Full title: Naturally-acquired protection against upper respiratory symptoms involving group A *Streptococcus* in a longitudinal cohort study

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Summary: Previous detection of a GAS *emm* type is associated with protection against typical symptoms at future acquisitions of the same type. Protection against partially-heterologous *emm* types within an *emm* cluster may broaden the strain coverage of *emm* type-based vaccines.

ABSTRACT

Background: Pharyngitis due to group A *Streptococcus* (GAS) represents a major cause of outpatient visits and antibiotic use in the United States. A leading vaccine candidate targets 30 of the >200 *emm* types of GAS. We aimed to assess natural protection conferred by GAS against respiratory symptoms.

Methods: In a 5-year study among school-aged children in Pittsburgh, Pennsylvania, pharyngeal cultures were obtained from children at 2-week intervals, and active surveillance was conducted for respiratory illnesses. We assessed protection via the relative odds of previous detection of homologous strains (defined by field-inversion gel electrophoresis banding pattern), *emm* types, and *emm* clusters at visits where GAS was detected with symptoms, versus visits where GAS was detected without symptoms. We used a cluster bootstrap of children to adjust estimates for repeated sampling.

Results: At visits where previously-detected GAS *emm* types were identified, we estimated 81.8% (95%CI: 67.1-91.7%) protection against typical pharyngitis symptoms among children re-acquiring the same strain, and 94.5% (83.5-98.6%) protection among children acquiring a distinct strain. We estimated 77.1% (33.7-96.3%) protection against typical symptoms among children acquiring partially-heterologous *emm* types belonging to a previously-detected *emm* cluster. Protection was evident after both symptomatic and asymptomatic detections of GAS. We did not identify strong evidence of protection against atypical respiratory symptoms.

Conclusions: Within a 5-year longitudinal study, previous detection of GAS *emm* types was associated with protection against typical symptoms when homologous strains were subsequently detected. Naturally-acquired protection against partially-heterologous types suggests *emm* type-based vaccines may have broader strain coverage than what has been previously assumed.

Key words: Group A streptococcus; pharyngitis; naturally-acquired protection; cohort study

INTRODUCTION

Group A *Streptococcus* (GAS; *Streptococcus pyogenes*) causes a spectrum of clinical manifestations encompassing infections of the skin and upper-respiratory tract as well as severe invasive infections, scarlet fever, rheumatic fever, rheumatic heart disease, and post-streptococcal glomerulonephritis. Of these, GAS pharyngitis is the most common illness, causing an estimated 13 cases per 100 children ages 5-12y annually and substantial antibiotic prescribing in settings where antibiotic treatment of GAS pharyngitis is recommended [1,2]. Children with GAS pharyngitis who do not receive antibiotics may spread infection and are at risk for suppurative and non-suppurative complications including rheumatic heart disease, which remains a prevalent cause of morbidity and mortality in lower-income settings [3].

An effective GAS vaccine would help to reduce GAS disease burden [4,5]. Among many GAS surface antigens, the M protein is the best-characterized and has received the greatest attention as a vaccine target. Over 200 *emm* types of GAS have been defined based on sequences of the hypervariable M protein-encoding gene [6]. Multiple co-circulating strains may encode each *emm* type, in addition to other GAS antigens and virulence factors [7,8]. Recently, *emm* types have been partitioned into 48 *emm* clusters based on shared molecular properties [9]. A leading vaccine candidate targets 30 *emm* types of GAS across 10 distinct *emm* clusters [10], which collectively account for the greatest share of GAS pharyngitis and invasive disease in western, high-income settings [11].

Evidence that natural exposure to a pathogen confers protection against recurrent infection or disease helps to establish the feasibility of an efficacious vaccine, and provides a baseline against which the degree of protection conferred by vaccination can be assessed [12]. Historical studies reported reduced likelihood of clinical symptoms among individuals who acquired M serotypes of GAS against which they had pre-existing antibodies [13–16]. While this mechanism may account for the lower incidence of GAS pharyngitis among adults than children [17], there have been few modern studies addressing protection and immunity following natural GAS acquisition [18–22].

A previously-conducted longitudinal study addressing GAS carriage and pharyngitis among school-aged children [23,24] presented an opportunity to assess evidence of naturally-acquired protection. We revisited data from this study aiming to estimate protection against symptomatic GAS detections.

METHODS

Cohort

We used data from a 5-year school-based longitudinal cohort study of children in Pittsburgh, Pennsylvania undertaken between October, 1998 and May, 2003. Study methods have been described previously [23]; briefly, all children (~285 per year) enrolled in a private, tuition-supported elementary school serving students in kindergarten through grade 8 were eligible to participate. As classroom placement within the school is based on readiness rather than age or grade level, significant social mixing occurs across age groups. Study participants came from all classrooms in the school, providing a representative illustration of transmission dynamics.

