

Supplemental Digital Content 1. Demographic characteristics and AOM risk factors of the study cohort.

Total Children		301		
Sex	Male	165	55%	
	Female	136	45%	
Ethnicity	Hispanic or Latino	33	11%	
	Non-Hispanic or Latino	268	89%	
Race	White	261	87%	
	African American	12	4%	
	Asian	4	1%	
	Other (more than one race)	24	8%	
Sibling	Yes (any age)	198	66%	
	<5 year of age	142	47%	
	No	103	34%	
Breastfeeding in the first month of life	Exclusively	174	58%	
	Partially	76	25%	
	None	51	17%	
Breastfeeding up to six months of life	Exclusively	89	30%	
	Partially	48	16%	
	None	164	54%	
Infant exposed to smoke	Yes	45	15%	
	No	92	31%	
	Not documented	164	54%	
Family member prone to ear infections	Yes	165	55%	
	No	136	45%	
Socioeconomic status surrogate*		BayCreek	Geneva	Total
	Medicaid/Uninsured	48%	61%	55%
	Commercial Insurance	52%	39%	45%
Geographic residence*	Urban	33%	51%	43%
	Rural	67%	49%	57%

Note: *Socioeconomic status and geographic residence information was collected from all the population in the practice, not specifically from enrolled children. Bay creek and Geneva are two different pediatric sites where samples were collected.

This was calculated using the 2020 Census List of Urban Areas in the United States, Puerto Rico and Island Areas. <https://www.federalregister.gov/documents/2022/12/29/2022-28286/2020-census-qualifying-urban-areas-and-final-criteria-clarifications>. As of the 2020 Census, area definitions were limited to Urban and Rural classifications.

Supplemental Digital Content 2. Nasopharyngeal isolation of *Streptococcus pneumoniae* (*Spn*), *Haemophilus influenzae* (*Hflu*), and *Moraxella catarrhalis* (*Mcat*) by age at 6 through 36 months of age at healthy visits.

Healthy visits	Total visits	# of visits with <i>Spn</i> (%)	# of visits with <i>Spn</i> oxacillin nonsusceptible (%)	# of visits with <i>Hflu</i> (%)	# of visits with <i>Hflu</i> β lactamase positive (%)	# of visits with <i>Mcat</i> (%)
6 months	86	18 (21%)	9 (50%)	6 (7%)	0 (0%)	36 (42%)
9 months	115	22 (19%)	13 (59%)	14 (12%)	5 (36%)	43 (37%)
12 months	120	32 (27%)	8 (25%)	11 (9%)	1 (9%)	55 (46%)
15 months	87	25 (29%)	6 (24%)	11 (13%)	4 (36%)	36 (41%)
18 months	80	21 (26%)	7 (33%)	15 (19%)	4 (27%)	37 (46%)
24 months	76	17 (22%)	9 (53%)	6 (8%)	1 (17%)	32 (42%)
36 months	88	24 (27%)	9 (38%)	3 (3%)	1 (33%)	30 (34%)
Total	652	159 (24%)	61 (38%)	66 (10%)	16 (24%)	269 (41%)

The proportion of detected potential pathogens was consistent across the age range.

Supplemental Digital Content 3. Antibiotic susceptibility of *S pneumoniae* isolates recovered during healthy and AOM visits, 2021-2023

Antibiotic Tested ¹	% of Non-susceptible (I+R)			P-value healthy NP vs AOM NP, healthy NP vs AOM MEF
	Healthy NP (N=177) ^{2,3}	AOM NP (N=79) ³	AOM MEF (N=24) ³	
Penicillin	36%	47%	40%	0.16, 0.81
Amoxicillin	10%	21%	30%	0.07, 0.09
Cefotaxime	3%	0%	0%	ns
Ceftriaxone	2%	0%	0%	ns
Ertapenem	4%	4%	9%	ns
Meropenem	21%	27%	36%	0.26, 0.11
Ofloxacin	2%	2%	0%	ns
Erythromycin	40%	54%	55%	0.06, 0.23
Tetracycline	5%	7%	0%	ns
TMP/SMX	18%	17%	5%	ns, 0.13
Clindamycin	2%	0%	0%	ns

1 Levofloxacin, Moxifloxacin, Telithromycin, Linezolid, Vancomycin, Chloramphenicol, and Tigecycline were also tested, but none or only one isolate(s) were non-susceptible.

2 AOM follow-up visits tested (n=20) are included with the healthy visits tested (n=157) for a total of 177 isolates

3 The denominator for the percentage non-susceptible is the total number of isolates tested for the respective antibiotic, which differs per antibiotic.

NP= nasopharynx, AOM= acute otitis media, MEF= middle ear fluid, ns = not significant.

