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MAJOR ARTICLE

Increase in Invasive Group A Streptococcal Disease and Emergence of Mucoid Strains in a Pediatric Population: February–June 2017

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Background. Infection with group A *Streptococcus* (GAS) can cause severe systemic and locally invasive disease. Invasive group A streptococcal (iGAS) disease incidence varies both seasonally and year-to-year, and it may exhibit clustered outbreaks. We observed an upswing in iGAS cases at a tertiary care Children's Hospital, prompting further characterization of local iGAS disease.

Methods. Cases of iGAS disease were abstracted from the medical record by manual chart review of all positive screening tests and cultures for GAS over a 4-year span. Incidence rates per 1000 hospital admissions and per 100 positive GAS tests were calculated and compared. Selected isolates were further characterized by whole-genome sequencing.

Results. Significant year-to-year differences in per-admission iGAS incidence rate were observed in February and June, although per-positive test incidence rates were not significantly different. Whole-genome sequencing revealed 2 dominant serotypes—emm3 and emm6—with high rates of mucoid phenotype and systemic bacteremia.

Conclusions. We document a significant but transient increase in iGAS disease incidence in 2 months of 2017. Genome sequencing revealed 2 dominant serotypes associated with mucoid phenotypes and severe disease, highlighting the dynamic nature of iGAS disease pattern.

Keywords: bacteremia; group A *Streptococcus*; iGAS; invasive disease; mucoid.

Group A *Streptococcus* (GAS) is an important pathogen in adult and pediatric populations, causing an estimated 500 000 annual deaths worldwide [1]. The clinical manifestations of GAS in humans are diverse, including asymptomatic pharyngeal colonization, superficial pharyngeal, and skin infections as well as invasive disease including bacteremia, abscesses, bone and joint infections, necrotizing fasciitis, and streptococcal toxic shock syndrome (reviewed in [2]).

Invasive group A streptococcal (iGAS) disease is known to exhibit routine seasonal variation in temperate climates, peaking along with pharyngitis in the winter and early spring, as well as episodic genetically related outbreaks [1, 3]. Predominant GAS strains in a population can change over time, driving variation in the distribution of clinical manifestations [4, 5]. The frequency of certain GAS disease manifestations in the United States has varied over time, with a decline in rheumatic fever incidence through the 20th century and a surge in necrotizing fasciitis in the late 1980s and 1990s [6, 7]. True clonal outbreaks of iGAS do occur, which can cause a specific disease manifestation in a local population. Introduction of bacteriophage-encoded virulence factors into circulating strains can also cause an increase in invasive disease [8, 9]. Ongoing local and national surveillance is critical to understanding trends in GAS disease.

In early 2017, a series of iGAS infections in children was observed at a 315-bed tertiary-care Children's hospital in Pittsburgh, Pennsylvania. The overall number of iGAS cases increased relative to previous years, and atypical presentations were observed (eg, sternal osteomyelitis identified in an otherwise healthy infant). Simultaneously, several clinical isolates of GAS from the same hospital displayed a mucoid colony morphology. We reviewed this cluster of iGAS cases, characterized representative GAS strains, and compared iGAS disease rates in recent years to determine whether the perceived increase was indicative of a change in local patterns of iGAS disease.

METHODS

Chart Review

A retrospective review was conducted to identify all iGAS infections from 1 July 2013 to 30 June 2017. All positive rapid antigen detection screening tests for *Streptococcus pyogenes* or cultures for GAS were identified by clinical surveillance

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software, and cases of iGAS were identified by manual chart review. Invasive group A streptococcal disease was defined as GAS "isolated from a normally sterile site, such as blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, pericardial fluid, bone, joint/synovial fluid, or internal body site (eg, lymph node, brain)" or from a wound culture accompanied by necrotizing fasciitis or *Streptococcal* toxic shock syndrome per Centers for Disease Control and Prevention (CDC) definitions [10]. By this definition, GAS causing simple pharyngitis, upper respiratory infection, impetigo, cellulitis, uncomplicated wound infection, or paronychia were excluded. Demographic data (age, gender), microbiological data, and site and nature of infection were manually extracted from the medical record.

Descriptive Epidemiology

Monthly rates of iGAS infection per 1000 hospital admissions and per 100 GAS cases were calculated for each month in the study period. The overall number of GAS cases was defined as the count of all individuals who tested positive for GAS (defined as any positive pharyngeal *Streptococcus* rapid antigen detection screen or GAS culture, from any site) in the hospital inpatient ward or emergency department. Year-to-year differences in monthly rates were evaluated for significance with the Fisher exact test, and year-to-year differences in count data were evaluated for significance with Grubb's test for outliers.

