



## Introduction

- Mutans streptococci (MS) are widely regarded as a group of cariogenic species of bacteria.
- However, MS are only a small fraction of the overall microflora in both non-carious patients and early carious lesions.
- Researchers have discovered non-MS species with similar acidogenic and aciduric properties as MS.
- There has been a renewed interest in *C. albicans* as a potential biomarker for ECC, which is often found in conjunction with high levels of *S. mutans* in children with ECC.
- The goal of this research project was to further explore the potential differences in amounts of: *C. albicans*, *S. mutans*, acid-tolerant streptococci and total streptococci relative to total cultivable count of dental plaque samples collected in children with and without caries.

## Materials and Methods

**Table 1.** Subject demographics including age in months and sex (male or female) of subjects with and without caries

With Caries			Without Caries		
Subject #	Age (months)	Sex	Subject #	Age (months)	Sex
1	46	F	1	44	F
2	32	M	2	33	M
3	36	F	3	36	F
4	26	M	4	26	M
5	36	M	5	37	M
6	46	M	6	47	M
7	40	F	7	40	F
8	39	M	8	39	M
9	21	M	9	22	M
10	46	M	10	47	M
11	46	M	11	39	M
12	43	M	12	43	M
Average	38			38	

## Results

**Table 2.** Percentages of *C. albicans*, *S. mutans*, acid-tolerant strains, and total streptococci relative to total cultivable counts

Subject	<i>Candida</i>	<i>S. mutans</i>	<i>Streptococcus</i>	Total	Acid tolerant
RMH.1	0.00E+00	4.30E+03	1.20E+07	6.30E+07	1.60E+04
RMH.2	6.40E+02	3.30E+05	2.10E+07	4.80E+07	7.60E+04
RMH.3	0.00E+00	1.60E+04	1.70E+07	3.30E+07	8.00E+03
RMH.4	1.40E+02	4.10E+05	1.60E+07	3.10E+07	4.50E+05
RMH.5	0.00E+00	0.00E+00	4.60E+07	5.40E+07	5.50E+05
RMH.6	6.30E+03	4.20E+05	1.30E+07	2.00E+07	4.60E+05
RMH.7	1.10E+03	0.00E+00	9.00E+06	1.90E+07	1.20E+04
RMH.8	4.00E+01	1.30E+05	6.00E+06	8.20E+06	1.70E+05
RMH.9	1.00E+04	0.00E+00	4.00E+06	6.40E+06	1.20E+04
RMH.10	1.70E+04	3.30E+05	1.70E+07	3.40E+07	4.20E+05
RMH.11	0.00E+00	1.50E+05	1.40E+06	5.00E+06	3.20E+04
RMH.12	9.60E+02	1.50E+05	3.00E+06	1.50E+07	2.50E+05

% of total	<i>Candida</i>	<i>S. mutans</i>	<i>Streptococcus</i>	Total	Acid tolerant
RMH.1	0.00	0.01	19.05	100.00	0.03
RMH.2	0.00	0.69	43.75	100.00	0.16
RMH.3	0.00	0.05	51.52	100.00	0.02
RMH.4	0.00	1.32	51.61	100.00	1.45
RMH.5	0.00	0.00	85.19	100.00	1.02
RMH.6	0.03	2.10	65.00	100.00	2.30
RMH.7	0.01	0.00	47.37	100.00	0.06
RMH.8	0.00	1.59	73.17	100.00	2.07
RMH.9	0.16	0.00	62.50	100.00	0.19
RMH.10	0.05	0.97	50.00	100.00	1.24
RMH.11	0.00	3.00	28.00	100.00	0.64
RMH.12	0.01	1.00	20.00	100.00	1.67

	<i>Candida</i>	<i>S. mutans</i>	<i>Streptococcus</i>	Acid tolerant
Average	0.02	0.89	49.76	0.90
p-value	0.59	0.005	0.45	0.016

