

Supplementary Figures

An ancient family of mobile genomic islands introducing cephalosporinase and carbapenemase genes in *Enterobacteriaceae*

Suruchi Nepal¹, Florian Bonn^{2,†}, Stefano Grasso¹, Tim Stobernack¹, Anne de Jong³, Kai Zhou^{1,4}, Ronald Wedema¹, Sigrid Rosema¹, Dörte Becher², Andreas Otto², John W. Rossen¹, Jan Maarten van Dijl^{1*#}, and Erik Bathoorn^{1*#}

¹University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Hanzeplein 1, 9700 RB Groningen, The Netherlands. E-mail: s.nepal@umcg.nl; s.grasso@umcg.nl; t.stobernack01@umcg.nl; r.wedema@pl.hanze.nl; s.rosema@umcg.nl; j.w.a.rossen@rug.nl; j.m.van.dijl01@umcg.nl; d.bathoorn@umcg.nl.

²Institute for Microbiology, Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany. E-mail: bonn@med.uni-frankfurt.de; dbecher@uni-greifswald.de; andreas.otto@uni-greifswald.de;

³Department of Molecular Genetics, University of Groningen, Groningen Biomolecular Sciences and Biotechnology Institute, 9747 AG Groningen, the Netherlands. E-mail: anne.de.jong@rug.nl.

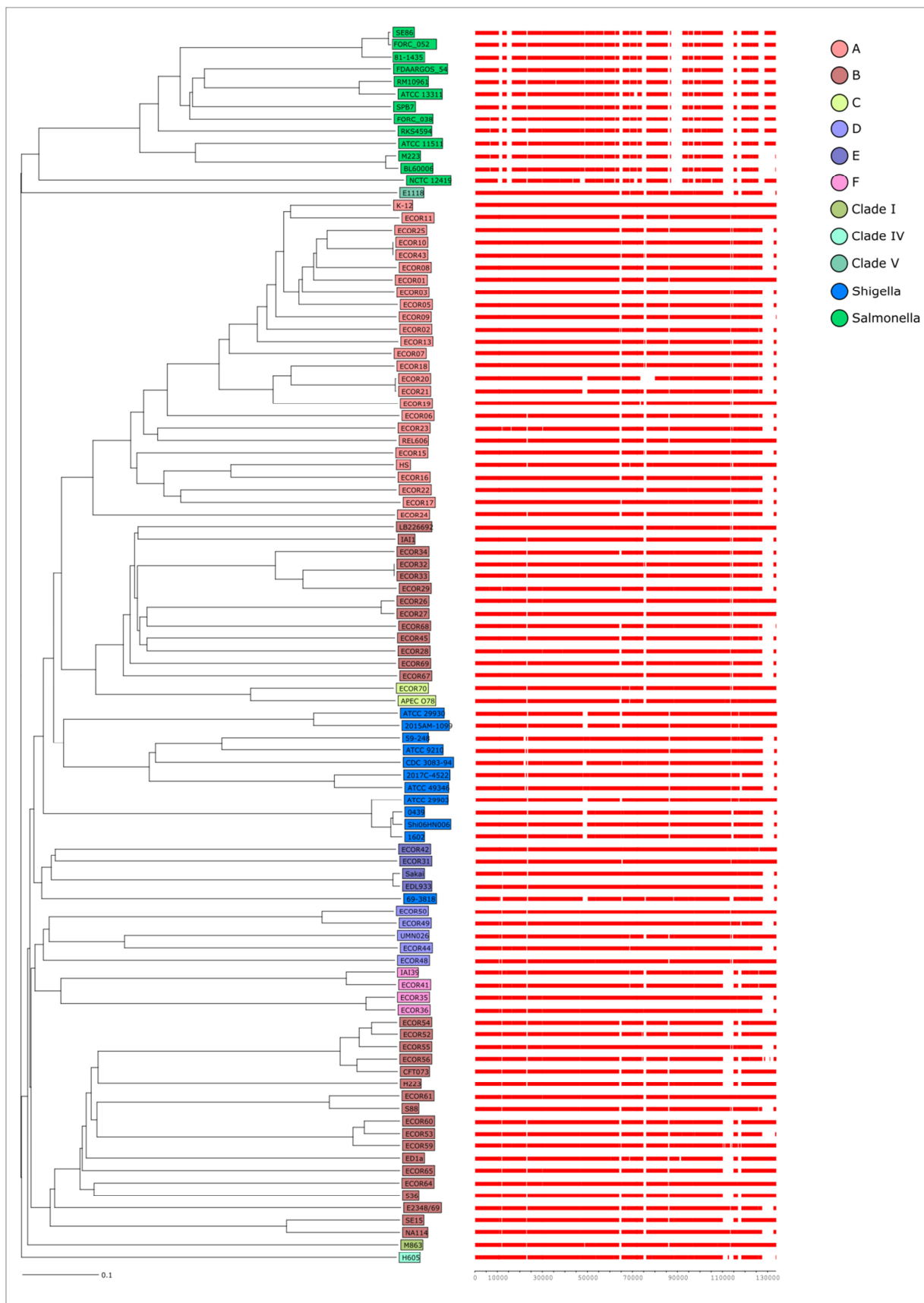
⁴State Key Laboratory for Diagnosis and Treatment of Infectious Disease, The First Affiliated Hospital, Zhejiang University, Hangzhou, China. E-mail: dr.kaizhou@qq.com.

