillin and 1 μg/ml erythromycin. All the bacterial strains included in the study were inhibited by 4 μg/ml clindamycin, a similar finding to the present investigation.

5.5 CONCLUSIONS

(i) Growth of the majority of bacteria isolated from acute dentoalveolar abscesses is inhibited by antibiotic concentrations which are obtained in serum following routine oral doses.

(ii) Resistance to the antibiotics used to treat acute dentoalveolar abscess is only demonstrated by occasional strains of facultative gram-positive cocci, anaerobic gram-positive cocci or anaerobic gram-negative bacilli encountered in these infections.

(iii) The MIC values obtained by the agar dilution method were similar to those obtained by the broth dilution method.

(iv) The MBC of each of the antibiotics was rarely more than 2 double dilutions higher than the MIC.

(v) Determination of MIC supported the susceptibiltity classification obtained by Stokes method.

(vi) Bacterial strains isolated from acute dentoalveolar abscess have similar susceptibility patterns to strains of the same bacterial species isolated from periodontal diseases or non-oral sites.

CHAPTER 6

SHORT-COURSE HIGH-DOSE AMOXYCILLIN COMPARED TO CONVENTIONAL PENICILLIN THERAPY IN THE TREATMENT OF ACUTE DENTOALVEOLAR ABSCESS

6.1 INTRODUCTION

The primary principle in the treatment of acute dentoalveolar abscess is the establishment of drainage of pus present by extraction of the affected tooth, incision of any soft tissue swelling or by opening the pulp chamber (Zallen and Black, 1976; Piecuch, 1983). When adequate drainage cannot be established or if the patient is showing signs of poor health then antibiotics are frequently prescribed. Although a number of different antibiotics have been used in these cicumstances penicillin has long been regarded the drug of choice, with erythromycin as the alternative for patients with a known hypersensitivity to penicillin (Sims, 1974). If an antibiotic is used it is common practice for it to be prescribed for either 5 or 7 days.

Out-patient compliance with antibiotic therapy is known to be poor especially after symptoms have resolved (Greenberg, 1984; Stewart and Cluff, 1972; Bailey and Abbott, 1977). The observation that many patients do not appear to suffer as a result of poor compliance has led to the suggestion, in a leading article of the British Medical Journal, that traditional regimens for common conditions should be scrutinised (Anonymous, 1979). The concept of a "complete course" of an antibiotic may have arisen due to a general belief that short antibiotic regimens encourage the emergence of

resistant bacterial strains. However Lacey (1984) has claimed that the idea of a "complete course" of antibiotic is obsolete and that therapy beyond the time of clinical cure enhances the development of resistance rather than decreasing it.

A short course of high-dose amoxycillin has been reported to be as effective as a longer traditional regimen of this antibiotic for the treatment of otitis media (Bain et al., 1985) and urinary tract infections (Bailey and Abbott, 1977). Amoxycillin has been recommended as a suitable drug for the treatment of dental infections (Greenberg et al., 1979; Morey et al., 1984). Further more the results of the antibiotic sensitivity tests presented in Chapters 4 and 5 of this thesis demonstrate that the vast majority bacteria isolated from acute dentoalveolar abscess are sensitive to amoxycillin. In addition animal experiments have shown that amoxycillin penetrates well into sites of acute infection (Boon et al., 1982). Taken together these observations suggest that amoxycillin may well be effective in the treatment of acute dentoalveolar abscess. High-dose amoxycillin, in the form of a 3 g dispersable sachet, already has an established role in dental surgery for the prevention of infective endocarditis. If short-course high-dose amoxycillin has a place in the treatment of acute dentoalveolar abscess then a regimen involving 3 g would seem convenient because of its availabilty and familiarity to dental surgeons.

Therefore the aim of this study was to compare the performance of short-course high-dose amoxycillin in the treatment of acute dentoalveolar abscess with conventional penicillin therapy.

6.2 MATERIALS AND METHOD

6.2.1 Selection of patients

Approval for the clinical trial was sought and granted from the Dental Ethics Committee of Glasgow Dental Hospital and School. Patients presenting with an acute dentoalveolar abscess (Figure 6.1) at the Casualty Receiving Clinic were asked to participate in the trial and informed consent was obtained from all patients. Patients were excluded if they had any concurrent illness, had received antibiotics in the preceding 3 months, were pregnant or reported an allergy to penicillin.

6.2.2 Clinical details

The sex and age of each patient was noted at the initial visit and assessments made of clinical symptoms. All patients were reviewed after 24 hours (Day 2) and 72 hours (Day 4) at which time the severity of symptoms was re-assessed. Those patients with symptoms on Day 4 were seen again after a further 72 hours (Day 7). All the patients were seen by the same dental surgeon at each visit.

The following clinical assessments were made;

Pain

Severity of pain was measured at all visits using a 10 cm visual analogue as described in Section 2.2.2.

Swelling

At the time of initial presentation patients were asked if they were aware of any swelling of the tissues in the area of the affected tooth and if present to state its duration. At review visits

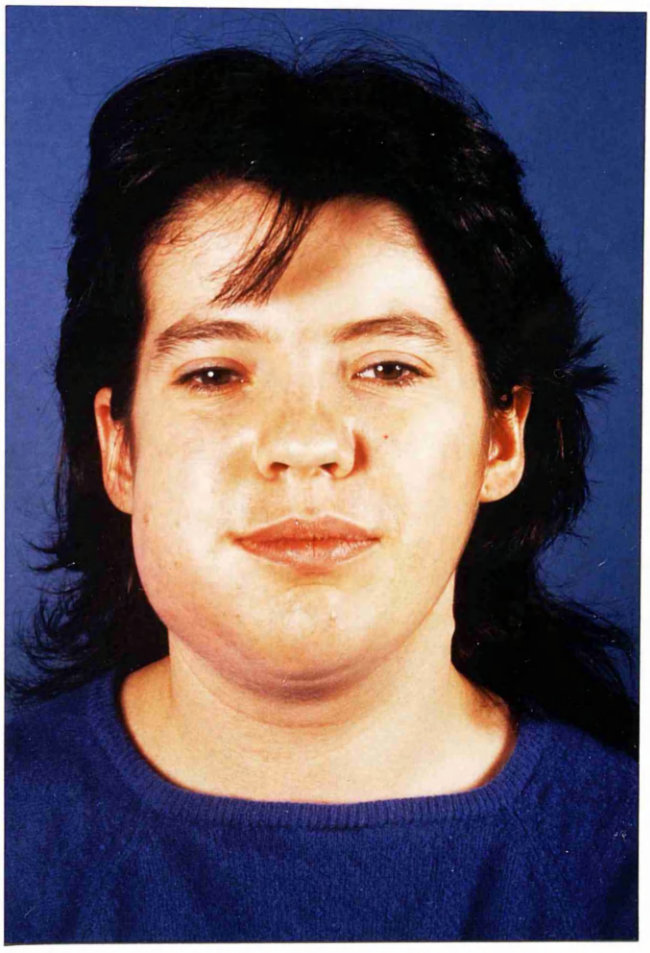


Figure 6.1: Diffuse swelling of the right cheek in a 23 year old female suffering from an acute dentoalveolar abscess of the upper right first molar.

the amount of residual swelling was assessed using a 10 cm linear analogue scale (Figure 6.2). One extremity of the line (0 cm) was labelled "No swelling" and the other (10 cm) "Swelling at last visit". Patients were asked to place a mark on the scale at the point which they thought corresponded to the amount of swelling they were experiencing. A numerical value of swelling at each visit was obtained by measuring the distance from zero to the mark.

Lymphadenopathy

Presence of lymphadenopathy was assessed at all appointments and was considered to be present if one or more cervical lymph nodes was firm and tender to palpation.

Temperature

Oral (sub-lingual) temperature and axillary temperature was measured at all visits using a standard clinical thermometer held in place for 30 seconds.

6.2.3 Treatment

Any area of obvious fluctuant swelling was aspirated for the presence of pus using the technique described in Section 2.2.3 then incised to establish surgical drainage. Whenever extraction of a tooth was indicated this was performed once adequate local anaesthesia could be achieved. If a tooth was to be retained the pulp was extripated and drainage established through the root canal. Patients received appropriate further treatment (re-incision, extraction or drainage through root canal) whenever indicated at review appointments.

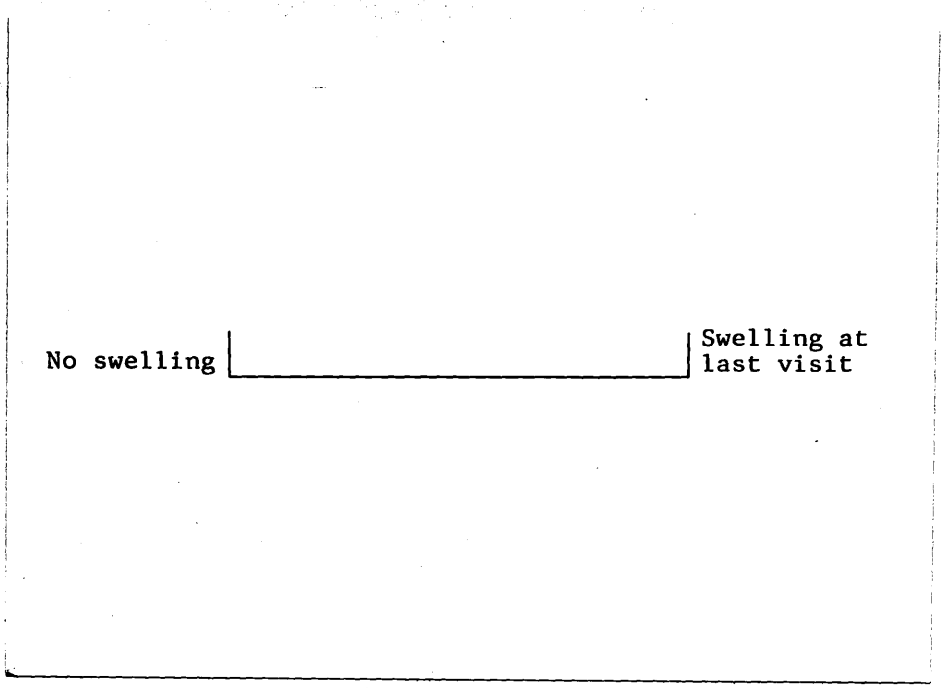


Figure 6.2: Design of a 10 cm linear analogue scale which was used to measure perception of swelling.

