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Prevalence, intensity and identity of bacteraemia following conservative dental procedures in children

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Introduction: The aim of this study was to investigate the prevalence, intensity and microbial identity of bacteraemia following conservative dental procedures. The procedures were placement of rubber dam, use of the fast drill, use of the slow drill and placement of a matrix band and wedge.

Method: Two hundred and five children and adolescents undergoing general anaesthesia for dental treatment at the Eastman Dental Hospital were recruited. Each subject was randomly allocated to one of the procedure groups. A baseline blood sample was taken before any dental treatment was carried out. A second blood sample was taken 30 s after a single conservative procedure. The blood samples were processed using lysis filtration. All bacterial isolates were identified using comparative 16 S ribosomal RNA gene sequencing. Oral S*treptococcus* spp. and coagulase-negative *Staphylococcus* spp. were further identified by comparative *sodA* gene sequencing.

Results: The prevalence of bacteraemia was significantly greater following placement of rubber dam ($P = 0.01$) and placement of matrix band and wedge, compared with baseline. The intensity of bacteraemia was significantly greater following placement of rubber dam ($P = 0.001$) and placement of matrix band and wedge ($P = 0.0001$). The most frequently isolated bacteria were *Streptococcus* spp. (56%), *Actinomyces* spp. (15%) and coagulase-negative *Staphylococcus* spp. (15%).

Conclusion: Conservative dental procedures are a significant cause of bacteraemia.

Key words: bacteraemia; conservative dentistry; *sodA* gene sequencing

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Until recently, it has been believed that the transient and short-lived bacteraemia following extraction of teeth has a significant role in the pathogenesis of infective endocarditis (15). Work investigating the prevalence of bacteraemia following other dentogingival manipulative procedures, for example placement of rubber dam, use of matrix band and wedge (33) and orthodontic separators (20) has shown clearly that treatment other than extractions causes a bacteraemia significantly greater than baseline. Current thinking has shifted towards the role of everyday procedures, for example tooth-brushing, in the pathogenesis of infective endocarditis. The guidelines for endocarditis prophylaxis from the American Heart Association (AHA) (39) were formulated on the premise that infective endocarditis is more likely to develop as a result of the frequent exposure to bacteraemia associated with daily activities, for example tooth-brushing, than a once or twice yearly dental procedure. Therefore, the routine administration of prophylactic antibiotics is not recommended. It is of note that the new UK guidelines published in 2006 (11) and the new AHA guidelines for 2007 (39) are very similar regarding the recommendations for dental prophylaxis. The most recent guidelines from the National Institute for Clinical Excellence (NICE) state

that there is no evidence that the development of infective endocarditis is a result of bacteraemia from dental treatment procedures (26). Antibiotic prophylaxis for dental treatment is no longer recommended (6).

The standard technique for detecting the prevalence of bacteraemia is broth culture. Although this method enables rapid bacterial identification, it does not provide information on the number of bacteria in the blood sample. The technique of lysis filtration, which has been developed for clinical purposes (12, 13), not only estimates the prevalence of bacteraemia but also estimates the intensity in colony-forming units per millilitre of blood. It is a sensitive method that provides a significantly higher bacterial yield from blood than other techniques (13). Lysis filtration has been shown to be successful in isolating organisms following dental extractions and other minor oral surgery procedures in adults (13). The technique has been validated by comparing test samples of known bacterial intensity (21). The time interval of 30 s after each dentogingival manipulative procedure was used for the second blood sample following earlier work (30, 34).

In clinical laboratories bacteria are generally identified using phenotypic tests. The inherent difficulties with phenotypic tests for identifying oral streptococci are that not all strains within a given species may be positive for a common trait (3, 17) and that the same species may exhibit biochemical variability (14, 36). Nucleic-acid-based technology has been developed resulting in improved bacterial characterization although a lack of discrimination of the 16 S ribosomal RNA (rRNA) genetic target for streptococci and coagulase-negative staphylococci has been reported (28, 29). For this investigation, the more discriminatory target, the *sodA* gene (28, 29) was used for species identification of oral streptococci and coagulase-negative staphylococci.

