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Streptococcus pyogenes emm Type 3.93 Emergence, the Netherlands and England

Appendix

Appendix Table 1.

| Sample ID | Country | Colonization type | <i>Emm</i> type | Collection year | Collection date period* | Source | BioProject | Sample accession |
|-----------|-------------|-------------------|-----------------|-----------------|-------------------------|---------------|--------------|------------------|
| 1138712 | Netherlands | Invasive | 3.93 | 2017 | 3 | Blood | PRJNA1125189 | SAMN41885169 |
| 4612210 | Netherlands | Invasive | 3.93 | 2017 | 3 | Blood | PRJNA1125189 | SAMN41885170 |
| 9877795 | Netherlands | Invasive | 3.93 | 2017 | 3 | CSF | PRJNA1125189 | SAMN41885171 |
| 4117681 | Netherlands | Invasive | 3.93 | 2019 | 4 | Blood | PRJNA1125189 | SAMN41885172 |
| 1221089 | Netherlands | Invasive | 3.93 | 2023 | 7 | Blood | PRJNA1125189 | SAMN41885173 |
| 6101976 | Netherlands | Invasive | 3.93 | 2024 | 7 | CSF | PRJNA1125189 | SAMN41885174 |
| 1157077 | Netherlands | Invasive | 3.93 | 2024 | 7 | Sputum | PRJNA1125189 | SAMN41885175 |
| 1111116 | Netherlands | Invasive | 3.93 | 2024 | 7 | CSF | PRJNA1125189 | SAMN41885176 |
| 1001106 | Netherlands | Carriage | 3.93 | 2017 | 3 | Nose | PRJNA1125189 | SAMN41885177 |
| 4610265 | Netherlands | Carriage | 3.93 | 2017 | 3 | Nose | PRJNA1125189 | SAMN41885178 |
| 1211118 | Netherlands | Invasive | 3.93 | 2016 | 3 | Blood | PRJNA1125189 | SAMN41885179 |
| 511605 | England | Invasive | 3.93 | 2017 | 3 | Blood | PRJNA1125189 | SAMN41885180 |
| 511606 | England | Invasive | 3.93 | 2017 | 3 | Blood | PRJNA1125189 | SAMN41885181 |
| 511608 | England | Invasive | 3.93 | 2018 | 4 | Blood | PRJNA1125189 | SAMN41885182 |
| 511614 | England | Invasive | 3.93 | 2018 | 4 | Blood | PRJNA1125189 | SAMN41885183 |
| 511619 | England | Invasive | 3.93 | 2018 | 4 | Blood | PRJNA1125189 | SAMN41885184 |
| 537090 | England | Invasive | 3.93 | 2018 | 4 | Blood | PRJNA1125189 | SAMN41885185 |
| 705173 | England | Invasive | 3.93 | 2018 | 4 | Tissue | PRJNA1125189 | SAMN41885186 |
| 1110171 | England | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1125189 | SAMN41885187 |
| 1112763 | England | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1125189 | SAMN41885188 |
| 1112829 | England | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1125189 | SAMN41885189 |
| 1154616 | England | Invasive | 3.93 | 2023 | 6 | Tissue | PRJNA1125189 | SAMN41885190 |
| 1154654 | England | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1125189 | SAMN41885191 |
| 1154693 | England | Invasive | 3.93 | 2023 | 6 | Tissue | PRJNA1125189 | SAMN41885192 |
| 1164292 | England | Invasive | 3.93 | 2023 | 6 | Abscess | PRJNA1125189 | SAMN41885193 |
| 1188675 | England | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1125189 | SAMN41885194 |
| 1193701 | England | Invasive | 3.93 | 2023 | 6 | Pus | PRJNA1125189 | SAMN41885195 |
| 1193716 | England | Non-invasive | 3.93 | 2023 | 6 | Wound swab | PRJNA1125189 | SAMN41885196 |
| 1200347 | England | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1125189 | SAMN41885197 |
| 1206953 | England | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1125189 | SAMN41885198 |
| 1219862 | England | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1125189 | SAMN41885199 |
| 1314957 | England | Invasive | 3.93 | 2023 | 7 | Blood | PRJNA1125189 | SAMN41885200 |
| 1329712 | England | Invasive | 3.93 | 2023 | 7 | Blood | PRJNA1125189 | SAMN41885201 |
| 1339736 | England | Invasive | 3.93 | 2023 | 7 | Blood | PRJNA1125189 | SAMN41885202 |
| 1339738 | England | Invasive | 3.93 | 2023 | 7 | Blood | PRJNA1125189 | SAMN41885203 |
| 1355993 | England | Invasive | 3.93 | 2023 | 7 | Blood | PRJNA1125189 | SAMN41885204 |
| 1355999 | England | Non-invasive | 3.93 | 2023 | 7 | Throat swab | PRJNA1125189 | SAMN41885205 |
| 1356031 | England | Invasive | 3.93 | 2023 | 7 | Blood | PRJNA1125189 | SAMN41885206 |
| 1377859 | England | Invasive | 3.93 | 2023 | 7 | Blood | PRJNA1125189 | SAMN41885207 |
| 1377860 | England | Invasive | 3.93 | 2023 | 7 | Blood | PRJNA1125189 | SAMN41885208 |
| 1380021 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885209 |
| 1380060 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885210 |
| 1380103 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885211 |
| 1389677 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885212 |
| 1389695 | England | Invasive | 3.93 | 2024 | 7 | CSF | PRJNA1125189 | SAMN41885213 |
| 1389698 | England | Invasive | 3.93 | 2024 | 7 | Pleural fluid | PRJNA1125189 | SAMN41885214 |