6.2.4 Antibiotic therapy

Using random sampling numbers (Bradford Hill, 1977) patients were allocated to treatment with either amoxycillin or penicillin. The amoxycillin regimen consisted of two 3 g sachets of amoxycillin (Amoxil, Bencard, Brentford). The first sachet was taken at the time of presentation and the second 8 hours later. The penicillin regimen was a conventional course of phenoxymethylpenicillin (Crystapen V, Glaxo, Greenford) consisting of a 250 mg tablet every 6 hours for 5 days.

Antibiotic therapy was dispensed with written instructions by the nursing staff and the the first dose was taken under supervision before the patient left the clinic. The dental surgeon was unaware of the antibiotic given to an individual patient but this information was readily available in case of emergency.

6.2.5 Microbial analysis

All pus samples were rapidly transported to the laboratory for microbiological processing. Specimens were either cultured according to the method described in Sections 2.2.4 and 2.2.5 or they were inoculated, using standard techniques, onto Columbia Blood Agar (Oxoid Ltd., Basingstoke) and incubated at 37°C aerobically and anaerobically for 48 hours. All isolates were identified using the methods described in Section 2.2.7.

Antibiotic sensitivity testing of primary cultures of the pus specimens was performed on Columbia Blood Agar (Oxoid Ltd., Basingstoke) using Stokes method (Stokes and Waterworth, 1972). The

control organism used was Staphylococcus aureus (NCTC 6571). Antibiotics were tested in the form of impregnated paper discs obtained comercially (Mast, Bootle) as follows; amoxycillin 10 g and penicillin G 2 units. Plates were incubated at 37°C in an anaerobic chamber (Appendix I) and read initially after 48 hours.

6.3 RESULTS

6.3.1 Patients

Sixty patients consisting of 47 males and 13 females were included in the trial. The mean age of the group was 33 years with a range of 14 to 65 years. Thirty patients received amoxycillin (A Group) and 30 patients penicillin (P Group). The ratio of males to females and age of patients in the two treatment groups were very similar (Table 6.1). Twenty-four of the patients, 11 of the P Group and 13 of the A Group, also participated in the study described in Chapter 2 of this thesis.

The clinical symptoms recorded at the time of initial presentation and at subsequent visits are divided into two tables, Table 6.2 contains details for the patients who received amoxycillin and Table 6.3 contains details of the patients who received penicillin.

	A Group	P Group
Sex ratio M/F	4•0	3•3
Mean Age (Years)	34•0	32•4
Age Range (Years)	16 - 65	18 - 65

Table 6.1: Sex ratio and mean ages of patients in amoxycillin treated group (A group) and penicillin treated group (P group).

Table 6.2: Clinical details at Day 1, 2, and 4 of 30 patients receiving amoxycillin.

Patient number	Visit day	Severity of pain	Residual swelling	Oral temp. (°C)	Axilla temp. (°C)	Tender nodes present	Pus obtained
1	1	4.0		37.0	36.8	Yes	No
	2	0.0	1.2	36.6	36.4	Yes	
	4	0.0	0.0	36.6	36.6	No	
2	1	4.8		37.4	37.0	No	Yes
	2	0.5	6.8	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
3	1	7.0		37.0	36.8	No	No
	2	0.0	1.6	37.0	36.8	No	
	4	0.0	0.0	36.8	36.4	No	
4	1	3.1		37.4	37.2	No	No
	2	0.0	3.2	37.0	36.8	No	
	4	0.0	0.0	37.0	36.8	No	
5	1	9.0		37.2	37.0	No	Yes
	2	2.6	6.1	36.8	36.4	No	
	4	0.0	1.9	36.8	36.5	No	
	7	0.0	0.0	36.8	36.5	No	
6	1	5.8		37.0	36.8	No	Yes
	2	0.3	1.1	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
7	1	5.8		38.0	37.3	No	Yes
	2	0.0	0.1	36.9	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
8	1	2.8		37.4	37.0	No	Yes
	2	0.0	3.1	36.8	36.4	No	
	4	0.0	0.0	36.6	36.4	No	
9	1	2.1		36.8	36.4	No	Yes
	2	0.0	2.3	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
10	1	9.8		36.8	36.4	No	Yes
	2	0.0	1.7	36.8	36.5	No	
	4	0.0	0.0	36.8	36.4	No	

Patient number	Visit day	Severity of pain	Residual swelling	Oral temp. (°C)	Axilla temp. (°C)	Tender nodes present	Pus obtained
11	1	4.6		36.9	36.5	No	Yes
	2	0.0	1.0	36.8	36.5	No	
	4	0.0	0.0	36.8	36.4	No	
12	1	3.7		36.8	6.5	No	Yes
	2	0.0	1.5	36.8	36.4	No	
	4	0.0	0.0	36.9	36.5	No	
13	1	8.2		37.5	37.3	No	Yes
	2	1.7	2.5	36.9	36.5	No	
	4	0.0	0.0	36.8	36.4	No	
14	1	7.0		36.8	36.4	No	No
	2	0.0	0.8	36.8	36.5	No	
	4	0.0	0.0	36.8	36.4	No	
15	1	5.0		37.9	37.3	Yes	No
	2	0.0	0.5	36.9	36.7	Yes	
	4	0.0	0.0	36.9	36.4	No	
16	1	7.9		36.8	36.4	No	Yes
	2	0.6	1.4	36.9	36.5	No	
	4	0.0	0.0	36.8	36.5	No	
17	1	7.2		36.8	36.4	No	Yes
	2	3.0	1.4	36.8	36.5	No	
	4	0.0	0.0	36.8	36.5	No	
18	1	9.7		37.4	37.0	No	Yes
	2	1.3	1.7	36.8	36.5	No	
	4	0.0	0.0	36.8	36.4	No	
19	1	4.1		36.8	36.6	No	Yes
	2	0.5	6.4	36.8	36.5	No	
	4	0.0	0.0	36.8	36.5	No	
20	1	0.5		37.0	36.5	No	No
	2	0.0	2.3	37.0	36.5	No	
	4	0.0	0.0	37.0	36.5	No	

Table 6.2 (Cont.)

Patient number	Visit day	Severity of pain	Residual swelling	Oral temp. (°C)	Axilla temp. (°C)	Tender nodes present	Pus obtained
21	1	3.5		36.7	36.5	No	Yes
	2	0.0	1.4	36.8	36.5	No	
	4	0.0	0.4	36.9	36.4	No	
	7	0.0	0.0	36.8	36.4	No	
22	1	6.0		36.8	36.5	No	No
	2	0.0	1.7	36.9	36.5	No	
	4	0.0	0.0	36.8	36.6	No	
23	1	1.6		36.7	36.4	No	Yes
	2	0.0	5.4	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
24	1	7.0		36.9	36.5	No	No
	2	0.0	0.5	36.8	36.5	No	
	4	0.0	0.0	36.8	36.5	No	
25	1	5.2		37.0	36.8	No	Yes
	2	0.0	4.6	37.0	36.7	No	
	4	0.0	0.0	37.0	36.7	No	
26	1	5.7		37.0	36.7	No	Yes
	2	0.0	0.3	36.9	36.6	No	
	4	0.0	0.0	36.9	36.6	No	
27	1	0.6		36.9	36.7	No	Yes
	2	0.6	4.2	37.0	36.6	No	
	4	0.0	0.0	36.9	36.6	No	
28	1	1.1		37.0	36.6	Yes	Yes
	2	0.0	3.4	37.0	36.6	Yes	
	4	0.0	0.0	37.0	36.6	No	
29	1	9.8		37.8	37.2	Yes	Yes
	2	0.3	3.4	36.8	36.5	Yes	
	4	0.0	0.7	36.8	36.6	No	
	7	0.0	0.0	36.9	36.6	No	
30	1	6.8		37.0	36.5	No	No
	2	2.2	3.9	36.9	36.5	No	
	4	0.0	0.0	36.9	36.5	No	

Table 6.2 (Cont.)

Table 6.3: Clinical details at Day 1, 2 and 4 of 30 patients receiving penicillin.

Patient number	Visit day	Severity of pain	Residual swelling	Oral temp. (°C)	Axilla temp. (°C)	Tender nodes present	Pus obtained
1	1	3.7		37.0	36.4	No	Yes
	2	0.7	3.9	37.0	36.8	No	
	4	0.0	0.0	37.0	36.8	No	
2	1	7.5		37.2	37.0	No	Yes
	2	0.0	5.1	36.8	36.8	No	
	4	0.0	0.8	36.8	36.6	No	
	7	0.0	0.0	36.8	36.6	No	
3	1	5.4		36.8	36.6	Yes	No
	2	0.0	2.8	36.8	36.3	Yes	
	4	0.0	0.0	36.9	36.4	No	
4	1	8.5		37.4	37.0	No	No
	2	3.3	3.3	37.6	37.0	No	
	4	0.0	0.0	37.0	36.7	No	
5	1	1.7		36.8	36.4	No	Yes
	2	0.0	3.0	36.8	36.4	No	
	4	0.0	0.0	37.0	36.6	No	
6	1	2.5		37.4	36.8	No	Yes
	2	1.0	3.0	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
7	1	3.5		37.4	37.0	No	Yes
	2	0.0	2.3	36.9	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
8	1	3.8		36.8	36.5	No	Yes
	2	0.0	4.8	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
9	1	4.7		36.8	36.4	No	Yes
	2	2.9	10.0	37.4	37.0	No	
	4	0.0	0.0	36.6	36.4	No	
	7	0.0	0.0	36.6	36.4	No	
10	1	3.5		36.8	36.5	No	No
	2	0.0	1.1	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	

Patient number	Visit day	Severity of pain	Residual swelling	Oral temp. (°C)	Axilla temp. (°C)	Tender nodes present	Pus obtained
11	1	5.2		37.3	36.8	Yes	Yes
	2	0.0	2.1	36.8	36.4	Yes	
	4	0.0	0.0	36.8	36.5	No	
12	1	4.8		37.0	36.6	No	No
	2	4.2	4.6	37.0	36.5	No	
	4	0.0	0.0	37.0	36.5	No	
13	1	6.7		37.5	37.0	Yes	Yes
	2	0.8	6.9	37.4	37.1	Yes	
	4	0.5	0.0	36.8	36.6	No	
	7	0.0	0.0	36.8	36.6	No	
14	1	2.9		36.8	36.4	No	Yes
	2	0.0	1.7	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
15	1	8.3		36.8	36.4	No	No
	2	1.0	1.9	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
16	1	8.0		36.8	36.4	No	Yes
	2	1.5	9.1	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
17	1	8.2		36.8	36.5	No	No
	2	0.8	0.5	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
18	1	6.2		37.6	37.4	No	Yes
	2	1.5	4.4	36.9	36.7	No	
	4	0.0	0.0	36.9	36.7	No	
19	1	2.8		36.7	36.6	No	Yes
	2	1.5	2.4	36.8	36.5	No	
	4	0.0	0.0	36.8	36.5	No	
20	1	4.7		37.0	36.6	No	No
	2	0.0	1.0	37.0	36.6	No	
	4	0.0	0.0	37.0	36.6	No	

Table 6.3 (Cont.)

