Sequential Quadriplex Real-Time PCR for Identifying 20 Common emm Types of Group A Streptococcus

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ABSTRACT

We developed a sequential quadriplex real-time PCR-based method for rapid identification of 20 emm types commonly found in invasive group A Streptococcus (iGAS) strains recovered through the Centers for Disease Control and Prevention’s Active Bacterial Core surveillance. Each emm real-time PCR assay showed high specificity and accurately identified the respective target emm type, including emm subtypes in the United States. Furthermore, this method is useful for rapid typing of GAS isolates and culture-negative specimens during outbreak investigations.

KEYWORDS

group A Streptococcus, M protein, vaccine, emm typing, multiplex real-time PCR

Group A Streptococcus (GAS) causes both mild infections (pharyngitis, anitis, and impetigo) and invasive diseases (acute rheumatic fever, rheumatic heart disease, and necrotizing fasciitis) worldwide (1). In the United States, nearly 24,000 invasive GAS infections were estimated in 2018 (https://www.cdc.gov/abcs/reports-findings/survreports/gas18.html). GAS is an important cause of severe, life-threatening illness among the elderly population, particularly those individuals residing in long-term-care facilities (LTCFs) (2) and skilled nursing facilities (SNFs) (3). Currently, multiple vaccines are in early stages of development to protect against this medically important pathogen (4).

The M protein, encoded by the emm gene, is a major GAS virulence factor traditionally targeted in serotyping GAS isolates (5). In-house antisera and agglutination methods were used to identify up to 80 classical emm serotypes (6). More than 25 years ago, a method for deducing M serotypes based on the 5'-variable region sequences of emm genes was described (7). This method was subsequently standardized at the CDC and became widely used worldwide (5). To avoid confounding with emm-like genes, the CDC’s current whole-genome-sequence-based approach relies upon identification of gene sequence that is linked to the 5'-situated primer 1 (7). This reference method, which relies upon a 180-bp emm sequence encoding the surface-exposed M protein N-terminal 50 residues and 10 residues of conserved signal peptide (8), has identified over 250 emm types and 2,200 subtypes (https://www2.cdc.gov/vaccines/biotech/strepblast.asp). Recently, a new reverse primer (CDC3) was designed and validated to replace the less specific primer 2 of the first emm typing scheme (7), with a resultant improvement of amplification specificity (9). Sequencing and whole-genome sequencing (WGS)-based methods (2, 3, 5, 10) are relatively costly compared to PCR approaches, especially for resource-limited settings, and are not always suitable for outbreak situations, in which fast results are important.

Here, we describe a TaqMan-based quadriplex real-time PCR strategy to rapidly identify 20 emm types (emm1, -2, -3, -4, -6, -11, -12, -28, -49, -59, -75, -76, -77, -81, -82,
-83, -87, -89, -92, and -118) that were the most common among invasive GAS (iGAS) recovered through CDC's year 2015 invasive GAS surveillance (11).

MATERIALS AND METHODS

Bacterial isolates. All bacterial isolates were cultured on Trypticase soy agar (TSA) with 5% sheep blood plates at 37°C with 5% CO2. Genomic DNA was extracted from isolates using the Qiagen DNA minikit (Qiagen, Valencia, CA) as previously described (12), with a prelysis step. Briefly, a loopful of bacteria grown overnight from a blood agar plate was suspended in 50 μl of lysis solution (lysozyme, 0.08 mg/ml, and mutanolysin, 150 U/ml) and incubated at 37°C for 1 h, followed by steps described in the kit manufacturer’s user manual.

