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MAJOR ARTICLE

Rapid Emergence of a New Clone Impacts the Population at Risk and Increases the Incidence of Type *emm89* Group A *Streptococcus* Invasive Disease

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Background. Invasive group A *Streptococcus* (iGAS) disease caused by type *emm89* strains has been increasing worldwide, driven by the emergence of an epidemic clonal variant (clade 3 *emm89*). The clinical characteristics of patients with *emm89* iGAS disease, and in particular with clade 3 *emm89* iGAS disease, are poorly described.

Methods. We used population-based iGAS surveillance data collected in metropolitan Toronto, Ontario, Canada during the period 2000–2014. We sequenced the genomes of 105 *emm89* isolates representing all *emm89* iGAS disease cases in the area during the period and 138 temporally matched *emm89* iGAS isolates collected elsewhere in Ontario.

Results. Clades 1 and 2 and clade O, a newly discovered *emm89* genetic variant, caused most cases of *emm89* iGAS disease in metropolitan Toronto before 2008. After rapid emergence of new clade 3, previously circulating clades were purged from the population and the incidence of *emm89* iGAS disease significantly increased from 0.14 per 100 000 in 2000–2007 to 0.22 per 100 000 in 2008–2014. Overall, *emm89* organisms caused significantly more arthritis but less necrotizing fasciitis than strains of the more common type *emm1*. Other clinical presentations were soft tissue and severe respiratory tract infections. Clinical outcomes did not differ significantly between *emm89* clades overall. However, clade 3 *emm89* iGAS disease was more common in youth and middle-aged individuals.

Conclusions. The rapid shift in *emm89* iGAS strain genetics in metropolitan Toronto has resulted in a significant increase in the incidence of *emm89* iGAS disease, with noticeably higher rates of clade 3 disease in younger patients.

Keywords. emerging strain genotype; group A *Streptococcus*; invasive disease; populations at risk; whole-genome sequencing.

Group A *Streptococcus* ([GAS] also known as *Streptococcus pyogenes*) causes a broad range of diseases, from pharyngitis and superficial skin infections to life-threatening necrotizing fasciitis and streptococcal toxic shock syndrome (STSS) [1]. After a decline in the mid-20th century, re-emergence of invasive GAS (iGAS) infections has led to disease incidences estimated at 1.5–4.3 cases per 100 000 in high-income countries [2–4]. Group A *Streptococcus* isolates can be differentiated into more than 200 types by deoxyribonucleic acid (DNA) sequence analysis of the region of the *emm* gene encoding the variable amino terminal portion of the M protein (a virulence factor with antiphagocytic properties) [5–7]. Invasive GAS disease burden is associated with a relatively small number of *emm* types in many high-income countries [8–10].

Investigative approaches that include whole-genome sequencing (WGS) of population-based samples have resulted in several important discoveries that have enhanced our understanding of iGAS diseases [11–14]. For example, analysis of >3500 *emm1* GAS genomes has uncovered allele variation due to recombination in the promoter spacer region of genes *nga* and *slo* encoding secreted toxins NAD+-glycohydrolase (NADase) and streptolysin O (SLO), respectively [15, 16]. Enhanced expression of these key virulence factors by a recombinant epidemic *emm1* clone led to the global spread of severe *emm1* iGAS infections in the 1980s [16, 17]. Similar WGS-based approaches have begun to identify contributors to the sudden increase in *emm89* iGAS disease reported in recent years in several countries [16, 18–21]. Analysis of genome-wide single-nucleotide polymorphisms (SNPs) defined 3 genetically distinct *emm89* clades, which differ in virulence in mouse and nonhuman primate models of infection [15, 16, 22]. The more virulent *emm89* clonal clade (named "clade 3") has the same polymorphisms in the promoter spacer region of the *nga* and *slo* gene cluster (also resulting in enhanced NADase and SLO toxin

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activity) as epidemic *emm1* strains [16]. Furthermore, clade 3 *emm89* strains lack the *has* genes required for the biosynthesis of the hyaluronic acid capsule typical of GAS [22]. Replacement of historic clades 1 and 2 by clade 3 temporally coincides with the increase in *emm89* iGAS disease that has been documented in the United States and some European countries [16, 18].

Early reports described associations of *emm89* GAS with skin infections [23], healthy children and young adult patients developing gastrointestinal symptoms and peritonitis [24, 25], and adults with necrotizing fasciitis and STSS [26]. Although our understanding of the biology of *emm89* organisms has greatly advanced [15, 16, 18–20, 27], *emm89* iGAS disease remains relatively poorly characterized clinically. Moreover, the clinical presentations and populations at risk for iGAS disease caused by the various *emm89* clades have not been thoroughly investigated. To better understand these public health issues, we analyzed comprehensive population-based surveillance data for *emm89* iGAS disease collected in metropolitan Toronto in the period 2000–2014, in combination with WGS analysis of the *emm89* isolates responsible for these infections.