Patient number	Visit day	Severity of pain	Residual swelling	Oral temp. (°C)	Axilla temp. (°C)	Tender nodes present	Pus obtained
21	1	5.1		36.8	36.6	No	No
	2	0.0	1.9	36.8	36.6	No	
	4	0.0	0.0	36.8	36.5	No	
22	1	6.5		37.1	36.6	No	No
	2	3.0	4.8	37.0	36.6	No	
	4	0.0	0.0	37.0	36.6	No	
23	1	2.9		37.0	36.7	No	Yes
	2	0.0	3.2	37.0	36.7	No	
	4	0.0	0.0	37.0	36.6	No	
24	1	6.0		36.5	36.2	No	Yes
	2	2.5	4.8	36.8	36.4	No	
	4	0.0	0.0	36.8	36.4	No	
25	1	5.9		37.0	36.7	No	Yes
	2	0.0	2.9	37.0	36.6	No	
	4	0.0	0.0	37.0	36.6	No	
26	1	7.2		37.0	36.6	No	Yes
	2	5.2	9.8	37.2	36.6	No	
	4	0.0	1.0	37.0	36.6	No	
	7	0.0	0.0				
27	1	9.8		38.4	38.0	Yes	Yes
	2	0.8	2.1	37.0	36.8	Yes	
	4	0.0	0.0	37.0	36.6	No	
28	1	4.0		37.4	36.8	No	Yes
	2	0.0	3.5	36.8	36.6	No	
	4	0.0	0.0	36.8	36.6	No	
29	1	8.2		37.0	36.6	No	No
	2	6.2	5.8	37.0	36.6	No	
	4	0.0	0.0	37.0	36.6	No	
30	1	6.5		37.4	36.9	No	Yes
	2	2.4	4.5	37.2	36.9	No	
	4	0.0	0.0	36.8	36.5	No	

Table 6.3 (Cont.)

6.3.2 Pain

All patients had pain at the time of initial presentation and the mean pain score for each of the groups is shown in Table 6.4. The distribution of pain scores was not normal and therefore the significance of the differences between the two treatment groups was evaluated using the Mann Whitney U test.

Many patients were free of pain on the second day, however 11 patients in the amoxycillin group and 17 in the penicillin group still experienced pain. Although the mean pain score in the penicillin group, 23·1, was higher than the mean score for the amoxycillin group, 12·3, the difference was not statistically significant ($p > 0·05$).

By Day 4 only one patient was still in pain. This person was in the penicillin group and was pain free by Day 7.

6.3.3 Swelling

The mean amount of swelling remaining in each treatment group at review visits during the trial period are presented in Table 6.5. All patients presented with swelling on Day 1 and all still had swelling on Day 2. The distribution of swelling scores was even in both treatment groups and therefore the significance of differences was evaluated using Student's t test. The mean score for residual swelling on Day 2 was 24·9 for the amoxycillin group and 39·0 for the penicillin group. The difference in these values was statistically significant ($p < 0·05$).

	Group 'A'		Group 'P'		Significance*
Day 1	30		30		
Mean severity (± SD)	53•1	(27•0)	54•8	(21•1)	NS
Day 2	11		17		
Mean severity (± SD)	12•3	(9•8)	23•1	(16•5)	NS
Day 4	0		1		
Severity	0		0•5		NS

* Mann Whitney U test.

Table 6.4: Number of patients and mean severity of pain on Day 1, 2 and 4 in each treatment group. (A, amoxycillin and P, penicillin).

	Group 'A'		Group 'P'		Significance*
Day 1	30		30		
Day 2	30		30		
Mean swelling remaining (± SD)	24·9	(18·5)	39·0	(24·5)	$p < 0·05$
Day 4	3		2		
Mean swelling remaining (± SD)	10·0	(7·9)	9·0	(1·4)	NS

* Student's t test.

Table 6.5: Number of patients and mean swelling remaining on Day 1, 2 and 4 in each treatment group. (A, amoxycillin and P, penicillin).

On Day 4 only 5 patients still had swelling, 3 in the amoxycillin group and 2 in the penicillin group. However this difference was not statistically significant. None of the patients seen on Day 7 had any swelling.

6.3.4 Lymphadenopathy

Four patients in each group had palpable cervical lymph nodes on Days 1 and 2. None of the patients in the penicillin group and only one patient in the amoxycillin group had palpable tender nodes on Day 4. No patient had palpable nodes on Day 7.

6.3.5 Temperature

The mean oral temperature and axillary temperature alongwith the number of patients in each group with raised temperatures at each visit are shown in Table 6.6. There were no significant differences in either temperature at any time of the trial.

6.3.6 Microbiological findings

Pus was obtained from 21 patients in the amoxycillin group and from 20 patients im the penicillin group. Bacteria were cultured from all the pus samples and a total of 137 bacterial strains isolated. The identity of facultative isolates from each treatment group is presented in Table 6.7 and of the strictly anaerobic isolates in Table 6.8. The spectrum of bacteria isolated from each group of patients was very similar.

The antibiotic sensitivity patterns of the pus samples to penicillin and amoxycillin are shown in Table 6.9. The vast majority

Visit day	Mean oral temperature °C ± SD		Mean axillary temperature °C ± SD	
	Amoxycillin group	Penicillin group	Amoxycillin group	Penicillin group
1	(9)* 37·0 ± 0·3	(10)* 37·0 ± 0·4	(9)+ 36·7 ± 0·3	(5)+ 36·7 ± 0·3
2	(0)* 36·9 ± 0·1	(3)* 36·9 ± 0·2	(2)+ 36·5 ± 0·1	(3)+ 36·6 ± 0·2
4	(0)* 36·8 ± 0·1	(0)* 36·9 ± 0·1	(0)+ 36·5 ± 0·1	(0)+ 36·5 ± 0·1

* Numbers of patients with oral temperature greater than 37·2°C.

\+ Numbers of patients with axillary temperature greater than 37·0°C.

Table 6.6: Mean oral temperature and axillary temperature on Day 1, 2 and 4 for 60 patients receiving either amoxycillin or penicillin.

Organism Identity	Group A	Group P	Total
Gram-positive cocci			
α-haemolytic streptococcus	3	4	7
β-haemolytic streptococcus	3	2	5
Streptococcus milleri	7	9	16
Gram-positive bacilli			
Lactobacillus species	2	2	4
Actinomyces species	0	2	2
Arachnia propionica	0	1	1
Gram-negative bacilli			
Haemophilus parainfluenzae	1	1	2
Capnocytophaga ochracea	1	0	1
Eikenella corrodens	1	1	2
Total	**18**	**22**	**40**

Table 6.7: Identity of 40 facultative microorganisms isolated from 41 acute dentoalveolar abscesses.

Organism Identity	Group A	Group P	Total
Gram-positive cocci			
Peptococcus species	13	12	25
Peptostreptococcus species	6	1	7
Unidentified	2	3	5
Gram-positive bacilli			
Eubacterium lentum	0	2	2
Gram-negative cocci			
Veillonella parvula	0	1	1
Gram-negative bacilli			
Bacteroides capillosus	2	1	3
Bacteroides corrodens	0	1	1
Bacteroides distasonis	1	0	1
Bacteroides gingivalis	6	5	11
Bacteroides melaninogenicus	10	3	13
Bacteroides oralis	7	8	15
Bacteroides ruminicola	1	3	4
Bacteroides uniformis	0	1	1
Fusobacterium species	1	1	2
Unidentified	3	3	6
Total	**52**	**45**	**97**

Table 6.8: Identity of 97 strictly anaerobic microorganisms isolated from 41 acute dentoalveolar abscesses.

	Antibiotic Sensitivity					
	Amoxycillin			Penicillin		
	S*	MS+	R++	S	MS	R
Amoxycillin Group	20	0	1	18	2	1
Penicillin Group	18	2	0	13	6	1

*S Sensitive; +MS Moderately sensitive; ++R Resistant

Table 6.9: Antibiotic sensitivity of 41 pus samples obtained from 41 patients subsequently treated with either amoxycillin or pencillin.

of pus samples were sensitive to amoxycillin and penicillin and only two pus samples demonstrated resistance. The pus specimen obtained from patient 21 in the amoxycillin group was resistant to both antibiotics while the sample obtained from patient 16 in the penicillin group was resistant to penicillin alone.

6.3.7 Side effects

No side effects were reported by any of the patients receiving penicillin however two patients receiving amoxycillin complained of nausea following the second dose.

6.4 DISCUSSION

6.4.1 Methodology

Assessing the effectiveness of an antibiotic in the treatment of acute dentoalveolar abscess is difficult since there are problems in quantifying the clinical symptoms of each patient, separating the effect of surgical drainage from the effect of the antibiotic therapy and ensuring that the microbiology of the two treatment groups is similar.

Measurement of pain is complicated partly by the fact that it is usually accompanied by other sensations and partly because of variations in the pain threshold between individual patients. Therefore in the past some clinical trials of antibiotic therapy in the treatment of acute dental abscesses have simply recorded presence or absence of pain, making no attempt to quantify the severity (Ingham et al., 1977; Hood, 1978; von Konow and Nord, 1983; Cumming et al., 1984). In order to obtain a more detailed recording of the

pain associated with acute dental abscesses over a period of time, graded scales from 1 to 3 (Schuen et al., 1974) or 1 to 5 (Davies and Balcom, 1969) have been used. Using these methods, a higher number on the scale indicates a greater severity of pain. It is difficult however to interpret differences in mean scores when catergorised scales are used since the patient is not given a continous scale for evaluation and may find it difficult to decide between two adjacent grades. Revill et al., (1976) have found that a linear analogue is a reproducable and more reliable method of recording severity of pain. The linear analogue offers the patient an unlimited range of pain perception and provides results which can be easily analysed. It has not been used previously to measure pain associated with dentoalveolar abscesses.