The purpose of this work was to determine not only the prevalence of bacteraemia following dental conservative procedures but also the intensity and microbial identity.

Materials and methods

Subjects

Ethical approval was granted by the Joint Research Ethics Committee of the Eastman Dental Institute and Hospital and the Research and Development Directorate of the University College London Hospitals (JREC No: 00/E039). Children and adolescents heavier than 17.5 kg undergoing general anaesthesia for dental treatment were recruited. Subjects with chronic medical disorders, predisposing cardiac lesions, known viral carriage, haemorrhagic disorders and difficult veins were excluded from the study. Written and verbal consent was obtained from the parents and verbal consent was obtained from the subjects.

Indices were recorded for dental plaque and gingival inflammation using a modification of the index of O'Leary (10) by visual examination of each tooth quadrisection (mesiobuccal, distobuccal, mesiolingual and distolingual) for the whole mouth and a separate score was recorded for the teeth involved in the procedure.

Groups

Using random number tables each subject was allocated into one of the following groups:

(i) Rubber dam and clamp: a clamp was placed on either a single, fully erupted maxillary or mandibular primary or permanent molar.
(ii) Fast drill: either a carious primary or permanent molar tooth was drilled for 1 min using a high-speed handpiece and a diamond bur with water irrigation.
(iii) Slow drill: either a carious primary or permanent molar tooth was drilled for 1 min using a slow-speed handpiece and a number 4 rosehead bur.
(iv) Matrix band and wedge: a matrix band was placed on either a mandibular or maxillary primary or permanent molar. A wooden wedge was pushed between the matrix band and the adjacent tooth.

To avoid any confounding bacteraemia, the allocated procedure was completed in isolation before the planned treatment was started.

Blood samples

Following attainment of general anaesthesia, a 21-gauge Y-cannula (Wallace, Watford, UK) was placed in a vein in either the right or left antecubital fossa using aseptic technique. The skin was prepared using 1% povidone iodine solution and 70% isopropyl alcohol BP. A 0.5-ml sample of blood was withdrawn and discarded to void any skin contaminants (32). Using a separate sterile syringe, 6 ml blood was withdrawn and placed immediately into a sterile universal bottle containing 1.23 ml 0.35% of sodium polyanetholesulfonate (SPS; Sigma, St Louis, USA) solution to prevent clotting and to inactivate the natural antibacterial action of the blood. This was the pre-procedure or baseline sample. The cannula was flushed with approximately 1 ml sterile physiological saline. Thirty seconds after the procedure, a further 6 ml blood was withdrawn (30) and placed into a second sterile universal bottle containing 1.23 ml 0.35% SPS solution. The cannula was removed and the planned dental treatment was carried out.

Laboratory procedures

Lysis filtration

All blood samples were processed, using lysis filtration (12, 13, 21), within 1 h of collection, in a class 1 microbiological safety cabinet (BioMat-1, Medical Air Technology, Oldham, UK). Each 6 ml blood sample was added to lysing solution. Two equal volumes of the solution were poured into a disposable, sterile filtration unit and drawn through a 0.45-μm pore filter by negative pressure of approximately 460 mm Hg. Each filter was inoculated onto brain–heart infusion agar (Becton & Dickinson, Oxford, UK) supplemented with 5% defibrinated horse blood. One filter was incubated aerobically and the other filter was incubated in an anaerobic chamber, for 10 days.

As a negative control, the sterile distilled water for preparation of the streptokinase/streptodornase was filtered. The filter was cut in half, and each half was inoculated onto brain–heart infusion agar and incubated as described above. Non-inoculated plates were also included with each filtration run. From the third day for a period of 10 days each filter was checked for bacterial growth using a stereomicroscope (Fig. 1).