Throat cultures were performed for each child enrolled in the study approximately every 2wk during the school year. In addition, study personnel were “on call” to obtain throat cultures from children within 1d of onset of any new respiratory illness; if children received care for respiratory illnesses from their personal clinician and if throat cultures were performed by the practitioner, they were retrieved by study personnel. For antibiotic-treated episodes, follow-up cultures were performed 2-4d after end of treatment; for new GAS detections not treated with antibiotics, because the child did not have respiratory symptoms, follow-up cultures were performed 1wk after the initial positive culture.

Throat specimens were obtained with a rayon-tipped swab (BBL Becton Dickinson, Sparks, MD) and processed the same day by previously-described standard culture techniques [24]. Briefly, swabs were transported in Amies medium without charcoal and plated within 2h on 5% sheep’s-blood agar with bacitracin disks for incubation at 37ºC in 5% CO_2, before isolation and sub-culturing of beta-haemolytic

colonies. Isolates were typed initially by field-inversion gel electrophoresis (FIGE; a version of pulsed-field gel electrophoresis [25]) using DNA bands within the 50-250kb range. Isolates with identical FIGE banding patterns were considered to represent a single strain. From each FIGE type, one or more representative isolates were sent annually for *emm* typing by the Centers for Disease Control and Prevention, as described previously [26].

At each visit, physical examination of the pharynx was performed, and children were questioned about symptoms using a standardized questionnaire. When respiratory illnesses occurred, parents were contacted for additional information regarding symptoms. Children reporting sore throat as a prominent clinical complaint were defined as exhibiting typical symptoms; symptoms were considered atypical if children reported rhinorrhea and/or cough without sore throat. Detection of GAS without accompanying respiratory symptoms was considered colonization.

Design

We aimed to estimate the association of prior GAS detection with protection against typical and atypical symptoms at future visits where the same *emm* type was detected (homologous), and at visits where a distinct *emm* type belonging to the same *emm* cluster was detected (partially-heterologous). We estimated protection in a case-control framework. Specifically, we compared the odds of previous detection of homologous or partially-heterologous *emm* types at GAS-positive visits where children experienced typical or atypical symptoms ("case" visits) to odds of previous homologous or partially-heterologous *emm* type detection at GAS-positive visits where children experienced no symptoms ("control" visits). We distinguished recurrent detections of the same *emm* type according to whether the same FIGE type, or a new FIGE type, was detected. To prevent misclassification of continuous carriage episodes, we defined recurrent detections of FIGE types as visits separated from previous detection of the same type by at least two GAS-negative culture results. Visits preceded by detection of the same FIGE type without two intervening GAS-negative specimens were excluded from analysis.

Because antibiotic treatment of GAS episodes may prevent seroresponse [15], we also aimed to assess whether receipt of antibiotics influenced the likelihood of protection. For these analyses, we defined independent variables as follows:

1. Previous detection ever occurring with an antibiotic prescription (versus no previous detection).
2. Previous detection never occurring with an antibiotic prescription (versus no previous detection).

We further stratified analyses according to whether children had ever experienced typical symptoms, or had never experienced symptoms, at previous detections of an *emm* type or *emm* cluster.

Because the study had an open cohort design with enrollment of new children each year, the risk of missing GAS acquisitions while children were not under surveillance varied among children and over time. We therefore constructed analysis strata within which children were matched on current GAS exposure and the extent of prior surveillance: visits were stratified by semester of occurrence (10 semesters over 5 school years) and the semester children entered the study, resulting in 100 distinct strata.

Statistical methods

We used the Chi-squared test to compare proportions of children with typical symptoms, atypical symptoms, and no symptoms at their first and second detection of FIGE types. For analyses of protection, we estimated Mantel-Haenszel (matched) odds ratios, accounting for strata, via conditional logistic regression. We defined protection estimates as one minus the matched odds ratio. Because children contributed multiple visits, we did statistical inference in a cluster bootstrap framework, resampling study participants at each iteration [27]. We coded the cluster bootstrap *de novo* and used the *survival* package [28] in R (version 3.5.3) to fit conditional logistic regression models.

As a sensitivity analysis, we repeated analyses of protection within a subset of visits occurring after children had contributed ≥2 years of observations. We expected this sub-sample would have reduced risk

of misclassification resulting from failure to detect GAS acquisitions that occurred outside the study period.

