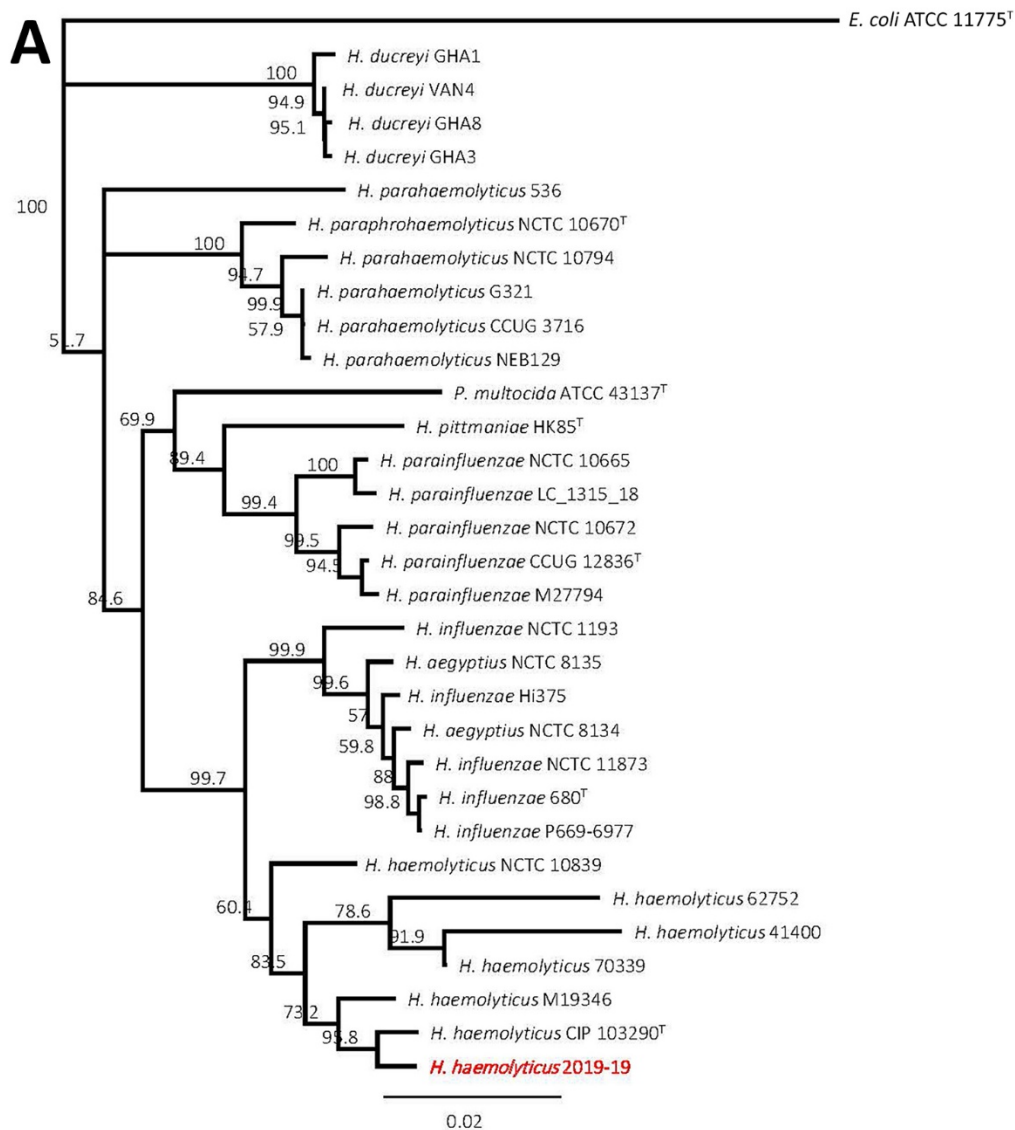
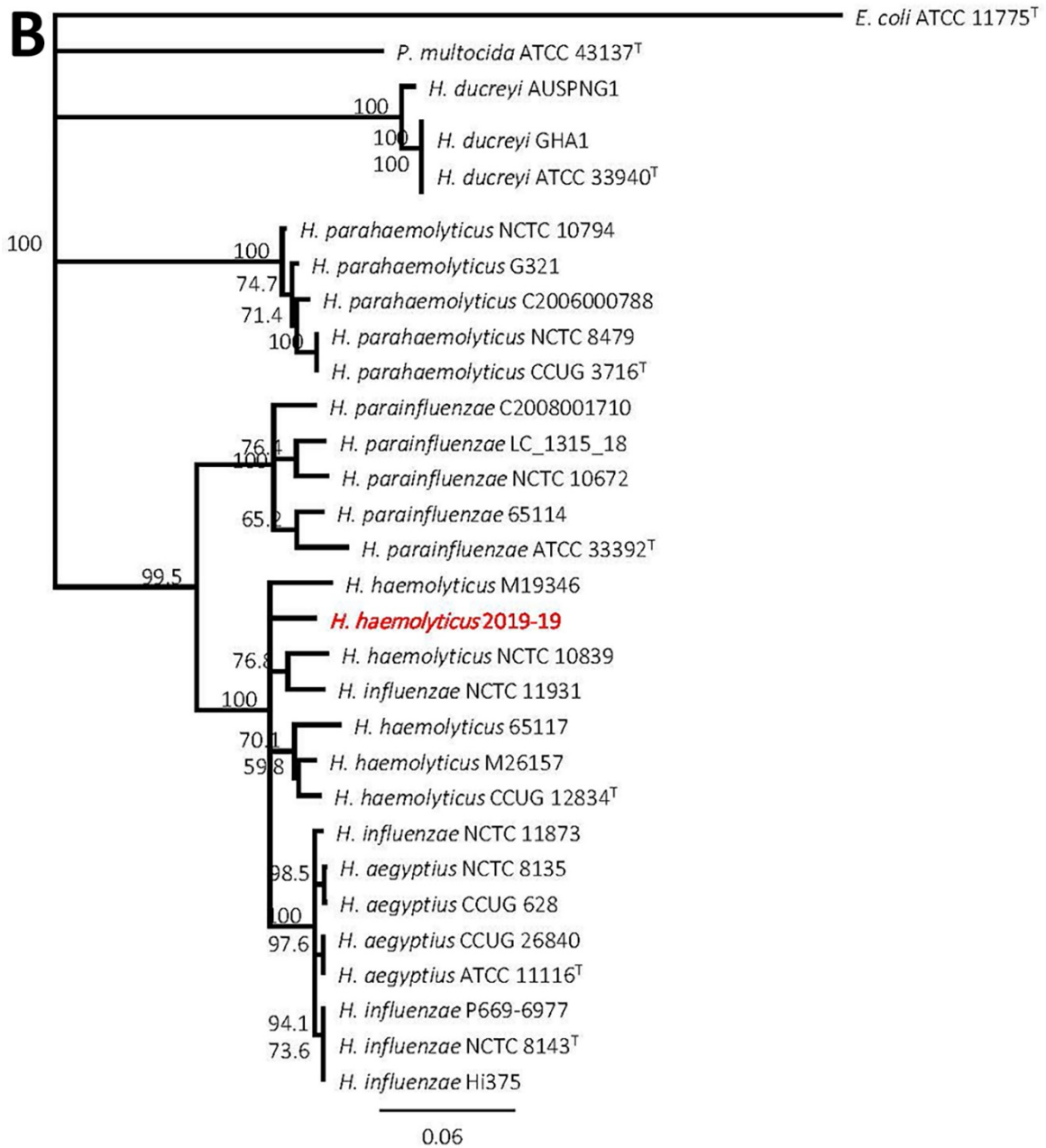
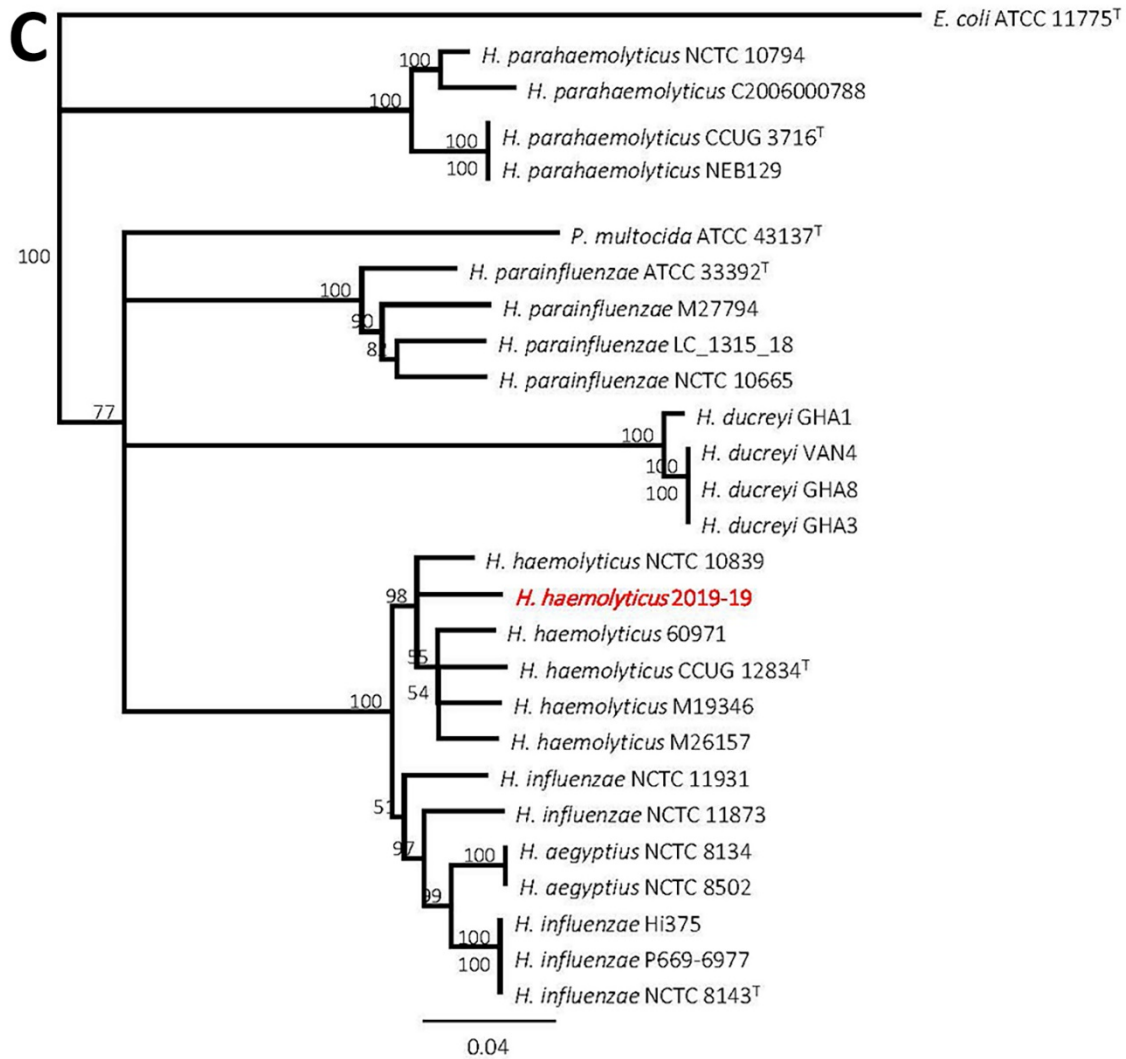