[†]Present Address: Institute of Biochemistry 2, Goethe University Medical School, Frankfurt, Germany.

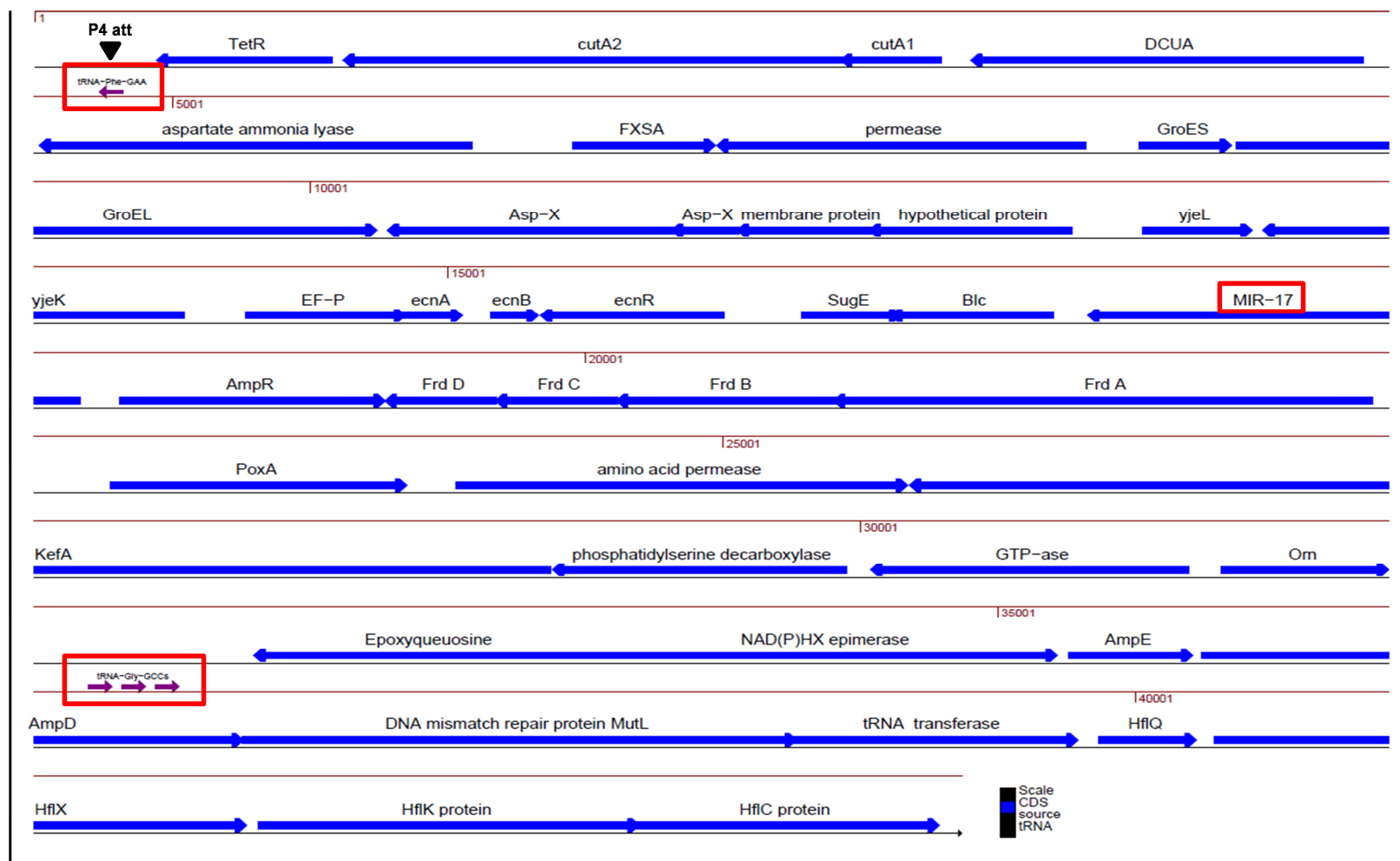
^{*}These authors contributed equally to this work.

