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Capsule-Negative *emm* Types Are an Increasing Cause of Pediatric Group A Streptococcal Infections at a Large Pediatric Hospital in Texas

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Abstract

Background.—Bacterial infections caused by group A *Streptococcus* (GAS) are common in childhood. Few study reports have provided data on pediatric-specific trends in the epidemiology and bacterial strain characteristics of GAS infections.

Methods.—We prospectively collected GAS isolates from the clinical microbiology laboratory at Texas Children's Hospital between July 1, 2013, and June 30, 2017. Patient characteristics and GAS disease categories were determined through chart review. GAS isolates were obtained from patients in either the inpatient or outpatient setting, and cases were defined as pharyngeal disease, skin and soft-tissue infection (SSTI), or invasive disease on the basis of predefined criteria. All isolates were *emm* typed to determine trends over time.

Results.—We identified 930 cases over the 4-year period, including 432 (46.4%) pharyngeal, 235 (25.3%) SSTI, and 263 (28.3%) invasive disease types. The most frequently encountered *emm* types were *emm1* (21.4%), *emm12* (15.7%), *emm89* (14.6%), *emm4* (9.2%), and *emm3* (8.2%). We observed significant changes over the 4-year period in the relative frequency of infections caused by *emm1* (−17.7%; $P = .046$), *emm4* (8.7%; $P = .023$), or *emm6* (−7.9%; $P = .024$). Using

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Supplementary Data

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bioinformatic analyses and targeted gene sequencing, we also discovered that all GAS *emm28* and *emm87* types harbored mutations that rendered them incapable of producing capsule. The relative frequency of GAS disease cases caused by capsule-negative GAS *emm* types (*emm4*, *emm22*, *emm28*, *emm87*, and *emm89*) increased over the 4-year period (32.2%–44.4%), although the difference was statistically significant for only nonpharyngeal disease types (27.1%–43.9%; $P = .038$).

Conclusions.—Our data suggest an evolving epidemiology of GAS in the Houston pediatric population characterized by an increase in the frequency of capsule-negative *emm* types.

Keywords

capsule; *emm* type; epidemiology; invasive; group A *Streptococcus*

Streptococcus pyogenes (group A *Streptococcus* [GAS]) is responsible for a wide array of disease. GAS most frequently causes relatively benign infections, such as pharyngitis (“strep throat”) and superficial skin infections (eg, impetigo). However, GAS infection also can result in severe life-threatening disease, such as necrotizing fasciitis (“flesh-eating disease”) and streptococcal toxic shock syndrome (STSS). The highest incidence rates have been reported in children aged <5 years and adults aged ≥65 years [1, 2]. Although a high proportion of cases in elderly patients result in death, case-fatality rates in children have been relatively low [1]. Although under development, no vaccine to prevent GAS infection is currently licensed for use [3].

Active prospective surveillance of GAS disease is crucial for identifying emergent GAS clones capable of increased disease frequency or severity. For example, pathogen-specific genetic changes beginning in the 1980s resulted in the emergence of an epidemic clone of M serotype 1 (M1 [*emm1*]) that is now the leading cause of invasive GAS disease [4]. More recently, *emm89* GAS has emerged as a significant cause of invasive disease, despite the fact that the emergent *emm89* GAS strains lack the genes necessary for capsule production, a virulence factor previously believed to be critical for causing GAS disease [5, 6].

Studies of the GAS capsule spanning the past century seemingly solidified its role as a key GAS virulence factor [7]. The GAS capsule consists of hyaluronic acid and is a major contributor to resistance to phagocytosis by human neutrophils [8-10]. However, results of studies have suggested that the capsule might be dispensable under certain conditions (eg, asymptomatic carriage) [11]. In addition, the discovery that certain *emm* types lack the genes necessary for capsule biosynthesis [12-14] yet are major contributors to pharyngeal [15] and invasive [1] GAS disease calls into question the role of the GAS capsule in pathogenicity. No studies have examined the frequency with which capsule-negative GAS strains cause disease in adult or pediatric populations.

We sought to better understand the epidemiology of GAS disease and the contribution of capsule-negative GAS in a pediatric population by determining epidemiologic GAS trends over 4 years at a freestanding children’s hospital in a large metropolitan area in Texas. Our data highlight the importance of GAS disease in children and document dramatic shifts in epidemiology, including the rise of capsule-negative GAS *emm* types.