A total of 253 bacterial isolates were used in this study to validate the typing scheme. The specificity and sensitivity of each of the five quadriplex assays were validated with respective emm type GAS isolates collected from Active Bacterial Core surveillance (ABCs). Twenty isolates of known S. pyogenes emm types, including emm1, -2, -3, -4, -5, -6, -11, -12, -28, -49, -59, -75, -76, -77, -81, -82, -83, -87, -89, -92, and -118, were used in assay optimization. Additionally, 189 isolates representing 179 subtypes of 80 emm types, 4 nontypeable GAS, and 6 group G streptococci (GGS) (Streptococcus dysgalactiae subsp. equisimilis) (6 subtypes of 5 emm types) were tested (see Table S1 in the supplemental material). The assays were further challenged for specificity with 44 non-S. pyogenes streptococcal and nontreptococcal isolates representing 41 species, including the streptococci Streptococcus acidominimus, S. agalactiae, S. bovis, S. canis, S. delphini, Streptococcus dysgalactiae subsp. dysgalactiae, S. dysgalactiae subsp. equisimilis, S. entericus, Streptococcus equi subsp. equi, Streptococcus equi subsp. zooepidemicus, S. gordini, S. hyointestinalis, S. iniae, S. intermedius, S. milleri, S. mitis, S. mutans, S. oralis, S. ovis, S. parauberis, S. phocae, S. plurimamillium, S. pneumoniae, S. porcinus, S. salivarius, S. sanguinis, S. sinensis, S. thalactis, S. urei, S. uvalensis, and S. pseudopneumoniae (n = 4), as well as Enterococcus faecalis, Enterococcus faecium, Haemophilus influenzae type b, Neisseria meningitidis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Pseudomonas aeruginosa. Streptococcal species were from the CDC reference collection and were identified using standard approaches (13).

Real-time PCR. The emm type-specific 5′ region for each emm type was selected by aligning all the available subtypes of each emm type from the CDC’s emm database (ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/teemm) using CLC Genomics Workbench 10.0. The oligonucleotides for individual emm types were designed by using PrimerQuest software (https://www.idtdna.com/pages/tools/ primerquest) to capture all the subtypes in each emm type. The primers and probes with different reporting dyes (6-carboxyfluorescein [FAM], 6-carboxy-2,4,4,5,7,7-hexachlorofluorescein [HEX], 6-carboxy-X-rhodamine [ROX], and 1′,1′-bis(3-hydroxypropyl)-3,3,3′,3′-tetrakis[4-chloro-3-([4-methoxyphenyl]-azo)-4-methyl-phenyl]-phthalazine [Cy5]) and appropriate quenchers were synthesized at the CDC Biotechnology Core Facility. The oligonucleotide sequences with their chemistry and optimal concentrations are provided in Table 1.

Optimal concentrations for primers and probes were determined by using SYBR green and TaqMan real-time PCR methods, respectively. Optimal concentrations of each oligonucleotide set were optimized with various concentrations (100, 200, 300, 400, and 500 nmol/liter) of each oligonucleotide against 10-fold dilutions of DNA from targeted emm type isolates in a Stratagene Mx3005P real-time PCR instrument (Agilent, Santa Clara, CA). The concentrations were optimized to obtain the highest DNA dilution yielding a cycle threshold (Ct) value of ≤35. Lower limits of detection for each oligonucleotide set were determined in both singleplex and multiplex formats using 10-fold serial dilutions of the targeted emm type control DNA. In total, 20 individual assays targeting 20 emm types were grouped into five quadruplex reactions (Table 2) based on their distribution in the United States (10). The reaction mixture (25 μl) contained 12.5 μl of 2× PerfeCTa multiplex qPCR supermix, QuantBio (WVR, Radnor, PA), optimal concentrations of oligonucleotides (Table 1), and PCR grade water and DNA (5 μl). Amplification was carried out with the following cycling conditions: 1 cycle of 95°C for 10 min and 40 cycles of 94°C for 15 s and 60°C for 1 min.

We also designed a synthetic DNA plasmid (Text S1) (Thermo Fisher Scientific; GeneArt), as described elsewhere (14–16), that was used as a positive control for all real-time PCRs. The synthetic DNA was designed to contain primer and probe binding regions for all 20 emm types, S. pyogenes-specific target spy as an internal positive control, and the Erwinia xeno assay for laboratory contamination control, all concatenated in a single plasmid (14).