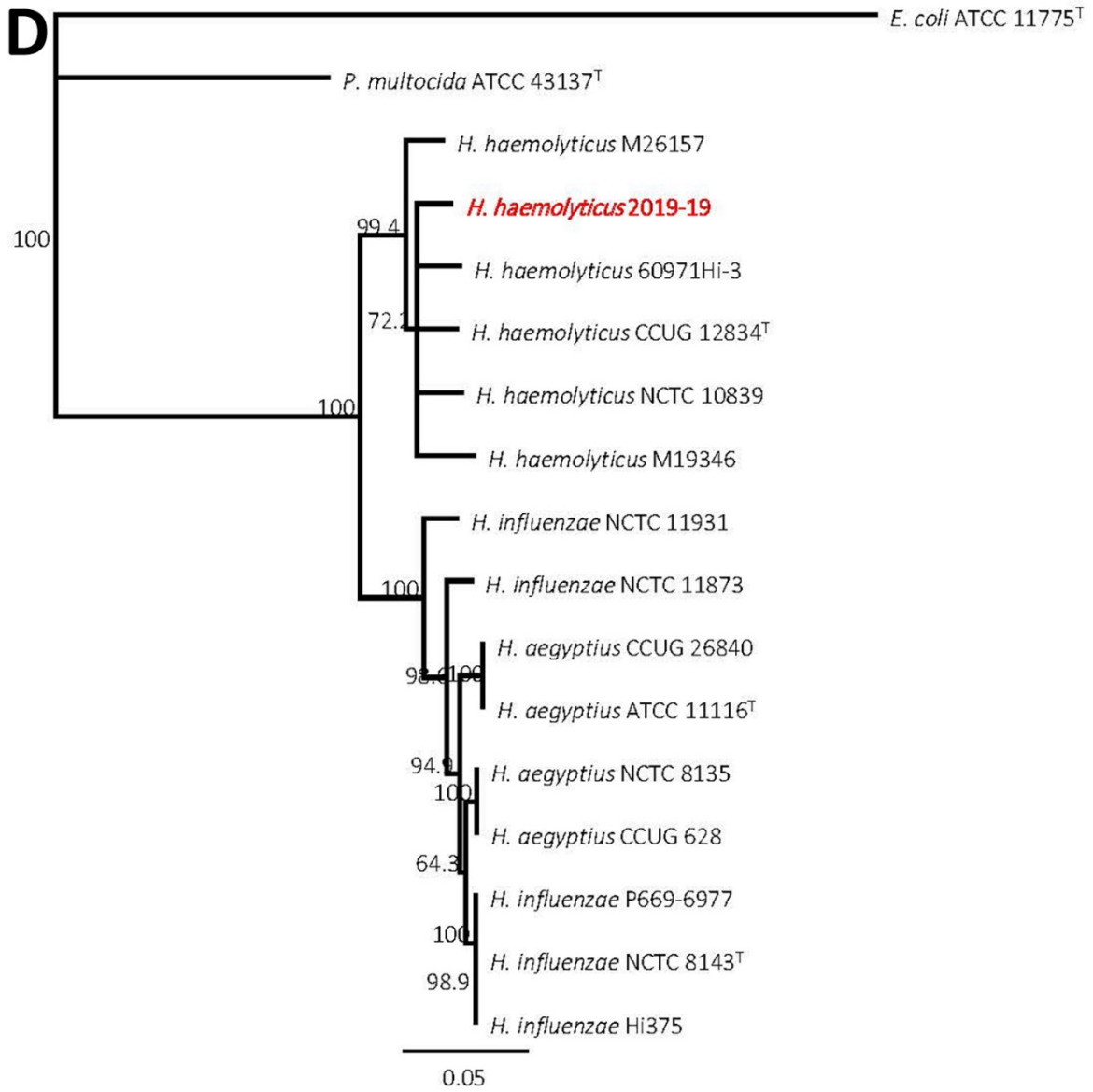
Appendix

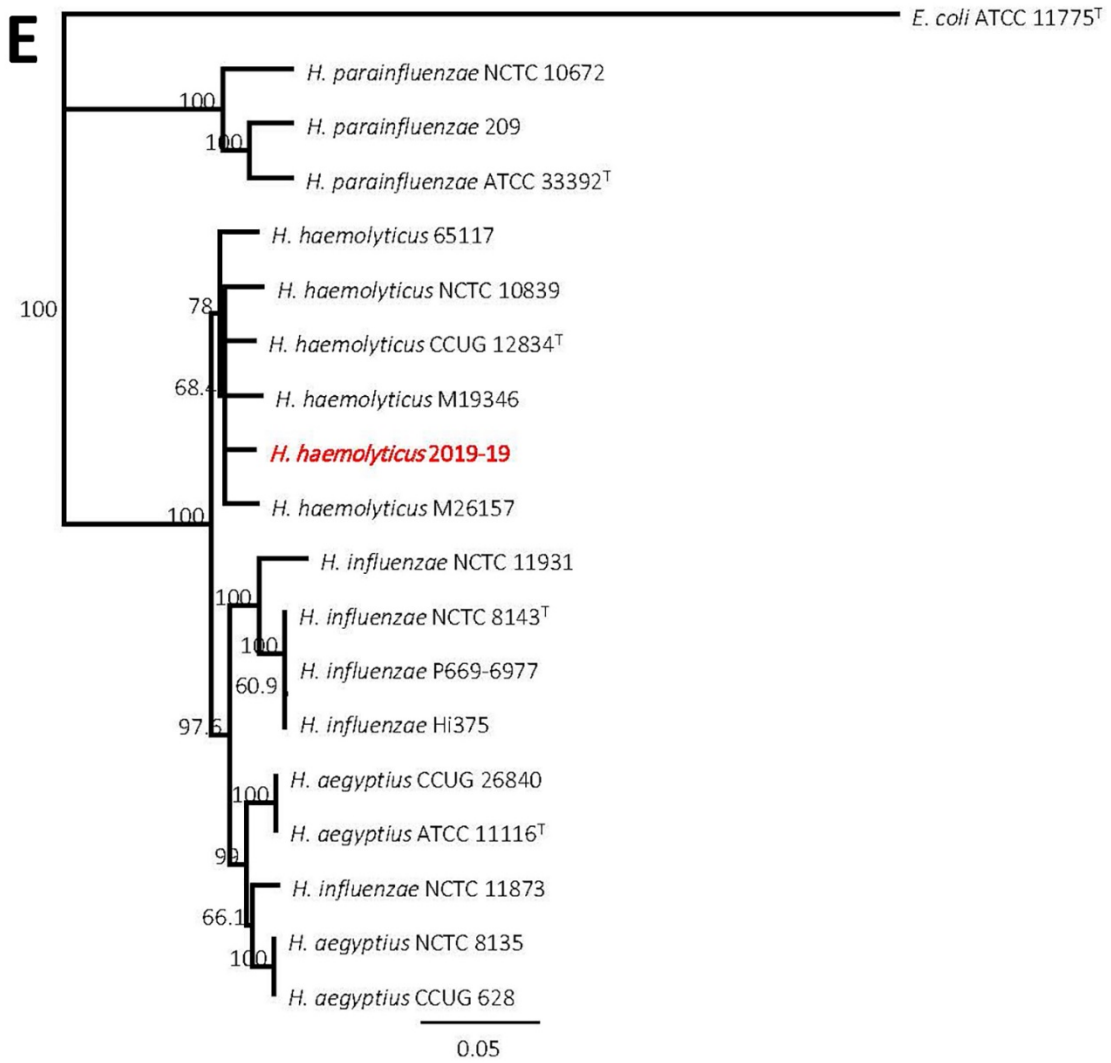
High-Level Quinolone-Resistant *Haemophilus haemolyticus* in a Pediatric Patient with No History of Quinolone Exposure

