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M3 rivR   CGGTAGATTCGCTACAATATATAGGATTATACAATTTAATTTAAGGAGAGAAAAATTACATGGTTAGATTATTATTGGA
M1 rivR   CGGTAGATTCGCTACAATATATAGGATTATACAATTTAATTTAAGGAGAGAAAAATTACATGGTTAGATTATTATTGGA
*****

M3 rivR   ATCCGATATTATTGACCAGAAAATCCTACTGGCTATTTTGTGTGACAGAAAAAGAACTGACGATGGCAGCAGCTTGTTCCTC
M1 rivR   ATCCGATATTATTGACCAGAAAATCCTACTGGCTATTTTGTGTGACAGAAAAAGAACTGACGATGGCAGCAGCTTGTTCCTC
*****

M3 rivR   AAACAACCTTTGACTGCTTTAAAAATAAAGCAGTATCTTCGACAAATTTAACGCCTTATTTAAAGGGTACTTACGTATTGAG
M1 rivR   AAACAACCTTTGACTGCTTTAAAAATAAAGCAGTATCTTCGACAAATTTAACGCCTTATTTAAAGGGTACTTACGTATTGAG
*****

M3 rivR   CTTATTAATCTCGTATTATTGTGAGGTATTAATGATAAGCGAGAAGCATTTTT-AAGACATTTTTCGCTTATCAGA
M1 rivR   CTTATTAATCTCGTATTATTGTGAGGTATTAATGATAAGCGAGAAGCATTTTTATGACATTTTTCGCTTATCAGA
*****
1bp deletion

M3 rivR   TACTCTAAAAATGTTGACATCTTATTACTAGATAATCCCAAACACAAATCAATCGCTGTTTATACACGAAAACAAGGGA
M1 rivR   TACTCTAAAAATGTTGACATCTTATTACTAGATAATCCCAAACACAAATCAATCGCTGTTTATACACGAAAACAAGGGA
*****

M3 rivR   TATCCAGTCAAAGCTTATCGTCTTATTCATAAATAAAACATTATTTACAAGACATTGGTTTAAATATTGTAGACAAT
M1 rivR   TATCCAGTCAAAGCTTATCGTCTTATTCATAAATAAAACATTATTTACAAGACATTGGTTTAAATATTGTAGACAAT
*****

M3 rivR   ACTGTTATCGGAGATGAACCTAAAATACGGTATTTGATGCTCTTTTACATAAAGAGTACGGCATTATATTATATGATAT
M1 rivR   ACTGTTATCGGAGATGAACCTAAAATACGGTATTTGATGCTCTTTTACATAAAGAGTACGGCATTATATTATATGATAT
*****

M3 rivR   ACAACCTGCAGACATTGAAACGATTCATGCTTTTATCTTTGCAGCACAAAAAACTTACAACCTTCTGCCTTTTAGATA
M1 rivR   ACAACCTGCAGACATTGAAACGATTCATGCTTTTATCTTTGCAGCACAAAAAACTTACAACCTTCTGCCTTTTAGATA
*****

M3 rivR   GACGCTTCTGTTTTTGTATGCTGTTGATGTTGACTTGAAGCGCCATCGCTATCTGTTTCAATTTGCCTCATTAGCT
M1 rivR   GACGCTTCTGTTTTTGTATGCTGTTGATGTTGACTTGAAGCGCCATCGCTATCTGTTTCAATTTGCCTCATTAGCT
*****

M3 rivR   CTTTTGAACATCTCAAATCCTTGCTATTTTGAACAATCAAACCATGCGGTGGATGACCCAGTACCTCGAACTCG
M1 rivR   CTTTTGAACATCTCAAATCCTTGCTATTTTGAACAATCAAACCATGCGGTGGATGACCTAGTACCTCGAACTCG
*****
SNP1

M3 rivR   TGTACATTTTCTGCTGATGATTTTACTATCTCTTTTAAATTTATTTAACGACTGATAACTCCTTCTGAGTGGGTACT
M1 rivR   TGTACATTTTCTGCTGATGATTTTACTATCTCTTTTAAATTTATTTAACGACTGATAACTCCTTCTGAGTGGGTACT
*****