METHODS

Clinical Data, Isolates, and Laboratory Methods

The Toronto Invasive Bacterial Diseases Network (TIBDN) is a collaboration of all hospitals, microbiology laboratories, and public health units serving the city of Toronto and Peel region, Ontario, Canada (hereafter designated "metropolitan Toronto"; population 4.2 million in 2014). The TIBDN uses standardized methods and forms to collect clinical data including demographic information, disease manifestations, and underlying medical conditions from all patients with iGAS disease in the geographical area. Data used in this study are from January 1, 2000 to December 31, 2014; collection and usage was approved by the Research Ethics Boards of participating TIBDN institutions. Invasive GAS cases were defined as those in which acute illness occurred in association with the isolation of GAS from a normally sterile site (blood, cerebrospinal, pleural, peritoneal, pericardial, or joint fluid [including bursa], bone, aspirates, and tissue specimens or swabs obtained during surgery). Streptococcal toxic shock syndrome and necrotizing fasciitis were defined as previously described [27, 28].

We included 1 strain from each of the 105 *emm89* iGAS disease cases recorded in metropolitan Toronto during the period (Table S1). We also analyzed clinical data and isolates from 138 additional *emm89* iGAS cases that occurred in the province of Ontario outside of metropolitan Toronto (Table S1), recovered by hospitals that report and submit iGAS isolates to TIBDN on a voluntary basis. Clinical data were limited for these cases. Isolates were cultured at 37°C with 5% CO_2 on Columbia blood agar plates containing 5% sheep blood or in Todd-Hewitt broth supplemented with 0.2% yeast extract. Isolates were confirmed to be GAS by β-hemolysis on sheep blood agar, grouping of carbohydrate antigen, large colony size, and bacitracin susceptibility [29].

Molecular Typing and Whole-Genome Sequencing of Group A *Streptococcus* Strains

Deoxyribonucleic acid was prepared from overnight cultures using the QIAamp DNA minikit (QIAGEN, Toronto, ON, Canada). *emm* typing was performed by polymerase chain reaction (PCR) and Sanger sequencing, as described previously [30]. We followed the procedures described by Nasser et al [17] to (1) prepare genomic libraries and perform paired-end Illumina genome sequencing of isolates, (2) identify polymorphisms against reference genomes, and (3) establish core-genome SNP-based phylogenies. We confirmed polymorphisms in the *nga/slo* promoter spacer region of all strains by PCR amplification and Sanger sequencing using previously reported primers [16]. Presence or absence of the 3-gene *has* locus was confirmed by PCR, as described previously [31].

Statistical Analysis

Statistical analysis was performed using SAS (version 9.3). Contingency tables were tested with 2-tailed χ^2 or Fisher's exact tests, as appropriate. Differences in disease incidence and length of hospitalization between *emm89* clades were evaluated with Poisson regression analysis and the Kruskal-Wallis test, respectively. P values <.05 were considered statistically significant.

RESULTS

The Incidence of *emm89* Invasive Group A *Streptococcus* Disease Significantly Increased in Metropolitan Toronto Commensurate With Emergence of Clade 3 Strains

One hundred five of the 1596 (7%) iGAS disease cases in metropolitan Toronto between 2000 and 2014 were caused by *emm89* strains. During this period, the overall incidence of iGAS infections remained relatively stable. In contrast, the incidence of *emm89* GAS infections increased significantly from 0.14 per 100 000 in 2000–2007 to 0.22 per 100 000 in 2008–2014 (χ^2, P = .021) (Table S2). To test the hypothesis that this increase in incidence was due to the emergence of clade 3 *emm89* strains, we used core-genome-SNP analysis to determine the phylogenetic relationships of the 105 *emm89* iGAS isolates. Results revealed a genetically diverse *emm89* population, including strains belonging to previously described clades 1, 2 and 3, which were genetically closely related to reference strains of those clades isolated elsewhere (Figure 1A). We also identified a fourth, distinct phylogenetic group, which we have named clade O. Strains of this clade were genetically distantly related to clades 1, 2, or 3 strains (Figure 1A), further supporting the notion that genetic diversity among *emm89* GAS is vastly higher than that observed in some other *emm* types [15]. We next studied the temporal distribution of the strains. Clade 1 strains (17% of the *emm89* iGAS isolates) predominated in the earlier years of this investigation (Figure 1B). Clade 2 strains (11% of the *emm89* iGAS

