Emergent Invasive Group A Streptococcus
dysgalactiae subsp. equisimilis,
United States, 2015–2018

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The Centers for Disease Control and Prevention’s Active Bacterial Core surveillance (ABCs) performs population-based surveillance of invasive group A Streptococcus (GAS) infections. Isolates collected from a population of ≥34 million persons are subjected to whole-genome sequence (WGS)–based characterization. We recently detected group A carbohydrate-positive S. dysgalactiae subsp. equisimilis (GCS) SE that were serologically group A through a single ancestral recombination event between a group C S. dysgalactiae subsp. equisimilis strain and a group A S. pyogenes strain.

The Study
During January 1, 2015–November 1, 2018, a total of 5,480 ABCs GAS isolates were subjected to WGS. We identified 35 atypical gacI-positive isolates; each yielded 1 of the M protein gene (emm) subtypes stG245.0, stG485.0, or stG652.0 commonly associated with SE (4–6). These 35 isolates lacked multilocus sequence types (MLSTs) inclusive of known S. pyogenes allelic designations. Lancefield grouping (7) and MLST (https://pubmlst.org/sdysgalactiae) (6) revealed the 35 isolates were serologically group A and MLST sequence type (ST) 128 (GAS/ST128/SE). We received 13 additional SE isolates recovered through ABCs GAS surveillance during this period that were found to be non–group A isolates (9 group G, 2 group C, and 2 group L) with MLSTs unrelated to ST128 (Figure 1). According to our normal protocol, these 13 non–group A SE isolates and 2 group G S. canis isolates that we also received were removed from the ABCs GAS database.

The Lancefield group A carbohydrate consists of a polyrhamnose chain with an immunodominant N-acetylglucosamine side chain (9) that functions in GAS pathogenesis (1). The group C carbohydrate also has a polyrhamnose backbone; however, its immunodominant side chain is the disaccharide N-acetylglucosaminosyl-N-acetylglu-
cosamine (9). Genomic comparison of the 12 gene group A carbohydrate synthetic cluster gacA-L (1) from S. pyogenes with the corresponding regions of the 35 GAS/ST128/SE revealed an upstream crossover point within the S. pyogenes gacE ABC transporter gene and a downstream crossover point within ebsA (Figure 2). The ancestral recipient SE strain was implicated as group C S. equisimilis (GCS/SE) by virtue of the near-identical sequence of the 1,363-bp sequence within GAS/ST128/SE encompassing sections of gacD and gacE homologs (designated as gccD and gccE) (Figure 2) with GCS and the marked divergence of this 1,363-bp sequence from group G SE (data not shown). This sequence is immediately adjacent to the upstream crossover point shown between S. pyogenes and GCS/SE (SP-5005 and SE-7136; Figure 2). We also found these same crossover points within the group carbohydrate gene cluster of the available genomic sequence from the previously described invasive GAS/SE strain AC-2713 recovered in 1999 (3). Subsequent genomic analysis revealed AC-2713 to be ST128 and emm type stG485.0. Phylogenetic analysis revealed that AC-2713 differed by 126 single-nucleotide polymorphisms from a pair of genetically indistinguishable GAS/ST128/SE recovered within the East Bay area of San Francisco, California, USA (Figure 1). These 2 isolates were from recurrent invasive GAS infections within the same patient that occurred 1.5 months apart.