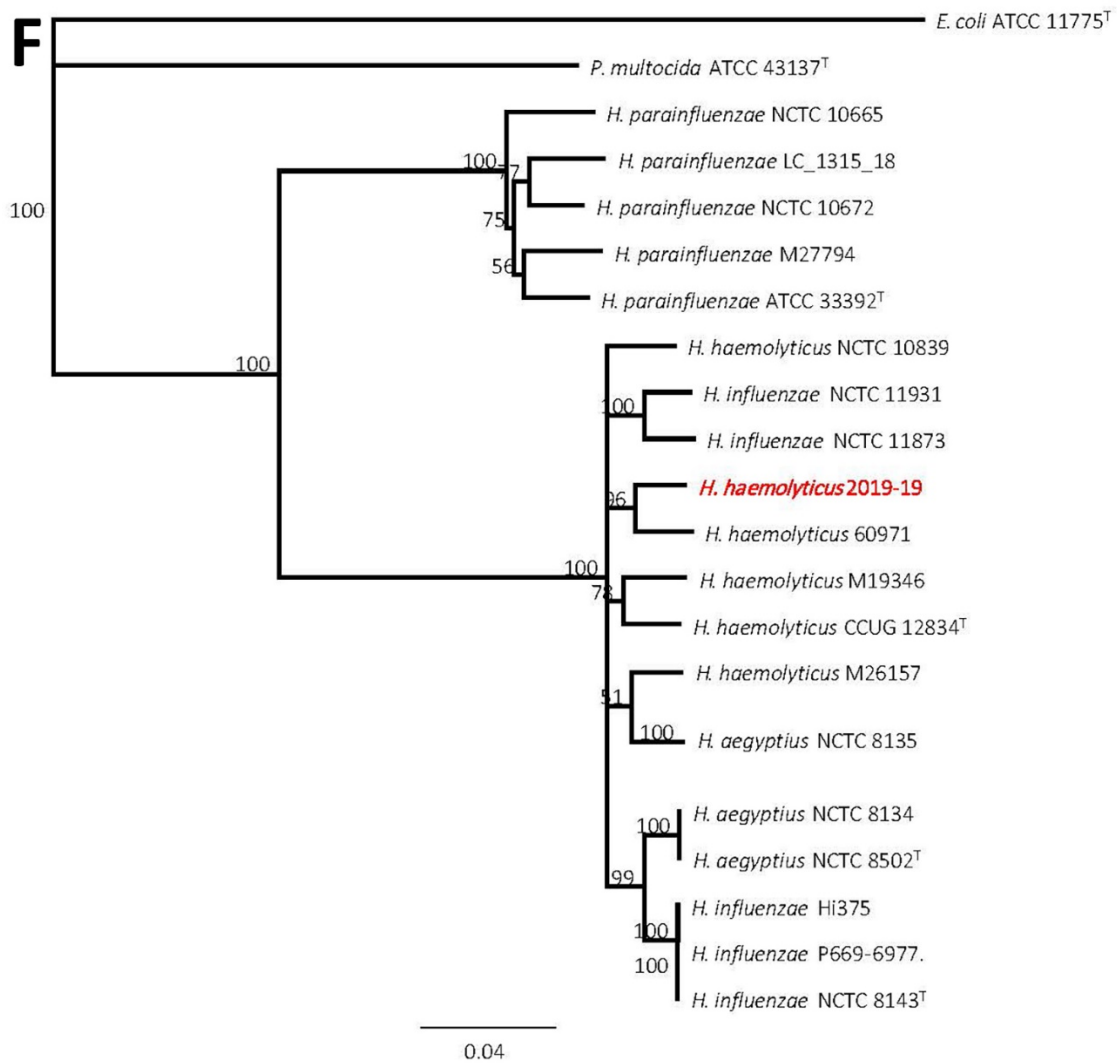


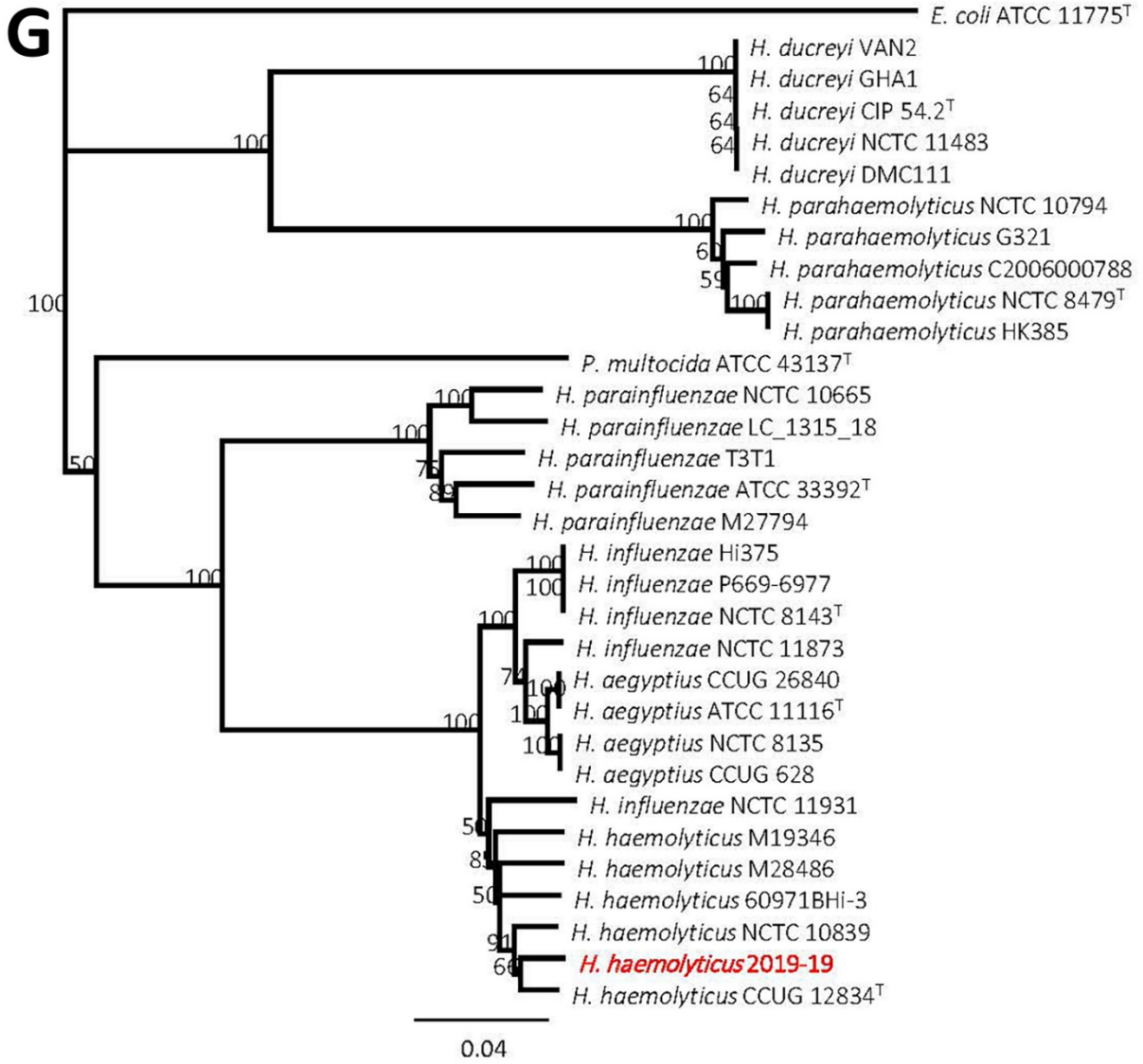


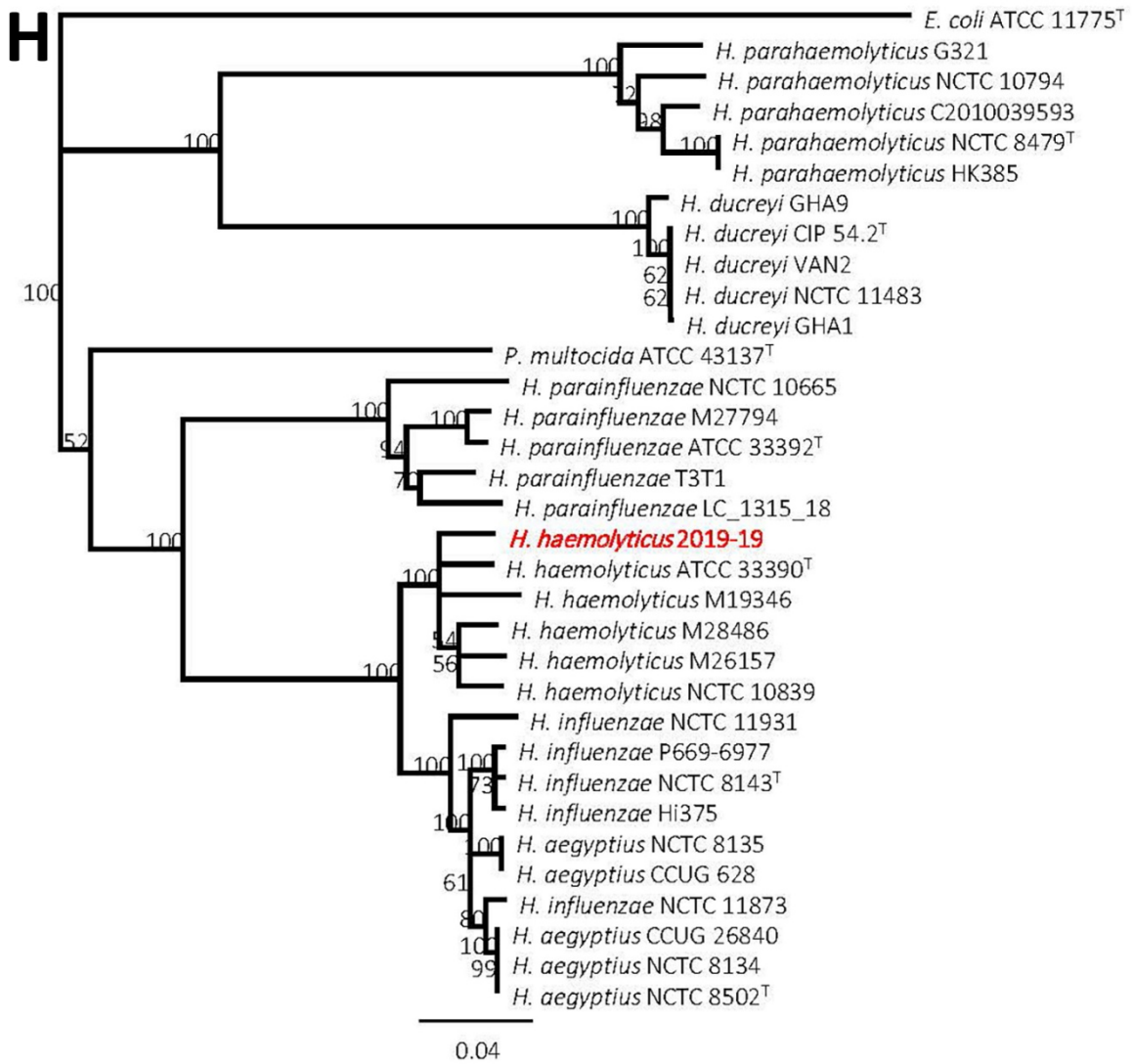


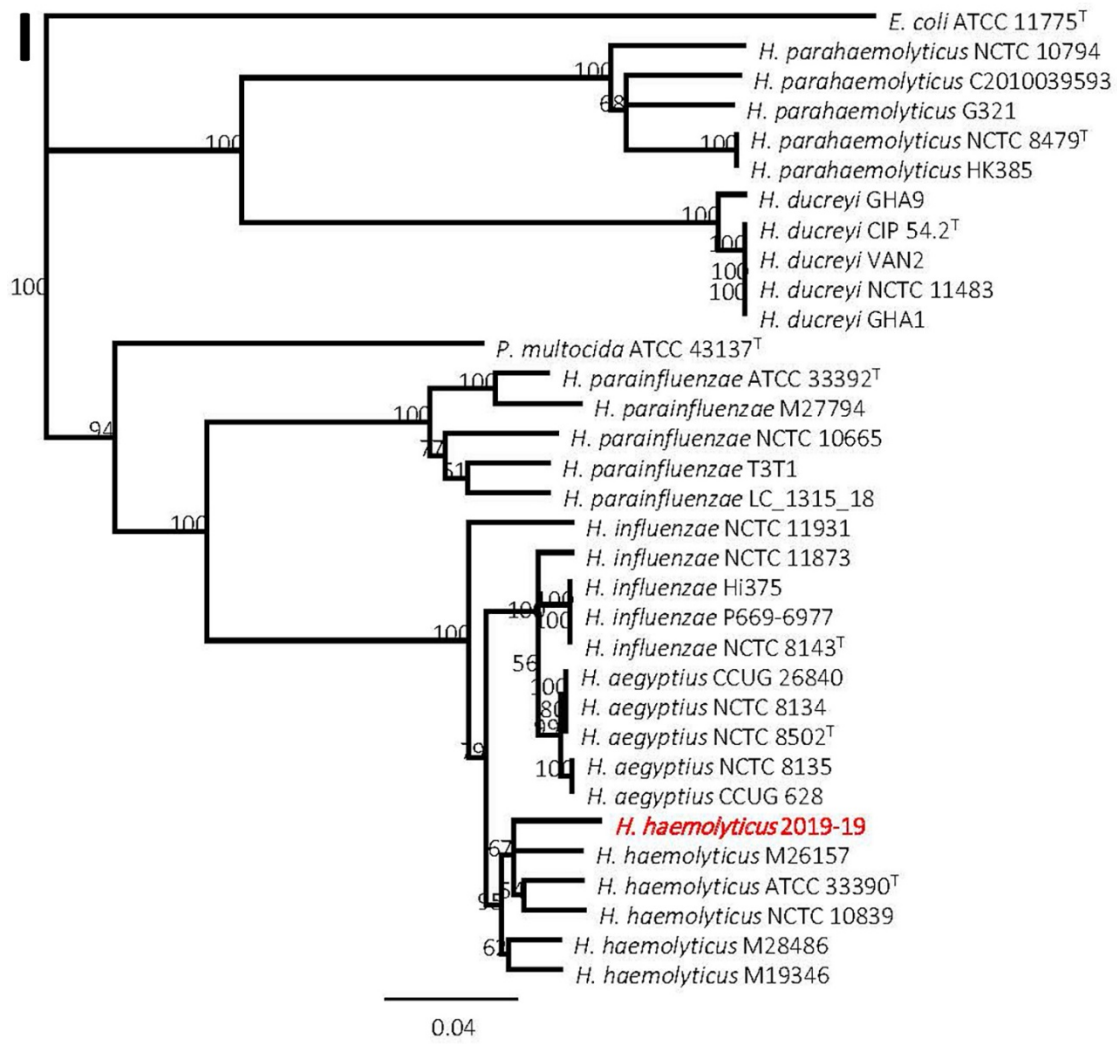


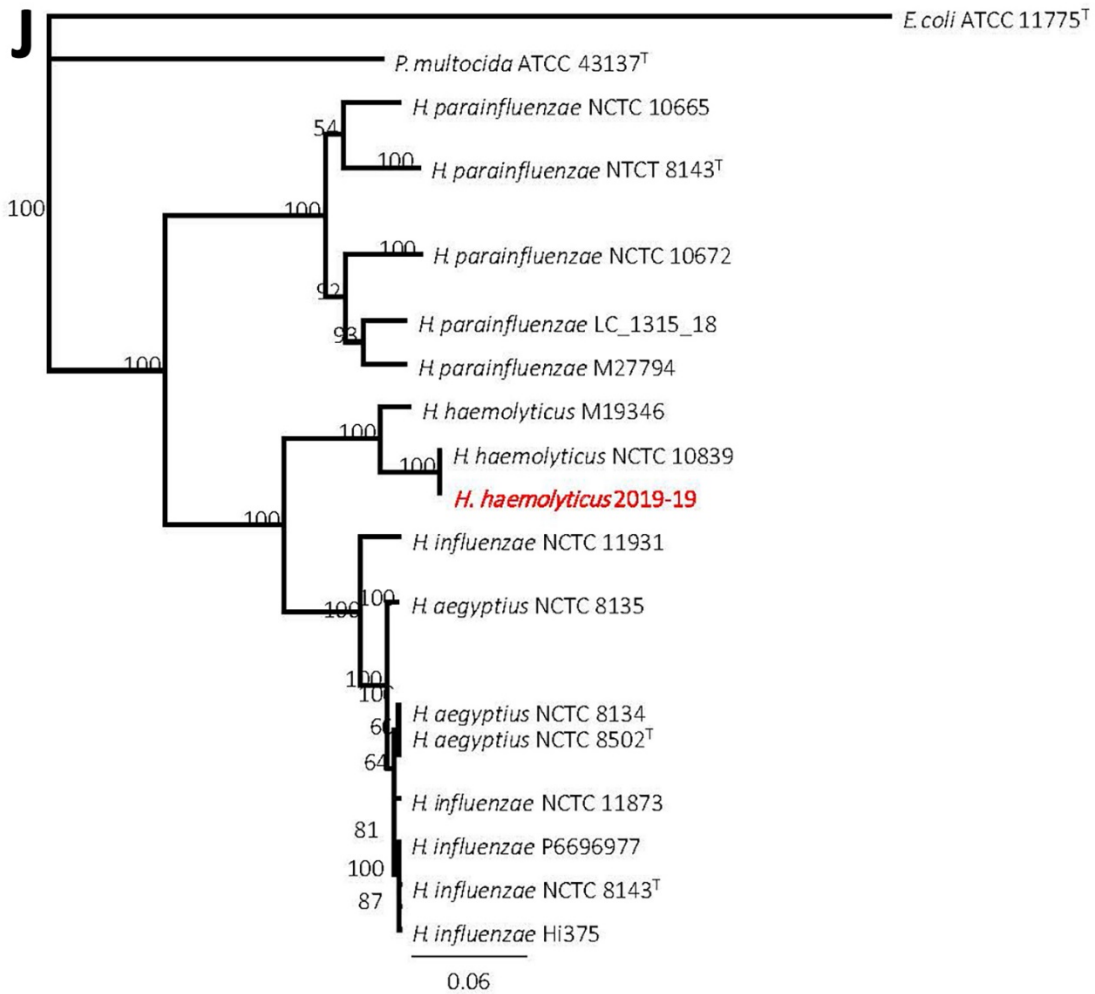




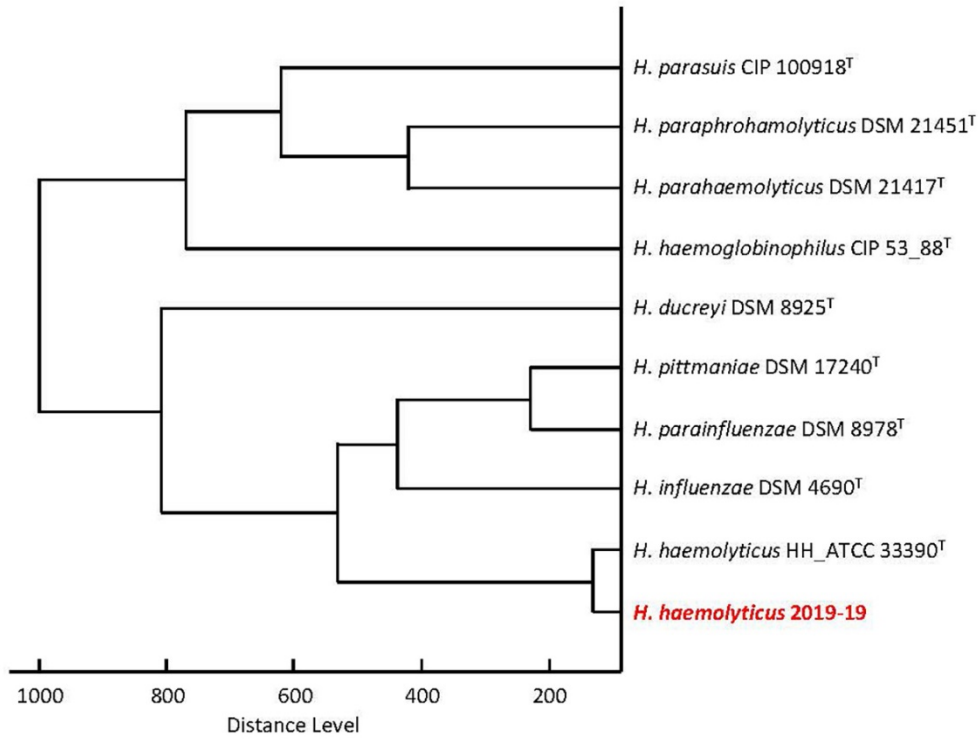








Appendix Figure 1. Neighbor-joining dendrograms based on nucleotide sequences. The dendrograms were drawn based on the nucleotide sequence of typical genes. They were drawn using the Clustal Omega program and neighbor-joining method using Geneious prime 2019 (Biomatters, <https://www.geneious.com>). *Escherichia coli* ATCC 11775^T was considered an outgroup. Panels A) 16S rDNA nucleotide sequences, B) *adk*, C) *pgi*, D) *recA*, E) *infB*, F) *gyrA*, G) *gyrB*, H) *parC*, I) *parE*, and J) *ftsI*. *H.*, *Haemophilus*



Appendix Figure 2. Main spectra library dendrogram of MALDI-TOF mass spectral profiles. The dendrogram was generated using MBT Compass Explorer 4.1.60 software (Bruker, <https://www.bruker.com>) on the basis of the MALDI-TOF mass spectral profiles of 10 *Haemophilus* spp. *H.*, *Haemophilus*

| | | | | |
|----------|--|-----|--|-----|