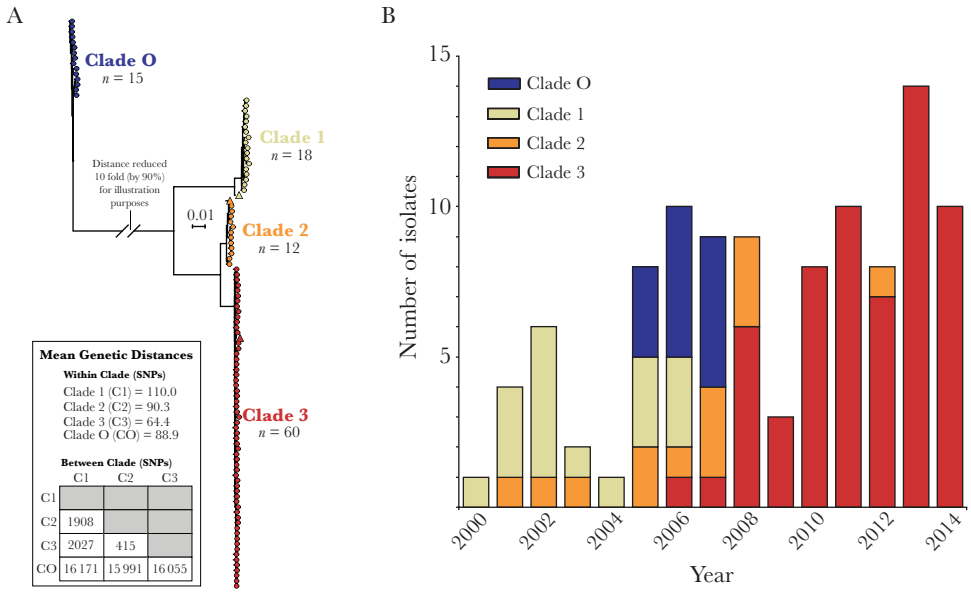

Figure 1. The increase in incidence of *emm89* invasive group A *Streptococcus* (iGAS) disease in metropolitan Toronto correlated with emergence of clade 3 *emm89* strains. (A) shows the inferred genetic relationships among *emm89* isolates causing iGAS disease in metropolitan Toronto. The neighbor-joining phylogenetic tree was constructed using 18 925 concatenated single nucleotide polymorphism (SNP) loci identified relative to the genome of reference strain MGAS23530 (clade 2; GenBank accession number CP013839). The analysis identified 4 distinct clades (1, 2, 3, and O) among isolates from metropolitan Toronto. The genetic and virulence differences between clades 1, 2, and 3 are shown and have been previously described [15]. Clade 1, 2, and 3 isolates from metropolitan Toronto were closely related to their respective reference strains (indicated in the tree by the triangles). Clade 1 strains, including reference strain MGAS11027 (GenBank accession number CP013838), differed pairwise on average by 110 SNPs. Clade 2 strains including reference strain MGAS23530 differed on average by 90 SNPs. Clade 3 strains including reference strain MGAS27061 (GenBank accession number CP013840) differed on average by 64 SNPs. Clade O strains differed on average by 89 SNPs. Clade O strains were distantly related to clades 1, 2, and 3 strains, differing on average by 16 171, 15 991, and 16 055 SNPs, respectively. Clade O strains were also distantly related to previously described *emm89* "distant and near outliers" [15] (see also Figure S1B). (B) shows the yearly distribution of *emm89* iGAS disease cases in metropolitan Toronto over the period 2000–2014. The different colors identify the genetic makeup of the *emm89* isolates causing each disease case. In earlier years, most cases were caused by clades 1 and 2. Clade O strains were only identified in years 2005 to 2007. Clade 3 strains were first observed in 2006. Clade 3 infections become more prevalent in 2008 and have since essentially replaced all historic clades. Clade 3 strains accounted for all but 1 of the 53 *emm89* iGAS isolates recovered after 2008.

isolates) were recovered primarily from 2005–2008. Clade O strains (14% of the total *emm89* isolates) were found only in 2005–2007. The circulating *emm89* strain population shifted dramatically in 2008, due to rapid expansion of clade 3 (57% of the total *emm89* isolates, and 94% of those recovered from 2008–2014; Figure 1B), which effectively purged historic clades 1, 2, and O from the population. To investigate whether a similar replacement of historic clades by clade 3 occurred in the rest of Ontario, we sequenced the genomes of 138 *emm89* isolates voluntarily submitted during the same time period (2000–2014) from areas of the province outside of metropolitan Toronto (Figure S1A). A similar increase in the number of *emm89* iGAS cases caused by clade 3 strains was observed in the rest of the province beginning in 2007 (Figure S1B and S1C).

Patients With Invasive Group A *Streptococcus* Disease Caused by Strains of the Different *emm89* Clades Differed in Comorbidities and Risk Factors

At least 1 underlying illness potentially predisposing to iGAS disease was present in 76% of *emm89*-infected patients, and 37% of patients had more than 1 underlying illness. Although there was no significant difference in the frequency of underlying illness between the different *emm89* clades, the distribution of underlying illness and risk factors was slightly different. Patients infected with clade 2 had higher rates of diabetes and cancer than patients infected with the other 3 clades (Table 1). This might be correlated with the observation that clade 2-infected patients had higher rates of STSS, intensive care unit (ICU) admission, and case fatality (Table 2). In contrast, we did not observe statistically significant differences in the length of hospitalization (median, 10 days) between patients infected with the different clades. More patients infected with clade 1 were admitted from nursing homes, whereas more patients infected with clade 2 and clade 3 strains were admitted from home than other clades (Table 1). Intravenous (IV) drug use and alcohol abuse was reported by 4% and 11% of *emm89*-infected patients, respectively, and all cases of self-reported IV drug use were associated with clades 1 or O strains (Table 1). None of these cases were clustered in space or time or associated with homelessness, although, as a group, clade O strains occurred more frequently among homeless patients (Table 1). Although we did not observe clade 3 *emm89* infections in metropolitan Toronto among IV drug users, 14 clade 3 patients from the rest of Ontario had a history of IV drug use (Table S3).