Measurement of facial swelling is also difficult, although the amount of soft tissue swelling following the removal of lower third molar teeth has been studied in some detail (For review, see Breytenbach, 1978). The methods used have included external measuring calipers, adjustable measuring frames attached to the teeth, photography and stereo-photogrammetry. Amin and Laskin (1983) described the use of a length of silk suture, held at each end in artery forceps, to record the distance from the outer canthus of the eye to the angle of the mandible and the distance from the corner of the mouth to the ear lobe. The two measurements are addded and the total divided by two to provide a value of facial swelling in the lower third molar region. While these objective assessments can provide some guide to the degree of extraoral swelling in the third molar region, none of the methods are suitable for measuring the

swelling associated with acute dentoalveolar abscess, which can occur at a wide range of sites related to the jaws. In the past therefore clinical trials evaluating the effect of antibiotic therapy in the treatment of dental abscesses have limited their assessment of swelling to the recording of its presence or absence (Hood, 1978, Ingham et al., 1977; von Konow and Nord, 1983) or the use of scales similar to those for the recording of pain (Davis and Balcon, 1969; Schuen et al., 1974). In the present study, although no attempt was made to quantify swelling objectively, a subjective measurement of perceived swelling was made by each patient, using a modification of the linear analogue scale used for pain. A linear analogue has not been used previously to assesss the amount of swelling associated with acute dentoalveolar abscess, however it was employed in the present study as it was felt that a recording of swelling perception was a more practical approach than attempting a direct measurement. In addition it was postulated that the patient would be the most percipient observer of own facial appearance.

White blood cell counts (WBC) and measurement of erythrocyte sedementation rate (ESR) have been used to monitor the severity of infection but they have proven to be poor indicators of the severity of acute dentoalveolar abscess (von Konow and Nord, 1983; Heimdahl et al., 1985). Therefore these haematological investigations were not included in the parameters measured in the present trial.

The antibiotics which have been subjected to clinical investigation for the treatment of oro-facial infections have included lincomycin (Davies and Balcom, 1969), cloxacillin (Hooley, 1969), clindamycin (Schuen et al., 1974), amoxycillin (Cruciani, 1978), cephalosporin (Cumming et al., 1984) and metronidazole (Ingham et al., 1977; Hood, 1978). Penicillin has usually been used as a comparative control treatment in studies assessing the effectiveness of new antibiotics although Cruciani (1978) assessed the performance of amoxycillin by a comparison with tetracycline. Two of the trials above did not employ a comparative antibiotic and effectiveness was determined by clinical resolution of symptoms rather than comparison with the effects of an established antibiotic regimens (Cumming et al., 1984; Hooley, 1978). Ideally a control group of patients receiving no antibiotic should be included in these studies to assess the contribution of surgical treatment to recovery. However, it would not be ethical to withhold antibiotic therapy from a patient who has clinical indications for its use and therefore it is not possible to use a control group who receive no antibiotic.

In any clinical trial comparing the effectiveness of a new antibiotic with that of an established therapy it is important to ensure the treatment groups of patients are similar with regard to presenting symptoms, treatment received and the microbiological species involved. In addition to ensuring similarity between the treatment groups it is important that clinical symptoms are measured in as much detail as is possible. Very little discussion has been made in other studies regarding the comparability of patient groups and the methods of assessing clinical symptoms. There has also been

wide variations in the number of patients investigated. For example whilst some trials have include between 60 and 68 patients (Schuen et al., 1974; von Konow and Nord, 1983) others, such as that of Cumming et al., (1984), have been limited to the study of only 10 patients. For one or more reasons therefore, the conclusions of some previous clinical studies should be viewed with some reservations.

6.4.2 Analysis of experimental findings

A comparison of the clinical details of the two experimental groups at the time of initial presentation in the present study showed them to be very similar. The surgical treatment each group received was the same and pus was obtained from approximately two thirds of the patients in each group. The identity of the bacterial species isolated and the antibiotic sensitivity of the pus samples obtained from each group was also very similar. Finally the spectrum of isolates compares well with other recent microbiological studies of acute dentoalveolar abscesses (Aderhold et al., 1981; Labriola et al., 1983; Williams et al., 1983; Heimdahl et al., 1985).

The similarities with regard to the clinical symptoms at the time of presentation, bacteriology of pus samples and treatment received in the two groups would suggest strongly that valid conclusions could be drawn from any significant differences noted in the results of the present study. The only statistically significant difference found between the two groups was the amount of residual swelling after the first 24 hours of treatment where the group of patients receiving amoxycillin recorded less swelling than those who had received penicillin. A possible explanation for this observation

is that since amoxycillin is known to penetrate into areas of acute infection (Boon et al., 1982) high dose therapy may achieve inhibitory or bactericidal concentrations of antibiotic at the site of infection which cannot be obtained in the first 24 hours of therapy by conventional doses of penicillin.

Although amoxycillin has been recommended for use in the treatment of dental infections on the basis of in vitro sensitivity tests (Greenberg et al., 1979; Morey et al., 1984) its performance has rarely been evaluated clinically. Cruciani (1978) compared the performance of accepted doses of amoxycillin (250 mg t.i.d) with tetracycline (250 mg q.i.d) in 40 patients with acute dental infections. Twenty patients received amoxycillin and a further 20 tetracycline for 3 days. Clinical symptoms of pain, swelling, lymphadenopathy, fever, trismus and erythema were measured and graded from 1 to 10 at each visit. Cruciani (1978) reported that the patients who received amoxycillin recorded a significantly greater improvement in symptoms than those who received tetracycline. It would appear therefore that a low dose of amoxycillin is effective but it is not known if it is as effective as a 3 g regimen. It would be important to determine the lowest effective dose of amoxycillin since Lacey (1984) has claimed that antibiotics should be prescribed for the shortest time at the lowest dose that produces clinical cure to minimise the emergence of resistant strains.

6.5 CONCLUSIONS

(i) The similarities in presenting clinical symptoms, microbiology of pus samples obtained and surgical treatment received by each of the two groups would indicate that any differences noted in recovery were due to the antibiotics.

(ii) Short-course high-dose amoxycillin is as effective as a conventional course penicillin in the treatment of acute dentoalveolar abscess.

(iii) Patients receiving a short-course of high-dose amoxycillin recorded a greater reduction in swelling during the first 24 hours of treatment than those receiving a conventional course of penicillin.

CHAPTER 7

CONCLUDING DISCUSSION

The purpose of this chapter is to inter-relate the results recorded in Chapters 2 to 6 and summarise the microbiological information which may be relevant to the management of acute dentoalveolar abscess and to highlight areas which require further research.

7.1 Bacterial flora

A quantitative bacteriological investigation of 50 acute dentoalveolar abscesses demonstrated that pus samples obtained from these infections usually contained viable microorganisms at a concentration of between 10^6 and 10^9 cfu/ml. A finding which agrees with the data provided for 6 abscesses by Williams et al., (1983). The likelihood of a numerical predominance of strict anaerobes within the microbial load in dentoalveolar abscess is a fashionable assumption by clinicians but the results presented in this thesis provide the first evidence that this is true, at least for an acute dentoalveolar abscess which has progressed to produce fluctuant swelling in the oro-facial soft tissues.

Three of the abscesses studied in Chapter 2 yielded facultative bacteria only (FO Group) whereas samples containing strictly anaerobic bacteria alone were isolated from 20 patients (AO Group). Although the number of patients in the FO group is too small to enable statistical analysis of the clinical symptoms recorded in

each group, examination of the results revealed that the patients in the FO Group reported a mean of 0•3 days of pain and 1•3 days of swelling prior to sampling whereas the equivalent values for the patients in the AO group were 2•1 and 2•3 respectively. Thus it would appear that a purely facultative flora was detected in patients who had a shorter history of infection than in patients from whom strict anaerobes alone were cultured. This finding tends to support the suggestion by Aderhold et al., (1981) that the flora of acute dentoalveolar abscess becomes progressively anaerobic with time. Since all the abscesses studied in this thesis had spread through alveolar bone into the surrounding soft tissues, they were likely to have been in a late phase of development and this may also partly explain why the number of abscesses with a purely facultative flora was small.

In order to investigate further the hypothesis that with time the microbial flora of an acute dentoalveolar abscess becomes progressively more anaerobic, it would be necessary to obtain pus from abscesses at different phases of development, especially early stages. However, this would be difficult to achieve since there are a number of problems in collecting samples from infections that are confined within alveolar bone. Firstly, as recorded in Chapter 2, there would be problems acquiring patients with early lesions since the majority of patients had suffered approximately 2 days pain and swelling prior to presentation. Secondly there is often a problem of accurately diagnosing apical infection before definite symptoms have developed. Thirdly a surgical approach to the apical area would probably be necessary because sampling of the apical area through the root canal

would be impractical due to the limited size of the apical foramen. However, surgery in the apical region is difficult due to problems of achieving anaesthesia in areas of acute inflammation and the risks of spreading infection further. For these reasons it is likely that an experimental animal abscess model, such as that described in Chapter 3, would be required to study any changes in the microflora of dentoalveolar abscess with time.

7.2 Antibiotic therapy

One of the aims of this thesis was to determine which antibiotic would be most effective in the treatment of acute dentoalveolar abscess. In vitro sensitivity tests revealed the vast majority of isolates to be sensitive to the antibiotics studied, though a small number of strains were resistant to each. Therefore none of the agents studied emerged as an "ideal" antibiotic. However, not all bacterial species are equally pathogenic and therefore it could be argued that if no uniformly effective agent is available then it would be more appropriate to employ an antibiotic directed specifically at the most pathogenic species. At present the relative pathogenicity of different isolates is uncertain, although the results of Chapter 3 indicate that strict anaerobes, such as bacteroides and fusobacteria, probably play a major role. Until it becomes possible to define more reliably which isolates from the mixed abscess flora are most pathogenic it is reasonable to prescribe an antibiotic which destroys the widest range of bacteria likely to be present.

The results of Chapter 4 demonstrated that the vast majority of bacterial strains isolated from acute dentoalveolar abscess are sensitive to amoxycillin, ampicillin, clindamycin, cephaloridine, erythromycin, penicillin or tetracycline. This finding suggests that any one of these antibiotics would be equally effective and selection can be dictated by other factors, such as cost, availability and the risk of known side effects. Phenoxymethylpenicillin has traditionally been regarded as the drug of choice for acute dental infections (Sims, 1974; Guralnick, 1985) because it is cheap and although allergy is well recognised, serious hypersensitivity reactions associated with anaphylaxis are rare (Ball, 1982). However concern has been expressed about the use of penicillin for these infections because clinical failures, believed to be due to the presence of beta-lactamase producing bacteroides, have been reported (Heimdahl et al., 1980; Bahn et al., 1981).

