Article:
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Table S1: Demographic and clinical data of teeth included in the study.*

						Soft tissue		Radiolucency		
Participants	**Age (years)**	**Gender[a]**	**Tooth[b]/Canal[c]**	**Infection**	**Discomfort/Pain**	**Sinus**	**swelling**	**Only Widening of PL[d]**	**<10mm**	**>10mm**
SUB-001	25	F	LR6/D	Primary	Yes	No	Yes	No	Yes	No
SUB-002	67	F	UR4/P	Secondary	No	No	No	No	Yes	No
SUB-003	34	F	LR7/D	Primary	Yes	No	Yes	No	Yes	No
SUB-006	28	F	UR1	Primary	No	No	No	No	Yes	No
SUB-007	49	F	LR6/D	Secondary	Yes	Yes	No	No	Yes	No
SUB-008	20	M	UL1	Primary	No	No	No	No	Yes	No
SUB-009	43	M	UR2	Secondary	Yes	No	Yes	No	No	Yes
SUB-010	57	F	LL5	Secondary	No	No	No	No	Yes	No
SUB-011	36	F	LL1	Secondary	No	No	No	No	Yes	No
SUB-012	35	F	UR4/P	Secondary	Yes	Yes	No	No	Yes	No
SUB-013	41	M	UR1	Primary	Yes	Yes	Yes	No	No	Yes
SUB-014	65	F	UR1	Primary	No	Yes	No	No	Yes	No
SUB-015	43	M	UR1	Secondary	Yes	No	Yes	No	No	Yes
SUB-017	26	F	UR1	Secondary	Yes	No	No	No	Yes	No
SUB-018	52	M	UR6/D	Primary	Yes	No	No	Yes	No	No
SUB-019	47	F	UL2	Primary	Yes	No	No	No	Yes	No
SUB-020	47	F	UL1	Primary	Yes	No	No	Yes	No	No
SUB-021	50	F	UL2	Secondary	No	No	No	No	Yes	No
SUB-022	50	F	UR2	Primary	No	No	No	No	Yes	No

a: F – female; M – male. **b**: U-upper, L-lower, R-right, L-left. **c**: D-distal, P-palatal. **d**: PL-periodontal ligament

***33** samples were used to carry out library preparation and sequencing procedures. 5 samples (011 S2, 017 S1 and S2, 019 S1 and S2) were discarded due to inadequate amplification, short library amplicon size and/or poor raw sequencing quality.