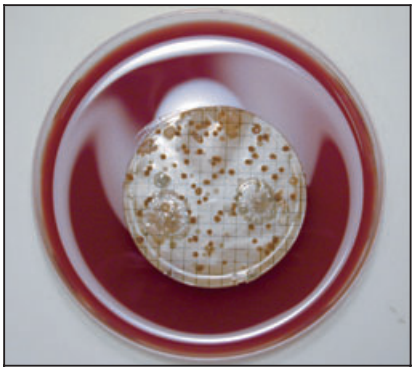

Fig. 1. Bacterial growth on filter after 10 days.

Detection limits

The detection limit for aerobic or anaerobic intensity was 0.33 colony-forming units (CFU)/ml. The detection limit for total bacterial intensity was 0.17 CFU/ml.

Outcome variables

These were: the prevalence (%) of bacteraemia; the intensity of bacteraemia (CFU/ml); the identity of bacteria.

Data analysis

All data were tested for normality using the Shapiro–Wilks test and found to be not normally distributed. McNemar's test was used to detect any difference in the proportion of positive blood cultures between baseline and postprocedure. The Wilcoxon signed ranks test was used to compare baseline and postprocedure intensities within each group. The statistics package used was SPSS for Windows version 11.0 (SPSS Incorporated, Chicago, IL).

Microbial identification

From each membrane filter representative colonies of each morphology type were subcultured. All bacteria were subjected to partial sequencing of the 16 S rRNA gene region. Oral *Streptococcus* spp. and coagulase-negative *Staphylococcus* spp. were further speciated by partial sequencing of the manganese-dependent *sodA* gene (28, 29).

16 S rRNA amplification and partial gene sequencing

Single bacterial colonies were suspended in a total reaction volume of 100 μl polymerase chain reaction (PCR) mastermix containing the following: 0.2 mM of each dNTP (dATP, dCTP, dGTP and dTTP; Promega, Madison, WI), 2.5 mM $MgCl_2$, 0.2 μM of each PCR primer 27f (5′-AGAGTTTGATCMTGGCTCAG) and 1492r (5′-TACGGYTACCTTGTTACGACTT) (Genosys, Cambridge, UK), 1 × reaction buffer [$(NH_4)_2SO_4$, Tris–HCl Tween-20; Bioline, London, UK] and 1 U *Taq* polymerase (Bioline).

PCR amplification was performed in a thermal cycler (Primus, MWG-Biotech AG, Ebersberg, Germany) according to the following reaction parameters: 94°C for 5 min, 29 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1.5 min, followed by an extension period of 72°C for 5 min. A negative control using sterile, nuclease-free water and a positive control of *Capnocytophaga ochracea* template were also prepared for each reaction.

PCR products of approximately 1500 base pairs (bp) were assessed using gel electrophoresis in a 1% agarose gel (Amresco, Solon, OH) made with Tris–acetate ethylenediaminetetraacetic acid buffer (TAE; Eppendorf, Hamburg, Germany) containing ethidium bromide (0.5 μg/ml) viewed under ultraviolet transillumination. The end well was loaded with a molecular weight marker (50–2000 bp; Amresco). The PCR products were cleaned using QIAquick PCR purification kit (Qiagen, Crawley, UK) and stored at −20°C until sequencing. The sequencing reaction was carried out by adding 3 μl sterile nuclease-free water, 2 μl of ABI Prism BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Warrington, UK) diluted 1 : 4 in 5 × sequencing buffer (400 mM Tris–HCl, 10 mM $MgCl_2$), 1 μl 357f primer (5′-CTCCTACGGGAGGCAGCAG; 5 pmol/μl; Genosys) and 1 μl DNA template to give a final reaction volume of 7 μl. The reaction was performed in a thermal cycler (Primus) according to the following programme; 99 cycles at 95°C for 10 s, 50°C for 5 s and 60°C for 4 min.

SodA PCR and gene aequencing for oral streptococci

The PCR master mix (50 μl) contained: 0.6 μM of each primer (D1 and D2), 0.4 M dNTPs (dATP, dCTP, dGTP, dTTP; Promega), 2.5 mM $MgCl_2$, 2 × reaction buffer and 1 U *Taq* polymerase (Bioline). PCR amplification was performed in a thermal cycler (Primus) according to the following conditions: 95°C for 3 min, 35 cycles at 95°C for 30 s, 47°C for 90 s, 72°C for 90 s, followed by an extension period of 72°C for 10 min.