The in vitro susceptibility studies reported in this thesis revealed that all the facultative bacteria (26 per cent of the isolates) were resistant to metronidazole, making it the least effective of the agents tested. This observation suggests that metronidazole would not be an effective agent for the treatment of dentoalveolar abscesses. However, since the findings in Chapter 2 and Chapter 3 indicate that strict anaerobes are the predominant bacteria in acute dentoalveolar abscesss and are also likely to be the major pathogens involved, the clinical use of metronidazole may be successful. Ingham et al., (1977) assessed the clinical performance of metronidazole in the treatment of acute oro-facial abscesses and reported that it was effective as penicillin. A finding which led

them to conclude that strict anaerobes are the sole pathogens in established acute dental abscesses since metronidazole is inactive against the facultative bacteria. This conclusion however neither takes account of the contribution that surgical drainage makes to the improvement of symptoms nor does it explain the minority of abscesses which apparently contain facultative bacteria only (Chapter 2).

Three of the abscesses studied in Chapter 2 yielded only facultative bacteria; Strep. milleri was isolated in pure culture from two pus samples and a mixture of Strep. milleri and Strep. mitis was obtained from the third. The results of Chapter 3 have revealed that oral strains of Strep. milleri are pathogenic since they produced experimental abscesses when inoculated in pure culture into mice. In addition Strep. milleri has been reported as a significant pathogen in infections at other body sites, such as the maxillary sinus, brain and lung (van der Auwera, 1985). All the strains of Strep. milleri studied in Chapter 4 were found to be resistant to metronidazole. Therefore it could be argued that metronidazole should not be regarded as a first choice agent for the treatment of dentoalveolar abscesses since a proportion are likely to contain Strep. milleri. The effectiveness of metronidazole in the treatment of an abscess containing Strep. milleri is not known since it is not possible to determine if Strep. milleri was involved in any of the patients included in the trial of metronidazole reported by Ingham et al., (1977).

Therefore there would appear to be no simple answer to the question of which is the most appropriate antibiotic to use in the management of acute dentoalveolar abscess. There are two possible solutions, one is to use more than one antibiotic and the second is to increase the dosage of a single antibiotic. In this thesis the only agent to which all the strict anaerobes were sensitive was metronidazole and the only antibiotic to which all the strains of facultative bacteria were sensitive was amoxycillin. These *in vitro* sensitivity results would therefore indicate that a combination of metronidazole and amoxycillin would be effective against all the bacterial species likely to be encountered. However, it is generally accepted that when possible it is preferable to administer one antibiotic rather than two. The sensitivity results in Chapter 4 indicate that amoxycillin was one of the most effective antibiotics and that the concentration expected in serum (4-6 μg/ml) following a standard oral dose inhibited the growth of all but one of the isolates (Chapter 5). The organism resistant to this concentration was a strain of *B. melaninogenicus*, for which the MIC of amoxycillin was 8 μg/ml. Serum concentrations as high as 24 μg/ml of amoxycillin (a level in excess of the MIC for all the bacteria tested in Chapter 5) can be safely achieved in patients following a single oral 3 g dose (Shanson *et al.*, 1980). Since animal experiments have shown that amoxycillin penetrates well into areas of acute infection (Boon *et al.*, 1982) a 3 g dose would probably achieve levels in pus which would be inhibitory for all the bacterial strains likely to be present. These facts together with the results of Chapter 6 indicate that 3 g amoxycillin is possibly the best single antibiotic regimen. High-doses of amoxycillin however are more expensive than conventional

regimens and this aspect of chemotherapy must be considered when formulating prescribing policies. A specific indication for the use of high-dose amoxycillin may be the situation where a patient presents as an emergency to general medical practitioners at times when a dental service is not be readily available. The doctor usually prescribes an antibiotic and analgesic, leaving the establishment of surgical drainage until a dental surgeon is available. High-dose amoxycillin could be useful in this situation since it may well achieve an antibiotic concentration that will limit further spread of infection even though surgical drainage is not achieved.

Although the results of this thesis have suggested that high-dose short-course amoxycillin can be an effective antibiotic regimen, a number of patients are hypersensitive to penicillins and therefore the use of alternative antibiotics is necessary. In vitro antibiotic sensitivity results indicate that erythromycin would be an effective second choice antibiotic.

7.3 Further work

The results of this thesis have established that acute dentoalveolar abscess contains high concentrations of viable bacteria and have confirmed the identity of the spectrum of bacterial species involved in addition to indicating the species likely to be the major pathogens. The thesis has also shown that the vast majority of bacteria present are susceptible to the range of antibiotics presently used to treat this acute dental infection. Further work is required regarding the development of acute dentoalveolar abscess

especially the source and pathogenicity of bacterial species involved. In addition further investigation into variations in the abscess microflora that may occur at different stages of development should be undertaken since this may have implications for the use of antibiotic therapy.

Development of acute dentoalveolar abscess

The experiments described in this thesis have confirmed that the bacterial species involved in acute dentoalveolar abscess are species which are also regarded as members of the commensal oral flora. Radiographs indicate that the majority of acute dentoalveolar abscesses develop from chronic inflammatory lesions at the root apex, which are believed to be produced by diffusion of toxins from necrotic pulp tissue. The similarity and close proximity of the abscess flora and microbial flora of the gingival crevice or necrotic pulp would support the proposals that bacteria spread directly into the apical region from either of these sites. It is also possible that organisms, which have gained access to the blood stream from other body sites, pass into the apical lesion via vessels of general circulation. Such bacterial invasion should usually be overcome by the host defence system. However, it is likely that situations arise when the host defence cannot adequately remove the bacteria and this eventually results in abscess development. Factors which may be predispose to abscess formation are probably the localised reduction of the host defence within an apical granuloma or the presence of bacteria with increased pathogenicity. These aspects of acute dentoalveolar abscess development are poorly understood and require further study.

An important aspect of bacterial pathogenicity that could be investigated is their ability to resist phagocytosis. In vitro experiments have shown that strict anaerobes, such as B. fragilis and B. melaninogenicus, can inhibit the phagocytosis of facultative organisms, such as E. coli (Ingham et al., 1981; Vel et al., 1985). Therefore it is possible that strict anaerobes isolated from acute dentoalveolar abscesses, such as B. oralis, B. melaninogenicus, B. gingivalis, may protect facultative organisms such as Strep. milleri from phagocytosis. This aspect of pathogenicity could be studied using the radiolabelled methods of Verhoef et al., (1977) as applied to B. assachrolyticus and B. fragilis by Jones and Gemmell (1986). It would be possible to use the bacterial strains isolated from the acute dentoalveolar abscesses examined in this thesis since fresh cultures of all the isolates have been stored in freeeze dried ampoules.

In vitro investigations should also be undertaken to determine the ability of bacterial species isolated from acute dentoalveolar abscesses to produce the toxins or enzymes which have been implicated in the pathogenicity of B. gingivalis and B. intermedius by Slots and Genco (1984) (see Tables 3.17 and 3.18). In addition, the presence of capsular material on bacteria isolated from acute dentoalveolar abscess should be determined since it is likely this structure plays an important role in resisting host defence systems (see Section 1.5.2).

An additional area that requires study is the inter-relationships which may exist between different bacterial species in the mixed flora. In the studies described in Chapter 3 pure cultures and combinations of 2 bacteria species were investigated however the results of Chapter 2 revealed that there was a mean of 3•3 bacterial species per abscess. It would therefore be more appropriate to investigate the infections produced by the inoculation of 3 or 4 bacterial strains. Not only would experiments involving these numbers of bacterial strains demonstrate the type of tissue reactions produced by inoculation of more than 2 bacterial strains but would also permit further study of any changes which may occur in the proportions of different members of a mixed flora with time.

Antibiotic therapy

Although penetration of antibiotics into areas of acute infection has been studied using experimental infections in animals, little information is available describing antibiotic concentrations achieved within the pus of acute dentoalveolar abscess in patients. Such study using standard doses of the antibiotics presently used to treat acute dentoalveoalr abscess would be useful. In addition, it would be helpful to determine the levels of amoxycillin achieved after a 3 g regimen since this would reveal if concentrations in excess of 8 μg/ml (the level that inhibited the growth of all the strains isolated in the present study) are reached. If this were the case it would support the proposal made in this thesis that high-dose therapy achieves inhibitory concentrations at the site of infection that cannot be achieved by standard dose regimens. This type of study

could be undertaken by giving patients, who had received no antibiotic prior to presentation, a known amount of antibiotic at a known period of time prior to sampling of the pus. Although it may be difficult to obtain a sufficient number of patients, the findings would have more clinical relevance and involve less laboratory time than the use of experimental animals.

In order to determine the therapeutic effect of amoxycillin in the situation where an abscess is not surgically drained it would be necessary to use an experimental animal model, such as described in Chapter 3, since it would not be ethical to withold surgical drainage from patients. The results obtained from this type of investigation would be helpful in establishing the effect of antibiotic therapy in cases where surgical drainage cannot be achieved either due to the diffuse nature of the infection or to the unavailability of a dental surgeon.

7.4 Summary

In conclusion the the investigations described in this thesis show that the microbiology of acute dentoalveolar abscess is complex. The findings have confirmed that;

1. The microbial flora usually contains 3 or 4 bacterial species at viable concentrations of 10^6 - 10^9 cfu/ml.

2. The bacterial species involved are predominantly strict anaerobes, although occasionally an abscess may have a purely facultative flora.

3. Anaerobic gram-negative bacilli are likely to be major pathogens however inter-relationships with other bacteria are also probably involved.

4. Only a minority of the bacteria encountered are resistant to the serum concentrations achieved following standard oral doses of the antibiotics presently used to treat acute dentoalveolar abscess.

5. High-dose short-course antibiotic therapy may have an important role in the management of acute dentoalveolar abscess.

The knowledge obtained from the studies in this thesis furthers the understanding of the microbiology of acute dentoalveolar abscess and has implications for the use of antibiotics in treatment. The findings indicate there is a need for further information on the source of the infecting organisms, the changes that may occur in the microbial flora with time and the mechanisms of bacterial pathogenicity.

APPENDICES

APPENDIX I

Anaerobic incubators

Two different anaerobic incubators were used during the three year period of study described in this thesis.

One incubator (Anaerobic System 1024), obtained from Raven Scientific Ltd., Suffolk, provided separate areas for incubation at 37°C and storage of plates at room temperature under anaerobic conditions. The second incubator (Figure 2.4), manufactured by Don Whitely Scientific Ltd., Shipely, consisted of a single chamber maintained at 37°C.

Both incubators contained an atmosphere of 85 per cent nitrogen, 10 per cent hydrogen and 5 per cent carbon dioxide.