Table 1. Underlying Conditions Associated With *emm89* and *emm1* iGAS Infections, and Location From Which iGAS Patients From Metropolitan Toronto Were Admitted to Hospital, 2000–2014

Underlying Condition	Clade O No. (%)	Clade 1 No. (%)	Clade 2 No. (%)	Clade 3 No. (%)	Total *emm89* No. (%)	*emm1* No. (%)
Total	15 (100)	18 (100)	12 (100)	60 (100)	105 (100)	397 (100)[d]
Diabetes mellitus	1 (7)	4 (22)	4 (33)	17 (25)	26 (25)	54 (14)[g]
Cardiac disease[a]	0 (0)	5 (28)	0 (0)	7 (12)	12 (11)	55 (14)
Pulmonary disease[b]	1 (7)	2 (11)	2 (17)	9 (15)	14 (13)	62 (16)
Renal disease	0 (0)	0 (0)	0 (0)	3 (5)	3 (3)	16 (4.1)
Liver disease	3 (20)	1 (6)	0 (0)	1 (2)	5 (5)	10 (2.6)
Immunodeficiency[c]	1 (7)	6 (33)	5 (42)	9 (15)	21 (20)	41 (11)[e]
Alcohol abuse	6 (40)	0 (0)	1 (8)	5 (8)	12 (11)	22 (5.7)[g]
IV drug use	3 (20)[f]	1 (6)[f]	0 (0)[f]	0 (0)[f]	4 (4)	6 (1.5)
Admission from						
Total	15 (100)	18 (100)	12 (100)	59 (100)	104 (100)[h]	393 (100)[i]
Home	10 (67)	12 (67)	11 (92)	48 (81)	81 (78)	342 (87)
Nursing home	0 (0)	4 (22)	0 (0)	5 (8)	9 (9)	16 (4.1)
Hospital	0 (0)	1 (6)	0 (0)	4 (7)	5 (5)	27 (6.9)
Retirement home/group home	1 (7)	1 (6)	1 (8)	2 (3)	5 (5)	6 (2)
Homeless	4 (27)	0 (0)	0 (0)	0 (0)	4 (4)	2 (1)

Abbreviations: HIV, human immunodeficiency virus; iGAS, invasive group A *Streptococcus*; IV, intravenous; SLE systemic lupus erythematosus.

[a]Includes cardiac disease and congestive heart failure.

[b]Includes asthma, chronic bronchitis, and other respiratory conditions such as interstitial lung disease and bronchiectasis.

[c]Includes previous organ/stem cell transplant, SLE, HIV infection, and cancer.

[d]Data available for 389 of 397 cases.

[e]Data for organ/stem cell transplant and SLE not available for *emm1*. Statistical analysis performed only for the comparison of HIV infection and cancer. No statistical difference found between *emm89* and *emm1*.

[f]$P < .05$ for the comparison between *emm89* clades clade O, clade 1, and clade 2, and the emerging clade 3.

[g]$P < .05$ for the comparison with all *emm89* patients.

[h]Data available for 104 of 105 patients.

[i]Data available for 393 of 397 patients.

Overall, Clade 3 *emm89* Strains Caused Invasive Group A *Streptococcus* Disease in Younger Patients

The 105 *emm89* iGAS disease cases from metropolitan Toronto occurred in patients aged 8 months to 96 years old, 53% of whom were female. The median age at infection was 51 years old for females and 48 years old for males. Males and females and patients of different ages had slightly different disease manifestations (Figure 2). Adult males had more arthritis, and soft tissue infections were more common among the elderly. The greatest incidence of infection occurred in those patients >75 years old, and this age

Table 2. Clinical Presentation and Outcomes of Patients With *emm89* and *emm1* iGAS Infections in Metropolitan Toronto, 2000–2014

Clinical Presentation/Outcome	Clade O No. (%)	Clade 1 No. (%)	Clade 2 No. (%)	Clade 3 No. (%)	*emm89* Total No. (%)	*emm1* No. (%)
Total	15 (100)	18 (100)	12 (100)	60 (100)	105 (100)	397 (100)
Arthritis	2 (13)	4 (22)	2 (17)	9 (15)	17 (16)	32 (8)[c]
Bacteremia without focus	0 (0)	3 (17)	3 (25)	7 (12)	13 (12)	61 (15)
Soft Tissue Infection						
Necrotizing fasciitis	0 (0)	0 (0)	0 (0)	2 (3)	2 (2)	36 (9)[c]
Other soft tissue	10 (67)	4 (22)	1 (8)	20 (33)	35 (33)	138 (35)
Respiratory Tract Infection						
Lower respiratory	2 (13)	3 (17)	5 (42)	8 (13)	18 (17)	79 (20)
Upper respiratory	1 (6)	1 (6)	1 (8)	7 (12)	10 (9)	20 (5)
Peripartum infection	0 (0)	1 (6)	0 (0)	4 (7)	5 (5)	8 (2)
Other[a]	0 (0)	2 (11)	0 (0)	3 (5)	5 (5)	23 (6)
STSS	1 (7)	5 (28)	5 (42)	14 (23)	25 (24)	117 (29)
Case fatality[b]	1 (7)	4 (22)	5 (42)	10 (17)	20 (19)	81 (20)
ICU admission	4 (27)	6 (33)	5 (42)	16 (27)	31 (30)	158 (40)

Abbreviations: ICU, intensive care unit; iGAS, invasive group A *Streptococcus*; STSS, streptococcal toxic shock syndrome.