| A | <i>H. haemolyticus</i> 2019-19 | 1 | MTDSIQSITPPVNIIEELKSYLDYAMSVIVGRALEDVRDGLKPVHRVRLVPSMDREGNTANKKYVKSARVSVIGKYPHGD AVY TIVRMAQPFSLR | 100 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 1 |S.....D..... | 100 |
| | <i>H. haemolyticus</i> NCTC 10839 | 1 |S.....D..... | 100 |
| | <i>H. haemolyticus</i> M19346 | 1 |S.....D..... | 100 |
| | <i>H. haemolyticus</i> M28486 | 1 |S.....D..... | 100 |
| | <i>H. haemolyticus</i> 2019-19 | 101 | YMLVDGGNFGSIDGDAPAAAMRYTEVRMOKITQALLTDLKRETVNFSFMYDGEIMLDPVLPTRIPALLANGSSGIAVGMATNIFPHNLNEVLNGCLAYID | 200 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 101 | | 200 |
| | <i>H. haemolyticus</i> NCTC 10839 | 101 | | 200 |
| | <i>H. haemolyticus</i> M19346 | 101 | | 200 |
| | <i>H. haemolyticus</i> M28486 | 101 | | 200 |
| | <i>H. haemolyticus</i> 2019-19 | 201 | NNEITIDELMQ IPGDFPTAALINGRKGIEEAYRTPRGRKVVYVRAATV NEKGRGQIIVSELPIYQUNKAKLVEKIAELIPEKKIEGINSNIDLSNKEG | 300 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 201 |H.....T..... | 300 |
| | <i>H. haemolyticus</i> NCTC 10839 | 201 |H.....T..... | 300 |
| | <i>H. haemolyticus</i> M19346 | 201 |H.....T..... | 300 |