M3 rivR   GGACAAGTCATCAGCGACAACAACCTTTACCATTAAATCACCAAGATCCCGATTATCATTTACTAATCCATCGTCTTCCAG
M1 rivR   GGACAAGTCATCAGCGACAACAACCTTTACCATTAAATCACCAAGATCCCGATTATCATTTACTAATCCATCGTCTTCCAG
*****

M3 rivR   GCAC TAGTGGAACTTTCGATATTCATGAGCATATCCCTAACTTAATTCCTTTTTTCAAAGAACTCTCTACAATTT
M1 rivR   GCAC TAGTGGAACTTTCGATATTCATGAGCATATCCCTAACTTAATTCCTTTTTTCAAAGAACTCTCTACAATTT
*****
SNP2

M3 rivR   ACAAACTTAATACCTTTGATGTTTATTTTGTATCAATATCAAGGCAACATGCTACTACTAGATAAGCTTGAACCG
M1 rivR   ACAAACTTAATACCTTTGATGTTTATTTTGTATCAATATCAAGGCAACATGCTACTACTAGATAAGCTTGAACCG
*****

M3 rivR   TTATCAAAGATTGGCTTTATGACACAGCCGTCAGGCGAGTATTAGTTCTGGTCACTTACACTTGTATGTTTATACCTT
M1 rivR   TTATCAAAGATTGGCTTTATGACACAGCCGTCAGGCGAGTATTAGTTCTGGTCACTTACACTTGTATGTTTATACCTT
*****

M3 rivR   GAGCAGATCTTGGAAAGCTCTATTGCTCCAATCAATATTACTGTTATTGAGAGCCAAGAACTGTTGTAACGTTATAGC
M1 rivR   GAGCAGATCTTGGAAAGCTCTATTGCTCCAATCAATATTACTGTTATTGAGAGCCAAGAACTGTTGTAACGTTATAGC
*****

M3 rivR   TAATTTTCATCACCTCAACTATCCCTTCTTACAAGTTGACTGTGTCACGGGTCATATCTTATCTGATAATATTATCCCT
M1 rivR   TAATTTTCATCACCTCAACTATCCCTTCTTACAAGTTAATCTGTGTCACGGGTCATATCTTATCTGATAATATTATCCCT
*****
SNP3

M3 rivR   ATGATAAACCTGTAGATCTGGTTTGTGACAGTGTGAGGCTCTTGCCTTTCTTAAAGAAGCTCGGTGTTTTTCCCAAAGAG
M1 rivR   ATGATAAACCTGTAGATCTGGTTTGTGACAGTGTGAGGCTCTTGCCTTTCTTAAAGAAGCTCGGTGTTTTTCCCAAAGAG
*****
SNP4

M3 rivR   ACCCGCTTGTGTTGAGTTTGGACTGTATCCAACAGCAGCGCGAAGATTTAATCAAACCATCTTAGCCTTGCACCA
M1 rivR   ACCCGCTTGTGTTGAGTTTGGACTGTATCCAACAGCAGCGCGAAGATTTAATCAAACCATCTTAGCCTTGCACCA
*****

M3 rivR   GAACCCTACCAAAAACGTTTAGAAGAAGTTGGAGAGTTCCTTCTTAA
M1 rivR   GAACCCTACCAAAAACGTTTAGAAGAAGTTGGAGAGTTCCTTCTTAA
*****

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Figure S1
Alignment of the *rivR* gene from the clinical strains MGAS2221 and MGAS10870. The *rivR* gene plus upstream 60 nucleotides were aligned between MGAS2221 (M1 *rivR*) and MGAS10870 (M3 *rivR*). Red asterisks highlight nucleotide identity while gaps and blue ovals highlight differences. The start codon (TTG) is colored green and the stop codon (TAA) is colored red.