[a]Other includes peritoneal infection, gynecological infection not associated with pregnancy.

[b]Case fatality was defined as death that could be attributed to GAS infection within 30 days of positive culture.

[c]$P < .05$ for the comparison with all *emm89* patients.

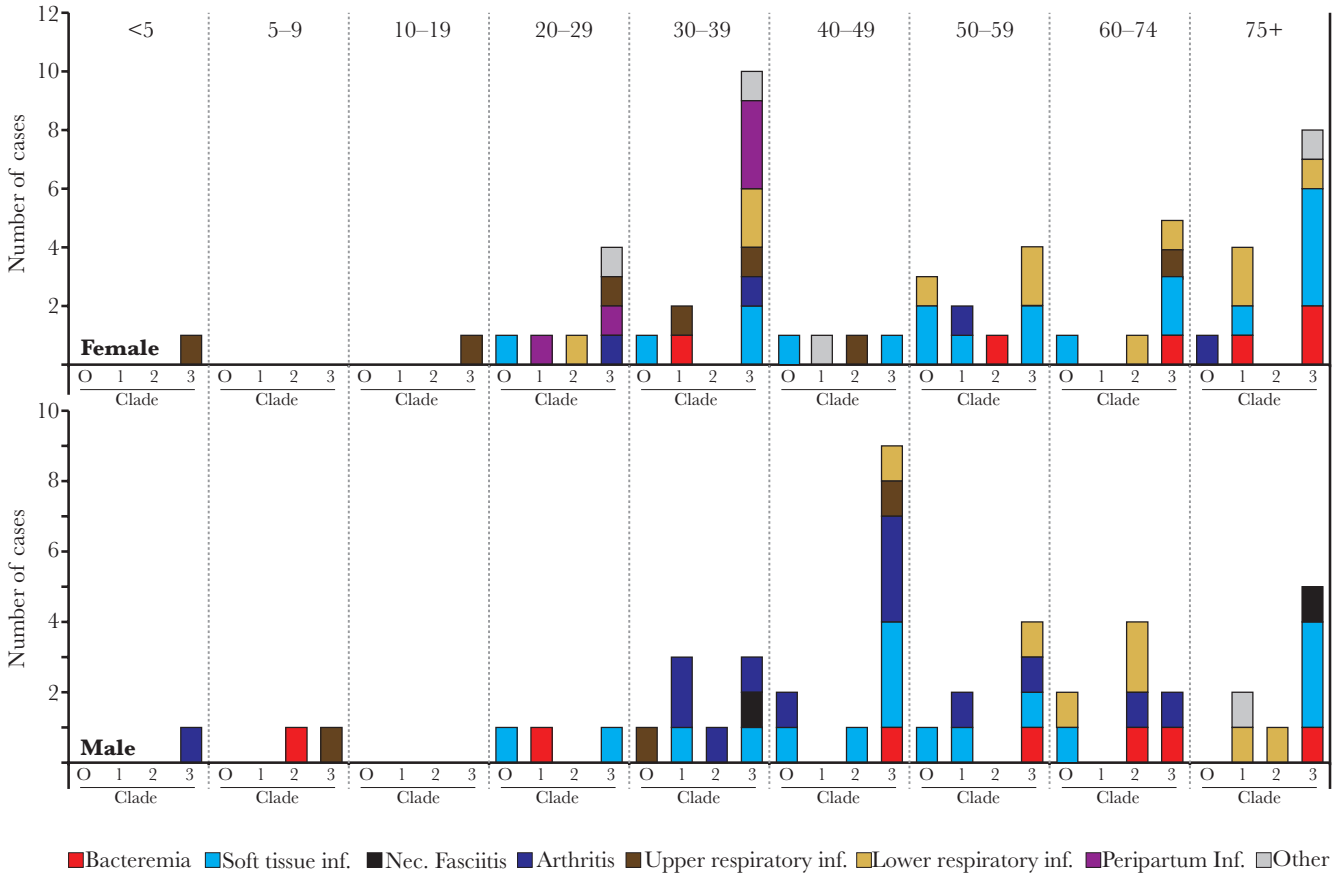

Figure 2. Clinical presentation of *emm89* invasive group A *Streptococcus* (iGAS) disease cases in metropolitan Toronto (2000–2014) by sex, age group, and *emm89* clade. The different clinical presentations are depicted in different colors as per the legend. Soft tissue and respiratory infections predominated among both female (top panel) and male patients (bottom panel) across all age groups and particularly in the elderly (75 years old or greater). Overall, more cases of clade 3 *emm89* iGAS occurred among children and younger adults. Arthritis was more common among adult males aged 30–49. "Other" includes peritoneal infections and gynecological infections not associated with pregnancy.

group had the highest case fatality rate (Table S4). The median age of patients infected with historic clades 1, 2, and O was 51.4 years (range 6–96 years), whereas among clade 3-infected patients the median age was 49.3 years (8 months–90 years). Relative to the historic clades, the incidence of clade 3 infections was higher across all age groups, and particularly among those <19 years old, and middle-aged patients (those in their 30s and 40s) (Table S4). Although our data do not allow the calculation of precise incidence rates in areas of Ontario outside of metropolitan Toronto, we observed that in these areas the largest proportion of patients infected with clade 3 strains was in the 30–39 age group. Relative to the historic clades, significantly more clade 3 strains were isolated from patients aged 20–49 (Figure S1D). Taking all isolates from Ontario together, clade 3 strains infected proportionally more patients aged 20–49 and fewer patients aged 75+, relative to historic clades (Table S5).