RESULTS

The sequential real-time PCR scheme consisted of five 4-plex reactions to identify 20 emm types, inclusive of all known subtypes within each type (see ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/teemm/ for a current listing of emm subtypes). Optimal concentrations of both primers and probes (Table 1) were determined for each assay. Multiplex real-time PCR of DNA from 106 GAS isolates from the 20 emm types showed that the assays specifically amplified all subtypes assigned to the 20 emm types, with the caveat that the emm49 reaction coidentified the closely related deletion derivative of emm49, emm151 (Table 1). No amplification was observed for the additional 66 emm types or for any of the 4 GAS emm nontypeable isolates or the 44 non-S. pyogenes species used to challenge the specificity of the assays. When 4
assays were multiplexed together in 5 individual reactions, we also observed no cross-reaction with any of the other *emm* types/subtypes (Table S1) or the non-*S. pyogenes* species. The multiplex reactions did not produce any false-positive or false-negative results. Among 209 clinical sterile-site GAS isolates (from ABCs) used in the

| Table 1 | Oligonucleotides used in the multiplex real-time PCR *emm* typing assays |
|---------|---------------------------------------------------------------|
| Name    | Oligonucleotide sequence (5'–3')                              | Optimal concn (nM) |
| Emm1-F  | GTGATGTGARTCCTCAGGRTAGT  | 400                 |
| Emm1-P  | FAM/TRCGGGA-BHQ1dT-TGTTTGTGCAAGADR/Spc6  | 100                 |
| Emm1-R  | CATTGACATCTCTATCTCGTCT  | 400                 |
| Emm2-F  | CAGTTAAGGACGAACGAACTAG  | 200                 |
| Emm2-P  | Cy5/AAATATTAG-BHQ2dT-GAAGCGAGTGATTCAGAACA/Spc6  | 200                 |
| Emm2-R  | GCTCTCTCCACATCTTATCTACCTTTGAAATA  | 200                 |
| Emm3-F  | GCAGAAGCTAAAGGGACGATG  | 300                 |
| Emm3-P  | ROX/TRGGAGTCT-BHQ2dT-AAATGGAGGTCTTCGAC/Gspc  | 300                 |
| Emm3-R  | CCGTGAATACAGCTCCAGTCT  | 300                 |
| Emm4-F  | CTGTTAACGCGYGGAACT  | 300                 |
| Emm4-P  | Cy5/CTRAARAA-BHQ2dT-TAACGCGGTATATGAGG/Spc6  | 200                 |
| Emm4-R  | TATCACTCCTCAGCCTGAR  | 300                 |
| Emm6-F  | TATTGGGCTAGAAATTTAAAACAGG  | 300                 |
| Emm6-P  | Cy5/TGAAGTAGG-BHQ2dT-GCAAGAGYGTTCCTGAC/A/Gspc  | 100                 |
| Emm6-R  | TGTAAAYTGCTTATTGAGCT  | 300                 |
| Emm11-F | GCCTTTGCAAYACACAAAAGAAG  | 300                 |
| Emm11-P | HEX/TCGAAGGAG-A/Gspc  | 300                 |
| Emm11-R | CTATCCTCAAGGACTTATATAGG  | 300                 |
| Emm12-F | TATTCGCTTAGAAAATTAAAAACAGG  | 200                 |
| Emm12-P | ROX/TTGGYAGAC-BHQ2dT-GAAACRGCDCC/TGAC/Gspc  | 300                 |
| Emm12-R | ATAGATGCTGTAAGGTTGDDTT  | 300                 |
| Emm28-F | GRCCTCTAATGGAARCTGRT  | 400                 |
| Emm28-P | FAM/AGYTGM-BHQ1dT-GYATACACAGATAGAA/A/Gspc  | 100                 |