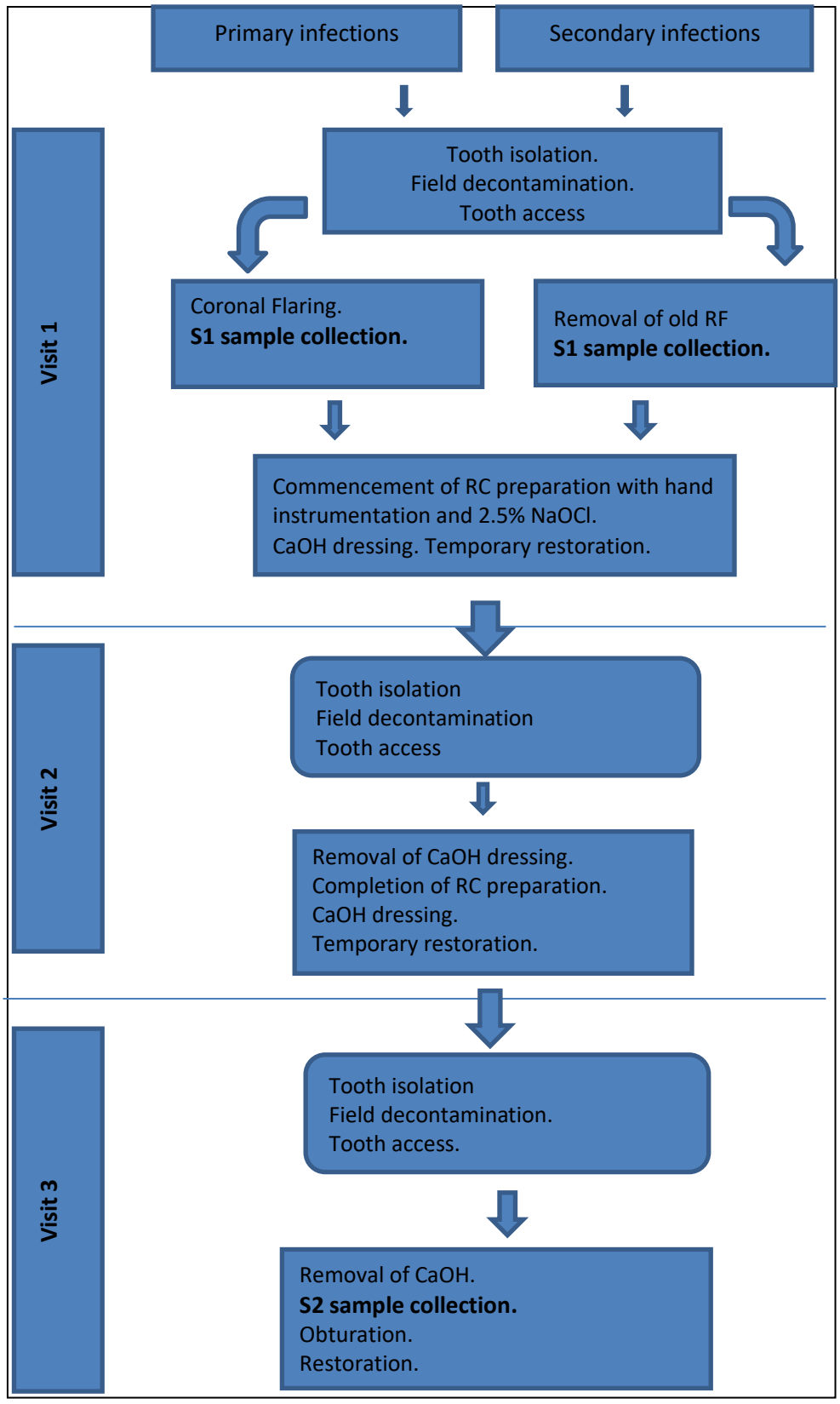

Figure S1: Flowchart of the clinical procedures undertaken. RF: root filling, RC: root canal. Participants who previously had root filled teeth may need more than one visit to complete intra-canal medication. This is because of the need to remove old root filling, which is standard procedure, and which explains the need for the second visit.

Table S2: Master mix agents and their volumes for each PCR tube.

Component	Volume (µl)
5X Q5 reaction buffer	5
10 mM dNTPs	0.5
10mM 347F primer	1.25
10 mM 803R primer	1.25
template DNA	2
High fidelity DNA polymerase	0.25
5X Q5 enhancer	5
Nuclease free water	9.75
Total	25

Table S3: Thermocycling conditions for the amplification of the partial 16S rRNA gene.

Step		Temperature	Time
Initial Denaturation		98 °C	30 seconds
35 cycles:	**Denaturation** **Annealing** **Extension**	98 °C 60 °C 72 °C	10 seconds 30 seconds 30 seconds
Final extension:		72 °C	2 minutes
Hold:		10 °C	

Table S4: Thermocycling conditions for the 'end repair' preparation.

Time	Temperature °C
30 minutes	20
30 minutes	65
Hold	4

Table S5: Thermocycling conditions for the addition of indexing primers.

Step	Temperature °C	Time	Cycles
Initial denaturation	98	30 seconds	1
Denaturation Annealing Extension	98 65 72	10 seconds 30 seconds 30 seconds	15
Final extension	72	5 minutes	1
Hold	4		

Table S6: Operational taxonomic unit (OTU) count per sample.