Clinical Characteristics of *emm89* Invasive Group A *Streptococcus* Infections

The most common manifestation of *emm89* iGAS disease was soft tissue infection, followed by lower respiratory infections and arthritis (Table 2). Streptococcal toxic shock syndrome occurred in 24% of *emm89*-infected patients. The overall case fatality rate was 19%. To assess the features of *emm89* disease, we compared the cohort of *emm89* patients to that of patients with *emm1* iGAS disease in the same population (Table 2). The most common disease manifestation among *emm1* strains was also soft tissue infection, followed by lower respiratory infections and bacteremia without focus. Significantly more cases of arthritis and significantly fewer cases of necrotizing fasciitis were observed among patients with *emm89* iGAS disease compared with those with *emm1* disease (Table 2). However, STSS, ICU admission, and case fatality rates were not significantly different between *emm89* and *emm1* iGAS cases (Table 2). There were no significant differences between clades with respect to the sites of isolation, although *emm89* iGAS strains were less frequently isolated from blood than *emm1* (Table 3). Overall, 76% of *emm89*-infected patients and 70% of *emm1*-infected patients in metropolitan Toronto had at least 1 underlying illness. A significantly greater proportion of *emm89*-infected patients had diabetes compared with *emm1*-infected patients (Table 1).

Table 3. Site of Isolation of *emm89* and *emm1* Isolates Causing iGAS Disease in Metropolitan Toronto, 2000–2014

Site	Clade O No. (%)[a]	Clade 1 No. (%)	Clade 2 No. (%)	Clade 3 No. (%)	Total *emm89* No. (%)	*emm1* No. (%)
Total	15 (100)	18 (100)	12 (100)	60 (100)	105 (100)	397 (100)
Blood and CSF[b]	10 (67)	9 (50)	9 (75)	44 (73)	72 (69)	312 (79)[d]
Other[c]	4 (27)	5 (28)	2 (17)	6 (10)	17 (16)	48 (12)
Synovial fluid	1 (7)	2 (11)	1 (8)	6 (10)	10 (10)	21 (5)
Peritoneal fluid	0 (0)	1 (6)	0 (0)	1 (2)	2 (2)	4 (1)
Pleural fluid	0 (0)	1 (6)	0 (0)	3 (5)	4 (4)	12 (3)

Abbreviations: CSF, cerebrospinal fluid; iGAS, invasive group A *Streptococcus*.

[a]Percentages may not add up to 100 due to rounding.

[b]One single CSF isolate was obtained from an *emm1*-infected patient.

[c]Other includes isolates obtained from abscesses, aspirates, and specimens obtained during surgical procedures.

[d]$P < .05$ for the comparison with all *emm89* patients.

DISCUSSION

Systems biology approaches combining in-depth genomic strain characterization with in vitro testing and experimental infection of nonhuman primates has unambiguously demonstrated that the recently emerged genetic clade 3 *emm89* GAS, which overexpresses the cytolytic toxins NADase and streptolysin O, is the main driver of the rapid worldwide increase in *emm89* iGAS disease [15, 16, 18, 22]. Here, using similar WGS-based approaches and temporal analysis, we show that in metropolitan Toronto the emergence and rapid expansion of clade 3 clonal progeny is responsible for the significant increase in incidence of *emm89* iGAS disease observed since 2008 in a context of relatively stable total iGAS disease burden. Emergence and rapid expansion of clade 3 in metropolitan Toronto was concurrent with rapid decline and apparent extinction of previously circulating *emm89* clades 1, and 2, and O.

The paucity of reports examining the clinical features of *emm89* iGAS disease is at odds with the magnitude of the increase in *emm89* iGAS disease reported here and in several other countries [15, 16, 18, 20, 32]. To begin to address this circumstance, we assessed comprehensively the clinical features of *emm89* iGAS disease using population-based surveillance data collected over 14 years. One of our findings was that *emm89* iGAS disease is characterized by the very frequent occurrence of soft tissue, severe respiratory infections, and arthritis. The prevalence of arthritis among *emm89* patients was significantly higher than among type *emm1* strains in metropolitan Toronto and similar to that reported previously in Europe [23, 33]. In contrast, *emm89* organisms caused significantly less necrotizing fasciitis than *emm1* strains. We next investigated the hypothesis that, similar to what has been described in animal models [16, 22], clade 3 *emm89* causes more severe disease in human patients than previously circulating clades. Although clade 3 caused slightly more necrotizing fasciitis, there were no significant differences in clinical diagnosis between patients infected with the different clades. Similar to a previous report [18], we also did not observe significant differences in 7- or 30-day mortality between clade 3 and other *emm89* clade types. We did not detect significant differences between *emm89* clades for predisposing conditions such as chronic illness or immune suppression. Although significantly fewer clade 3-infected patients were IV drug users in metropolitan Toronto, data from the rest of Ontario showed that IV drug usage was significantly associated with clade 3 strains.