Comparison of the S. pyogenes gacA-L cluster with the corresponding gcc loci from group C SE strains (SE-7136; Figure 2) revealed that GCS/SE genes shared homology with all 12 gacA-L genes (56%–89% sequence identity). The weakest conservation was observed between the gac/gccJK genes (56%–69% identity), consistent with the requirement of gccJK for the group A immunodominant
Figure 1. Analyses of invasive group A Streptococcus dysgalactiae subsp. equisimilis and conserved genomic pepD gene insertion site of highly related exotoxin speC gene–containing prophages found within group A ST128 S. equisimilis strain and S. pyogenes strain SP1336. Methods are described in the Appendix (https://wwwnc.cdc.gov/EID/article/25/8/18-1758-App1.pdf). A) Phylogenetic tree of 35 invasive group A S. dysgalactiae subsp. equisimilis (GAS/SE/MLST128 [ST128] complex) isolates and 13 unrelated group C, G, and L SE isolates recovered through the Centers for Disease Control and Prevention’s Active Bacterial Core surveillance during January 1, 2015–November 1, 2018. Trees are drawn to scale; branch lengths indicate number of substitutions per site. Surveillance areas (https://www.cdc.gov/abcs/reports-findings/surv-reports.html) are indicated: EB, East Bay San Francisco area, California; NY, New York; NM, New Mexico; CA, San Francisco Bay area, California; OR, Oregon; CO, Colorado; GA, Georgia; CT, Connecticut. Different counties and years of isolation are indicated (e.g., EB1–15 indicates county 1 in East Bay area and year 2015). The left tree depicts all 49 isolates and the right includes only the subset of the 36 GAS/ST128/SE (also including GAS/ST128/SE described by Brandt et al. [3] and assigned GenBank accession no. HE858529). Three pairs of isolates differing by 13 or fewer single-nucleotide polymorphisms are shown in red. Single-locus variants of the indicated multilocus sequence types are indicated with asterisks. B) Conserved genomic pepD gene insertion site of highly related exotoxin speC gene–containing prophages found within group A ST128 S. equisimilis strain (middle) and S. pyogenes strain SP1336 (GenBank accession no. CP031738). The nonfunctional pepD structural genes lacking bases 1–4 are depicted in the 2 prophage-containing strains. Nucleotide sequence identity is scaled from 70% (yellow) to 100% (green). The S. equisimilis prophage also contained the virulence-associated DNase gene spd1 as shown and previously described for the depicted SP1336 phage shown (8). Within both species, the pepD insertion site lies within a region between the conserved bacterial cell division genes ftsE/ftsX and the small ribosomal protein gene rpsL31b (GenBank accession no. for S. equisimilis AC2713 is HE858529).
N-acetylglucosamine side chain but not for synthesis of the polyrhamnose core (1). Two additional genes, designated gccM (glycotransferase gene) and gccN (UDP-monosaccharide epimerase gene), were evident within the gcc gene cluster. In the ancestral recombination event, an 11,527-bp GAS (S. pyogenes) chromosomal segment composed of the gacE 3′ portion, along with the gacF-L genes and a 5′ portion of ebsA, replaced the corresponding 13,813 bp of a GCS/ST128/SE strain, resulting in the recombinant GAS/ST128/SE lineage (Figure 2). This fragment encompasses the intact 7-gene gacF–gacL segment; each gene shared 99.4%–99.7% sequence identity with counterparts in S. pyogenes. The evident functionality of the hybrid gac/gcc cluster within the GAS/ST128/SE progeny lineage is consistent with identical roles of the first 3 genes of the cluster (gac/gccA–C) in the biosynthesis of the polyrhamnose core (1) that is present within the groups A, C, and G carbohydrates (9). Each of these 3 genes are also required for S. pyogenes viability (1).

The occurrence of multiple emm types within the same MLST is common in SE (5,6) and differs from emm/MLST associations within S. pyogenes, where an MLST is nearly always definitive of a single emm type (2,10). The presence of 3 different emm types and 8 macrolide-resistant isolates within GAS/ST128/SE (Figure 1) is indicative of a long-standing successful lineage. A single isolate of this lineage was positive for the exotoxin gene speC (Figure 1) that was carried on a prophage highly similar to a previously described speC-positive S. pyogenes strain (8). The relative genomic positions of the prophages are exactly conserved between the 2 species, inserted within the pepD gene in the genomic region that lies between the bacterial cell division genes fisE/fisX and the ribosomal protein gene rpsL31B (Figure 1). The number of single-nucleotide polymorphism differences between individual GAS/ST128/SE core genomes ranged from 0 to 613 (Figure 1). The GAS/ST128/SE strain AC-2713 recovered 20 years ago (3) is also indicative of a long-established lineage.

