Analysis Of PCR Products From Using Emm Primers For Different Streptococcus Pyogenes Strains

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Abstract

Streptococcus pyogenes is a small bacterium causing problematic infectious diseases. S. pyogenes expresses important virulence factors like the antiphagocytic M protein, the complement factor-inactivating C5a peptidase and the immunoglobulin-Fc-binding proteins on its surface. The corresponding emm and emm-related (fcrA, ennX) genes are adjacently encoded on the genome. The characterization of emm from the S. pyogenes revealed some discrepancies with serotyping, illustrating the difficulty in serotype determination when cross-reactions occur. Here, the author performed an analysis of PCR products from using emm primers for different Streptococcus pyogenes strains. Based on the PCR amplification, the electronic analysis of the PCR product for each S. pyogenes strain was identified. The reported sequence might be used in further development of molecular-based diagnostic tools.

INTRODUCTION

Streptococcus pyogenes (or group A beta hemolytic streptococcus) is a small bacterium causing problematic infectious diseases [1]. It is a pathogenic bacterium that can give rise to a range of invasive and autoimmune diseases, although it is more widely known as the cause of tonsillitis [1]. Resistance to erythromycin and lincomycin by S. pyogenes has been noted for many years and becomes an important problem in infectious medicine [2]. A significant increase in erythromycin resistance was observed with clinical S. pyogenes [3].

S. pyogenes expresses important virulence factors like the antiphagocytic M protein, the complement factor-inactivating C5a peptidase and the immunoglobulin-Fc-binding proteins on its surface [4]. The corresponding emm and emm-related (fcrA, ennX) genes are adjacently encoded on the genome [4]. Podbielski et al. first applied the polymerase chain reaction (PCR) to study the M protein gene family in beta-hemolytic streptococci in 1991 [5]. Musser et al noted that the occurrence of the same emm alleles in strains that are well differentiated in overall chromosomal character demonstrates that horizontal transfer and recombination play a fundamental role in diversifying natural populations of S. pyogenes [6]. Relf et al.noted that characterization of emm from S. pyogenes revealed some

discrepancies with serotyping, illustrating the difficulty in serotype determination when cross-reactions occur [7]. Here, the author performed an analysis of PCR products from using emm primers for different Streptococcus pyogenes strains.

MATERIALS AND METHODS

EMM PRIMERS AND STUDIED S. PYOGENES STRAIN

The emm primers used in this study are quoted from those developed by Pimtanothai et al [8]. The primers are 5'TATTCCCTTAGAAAATTAA and 5' GCAAGTTCTTCAGCTTGTTT, respectively. There are 5 observed S. pyogenes strains in this study. The studied strains include M1 GAS, MGAS8232, MGAS315, SSI-1 and MGAS10394.

PCR AMPLIFICATION TESTING AND SEQUENCING OF THE PCR PRODUCT

The PCR amplification was performed using the standard protocols proposed by Pimtanothai et al.[8]. The new electronic tool by Bikandi et al. [9] was used for amplification and sequencing of the PCR products Briefly, this tool allows getting the PCR results for a regular PCR amplification [8]. Resulting page will show an electronic list of amplified bands and a DNA electrophoresis of the bands

[₉]. DNA sequence of each band and the identity of amplified genes are automatically available on the resulting page [₉].

RESULTS

According to the PCR amplification, the electronic analysis of the PCR product for each S. pyogenes strain is shown in Table 1.

Figure 1

Table 1: Sequences of the bands from amplification for each Streptococcus pyogenes strain.