RESULTS

Enrollment and GAS detection

The study enrolled 145 children, among whom GAS was detected in 110 (**Table 1**; **Tables S1-S3**). Children enrolled during years 1-5 contributed specimens over, on average, 34.0 (range: 8-46), 26.0 (range: 8-34), 20.8 (range: 8-28), 11.9 (range: 3-19), and 8.6 (range: 3-9) distinct months (**Figure S1**). In total, cultures were collected at 7241 visits, and GAS was detected at 1120 visits (15.5%). Typical symptoms among GAS-positive children occurred at 194 visits (82 children), while atypical symptoms among GAS-positive children occurred at 82 visits (among 54 children). In addition, GAS was detected without respiratory symptoms at 844 visits (among 78 children).

Dynamics of *emm* types

Descriptions of GAS carriage and disease in the cohort have been reported previously [23]. Briefly, predominant *emm* types and FIGE types varied by year (**Table 2**); the majority of isolates belonged to *emm*29 and *emm*4 in 1998-99, and to *emm*28 and *emm*89 in 1999-2000 (**Figure S2**). Detections of *emm*6 became prominent in the winter of 2000, and this type became the most prevalent over all subsequent years of the study, accounting for 49.1%, 54.9%, and 30.4% of GAS isolates in 2000-2001, 2001-2002, and 2002-2003, respectively. Additional prevalent types over these years included *emm*89, *emm*12, *emm*28, *emm*5, and *emm*1.

The contribution of each type to disease and colonization further varied by year (**Figure S2**). For instance, typical symptoms occurred with 22.4% (35/156) of *emm*6 detections in 2000-2001, but only 9.2% (12/130) and 4.3% (3/70) of *emm*6 detections in 2001-2002 and 2002-2003, respectively. Similarly,

typical symptoms occurred with 21.2% (7/33) of *emm*89 detections in 1999-2000, but only 10.5% (6/58) of *emm*89 detections in 2000-2001. In 2001-2002, typical symptoms occurred with 31.6% (6/19) and 36.3% (21/130) of *emm*1 and *emm*12 detections, respectively, versus 7.1% (1/14) and 23.2% (14/70) of detections of these same types in 2002-2003.

Recurrent detections

We identified 280 visits where a FIGE type was newly detected in a child without history of carriage or disease involving the same type (**Table 3**). Typical and atypical symptoms were noted at 112 (40.0%) and 41 (14.6%) of these visits, respectively; children had no respiratory symptoms at the remaining 127 (45.4%) visits. A second detection of the same FIGE type, separated by ≥2 GAS-negative cultures, occurred in 83 instances. Of these second detections, 17 (20.5%), 8 (9.6%), and 58 (69.9%) presented with typical symptoms, atypical symptoms, and no symptoms, respectively, representing a notable departure from the distribution of symptoms on first detections ($\chi^2_{\mathrm{df}=2} = 15.6; p = 0.004$). We did not identify differences in symptoms at second detections among children whose first detections involved typical symptoms, atypical symptoms, or no symptoms ($\chi^2_{\mathrm{df}=4} = 3.3; p = 0.5$). These results suggest symptomatic and asymptomatic GAS acquisitions conferred similar protection against symptoms at future detections of the same FIGE type.

Protection

We estimated 81.8% (95%CI: 67.1-91.7%) protection against typical symptoms associated with previous detections of the same FIGE type, separated by ≥2 intervening GAS-negative cultures (**Table 4**). In an analysis limited to visits preceded by ≥2y of surveillance, we estimated 85.9% (47.1-97.8%) protection. Previous detections of a distinct FIGE type, belonging to the same *emm* type, were associated with 94.5% (83.5-98.6%) protection against typical symptoms in the full sample and 82.7% (27.3-97.1%) protection at visits preceded by ≥2y of surveillance. In these analyses, differences in estimated protection against the same and distinct FIGE types were not statistically meaningful at the $p \leq 0.05$ threshold. We

estimated 77.1% (33.7-96.3%) protection against typical symptoms associated with previous detections of a partially-heterologous *emm* type (**Table 4**). Within the sample of visits preceded by ≥2y of surveillance, data were available from only 16 visits where a partially-heterologous *emm* type was previously detected. We did not identify strong evidence of protection against atypical symptoms associated with previous detection of the same *emm* type or *emm* cluster (**Table 5**).