The 34 GAS/ST128/SE isolates for which information was available (32 from blood, 1 from a joint, and 1 from a surgical wound) recovered in ABCs since January 1, 2015, were recovered from older adults (age range 22–93

Figure 2. Ancestral recombination event depicting Streptococcus pyogenes group A carbohydrate gene donor (GAS/SP-5005; GenBank accession no. NC007297), group C S. dysgalactiae subspp. equisimilis recipient (GCS/SE7136; GenBank accession no. NCTC7136), and progeny group A S. dysgalactiae subspp. equisimilis progeny (GAS/SE/ST128) described in study of emergent invasive group A Streptococcus dysgalactiae subspecies equisimilis, United States, 2015–2018. The deduced crossover points between the group A gene cluster (red) donor and group C (green) recipient strains are shown. The 3 genes required for inclusion of the immunodominant N-acetylglucosamine side chain within the group A carbohydrate (gacI, gacJ, and gacK) are shown in red. The coordinates of the fragment transferred that is highly conserved between the donor and the progeny are indicated. The length of the 3 genomic regions are indicated. The gacE/gccE and ebsA genes are shown as green/red hybrids. The extra gcc cluster genes not conserved within the gcc cluster are shown in blue. The relative sequence identities of the 3 different regions of progeny (bottom) gac cluster genes with the group A S. pyogenes donor (top) and group C S. equisimilis recipient (middle) are indicated. The middle segment (asterisk) indicates a range of 56%–77% sequence identitybetween each of the 8 structural genes (gacF–pepT) that were received intact from the S. pyogenes donor. The gac cluster genes are described in more detail in van Sorge et al. (1). Gene assignments are as follows: dnaG, DNA primase; rpoD, major RNA polymerase sigma factor; mscF, metal sulfur complex assembly factor; gacA-L, group A carbohydrate biosynthetic genes (putative functions described in van Sorge et al. [1]); gccA-N, group C carbohydrate biosynthetic genes. gccA-L are functional homologs of gacA-L. gccM and gccN putatively encode an additional glycosyl transferase and UDP-monosaccharide 4-epimerase, respectively; ebsA, pore-forming protein; fd, ferredoxin (complement strand); ctkK, cytidylyl kinase; infC, translation initiation factor IF-3.
years; mean age 63 years) from 8 ABCs sites; most (85%) patients were men. Most patients had underlying medical conditions (data not shown), including 16 with diabetes, 15 with cellulitis (including 1 who had necrotizing fasciitis), 8 with pneumonia, and 6 with septic shock. One patient with bacteremia died.

Conclusions
ABCs identifies invasive infections caused by GAS without identification of isolates to the species level. Since 2015, when we implemented WGS as our primary platform for GAS characterization, we have identified rarely occurring non–S. pyogenes isolates through our bioinformatics pipeline automated MLST function rather than previously employed phenotypic testing. Of ≈16,000 GAS isolates recovered from ABCs during 1994–2014, only 11 had emm types characteristic of SE. All 11 were collected during 2011–2014 and were of the 3 emm types found among the 35 GAS/ST128/SE isolates from this study. Genomic analysis verified the GAS/ST128/SE lineage of these 11 older isolates (data not shown). Finding 35 additional invasive isolates of this lineage recovered during January 1, 2015–November 1, 2018, through ABCs suggests a level of expansion attributable to strain adaptation and fitness or to a more susceptible population. Attempts to identify circulating ST128/SE strains of the original group C have been unsuccessful, including an examination of a population-based sampling of SE (3).

Because group A SE is suspected to be rare, these findings raise the question of whether invasive disease attributable to SE of groups C, G, and L is also increasing. A 2-year population-based study of β-hemolytic streptococcal disease attributable to Lancefield groups other than A and B within 2 ABCs sites during 2002–2004 revealed that 80% of such isolates were SE (11), with clinical manifestations and targeted susceptible populations similar to S. pyogenes. Incidence of invasive disease attributable to non–group A SE during this period was estimated at 2.5 cases/100,000 population, similar to the incidence of GAS infections (2.89 cases/100,000 population) in these same 2 sites. The incidence of overall invasive GAS disease in the United States has also markedly increased during recent years, from 3.4 cases/100,000 population in 2012 to 7.2 cases/100,000 population in 2017 (https://www.cdc.gov/abc/). This study used the S. dysgalactiae subsp. equisimilis MLST website (http://pubmlst.org/sdysgalactiae) at the University of Oxford. The development of this site has been funded by the Wellcome Trust.

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Appendix

Construction of Maximum-Likelihood Trees

We constructed maximum-likelihood trees (Figure 1, https://wwwnc.cdc.gov/EID/article/25/8/18-1758-F1.htm) by using kSNP3.0 (1) with a kmer size of 19 and used MEGA 7 for evolutionary analysis (2). We inferred the evolutionary history by using the maximum-likelihood method on the basis of the general time reversible model (3). The trees with the highest log likelihood (−124033.37 for left tree and −13853.47 for right tree) are shown. We obtained initial trees for the heuristic search automatically by applying neighbor-join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log likelihood value (2).