| Sample ID | Country | Colonization type | Emm type | Collection year | Collection date period* | Source | BioProject | Sample accession |
|--------------|---------|-------------------|----------|-----------------|-------------------------|------------------|--------------|------------------|
| 1389710 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885215 |
| 1389712 | England | Non-invasive | 3.93 | 2024 | 7 | Ear swab | PRJNA1125189 | SAMN41885216 |
| 1389743 | England | Invasive | 3.93 | 2024 | 7 | Tissue | PRJNA1125189 | SAMN41885217 |
| 1397800 | England | Non-invasive | 3.93 | 2024 | 7 | Vaginal swab | PRJNA1125189 | SAMN41885218 |
| 1397823 | England | Non-invasive | 3.93 | 2024 | 7 | Wound swab | PRJNA1125189 | SAMN41885219 |
| 1397858 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885220 |
| 1397882 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885221 |
| 1413895 | England | Non-invasive | 3.93 | 2024 | 7 | Wound swab | PRJNA1125189 | SAMN41885222 |
| 1414070 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885223 |
| 1417878 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885224 |
| 1417895 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885225 |
| 1420627 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885226 |
| 1420644 | England | Invasive | 3.93 | 2024 | 7 | Pleural fluid | PRJNA1125189 | SAMN41885227 |
| 1420649 | England | Non-invasive | 3.93 | 2024 | 7 | Skin swab | PRJNA1125189 | SAMN41885228 |
| 1426265 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885229 |
| 1426268 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885230 |
| 1426286 | England | Non-invasive | 3.93 | 2024 | 7 | Wound swab | PRJNA1125189 | SAMN41885231 |
| 1426295 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885232 |
| 1426298 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885233 |
| 1426315 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885234 |
| 1427257 | England | Invasive | 3.93 | 2024 | 7 | Aspirate | PRJNA1125189 | SAMN41885235 |
| 1433694 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885236 |
| 1441513 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885237 |
| 1441515 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885238 |
| 1441543 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885239 |
| 1441558 | England | Invasive | 3.93 | 2024 | 7 | Blood | PRJNA1125189 | SAMN41885240 |
| 1441578 | England | Invasive | 3.93 | 2024 | 7 | Post mortem swab | PRJNA1125189 | SAMN41885241 |
| 1822122 | England | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1125189 | SAMN41885242 |
| 1904308 | England | Invasive | 3.93 | 2018 | 4 | Pleural fluid | PRJNA1125189 | SAMN41885243 |
| C_CC09_C_007 | England | Non-invasive | 3.93 | 2019 | 4 | Cough plate | PRJEB43915 | SAMEA8426085 |
| C_CC09_H_010 | England | Non-invasive | 3.93 | 2019 | 4 | Hand swab | PRJEB43915 | SAMEA8426086 |
| C_CC09_T_007 | England | Non-invasive | 3.93 | 2019 | 4 | Throat swab | PRJEB43915 | SAMEA8426087 |
| C_CC38_T_010 | England | Non-invasive | 3.93 | 2019 | 4 | Throat swab | PRJEB43915 | SAMEA8426108 |
| C_CC42_T_010 | England | Non-invasive | 3.93 | 2019 | 4 | Throat swab | PRJEB43915 | SAMEA8426116 |
| C_E1_S_010 | England | Non-invasive | 3.93 | 2019 | 4 | Settle plate | PRJEB43915 | SAMEA8426124 |
| C_E3_S_001 | England | Non-invasive | 3.93 | 2019 | 4 | Settle plate | PRJEB43915 | SAMEA8426125 |
| C_E4_S_007 | England | Non-invasive | 3.93 | 2019 | 4 | Settle plate | PRJEB43915 | SAMEA8426126 |
| C_E4_S_010 | England | Non-invasive | 3.93 | 2019 | 4 | Settle plate | PRJEB43915 | SAMEA8426127 |
| C_H2_T_010 | England | Non-invasive | 3.93 | 2019 | 4 | Throat swab | PRJEB43915 | SAMEA8426128 |
| ERR1359363 | England | Non-invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3930736 |
| ERR1359409 | England | Invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3930992 |
| ERR1359410 | England | Invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3931030 |
| ERR1359485 | England | Non-invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3930732 |
| ERR1359601 | England | Non-invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3930878 |