	10	20	30	40	50	60
M1 RivR	MLDYYLESDIIDQKILLAILLTEKELTMAAACSQTTLTALKIKQYLRQFNALFKGYLRIE					
M3 RivR	MLDYYLESDIIDQKILLAILLTEKELTMAAACSQTTLTALKIKQYLRQFNALFKGYLRIE					

	70	80	90	100	110	120
M1 RivR	LIKSRIYCEVLNDKREAFFYDIFALSDTLKMLTFLLLDNPKHKSIAVYTRKQGISQSKAY					
M3 RivR	LIKSRIYCEVLNDKREAFFYDIFALSDTLKMLTFLLLDNPKHKSIAVYTRKQGISQSKAY					

	130	140	150	160	170	180
M1 RivR	RLIHKLKHYLQDIGLNIVDNTVIGDELKIRYLIALLHKEYGIILYDIQPADIETIHAFIF					
M3 RivR	RLIHKLKHYLQDIGLNIVDNTVIGDELKIRYLIALLHKEYGIILYDIQPADIETIHAFIF					

	190	200	210	220	230	240
M1 RivR	AAQKNLQPSAFLDRRFLFFDVLLMLTWKRHRYPVHLPALFEHLKSLPIFDNIKTIAVD					
M3 RivR	AAQKNLQPSAFLDRRFLFFDVLLMLTWKRHRYPVHLPALFEHLKSLPIFDNIKTIAVD					

	250	260	270	280	290	300
M1 RivR	ELAPRTRVTFSSDDFDYLFLLIYLTDDNSFLSGYWTSHQRQQLYHLITKDPDYHLLIHLRQ					
M3 RivR	EPAPRTRVTFSSDDFDYLFLLIYLTDDNSFLSGYWTSHQRQQLYHLITKDPDYHLLIHLRQ					

	310	320	330	340	350	360
M1 RivR	ALVGVKYSIDIEHIPNLIPIFFKRTLYNLQTLITFDGYYFDQYQGNMLLLDKLETVIKDWLY					
M3 RivR	ALVGNYSIDIEHIPNLIPIFFKRTLYNLQTLITFDGYYFDQYQGNMLLLDKLETVIKDWLY					

	370	380	390	400	410	420
M1 RivR	DTGRQGSISSGHLHLMCLYLEQILESSIAPINITVIESQETVGNVIANFITSTIPSYKVK					
M3 RivR	DTGRQGSISSGHLHLMCLYLEQILESSIAPINITVIESQETVGNVIANFITSTIPSYKVE					

	430	440	450	460	470	480
M1 RivR	LSRVNILSDNIYPYDKPVDLVVTSQKLLPFLKELGVFPKETRLFGLSLDCIQQREDLIK					
M3 RivR	LSRVNILSDNIYPYDKPVDLVVTSQKLLPFLKELGVFPKETRLFGLSLDCIQQREDLIK					

	490	500				
M1 RivR	TILALHQNHYQKRLEELWRVPS					
M3 RivR	TILALHQNHYQKRLEELWRVPS					

Figure S2

RivR amino acid alignment between strains MGAS2221 and 10870rivR^{+1bp}. Fixing the 1 bp deletion in the *rivR* gene of M3 GAS results in a protein with three amino acid substitutions relative to the RivR protein of MGAS2221 (highlighted with ovals). An asterisk highlights the location of the truncation, caused by the 1 bp deletion, in clinical M3 GAS *rivR* alleles.