**Caries Subjects**

Subject	<i>Candida</i>	<i>S. mutans</i>	<i>Streptococcus</i>	Total	Acid tolerant
105	0.00E+00	0.00E+00	2.00E+07	3.40E+07	4.40E+03
18	0.00E+00	4.00E+01	1.08E+07	1.96E+07	4.40E+04
96	0.00E+00	0.00E+00	1.20E+07	2.02E+07	3.40E+03
91	0.00E+00	0.00E+00	5.20E+07	5.50E+07	3.70E+03
1	0.00E+00	1.60E+05	1.54E+07	2.48E+07	6.22E+03
12	9.99E+01	4.00E+01	1.44E+06	7.60E+06	2.36E+04
38	0.00E+00	4.20E+04	2.18E+06	1.65E+07	3.16E+05
28	0.00E+00	7.80E+04	1.93E+07	5.05E+07	6.80E+04
50	0.00E+00	0.00E+00	1.30E+07	1.70E+07	4.20E+04
81	0.00E+00	4.60E+03	8.80E+06	6.10E+07	3.70E+03
114	0.00E+00	0.00E+00	3.30E+07	4.60E+07	2.00E+02
32	0.00E+00	2.00E+03	1.98E+07	9.72E+07	9.59E+04

% of total	<i>Candida</i>	<i>S. mutans</i>	<i>Streptococcus</i>	Total	Acid tolerant
105	0.00	0.00	58.82	100.00	0.01
18	0.00	0.00	55.10	100.00	0.22
96	0.00	0.00	59.41	100.00	0.02
91	0.00	0.00	94.55	100.00	0.01
1	0.00	0.65	62.10	100.00	0.03
12	0.00	0.00	18.95	100.00	0.31
38	0.00	0.25	13.21	100.00	1.92
28	0.00	0.15	38.22	100.00	0.13
50	0.00	0.00	76.47	100.00	0.25
81	0.00	0.01	14.43	100.00	0.01
114	0.00	0.00	71.74	100.00	0.00
32	0.00	0.00	20.37	100.00	0.10

	<i>Candida</i>	<i>S. mutans</i>	<i>Streptococcus</i>	Acid tolerant
Average	0.00	0.09	48.61	0.25
<i>p-values compared to the same outcomes between caries and caries-free groups</i>				

**Caries-free Subjects**

- *C. albicans* was recovered in 8 of 12 individuals with caries and one of the 12 patients without caries.
- There was a statistically significant difference in recoveries of *S. mutans* and acid-tolerant bacterial strains.
- Individuals with caries had significantly higher proportions of *S. mutans* and acid-tolerant strains than their caries-free counterparts.
- Additional research in our lab and contemporary literature suggests that these changes in the microflora may begin as early as 12 to 18 months prior to diagnosis of caries
- At the individual level, all 12 toddlers with caries had detectable *Candida*, MS at  $\geq 0.01\%$  of the total count, and/or an acid tolerant recovery of  $\geq 1\%$  of the total count, whereas only 5 of 12 caries-free subjects met at least one of these criteria.

## Conclusions

- In children under age 48 months, we noticed significantly higher proportions of *S. mutans* and acid-tolerant bacterial strains in children with caries vs. those without caries.
- *C. albicans* was recovered in 8 of 12 children with caries, but only one of the 12 children without caries. Detection was more discriminatory than quantitative level.
- In addition to *S. mutans*, *C. albicans* and other acid-tolerant bacterial strains may serve as important biomarkers for elevated caries risk and could therefore be useful as future predictors of caries.

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## References

1. Banas JA, Takanami E, Hemsley RM, Villhauer A, Zhu M, Qian F, Marolf A, Drake DR. Evaluating the relationship between acidogenicity and acid tolerance for oral streptococci from children with or without a history of caries. *J Oral Microbiol.* 2019 Nov 7;12(1):1688449. doi: 10.1080/20002297.2019.1688449. Erratum in: *J Oral Microbiol.* 2019 Dec 2;12(1):1699376. PMID: 31893016; PMCID: PMC6844425.
2. Hemadi AS, Huang R, Zhou Y, Zou J. Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment. *Int J Oral Sci.* 2017 Nov 10;9(11):e1. doi: 10.1038/ijos.2017.35. PMID: 29125139; PMCID: PMC5775330.
3. Xiao J, Moon Y, Li L, Rustchenko E, Wakabayashi H, Zhao X, Feng C, Gill SR, McLaren S, Malmstrom H, Ren Y, Quivey R, Koo H, Kopycka-Kedzierawski DT. *Candida albicans* Carriage in Children with Severe Early Childhood Caries (S-ECC) and Maternal Relatedness. *PLoS One.* 2016 Oct 14;11(10):e0164242. doi: 10.1371/journal.pone.0164242. PMID: 27741258; PMCID: PMC5065202.
4. Banas JA, Drake DR. Are the mutans streptococci still considered relevant to understanding the microbial etiology of dental caries? *BMC Oral Health.* 2018 Jul 31;18(1):129. doi: 10.1186/s12903-018-0595-2. PMID: 30064426; PMCID: PMC6069834.