Whole-Genome Sequencing Analysis

To assess for potential genetic relatedness among identified streptococcal strains, 15 iGAS isolates were analyzed by the CDC *Streptococcus* Laboratory via whole-genome sequencing (WGS). Isolates selected for WGS analysis included samples from February to June of 2017 including several from the initial disease cluster. A representative selection of 15 mucoid and nonmucoid invasive isolates from various anatomical locations were selected for WGS, because resources for sequencing were limited and retrospectively identified iGAS infections were not available for analysis. Preparation of GAS chromosomal deoxyribonucleic acid library construction, WGS determination, and bioinformatic analysis were performed as described previously [11, 12], with the exception that the query for the *hasA* determinant required for hyaluronate (capsule) expression was expanded to detect frame-shifts as well as presence/absence (updates available at https://github.com/BenJamesMetcalf). Data examined included *emm*- and T-antigen subtypes, presence of virulence factors, and single-nucleotide polymorphism (SNP) analysis of core genome sequences using kSNP3.0 [13]. Dendrograms showing strain similarity by SNP analysis were created using the hierarchical clustering function in R [14]. This study was approved by the University of Pittsburgh Institutional Review Board.

RESULTS

Twenty-nine cases of iGAS disease were observed during the suspected outbreak of February–June 2017 and are enumerated in Table 1. The first 15 cases represent the initial cluster occurring in February and March that prompted further inquiry into patterns of iGAS disease. A distinct (but overlapping) set of 15 cases was selected for WGS, including isolates from multiple anatomic locations and both mucoid and nonmucoid strains. In this period, 38% of strains were observed to have a mucoid phenotype, a finding not previously noted by our microbiology staff. Some cases of iGAS disease were identified during this period by retrospective chart review, and therefore their capsule phenotype is not known because no bacterial sample was saved for further characterization.

The 4-year chart review yielded 98 total cases of iGAS infections from July 2013 to June 2017. Fifty-three percent of patients were male, mean age was 6 years, and 60% of patients had head and neck infections (largely peritonsillar and retropharyngeal abscesses). The annual case rate for fiscal year (FY) 2014–2016 ranged from 1.4 to 2.1 iGAS cases per month with an increase to 3.0 cases/month in FY 2017 and 5.8 cases/month during the period of interest February–June 2017. Characteristics of iGAS cases during the study period are summarized in Table 2. Of the 98 invasive cases, the majority (70%) had no antibiotic testing performed, making trends in resistance patterns difficult to assess. No patients with iGAS disease died from any cause during the study period.

Monthly rates of iGAS disease per overall hospital admission are summarized in Figure 1 for December–June for FY 2014–17. A significant year-to-year difference was observed for the months of February and June ($P < .05$). No statistically significant differences were observed when comparing monthly rates of iGAS cases per total positive GAS tests (including noninvasive pharyngitis and superficial skin infection) (Figure 2). Monthly proportions of positive GAS tests were calculated by dividing the number of positive tests by the number of total tests sent (Figure 3). Only one record per individual per day was included in this analysis—ie, an individual with multiple negative tests on a single day counts as a single negative, whereas an individual with any positive test was counted as a single positive.

Whole-genome sequence analysis of 15 iGAS strain demonstrated a preponderance of emm3/T3 and emm6/T6 strains, comprising 11 of 15 sequenced strains (Table 1). All emm3 and emm6 strains possessed the intact *hasA* hyaluronic acid synthetase operon determinant, which is responsible for capsule expression [15]. None of the 4 non-emm3/emm6 isolates contained a functional *hasA* determinant (the *emm89* isolates completely lack the *hasA* structural gene, whereas the *emm28* and *emm87* isolates contain frame-shifted *hasA* alleles). The mucoid colony phenotype, associated with increased capsule expression, was seen in

Table 1. Characteristics of 15 Invasive Group A *Streptococcus* (iGAS) Patients From February to March 2017 Prompting Investigation into iGAS Cluster