| Emm28-R | ACTACAGCAGGTTCTCAGTT  | 400                 |
| Emm49/151-F* | TGGCGGATATGCTMTA  | 400                 |
| Emm49/151-P | HEX/CTATAGGAG-A/Gspc  | 300                 |
| Emm49/151-R | CTTACCTATGTCCTCAGTCT  | 400                 |
| Emm59-F | CTGCGAAACAACACAGAATGTAAG  | 200                 |
| Emm59-P | FAM/AAATACAG-BHQ1dT-GYATACGATGAAATGACTGCTTCTC/Gspc  | 100                 |
| Emm59-R | TATATTTATATCTCCTTCCTCTCT  | 200                 |
| Emm75-F | TAGAGAAGTARATAACAGATCG  | 300                 |
| Emm75-P | FAM/AAATACAG-BHQ1dT-GAAGCTAGCATTAGATACTAATGCTTCTC/Gspc  | 300                 |
| Emm75-R | CTAATCTCTGAAGTCTTCTGTC  | 300                 |
| Emm76-F | TGCAAACAACAGAAGTTAAG  | 300                 |
| Emm76-P | ROX/TGACTCATGGGC-BHQ2dT-GAATGATAAAGYT/Gspc  | 300                 |
| Emm76-R | GTTCACTAATGCTCTGATY  | 300                 |
| Emm77-F | TGCAAAAAGCAACAGAAGTARG  | 300                 |
| Emm77-P | ROX/AGTGTGCA-BHQ2dT-CGGAATCTACAGAAACC/Gspc  | 200                 |
| Emm77-R | AAGTGCATGTAATCGGGTATG  | 300                 |
| Emm81-F | TCGAAACACACAGAAGTTAAG  | 300                 |
| Emm81-P | HEX/TGCGGG-BHQ1dT-YCAAGAAGAARATGACY/Gspc  | 300                 |
| Emm81-R | TTSAAAGTGGTCTGATATKTCGG  | 300                 |
| Emm82-F | ATCAGTACGAGGCTGATCTA  | 300                 |
| Emm82-P | Cy5/TGAAAGAC-BHQ2dT-ARGTTTGTACGACGACA/Gspc  | 300                 |
| Emm82-R | TTTCACTCTGTTGCTCTACCT  | 300                 |
| Emm83-F | CCAGAAGGCGAAGCTTGGGCTTA  | 300                 |
| Emm83-P | Cy5/CTATGAGG-BHQ2dT-AACACAGGGAACGRC/Gspc  | 200                 |
| Emm83-R | TCCTATACCTGAAATCTCTGGAAG  | 400                 |
| Emm87-F | CYAGAGAAAGTARCCARCAGA  | 200                 |
| Emm87-P | FAM/TGCGTATG-BHQ1dT-TCGARTYGAAGAA/A/Gspc  | 200                 |
| Emm87-R | CTGTTACCTTATCAGGATACC/Gspc  | 200                 |
| Emm89-F | TAAGGCGAAGAATCGAA/A/Gspc  | 300                 |
| Emm89-P | HEX/TGCTCTTG-BHQ1dT-CRAAGATAATGAAATGACAC/Gspc  | 300                 |
| Emm89-R | CATCTTCTCTATTATGCTTCT  | 300                 |
| Emm92-F | GATGACGCGGCGGACTGACT  | 200                 |
| Emm92-P | HEX/AATAGGCTG-BHQ1dT-AGCGTACGACY/Gspc  | 300                 |
| Emm92-R | AGCTCAACATGGTCCAAT  | 200                 |
| Emm118-F | GCGACAGAATAGGCG  | 300                 |
| Emm118-P | ROX/CTAGGCTG-BHQ2dT-GCAAGGACTTACAAACC/A/Gspc  | 200                 |
| Emm118-R | CTCCGTTTTTATCTGGAAGTCG  | 300                 |

*a*Assay targets both types *emm49* and *emm151*. 
validation, the real-time PCR emm typing assays correctly identified respective emm types and had 100% concordance with conventional PCR and sequencing and/or WGS results. While we only tested the subtypes for the 21 emm types (including emm151) that were available within ABCs, we infer based on in silico alignment of all available subtypes in the emm database for the primer and probe designs that the PCR assays would detect all known subtypes.