METHODS

Surveillance

GAS isolates were collected between July 1, 2013, and June 30, 2017, under a protocol approved by the institutional review board at Baylor College of Medicine. All GAS isolates were identified in the clinical microbiology laboratory at Texas Children's Hospital (TCH) in Houston. TCH includes a 651-bed freestanding hospital located in the Texas Medical Center and 2 satellite hospitals, West Campus and The Woodlands, that opened in 2010 and 2017, respectively. All 3 hospitals have outpatient, emergency, and inpatient services. Microbiologic specimens are received and processed at the main hospital in the Texas Medical Center, which facilitates the collection of GAS isolates from all inpatient and outpatient (emergency and outpatient clinic) encounters.

Definitions

Medical records of patients with a GAS-positive culture result were reviewed to determine their diagnosis and categorize their GAS disease. We modified the case-report form used for active laboratory- and population-based surveillance conducted by the Centers for Disease Control and Prevention (CDC) Active Bacterial Core surveillance (ABCs) system (https://www.cdc.gov/abcs/downloads/abcs-case-rpt-form.pdf, accessed 6 June 2018) to define infection types with the following exceptions: abscesses were subcategorized as being derived from lymph node, peritonsillar, retropharyngeal, or an other site; we included distinct categories for impetigo and erysipelas, because these diagnoses were identified frequently. We categorized infection types into 1 of 3 groups: invasive, skin and soft-tissue infection (SSTI), or pharyngeal. Invasive disease was defined as illness in a patient from whom GAS was isolated from a normally sterile site (including blood, bone, joint fluid, cerebrospinal fluid, pleural fluid, or deep tissue) or cultured from any site/source from a patient who had STSS or necrotizing fasciitis. GAS disease was categorized as an SSTI if the bacteria were cultured from skin or subcutaneous tissue and were not from a patient who had a disease known to be invasive. Any GAS isolate cultured from the throat was considered pharyngeal. GAS isolates cultured from the throat of patients in the absence of symptoms (n = 74) were excluded. A GAS case was defined as any 1 of these 3 types of infections.

Inpatient encounters were defined as admission to 1 of the hospital units for ≥24 hours. Outpatient encounters included any emergency center or outpatient clinic visit for which the patient was not admitted. Outpatient encounters that resulted in hospital admission in <48 hours after the initial outpatient encounter with the same diagnosis were considered inpatient.

Microbiology and *emm* Typing

Growth and stocking of isolates was performed as described previously [16]. Genomic DNA was extracted from GAS isolates as described previously using the DNeasy blood and tissue kit (Qiagen, Germantown, MD, USA) with modifications for Gram-positive bacteria [17]. Polymerase chain reaction amplification and sequencing of the *emm* gene were performed according to the CDC *Streptococcus* laboratory protocol (https://www.cdc.gov/

streplab/protocol-emm-type.html, accessed 6 June 2018). Specific *emm* types were assigned using the basic local alignment search tool (BLAST) [18] to identify the *emm* reference sequence downloaded from the CDC *Streptococcus* laboratory. We categorized GAS *emm* types further to establish the following GAS tissue tropism patterns: pattern AC (throat), pattern D (skin), or pattern E (no obvious [generalist] site of infection preference) [19, 20]. We also categorized GAS strains on the basis of *emm* cluster definitions, as provided by Sanderson-Smith et al [21].

Bioinformatic Analyses and *hasA* Gene Sequencing

We interrogated the essential genes for capsule synthesis, *hasAB* [5], from all publicly available GAS genomes (n = 58, excluding 2 M89 and 2 M4 genomes lacking *hasAB*). Alignment of the *hasAB* genes (including the *hasA* promoter) was performed using Geneious 11 (Biomatters, Inc., Newark, New Jersey). Nucleotide differences were identified by visual inspection. De novo assembly of and polymorphism identification in the *hasAB* genes of previously sequenced *emm28* [22] and *emm87* [17, 22] GAS strains was performed using SPAdes [23] and CLC Genomics Workbench 11 (Qiagen). The first gene of the capsule operon (*hasA*) of all *emm28* isolates identified in our study was polymerase chain reaction amplified and sequenced as described previously [12].