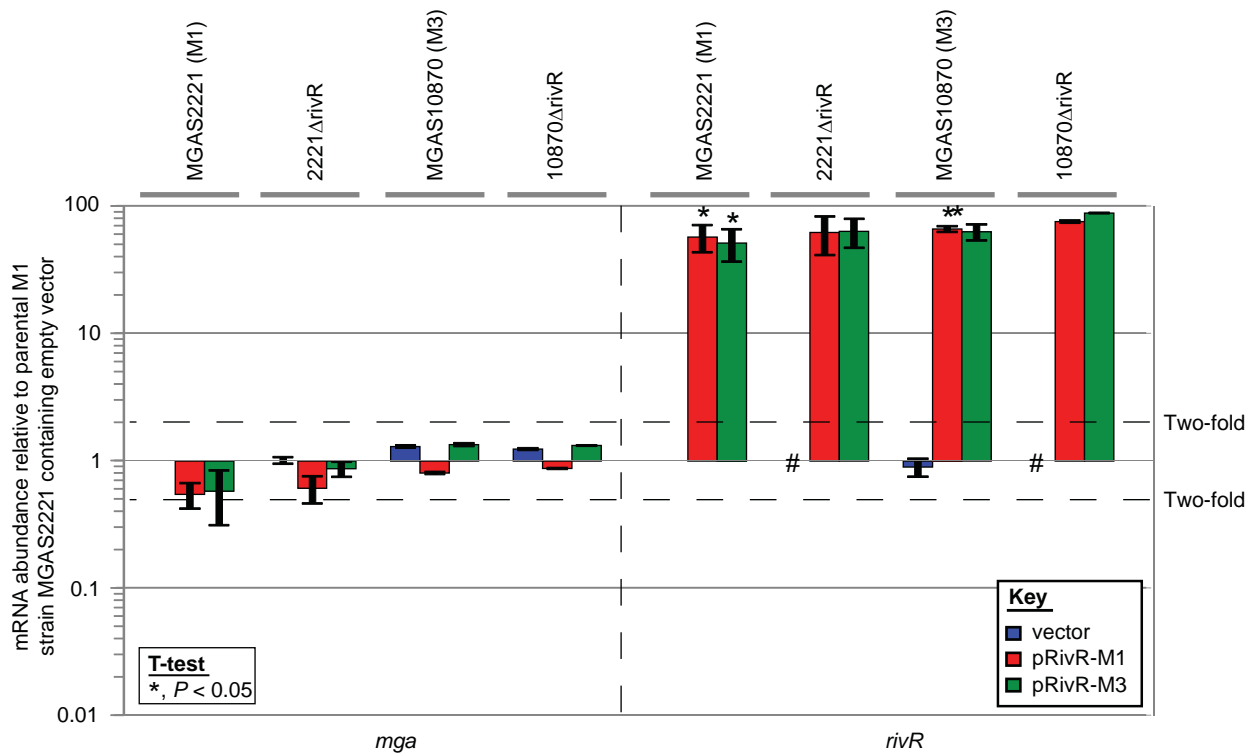


Figure S3

Analysis of *mga* and *rivR* mRNA levels between M1 and M3 GAS isolates containing different plasmid-encoded *rivR* alleles.

Parental (MGAS2221) and full *rivR* deletion mutant (2221Δ*rivR*) M1 isolates, and parental (MGAS10870) and full *rivR* deletion mutant (10870Δ*rivR*) M3 isolates, were compared following transformation with either empty vector, a plasmid containing *rivR* from M1 GAS strain MGAS2221 (pRivR-M1), or a plasmid containing *rivR* from M3 GAS strain MGAS10870 (pRivR-M3). RNA from exponential phase cultures of the indicated strains were analyzed for *mga* and *rivR* mRNA levels by quantitative RT-PCR. Experiment was performed in triplicate with mean (± standard deviation) shown. Hashes (#) highlight the lack of detectable *rivR* mRNA in the two *rivR* mutant strains containing empty vector. Asterisks (*) highlight those samples that were statistically significantly different, via T-test, relative to the respective empty vector-containing strain.

A

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      10      20      30      40      50      60      70      80      90     100
M1 grab    ATGATATCCTAACTAATATTATTAATTTTAATGCTAAAATAATGAGTTGACGGTAGTAAAGTGATAAATAAACACTCCTTAAAATAATTATTTTATTAAT
M3 grab    ATGATATCCTAACTAATATTATTAATTTTAATGCTAAAATAATGAGTTGACGGTAGTAAAGTGATAAATAAACACTCCTTAAAATAATTATTTTATTAAT
*****

      110     120     130     140     150     160     170     180     190     200
M1 grab    TTTTAAAAAATAATATATAAATTAGATAATAAACTTGATTTTATGCAATTAATCTGTATAATAAAAGATGGAATTATAATTATAATTCCAAATTAT
M3 grab    TTTTAAAAA-TAATATATAAATTAGATAATAAACTTGATTTTATGCAATTAATCTGTATAATAAAAGATGGAATTATAATTATAATTCCAAATTAT
*****

      210     220     230     240     250
M1 grab    CGGTAATTTAATATGCTAATGCATATAAAAATAAAAAGGAGAAACAATG
M3 grab    CGGTAATTTAATATGCTAATGCATATAAAAATAAAAAGGAGAAACAATG
*****