The sensitivity of the real-time PCR assays was determined with 10-fold serial dilutions of DNA extracted from all 21 positive-control isolates used in the validation. All newly validated assays had high sensitivity of detection (<10 genome copies/assay) in both monoplex and multiplex formats.

**DISCUSSION**

The CDC's Streptococcus Lab serves as a reference laboratory for streptococci and assists clinical and state public health laboratories by providing support and characterization of GAS, particularly from LTCFs and SNFs, where outbreaks among residents commonly occur (2, 3, 17, 18). Traditionally, PCR combined with sequencing (5) along with other genotyping methods, such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), have been extensively used to study the clonal relatedness of GAS isolates for epidemiological investigations (3, 17). Recently, WGS has been used for higher discriminatory genetic analysis using single nucleotide polymorphisms (SNPs) to increase resolution in GAS outbreak investigations (2, 18). While all these methods provide valuable information, they either are costly or take several days to complete, and many use equipment and reagents not readily available in most laboratories. The real-time-PCR-based emm typing method developed in this study has an advantage over other methods in that it is more rapid, with results generated in a few hours rather than days. Our method identifies four emm types in a single reaction and utilizes a sequential multiplex-approach which does not require post-PCR steps for confirmation, saving time and cost. Furthermore, it employs real-time PCR, which is a technology available in many clinical, public health, and reference laboratories. This approach can provide information on the relatedness of GAS isolates circulating in a particular setting and allows for a more rapid response. We have recently found this assay very useful for typing of GAS disease cluster isolates, and we find this approach quite useful prior to proceeding with higher-resolution genomic sequencing of strains sharing the same emm types (our unpublished data). Also, this PCR-based approach could further detect GAS from culture-negative or low-DNA-copy-number clinical specimens employing the real-time PCR for the GAS-specific target gene spy (19), in which many specimens would be projected to yield emm type information from included emm type-specific targets.

In summary, a sequential quadruplex real-time PCR scheme was developed that was highly sensitive and specific for the identification of the most frequently occurring 20 emm types covering ~93% of iGAS isolates in the United States collected through CDC's ABCs program in 2015 (10). Also, the current real-time PCR scheme overlaps 60% of emm types (18 of 30 emm types) included in a GAS vaccine (4, 20) that has good phase I clinical trial data and targets common M serotypes in the United States, Canada, and Europe. Most clinical and public health laboratories

**TABLE 2 Quadruplex reactions for identification of 20 emm types of iGAS commonly found in the United States**

| emm typing reaction | emm types<sup>a</sup>                  |
|---------------------|--------------------------------------|
| 1                   | emm1, emm89, emm12, emm82            |
| 2                   | emm28, emm92, emm77, emm4            |
| 3                   | emm59, emm11, emm3, emm2             |
| 4                   | emm87, emm81, emm118, emm6           |
| 5                   | emm75, emm49/151, emm76, emm83      |

<sup>a</sup>Each target assay identifies known subtypes tested within the emm type.

<sup>b</sup>emmm49/151 is considered here as one type. emm151 is a rarely encountered 11-codon deletion derivative of emm49 that occurs within the same MLST type as emm49 (ST433).
routinely isolate and identify GAS but do not perform DNA-based emm typing, as the method is labor-intensive. This method will provide typing capability for these laboratories for outbreak investigation support where emm typing may be useful to determine genetic relatedness of isolates to help guide response efforts. A limitation of the method is that the real-time PCR assays do not target all known emm types and subtypes, and all subtypes within each of the 20 emm types targeted were not tested. However, while this method was developed using the most commonly occurring emm types in the United States, the assay also encompasses a large proportion of types globally (21–23) and can be adapted for use in any country where these emm types are common.

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 0.04 MB.

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**REFERENCES**

1. Bessen DE, Smeesters PR, Beall BW. 2018. Molecular epidemiology, ecology, and evolution of group A streptococci. Microbiol Spectr 6:CPP3-0009-2018. https://doi.org/10.1128/microbiolspec.CPP3-0009-2018.