| | <i>H. haemolyticus</i> M28486 | 201 |H.....T..... | 300 |
| | <i>H. haemolyticus</i> 2019-19 | 301 | IRIEIDIKRDAVGEVVLNHLVSLTMCQVTFGINMVALDHGQPLFLNKEIE FVLHRRVVTRRSIFELFKARERTHILEGLAVARNIDEMIAITRNS | 400 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 301 | | 400 |
| | <i>H. haemolyticus</i> NCTC 10839 | 301 |A..... | 400 |
| | <i>H. haemolyticus</i> M19346 | 301 |A..... | 400 |
| | <i>H. haemolyticus</i> M28486 | 301 |A..... | 400 |
| | <i>H. haemolyticus</i> 2019-19 | 401 | KNREBAATASRSRMTLHSDIINLLDVSARPPDLEENLGIQGGQVLYSPAQUNALIELRLHRLTGLAFEEVVEVEELLVKADLHLHLSAERLMEVIR | 500 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 401 | | 500 |
| | <i>H. haemolyticus</i> NCTC 10839 | 401 | | 500 |
| | <i>H. haemolyticus</i> M19346 | 401 | | 500 |
| | <i>H. haemolyticus</i> M28486 | 401 | | 500 |
| | <i>H. haemolyticus</i> 2019-19 | 501 | EELVEVKAQFGDRLTEITAASGDIDLEDLIAQEDVVVTLSEHGVKYPQLTDYEAQRGGKGSATFMKEEDFIEKLLVANHTDITLFCSSRGLVWLK | 600 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 501 | | 600 |
| | <i>H. haemolyticus</i> NCTC 10839 | 501 | | 600 |
| | <i>H. haemolyticus</i> M19346 | 501 | | 600 |
| | <i>H. haemolyticus</i> M28486 | 501 | | 600 |