Statistics

Statistical calculations were performed using Prism (GraphPad, San Diego, California). The Fisher exact test was used to compare categorical variables. Linear regression (goodness of fit [R^2]) was used to identify trends in the relative frequency of GAS *emm* types over the 4 years. A P value of <.05 was considered significant for all comparisons.

RESULTS

Demographics and Clinical Characteristics

A total of 930 cases of GAS were identified during the 4-year period, for an average of 232 cases per year. There was a slight male predominance (54.2%) (Table 1). The majority of GAS isolates were derived from patients with a disease type classified as pharyngeal (n = 432 [46.5%]), and the others had a disease type of invasive (n = 263 [28.3%]) or SSTI (n = 235 [25.3%]) (Table 1). Only 34 (6.8%) cases were among patients who received care in the intensive care unit; 17 (50%) cases were in patients diagnosed with STSS or septic shock. Two deaths were identified, both of which were of children diagnosed with STSS.

emm Sequence Types

All 930 GAS isolates were *emm* typed successfully. Over the 4-year period, the 5 most frequently encountered *emm* types were *emm1* (21.4%), *emm12* (15.7%), *emm89* (14.6%), *emm4* (9.2%), and *emm3* (8.2%) (Figure 1A). However, when examining the frequency of *emm* type according to 12-month period, we observed significant differences in the relative frequencies of multiple *emm* types over the 4 years (Supplementary Tables S1 and S2). Cases of GAS of *emm* type 1 significantly decreased in frequency every year according to our examinations of all infections (R^2 = 0.911; P = .046) and of invasive (R^2 = 0.936; P = .033) and SSTI (R^2 = 0.936; P = .032) disease separately (Supplementary

Table S2). We observed a similar significant decrease in the overall annual frequency of *emm6* GAS (R^2 = 0.963; *P* = .019); however, this decrease was not statistically significant when stratified according to source (Supplementary Table S2). In contrast, we observed a significant overall annual increase in the frequency of *emm4* (R^2 = 0.955; *P* = .023), but that trend was statistically significant only among pharyngeal source isolates (R^2 = 0.962; *P* = .019) (Supplementary Table S2).

When we used the *emm*-typing results to categorize GAS strains according to *emm* cluster, we identified 9 *emm* clusters, and >90% of them were assigned to the AC or E clusters (Figure 1B). We observed a significantly greater frequency of E1 (*emm4*) cluster isolates among cases defined as SSTI than among those defined as invasive (15.7% vs 3.4%, respectively; *P* < .0001) or pharyngeal (15.7% vs 9.3%, respectively; *P* = .0011). In addition, a significantly greater proportion of GAS isolates from cases defined as invasive than from those defined as SSTI were of the AC5 cluster (*emm3*) (50.2% vs 40%, respectively; *P* = .023). When we examined seasonal trends of *emm* clusters, we observed that isolates defined as cluster E peaked in the mid- to late summer months (quarter 3 [Q3], July to September), whereas AC cluster isolates peaked anywhere between October and June (Figure 1C). We also observed a significant increase in quarterly trends for *emm4* beginning in Q4 of 2013 through Q2 of 2017 (R^2 = 0.411; *P* = .01) (Figure 1C).

Increasing Frequency of Capsule-Negative *emm* Types

Among the *emm* types identified, several of the more prominent types are known to lack capsule production (eg, *emm4*, *emm22*, and *emm89*). Thus, we next sought to identify additional GAS emm types incapable of producing hyaluronic acid capsule using bioinformatic methods. Comparing *hasAB* genes from all completed reference genomes, we discovered a single-base-pair insertion in *hasA* of *emm87* GAS not identified in a previous study of *emm87* [17] that resulted in a frameshift mutation and early truncation of the HasA protein (Supplementary Figure S1 and Table S3). Analysis of whole-genome sequencing of the *emm87* GAS isolates (n = 30) confirmed the identical *hasA* insertion in all of them (Supplementary Table S4) [17].

In addition to the frameshift mutation in *hasA* of *emm87* GAS, our analysis revealed that all 6 M28 GAS reference genomes had an identical single-base-pair insertion in *hasA* that results in a reading frameshift and early truncation (Supplementary Figure S1 and Table S3). The *emm28* GAS reference genomes are temporally and geographically diverse [24-28]. The *hasA* disruption identified in the *emm28* GAS reference genomes was present in all 27 *emm28* GAS isolates in our study (Supplementary Table S3). We also discovered the same frameshift mutations in *hasA* identified in our analyses among 95 *emm28* and 26 *emm87* isolates recently sequenced by the CDC [22] (data not shown).