Strain	Length	Sequence
M1 GAS	1175	GCAAGTTCTT CAGCTTGTTT CGCTAATTGT TCTTTGAGTG
		CTTTTGCTTC TGCTTCAAGT TTTGCTTGTA GTTCAGCTTT
		TTCTTTTTCT GTTAATTTCT 1684139 TGCTTTCTTC
		AAGCTCTTTG TTAAGTTTTT CAAGAGCAGC TAATTTGCTG
		TITGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT
		CACGTGATGC 1684239 GTCCAAGTCA CGGCGAAGGC
		CTTGACGGCT TGCGTCTGAG ATTTGTTTT CTTCTTTAAC
		CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA 1684339 ACCTGTTTCT TAGCTTCACG TGATGCGTCC
		AAGTCACGGC GAAGGCCTTG ACGGCTTGCG
		TCTGAGATTT GTTTGTCTTC TTTAACCTTA TCAAGTTCAG
		1684439 CAGTCAAGTT TGCTAAATCT TTTTCAACCT
		GTTTCTTAGC TTCACGTGAT GCGTCCAAGT CACGACGAAG
		GCTTTGACGA CTTGCGTCTG AGATTTGTTT 1684539
		TTCTTCCTCA AGTTTTGCTT TTTCGATCGT TAGCTGCTCT
		TTTTCAGATG AAAGTTGATC AAGTTCAAGT TTTGCATTGC
		CTAAAAGATT ACGATTAATC 1684639 TCTTGTTCTC
		TAGTAATCGT TTCTAACTCT TTTTCTAAGA CGTTAGCTCT
		ATTATAGTCC TGACTCGCTT GGTCTATCGC TAATTCAAGA
		GCTITCTTTT 1684739 TCTCTTCTAA CTCTTTTTCT
		AAAGCGGTAG CTCTATGGTA GTCCCGACTT
		GCCTGGTCTA TCGCTAATTC AAGAGCTTCC TTTTTCTCTT
		CTAACTCTTT 1684839 TTCAAGTCTT TGTCTATCCC
		AACTTGTTGA TTCCTTTGCT AAGTCATAGT CTTGTTGTAG
		TTCTTTTAAT TTAGTTTCTA AATCTTTACG CTGGTCTTCT 1684939 AAGGCTTGTT TTGCTTTTTC AAGTTCTTCA
		GCTCTCTTAA AATCTCTTCC TGCAACTTCC ATTGCATTCT
		CTAATCTCGC TTTTAAGTCC TTGTTTTCGT 1685039
MGAS82	926	AACGTAAACG TATATITTGT ATTGCGGGAT TGTTTGCTGC
32		AAGATCTTCT ATAACTTCCC TAGGATTACC ATCACCGTTA
		GCCTTAACCT CTGTTTGATT 1685139 CGCAAAACCT
		GCCCCTAAAA CAGTCAAAGC TACCGCTACT
		GAAGCCGTTC CTGTTTTTAA TTTTCTAAGC GAATA
		GCAAGTTCTT CAGCTTGTTT TGCTAATTGT TCTTTGAGTG
		CTITTGCTTC TGCTTCAAGT TTTGCTTGTA GCTCAGCTTT TTCTTTTCT GTTAATTTCT 1739831 TGCTTTCTTC
		AAGCTCTTTG TTAAGTTTTT CAAGAGCAGC TAATTTGCTG
		TTTGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT
		CACGTGATGC 1739931 GTCCAAGTCA CGGCGAAGAC
		CTTGACGGCT TGCGTCTGAG ATTTGTTTTT CTTCTTTAAC
		CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA
		1740031 ACTTGTTTCT TAGCTTCACG TGATGCATCT
		AAGTCACGAC GAAGACCTTT ACGACTTGCT TCTGAAATTT
		TGTTTTGTTC TTCTTTTTA GCCAATTCTT 1740131
		GTITAAGGGC ACCAAAGTCT TGTITACTTT TITGCTCTCT
		CGCAATTTTA TCTTTTACTG TCTTTTCCAA GAGTTCATTA
		AGGGTCTTTT CATTTTCTTT 1740231 ACTCTCTTGC
		TGTTTATTTG CTAATTCTTG TCGAGTTTCA TTCAACTTT