| Sample ID | Country | Colonization type | <i>Emm</i> type | Collection year | Collection date period* | Source | BioProject | Sample accession |
|------------|-------------|-------------------|-----------------|-----------------|-------------------------|--------|--------------|------------------|
| ERR1359650 | England | Invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3931105 |
| ERR1359731 | England | Non-invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3930761 |
| ERR1359759 | England | Non-invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3930584 |
| ERR1359763 | England | Non-invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3930723 |
| ERR1359862 | England | Non-invasive | 3.93 | 2014 | 2 | NA | PRJEB13551 | SAMEA3930781 |
| 23GA0015 | New Zealand | Non-invasive | 3.93 | 2023 | 6 | Throat | PRJNA1100230 | SAMN41032505 |
| 23GA0506 | New Zealand | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1100230 | SAMN41032978 |
| 23GA0008 | New Zealand | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1100230 | SAMN41032498 |
| 23GA0256 | New Zealand | Non-invasive | 3.93 | 2023 | 6 | Throat | PRJNA1100230 | SAMN41032739 |
| 23GA0600 | New Zealand | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1100230 | SAMN41033069 |
| 23GA0688 | New Zealand | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1100230 | SAMN41033151 |
| 23GA0435 | New Zealand | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1100230 | SAMN41032912 |
| 23GA0458 | New Zealand | Invasive | 3.93 | 2023 | 6 | Blood | PRJNA1100230 | SAMN41032934 |
| 23GA0432 | New Zealand | Non-invasive | 3.93 | 2023 | 6 | Tissue | PRJNA1100230 | SAMN41032909 |

*1 = 2009–2011; 2 = 2012–2014; 3 = 2015–2017; 4 = 2018–2019; 5 = 2020–2021; 6 = 2022–Oct 2023; 7 = Nov 2023–2024

Appendix Table 2. Lineage-specific single nucleotide polymorphisms (SNPs). Clade-specific SNP annotations against reference strain (Ref) MGAS315 (NC_004070.1), determined with Snippy.