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B

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      10      20      30      40      50      60      70      80      90     100
M1 hasA    TTTTTCACAAAATTAGATAAACTAATTTGAAAAGTTTCTAAAATAATTTATAACAATTCAATTATTTTGAGATAAATTTATATTAATAAAATTATCCT
M3 hasA    TTTTTCATAAAATTAGATAAACTCTTGAAAAGTTTACTAAAATAATTTATAACAATTCAA-----TTATCCT
*****

      110     120     130     140     150     160     170     180     190     200
M1 hasA    GATTTTCTTTTTTGGGGGAAATTTTTTAATTAAATACATTTTTATTAATAAATATTTCTATGACTAGTTGACATTACCCCTTATTTATATTAGAATAT
M3 hasA    GATTTTCTTTTTCGGGGAAATTTTTTAATTGAAACACAATTTTATTAATAAATATCTCTATGCTAGTTGACATTATTTCTTATTTATATTAGAATAT
*****

      210     220     230     240     250
M1 hasA    CGAGGTCCTGTCTTTCAAGGAAATAAAAAGAAAGAGGTGTAATTGTG
M3 hasA    TGAGGCCCTTA-CITTCAGGAAATAAAAAGAAAGAGGTGTAATTGTG
*****

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Figure S4. Alignment of the nucleotides upstream of the *grab* and *hasA* mRNAs in strains MGAS2221 and MGAS10870. Nucleotides upstream of *grab* (A) or *hasA* (B) in the serotype M1 strain MGAS2221 and the serotype M3 strain MGAS10870 were aligned. Red nucleotides and asterisks highlight identity while black nucleotides highlight differences. The start codons of *grab* and *hasA* are shown in blue.