We estimated 89.6% (66.3-97.1%) and 42.0% (–168.8-87.0%) protection against typical and atypical symptoms, respectively, associated with previous detections of the same FIGE type at visits where antibiotics were not prescribed (**Table 6**). For previous detections resulting in antibiotic prescriptions, we estimated 71.2% (37.8-89.4%) protection against typical symptoms. We estimated 95.1% (77.3-99.1%) and 71.1% (–120.0-95.7%) protection against typical and atypical symptoms, respectively, associated with previous detections of a distinct FIGE type belonging to the same *emm* type and occurring without an antibiotic prescription. Previous detections of a distinct FIGE type belonging to the same *emm* type, when accompanied by an antibiotic prescription, were associated with 94.1% (71.0-98.9%) protection against typical symptoms. Previous detections of a partially-heterologous *emm* type were associated with 86.7% (38.0-97.0%) protection against typical symptoms when antibiotics were not prescribed, and with 48.8% (–75.4-88.4%) protection against typical symptoms when antibiotics were prescribed.

Previous detections of the same *emm* type occurring with typical symptoms and resulting in an antibiotic prescription were associated with 73.2% (36.3-91.6%) against typical symptoms involving the same FIGE type, and 94.7% (77.2-98.9%) protection against typical symptoms involving a distinct FIGE type (**Table 7**). Similarly, we estimated 76.2% (33.6-94.7%) and 92.8% (66.8-98.8%) protection against typical symptoms involving the same and distinct FIGE types, respectively, associated with previous asymptomatic detections of the same *emm* type where no antibiotic prescription occurred. Differences in estimated protection against matched and distinct FIGE types were again not statistically meaningful. Previous detection of a partially-heterotypic *emm* type was associated with 47.5% (–234.0-93.8%) protection against typical symptoms when earlier visits occurred with typical symptoms and an antibiotic

prescription, and 84.9% (25.9-96.8%) protection when earlier visits occurred without symptoms or an antibiotic prescription.

DISCUSSION

Evidence of naturally-acquired protection against a pathogen strengthens the rationale for vaccine development and provides a benchmark for assessing vaccine efficacy. We identify that prior detection of GAS is associated with protection against typical symptoms at future detections of the same *emm* type or cluster. Our finding of protection after detections of distinct FIGE types belonging to the same *emm* type is consistent with the hypothesis of protective responses to the M protein. These findings support the biological plausibility of preventing symptomatic GAS upper respiratory infections using *emm* type-based vaccines.

Whereas it has previously been thought that GAS carriage does not cause immune responses [21], we find evidence of type-specific protection against typical symptoms after asymptomatic GAS detections. These findings are consistent with those of a recent longitudinal study, wherein 70% of new asymptomatic GAS acquisitions resulted in detectable antibody responses [18]. Previous studies have also demonstrated reduced likelihood of seroconversion when GAS episodes are treated with antibiotics [15]. The fact that most antibiotic-treated episodes presented with typical symptoms makes it difficult to disentangle effects of previous symptoms and antibiotic treatment on children's likelihood of future protection in this study. We obtain lower point estimates of protection against typical symptoms associated with detections occurring with an antibiotic prescription, as compared to detections without an antibiotic prescription. While greater differences in our estimates of protection against atypical symptoms are evident when comparing previous detections that occurred with or without an antibiotic prescription, our estimates are underpowered in these strata.

Our findings agree with those of previous studies. In US army cohorts, presence of anti-M antibody predicted reduced risk of prolonged colonization and of respiratory symptoms upon reacquisition of

homologous GAS M-serotypes [13,14]. In a multi-year study of institutionalized children, GAS pharyngitis epidemics tended to involve M serotypes that had not been detected previously within the population [29]; year-to-year variation in *emm* types causing pharyngitis and other conditions has also been reported in modern studies [30,31], and suggests type-specific protection influences disease dynamics within the community. Age-related increases in the diversity of *emm* types causing pharyngitis [32], alongside increasing prevalence of antibody against common *emm* types [33], further suggests that type-specific protection acquired over successive GAS exposures in childhood reduces the incidence of GAS pharyngitis among teens and adults [15,17,34]. Contributions of type-specific protection to other features of GAS epidemiology, including differences in the composition and diversity of disease-causing *emm* types [11,35], remain important to investigate.

Notably, we identify naturally-acquired protection against previously-encountered *emm* types as well as partially-heterologous *emm* types belonging to previously-encountered *emm* clusters. Several lines of evidence support the biological plausibility of this finding. Immune cross-opsonization within *emm* clusters occurs in animals [10] and humans [19]. In animals, synthetic proteins emulating shared structural components of M protein variants at the level of the *emm* cluster elicit cross-reactive antibodies and bactericidal activity against partially-heterologous *emm* types [36]. While our study observed only 10 of the 48 *emm* clusters, the *emm* types identified account for approximately 70% of GAS isolates in high-income countries [11]. Individual types and clusters may vary in the likelihood of exhibiting cross-protection. Our findings nonetheless suggest the strain coverage of *emm* type-based vaccines may be greater than what has been previously assumed [11].