Figure 2

		TAGTTAAGTC ATCATTATTA ATGTTTAACG TTTCATTCTT
	1466	GTGTTTCGCT 1740331 TCCGCTACTT CTTTTCAAG
MGAS31		ATTITCTITC TGAGTAGCTA ACTICTCACT CCGTTGTTCT
5		AACTGATCCT TCTCAGTTTT TAAATCATCA TTCTCTTTTG
		1740431 TTAACTGTTC CTTATCAATT TTTAATTTTT
		TATTCTCAAC TGTTAACTGA TGGTTCTGTA TCTCATAACC
		GTTAGCTCTT TTTATTAATT CGTCTTTATT 1740531
		GTCTGCTGTA GCTCGAGTAA GAGGTGCTGC
		GCTAACTTCT GTTTGGTTAA CCGCTAATCC TACCCCTAAG
		GCACTCAAAG CAACCGCTAC TGAAGCAGTA 1740631
111111		CTTTTTTTTA ATTTTCTAAG CGAATA
		GCAAGTTCTT CAGCTTGTTT TGCTAATTGT TCTTTGAGTG
		CTITTGCTTC TGCTTCAAGT TTTGCTTGTA GCTCAGCTTT
		TTCTTTTTCT GTTAATTTCT 1746554 TGCTTTCTTC
		AAGCTCTTTG TTAAGTTTTT CAAGAGCAGC TAATTTGCTG
		TTTGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT
		CACGTGATGC 1746654 GTCCAAGTCA CGGCGAAGAC
		CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA
		1746754 ACTTGCTTCT TAGCTTCACG TGATGCGTCC
		AAGTCACGGC GAAGACCCTT ACGGCTTGCT
		TCTGAAATCC TGTTTTGTTC TTCAAGTTGT TTGAGAGCAG
		1746854 CTTCAACTTG TGCTTTTGCT TGGCGAACTG
		CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA
		GATTTGTTTT TGTTCTGTAA CTTTTGCAAG 1746954
		CTCTGCTTTG AGGTTGTTTA ATTCAGCTTC CGTAGCTTTT
		TTAGCTTGGC GAACAGCTTC AAGGTCTCTT GCTGTACCTT
		TACGGCTAGC ATCTAGGATT 1747054 TGTTTTTGCT
		CTTCTAGTTG TTTAATTTGA TCATCTTTTT CTGCTAGTTT
		TGCTTGATAC TCATTATGAG CATGTTGGTG ACCTAATGCT
		GCTAGTTCAA 1747154 AATCTTCCTT TGCCTCTTTT
		AGTTTTGTTT CTAAATCGGC AATCTTAGCT TCTAACTTTT
		GTTTATCAGC GGCATTAGCT AATGCTGCTA GTTCAAAATC
		1747254 TTCTTTAGCA TTTTGTTTAG TAGTAGCTAA
	1466	TTCTTGCTCT AACTGAGAAA CACGATTTTG TTTTTCAGTT
		AAATCTTGCA TAGTACTTTC AACTTTTTGT 1747354
SSI-1		TTAACTGTCT CTAGCTCTTC GCTAATCTGG CCAACTTTAG
		CAGTTGCTTC TGTAACTTTC TTTTCTTTTT CTTCTAGTTC
		TTGTTGATGT CTTTTATCTG 1747454 ATAAAACATA
		CCCCTGTTTG GCCAAGTCAA AATCTTTATC TAAGTCCTGA
		TATTCCTTTT CCTTTTTGTC TATTTTTTCC TTAAGTTCTT
		TAACCTCCTT 1747554 TTCTAAATCA TCTTTTCAA
		AAGCCACTTT ACCTAAAACT TTTTTTACAT CTTCTCCATT
		TAACTCTTGT AACAGCCTCT CAGCCCAATC ATTAAGGCCT
		1747654 TITAGATATT CAGCCTTITG TCTCAGGTCA
		AGTCTGCCAG CTTGTGCATT ATATTGTTGG TAATTACTAT
		TATGTTTAGT ATATAATTGT GTAACCTGAT 1747754
		CTAACAAGTT CTCAATTTCA TTTTTTAATT TAACATGTCT
		AGGAAACTCT CCATTAACAC TCCTAGCATC TGCCTTTACT