Primer name	Sequence (5' - 3')	Role
FASXNF	CTTATTACGACTCACTATAGGGAGCAAAACAAAGACAACACTGACATCG	Forward primer containing T7 promoter sequence that is used to generate template for <i>in vitro</i> transcription of a FasX-specific RNA probe
FASXNR	GAAGTCATGAGTTTATCGAG	Reverse primer that is used to generate template for <i>in vitro</i> transcription of a FasX-specific RNA probe
5SF	CTTAATACGACTCACTATAGGGAGCTAAGCGACTACCTTATCTC	Forward primer containing T7 promoter sequence that is used to generate template for <i>in vitro</i> transcription of a 5S RNA-specific RNA probe
5SR	GTTAAGTGACGATAGCCTAG	Reverse primer that is used to generate template for <i>in vitro</i> transcription of a 5S RNA-specific RNA probe
FASCA	CTTGCTTATCTGCTGATTTTCC	Used in construction of the <i>fasC</i> mutant strain 2221Δ <i>fasC</i> (left flank primer)
FASCB	GTTATAGTTATTAAACATGTAATGGTAGCATAGAATAAAGCCATAC	Used in construction of the <i>fasC</i> mutant strain 2221Δ <i>fasC</i> (left flank primer)
FASCC	CTATTAAATAACAGATTAAAAAATTAAAGCGCTTTCGACTAAAAGTGACC	Used in construction of the <i>fasC</i> mutant strain 2221Δ <i>fasC</i> (right flank primer)
FASCD	CCAAACAAGACAACACTGACATC	Used in construction of the <i>fasC</i> mutant strain 2221Δ <i>fasC</i> (right flank primer)
FASCSPECF	GTATGGCTTATTCTATGCTACCAATACATGTTATAATAACTATAAC	Used in construction of the <i>fasC</i> mutant strain 2221Δ <i>fasC</i> (spec cassette primer)
FASCSPECR	GGTCACTTTTAGTCGAAAGCGCTTATAATTTTTTAAATCTGTTATTTAAATAG	Used in construction of the <i>fasC</i> mutant strain 2221Δ <i>fasC</i> (spec cassette primer)
FASCE	TTATTAATATATGATTGGAAG	Used to verify the <i>fasC</i> mutant strain 2221Δ <i>fasC</i>
FASCF	GAGCCATCATATCAAAAATAC	Used to verify the <i>fasC</i> mutant strain 2221Δ <i>fasC</i>
M3RIVR1	caaaatttcacagctcagcttcGAGGTGATGAAATTAGCTATAACGTACC	Used with M3RIVR5 to generate the downstream flank used to fix the 1bp deletion in <i>rivR</i> that is present in serotype M3 isolates
M3RIVR2	GAGTATCTGATAAGGCAAAAATGTCAAAAATGCTTCTCGCTTATCATTAAATACCTC	Used with M3RIVR5 to generate the upstream flank used to fix the 1bp deletion in <i>rivR</i> that is present in serotype M3 isolates
M3RIVR3	GAGGTATTAAATGATAAGCGAGAAGCATTITTTATGACATTTTTGCGCTTATCAGATACTC	Used with M3RIVR1 to generate the downstream flank used to fix the 1bp deletion in <i>rivR</i> that is present in serotype M3 isolates
M3RIVR5	CTTGCAATGCTATAAAAAATTAAGCCTG	Used with M3RIVR2 to generate the upstream flank used to fix the 1bp deletion in <i>rivR</i> that is present in serotype M3 isolates
M3RIVR8	GGTTTATCATAGGGATAAATATTATCAG	Used to verify that that 1bp deletion in the <i>rivR</i> gene has been fixed in isolates 10870 <i>rivR</i> ^{+1bp} and 10870 <i>fasC</i> ^{Comp} <i>rivR</i> ^{+1bp}
M3RIVR9	GCTCTCGACGATTTGACCAAC	Used to verify that that 1bp deletion in the <i>rivR</i> gene has been fixed in isolates 10870 <i>rivR</i> ^{+1bp} and 10870 <i>fasC</i> ^{Comp} <i>rivR</i> ^{+1bp}
M3FIXA	gctaggatccTGCAGATAAATAAAAAGAGACTTAC	Used with M3FIXB to generate the upstream flank used to fix the <i>fasC</i> mutation that is present in serotype M3 isolates
M3FIXB	CACAATAACCCAAATAAAAAAGCTAAATAAATAAATACTAAAATAGACCGGTAC	Used with M3FIXA to generate the upstream flank used to fix the <i>fasC</i> mutation that is present in serotype M3 isolates
M3FIXC	GTACCGGTCTATTTAGTATTTATTTAGCTATTTTTATTGGGTTATTGTG	Used with M3FIXD to generate the downstream flank used to fix the <i>fasC</i> mutation that is present in serotype M3 isolates
M3FIXD	gctatctagaTTTGTGGAATAGCTGCAGCTAATTC	Used with M3FIXC to generate the downstream flank used to fix the <i>fasC</i> mutation that is present in serotype M3 isolates