| Clade | MGAS315 Location | Locus tag | Gene | Product | S/NS | Amino acid change | Ref | SNP/indel |
|-----------------------|------------------|---------------|-------------|---|------------|-------------------|-----|-----------|
| Clade 1 (26 isolates) | 298891 | SPYM3_RS01565 | <i>yidC</i> | membrane protein insertase YidC | NS | Arg305Gln | C | T |
| | 347532 | Intergenic | NA | NA | Intergenic | NA | CA | C |
| | 401009 | SPYM3_RS02125 | <i>ccpA</i> | catabolite control protein A | NS | Ala167Asp | C | A |
| | 433606 | SPYM3_RS10295 | NA | DUF2326 domain-containing protein | Frameshift | Lys158fs | AT | A |
| | 529296 | Intergenic | NA | NA | Intergenic | NA | A | G |
| | 591410 | Intergenic | NA | NA | Intergenic | NA | AG | A |
| | 700812 | SPYM3_RS03535 | <i>recJ</i> | single-stranded-DNA-specific exonuclease RecJ | NS | Met315Val | A | G |
| | 799621 | SPYM3_RS04090 | NA | response regulator transcription factor | NS | Gly82Ser | G | A |
| | 810674 | SPYM3_RS04135 | <i>pbp3</i> | D-alanyl-D-alanine carboxypeptidase PBP3 | NS | Thr265Ser | C | G |
| | 947767 | SPYM3_RS04805 | NA | restriction endonuclease subunit S | S | Gly48Gly | G | C |
| | 1068134 | SPYM3_RS05460 | NA | methionyl aminopeptidase | S | Leu255Leu | T | C |
| | 1217111 | Intergenic | NA | NA | Intergenic | NA | T | C |
| | 1434583 | SPYM3_RS07490 | NA | PBSX family phage terminase large subunit | NS | Phe266Tyr | A | T |
| | 1581156 | SPYM3_RS08200 | NA | magnesium transporter CorA family protein | S | Leu262Leu | A | G |
| | 1609622 | SPYM3_RS08345 | NA | MerR family transcriptional regulator | S | Asp116Asp | G | A |
| Clade 2 (11 isolates) | 184143 | SPYM3_RS01030 | <i>speG</i> | streptococcal pyrogenic exotoxin SpeG | S | Ser205Ser | T | C |
| | 642839 | SPYM3_RS03245 | <i>ideS</i> | immunoglobulin G-degrading enzyme IdeS | NS | Glu66Lys | C | T |
| | 833981 | Intergenic | NA | NA | Intergenic | NA | T | TA |
| | 1147260 | SPYM3_RS05820 | NA | hypothetical protein | NS | Thr59Ile | G | A |
| | 1524461 | SPYM3_RS07970 | <i>grpE</i> | nucleotide exchange factor GrpE | NS | Leu103Ile | G | T |
| | 1740613† | SPYM3_RS08960 | NA | pneumococcal-type histidine triad protein | NS | Ala172Val | G | A |
| | 1763604 | SPYM3_RS09050 | <i>ropB</i> | quorum-sensing system transcriptional regulator RopB/Rgg1 | NS | Asp24His | G | C |
| Clade 3 (14 isolates) | 341961 | SPYM3_RS01800 | <i>metG</i> | methionine-tRNA ligase | S | Pro229Pro | A | C |
| | 370021‡ | SPYM3_RS01945 | <i>msrA</i> | peptide-methionine (S)-S-oxide reductase MsrA | NS | Ala158Thr | G | A |