Strengths of our study include access to data from children within a single school and community, long-term follow-up with up to 5y per child to characterize history of GAS detections, and active surveillance of all respiratory symptoms. However, certain limitations should be considered. Children who were GAS-culture positive with typical symptoms may not have GAS pharyngitis, as viral co-infections could cause symptoms among GAS culture-positive children. Misclassification may also arise from our inability to account for GAS acquisitions preceding enrollment or occurring during the summers, when sampling did

not occur. However, we obtain similar results in analyses restricted to visits preceded by ≥2y of surveillance, suggesting any resulting bias is small. While FIGE typing enabled us to define unique strains transmitted among children in this school-based study, whole-genome sequencing would offer greater resolution for characterizing strains, and could enable assessments of protection associated with non-M antigens [7]. We could not fully disentangle differences in protection associated with previous symptoms and antibiotic prescribing. Larger studies, studies undertaken in settings with higher transmission rates, or studies in settings with differing treatment protocols for GAS pharyngitis [37] may better indicate how symptom severity and antibiotics influence naturally-acquired protection. Last, our analysis considers only protection against symptoms given new GAS detection, and not protection against colonization. In our study, year-to-year changes in circulating GAS *emm* types suggest natural protection may also prevent acquisition of GAS in the upper respiratory tract [38]. Though historical studies presented conflicting evidence of protection against GAS colonization [13,14,16], investigational M protein vaccines have conferred type-specific protection against GAS acquisition in the respiratory tract upon challenge [39,40].

To conclude, we identify that natural GAS acquisition confers strong type-specific protection against future respiratory symptoms when detected a second time during the span of a 5-year longitudinal study. These findings support the plausibility of preventing symptomatic GAS pharyngitis using *emm* type-based vaccines.

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CONFLICTS OF INTEREST

No authors report potential conflicts of interest.

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Table 1: Study enrollment and observations.

Entry year	Observation	Academic year					
		1998-99	1999-2000	2000-01	2001-02	2002-03	All years
1998-99							
	Total enrollment	48	45	41	34	26	48
	Samples obtained	829	800	736	601	466	3432
	Children with GAS detection without symptoms	24	17	16	13	7	30
	Children with GAS-positive typical symptoms	12	15	16	8	5	28
	Children with GAS-positive atypical symptoms	8	6	5	5	2	18
	Visits with GAS detection without symptoms	92	68	111	69	49	389
	Visits with GAS-positive typical symptoms	12	21	26	10	8	77
	Visits with GAS-positive atypical symptoms	11	7	5	7	2	32
1999-2000							
	Total enrollment	– –	30	26	22	18	30
	Samples obtained	– –	520	470	410	316	1716
	Children with GAS detection without symptoms	– –	15	12	6	3	21
	Children with GAS-positive typical symptoms	– –	11	11	10	7	21
	Children with GAS-positive atypical symptoms	– –	8	5	1	2	13
	Visits with GAS detection without symptoms	– –	89	77	39	23	228
	Visits with GAS-positive typical symptoms	– –	15	17	14	8	54
	Visits with GAS-positive atypical symptoms	– –	11	6	1	3	21
2000-01							
	Total enrollment	– –	– –	32	26	20	32
	Samples obtained	– –	– –	540	478	371	1389
	Children with GAS detection without symptoms	– –	– –	10	9	6	12
	Children with GAS-positive typical symptoms	– –	– –	14	11	7	23
	Children with GAS-positive atypical symptoms	– –	– –	7	5	0	11
	Visits with GAS detection without symptoms	– –	– –	58	55	36	149
	Visits with GAS-positive typical symptoms	– –	– –	21	15	11	47
	Visits with GAS-positive atypical symptoms	– –	– –	7	7	0	14
2001-02							
	Total enrollment	– –	– –	– –	15	8	15
	Samples obtained	– –	– –	– –	216	148	364
	Children with GAS detection without symptoms	– –	– –	– –	5	4	8
	Children with GAS-positive typical symptoms	– –	– –	– –	4	3	6
	Children with GAS-positive atypical symptoms	– –	– –	– –	4	5	9
	Visits with GAS detection without symptoms	– –	– –	– –	19	27	46
	Visits with GAS-positive typical symptoms	– –	– –	– –	5	6	11
	Visits with GAS-positive atypical symptoms	– –	– –	– –	5	7	12
2002-03							
	Total enrollment	– –	– –	– –	– –	20	20
	Samples obtained	– –	– –	– –	– –	340	340
	Children with GAS detection without symptoms	– –	– –	– –	– –	7	7
	Children with GAS-positive typical symptoms	– –	– –	– –	– –	4	4
	Children with GAS-positive atypical symptoms	– –	– –	– –	– –	3	3
	Visits with GAS detection without symptoms	– –	– –	– –	– –	32	32
	Visits with GAS-positive typical symptoms	– –	– –	– –	– –	5	5
	Visits with GAS-positive atypical symptoms	– –	– –	– –	– –	3	3
Full cohort							
	Total enrollment	48	74	99	97	92	145
	Samples obtained	829	1320	1746	1705	1641	7241
	Children with GAS detection without symptoms	24	32	38	33	27	78
	Children with GAS-positive typical symptoms	12	26	41	33	26	82
	Children with GAS-positive atypical symptoms	8	14	17	15	12	54
	Visits with GAS detection without symptoms	92	157	246	182	167	844
	Visits with GAS-positive typical symptoms	12	36	64	44	38	194
	Visits with GAS-positive atypical symptoms	11	18	18	20	15	82