N	Sex	Age	Month	Source	Diagnosis	emm-Type	T-Type	Mucoid
1[a]	F	2 years	February	Abscess	Peritonsillar abscess	[b]	[b]	Unk
2[a]	M	8 years	February	Abscess	Neck abscess	[b]	[b]	Unk
3[a]	F	10 years	February	Blood	Wound infection with bacteremia	28	T28	No
4[a]	F	6 years	February	Abscess	Retropharyngeal abscess	[b]	[b]	Unk
5[a]	M	5 years	February	Abscess	Peritonsillar abscess	[b]	[b]	Unk
6[a]	M	10 years	February	Abscess	Orbital abscess	[b]	[b]	Unk
7[a]	F	9 years	February	Abscess	Mastoiditis	3.1	T3	Yes
8[a]	M	3 years	February	Blood	Bacteremia	3.1	T3	Yes
9[a]	F	9 years	February	Joint fluid	Septic arthritis of hip	6.1	T6	No
10[a]	M	5 years	March	Abscess	Mastoiditis	6.4	T6	Yes
11[a]	M	8 years	March	Blood	Bacteremia	6.4	T6	Yes
12[a]	M	1 month	March	Bone biopsy	Osteomyelitis of sternum	28	T28	No
13[a]	F	8 years	March	Abscess	Peritonsillar abscess	[b]	[b]	Unk
14[a]	M	10 years	March	Blood	Bacteremia	3.1	T3	Yes
15[a]	M	3 years	March	Abscess	Neck abscess	28	T28	No
16	M	3 years	April	Abscess	Suppurative adenitis	6.4	T6	No
17	M	6 years	April	Abscess	Retropharyngeal abscess	[b]	[b]	Yes
18	F	2 years	April	Abscess	Premaxillary subperiosteal abscess	[b]	[b]	Unk
19	F	3 years	April	Abscess	Surgical site abscess	[b]	[b]	No
20	F	8 years	May	Abscess	Orbital abscess	3.1	T3	Yes
21	F	6 years	May	Abscess	Temporal abscess	6.115	T6	No
22	M	2 weeks	May	Blood	Cellulitis, bacteremia	[b]	[b]	Yes
23	M	7 months	May	Abscess	Retropharyngeal abscess	[b]	[b]	Yes
24	F	8 years	May	Abscess	Peritonsillar abscess	3.1	T3	Yes
25	F	11 years	June	Abscess	Lymphatic malformation infection	[b]	[b]	Unk
26	M	13 years	June	Abscess	Myofascitis, muscle abscess	3.1	T3	Yes
27	F	17 years	June	Abscess	Peritonsilar abscess	[b]	[b]	Unk
28	F	11 years	June	Ascites	Peritoneal fluid infection	[b]	[b]	No
29	M	3 years	June	Joint fluid	Septic arthritis of knee	89	T89	No

Abbreviations: Unk, unknown.

[a]Member of the initial cluster of 15 iGAS cases.

[b]No serotype determined.

all emm3 strains and 2 of 5 emm6 strains but none of the non-emm3/emm6 isolates. Dendrograms showing the level of core-genome relatedness amongst emm3 and 6 strains are shown in Figure 4.

DISCUSSION

In February and June of 2017, we observed a significantly increased per-admission incidence rate of iGAS disease relative to recent years. Patterns in the overall number of GAS cases

Table 2. Characteristics of Invasive Group A *Streptococcus* (iGAS) Infections Fiscal Year (FY) 2014–2017[a]

Selected Group	FY14	FY15	FY16	FY17	Suspected Outbreak Period	Mucoid Strains Only	Total
Months	July-June	July-June	July-June	July-June	February-June	February-June	NA
No. of cases	17	20	25	36	29	11	98
Cases per month	1.4	1.7	2.1	3.0	5.8	2.2	2.0
Gender (%male)	53	50	52	56	52	73	53
Age (mean [range])	5 [0–14]	6 [1–18]	6 [0–18]	7 [0–17]	7 [0–17]	7 [0–14]	6 [0–18]
Site of Infection							
Head/neck (N [%])	11 [65]	13 [65]	15 [60]	20 [56]	17 [59]	6 [55]	59 [60]
Bacteremia/NF (N [%])	1 [6]	1 [5]	3 [12]	5 [14]	4 [14]	4 [36]	10 [10]
Deep skin/soft tissue (N [%])	3 [18]	1 [5]	2 [8]	4 [11]	3 [10]	1 [9]	10 [10]
Bone/joint (N [%])	2 [12]	1 [5]	4 [16]	3 [8]	3 [10]	0 [0]	10 [10]
Other (N [%])	0 [0]	4 [20]	1 [4]	4 [11]	2 [7]	0 [0]	9 [9]

Abbreviations: NA, not applicable; NF, necrotizing fasciitis.