2. Nanduri SA, Metcalf BJ, Arwady MA, Edens C, Lavin MA, Morgan J. Clegg W, Beron A, Albertson JP, Link-Gelles R, Ogundimu A, Gold J, Jackson D, Chochua S, Stone N, Van Beneden C, Fleming-Dutra K, Beall B. 2019. Prolonged and large outbreak of invasive group A Streptococcus disease within a nursing home: repeated intrafacility transmission of a single strain. Clin Microbiol Infect 25:248.e1–248.e7. https://doi.org/10.1016/j.cmi.2018.04.034.

3. Dooling KL, Crist MB, Nguyen DB, Bass J, Lorentson L, Toews KA, Pondo T, Stone ND, Beall B, Van Beneden C. 2013. Investigation of a prolonged group A streptococcal outbreak among residents of a skilled nursing facility, Georgia, 2009–2012. Clin Infect Dis 57:1562–1567. https://doi.org/10.1093/cid/cit558.

4. Dale JB, Walker MJ. 2020. Update on group A streptococcal vaccine development. Curr Opin Infect Dis 33:244–250. https://doi.org/10.1097/QCO.0000000000000646.

5. Beall B, Facklam R, Thompson T. 1996. Sequencing emm-specific PCR products for routine and accurate typing of group A streptococci. J Clin Microbiol 34:953–958. https://doi.org/10.1128/JCM.34.4.953-958.1996.

6. Lancefield RC. 1962. Current knowledge of the type specific M antigens of group A streptococci. J Immunol 89:307–313.

7. Whatmore AM, Kapur V, Sullivan DJ, Musser JM, Kehoe MA. 1994. Non-congruent relationships between variation in emm gene sequences and the population genetic structure of group A streptococci. Mol Microbiol 14: 619–631. https://doi.org/10.1111/j.1365-2958.1994.tb01301.x.

8. Li Y, Sakota V, Jackson D, Franklin AR, Beall B, Active Bacterial Core Surveillance/Emerging Infections Program Network. 2003. Array of M protein gene subtypes in 1064 recent invasive group A streptococcus isolates recovered from the active bacterial core surveillance. J Infect Dis 188:1587–1592. https://doi.org/10.1086/379050.

9. Frost HR, Davies MR, Velusamy S, Delforge V, Erhart A, Darboe S, Steer A, Walker MJ, Beall B, Botteaux A, Smeesters PR. 2020. Updated emm typing protocol for Streptococcus pyogenes. Clin Microbiol Infect 26: 946.e5–946.e8. https://doi.org/10.1016/j.cmi.2020.02.026.

10. Long SW, Kachroo P, Musser JM, Olsen RJ. 2017. Whole-genome sequencing of a human clinical isolate of emm28 Streptococcus pyogenes causing necrotizing fasciitis acquired contemporaneously with hurricane Harvey. Genome Announc Sci 01269-17. https://doi.org/10.1128/genomeA.01269-17.

11. Chochua S, Metcalf BJ, Li Z, Rivers J, Mathis S, Jackson D, Gertz RE, Jr, Srinivasan V, Lynfield R, Van Beneden C, McGee L, Beall B. 2017. Population and whole genome sequence based characterization of invasive group A streptococcus recovered in the United States during 2015. mBio 8:e01422-17. https://doi.org/10.1128/mBio.01422-17.

12. da Gloria Carvalho M, Pimenta FC, Jackson D, Roundtree A, Ahmad Y, Millar EV, O'Brien LO, Whitney CG, Cohen AL, Beall BW. 2010. Revisiting pneumococcal carriage using broth-enrichment and PCR techniques for enhanced detection of carriage and serotypes. J Clin Microbiol 48: 1611–1618. https://doi.org/10.1128/JCM.02243-09.

13. Facklam R. 2002. What happened to the streptococci: overview of taxonomy and nomenclature changes. Clin Microbiol Rev 15:613–630. https://doi.org/10.1128/cmr.15.4.613-630.2002.