The PCR tubes containing 50 μl of PCR product were purified using the Qiagen purification kit (Qiagen) and sequenced using D1 primer (Oswel Company, Eurogentec, Romsey, UK). The resulting electrophoretograms were analysed in the same manner as described earlier using CHROMAS version 1.45. Stringent criteria were used and only sequences with 300 or more bases, tall peaks, low background noise and a minimal number of unidentified bases (Ns) were submitted to the BLAST analysis database (1, 2). The matches with the highest alignment scores were accepted as final species identification.

Results

Subjects

From a preliminary sample of 247 subjects, 205 were recruited for the study. Thirty-six subjects refused to participate and a further six were excluded because of failed venepuncture. There were 102 boys and 103 girls and the mean age was 10.8 years (SD 3.67), range 4–17.5 years.

Dental plaque and gingivitis scores

The mean plaque and gingivitis scores for the whole group are shown in Table 1.

Prevalence of bacteraemia

There was a significantly greater prevalence of bacteraemia following both placement of rubber dam ($P = 0.01$) and placement of matrix band and wedge ($P = 0.001$) compared with baseline (Table 2).

Intensity of bacteraemia

There was a significantly greater number of CFU/ml of bacteria isolated aerobically following placement of rubber dam ($P = 0.005$), use of the slow drill

Table 1. Dental plaque and gingivitis scores and indices: whole mouth; ($n = 205$)

	Mean	SD	Median	Min–Max
Plaque score	11.8	8.9	10.0	0–60
Plaque index	12.6	9.2	11.4	0–54
Gingivitis score	1.2	3.4	0	0–32
Gingivitis index	1.2	3.3	0	0–30

n = number of subjects.

Table 2. Prevalence of bacteraemia (%): baseline and following each dentogingival manipulative procedure ($n = 41$)

	Baseline		Postprocedure		
Procedure	%	Number	%	Number	Significance
Rubber dam	29	12	54	22	$P = 0.01$
Fast drill	15	6	22	9	$P = 0.5$
Slow drill	10	4	22	9	$P = 0.2$
Matrix band and wedge	32	13	66	27	$P = 0.001$

Table 3. Aerobic intensity of bacteraemia (CFU/ml): baseline and following each dentogingival manipulative procedure (n = 41)

	Baseline				Postprocedure				
Procedure	Median[1]	N^{DB}	Median	Min–Max[2]	Median	N^{DB}	Median	Min–Max[2]	Significance
Rubber dam	ND	7	0.33	0.33–0.67	ND	17	0.33	0.33–6.33	P = 0.005
Fast drill	ND	4	0.5	0.33–1.33	ND	7	0.33	0.33–0.67	P = 0.6
Slow drill	ND	2	0.33	NC	ND	9	0.33	0.33–0.67	P = 0.02
Matrix band and wedge	ND	9	0.33	0.33–2.0	ND	18	0.5	0.33–31.7	P = 0.002

ND, not detected; N^{DB}, number of subjects with a detectable bacteraemia (≥0.33 CFU/ml); n = number of subjects; NC, not calculable.
[1]Median for all subjects (n = 41).
[2]Median and min-max for subjects with a detectable bacteraemia.

Table 4. Anaerobic intensity of bacteraemia (CFU/ml) at baseline and following each dentogingival manipulative procedure (n = 41)

	Baseline				Postprocedure				
Procedure	Median[1]	N^{DB}	Median	Min–Max[2]	Median	N^{DB}	Median	Min–Max[2]	Significance
Rubber dam	ND	6	0.33	NC	ND	16	0.83	0.33–10.33	P = 0.001
Fast drill	ND	4	0.33	NC	ND	5	0.33	0.33–4.67	P = 0.4
Slow drill	ND	2	0.5	0.33–0.67	ND	1	0.5	NC	P = 1.0
Matrix band and wedge	ND	6	0.33	NC	0.33	21	0.67	0.33–24.7	P = 0.0001

ND, not detected; N^{DB}, number of subjects with a detectable bacteraemia (≥0.33 CFU/ml); n = number of subjects; NC, not calculable.
[1]Median for all subjects (n = 41).
[2]Median and min-max for subjects with a detectable bacteraemia.