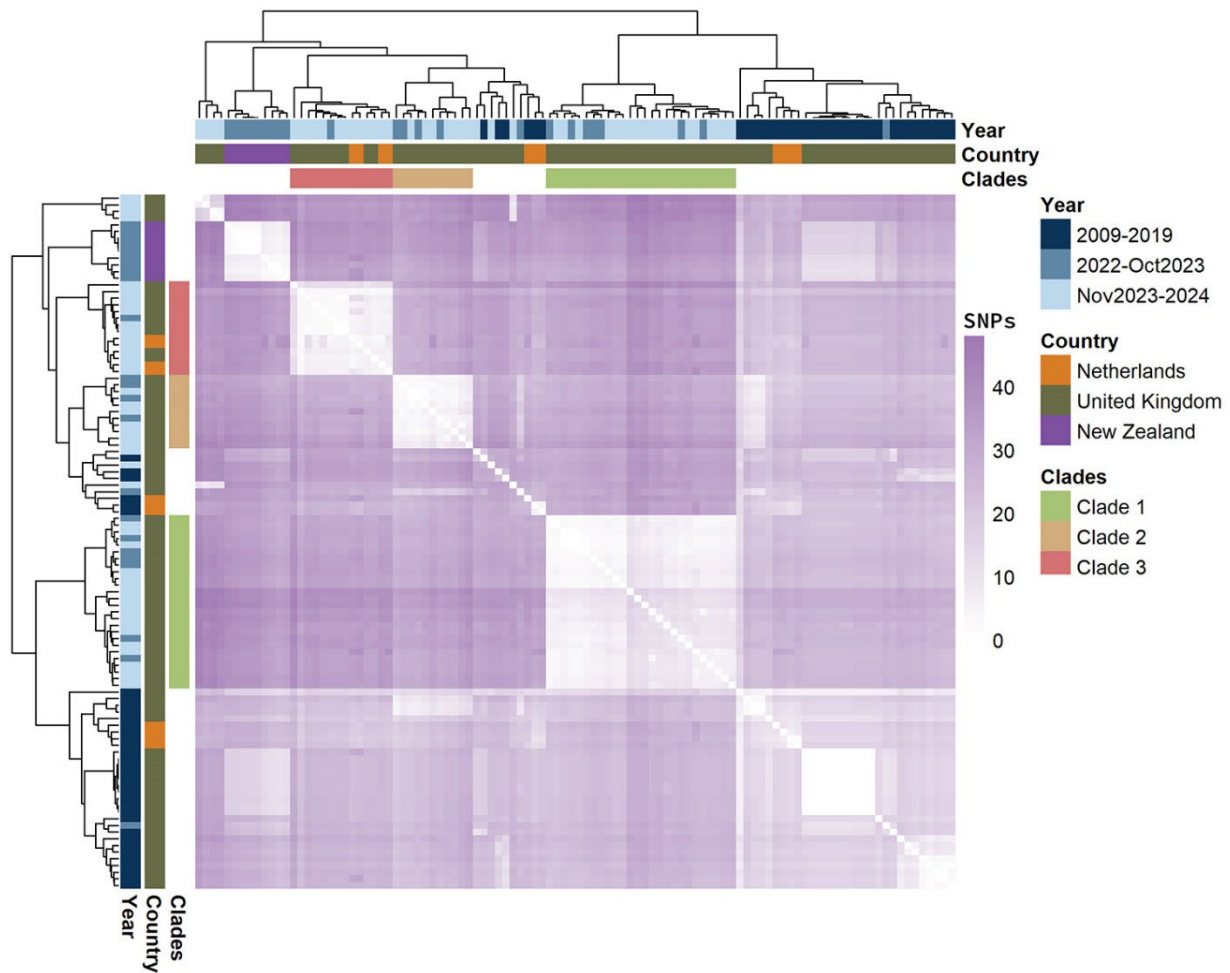
| Clade | MGAS315 Location | Locus tag | Gene | Product | S/NS | Amino acid change | Ref | SNP/indel |
|-------|------------------|---------------|------|---|------|--------------------|----------|-----------|
| | 427400 | SPYM3_RS02235 | NA | serine kinase | NS | Asp145Gly | A | G |
| | 502485 | SPYM3_RS02620 | NA | (S)-acetoin forming diacetyl reductase | S | Tyr150Tyr | C | T |
| | 629815 | SPYM3_RS03180 | NA | cation diffusion facilitator family transporter | NS | Pro200Ser | G | A |
| | 987708 | SPYM3_RS05000 | NA | phage tail spike protein | S | Arg79Arg | C | T |
| | 987714 | SPYM3_RS05000 | NA | phage tail spike protein | S | delAGTGinsGG TA | CAC T | TACC |
| | 1270371 | SPYM3_RS06610 | NA | hypothetical protein | S | Asp117Asp | C | T |
| | 1281283 | SPYM3_RS06655 | NA | shikimate kinase | NS | Asn171Lys | G | C |
| | 1300051 | SPYM3_RS06745 | NA | ROK family protein | S | Asn105Asn | T | C |
| | 1802116 | SPYM3_RS09230 | NA | APC family permease | NS | Thr122Ile | C | T |