After adding *emm28* and *emm87* to the list of capsule-non-producing GAS types, 4 of the 10 *emm* types that increased in relative frequency over the study period are known or predicted to lack capsule production (*emm4*, *emm22*, *emm28*, and *emm87*) (Supplementary Table S1). We observed an increase in the overall percentage of GAS disease caused by capsule-negative *emm* types (*emm4*, *emm22*, *emm28*, *emm87*, and *emm89*) over the 4 seasons (32.2%–44.4%) (Figure 2A). However, this trend was statistically significant only

after excluding isolates from a pharyngeal source (Figure 2B). The increase was not caused by the most prevalent capsule-negative *emm* type (*emm89*); rather, the increase was a result primarily of increases in the frequency of *emm4* and *emm28*. Moreover, all capsule-negative *emm* types are designated cluster E and occurred most frequently in cases defined as SSTI (Figure 1B). In contrast, cluster AC GAS strains (predicted to be encapsulated) were associated with more severe disease. Approximately 75% (25 of 34) of GAS cases in patients cared for in the intensive care unit were from GAS strains predicted to produce capsule, the majority of which, including those involved in the 2 deaths, were *emm1* (14 of 25).

DISCUSSION

Disease caused by GAS remains a significant cause of morbidity in pediatric populations. To date, our study is one of the largest epidemiologic studies of pediatric GAS disease. We identified more than 900 cases of GAS infection over a 4-year period in both inpatient and outpatient settings. Important to note is that in addition to invasive disease, our study included a large number of pharyngeal infections and SSTIs identified primarily in the outpatient setting. Overall, our study results provide several novel observations, including annual decreases in the frequency of *emm1* GAS and concomitant increases in the prevalence of capsule-negative GAS *emm* types.

Our observation of a marked decrease in both the total number and percentage of cases of GAS disease caused by *emm1* was surprising. *emm1* GAS has consistently been the leading cause of GAS infection in the United States, has accounted for approximately 20% of cases of GAS disease in pediatric and adult populations since 2000 [1, 2]. It is not surprising that in the first year-long period of our study, more than 30% of GAS diseases was attributable to *emm1*. However, a progressive and sharp decline in the frequency of disease caused by *emm1* GAS was observed every year, which resulted in a cumulative decrease of close to 20% over the study period. To our knowledge, this is the first carefully described decrease in disease caused by *emm1* GAS over multiple years in a US population since the emergence of *emm1* GAS as a major cause of invasive disease in the 1980s. On further investigation of ABCs trends, although *emm1* has remained the dominant *emm* type across combined ABCs sites since 1997, transient declines in the predominance of *emm1* for 2–3 years have been seen within individual ABCs sites (see https://www.cdc.gov/abcs/reports-findings/surv-reports.html, accessed 6 June 2018). ABCs site-specific observations have likely been masked by combining the 10 surveillance regions. Important to note is that we did not observe a dominant GAS *emm*-type replacement. Rather, collective increases in prevalence of multiple GAS *emm* types were responsible for maintaining stable numbers of GAS disease in the population. Similar *emm*-type variability has been found in surveillance of pediatric GAS pharyngitis [29]. Given that *emm1* GAS is known to cause severe GAS infection [1, 2], it is possible that the decline in *emm1* GAS infections will result ultimately in a decrease in the incidence of severe GAS disease. Whether host, pathogen, or a combination of factors is responsible for the decline in the incidence of *emm1* GAS, and whether that decline will persist, remains to be elucidated.

Alongside the decrease in the frequency of *emm1* disease, we discovered a significant increase in the frequency of GAS *emm* types known to lack capsule production. Only in the past 5 years has it been recognized that GAS *emm* types that completely lack the genes essential for capsule production (eg, *emm4*) are major contributors to invasive disease [12-14]. For decades, the GAS capsule (composed of hyaluronic acid identical to that produced in humans) was believed to be a critical virulence factor [5, 6]. Discovered more recently is the prevalence of type *emm89* GAS increased dramatically world-wide beginning in the early 2000s, and this increase was linked to clonal replacement with a strain that lacks the essential capsule genes [13, 14]. Collectively, we observed a 12% increase in the prevalence of capsule-negative GAS *emm* types (ie, *emm4*, *emm22*, *emm28*, *emm87*, and *emm89*) over the 4-year study period, and close to 50% of all GAS isolates in the 2016–2017 period were capsule negative. It is intriguing that the capsule-negative *emm4* GAS isolates had a significant increase in frequency over the 4-year period and that they were represented disproportionately among SSTI and pharyngeal sources (77 of 86 [89.5% of total]). Continued surveillance is essential for determining if the observed changes in the frequency of capsule-negative GAS are sustained over time.