(P = 0.02) and placement of matrix band and wedge compared with baseline (P = 0.002) (Table 3). A significantly greater number of CFU/ml were isolated anaerobically following both placement of rubber dam (P = 0.001) and matrix band and wedge compared with baseline (P = 0.0001) (Table 4).

Identity of bacteria isolated

A total of 628 bacterial isolates were recovered from the membrane filters of which 53 were from baseline blood samples and 575 from postprocedure samples The greatest number of bacterial colonies were isolated following placement of matrix band and wedge (n = 357). One hundred and thirty-seven colonies were isolated following rubber dam placement, 27 following use of the fast drill and 16 following use of the slow drill (Table 5). Of these, S*treptococcus* spp. comprised 3.8% and 52%, *Staphylococcus* spp. comprised 49% and 18.3% at baseline and postprocedure respectively, and *Actinomyces* spp. 14% postprocedure only (Table 5).

Discussion

The technique commonly used for processing blood cultures is broth culture. The prevalence of bacteraemia and bacterial identification are available within a few hours but broth culture does not provide information on the number of bacteria in the blood sample. Lysis filtration has been developed for clinical purposes (12, 13) and provides both an estimate of the prevalence of bacteraemia and the intensity in CFU/ml blood. It is a sensitive method that provides both a significantly higher prevalence of bacteraemia (13) and a higher bacterial yield from blood than other techniques.

Other workers have reported a significant bacteraemia following fillings, stainless steel crown placement and extractions (4), endodontic treatment (9) and restorative treatment under general anaesthesia (19). Although these are, in general, supportive of the present findings, interpretation is difficult because the samples taken were following a series of other procedures, which is likely to have confounded the results.

The range of bacteria isolated was similar to that from a variety of other dental treatment procedures using lysis filtration (13, 20, 31, 33).

The most frequently implicated oral microorganisms in the development of infective endocarditis have been *Streptococcus* species (18, 22, 37, 40). The isolation of *Staphylococcus* species, particularly *Staphylococcus aureus*, from individuals with infective endocarditis has increased, causing almost 50% of cases of infective endocarditis (7, 25, 35, 38). In the investigation reported here, several species of coagulase negative staphylococci that have been isolated after conservative dental procedures have been implicated in both native and prosthetic valve endocarditis (8). These include *Staphylococcus capitis*, *Staphylococcus epidermidis* and *Staphylococcus hominis*. Coagulase negative staphylococci have generally been regarded as skin contaminants. In the present study this is unlikely because early work with lysis filtration demonstrated that insertion of the venepuncture needle only through the skin and not into the vein, using aseptic technique, resulted in only two positive blood cultures for *S. epidermidis* from a total of 100 cases (41). Subgingival *Staphylococcus* spp. have been isolated from both children (23) and 30–70% of healthy adults (16, 24, 27).

Even though sequencing of the 16 S rRNA gene region is widely used for bacterial identification, difficulties arise with very closely related taxa such as mitis group streptococci and coagulase negative staphylococci. In these cases the resolution of the 16 S rRNA gene is not sufficient to allow species level identification. *Streptococcus* and *Staphylococcus* spp. isolated in this study showed low discrimination in 16 S rRNA sequences between the species with identical alignment scores and expect values (e-values) when analysed by BLAST (1, 2). By using an alternative sequencing target, *soda* (28, 29), identification of these 'difficult' groups was achieved and has shown that a large number of different species were isolated. The main disadvantage is the necessity for an additional amplification and sequencing step following the 16 S rRNA gene sequencing.

