Fig. S1: Microbiology test results for the identification of *E. faecalis* and *S. anginosus*. The *E. faecalis* strain demonstrated large alpha haemolytic white colonies (1-2mm in size), chains of Gram-positive cocci approximately 1-2μm in diameter, was catalase negative, able to grow on MacConkey agar with a pink colony colour, able to grow on bile aesculin agar with a characteristic black agar colour change and belonged to the group D Lancefield grouping. The *S. anginosus* strain formed small alpha haemolytic colonies (<0.5mm) on blood agar, consisted of chains of Gram-positive cocci approximately 1-2μm in diameter, was catalase negative, unable to grow on MacConkey and bile aesculin agar and was positive for Group F Lancefield grouping. No growth of *S. anginosus* was detected after heat treatment and on bile aesculin agar supplemented with 6.5%w/w sodium chloride (data not shown).
D88 (forward) sequence (query) alignment with *Enterococcus faecalis* strain CAU:180 16S ribosomal RNA gene, partial sequence (Accession number: MF369839.1; subject).
B

E. faecalis clinical isolate E94 sequence showed a 97% sequence identity with E. faecalis

E94 (reverse) sequence (query) alignment with Enterococcus faecalis partial 16S rRNA gene, isolate BS3-1 (Accession number HG798397.1; subject).

 Query  850  CGCATTAAGCACTCCGCCCTGGGGAGTACGACCGCAAG-T-GAACTTCAAAGGAA-TGAC  906
                  ^                                                ^
 Sbjct  847  CGCATTAAGCACTCCGCCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAATTGAC  905
 Query  907  GGGGGCCCGCCACA-GCGTTG-AGCATGGTGGATTTATTCGA-GCAACGCGA-GA-C-T-  959
                  ^                                                ^
 Sbjct  906  GGGGGCCCGC-ACAAGCGGTGGAGCATG-TGGTTTAATTCGAAGCAACGCGAAGAACCTT  963
 Query  960  AC-AG-TCTTGACACTCTCTTTGA  981
                  ^                                                ^
 Sbjct  964  ACCAGGTCTTGACA-TC-CTTTGA  985

E. faecalis
Query | 732 | GTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCGTCGCC | 791
Sbjct | 217 | GTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCGTCGCC | 158

Query | 792 | ACTGGTGTTCCTCCATATATCTACGCATTTCAC-GCTACACATGGAGT-C-ACTCT-CTC | 847
Sbjct | 157 | ACTGGTGTTCCTCCATATATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTC | 98

Query | 848 | TTCTGCACTCAAGTCTCCCAGTTTCCAATGA-CCCTCCCCGGTTGAGCCGGGGGCTTTCTC | 904
Sbjct | 97 | TTCTGCACTCAAGTCTCCCAGTTTCCAATGA-CCCTCCCCGGTTGAGCCGGGGGCTTTCTC | 39

Query | 905 | CATCCCAACTTAA-AAACGCCTGCGCGTGCTT | 936
Sbjct | 38 | CATCCCAACTTAA-AAACGCCTGCGCGTGCTT | 8

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C

*S. anginosus* clinical isolate D88 sequence showed 98% sequence identity with *S. anginosus*

D88 (forward) sequence (query) alignment with Streptococcus anginosus strain ChDC B407-l 16S ribosomal RNA gene, partial sequence (Accession number KC569583.1; subject).

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Query | 8 | GGCG-GGCT-ATA-ATGCA-GTAGGACGCACAGTTTATACCGTAGCTTGCTACACCATAG | 63
Sbjct | 22 | GGCG-TGCTCCAATACATGCAGGTTAGGACGCACAGTTTATACCGTAGCTTGCTACACCATAG | 81

Query | 64 | ACTGTGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTATTAGAGGGGGATAAC | 123
Sbjct | 82 | ACTGTGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTATTAGAGGGGGATAAC | 141

Query | 124 | TATGGAAACGATAGCTAATACCCTGCTACTGCTACTGCT ACTTGAAG | 183
Sbjct | 142 | TATGGAAACGATAGCTAATACCCTGCTACTGCTACTGCT ACTTGAAG | 201

Query | 184 | ATGCAATTGCATCGCTAGTAGATGGACCTGCGTTGTATTAGCTAGTAGGTAGGGTAATGG | 243
Sbjct | 202 | ATGCAATTGCATCGCTAGTAGATGGACCTGCGTTGTATTAGCTAGTAGGTAGGGTAATGG | 261

Query | 244 | CCTACCTAGGGCAGCGATACATAGCGACCTGAGAGGGGTAGTGAACCTGCCACACTTGGACCTG | 303
Sbjct | 262 | CCTACCTAGGGCAGCGATACATAGCGACCTGAGAGGGGTAGTGAACCTGCCACACTTGGACCTG | 321