Figure 3

GTCTGCCCTG CTACCAGTCC 1747854 TGTCCCTAAA ACTGTCAAAG CAACCGCTAC TGAAGCCGTT CCTGTTTT/ ATTITCTAAG CGAATA GCAAGTICTT CAGCTTGTTT TGCTAATTGT TCTTTGAGTG CTTTGCTTC TGCTTCAAGT TTTGCTTGTA GCTCAGCTTT TTCTTTTTCT GTTAATTTCT 1740340 TGCTTCTTC AAGCTCTTG TTAAGTTTT CAAGAGCAGC TAATTTGCTG TTTGCTTCT CTAAAGCTTT TCCAAGCTA CGGCGAAGAAC CTTGACGGCT TGCGTCTGAG ATTTGTTTT TCTTTTCA CACGTGATGC 1740440 GTCCAAGCTA CGGCGAAGAAC CTTGACGGCT TGCGTCTGAG ATTTGTTTT CTTTTAACT CACGTGATGC 1740440 GTCCAAGCTA CGGCGAAGAAC CTTGACGGCT GCGCTCTGAG ATTTGTTTT CTTTTTAAC 1740540 ACTTGCTTCT TAGCTT CACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTGCT TCTGAAATCC TGTTTTGTT TCAAGTTGT TTGAGAGCAGG 1740640 CTTCAACTTG TGCTTTTGCT TGGCGAACTG CTTCAAGATC TCTGCTGAA CTTTTGCAGC TAGCATCTAA GATTTGTTTT AGTTCTGAA CTTTGCAGC TAGCATCTAA GATTTGTTTT AGGTGCTAA ACTTTGCTCT GCGAGCTG TTCGAGATC TCTTGCTAA CTTTGCCAGC TAGCATCTAA GATTTGTTTT AGGTGCTAA ACTTTGCTC GTAGCTTCCAGC TTCCAGGC GAACAGCTCC AGGCTCCT GCTGCTCT TAGCTGCG GAACAGCTC AGGCTCTT GCTGTACTT TAGCTGCC TGC AGCAGCTC AGGCTCTT GCTGTACTT
ATTITICTAAG CGAATA GCAAGTICTT CAGCTIGTIT IGCTAATIGT TCTITGAGTG CTITIGCTTC TGCTTCAAGT TITGCTTGTA GCTCAGCTTT TTCTITTICT GITAATTICT 1740340 TGCTTCTTC AAGCTCTTTG ITAAGTTTIT CAAGAGCAGC TAATTIGCTG TITGCTTCTI CTAAAGCTTI TICAACTTG TICTTAGCTT CACGTGATGC 1740440 GTCCAAGTCA CGGCGAAGAC CTIGACGGCT IGCGTCTGAG ATTIGTTTIT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTITGCTAA ATCTITTCA 1740540 ACTTGCTTCT TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTIGCT TCTGAAATCC TGTTTGTTC TTCCAAGTTGT TTGAGAGCAGG 1740640 CTTCCAACTTG TGCTTACGAC TAGCATCTAA GATTIGTTTI GTTCTGTAA CTTTTGCAG GTAGCATCTAA GATTIGTTTT GATCTTGTAA CTTTGCCAGGCTAGCATCTAA GATTIGTTTT GATCTGTGTAA CTTTGCCAGGCTAGCATCTAA GATTIGTTTT GATCTGTGTTAA ATCAGCTCC CGTAGCTTTI 968 TTAGCTTGGC GAACAGCTTC AAGGTCCTT GCTGTACCT
GCAAGTICTT CAGCTTGTTT TGCTAATTGT TCTTTGAGTG CTTTGCTTC TGCTTCAAGT TTTGCTTGTA GCTCAGCTTT TTCTTTTTCT GTTAATTTCT 1740340 TGCTTTCTC AAGCTCTTTG TTAAGTTTT CAAGAGCAGC TAATTTGCTG TTTGCTTCTT CTAAAGCTTT TCCAACTTGT TCTTAGCTT CACGTGATGC 1740440 GTCCAAGTCA CGGCGAAGAC CTTGACGGCT TGCGTCTGAG ATTTGTTTT CTTCTTTAAC CTTGACGGCT TGCGCGTCTGAG ATTTGTTTT CTTCTTTAC 1740540 ACTTGCTTCT TAGCTTCCAG GGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTGCT TCTGAAATCC TGTTTTTTTTCT TCTGAGAGCAG 1740640 CTTCAACTTG TGCTTTACGAC TGGCGAACTG CTTCAAGATT CTTGTGTA CCTTTACGAC AGATCGCTCAA GATTGTTTT GTCTTGTA CCTTTACGAC 1740740 CTCTGCTTG AGGTGTTAA ATTCAGCTTC CGTAGCTTTA
CTITIGCTTC TGCTTCAAGT TTTGCTTGTA GCTCAGCTTT TTCTTTTTCT GTTAATTTCT 1740340 TGCTTTCTC AAGCTCTTTG TTAAGTTTTT CAAGAGCAGC TAATTTGCTG TTTGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT CACGTGATGC 1740440 GTCCAAGTCA CGGCGAAGAC CTTGCAAGGTCT GCGTCTGAG ATTTGTTTT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA 1740540 ACTTGCTTCT TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTGCT TCTGAAATCC TGTTTGTTC TTCAAGTTGT TTGAGAGCAGG 1740640 CTTCAACTTG TGCTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTTGTTCTTGAA CTTTTGCAGG 1740740 CTCTGCTTTG AGGTTGTTTA ATCAGCTTC CGTAGCTTT