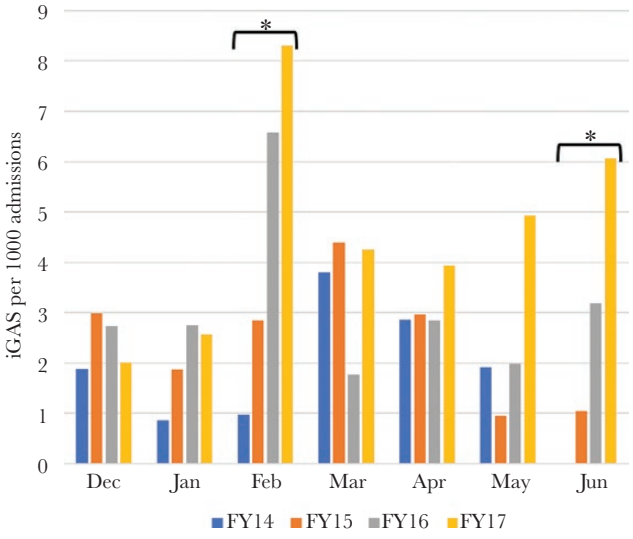

Figure 1. Rates of invasive group A *Streptococcus* (iGAS) cases per 1000 hospital admissions by fiscal year ([FY] July to June). Significant differences in yearly rates were observed in February and June. (*, $P < .05$)

(including pharyngitis and superficial skin infections) indicate an overall surge in GAS incidence in the community (Figure 3A and B), with significantly higher absolute numbers of GAS cases in April, and higher proportion of positive GAS tests in April, May, and June. This upsurge in invasive as well as overall GAS disease suggests a change in either the susceptibility of the population or the virulence or infectivity of the circulating GAS strains. At the same time, no significant difference in total number of GAS tests sent was observed (Figure 3C). This suggests a true increase in community incidence of GAS disease (including, but not limited to, invasive cases), rather than changes in referral or testing patterns.

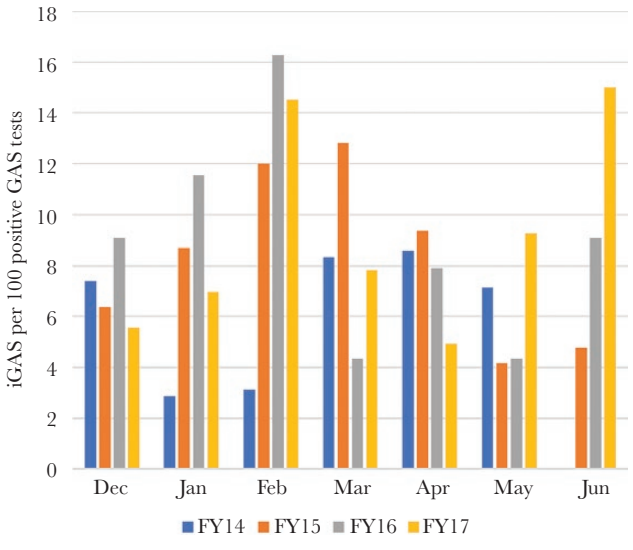

Figure 2. Rates of invasive group A *Streptococcus* (iGAS) cases per 100 positive GAS tests by fiscal year ([FY] July to June). All comparisons $P > .05$.

Given the observed increase in iGAS incidence, we assessed a selected subset of isolates for genetic diversity using WGS. Although we did not observe a clonal outbreak, sequenced isolates were predominantly emm3 and emm6. These 2 emm-types comprised 73% of sequenced strains in this group but only 4% of isolates in a nationwide surveillance network in 2015 [11]. The epidemiology of GAS disease is complex, and various factors can contribute to changes in local disease patterns. True clonal outbreaks of novel strains causing invasive disease have been reported in association with changes in local colonizing strains [9]. Bacteriophage-encoded genetic factors can also confer increased virulence to an already circulating GAS strain, as seen in a 2009 outbreak of iGAS in the United Kingdom [8]. Finally, changes in referring population, disease surveillance methods, or testing approaches can drive changes in apparent incidence rates. In this study, WGS was performed on a non-random sample of iGAS isolates. Even in the unlikely case that none of the unsequenced isolates were emm3 or emm6, these 2 strains would still comprise 38% of iGAS cases identified during this suspected outbreak, substantially more than the 4% reported elsewhere.