The discovery of a conserved mutation in *hasA* among *emm28* GAS isolates is of considerable interest. *emm28* GAS is consistently among the top 5 *emm* types surveyed in the CDC ABCs over the past 20 years (see https://www.cdc.gov/abcs/reports-findings/surv-reports.html, accessed 6 June 2018). The presence of the R28 antigen gene, a homolog of an adhesin found in group B *Streptococcus* [30], is believed to a major factor that leads to increased frequency of *emm28* GAS involved in postpartum invasive GAS infections [31, 32], a diagnosis not encountered in our pediatric data set. In contrast to the *emm89* epidemic, in which the loss of capsule production was linked to increased expression of the secreted toxins nicotinamide adenine dinucleotide-glycohydrolase (NADase) and streptolysin O (SLO) (encoded by *nga* and *slo*, respectively) [14], currently circulating *emm28* GAS strains do not possess the same promoter mutation [22]. In addition, almost half (39 of 95) of the 2015 CDC *emm28* isolates possessed a mutation in *nga* that rendered it inactive [33]. Thus, it seems that 2 populations of *emm28* GAS isolates are circulating currently in the United States. Evidence that this conserved *hasA* frameshift has been a long-standing feature of *emm28* strains is the fact that it was found within 5 observed single-locus variants (2 with inactive *nga* and 3 with active *nga*) of the major *emm28*/sequence type 52 complex recovered during an invasive GAS disease surveillance in 2015. Whether we are in the midst of a new *emm28* clone emergence is unclear. Although we did not assess *emm28* GAS for the production of capsule, given the nature of the mutation (frameshift in *hasA*), the studies that have demonstrated the essentiality of *hasA* for capsule production [5], and the effect of similar frameshift mutations in *hasA* on capsule production [11, 17], it is unlikely that *emm28* GAS produces hyaluronic acid capsule. More detailed investigations using temporally diverse strains are needed to determine the molecular mechanisms behind the disease success of capsule-negative *emm28* GAS.

Our study had several limitations. We included only 1 hospital system and metropolitan area, but because TCH serves as a major referral center for all 51 associated outpatient clinics and includes a catchment area of >6 million persons, our sample is likely representative of the greater Houston area. It is possible that the inclusion of additional sites could have

influenced the rate of GAS disease and *emm* type distributions, among other factors. We did not apply strict criteria to identifying GAS pharyngitis. Thus, it is possible that some GAS cases defined as pharyngeal represent carriage, but only GAS isolates from patients with symptoms were included in the study. Likewise, because most cases of GAS pharyngitis are diagnosed in the outpatient community setting, our hospital-based collection might not be representative of the true *emm*-type-specific prevalence in GAS pharyngitis. Some subjects identified as outpatients could have received inpatient care at an outlying hospital, which could have contributed to misclassification bias. Last, the referral base for TCH extends beyond the Houston metropolitan area, which means that we were unable to perform accurate incidence calculations using these data.