CTITIGCTTC TGCTTCAAGT TTTGCTTGTA GCTCAGCTTT TTCTTTTTCT GTTAATTTCT 1740340 TGCTTTCTC AAGCTCTTTG TTAAGTTTTT CAAGAGCAGC TAATTTGCTG TTTGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT CACGTGATGC 1740440 GTCCAAGTCA CGGCGAAGAC CTTGCAAGGTCT GCGTCTGAG ATTTGTTTT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA 1740540 ACTTGCTTCT TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTGCT TCTGAAATCC TGTTTGTTC TTCAAGTTGT TTGAGAGCAGG 1740640 CTTCAACTTG TGCTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTTGTTCTTGAA CTTTTGCAGG 1740740 CTCTGCTTTG AGGTTGTTTA ATCAGCTTC CGTAGCTTT
TTCTTTTCT GTTAATTTCT 1740340 TGCTTTCTTC AAGCTCTTTG TTAAGTTTTT CAAGGAGCAGC TAATTTGCTG TTTGCTTCTT CTAAAGCTTT TCCAACTTGT TCTTAGCTT CACGTGATGC 1740440 GTCCAAGTCA CGGCGAAGAC CTTGACGGCT TGCGTCTGAG ATTTGTTTT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA 1740540 ACTTGCTTCT TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTTGCT TCTGAAATCC TGTTTGTTC TTCAAGTTGT TTGGGAGCAGG 1740640 CTTCAACTTG TGCTTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTT TGTTCGTAA CTTTTGCAGG 1740740 CTCTGCTTTG AGGTTGTTTA ATTCAGCTTCT CGTGACCTTI
AAGCTCTTTG TTAAGTTTTT CAAGAGCAGC TAATTTGCTG TTTGCTTCTT CTAAAGCTTTT TCAACTTGT TTCTTAGCTT CACGTGATGC 1740440 GTCCAAGTCA CGGCGAAGAC CTTGACGGCT TGCGTCTGAG ATTTGTTTTT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA 1740540 ACTTGCTTCT TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTTGCT TCTGAAATCC TGTTTTGTTC TTCAAGTTGT TTGAGAGCAG 1740640 CTTCAACTTG TGCTTTACGAC TAGCACCG CTTCAAGATC TCTTGCTGTA CCTTTACGAC AGCATCA GATTGTTTT GTTCTGTAA CTTTACGAC 1740740 CTCTGCTTTG AGGTGTTTA ATTCAGCTTC CGTAGCTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCCTT GCTGTACCT
TTTGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT CACGTGATGC 1740440 GTCCAAGTCA CGGCGAAGAC CTTGACGGCT TGCGTCTGAG ATTTGTTTT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA 1740540 ACTTGCTTCT TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTTGCT TCTGAAATCC TGTTTGTTC TTCAAGTTGT TTGAGAGCAG 1740640 CTTCAACTTG TGCTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTT GTTCTGTAA CTTTTGCAAG 1740740 CTCTGCTTTTA AGGTTGTTTA ATTCAGCTTC CGTAGCTTTI 968 TTAGCTTGGC GAACAGCTTC AAGGTCCTT GCTGTACCT
CACGTGATGC 1740440 GTCCAAGTCA CGGCGAAGAC CTTGACGGCT TGCGTCTGAG ATTTGTTTT CTTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA 1740540 ACTTGCTTCT TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTGCT TCTGAAATCC TGTTTTGTT TCAAGTTGT TTGAGAGAGGAG 1740640 CTTCAACTTG TGCTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTT GTTCTGTAA CTTTGCAG 1740740 CTCTGCTTTG AGGTTGTTTA ATTCAGCTTCT CGTAGCTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCTTT GCTGTACCT
CTTGACGGCT TGCGTCTGAG ATTTGTTTT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA 1740540 ACTTGCTTC TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTTGCT TCTGAAATCC TGTTTTGTTC TTCAAGTTGT TTGAGAGCAG 1740640 CTTCAACTTG TGCTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTT GTTCTGTAA CTTTACGAC 1740740 CTCTGCTTTG AGGTGTTTA ATTCAGCTCT CGTAGCTTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCCTT GCTGTACCT
CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTCA 1740540 ACTTGCTTCT TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTTGCT TCTGAAATCC TGTTTTGTTC TTCAAGTTGT TTGAGAGCAG 1740640 CTTCAACTTG TGCTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTT GTTCTGTAA CTTTGCAG 1740740 CTCTGCTTTG AGGTTGTTAA ATTCAGCTC CGTAGCTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCCTT GCTGTACCT
1740540 ACTTGCTTCT TAGCTTCACG TGATGCGTCC AAGTCACGGC GAAGACCCTT ACGGCTGCT TCTGAAATCC TGTTTGTTC TTCAAGTTGT TTGAGAGCAG 1740640 CTTCAACTTG TGCTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTI GTTTT GTTCTGTAA CTTTGCCAG 1740740 CTCTGCTTTG AGGTTGTTTA ATTCAGCTTC CGTAGCTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCTCTT GCTGTACCT
AAGTCACGGC GAAGACCCTT ACGGCTTGCT TCTGAAATCC TGTTTGTTC TTCAAGTTGT TTGAGAGCAG 1740640 CTTCAACTTG TGCTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTT TGTTCTGTAA CTTTTGCAAG 1740740 CTCTGCTTTG AGGTTGTTTA ATTCAGCTTC CGTAGCTTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCTCTT GCTGTACCT
TCTGAAATCC TGTTTGTTC TTCAAGTTGT TTGAGAGCAG 1740640 CTTCAACTTG TGCTTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTT TGTTCTGTAA CTTTTGCAAG 1740740 CTCTGCTTTG AGGTTGTTTA ATTCAGCTTC CGTAGCTTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCTCTT GCTGTACCT
1740640 CTTCAACTTG TGCTTTTGCT TGGCGAACTG CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTT TGTTCTGTAA CTTTTGCAAG 1740740 CTCTGCTTTG AGGTTGTTTA ATTCAGCTTC CGTAGCTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCTCTT GCTGTACCT
CTTCAAGATC TCTTGCTGTA CCTTTACGAC TAGCATCTAA GATTTGTTTT TGTTCTGTAA CTTTGCCAG 1740740 CTCTGCTTTG AGGTTGTTTA ATTCAGCTCT CGTAGCTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCTCTT GCTGTACCT
GATTTGTTTT TGTTCTGTAA CTTTTGCAAG 1740740 CTCTGCTTTG AGGTTGTTTA ATTCAGCTTC CGTAGCTTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCTCTT GCTGTACCT
968 CTCTGCTTTG AGGTTGTTTA ATTCAGCTTC CGTAGCTTTT 968 TTAGCTTGGC GAACAGCTTC AAGGTCTCTT GCTGTACCT
968 TTAGCTTGGC GAACAGCTTC AAGGTCTCTT GCTGTACCT
TACGGCTAGC ATCTAGGATT 1740840 TGTTTTGCT
MGAS10 CTTCTAGTTG TTTAATTTGA TCATCTTTTT CTGCTAGTTT
394 TGCTTGATAC TCATTATGAG CATGTTGGTG ACCTAATGCT
GCTAGTTCAA 1740940 AATCTTCCTT TGCCTCTTTT
AGTITTGTTT CTAAATCGGC AATCTTAGCT TCTAACTITT
GTTTATCAGC GGCATTAGCT AATGCTGCTA GTTCAAAATC
1741040 TTCTTTAGCA TTTTGTTTAG TAGTAGCTAA
TTCTTGCTCT AACTGAGAAA CACGATTTTG TTTTTCAGTT AAATCTTGCA TAGTACTTTC AACTTTTGT 1741140
TTAACTGTCT CTAGCTCTTC GCTAATCTGG CCAACTTTAG
CAGTIGCTIC TGTAACTITC TTTCTTTT CTTCTAGTIC
TTGTTGATGT CTTTTATCTG 1741240 ATAAAACATA
CCCCTGTTTG GCCAAGTCAA AATCTTATC TAAGTCCTGA
TATTCCTTT CCTTTTGTC TATTTTTCC TTAGGTCTT
TAACCTCCTT 1741340 TTCTAAATCA TCTTTTTCAA
AAGCCACTTT ACCTAAAACT TTTTTTACAT CTTCTCCATT
TAACTCTTGT AACAGCCTCT CAGCCCCAATC ATTAAGGCCT
1741440 TITAGATATT CAGCCTTTTG TCTCAGGTCA
AGTCTGCCAG CTTGTGCATT ATATTGTTGG TAATTACTAT
TATGTTTAGT ATATAATTGT GTAACCTGAT 1741540
CTAACAAGTT CTCAATTTCA TTTTTTAATT TAACATGTCT
AGGAAACTCT CCATTAACAC TCCTAGCATC TGCCTTTACT
GTCTGCCCTG CTACCAGTCC 1741640 TGTCCCTAAA
ACTGTCAAAG CAACCGCTAC TGAAGCCGTT CCTGTTTT
ATTTTCTAAG CGAATA
GCAAGTTCTT CAGCTTGTTT CGCTAATTGT TCTTTGAGTG
CTITTGCTTC TGCTTCAAGT TITGCTTGTA GCTCAGCTTT
TTCTTTTTCT GTTAATTTCT 1729903 TGCTTTCTTC
AAGCTCTTTG TTAAGTTTTT CAAGAGCAGC TAATTTGCTG