[#]Corresponding Authors.

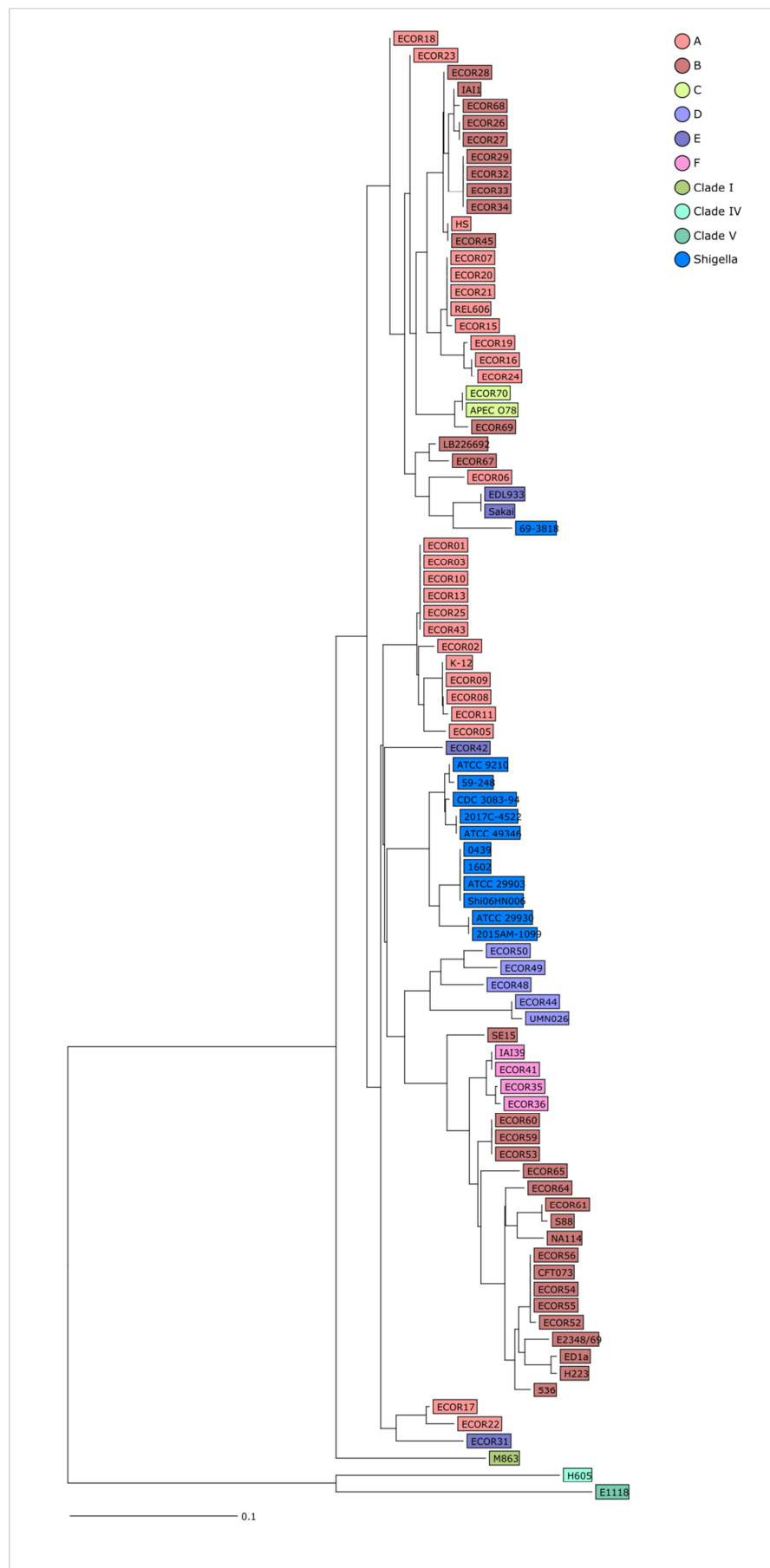
Running title: Cephalosporinase islands in *Enterobacteriaceae*