We next examined whether clade 3 emergence and rapid expansion correlated with enhanced ability of the strains to cause disease in different groups of individuals than previously circulating clades, and we made several interesting observations. First, although studies of iGAS incidence by age are frequently bimodal, with rates peaking in children <2 years old and highest rates in those >85 years of age [26, 27, 34], we identified very few cases of historic clades 1, 2, and O in children <5 years old and in those aged 5–19, age groups in which clade 3 *emm89* iGAS disease was more frequent. In addition, clade 3 cases were significantly more common in patients aged 20–49 years old, who less frequently reported underlying comorbidities. Thus, since the emergence of clade 3, there is a trend towards a profile of *emm89* GAS infected patient that is younger, slightly healthier, and with a lower rate of underlying illness.

CONCLUSIONS

Clonal replacement among iGAS is a well described phenomenon [35, 36]. Given that the replacement of historic *emm89* clades by clade 3 has occurred simultaneously in several unrelated countries, which have different models of access to healthcare, and whose populations are dissimilar in many social and human factors [16, 18], we speculate that emergence of this clone is dependent primarily on bacterial factors such as enhanced ability to persist and infect naive hosts rather than on host factors. The use of nonhuman primate models of infection has shown that enhanced NADase and SLO toxin activity has endowed clade 3 strains with increased fitness in the upper respiratory tract [15, 16]. It is interesting to note that *emm89* pharyngitis also strikingly increased in frequency in 2007 in Ontario [21], likely related to the emergence of clade 3. We speculate that this enhanced ability to persist in

the upper respiratory tract may be one key contributor to the rapid dissemination of clade 3 and its ability to readily infect younger, healthier patients. Data presented here, data from the Centers for Disease Control and Prevention Active Bacterial Core surveillance, and our unpublished data for Ontario for the period 2015–2016 suggest that *emm89* iGAS cases continue to occur in high numbers in North America. Very recent reports from Finland have shown that diversifying clonal variants (subclades) of clade 3 *emm89* have appeared that are associated with increased mortality [37]. Continued monitoring of changes in *emm89* genetic diversity and associated iGAS disease is warranted.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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References