Supplemental Digital Content 4. Proportion of antibiotic non-susceptible *S. pneumoniae* isolates by serotype¹

		Penicillin	Amoxicillin	Cefotaxime	Ceftriaxone	Ertapenem	Meropenem	Ofloxacin	Erythromycin	Vancomycin	Tetracycline	Chloramphenicol	TMP/SMX	Clindamycin
		# (%) ²	# (%)	# (%)	# (%)	# (%)	# (%)	# (%)	# (%)	# (%)	# (%)	# (%)	# (%)	# (%)
PCV13 Serotypes														
3	n=6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.3)	0 (0)	0 (0)	1 (16.7)	1 (16.7)	0 (0)	0 (0)
23F	n=1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
PCV20, non-PCV13 Serotypes														
15B	n=18	4 (22.2)	2 (11.1)	1 (5.6)	0 (0)	0 (0)	2 (11.1)	0 (0)	9 (50)	0 (0)	5 (27.8)	0 (0)	4 (22.2)	0 (0)
11A	n=13	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (23.1)	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)
Non-PCV Serotypes														
35B	n=63	56 (88.9)	14 (22.2)	0 (0)	0 (0)	5 (7.9)	42 (66.7)	0 (0)	50 (79.4)	0 (0)	3 (4.8)	0 (0)	13 (20.6)	0 (0)
23A	n=25	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (24)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
23B	n=25	5 (20)	0 (0)	1 (4)	1 (4)	0 (0)	2 (8)	0 (0)	6 (24)	0 (0)	0 (0)	0 (0)	5 (20)	0 (0)
35F	n=23	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.7)	0 (0)	2 (8.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
35D	n=13	10 (76.9)	2 (15.4)	0 (0)	0 (0)	0 (0)	6 (46.2)	0 (0)	9 (69.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
21	n=12	4 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (16.7)	0 (0)	0 (0)	0 (0)	1 (8.3)	0 (0)
Non-Typeable	n=12	6 (50)	4 (33.3)	3 (25)	2 (16.7)	2 (16.7)	7 (58.3)	1 (8.3)	8 (66.7)	1 (8.3)	2 (16.7)	0 (0)	9 (75)	0 (0)
15C	n=10	4 (40)	2 (20)	0 (0)	0 (0)	0 (0)	2 (20)	0 (0)	5 (50)	0 (0)	3 (30)	0 (0)	4 (40)	0 (0)
16, 36, or 37	n=8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
7 (B or C)	n=6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	2 (33.3)	0 (0)
15A	n=5	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	1 (20)	0 (0)	0 (0)	1 (20)
6C	n=5	4 (80)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (80)	0 (0)	0 (0)	0 (0)	2 (40)	0 (0)
10	n=4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)
13	n=3	2 (66.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)

24, 31, or 40	n=2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
27, 32, or 41	n=2	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
7A	n=2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
33B	n=1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
Not Serotyped	n=2	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

1 Antibiotics and serotypes for which there were no non-susceptible isolates are not included in this table.

2 The denominator is the total number of isolates per serotype.

Supplemental Digital Content 5. Antibiotic Non-Susceptibility of *H. influenzae* isolates, 2021-2023

Antibiotic Tested	% of Non-susceptible (I+R)			P-value healthy NP vs AOM NP, healthy NP vs AOM MEF
	Healthy NP (N=94) ¹	AOM NP (N=72)	AOM MEF (N=54)	
Ampicillin	21%	22%	20%	ns
TMP/SMX	37%	35%	35%	ns
Cefaclor	6%	19%	22%	0.02, 0.01
Cefuroxime	2%	6%	6%	ns
Cefprozil	14%	22%	22%	ns
Cefdinir ²	0%	15%	11%	ns
Cefixime	0%	1%	2%	ns
Cefpodoxime	0%	1%	0%	ns
Ceftriaxone	1%	1%	2%	ns
Erythromycin ³	56%	57%	52%	ns
Clarithromycin	6%	7%	7%	ns
Amoxicillin/clavulanate	0%	7%	6%	0.01, 0.04

1 AOM Follow-up visits tested (n=28) are included with the healthy visits tested (n=66) for a total of 94 isolates.

2 Cefdinir disks were discontinued and a replacement product could not be found (therefore very few isolates, n=28, were tested). Given that 5 other cephem group antibiotics are being tested, we chose to switch testing to another antibiotic group and tested meropenem. None of the isolates showed meropenem or azithromycin non-susceptibility (not shown).

3 Erythromycin disc breakpoints are not provided in CLSI-2018 M100 guidelines for *H. influenzae*. We used breakpoint limit of *Streptococcus sp.*

NP= nasopharynx, AOM= acute otitis media, MEF= middle ear fluid, ns = not significant.