In summary, our study provided a comprehensive examination of pediatric GAS infections in a large metropolitan area. We are witnessing a potential epidemiologic shift in GAS *emm* types and an increasing prevalence of capsule-negative strains. Continued surveillance is critical for following the trends in *emm1* and other *emm*-type frequencies to determine if these shifts persist and to better understand the disease-causing potential of GAS strains that lack the genes necessary for capsule production.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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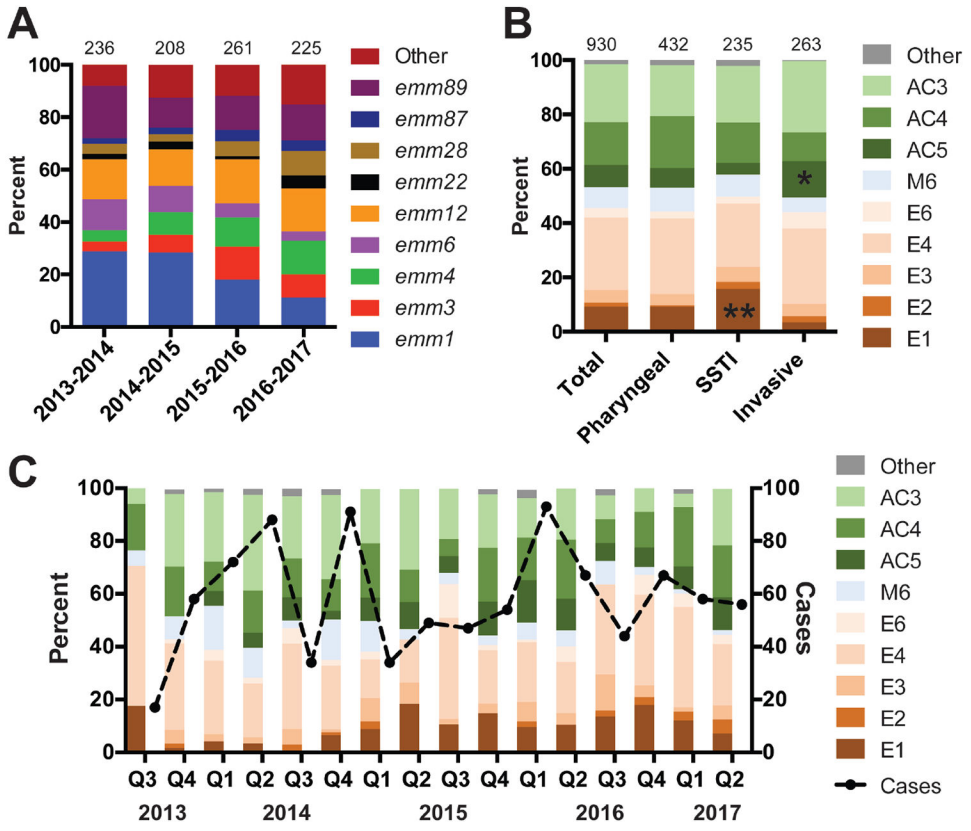

Figure 1.

Distribution of group A Streptococcus (GAS) according to *emm* type and cluster. (A) All GAS *emm* types per 12-month period (2013–2017). A 12-month period was defined as July 1 to June 30 of the following year. The total number of isolates is indicated above the bar for each 12-month period. (B) GAS *emm* clusters according to disease type. The number of cases is indicated above each bar. **, Significant difference ($P < .05$, Fisher exact test) in the frequencies of cluster E1 (*emm4*) isolates obtained from children with a skin and soft-tissue infection (SSTI) compared to those obtained from children with an invasive or pharyngeal infection; *, significant difference ($P < .05$, Fisher exact test) in the frequencies of cluster AC5 (*emm3*) isolates obtained from children with an invasive infection compared to those with only an SSTI. (C) Distribution of *emm* clusters according to 3-month period (ie, quarter) for the entire study. The x-axis indicates quarterly period (eg, Q1 indicates January through March). The total number of cases per quarter is shown using black dots and a dashed line and on the right y-axis.

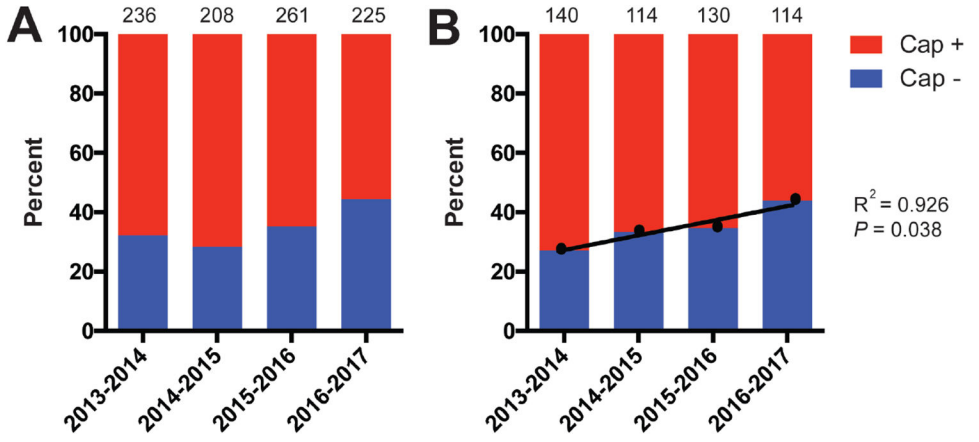

Figure 2.