APPENDIX II

Standardisation of bacterial suspensions

A spectrophotometer (model SP 8-100, Pye Unicam, Cambridge), set at a wavelenght of 570 nm, was used to standardise the concentration of bacterial suspensions of a representative strain of each bacterial group used in the pathogenicity studies described in Chapter 3 and the antibiotic sensitivity tests described in Chapter 5. The bacterial strains used were Strep. milleri 036 for facultative gram-positive cocci, Peptococcus species 006 for anaerobic gram-positive cocci and F. nucleatum 013 for anaerobic gram-negative bacilli. Standard concentrations were also determined for the antibiotic sensitivity control strains, C. perfringens NCTC 8237 and B. fragilis NCTC 9343.

A culture of each organism was prepared by inoculating 40 to 50 colonies from a Columbia Blood Agar plate (Oxoid Ltd., Basingstoke) of pure culture into 20 ml Anaerobic Blood Broth (ABB) (Gibco Europe, Paisley). All broths were incubated at 37°C in an anaerobic chamber (Appendix I). Broths containing Strep. milleri were incubated for 24 hours and those containing a strictly anaerobic strain for 48 hours. Following this a series of dilutions of each broth was made in further ABB (Gibco Europe, Paisley) as below,

Bijou		Dilution
1	9•0 ml ABB + 3 ml culture	1 : 4
2	1•5 ml ABB + 3 ml Bijou 1	1 : 6
3	5•0 ml ABB + 5 ml Bijou 1	1 : 8
4	1•5 ml ABB + 3 ml Bijou 3	1 : 12
5	3•0 ml ABB + 3 ml Bijou 3	1 : 16
6	2•0 ml ABB + 2 ml Bijou 3	1 : 32

When placed in plastic disposable cuvettes with a path lenght of 10 mm (Sarstedt Ltd., Leicester) these dilutions produced a series of bacterial suspensions with absorbance ranging from 0•05 to 0•6. The turbity of each dilution was measured by comparison with uninoculated ABB (Gibco Europe, Paisley). Following measurement of absorbance the broth in Bijou 3 was diluted 1:100 and 1:1000 in ABB (Gibco Europe, Paisley) and 50 μl of each dilution inoculated onto Columbia Blood Agar (Oxoid Ltd., Basingstoke) using a spiral plater (Don Whitely Scientific, Shipley). Plates were incubated anaerobically at 37°C for 48 hours after which time the viable count was determined according to the method described in Section 2.2.6. Viable counts for the remaining bijous were calculated by adjusting the concentration obtained for Bijou 3 by the appropriate dilution factor.

Each organism was tested in triplicate and a growth curve constructed from each test. An example of a growth curve for Strep. milleri 036 is shown in Figure A.1 and and an example for F. nucleatum 013 shown in Figure A.2. The absorbance that corresponded to a concentration of 2×10^7 cfu/ml was measured on each

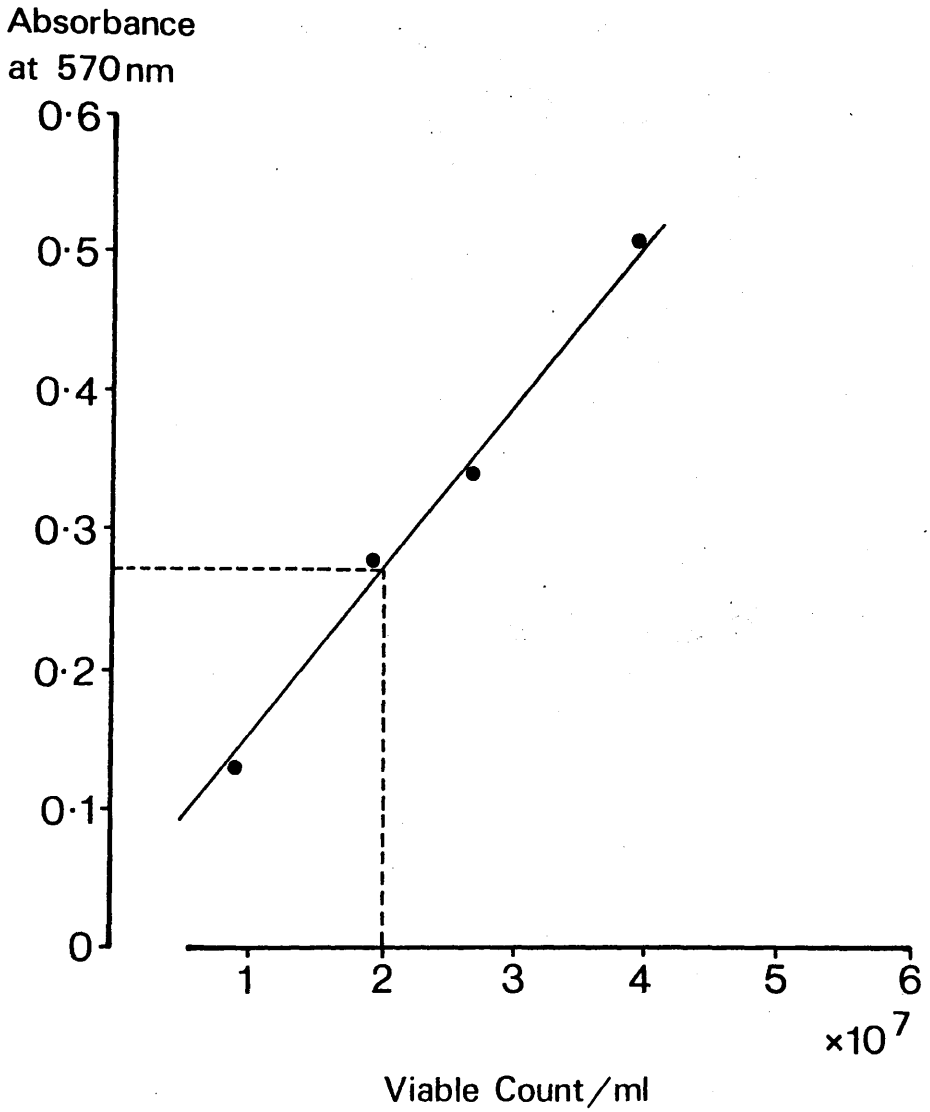


Figure A.1: Absorbance growth curve for Strep. milleri 036. In this test a concentration of 2 x 10^7 cfu/ml corresponed to an absorbance of 0·28.

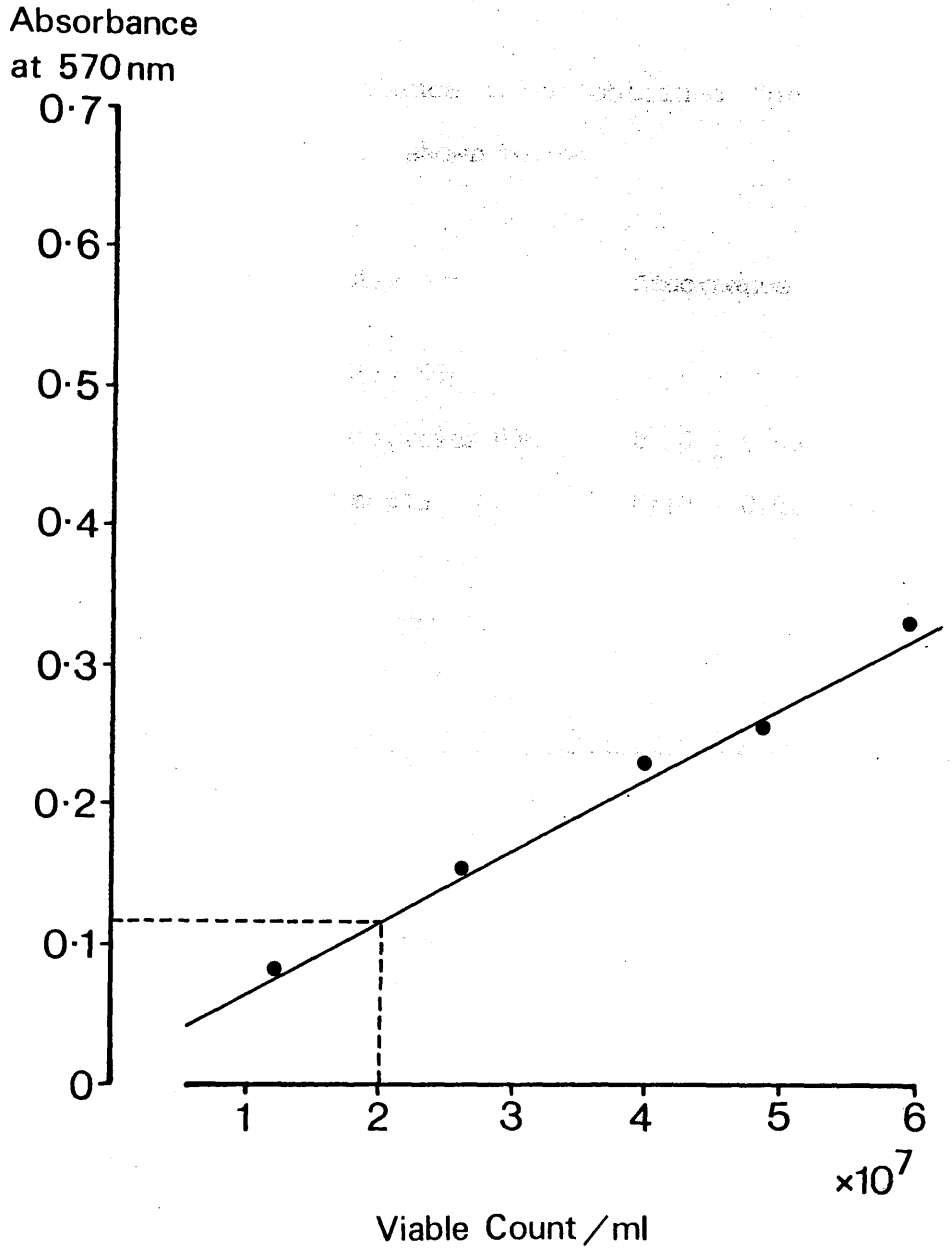


Figure A.2: Absorbance growth curve for F. nucleatum 013. In this test a concentration of 2 X 10^7 cfu/ml corresponded to an absorbance of 0•11.

of the 3 curves obtained for each representative strain and subsequently a mean was calculated.

The mean absorbance value obtained for each of the 5 bacterial strains tested is shown below.

Bacterial strain	Absorbance ± SD
Strep. milleri 036	0•26 ± 0•01
Peptococcus species 006	0•26 ± 0•05
F. nucleatum 013	0•10 ± 0.02
C. perfringens NCTC 8237	0•25 ± 0•03
B. fragilis NCTC 9343	0•10 ± 0•02

The value obtained for the representative strain of each bacterial group was used subsequently for all the test strains belonging to that group.