GAS: Group A *Streptococcus*

Table 2: GAS types observed during study period.

emm Cluster	*emm* type	FIGE type	Year					
			1998-99	1999-2000	2000-01	2001-02	2002-03	All years
E1								
	4	1	35	19	0	0	4	58
E3								
	58	2	0	0	0	0	9	9
E4								
	22	3	0	0	1	0	0	1
	28	4	0	91	33	12	0	136
	77	5	0	0	3	0	0	3
	89	6	0	34	57	9	36	136
		7	0	0	0	6	0	6
		8	0	0	0	0	19	19
E6								
	75	9	3	11	7	2	10	33
	94	10	0	1	0	0	0	1
A-C3								
	1	11	9	6	6	19	5	45
		12	0	0	0	2	7	9
A-C4								
	12	13	0	1	0	12	57	70
		14	0	0	0	25	0	25
A-C5								
	3	15	0	6	16	0	0	22
		16	0	0	0	18	0	18
5								
	5	17	0	0	0	3	0	3
		18	6	5	25	7	5	48
		19	7	0	0	0	0	7
6								
	6	20	2	20	156	4	0	182
		21	0	0	0	5	35	40
		22	0	0	0	126	0	126
		23	0	0	0	3	27	30
29								
	29	24	53	17	11	0	0	81

GAS: Group A *Streptococcus*
FIGE: Field inversion gel electrophoresis; FIGE types are defined by matched banding patterns.
Cell values indicate the number of isolates, from each year, belonging to each FIGE type, grouped in the left-hand columns by the associated *emm* types and *emm* clusters.

Table 3: Summary of recurrent detections of GAS strains.

First occurrence of any FIGE type		**Second occurrence of same FIGE type, separated by ≥2 negative cultures**		
Symptoms	*N* (%)	*Symptoms*	*N* (%)	
			Among children with first detection	Among children with recurrence
Typical symptoms[1,2]	112 (40.0)			
		Typical symptoms	9 (8.0)	9 (27.3)
		Atypical symptoms	3 (2.7)	3 (9.1)
		Without symptoms	21 (18.8)	21 (63.6)
		Any second detection	33 (29.5)	33 (100)
		No second detection	79 (70.5)	– –
		Total	112 (100)	– –
Atypical symptoms[1,2]	41 (14.6)			
		Typical symptoms	2 (4.9)	2 (20.0)
		Atypical symptoms	2 (4.9)	2 (20.0)
		Without symptoms	6 (14.6)	6 (60.0)
		Any second detection	10 (24.4)	10 (100)
		No second detection	31 (75.6)	– –
		Total	41 (100)	– –
No symptoms[1,2]	127 (45.4)			
		Typical symptoms	6 (4.7)	6 (15.0)
		Atypical symptoms	3 (2.4)	3 (7.5)
		Without symptoms	31 (24.4)	31 (77.5)
		Any second detection	40 (31.5)	40 (100)
		No second detection	87 (68.5)	– –
		Total	127 (100)	– –
Any symptoms status[2]	280 (100)			
		Typical symptoms	17 (6.1)	17 (20.5)
		Atypical symptoms	8 (2.9)	8 (9.6)
		Without symptoms	58 (20.7)	58 (69.9)
		Any second detection	83 (29.6)	83 (100)
		No second detection	197 (70.4)	– –
		Total	280 (100)	– –

GAS: Group A *Streptococcus*

FIGE: Field inversion gel electrophoresis; FIGE types are defined by matched banding patterns.