	Sample ID	No of OTUs (S1)	No of OTUs (S2)
1	001	14341	16209
2	002	33376	20738
3	003	18658	87509
4	006	36925	42531
5	007	30183	30347
6	008	13759	23073
7	009	34225	21377
8	010	33507	31376
9	011	24386	Discarded
10	012	26380	34477
11	013	39936	30212
12	014	24305	52847
13	015	36235	35513
14	017	Discarded	Discarded
15	018	61264	45153
16	019	Discarded	Discarded
17	020	48304	37154
18	021	43550	35342
19	022	35823	49843

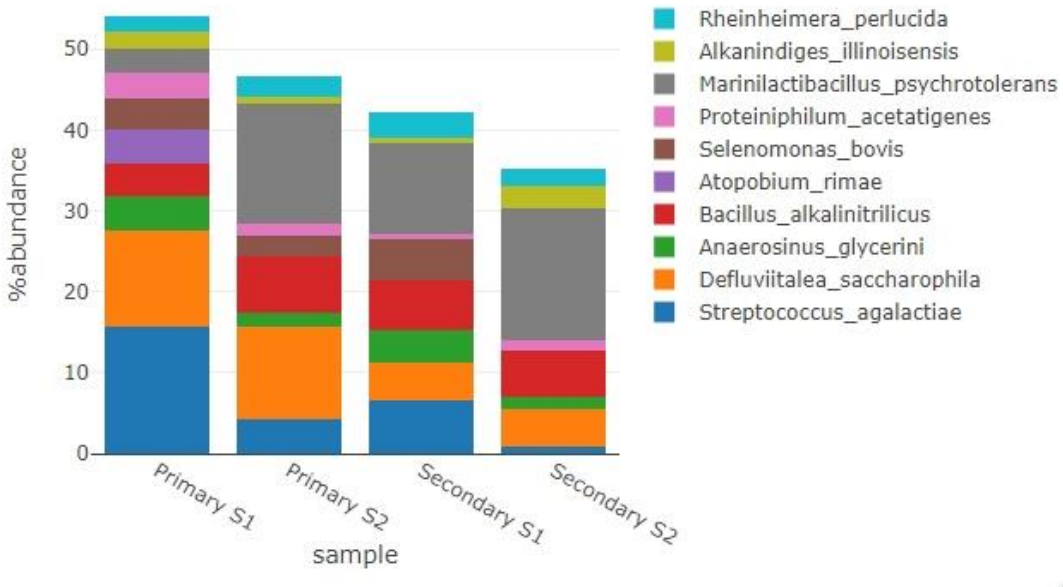

Figure S2: Ten of the most abundant species in primary S1 samples and their corresponding abundance in other sample groups.

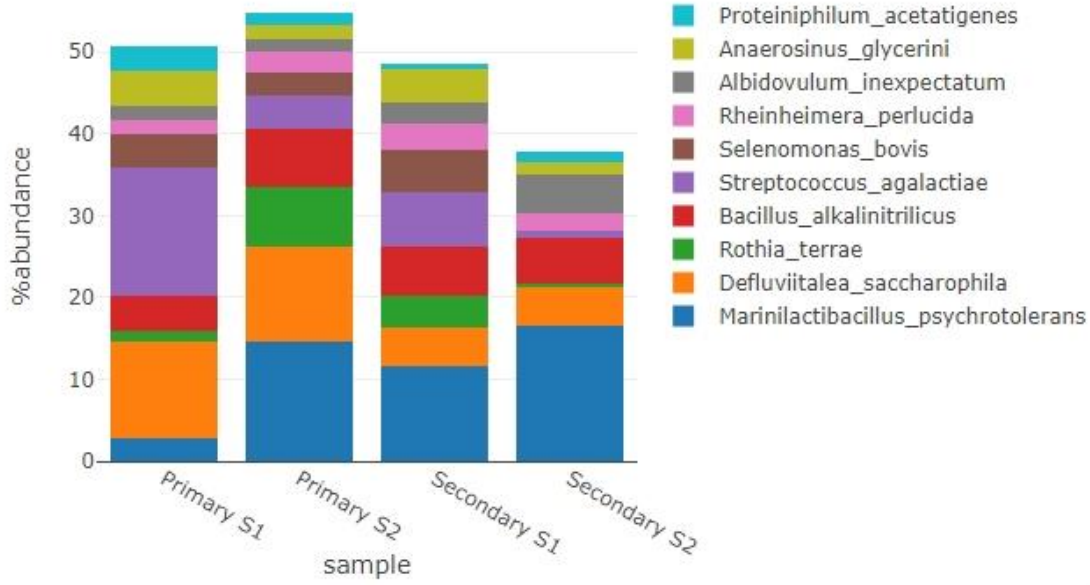

Figure S3: Ten of the most abundant species in primary S2 samples and their abundance in the other groups.