APPENDIX III

Culture media

Anaerobic Blood Broth

(Gibco Europe, Paisley, Scotland)

Formula of base:

Tryptone	10 g
Beef extract	2 g
Liver extract	3 g
Yeast extract	5 g
Glucose	5 g
Sodium chloride	5 g
Menadione	0·005 g
Haemin	0·005 g
Cysteine hydrochloride	1 g
Dithiothreitol	0·1 g
Sodium bicarbonate	0·9 g

1. Dissolve base in 1 litre distilled water at room temperature.
2. Aliquot 20 ml volumes into sterile glass McCartney bottles.
3. Autoclave at 121°C for 15 minutes.

Columbia Blood Agar

(Oxoid Ltd., Basingstoke, England)

Formula of base:

Special peptone	23 g
Starch	1 g
Sodium chloride	5 g
Agar No. 1	10 g

1. Dissolve base in 1 litre distilled water at 100°C.
2. Adjust pH to 7·3
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C and add 50 ml sterile defibrinated horse blood (Gibco Europe, Paisley).
5. Aliquot 15 ml into 90 mm sterile petri dish (Sterilin Ltd., Teddington).

Modified Campylobacter Selective Medium

(Gibco Europe, Paisley, Scotland)

Formula:

Tryptone	15 g
Soya peptone	5 g
Sodium chloride	5 g
Agar	15 g

1. Dissolve at 100°C in 1 litre distilled water
2. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C and add 10 ml vitamin K and haemin* (Gibco Europe, Paisley), 50 ml defibrinated sterile horse blood (Gibco Europe, Paisley) and 1 ml campylobacter antibiotic mix** (Gibco Europe, Paisely).
5. Pour 15 ml into sterile 90 mm Petri dish (Sterilin Ltd., Teddington).

* Vitamin K and haemin mixture:

Vitamin K	0•0005 g/ml
Haemin	0•0005 g/ml

** Campylobacter antibiotic mix

Vancomycin	0•010 g/l
Trimethoprim	0•005 g/l
Polymixin B	2,500 IU/l

Modified Wilkins-Chalgren Anaerobe Broth

(Gibco Europe, Paisley, Scotland)

Formula of base:

Tryptone	10 g
Gelatin peptone	10 g
Yeast extract	5 g
Dextrose	1 g
Sodium chloride	5 g
L - Arginine	1 g
Sodium pyruvate	1 g
Menadione	0·005 g
Haemin	0·005 g

1. Dissolve at room temperature in 1 litre distilled water.
2. Adjust pH to 7·1
3. Aliquot 250 ml volumes into sterile glass bottles.
4. Autoclave at 121°C for 15 minutes.
5. Cool to room temperature and add 2·5 ml inactivated horse serum (Gibco Europe, Paisley) to each bottle.

Modified Wilkins-Chalgren Anaerobe Agar

(Gibco Europe, Paisley, Scotland)

Formula of base:

Tryptone	10 g
Gelatin phosphate	10 g
Yeast extract	5 g
Dextrose	1 g
Sodium chloride	5 g
L - Arginine	1 g
Sodium pyruvate	1 g
Menadione	0•005 g
Haemin	0•005 g
Agar	10 g

1. Dissolve at 100°C in 1 litre distilled water.
2. Adjust pH to 7•1
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C and add 50 ml sterile lysed horse blood (Gibco Europe, Paisley).
5. Pour 18 ml into 90 mm sterile petri dish (Sterilin Ltd., Teddington). For the preparation of an antibiotic sensitivity plate, 2 ml of antibiotic solution was added at this stage and the plate gently swirled to ensure even mixing.

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Quantitative bacteriology of acute dento-alveolar abscesses

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Summary. A qualitative and quantitative bacteriological study was performed on pus specimens otained 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 $10^{6\cdot9\pm0\cdot2}$. The mean concentration of anaerobic bacteria was $10^{6\cdot2\pm0\cdot1}$ and of facultatively anaerobic bacteria $10^{5\cdot7\pm0\cdot2}$.

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. 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.

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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-μl 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 CO_2 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
Streptococcus milleri	25	*Peptostreptococcus* spp.	14
Streptococcus mitior	3	*Peptococcus* spp.	32
Streptococcus sanguis	3	*Streptococcus intermedius*	3
Streptococcus mutans	1	*Streptococcus constellatus*	1
Lactobacillus fermentum	2	*Propionibacterium acnes*	1
Lactobacillus salivarius	1	*Eubacterium lentum*	1
Actinomyces odontolyticus	1		
Actinomyces naeslundi	1		
Actinomyces meyeri	1		
Arachnia propionica	1		
		Veillonella parvula	3
Haemophilus parainfluenzae	2	*Bacteroides oralis*	20
Capnocytophaga ochracea	1	*Bacteroides gingivalis*	14
Eikenella corrodens	1	*Bacteroides melaninogenicus*	12
		Bacteroides intermedius	5
		Bacteroides ruminicola	6
		Bacteroides distasonis	1
		Bacteroides ureolyticus	1
		Bacteroides capillosus	1
		Bacteroides uniformis	1
		Fusobacterium nucleatum	6
		Fusobacterium mortiferum	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 Aderhold *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 CO_2, but precise data were not presented. Williams

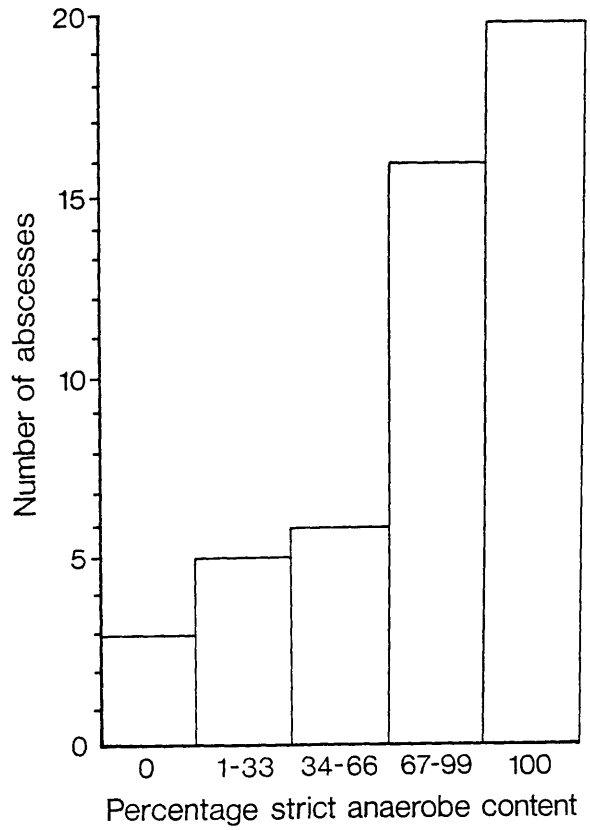

Figure. Distribution of abscesses according to percentage of content that was strictly anaerobic.

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 $(3{\cdot}3 \times 10^6)$–$(1{\cdot}2 \times 10^9)$ cfu/ml of pus, figures similar to ours. Interestingly, the mean concentration of $10^{6{\cdot}9}$ 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 ($10^{7{\cdot}7}$/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 CO_2-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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Oral Surgery

Short-course high-dosage amoxycillin in the treatment of acute dento-alveolar abscess

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In a randomised operator-blind clinical trial, the efficacy of a short course (one day) of high-dose amoxycillin was compared to a conventional regimen (five days) of phenoxymethylpenicillin in the treatment of 60 adult patients presenting with acute dento-alveolar abscess. Subjective and objective assessments were made of each patient's clinical symptoms on four occasions over the subsequent seven days. The spectrum of bacterial isolates and the antibiotic sensitivity patterns of the pus samples obtained from each treatment group were very similar. Patients receiving amoxycillin recorded a significantly greater reduction in swelling ($P < 0.05$) during the first 24 hours of treatment than patients receiving penicillin. There were no other significant differences between the two treatment groups at any time. Short-course high-dose amoxycillin was as effective as conventional penicillin therapy.

The management of acute dento-alveolar infections is influenced by the severity of infection, the presence of trismus, the patient's general health, attitudes to tooth loss, and the circumstances of dental practice. A primary principle is the establishment and maintenance of drainage by one or more methods, including tooth extraction, incision of soft tissue swelling or drainage through the root canal by opening into the pulp chamber. Many cases can be managed by drainage alone, but sometimes this cannot easily be achieved, for example, if swelling is so diffuse that there is no obvious point of incision, if extraction of the tooth is complicated by impaction, or if local anaesthesia cannot be obtained and general anaesthesia is either contra-indicated or not immediately available. If adequate drainage cannot be established, antibiotics are commonly prescribed until drainage is possible. They may also be prescribed in addition to drainage for patients who show signs of systemic toxicity or are in poor health.

Penicillin has long been the drug of choice in these circumstances as it is effective against most of the species of facultative and strictly anaerobic organisms in orofacial infections.[1] It is common practice to prescribe a five-day course of oral phenoxymethylpenicillin. However, it has been suggested that traditional regimens such as this should be scrutinised as there is doubt concerning the optimum duration of therapy,[2] and it is well recognised that many patients fail to complete a course of penicillin.[3] The idea of a 'complete course' of antibiotic has been challenged, and it has been proposed that therapy should be given for the shortest period at the lowest dose necessary for clinical cure.[4]

Recently, short-course high-dose amoxycillin has been used to treat otitis media,[5] urinary tract infections[6] and gonorrhoea[7] with as good results as longer conventional regimens.

Amoxycillin has also been recommended for the treatment of dental infections, as it has been shown to be effective against the vast majority of organisms isolated from acute dento-alveolar abscess[8] and high concentrations are achieved at sites of acute infection.[9]

The aim of this study was to evaluate the role of a short course of high-dose amoxycillin in the treatment of dento-alveolar abscess using conventional penicillin as a comparison.

Materials and method

Approval for the trial was granted by the Dental Ethics Committee of Glasgow Dental Hospital. Adult patients presenting at the Casualty Receiving Clinic with acute dento-alveolar abscesses were included in the study, subject to their informed consent. Patients were excluded if they had received antibiotics in the preceding three months, had any concurrent illness, were pregnant, or reported an allergy to penicillin. At initial presentation, objective and subjective assessments were made of each patient's symptoms. Whenever possible, tissue swellings were aspirated to collect pus and surgical drainage established either by extraction of the affected tooth, incision of an obvious tissue swelling or by opening of the pulp chamber, as appropriate.