| | <i>H. haemolyticus</i> 2019-19 | 601 | VYQLPQASRGARGPVNLPLQENRITAIIEVSAVBEKDFVUMTAGGIVKIKIALTEFSPRSPNGIALLNLRDEDELIGVDITDGSNEMIMFSSQGRV | 700 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 601 | | 700 |
| | <i>H. haemolyticus</i> NCTC 10839 | 601 | | 700 |
| | <i>H. haemolyticus</i> M19346 | 601 | | 700 |
| | <i>H. haemolyticus</i> M28486 | 601 | | 700 |
| | <i>H. haemolyticus</i> 2019-19 | 701 | VRPABNAVAMGRLATVGRGIKALITNDISDESAVEIE ISDDNABASLDLNDIKVUSLVVPEKGAITATONGYKRTQL EYPTKSRNTKGVISIR | 800 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 701 |D.....S..... | 800 |
| | <i>H. haemolyticus</i> NCTC 10839 | 701 |D.....S..... | 800 |
| | <i>H. haemolyticus</i> M19346 | 701 |D.....S..... | 800 |
| | <i>H. haemolyticus</i> M28486 | 701 |D.....S..... | 800 |
| | <i>H. haemolyticus</i> 2019-19 | 801 | VSRNGKVAAZQVEETDQIMLITDAGTLVRRVRSVIVGRNTGGVLRIRADDEHVUSLVRCDADSEDSLEESGSEE | 880 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 801 | | 880 |
| | <i>H. haemolyticus</i> NCTC 10839 | 801 | | 880 |
| | <i>H. haemolyticus</i> M19346 | 801 | | 880 |
| | <i>H. haemolyticus</i> M28486 | 801 | | 880 |