Multilocus sequence type, emm type determination, and grouping traits were determined as described previously (4–6). By using the CDC S. pyogenes whole-genome sequencing bioinformatics pipeline (4), we found that all but 7 of the strains shown were positive for the sfb1 fibronectin binding protein gene (data not shown). Three of the 7 sfb1-negative strains included AC2713 and the 2 identical EB1–5 isolates. All strains except for the 2 group L strains (left tree) were positive for the query for the S. pyogenes nga determinant encoding the extracellular NAD+ glycohydrolase virulence factor (data not shown). As shown, 13 strains were positive for erm– or mef– encoded macrolide-resistance determinants, including a cluster of 8 GAS/ST128 isolates of emm subtype stG245.0. A single group ermT-positive group L strain had the ParC S79Y substitution associated with fluoroquinolone-resistance. In addition, a single group G/ST277 strain was positive for the pilus determinant T58. Single isolates were positive for the CDC pipeline queries for exotoxin genes smeZ (group L in left tree) and speC (GAS/ST128/stG652.0). All strains were β-hemolytic because of expression of the previously
characterized homologue of the \textit{S. pyogenes} streptolysin S determinant (data not shown) (7). A total of 19,701 positions (for 49 isolates of left tree) and 2,108 (36 isolates of right tree) are included in the final datasets. The distance reference is indicative of 394 single nucleotide polymorphisms based on 19,701 positions within the 48 genomes (left tree) or 42 positions based upon 2,108 positions within 36 genomes (right tree). Genome fastQ accession numbers are provided for the 48 isolates (Appendix Table) and are associated with laboratory identifiers as well as the information provided from CDC \textit{S. pyogenes} bioinformatics pipeline as described in Chochua et al. (4) and Appendix (Construction of Maximum-Likelihood Trees).
### Appendix Table. SRA accession numbers, laboratory identifiers, and other features of *Streptococcus dysgalactiae* subsp. *equisimilis* study isolates