Concurrent with the increased incidence of iGAS, several isolates with mucoid appearance were observed, including 5 of the initial 15 strains. The expression of a mucoid capsule has been reported to be associated with pathogenic adaptations and outbreak strains in GAS [16, 17]. Of note, 36% of invasive infections with mucoid strains in this series presented with bacteremia—greater than the 10% rate seen amongst all iGAS cases in the study period (Table 2). Amongst the isolates analyzed by WGS, the mucoid phenotype was limited to emm3 and emm6 strains. All emm3/emm6 strains sequenced appeared to have the capacity to produce mucoid capsule, because they all contained the *hasA* locus determinant. All emm3 isolates were phenotypically mucoid, whereas 2/5 emm6 demonstrated this phenotype, suggesting variable expression of capsule in the emm6 strains. The mucoid phenotype is sometimes associated with mutations within the *covRS* 2 component regulatory system [18, 19]; however, no obvious null mutations were observed within the *covRS* locus from the 7 mucoid strains that were examined (data not shown). The production of capsule, particularly in emm3 strains, has been linked with more severe disease and risk of death [1, 16, 20]. Single-nucleotide polymorphism-based analysis of these strains demonstrated 3 small closely related clusters: 4 of the 6 *emm3* isolates were genetically near-identical (0–2 SNPs difference), and the *emm6* isolates segregated into 2 distinct clusters containing 3 (2–5 SNPs difference) and 2 (2 SNPs difference) isolates, respectively (Figure 4). Although SNP analysis did not reveal a clonal outbreak, the close genetic relationships within these clusters suggest that each cluster may have arisen from a close common ancestor.

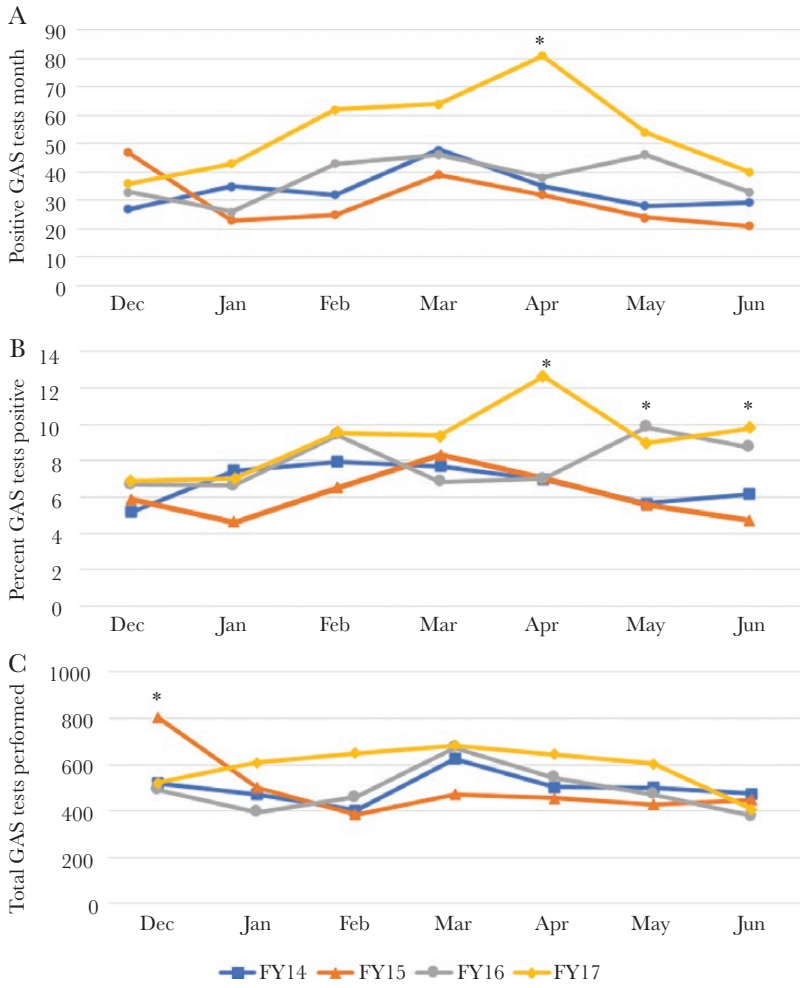

Figure 3. (A) Total number of positive group A *Streptococcus* (GAS) tests per month by fiscal year ([FY] July to June). (B) Percentage of all GAS tests (screening and culture) positive by FY and month. (C) Total number of GAS tests (screening and culture) performed by FY and month. One test per individual per calendar day is counted; scored as negative if all tests are negative or positive if 1 or more is positive. *, $P < .05$.