| | | | | |
|----------|--|-----|---|-----|
| B | <i>H. haemolyticus</i> 2019-19 | 1 | MSSETNDNYGASSIKVLRGLDAVRRRPGRYIGDPTDGGTGLHMMVFEVVDNMAIDBALAGHCSDIIVTIHDDNSVSVQDDGRGIPVDIMPEBEGVSAAEVIMT | 100 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 1 | | 100 |
| | <i>H. haemolyticus</i> NCTC 10839 | 1 | | 100 |
| | <i>H. haemolyticus</i> M19346 | 1 | | 100 |
| | <i>H. haemolyticus</i> M28486 | 1 | | 100 |
| | <i>H. haemolyticus</i> 2019-19 | 101 | VHAGGKPFDDNYKVSQGLHSGVSVVNALSDKLGTLTRQGHVHQPYHLGEPQAPLTVIGDTEATGTTVRFWFS SDIFAITTDPYKLLAKRLBELSPL | 200 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 101 | | 200 |
| | <i>H. haemolyticus</i> NCTC 10839 | 101 | | 200 |
| | <i>H. haemolyticus</i> M19346 | 101 | | 200 |
| | <i>H. haemolyticus</i> M28486 | 101 | | 200 |
| | <i>H. haemolyticus</i> 2019-19 | 201 | NSGVSRILDKRDGSDHFFHYEGGIQAFVYLNKKNMIPHPKPYFTAERDKGIGVEVALQNDGVNENVYCFPTNNIPQRDGGTHLAGFRGALTRSLNSYM | 300 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 201 | | 300 |
| | <i>H. haemolyticus</i> NCTC 10839 | 201 | | 300 |
| | <i>H. haemolyticus</i> M19346 | 201 | | 300 |
| | <i>H. haemolyticus</i> M28486 | 201 | | 300 |
| | <i>H. haemolyticus</i> 2019-19 | 301 | ENSGMLKKEKVATSGDDAREGLVAILSVKVPDPKFFSQKDKLVSSEVKSVAESAMNEKMQEYLLNFPADAKIIVNQIIMAAARAAARKAREMTRRKA | 400 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 301 | | 400 |
| | <i>H. haemolyticus</i> NCTC 10839 | 301 | | 400 |
| | <i>H. haemolyticus</i> M19346 | 301 | | 400 |
| | <i>H. haemolyticus</i> M28486 | 301 | | 400 |
| | <i>H. haemolyticus</i> 2019-19 | 401 | LDIAGLPGKLDACCQEKDFALSELYLVEGDSAGSARKVGRDRKTCALLKGLKILNVEKARFDMLS SQEVTGLITLALGCGIGRDEYNPKLRYHHILIMT | 500 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 401 | | 500 |
| | <i>H. haemolyticus</i> NCTC 10839 | 401 | | 500 |
| | <i>H. haemolyticus</i> M19346 | 401 | | 500 |
| | <i>H. haemolyticus</i> M28486 | 401 | | 500 |
| | <i>H. haemolyticus</i> 2019-19 | 501 | DADVDSGSHIRTLLETPFVYRQMPLEIERSGVYVIAQPELYVYKRGKQSRVIRADADEMQEYELTALDG ELHISANAPANHALVFEKLVAEYHSVQKLIQRL | 600 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 501 |A..... | 600 |
| | <i>H. haemolyticus</i> NCTC 10839 | 501 |A..... | 600 |
| | <i>H. haemolyticus</i> M19346 | 501 |A..... | 600 |
| | <i>H. haemolyticus</i> M28486 | 501 |A..... | 600 |
| | <i>H. haemolyticus</i> 2019-19 | 601 | SRHYFAPVLQGLIYQSPISVEMKDESAVE NGSKSFVQLTAKETAHQVSVRTQFNAERQVYEA VITVYRKHGIDTDYFLNDFPVHNEYAKIVSLNKLQL | 700 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 601 |N..... | 700 |
| | <i>H. haemolyticus</i> NCTC 10839 | 601 |N..... | 700 |
| | <i>H. haemolyticus</i> M19346 | 601 |N..... | 700 |
| | <i>H. haemolyticus</i> M28486 | 601 |N..... | 700 |
| | <i>H. haemolyticus</i> 2019-19 | 701 | NGLLEBAGYVTRGEKVQ VRSFEQ VEWLVKE SRKLEVQRVYKGLGEMNADQLWETTMDPNSRRMLKVSIKDAVAADQLFTLMLGDEVEPRREFIELNAL | 800 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 701 |P.....A..... | 800 |
| | <i>H. haemolyticus</i> NCTC 10839 | 701 |P.....A..... | 800 |
| | <i>H. haemolyticus</i> M19346 | 701 |P.....A..... | 800 |
| | <i>H. haemolyticus</i> M28486 | 701 |P.....A..... | 800 |
| | <i>H. haemolyticus</i> 2019-19 | 801 | RANLDV | 806 |
| | <i>H. haemolyticus</i> CCUG 12834 ^T | 801 | | 806 |
| | <i>H. haemolyticus</i> NCTC 10839 | 801 | | 806 |
| | <i>H. haemolyticus</i> M19346 | 801 | | 806 |
| | <i>H. haemolyticus</i> M28486 | 801 | | 806 |