| Biosample accession no. | LABID* | Group† | MLST | County, year‡ | emm subtype§ | Features detected from S. pyogenes WGS bioinformatics pipeline¶ |
|-------------------------|--------|--------|------|--------------|--------------|-------------------------------------------------|
| SAMN09848964            | 20173110 A 128 NM2, 2016 | stG652.0 | sfb1, nga |
| SAMN09848983            | 20173524 A 128 NM2, 2017 | stG652.0 | sfb1, nga |
| SAMN07153383            | 20154682 A 128 NM2, 2015 | stG652.0 | sfb1, nga |
| SAMN08690859            | 20161483 A 128 NM1, 2016 | stG652.0 | sfb1, nga |
| SAMN08691225            | 20171682 A 128 NM3, 2016 | stG652.0 | sfb1, nga |
| SAMN09848571            | 20182387 A 128 NM3, 2017 | stG652.0 | sfb1, nga |
| SAMN09849325            | 20181492 A 128 NM4, 2017 | stG652.0 | sfb1, nga |
| SAMN07153783            | 20156439 A 128 EB1, 2015 | stG652.0 | sfb1, nga |
| SAMN07154238            | 20162102 A 128 EB2, 2015 | stG652.0 | sfb1, nga |
| SAMN08691109            | 20165290 A 128 EB1, 2016 | stG652.0 | sfb1, nga |
| SAMN08691691            | 20170764 A 128 NM3, 2016 | stG652.0 | sfb1, nga |
| SAMN08691433            | 20165326 A 128 CA1, 2016 | stG652.0 | sfb1, nga |
| SAMN08692346            | 20173686 A 128 ER2, 2016 | stG652.0 | sfb1, nga |
| SAMN08691545            | 20170128 A 128 CO1, 2016 | stG652.0 | sfb1, nga |
| SAMN07154260            | 20162127 A 128 CA1, 2015 | stG652.0 | sfb1, nga |
| SAMN08691583            | 20170168 A 128 CO2, 2016 | stG652.0 | sfb1, nga |
| SAMN08691937            | 20171717 A 128 OR2, 2016 | stG652.0 | sfb1, nga |
| SAMN09849664            | 20182677 A 128 OR1, 2017 | stG652.0 | sfb1, nga |
| SAMN07153796            | 20156452 A 128 EB1, 2015 | stG652.0 | sfb1, nga |
| SAMN07154052            | 20160976 A 128 NY2, 2015 | stG485.0 | sfb1, nga |
| SAMN08690593            | 20162522 A 128 GA5, 2016 | stG245.0 | sfb1, nga |
| SAMN08691696            | 20170850 A 128 TN1, 2016 | stG245.0 | sfb1, nga |
| SAMN07154389            | 20163069 A 128 CT2, 2015 | stG652.0 | sfb1, nga |
| SAMN08691781            | 20171173 A 128 GA2, 2016 | stG652.0 | sfb1, nga, specC |
| SAMN09849027            | 20176966 A 128 CO3, 2017 | stG485.0 | sfb1, nga |
| SAMN08691278            | 20165552 A 128 TN2, 2016 | stG245.0 | sfb1, nga, ermB |
| SAMN07153678            | 20156257 A 128 NY1, 2015 | stG245.0 | sfb1, nga, mef |
| SAMN07153032            | 20154376 A 128 NY3, 2015 | stG245.0 | sfb1, nga, mef |
| SAMN07153560            | 20155412 A 128 NY1, 2015 | stG245.0 | sfb1, nga, mef |
| SAMN09848910            | 20163223 A 128 NY1, 2016 | stG245.0 | sfb1, nga, mef |
| SAMN08691739            | 20170914 A 128 NY1, 2016 | stG245.0 | sfb1, nga, mef |
| SAMN08691478            | 20166757 A 128 NY2, 2016 | stG245.0 | sfb1, nga, mef |
| SAMN010342858           | 20185475 A 128 NY2, 2018 | stG245.0 | sfb1, nga, mef |
| SAMN07154253            | 20162120 A 128 EB1, 2015 | stG485.0 | sfb1, nga |
| SAMN07154273            | 20162140 A 128 EB1, 2015 | stG485.0 | sfb1, nga |
| SAMN09848906            | 20161329 G 48 NM3, 2015 | stG643.0 | nga, mef |
| SAMN09849191            | 20180052 G 127 MD2, 2017 | stG245.0 | nga, tetM |
| SAMN010342859           | 20162531 G 35 GA5, 2016 | emm23.4 | sfb1, nga, mef, tetM, cat |
| SAMN010342860           | 20170556 G 277 OR2, 2016 | stG6.0 | nga, teeS8 |
| SAMN08692801            | 20175240 C 20 NM3, 2017 | stG6247.0 | nga |
| SAMN08691815            | 20171682 C 17 NM1, 2016 | stG74A.0 | sfb1, nga, ermTR |
| SAMN09848934            | 20170580 G 17 OR3, 2016 | stG485.0 | sfb1, nga |
| SAMN08691738            | 20170893 G 206 CT1, 2016 | stG245.0 | sfb1, nga |
| SAMN08690595            | 20162524 G 17 GA1, 2016 | stG245.1 | sfb1, nga |
| SAMN09849303            | 20177043 G 17 GA3, 2017 | stG6792.0 | sfb1, nga |
| SAMN010342861           | 20185314 G 470 OR2, 2018 | stG840.0 | sfb1, nga |
| SAMN010342862           | 20161734 L 1 MD1, 2016 | stl1921.0 | sfb1, smeZ |
| SAMN10342863            | 20163969 L 467 MN1, 2016 | stl1929.1 | sfb1, emrT, ParC-S79Y |

*These isolates are listed in order (top to bottom) as in Figure 1, panel A ([https://wwwnc.cdc.gov/EID/article/25/8/18-1758-F1.htm](https://wwwnc.cdc.gov/EID/article/25/8/18-1758-F1.htm)).
†The group A strains were positive for the group A carbohydrate cluster gene I (gacI). The group carbohydrate for these and the strains of groups C, G, and L were also determined serologically.
‡County and year of isolation. For example, NM2, 2016 refers to New Mexico county 2 and isolation during 2016.
§emm subtypes provided from CDC M protein gene database at [ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/seqemn](ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/seqemn).
¶Additional features obtained from CDC whole genome sequencing bioinformatics pipeline as described in Chochua et al. (4) and this Appendix (Construction of Maximum-Likelihood Trees).
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