*Also present in the 2 most related isolates to the clade

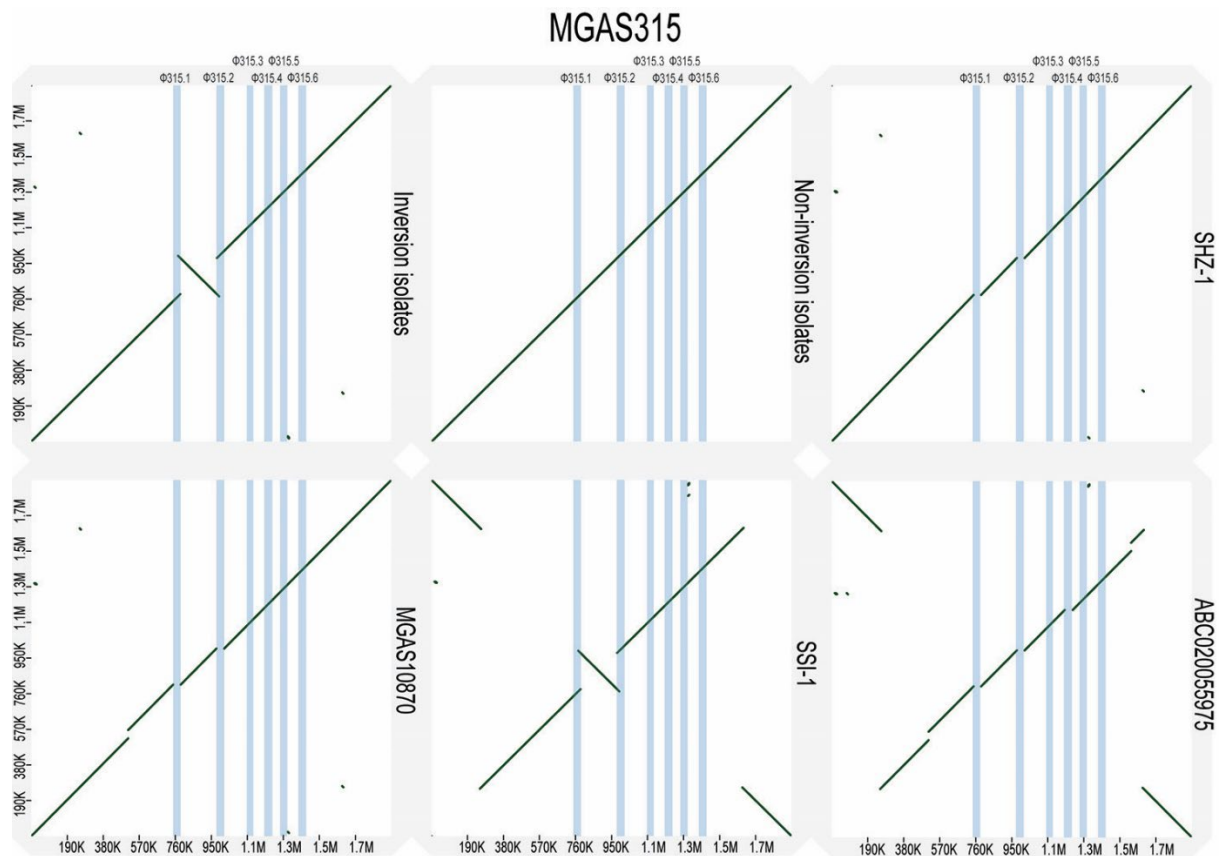
†Missing from 1 isolate in the clade

‡Missing from 2 isolates in the clade

S refers to synonymous and NS refers to non-synonymous mutations



Appendix Figure 1. Core-SNP distance matrix of the core SNP alignment of 104 *emm3.93* genomes from the Netherlands, United Kingdom and New Zealand between 2009–2024. Color in the heatmap indicates the number of SNPs between isolates, with highly similar isolates in white and shifting toward purple as number of SNPs increases. Year, country, and *emm3.93* clades are visualized by both dendrograms, which have been clustered with default hierarchical clustering from R package pheatmap.



Appendix Figure 2. Dotplots representing pairwise genome alignment of MGAS315 reference genome (NC_004070; x-axes) against either inversion isolates, non-inversion isolates or four publicly available *emm3.1* complete genomes (y-axes). An upwards diagonal black line represents region of shared sequence in the same orientation, whereas a downwards diagonal black line represents region of shared sequence in the opposite orientation. Six known MGAS315 prophages (Φ 315.1, Φ 315.2, Φ 315.3, Φ 315.4, Φ 315.5, Φ 315.6) are visualized as blue vertical lines. White gaps represent no sequence similar shared between the genomes. SSI-1 genomic configuration is similar to NIH34, M3-b and STAB902 and therefore not displayed. Plots were generated by D-GENIES.