Query | 304 | ACAACGGCCCAAGCTCTCCAAGGAGCGAGCACTGGAATCTTCCGCAATGGGGGGAACCC | 363
Sbjct | 322 | ACAACGGCCCAAGCTCTCCAAGGAGCGAGCACTGGAATCTTCCGCAATGGGGGGAACCC | 381

Query | 364 | TGACCAGCAACGCGCGCGTGAGTAAGAGAGGTTTTCGACGATGTAAGCTCTTGTTGAAG | 423
Sbjct | 382 | TGACCAGCAACGCGCGCGTGAGTAAGAGAGGTTTTCGACGATGTAAGCTCTTGTTGAAG | 441

Query | 424 | GAAGAAACGAGGTGGGAGAAGAGATTTCTACTGCTAGGCTACTTACCAGAAAGGGGACG | 483
Sbjct | 442 | GAAGAAACGAGGTGGGAGAAGAGATTTCTACTGCTAGGCTACTTACCAGAAAGGGGACG | 501

Query | 484 | GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGATCTCCGCGGATTTATT | 543
Sbjct | 502 | GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGATCTCCGCGGATTTATT | 561

Query | 544 | GGCGTAAAGCGAGCGCAGCAGCGGGTTGAGAAGATCTCGAAGTAGAAGAGCGAGTGCTCAACCA | 603
Sbjct | 562 | GGCGTAAAGCGAGCGCAGCAGCGGGTTGAGAAGATCTCGAAGTAGAAGAGCGAGTGCTCAACCA | 621
**D**

*S. anginosus* clinical isolate E94 sequence showed a 96% sequence identity with *S. anginosus*.

E94 (reverse) sequence (query) alignment with Streptococcus anginosus strain SK52 16S ribosomal RNA, complete sequence (Accession number NR_041722.2; subject).
Fig. S2: Sequence alignments for *E. faecalis* and *S. anginosus*. Bacterial 16S rRNA DNA sequence reactions were performed using DNA from clinical isolates of *E. faecalis* (A and B) and *S. anginosus* (C and D) with universal primers D88 (A and C) and E94 (B and D). The 16S rRNA sequencing revealed that the clinical isolates used in this study had an average of 97% sequence identity with *E. faecalis* and *S. anginosus* species respectively. The microbiological tests and the sequencing results confirmed that the clinically isolated species of bacteria used in this study were *E. faecalis* and *S. anginosus*. 
Fig. S3: Immunohistochemistry controls for TNF-α and IL-1β for the mixed species infection experiment. P represents the dental pulp, O the odontoblast region and D the dentine. Representative images from three experimental repeats shown.
Fig. S4: SDS-PAGE and silver stain of bacterial (A) water soluble cell wall proteins and (B) culture supernatants: (i) *S. anginosus*, (ii) *E. faecalis*, (iii) 50:50 *S. anginosus* : *E. faecalis* and (iv) 90:10 *S. anginosus* : *E. faecalis*. Arrow highlights a difference in supernatant proteins at approximately 35kDa.
Fig. S5: Immunohistochemistry controls for TNF-α and IL-1β for the *E. faecalis* heat killed and supernatant experiment. *P* represents the dental pulp, *O* the odontoblast region and *D* the dentine. Representative images from three experimental repeats shown.
Fig. S6: Method of counting pulpal cells using ImageJ software.
Fig. S7: Method of fluorescence quantification using ImageJ software.

% of pulp covered by bacteria = (measured area/area of pulp) x 100
Fig. S8: RNA integrity for (a) Control, (b) *E. faecalis*, (c) *S. anginosus*, (d) 50:50 *S. anginosus*: *E. faecalis* and (e) 90:10 *S. anginosus*: *E. faecalis* samples showing 28S and 18S RNA bands.
Fig. S9: Representative melt curve peaks for (A) GAPDH, (B) β-actin, (C) HPRT-1, (D) RPL13a, (E) 18s rRNA, (F) TNF-α and (G) IL-1β (red line represents a samples whilst the blue line represents a no template control).
Fig. S10: Representative qPCR products run on gel for one *E. faecalis* infected tooth sample (top row) and the no template control (bottom row): (A) GAPDH, (B) β-actin, (C) HPRT-1, (D) RPL13a, (E) 18s rRNA, (F) TNF-α and (G) IL-1β.
Fig. S11: Primer efficiency curves for (A) GAPDH, (B) β-actin, (C) HPRT-1, (D) RPL13a, (E) 18s rRNA, (F) TNF-α and (G) IL-1β.
Fig. S12: NormFinder results for qPCR reference gene selection.