M3FIXE	TATGCCCAAACGGTTTATGAAAGC	Used to verify that the <i>fasC</i> gene has been fixed in isolates 10870 <i>fasC</i> ^{Comp} , 315 <i>fasC</i> ^{Comp} , and 10870 <i>fasC</i> ^{Comp} <i>rivR</i> ^{+1bp}
M3FIXF	CGTAAGTATATCGGATACATTC	Used to verify that the <i>fasC</i> gene has been fixed in isolates 10870 <i>fasC</i> ^{Comp} , 315 <i>fasC</i> ^{Comp} , and 10870 <i>fasC</i> ^{Comp} <i>rivR</i> ^{+1bp}
RIV2	CAGAACATGGGAGCGCACTTTTtagagatgcttccatctacg	Used with RIVB4 to generate the upstream flank used to delete <i>rivR</i> from the genome of M3 isolate MGAS10870
RIV5	CAAAATTTTCATCAAGCTCTAGTTCCcagataaagtgggtgctgcttc	Used with RIV13 to generate the downstream flank used to delete <i>rivR</i> from the genome of M3 isolate MGAS10870
RIV6	GTAGAAGCTGACGCTAACACTG	Used to verify that the <i>rivR</i> gene has been deleted in strain 10870Δ <i>rivR</i>
RIV13	cgtagaatgaaacatcctctaaaaAAAGTGCCTCCCATGTTCTG	Used with RIV5 to generate the downstream flank used to delete <i>rivR</i> from the genome of M3 isolate MGAS10870
RIVB4	TAGTTGGAAACTACTACTCAGTAACG	Used with RIV2 to generate the upstream flank used to delete <i>rivR</i> from the genome of M3 isolate MGAS10870
RIVB6	TACCATAGCTCACTAAAGCCAACTTC	Used to verify that the <i>rivR</i> gene has been deleted in strain 10870Δ <i>rivR</i>
PROSTMP	TACCAATGCCGTTGCAGGAGCTAACAA	<i>proS</i> taqman probe
PROSTMF	GCTGACCGCAAAGTGCAAA	<i>proS</i> taqman primer
PROSTM	TGACTCTGTCTATGGAACCA	<i>proS</i> taqman primer
SKATMP	CGGCTACTTTGAGGTCAATTGATT	<i>ska</i> taqman primer
SKATMR	CCGAACCATCTTTGTCAGCAA	<i>ska</i> taqman primer
SKATMP	CAAGCGATGCAACCATTAAGTATGCAAAAC	<i>ska</i> taqman probe
GRABTMP	TGTTGACTCACCTATCGAACAGCCTCGA	<i>grab</i> taqman probe
GRABTMF	GCATCAGTATTAGTCGGTTTCAAGT	<i>grab</i> taqman primer
GRABTMR	GGTCCGCCATTTGGAATAA	<i>grab</i> taqman primer
HASATMP	ACGCACTGTCTACCAATCAACAGCTAGATGTG	<i>hasA</i> taqman probe
HASATMF	ATGATCGATGTTTAAACAAATTATGCTATTG	<i>hasA</i> taqman primer
HASATMR	TTAAAATAACTTTTAAATTTGGAAAGGTACATCAG	<i>hasA</i> taqman primer
MGATMP	ACGTAAACAGAAAACGCTGACGCCATTG	<i>mga</i> taqman probe
MGATMF	CAAGTCAACAGTGGAGAGAACTAAATTT	<i>mga</i> taqman primer
MGATMR	ATGGAGATGTTGAGAGCTTTGCT	<i>mga</i> taqman primer
RIVRTMP	ACTGTATCCAACAGCAGCGGAAGATT	<i>rivR</i> taqman probe
RIVRTMF	CCCGCTTGTTTGGTTTGAGT	<i>rivR</i> taqman primer
RIVRTMR	GGTGCAAGGCTAAGATGTTTT	<i>rivR</i> taqman primer
RIVRCP1	gctaggatccAACAGCAAAAATATGATTTTC	Used with RIVRCP2 to clone the <i>rivR</i> gene from different GAS strains and clone them into vector pDC123
RIVRCP2	gctaatgcatAAAGACCCTCCTAGACTGTG	Used with RIVRCP1 to clone the <i>rivR</i> gene from different GAS strains and clone them into vector pDC123
RIVRSEOF	GATCAAAAATAAACCATCAAAGG	Used to amplify and sequence an internal region of the <i>rivR</i> gene to determine whether the allele contains the 1 bp deletion
RIVRSEQR	CTTGTCTCAACCACTTTGAC	Used to amplify and sequence an internal region of the <i>rivR</i> gene to determine whether the allele contains the 1 bp deletion
RIV7	GTCAAAAAGACTTCTCTTGTAC	Used to amplify the whole <i>rivR</i> gene prior to sequencing
C2RIV4	GTTTAAACCTGACCAAGTAAACC	Used to amplify the whole <i>rivR</i> gene prior to sequencing
M3RIVR8	GGTTTATCATAGGGATAAATATTATCAG	Used to sequence the <i>rivR</i> gene
C2D	TAAAAGGCGAAGGTTGTAAG	Used to sequence the <i>rivR</i> gene
C2RIV5	GACAATACTGTTATCGGAGATG	Used to sequence the <i>rivR</i> gene
FASC87	CAGCTGCTTAGGATTAGCATTGGTC	Used to amplify and sequence an internal region of the <i>fasC</i> gene to determine whether the allele contains the 4 bp deletion
FASC88	GTTGCCGATTGCTGAATAAC	Used to amplify and sequence an internal region of the <i>fasC</i> gene to determine whether the allele contains the 4 bp deletion