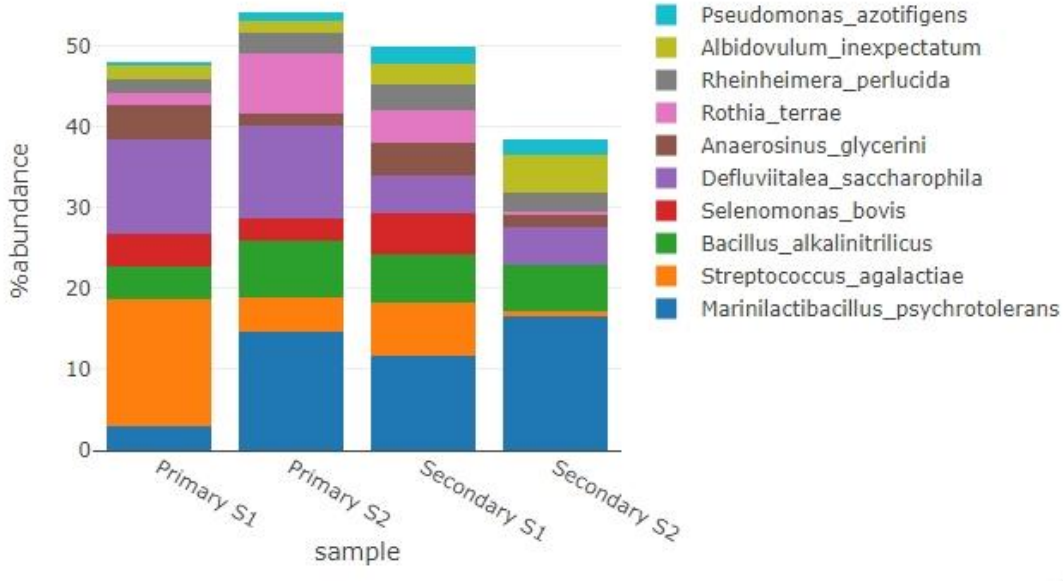

Figure S4: Ten of the most abundant species in secondary S1 samples and their abundance in the other groups.

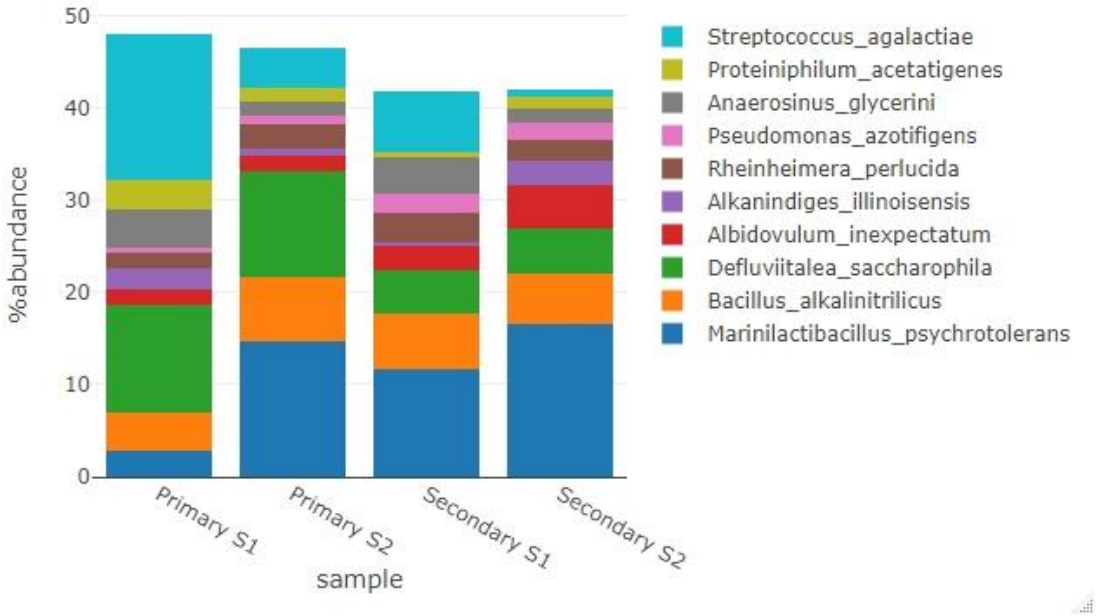

Figure S5: Ten of the most abundant species in secondary S2 samples and their abundance in the other groups.