Entries indicate the number of distinct FIGE types detected and re-detected after ≥2 intervening GAS-negative cultures, for each child, summed over all children.

1. We do not identify strong evidence of a difference in the proportion of children with typical symptoms, atypical symptoms, and no symptoms on their second detection of a FIGE type among those who experienced typical symptoms, atypical symptoms, or no symptoms on their first detection of the same PFGE type ($\chi^2_{\mathrm{df}=4} = 3.3$; p=0.5).
2. The distribution of typical symptoms, atypical symptoms, and no symptoms on second detections of a FIGE type differs significantly from the distribution of typical symptoms, atypical symptoms, and no symptoms on first detections ($\chi^2_{\mathrm{df}=2} = 15.6$; p=0.004)

Table 4: History of homologous-type detection at GAS-positive visits with typical symptoms and no symptoms.

Outcome	Previously-detected FIGE type		Distinct FIGE type belonging to previously-detected *emm* type		Heterologous *emm* type belonging to previously-detected *emm* cluster	
	No previous detection[1]	Previous detection	No previous detection[1]	Previous detection	No previous detection[1]	Previous detection
All visits						
GAS-positive without symptoms, number of visits	93	68	503	192	503	69
GAS-positive typical symptoms, number of visits	112	18	150	15	150	7
Est. protection against typical symptoms (95% CI), %	ref.	81.8 (67.1, 91.7)	ref.	94.5 (83.5, 98.6)	ref.	77.1 (33.7, 96.3)
Visits preceded by ≥2y surveillance						
GAS-positive without symptoms, number of visits	35	39	202	68	202	11
GAS-positive typical symptoms, number of visits	44	8	58	4	58	5
Est. protection against typical symptoms (95% CI), %	ref.	85.9 (47.1, 97.8)	ref.	82.7 (27.3, 97.1)	ref.	–21.7 (–1519.1, 85.5)

GAS: Group A *Streptococcus*

FIGE: Field inversion gel electrophoresis; FIGE types are defined by matched banding patterns.

Estimates of protection are obtained as one minus the matched odds ratio, accounting for matching strata. We derive 95% confidence intervals via cluster-bootstrap resampling of children.

1. We define “no previous detection” as no prior detection of any isolate belonging to the same FIGE type, *emm* type, or *emm* cluster. Fewer observations are available for analyses of prior detection of the same FIGE type because we exclude second detections not separated from prior detections by ≥2 GAS-negative swabs.

Table 5: History of homologous-type detection at GAS-positive visits with atypical symptoms and no symptoms.

Outcome	Previously-detected FIGE type		Distinct FIGE type belonging to previously-detected *emm* type		Heterologous *emm* type belonging to previously-detected *emm* cluster	
	No previous detection[1]	Previous detection	No previous detection[1]	Previous detection	No previous detection[1]	Previous detection
All visits						
GAS-positive without symptoms, number of visits	93	68	503	192	503	69
GAS-positive atypical symptoms, number of visits	28	12	42	15	42	7
Est. protection against atypical symptoms (95% CI), %	ref.	8.3 (–133.0, 67.9)	ref.	46.5 (–353.2, 90.00	ref.	–84.8 (–364.5, 46.1)
Visits preceded by ≥2y surveillance						
GAS-positive without symptoms, number of visits	35	39	202	68	202	11
GAS-positive atypical symptoms, number of visits	5	5	10	3	10	0
Est. protection against atypical symptoms (95% CI), %	ref.	13.3 (–∞, 82.7)	ref.	32.3 (–423.2, 90.0)	ref.	– –

GAS: Group A *Streptococcus*

FIGE: Field inversion gel electrophoresis; FIGE types are defined by matched banding patterns.

Estimates of protection are obtained as one minus the matched odds ratio, accounting for matching strata. We derive 95% confidence intervals via cluster-bootstrap resampling of children.

1. We define “no previous detection” as no prior detection of any isolate belonging to the same FIGE type, *emm* type, or *emm* cluster. Fewer observations are available for analyses of prior detection of the same FIGE type because we exclude second detections not separated from prior detections by ≥2 GAS-negative swabs.

Table 6: History of homologous-type detection and antibiotic receipt at GAS-positive visits with typical symptoms, atypical symptoms, and no symptoms.