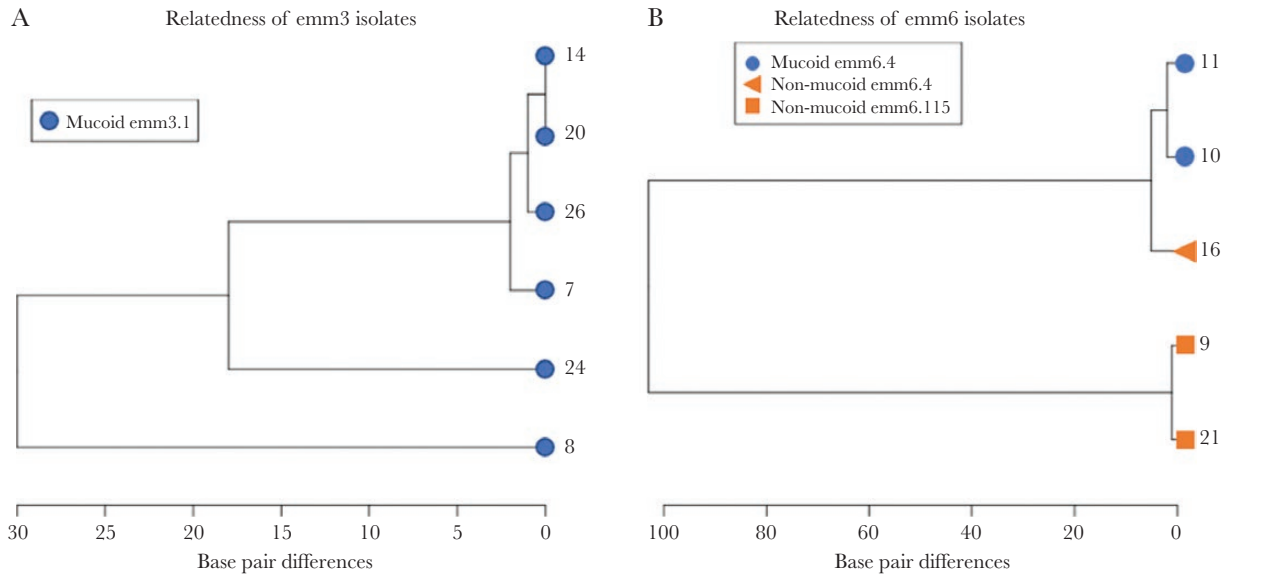

Figure 4. Relatedness of invasive group A *Streptococcus* strains by core genome single-nucleotide polymorphism differences. Numbers at dendrogram tips correspond to rows in Table 1. (A) emm type-3 strains; (B) emm type-6 strains.

In an effort to determine whether this increase in iGAS incidence was transient or sustained, we did a subsequent review of iGAS cases from FY 2018. Between July 2017 and June 2018, there was a rate of 1.75 iGAS cases per month, in line with pre-FY 2017 rates of disease. Anecdotally, our microbiology department has not observed further mucoid GAS isolates, although this has not been reviewed systematically. This further highlights the transient and frequently shifting nature of streptococcal epidemiology.

There are several limitations to this study. Although we suspect mucoid strains may be contributing to the observed changes in iGAS rates, it is unknown what proportion of asymptomatic colonization or noninvasive disease was due to mucoid strains during this study period. Invasive group A streptococcal isolates from previous years were not available for further analysis, although it is notable that mucoid GAS isolates from sites of invasive disease had not been noted in previous years by our hospital's microbiology laboratory. The cases sent for WGS were selected to represent a range of disease and capsule phenotype, but they represented a nonrandom subset of isolates. Given the stable population base of our institution and lack of significant difference in numbers of GAS tests sent, it is unlikely that changes in referral, testing, or surveillance patterns are driving the year-to-year changes seen here; however, this is difficult to fully exclude in a retrospective study.

CONCLUSIONS

In this study, we report a period of increased incidence of iGAS disease associated with emergence of a mucoid phenotype. Sequenced strains observed during this cluster were largely type emm3 and emm6, strains known to be associated with severe disease but only responsible for a small fraction of invasive cases in the United States. Although it is difficult to prove conclusively within the limitations of this study, results suggest that this cluster of disease may have been due to introduction of emm3/emm6 GAS strains with a capsule virulence factor into this population. Invasive group A *Streptococcus* remains an important pediatric pathogen with significant morbidity, and identification of outbreaks of invasive disease remains important to understanding regional and individual patterns of disease.

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