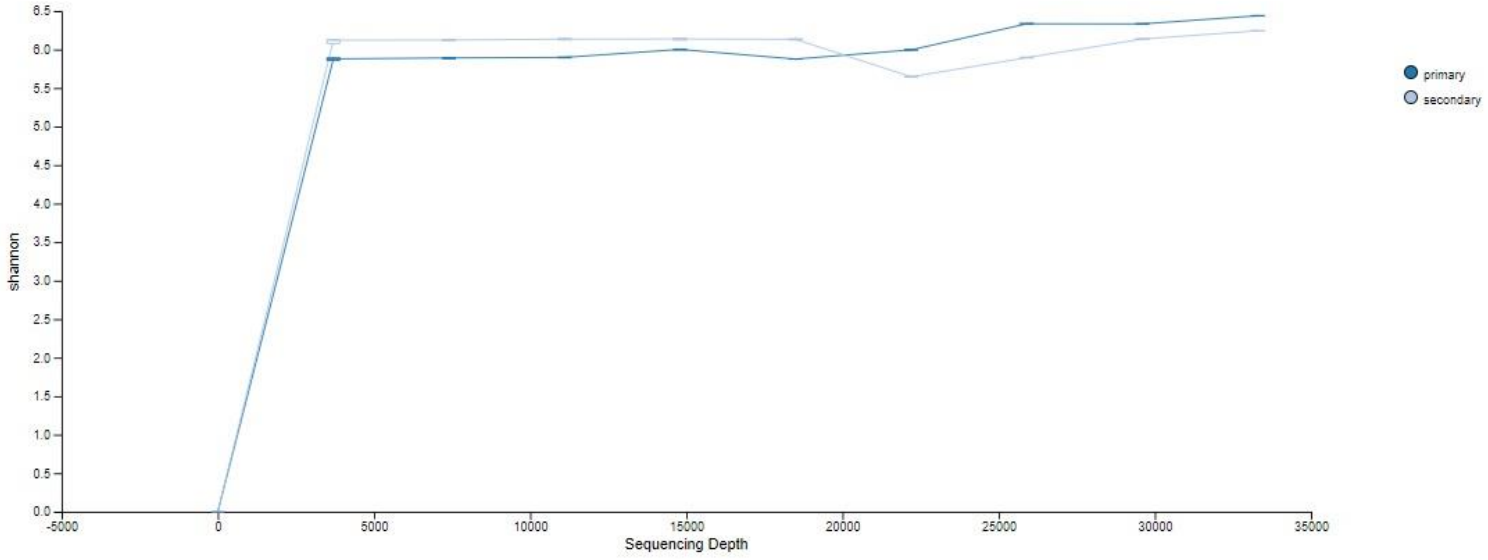

Figure S6: Alpha diversity rarefaction curve of observed species in primary and secondary infection samples.