Using random number tables,[10] patients were allocated to treatment with either short-course high-dose amoxycillin (one 3 g sachet Amoxil (Bencard) followed by a second sachet 8 hours after the first) or phenoxymethylpenicillin (Glaxo) (250 mg tablet 6-hourly for 5 days). Antibiotics were dispensed in coded envelopes by the general nursing staff. All patients were assessed on days 1, 2 and 4 by the same surgeon, who was unaware of the antibiotic therapy given to an individual patient, although access to this information was immediately available in case of emergency. On days 2 and 4, reduction in perceived swelling was also recorded. Patients received appropriate further treatment (re-incision, extraction or root canal drainage) whenever indicated. Patients with symptoms remaining on day 4 were seen again on day 7.

Pain measurement

On days 1, 2 and 4, patients registered the severity of their pain by making a mark on a horizontal 10-cm visual analogue scale.[11] One extremity (0 cm) of the line was labelled 'no pain', the other (10 cm) 'unbearable pain'. A scale value of pain was obtained by measuring the distance (mm) from zero to the mark placed by the patient.

Swelling measurement

On days 2 and 4, patients registered the reduction of their swelling using a horizontal 10-cm visual analogue scale. The boundaries of this scale were 'no swelling' (0 cm) and 'swelling at first visit' (10 cm). A scale value of swelling remaining was obtained by measuring the distance from zero to the mark placed by the patient.

Temperature measurement

Since infection may produce a local temperature effect, axillary temperature was recorded, in addition to oral temperature, at all visits using a mercury thermometer held in place for 90 seconds.

Lymphadenopathy

A positive score was recorded when one or more cervical lymph nodes were tender to palpation.

Statistical analysis

The distribution of results was examined and appropriate statistical tests applied to assess their significance. Since differences in pain values between the two groups were not distributed evenly, they were evaluated using Mann–Whitney *U* test.[10]

Differences in reduction of swelling, however, did have a normal distribution and were calculated using Student's *t*-test.[10]

Microbiological analysis

Pus samples were obtained by needle aspiration and transferred immediately to the laboratory for processing. Specimens were inoculated onto Columbia Blood Agar (Oxoid, Basingstoke) and modified campylobacter selective medium (Gibco Europe, Paisley) containing 0·1 ml campylobacter antibiotic mix[12] per 100 ml. Plates were incubated at 37°C aerobically and in an anaerobic chamber (Forma Scientific, Ohio) for 48 hours. Facultative Gram-positive cocci were identified using the API 20 system (API Laboratory Products, Basingstoke) for streptococci. Other facultative anaerobes were identified using standard laboratory methods.[13-15]

Strictly anaerobic isolates were identified using the Minitek System (Becton-Dickinson UK Ltd, Oxford). Anaerobic Gram-positive cocci were initially differentiated as peptococci or peptostreptococci by their susceptibility to a 5 μg novobiocin disc.[16]

Antibiotic sensitivity testing of the pus was performed on Columbia Blood Agar using Stokes' method,[17] the control organism being *Staphylococcus aureus* NCTC 6571. Antibiotic discs (Mast, Bootle) were used at the following concentrations: amoxycillin 10 μg and penicillin G 2 units. Plates were incubated at 37°C in an anaerobic chamber and read after 48 hours.

Results

Sixty patients completed the trial, of whom 30 received amoxycillin (Group A) and 30 penicillin (Group P). The two groups were closely matched with regard to sex and age (Table I).

Pain, swelling, temperature and lymphadenopathy in the two treatment groups are compared in Table II. With one exception, these clinical features did not differ significantly between the groups at the time of initial presentation or at any time during the study. Patients receiving amoxycillin reported a greater reduction in swelling in the first 24 hours of treatment than those receiving penicillin ($P < 0{\cdot}05$). The apparent greater reduction in pain in the same group over the same period was not statistically significant. All patients were symptom-free by day 7. No side-effects were reported by any of the patients receiving penicillin, while two patients in the amoxycillin group complained of nausea following the second 3 g dose.

Pus was obtained from 21 patients in the amoxycillin group and 20 patients in the penicillin group.

One hundred and thirty-seven isolates were obtained from 41 dento-alveolar abscesses: 97 (71%) were strict anaerobes and 40 (29%) were facultative anaerobes. The mean number of bacterial species was 3·3 per abscess. The identity of the 137 isolates is shown in Table III. The spectrum of bacterial species was very similar for both treatment groups. The anaerobic species most commonly isolated were *Bacteroides* spp. and *Peptococcus* spp. The majority of facultative bacteria were Gram-positive cocci, the commonest isolate being *Streptococcus milleri*.

The antibiotic sensitivity of the pus specimens is shown in Table IV. Both penicillin and amoxycillin were effective against the vast majority of samples. Two pus samples demonstrated resistance to the antibiotics; one in the amoxycillin group was resistant to both drugs, and one in the penicillin group was resistant to penicillin only.

Discussion

The object of systemic antibiotic therapy is to attain an effective drug concentration at the site of infection and to maintain this until the infection has been overcome. In the management of dento-alveolar abscess, this is frequently achieved by prescribing penicillin for a minimum of five days, even if symptoms resolve. Although there is no scientific evidence to support the concept of a complete course of antibiotic, it has probably arisen from the clinician's fear of encouraging the emergence of resistant bacterial strains if a course of antibiotics is not completed. On the contrary, it has been suggested that therapy beyond the time of clinical cure actually enhances the development of resistance.[4]

Short-course therapy has been shown to be satisfactory in the treatment of a number of common infections,[5-7] with the added advantages of better patient compliance, reduced accumulation of potentially harmful drugs at home and reduced chance of alteration of the resident microflora.

It is difficult to assess the effectiveness of an antibiotic in the treatment of dento-alveolar abscess because (1) clinical symptoms are not easy to quantify; (2) the effect of surgical drainage has to be separated from the effect of antibiotics; (3) there may be wide variations in the identity and antibiotic sensitivity patterns of the causative microorganisms between

Table I Comparability of amoxycillin-treated (A Group) and penicillin-treated (P group) groups

	A group	P group
Sex ratio M/F	4·0	3·3
Mean age	34·0	32·4
Age range (years)	14-65	18-65

Table II Comparison of the clinical features in the two treatment groups

	Amoxycillin			Penicillin			
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 4	Significance
Pain							
Yes	30	11	0	30	17	1	NS
Mean severity (±SD)	53·1±27·0	12·3±9·8	0	54·8±21·1	23·1±16·5	0·5	NS
Swelling							
Yes	30	30	3	30	29	2	NS
Mean value (±SD)	—	24·9±18·5	10·0±7·9	—	39·0±24·5	9·0±1·4	$P<0·05$[a], NS[b]
Temperature (°C)							
Oral 37·2+	9	0	0	10	3	0	NS
Axillary 37·0+	8	1	0	5	1	0	NS
Lymphadenopathy							
1 or more tender cervical nodes	4	4	1	4	4	0	NS
Pus obtained	21			20			

[a]Day 2
[b]Day 4

Table III Identity of 137 microorganisms isolated from 41 acute dento-alveolar abscesses

Facultative anaerobes				Strict anaerobes			
Organism identity	Group A	Group P	Total	Organism identity	Group A	Group P	Total
Gram-positive cocci				**Gram-positive cocci**			
α-Haemolytic streptococcus	3	4	7	*Peptococcus* spp.	13	12	25
β-Haemolytic streptococcus	3	2	5	*Peptostreptococcus* spp.	6	1	7
Streptococcus milleri	7	9	16	Unidentified	2	3	5
Gram-positive bacilli				**Gram-positive bacilli**			
Lactobacillus spp.	2	2	4	*Eubacterium lentum*		2	2
Actinomyces spp.		2	2				
Arachnia propionica		1	1				
				Gram-negative cocci			
				Veillonella parvula		1	1
Gram-negative bacilli				**Gram-negative bacilli**			
Haemophilus parainfluenzae	1	1	2	*Bacteroides capillosus*	2	1	3
Capnocytophaga orchracea	1		1	*Bacteroides corrodens*		1	1
Eikenella corrodens	1	1	2	*Bacteroides distasonis*	1		1
				Bacteroides gingivalis	6	5	11
				Bacteroides melaninogenicus	10	3	13
				Bacteroides oralis	7	8	15
				Bacteroides ruminicola	1	3	4
				Bacteroides uniformis		1	1
				Fusobacterium spp	1	1	2
				Unidentified	3	3	6
Total	18	22	40	Total	52	45	97

Table IV Antibiotic sensitivity of 41 pus samples obtained from the two treatement groups

	Antibiotic sensitivity[a]					
	Amoxycillin			Penicillin		
	S	MS	R	S	MS	R
Amoxycillin group	20	0	1	18	2	1
Penicillin group	18	2	0	13	6	1

[a]S = sensitive; MS = moderately sensitive; R = resistant

the treatment groups. The present study was designed to take account of these problems as far as possible. A comparison of clinical details for the two groups at the time of initial presentation strongly suggests that they were sufficiently similar for valid conclusions to be drawn. The surgical treatment each group received was the same, and pus was obtained from approximately two-thirds of the patients in each group. The identity of the microorganisms isolated and the antibiotic sensitivity of the pus samples was also very similar. The majority of isolates were strict anaerobes, which is in agreement with other recent microbiological studies of acute dento-alveolar abscess.[18-21]

It appears that a short course of high-dose amoxycillin is as effective as a conventional five-day course of penicillin for the treatment of acute dento-alveolar abscess. Our observation that swelling appears to be reduced more rapidly in the first 24 hours after high-dose amoxycillin is interesting and could be explained by the fact that high levels of amoxycillin can be achieved in localised abscesses.[9] It follows that high-dose therapy may be achieving inhibitory or bactericidal concentrations of antibiotic at the site of infection sooner than conventional doses of penicillin.

Patients with dental abscesses commonly present as an emergency to general medical practitioners at times when a dental service may not be readily available. The doctor usually prescribes an antibiotic, leaving the establishment of drainage until a dental surgeon is available. The results of this study suggests that the use of short-course high-dose amoxycillin could be useful in this situation, for if infection was at an early stage, gross abscess formation might be prevented. If an abscess has already formed, the high dosage may decrease the likelihood of any further spread of infection.

We do not know if a dosage smaller than 3 g would have been equally effective in the treatment of dento-alveolar infections. However, a 3 g dose was used in this study because dental practitioners are already familiar with this preparation owing to its established role in the prophylaxis of infective endocarditis following dental procedures. Indeed we know that the familiarity and the availability of 3 g amoxycillin in the dental surgery have already led to some practitioners using the preparation in the treatment of acute dental infections. The observations made in the study indicate that this practice is justificable.

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