1. Cole JN, Barnett TC, Nizet V, Walker MJ. Molecular insight into invasive group A streptococcal disease. Nat Rev Microbiol **2011**; 9:724–36.
2. Hoge CW, Schwartz B, Talkington DF, et al. The changing epidemiology of invasive group A streptococcal infections and the emergence of streptococcal toxic shock-like syndrome. A retrospective population-based study. JAMA **1993**; 269:384–9.
3. Ralph AP, Carapetis JR. Group a streptococcal diseases and their global burden. Curr Top Microbiol Immunol **2013**; 368:1–27.
4. Laupland KB, Ross T, Church DL, Gregson DB. Population-based surveillance of invasive pyogenic streptococcal infection in a large Canadian region. Clin Microbiol Infect **2006**; 12:224–30.
5. Lancefield RC. The antigenic complex of *Streptococcus haemolyticus*: I. Demonstration of a type-specific substance in extracts of *Streptococcus haemolyticus*. J Exp Med **1928**; 47:91–103.
6. Scott JR, Pulliam WM, Hollingshead SK, Fischetti VA. Relationship of M protein genes in group A streptococci. Proc Natl Acad Sci U S A **1985**; 82:1822–6.
7. Manjula BN, Acharya AS, Fairwell T, Fischetti VA. Antigenic domains of the streptococcal Pep M5 protein. Localization of epitopes crossreactive with type 6 M protein and identification of a hypervariable region of the M molecule. J Exp Med **1986**; 163:129–38.
8. Walker MJ, Barnett TC, McArthur JD, et al. Disease manifestations and pathogenic mechanisms of group A *Streptococcus*. Clin Microbiol Rev **2014**; 27:264–301.
9. Steer AC, Law I, Matatolu L, et al. Global *emm* type distribution of group A streptococci: systematic review and implications for vaccine development. Lancet Infect Dis **2009**; 9:611–6.
10. Smeesters PR, McMillan DJ, Sriprakash KS, Georgousakis MM. Differences among group A *Streptococcus* epidemiological landscapes: consequences for M protein-based vaccines? Expert Rev Vaccines **2009**; 8:1705–20.
11. Musser JM, Shelburne SA 3rd. A decade of molecular pathogenomic analysis of group A *Streptococcus*. J Clin Invest **2009**; 119:2455–63.
12. Carroll RK, Shelburne SA 3rd, Olsen RJ, et al. Naturally occurring single amino acid replacements in a regulatory protein alter streptococcal gene expression and virulence in mice. J Clin Invest **2011**; 121:1956–68.
13. Olsen RJ, Sitkiewicz I, Ayeras AA, et al. Decreased necrotizing fasciitis capacity caused by a single nucleotide mutation that alters a multiple gene virulence axis. Proc Natl Acad Sci U S A **2010**; 107:888–93.
14. Bessen DE, McShan WM, Nguyen SV, et al. Molecular epidemiology and genomics of group A *Streptococcus*. Infect Genet Evol **2015**; 33:393–418.
15. Beres SB, Kachroo P, Nasser W, et al. Transcriptome remodeling contributes to epidemic disease caused by the human pathogen *Streptococcus pyogenes*. MBio **2016**; 7 pii: e00403-16.
16. Zhu L, Olsen RJ, Nasser W, et al. A molecular trigger for intercontinental epidemics of group A *Streptococcus*. J Clin Invest **2015**; 125:3545–59.
17. Nasser W, Beres SB, Olsen RJ, et al. Evolutionary pathway to increased virulence and epidemic group A *Streptococcus* disease derived from 3615 genome sequences. Proc Natl Acad Sci U S A **2014**; 111:1768–76.
18. Turner CE, Abbott J, Lamagni T, et al. Emergence of a new highly successful acapsular group a *Streptococcus* clade of genotype *emm89* in the United Kingdom. MBio **2015**; 6:e00622.
19. Ikebe T, Tominaga K, Shima T, et al. Increased prevalence of group A *Streptococcus* isolates in streptococcal toxic shock syndrome cases in Japan from 2010 to 2012. Epidemiol Infect **2015**; 143:864–72.
20. Friães A, Machado MP, Pato C, et al. Emergence of the same successful clade among distinct populations of *emm89 Streptococcus pyogenes* in multiple geographic regions. MBio **2015**; 6:e01780–15.
21. Shea PR, Ewbank AL, Gonzalez-Lugo JH, et al. Group A *Streptococcus emm* gene types in pharyngeal isolates, Ontario, Canada, 2002–2010. Emerg Infect Dis **2011**; 17:2010–7.
22. Zhu L, Olsen RJ, Nasser W, et al. Trading capsule for increased cytotoxin production: contribution to virulence of a newly emerged clade of *emm89 Streptococcus pyogenes*. MBio **2015**; 6:e01378–15.
23. Luca-Harari B, Darenberg J, Neal S, et al. Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. J Clin Microbiol **2009**; 47:1155–65.
24. Gillespie RS, Hauger SB, Holt RM. Primary group A streptococcal peritonitis in a previously healthy child. Scand J Infect Dis **2002**; 34:847–8.
25. Ekelund K, Lemcke A, Konradsen HB. Evaluation of gastrointestinal symptoms as primary sign of severe invasive group A streptococcal infections. Indian J Med Res **2004**; 119:179–82.
26. Plainvert C, Doloy A, Loubinoux J, et al. Invasive group A streptococcal infections in adults, France (2006–2010). Clin Microbiol Infect **2012**; 18:702–10.
27. Davies HD, McGeer A, Schwartz B, et al. Invasive group A streptococcal infections in Ontario, Canada. Ontario Group A Streptococcal Study Group. N Engl J Med **1996**; 335:547–54.
28. Defining the group A streptococcal toxic shock syndrome. Rationale and consensus definition. The Working Group on Severe Streptococcal Infections. JAMA **1993**; 269:390–1.
29. Facklam RR, Washington JA II. *Streptococcus* and related catalase-negative Gram-positive cocci. In: Balows A, Hausler WJ Jr, Herrmann KL, et al (eds). Shadomy Manual *of Clinical Microbiology*, 5th ed. Washington, D.C.: American Society for Microbiology; **1991**: pp 238–57.
30. Beall B, Gherardi G, Lovgren M, et al. *emm* and *sof* gene sequence variation in relation to serological typing of opacity-factor-positive group A streptococci. Microbiology **2000**; 146:1195–209.
31. Flores AR, Jewell BE, Fittipaldi N, et al. Human disease isolates of serotype M4 and M22 group a *streptococcus* lack genes required for hyaluronic acid capsule biosynthesis. MBio **2012**; 3:e00413-12.
32. Latronico F, Nasser W, Puhakainen K, et al. Genomic tracks behind spread of bacteremic Group A *Streptococcus* type *emm89* in Finland, 2004–2014. J Infect Dis **2016**; 214:1987–95.
33. Olafsdottir LB, Erlendsdottir H, Melo-Cristino J, et al. Invasive infections due to *Streptococcus pyogenes*: seasonal variation of severity and clinical characteristics, Iceland, 1975 to 2012. Euro Surveill **2014**; 19:5–14.
34. Nelson GE, Pondo T, Toews KA, et al. Epidemiology of invasive group A streptococcal infections in the United States, 2005–2012. Clin Infect Dis **2016**; 63:478–86.
35. Athey TB, Teatero S, Sieswerda LE, et al. High incidence of invasive group A *Streptococcus* disease caused by strains of uncommon *emm* types in Thunder Bay, Ontario, Canada. J Clin Microbiol **2016**; 54:83–92.
36. Fittipaldi N, Beres SB, Olsen RJ, et al. Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A *Streptococcus*. Am J Pathol **2012**; 180:1522–34.
37. Latronico F, Nasser W, Puhakainen K, et al. Genomic tracks behind spread of bacteremic group A *Streptococcus* type *emm89* in Finland, 2004–2014. J Infect Dis **2016**; 214:1987–95.