C

| | | | |
|--|-----|--|-----|
| <i>H. haemolyticus</i> 2019-19 | 1 | MTNINYGIEQMPLEPTFERAYLVNYSVMVMDRALPTDGLKPVORRIVVAMSBLGNATARKYKKSARTVSDVLDGKPHDGD ACYEAUMVMAQPFYSR | 100 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 1 |S..... | 100 |
| <i>H. haemolyticus</i> NCTC 10839 | 1 |S..... | 100 |
| <i>H. haemolyticus</i> M19346 | 1 |S..... | 100 |
| <i>H. haemolyticus</i> M28486 | 1 |S..... | 100 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 101 | YPLVDGQGNWAPDDPKSFAAMRYTESRLSKISEILL ELGQGTVDYQPNFDGTLAEPQYLPAFLPHILLNGTGTGIAVGMATDIPPHNINEIDAAMVLL | 200 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 101 |S..... | 200 |
| <i>H. haemolyticus</i> NCTC 10839 | 101 |S..... | 200 |
| <i>H. haemolyticus</i> M19346 | 101 |S..... | 200 |
| <i>H. haemolyticus</i> M28486 | 101 |S..... | 200 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 201 | DNFKA LDDVLEI QGFDPFTEAEIISPKSEIRKIYBOSRGSINRAATWKKEDGSIILSALPHQSSPSK IAQIABQMTAKKLPNLEDIADADHENFIR | 300 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 201 |G.....V..... | 300 |
| <i>H. haemolyticus</i> NCTC 10839 | 201 |G.....V..... | 300 |
| <i>H. haemolyticus</i> M19346 | 201 |G.....V..... | 300 |
| <i>H. haemolyticus</i> M28486 | 201 |G.....V..... | 300 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 301 | IVLVPRSNRVDTDALMAHLFATDLEKSYRVMNMMI GLDHPAVKGLLEILNEMIL FRRTVTVRLQVRLDKVLSRLHLEGLMIAPLMDIWEVYIIRHE | 400 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 301 |T..... | 400 |
| <i>H. haemolyticus</i> NCTC 10839 | 301 |T..... | 400 |
| <i>H. haemolyticus</i> M19346 | 301 |T..... | 400 |
| <i>H. haemolyticus</i> M28486 | 301 |T..... | 400 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 401 | DDFKAEIMARFNLSDEQADAILNLRHLAKLEENQKABQ ELEKERLNLEALIGSERRLNTLIKKEIQEDAKKYASPPMSQOLVEREAPMISESDMTF | 500 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 401 |D..... | 500 |
| <i>H. haemolyticus</i> NCTC 10839 | 401 |D..... | 500 |
| <i>H. haemolyticus</i> M19346 | 401 |D..... | 500 |
| <i>H. haemolyticus</i> M28486 | 401 |D..... | 500 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 501 | ABPVTVLSENGWURCAKGHIDIPKESLYKAGDNYRAHACGSKSNQAVVFD STGRSYALDELSPSARSQSEELTQKLNLEAGATIEYVW ASBEOQLLM | 600 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 501 |M..... | 600 |
| <i>H. haemolyticus</i> NCTC 10839 | 501 |M..... | 600 |
| <i>H. haemolyticus</i> M19346 | 501 |M..... | 600 |
| <i>H. haemolyticus</i> M28486 | 501 |M..... | 600 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 601 | ASDAGYGFICKFEDLIARNKAGKALISLEFNKAVMPEKTL NATALVVMATSGRMLIFP KDLEALSKGKGNKI VTI PAANAKERSSELLV LLLISDQA | 700 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 601 |A.....A..... | 700 |
| <i>H. haemolyticus</i> NCTC 10839 | 601 |A.....A.....R..... | 700 |
| <i>H. haemolyticus</i> M19346 | 601 |A.....A.....R..... | 700 |
| <i>H. haemolyticus</i> M28486 | 601 |A.....A.....R..... | 700 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 701 | SLEPHSGRRKIVLKPEDLQKFRPAERGRKGGSTLPRGLHTN EIVVIEPQHNT | 751 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 701 | | 751 |
| <i>H. haemolyticus</i> NCTC 10839 | 701 |V..... | 751 |
| <i>H. haemolyticus</i> M19346 | 701 |V..... | 750 |
| <i>H. haemolyticus</i> M28486 | 701 |V..... | 751 |

D

| | | | |
|--|-----|--|-----|
| <i>H. haemolyticus</i> 2019-19 | 1 | MTNINYSQIEITVLKDLPEVQIRFGMYTDTTRNHLAQEVIDNSVDEALAGFATKIEVILHFDQSIKVTNDRGMFVDIHPTEGVSQVEVILT KLHAGGKF | 100 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 1 | | 100 |
| <i>H. haemolyticus</i> NCTC 10839 | 1 | | 100 |
| <i>H. haemolyticus</i> M28486 | 1 | | 100 |
| <i>H. haemolyticus</i> M19346 | 1 | | 100 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 101 | SNKNYEFAGGLHGVGI SVVNALSERVDIQVRRNGEVYKIAFENGRNVBELEVI GTGSRRTTGTTVHF KENKPYFDSAKFVSRLRHLRAKAVLCSGLIEI | 200 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 101 | | 200 |
| <i>H. haemolyticus</i> NCTC 10839 | 101 | | 200 |
| <i>H. haemolyticus</i> M28486 | 101 | | 200 |
| <i>H. haemolyticus</i> M19346 | 101 | | 200 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 201 | KFIDKVNNTQDIWLYEDGLSDVYLIEAVNGPETLPEKFFVGEFGKTNBAVSWALLWLPPEGSELIGESYVNLIPITIQGGTHVNGLRQGLLDAREFCFRNL | 300 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 201 | | 300 |
| <i>H. haemolyticus</i> NCTC 10839 | 201 | | 300 |
| <i>H. haemolyticus</i> M28486 | 201 | | 300 |
| <i>H. haemolyticus</i> M19346 | 201 | | 300 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 301 | LERGVKLTADDIWDRCYIILSKMQDAQFAGQTKERLSSRQSAVEVSGVLKDAFSLMNLGNVQDAKRLAEIAISSAQRRLRAAKVVRKILVSGPALPGK | 400 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 301 | | 400 |
| <i>H. haemolyticus</i> NCTC 10839 | 301 | | 400 |
| <i>H. haemolyticus</i> M28486 | 301 | | 400 |
| <i>H. haemolyticus</i> M19346 | 301 | | 400 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 401 | LADCGSQDLKTELEFLVEGDSAGGSARQARDREYQAIL LRGKIINTWEVSPDQVLSGTEIHDAIVALGIDPDSNDLSQLRYKVCILADADSDGLHIAT | 500 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 401 |P..... | 500 |
| <i>H. haemolyticus</i> NCTC 10839 | 401 |P..... | 500 |
| <i>H. haemolyticus</i> M28486 | 401 |P..... | 500 |
| <i>H. haemolyticus</i> M19346 | 401 |P..... | 500 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 501 | I CALFLRHFPKLVQDGHVIVAMPPLRYRIDLNKEVYFALDESEKBAILDRLKNNKKGKENVQREKGLGEMNPSQLRETTMDNTRRLVQLTYDLEE QG D | 600 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 501 |L.....D.....A..... | 600 |
| <i>H. haemolyticus</i> NCTC 10839 | 501 |L.....D.....A..... | 600 |
| <i>H. haemolyticus</i> M28486 | 501 |L.....D.....A..... | 600 |
| <i>H. haemolyticus</i> M19346 | 501 |L.....D.....A..... | 600 |
| | | | |
| <i>H. haemolyticus</i> 2019-19 | 601 | TLELMDMLLAKKRSSEDRKNWLQAKGDQVDSLVS | 632 |
| <i>H. haemolyticus</i> ATCC 33390 ^f | 601 | | 632 |
| <i>H. haemolyticus</i> NCTC 10839 | 601 | | 632 |
| <i>H. haemolyticus</i> M28486 | 601 | | 632 |
| <i>H. haemolyticus</i> M19346 | 601 | | 632 |

Appendix Figure 3. Amino acid alignments of quinolone target proteins. These alignments were created using Genetyx version 10 (<https://www.genetyx.co.jp>). The amino acid substitutions are shown as single character codes. These data are the basis for Table 3. Panels A) *gyrA*, B) *gyrB*, C) *parC*, and D) *parE*. *H., Haemophilus*