Table S1
Table of primers and probes used in this study.

Strain	Serotype	Isolation year	Isolation location	<i>fasC</i> allele	<i>rivR</i> allele
MGAS1264	M1	1973	Denmark	Wild-type	Wild-type
SF370	M1	1985	Unknown	Wild-type	Wild-type
MGAS1508	M1	1985	Czechoslovakia	Wild-type	Wild-type
MGAS2221	M1	1988	Australia	Wild-type	Wild-type
MGAS313	M1	Late 1980s	Wyoming, USA	Wild-type	Wild-type
MGAS3350	M1	1995	Minnesota, USA	Wild-type	Wild-type
MGAS5005	M1	1996	Ontario, Canada	Wild-type	Wild-type
MGAS5804	M1	1997	Finland	Wild-type	Wild-type
MGAS22259	M1	2010	Alberta, Canada	Wild-type	Wild-type
MGAS1251	M3	1920s	UK	4bp deletion	SNPs
MGAS1254	M3	1937	New York, USA	4bp deletion	1bp deletion and SNPs
MGAS182	M3	1940s	Ottawa, Canada	4bp deletion	1bp deletion and SNPs
MGAS174	M3	1940s	Ottawa, Canada	4bp deletion	1bp deletion and SNPs
MGAS1372	M3	1969	Berlin, Germany	4bp deletion	1bp deletion and SNPs
MGAS1385	M3	1971	Madgeburg, Germany	4bp deletion	1bp deletion and SNPs
MGAS1392	M3	1972	Leipzig, Germany	4bp deletion	1bp deletion and SNPs
MGAS1404	M3	1973	Madgeburg, Germany	4bp deletion	1bp deletion and SNPs
MGAS1428	M3	1974	Cottbus, Germany	4bp deletion	1bp deletion and SNPs
MGAS1308	M3	1981	Schwerin, Germany	4bp deletion	1bp deletion and SNPs
MGAS1319	M3	1982	Chemnitz, Germany	4bp deletion	1bp deletion and SNPs
MGAS1344	M3	1983	Rostock, Germany	4bp deletion	1bp deletion and SNPs
MGAS1519	M3	1987	Moscow, Russia	4bp deletion	1bp deletion and SNPs
MGAS315	M3	1980s	Texas, USA	4bp deletion	1bp deletion and SNPs
MGAS157	M3	Late 1980s	Minnesota, USA	4bp deletion	1bp deletion and SNPs
MGAS159	M3	Late 1980s	Utah, USA	4bp deletion	1bp deletion and SNPs
MGAS1610	M3	Late 1980s	USA	4bp deletion	1bp deletion and SNPs
MGAS1638	M3	Late 1980s	USA	4bp deletion	1bp deletion and SNPs
MGAS491	M3	Pre-1991	UK	4bp deletion	1bp deletion and SNPs
MGAS3370	M3	1992	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3664	M3	1992	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10051	M3	1993	Ontario, Canada	4bp deletion	1bp deletion and SNPs
SSI-1	M3	1994	Japan	4bp deletion	1bp deletion and SNPs
MGAS3374	M3	1994	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3376	M3	1994	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3378	M3	1994	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3382	M3	1994	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10002	M3	1994	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10003	M3	1994	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10006	M3	1994	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10738	M3	1994	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3318	M3	1995	Minnesota, USA	4bp deletion	1bp deletion and SNPs
MGAS3385	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3392	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3394	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3397	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3452	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS3475	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10021	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10024	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10025	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10029	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10742	M3	1995	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10041	M3	1996	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS10044	M3	1996	Ontario, Canada	4bp deletion	1bp deletion and SNPs

MGAS15009	M3	2004	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15013	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15017	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15021	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15025	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15027	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15028	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15029	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15034	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15035	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15040	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15043	M3	2005	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15045	M3	2006	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15049	M3	2006	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15051	M3	2006	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15053	M3	2006	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15057	M3	2006	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15058	M3	2006	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15061	M3	2006	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15184	M3	2006	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15188	M3	2007	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15192	M3	2007	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15196	M3	2007	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS15200	M3	2007	Ontario, Canada	4bp deletion	1bp deletion and SNPs
MGAS22283	M3	2010	Alberta, Canada	4bp deletion	1bp deletion and SNPs
MGAS22440	M3	2010	Alberta, Canada	4bp deletion	1bp deletion and SNPs

Table S2
Clinical GAS isolates used in this study.