However, during the last 2 or 3 years there has been a radical change in thinking regarding any link between dental treatment procedures and the development of infective endocarditis. The British Society for Antimicrobial Chemotherapy (11) and

Table 5. Bacterial species isolated from blood samples

Bacteria	Baseline (n = 53)	Postprocedure (n = 575)				
		RD	FD	SD	MBW	Total CFU
Streptococci	2 (3.8%)	86	3	1	209	301 (52%)
S. oralis	–	9	2	–	124	135
S. mitis	–	44	–	1	39	84
S. gordonii	1	3	–	–	3	7
S. sanguinis	–	6	–	–	11	17
S. parasanguinis	–	12	1	–	–	13
S. pneumoniae	–	1	–	–	4	5
S. infantis	1	1	–	–	–	2
S. australis	–	4	–	–	–	4
S. peroris	–	2	–	–	–	2
S. constellatus	–	–	–	–	4	4
S. salivarius	–	–	–	–	3	3
S. mutans	–	–	–	–	9	9
Unidentified streptococci	–	4	–	–	12	16
Staphylococci	**26 (49%)**	**29**	**11**	**5**	**34**	**105 (18.3%)**
S. epidermidis	10	16	2	1	8	37
S. hominis	13	10	5	4	18	50
S. warneri	3	2	3	–	4	12
S. capitis	–	1	–	–	–	1
S. cohnii	–	1	1	–	1	3
S. haemolyticus	1	–	–	–	–	–
S. saprophyticus	–	–	–	–	1	1
S. schleiferi	–	–	–	–	1	1
Actinomyces	–	**7**	–	–	**74**	**81 (14%)**
A. odontolyticus	–	–	–	–	55	55
A. naeslundii	–	6	–	–	7	13
A. viscosus	–	1	–	–	3	4
A. georgiae	–	–	–	–	2	2
A. lingnae	–	–	–	–	1	1
A. gerencseriae	–	–	–	–	6	6
Rothia	**1**	**2**	–	**1**	**3**	**7**
R. mucilaginosus	–	1	–	1	2	4
R. dentocariosa	1	1	–	–	1	3
Neisseria spp.	–	**2**	–	**2**	**4**	8
N. flava-like	–	2	–	2	1	5
N. pharynges	–	–	–	–	3	3
P. acnes	**3**	**4**	**10**	**2**	3	22
Micrococcus luteus	**9**	**2**	**2**	**1**	2	16
Haemophilus parainfluenza	–	–	–	–	21	21
Aerococcus viridans	–	**1**	–	–	–	1
Veillonella parvula	–	–	–	–	2	2
Corynebacterium spp.	**1**	–	–	–	2	3
Brachybacterium spp.	**3**	–	–	**1**	1	5
Other	5	–	–	2	2	**9**
Unidentified	3	5	–	1		**9**

RD, rubber dam + clamp; FS, fast drill; SD, slow drill; MBW, matrix band + wedge.

the AHA (39) have reduced the cardiac-risk patients to only three categories. These are previous infective endocarditis, prosthetic valve, and surgically constructed conduits and shunts; these individuals should be prescribed antibiotic prophylaxis for all dentogingival manipulative procedures except a simple examination with a mirror. This means that a smaller number of patients should receive antibiotic prophylaxis for a greater range of treatment procedures. Furthermore, the AHA considered that the development of infective endocarditis was more likely as a result of everyday activities, for example, tooth-brushing than treatment carried out at an 'isolated' visit to the dentist. NICE have produced evidence-based draft guidelines (26) that support the AHA view that individuals are more at risk from everyday activities than isolated episodes of dental treatment. In addition, only an extremely small number of cases of infective endocarditis might be prevented by antibiotic prophylaxis even if prophylaxis were 100% effective.

The NICE guidelines were published recently (March 2008) and indicate that antibiotic prophylaxis, or chlorhexdine mouth rinses, are no longer recommended for any dental treatment, even for those individuals who have until now been considered to be at high risk of developing infective endocarditis (26).

So where does this leave us? The single most important activity is probably the reduction of oral bacterial loading for all these subjects by effective tooth-brushing and interdental cleaning. This will be the greatest challenge and objective data are needed to support this strategy. Current work is in progress on the estimation of a change in odontogenic bacteraemia following personalised tooth-brush instruction with or without the use of dental floss.

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