14. Kodani M, Winchell JM. 2012. Engineered combined-positive-control template for real-time reverse transcription-PCR in multiple-pathogen-detection assays. J Clin Microbiol 50:1057–1060. https://doi.org/10.1128/JCM.05987-11.

15. Schembri J, Gillis HD, Lang ALS, Warhuus M, Martin I, Demczuk W, ElSherif M, McNeil SA, LeBlanc JJ. 2018. Multi-target plasmid controls for conventional and real-time PCR-based serotyping of Streptococcus pneumoniae. Plasmid 98:45–51. https://doi.org/10.1016/j.plasmid.2018.09.005.

16. Velusamy S, Tran T, Mongkolrattanothai T, Walker H, McGee L, Beall BW. 2020. Expanded sequential quadruplex real-time polymerase chain reaction (PCR) for identifying pneumococcal serotypes, penicillin susceptibility, and resistance markers. Diagn Microbiol Infect Dis 97:115037. https://doi.org/10.1016/j.diagmicrobio.2020.115037.

17. Arnold KE, Schweitzer JL, Wallace B, Salter M, Neerman R, Hlady WG, Beall B. 2006. Tightly clustered outbreak of group A streptococcal disease at a long-term care facility. Infect Control Hosp Epidemiol 27:1377–1384. https://doi.org/10.1086/508820.

18. Saavedra-Campos M, Simone B, Balasegaram S, Wright A, Usdin M, Lamagni T. 2017. Estimating the risk of invasive group A Streptococcus infection in care home residents in England, 2009–2010. Epidemiol Infect 145:2759–2765. https://doi.org/10.1017/S0950268817001674.

19. Kodani M, Yang G, Conklin LM, Travis TC, Whitney CG, Anderson LJ, Schrag SJ, Taylor TH, Jr, Beall BW, Breiman RF, Felkin DR, Njenga MK, Mayer LW, Oberste S, Tondella MLC, Winchell JM, Lindstrom SL, Erdman DD, Fields BS. 2011. Application of TaqMan low-density arrays for simultaneous detection of multiple respiratory pathogens. J Clin Microbiol 49:2173–2182. https://doi.org/10.1128/JCM.02270-10.

20. Dale JB, Penfound TA, Chiang EY, Walton WJ. 2011. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. Vaccine 29:8175–8178. https://doi.org/10.1016/j.vaccine.2011.09.005.

21. Steer AC, Law I, Matatolu L, Beall BW, Carapetiis JR. 2009. Global emm...
type distribution of group A streptococci: systematic review and implications for vaccine development. Lancet Infect Dis 9:611–616. https://doi.org/10.1016/S1473-3099(09)70178-1.

22. Davies MR, McIntyre L, Mutreja A, Lees JA, Towers RJ, Duchene S, Smeesters PR, Frost HR, Price DJ, Holden MTG, David S, Giffard PM, Worthing KA, Seale AC, Berkley JA, Harris SR, Rivera-Hernandez T, Berking O, Cork AJ, Torres RSLA, Lithgow T, Strugnell RA, Bergmann R, Nitsche-Schmitz P, Chhatwal GS, Bentley SD, Fraser JD, Moreland NJ, Carapetis JR, Steer AC, Parkhill J, Saul A, Williamson DA, Currie BJ, Tong SYC, Dougan G, Walker MJ. 2019. Atlas of group A streptococcal vaccine candidates compiled using large-scale comparative genomics. Nat Genet 51:1035–1043. https://doi.org/10.1038/s41588-019-0417-8. (Author’s correction, 51:1295, https://doi.org/10.1038/s41588-019-0482-z.)

23. Frost HR, Davies MR, Delforge V, Lakh loufi D, Smith MS, Srinivasan V, Steer AC, Walker MJ, Beall B, Botteaux A, Smeester PR. 2020. Analysis of global collection of group A Streptococcus genomes reveals that the majority encode a trio of M and M-Like proteins. mSphere 5:e00806-19. https://doi.org/10.1128/mSphere.00806-19.