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 TTTGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT CACGTGATGC **1684239** GTCCAAGTCA CGGCGAAGGC CTTGACGGCT TGCGTCTGAG ATTTGTTTTT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTTCA **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 GCTTTCTTTT **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**
MGAS8232	926	AACGTAAACG TATATTTTGT ATTGCGGGAT TGTTTGCTGC AAGATCTTCT ATAACTTCCC TAGGATTACC ATCACCGTTA GCCTTAACCT CTGTTTGATT **1685139** CGCAAAACCT GCCCCTAAAA CAGTCAAAGC TACCGCTACT GAAGCCGTTC CTGTTTTTAA TTTTCTAAGC GAATA GCAAGTTCTT CAGCTTGTTT TGCTAATTGT TCTTTGAGTG CTTTTGCTTC TGCTTCAAGT TTTGCTTGTA GCTCAGCTTT TTCTTTTTCT GTTAATTTCT **1739831** TGCTTTCTTC AAGCTCTTTG TTAAGTTTTT CAAGAGCAGC TAATTTGCTG TTTGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT CACGTGATGC **1739931** GTCCAAGTCA CGGCGAAGAC CTTGACGGCT TGCGTCTGAG ATTTGTTTTT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTTCA **1740031** ACTTGTTTCT TAGCTTCACG TGATGCATCT AAGTCACGAC GAAGACCTTT ACGACTTGCT TCTGAAATTT TGTTTTGTTC TTCTTTTTTA GCCAATTCTT **1740131** GTTTAAGGGC ACCAAAGTCT TGTTTACTTT TTTGCTCTCT CGCAATTTTA TCTTTTACTG TCTTTTCCAA GAGTTCATTA AGGGTCTTTT CATTTTCTTT **1740231** ACTCTCTTGC TGTTTATTTG CTAATTCTTG TCGAGTTTCA TTCAACTTTT
MGAS315	1466	TAGTTAAGTC ATCATTATTA ATGTTTAACG TTTCATTCTT GTGTTTCGCT **1740331** TCCGCTACTT CTTTTTCAAG ATTTTCTTTC TGAGTAGCTA ACTTCTCACT CCGTTGTTCT 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** CTTTTTTTTA ATTTTCTAAG CGAATA GCAAGTTCTT CAGCTTGTTT TGCTAATTGT TCTTTGAGTG CTTTTGCTTC TGCTTCAAGT TTTGCTTGTA GCTCAGCTTT TTCTTTTTCT GTTAATTTCT **1746554** TGCTTTCTTC AAGCTCTTTG TTAAGTTTTT CAAGAGCAGC TAATTTGCTG TTTGCTTCTT CTAAAGCTTT TTCAACTTGT TTCTTAGCTT CACGTGATGC **1746654** GTCCAAGTCA CGGCGAAGAC CTTGACGGCT TGCGTCTGAG ATTTGTTTTT CTTCTTTAAC CTTATCAAGT TCAGCAGTCA AGTTTGCTAA ATCTTTTTCA **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
SSI-1	1466	TTCTTGCTCT AACTGAGAAA CACGATTTTG TTTTTCAGTT AAATCTTGCA TAGTACTTTC AACTTTTTGT **1747354** TTAACTGTCT CTAGCTCTTC GCTAATCTGG CCAACTTTAG CAGTTGCTTC TGTAACTTTC TTTTCTTTTT CTTCTAGTTC TTGTTGATGT CTTTTATCTG **1747454** ATAAAACATA CCCCTGTTTG GCCAAGTCAA AATCTTTATC TAAGTCCTGA TATTCCTTTT CCTTTTTGTC TATTTTTTCC TTAAGTTCTT TAACCTCCTT **1747554** TTCTAAATCA TCTTTTTCAA AAGCCACTTT ACCTAAAACT TTTTTTACAT CTTCTCCATT TAACTCTTGT AACAGCCTCT CAGCCCAATC ATTAAGGCCT **1747654** TTTAGATATT CAGCCTTTTG TCTCAGGTCA AGTCTGCCAG CTTGTGCATT ATATTGTTGG TAATTACTAT TATGTTTAGT ATATAATTGT GTAACCTGAT **1747754** CTAACAAGTT CTCAATTTCA TTTTTTAATT TAACATGTCT AGGAAACTCT CCATTAACAC TCCTAGCATC TGCCTTTACT

Figure 2

Figure 3

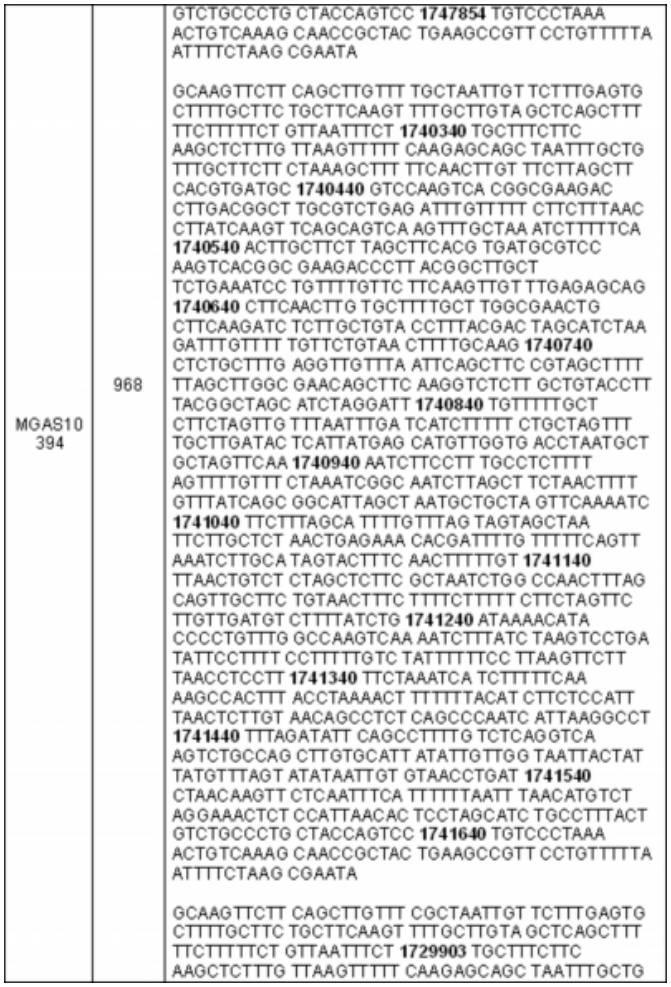
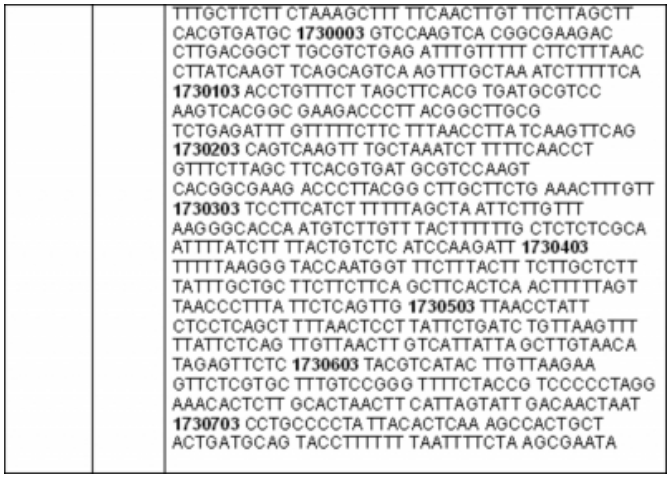


Figure 4



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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