Proportion of group A *Streptococcus* (GAS) disease stratified according to predicted capsule status for all cases (A) and excluding cases of pharyngeal (ie, invasive and skin and soft-tissue infection [SSTI] combined) (B). Cases of capsule-negative GAS (Cap-, blue bars) included *emm4*, *emm22*, *emm28*, *emm87*, and *emm89*, and those of capsule-positive GAS (Cap+, red bars) are all other identified *emm* types. The superimposed line represents goodness of fit (R^2) determined by linear regression. The number of cases is indicated above the bar for each 12-month period.

Table 1.

Characteristics of GAS Cases at Texas Children's Hospital

Infection Type[a]	Total Cases (n [%])	Inpatient Cases (n [%])	Invasive Cases (n [%])	Male Sex (n [%])	Patient Age[b] (mean [range]) (years)	Patients in ICU[c] (n [%])
Pharyngeal	432 (46.5)	4 (1.3)	—	224 (51.9)	7.7 (0.4–21.3)	—
SSTI	242 (26.0)	111 (36.9)	7 (2.9)	138 (57.0)	6.5 (0.02–23.8)	2 (0.8)
Cellulitis	109	62	4	61	6.5 (0.1–23.4)	1
Impetigo/erysipelas	69	19	2	36	5.5 (0.02–17.2)	—
Abscess (skin)	64	30	1	41	6.6 (1.1–17.7)	1
Abscess	105 (11.3)	79 (26.2)	105 (39.9)	54 (51.4)	8.1 (0.6–21.5)	5 (4.8)
Peritonsillar	51	29	51	26	11.1 (3.1–18.0)	—
Retropharyngeal	32	29	32	13	4.8 (0.7–14.1)	5
Lymph node	22	21	22	15	5.8 (0.6–21.5)	—
OM/mastoiditis[d]	51 (5.5)	17 (5.6)	51 (19.4)	33 (64.7)	5.0 (0.1–18.0)	—
Osteomyelitis/SA[e]	26 (2.8)	26 (8.6)	26 (9.9)	13 (50.0)	6.9 (0.05–12.5)	1 (3.8)
Septic shock/STSS[f]	24 (2.6)	24 (8.0)	24 (9.1)	15 (62.5)	6.4 (0.1–21.3)	17 (70.8)
Pneumonia/empyema[g]	15 (1.6)	14 (4.7)	15 (5.1)	7 (46.7)	7.3 (0.7–16.8)	4 (26.7)
Bacteremia (without a focus)	13 (1.4)	12 (4.0)	13 (4.9)	10 (76.9)	5.8 (0.8–14.0)	1 (7.7)
Necrotizing fasciitis	2 (0.2)	2 (0.7)	2 (0.7)	1 (50.0)	9.9 (3.2–16.6)	1 (50.0)
Other[h]	20 (2.2)	12 (4.0)	20 (8.3)	9 (55.0)	8.5 (0.6–21.5)	3 (17.6)
Total	930	301 (32.4)	263 (28.3)	504 (54.2)	7.2 (0.02–23.8)	34 (3.6)

Abbreviations: GAS, group A *Streptococcus*; ICU, intensive care unit; OM, otitis media; SA, septic arthritis; SSTI, skin and soft-tissue infection; STSS, streptococcal toxic shock syndrome; —, not applicable.

[a] Infection type was determined by discharge diagnosis and chart review (see "Methods").

[b] Some ages are displayed as a decimal number (eg, 6.2 years is 6 years 2 months and 12 days).

[c] Hospitalizations that included admission to the ICU.

[d] Mastoiditis was diagnosed in 12 of 51 children.

[e] Osteomyelitis was diagnosed in 18 of 26 children.

[f] STSS was diagnosed in 19 of 24 children, 2 of whom died.

[g] Empyema was diagnosed in 6 of 15 children.

[h] Includes abscess (not otherwise defined) (n = 3), epiglottitis (n = 1), meningitis (n = 2), pericarditis (n = 1), peritonitis (n = 2), sinusitis (n = 5), and urinary tract infection (n = 6).