Figure 4

TITIGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT
CACGTGATGC 1730003 GTCCAAGTCA CGGCGAAGAC
CTTGACGGCT TGCGTCTGAG ATTTGTTTTT CTTCTTTAAC
CTTATCAAGT TCAGCAGTCA AGTITGCTAA ATCTTITTCA
1730103 ACCTGTTTCT TAGCTTCACG TGATGCGTCC
AAGTCACGGC GAAGACCCTT ACGGCTTGCG
TCTGAGATTT GTTTTTCTTC TTTAACCTTA TCAAGTTCAG
1730203 CAGTCAAGTT TGCTAAATCT TTTTCAACCT
GTTTCTTAGC TTCACGTGAT GCGTCCAAGT
CACGGCGAAG ACCCTTACGG CTTGCTTCTG AAACTTTGTT
1730303 TCCTTCATCT TTTTTAGCTA ATTCTTGTTT
AAGGGCACCA ATGTCTTGTT TACTTTTTTG CTCTCTCGCA
ATTITATCTT TTACTGTCTC ATCCAAGATT 1730403
TITTTAAGGG TACCAATGGT TTCTTTACTT TCTTGCTCTT
TATTTGCTGC TTCTTCTTCA GCTTCACTCA ACTTTTAGT
TAACCCTITA TTCTCAGTTG 1730503 TTAACCTATT
CTCCTCAGCT TTTAACTCCT TATTCTGATC TGTTAAGTTT
TTATTCTCAG TTGTTAACTT GTCATTATTA GCTTGTAACA
TAGAGTTCTC 1730603 TACGTCATAC TTGTTAAGAA
GTTCTCGTGC TTTGTCCGGG TTTTCTACCG TCCCCCTAGG
AAACACTCTT GCACTAACTT CATTAGTATT GACAACTAAT
1730703 CCTGCCCCTA TTACACTCAA AGCCACTGCT
ACTGATGCAG TACCTTTTTT TAATTTTCTA AGCGAATA

DISCUSSION

In choosing the appropriate antimicrobial therapy, one must take into account the resistance profile of the target pathogen, the agent's antibacterial profile and the intrinsic activity against the target pathogen [$_{10}$]. Cunningham noted

that emm gene sequencing had changed serotyping, and new virulence genes and new virulence regulatory networks have been defined [11]. Cunningham also noted that the emm gene superfamily had expanded to include antiphagocytic molecules and immunoglobulin-binding proteins with common structural features [11].

During recent years, various new techniques have been adapted for the diagnosis of S. pyogenes infection, notably in the field of molecular biology and standard PCR is currently the method of choice for emm typing. In this study, the author analyzed the PCR products from using emm primers for five different Streptococcus pyogenes strains based on a new electronic tool. Findings from the electronic analysis of the product revealed that a specific produce for each strain can be separated. Of interest, these sequences have never been noted before. Here, the sequences of the detected products are also analyzed and presented. The reported sequence might be used in further development of molecular-based diagnostic tools.

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