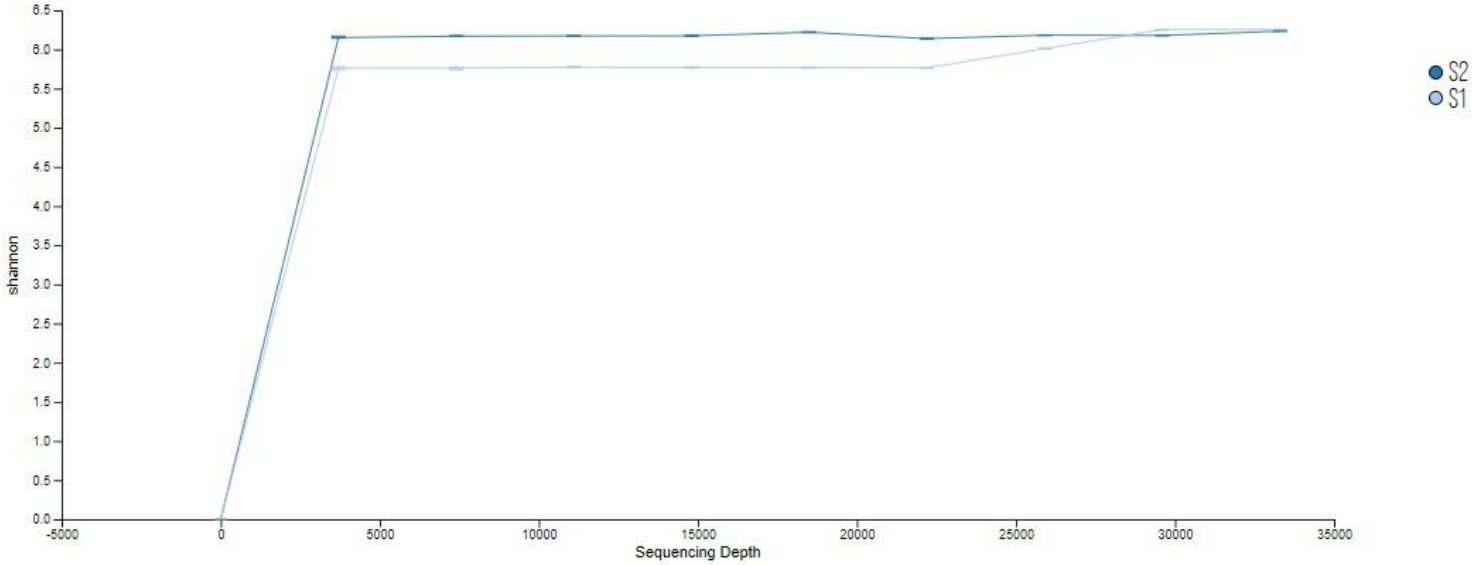

Figure S7: Alpha diversity rarefaction curve of observed species in samples before canal preparation (S1) and after canal preparation (S2).

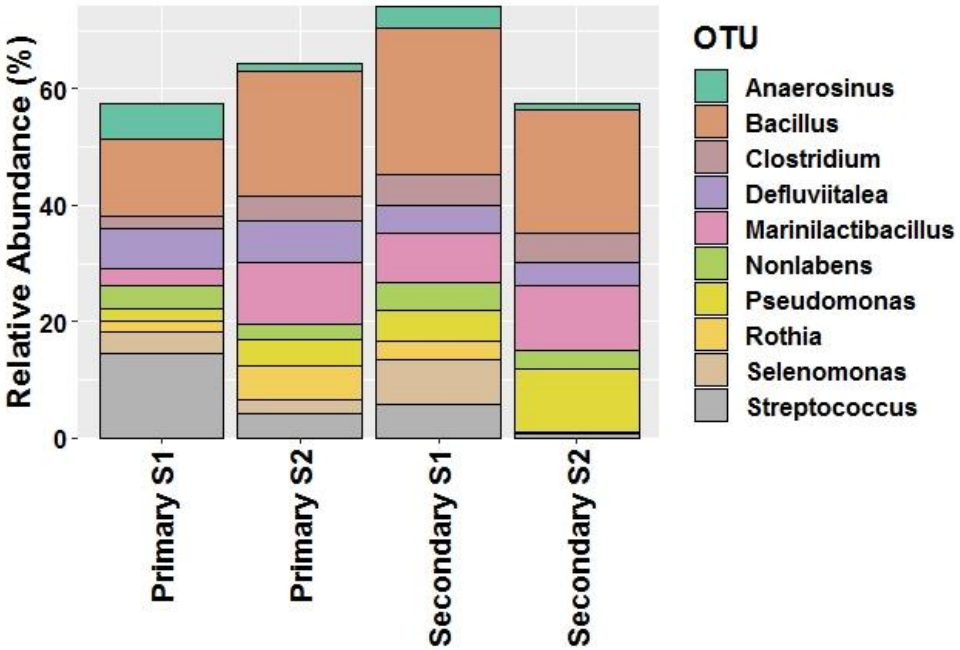

Figure S8: Genera abundance in Primary S1, Primary S2, Secondary S1 and Secondary S2 samples.