Outcome	Previously-detected FIGE type			Distinct FIGE type belonging to previously-detected *emm* type			Heterologous *emm* type belonging to previously-detected *emm* cluster		
	No previous detection[1]	Previous detection		No previous detection[1]	Previous detection		No previous detection[1]	Previous detection	
		No antibiotic prescribed	*Antibiotic prescribed*		*No antibiotic prescribed*	*Antibiotic prescribed*		*No antibiotic prescribed*	*Antibiotic prescribed*
GAS-positive without symptoms, number of visits	93	39	29	503	125	67	503	45	24
GAS-positive typical symptoms, number of visits	112	6	12	150	6	9	150	2	5
GAS-positive atypical symptoms, number of visits	28	3	9	42	6	9	42	3	4
Est. protection against typical symptoms, from any previous detection, % (95% CI)	ref.	89.6 (66.3, 97.1)	71.2 (37.8, 89.4)	ref.	95.1 (77.3, 99.1)	94.1 (71.0, 98.9)	ref.	86.7 (38.0, 97.0)	48.8 (–75.4, 88.4)
Est. protection against atypical symptoms, from any previous detection, % (95% CI)	ref.	42.0 (–168.8, 87.0)	–51.9 (–363.0, 56.6)	ref.	71.1 (–120.0, 95.7)	–20.6 (–1705.0, 86.1)	ref.	–25.8 (–379.1, 64.0)	– –

GAS: Group A *Streptococcus*

FIGE: Field inversion gel electrophoresis; FIGE types are defined by matched banding patterns.

Estimates of protection are obtained as one minus the matched odds ratio, accounting for matching strata. We derive 95% confidence intervals via cluster-bootstrap resampling of children.

1. We define “no previous detection” as no prior detection of any isolate belonging to the same FIGE type, *emm* type, or *emm* cluster. Fewer observations are available for analyses of prior detection of the same FIGE type because we exclude second detections not separated from prior detections by ≥2 GAS-negative swabs.

Table 7: History of homologous-type detection, symptoms, and antibiotic receipt at GAS-positive visits with typical symptoms, atypical symptoms, and no symptoms.

Exposure	Outcome	Previously-detected FIGE type			Distinct FIGE type belonging to previously-detected *emm* type			Heterologous *emm* type belonging to previously-detected *emm* cluster		
		No previous detection[1]	Previous detection		No previous detection[1]	Previous detection		No previous detection[1]	Previous detection	
			No antibiotic prescribed	*Antibiotic prescribed*		*No antibiotic prescribed*	*Antibiotic prescribed*		*No antibiotic prescribed*	*Antibiotic prescribed*
Previous detection with typical symptoms										
	GAS-positive without symptoms, number of visits	93	– –	25	503	– –	65	503	– –	13
	GAS-positive typical symptoms, number of visits	112	– –	10	150	– –	6	150	– –	3
	GAS-positive atypical symptoms, number of visits	28	– –	6	42	– –	8	42	– –	1
	Est. protection against typical symptoms, % (95% CI)	ref.	– –	73.2 (36.3, 91.6)	ref.	– –	94.7 (77.2, 98.9)	ref.	– –	47.5 (–234.0, 93.8)
	Est. protection against atypical symptoms, % (95% CI)	ref.	– –	15.5 (–169.7, 81.1)	ref.	– –	38.6 (–238.8, 90.0)	ref.	– –	– –
Previous detection without symptoms only										
	GAS-positive without symptoms, number of visits	93	18	– –	503	88	– –	503	37	– –
	GAS-positive typical symptoms, number of visits	112	5	– –	150	6	– –	150	2	– –
	GAS-positive atypical symptoms, number of visits	28	3	– –	42	3	– –	42	2	– –
	Est. protection against typical symptoms, % (95% CI)	ref.	76.2 (33.6, 94.7)	– –	ref.	92.8 (66.8, 98.8)	– –	ref.	84.9 (25.9, 96.8)	– –
	Est. protection against atypical symptoms, % (95% CI)	ref.	–6.3 (–420.5, 81.9)	– –	ref.	77.9 (–105.2, 95.8)	– –	ref.	16.3 (–161.7, 73.4)	– –

GAS: Group A *Streptococcus*

FIGE: Field inversion gel electrophoresis; FIGE types are defined by matched banding patterns.

Estimates of protection are obtained as one minus the matched odds ratio, accounting for matching strata. We derive 95% confidence intervals via cluster-bootstrap resampling of children.

1. We define “no previous detection” as no prior detection of any isolate belonging to the same FIGE type, *emm* type, or *emm* cluster. Fewer observations are available for analyses of prior detection of the same FIGE type because we exclude second detections not separated from prior detections by ≥2 GAS-negative swabs.