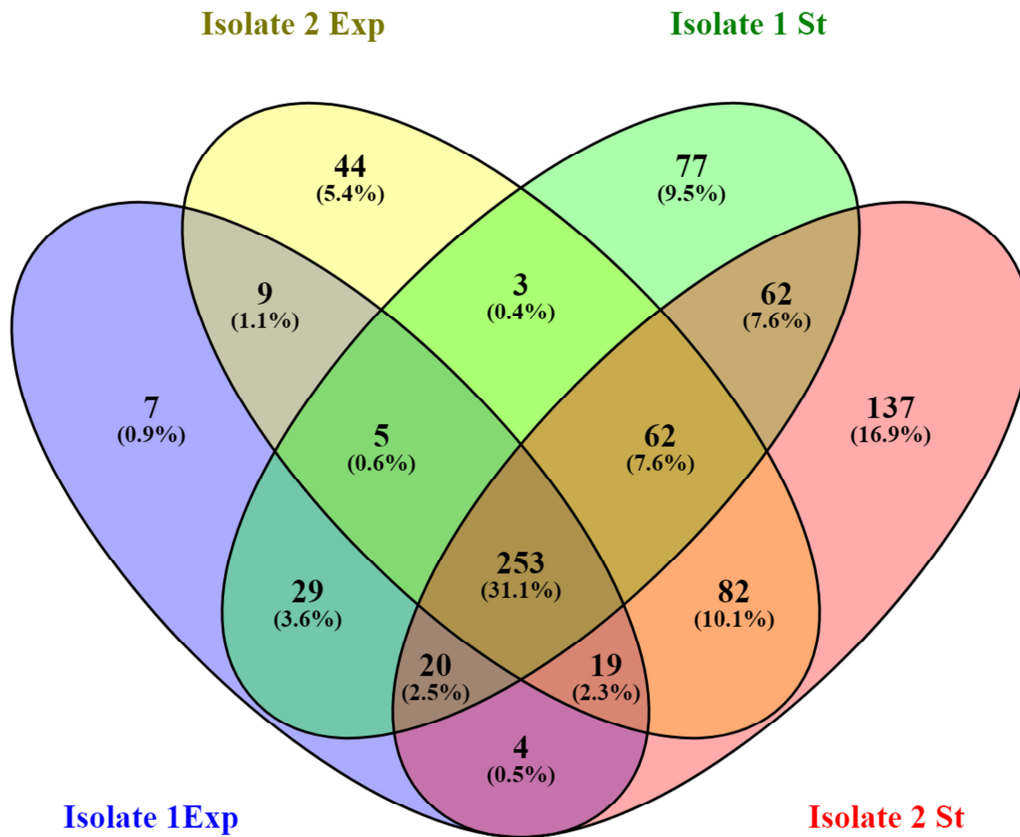
Supplemental Figure S1. Phylogenetic tree of *E. coli*, *Salmonella* and *Shigella*, and conservation of the MIR17-GI mobile genomic island. The phylogenetic tree of *E. coli* isolates, and *Salmonella* and *Shigella* species was created with the Ridom SeqSphere+ software v4.1.9 using a neighbor joining algorithm. It is built on a cgMLST analysis of 3839 targets from the reference genome of the *E. coli* K12 strain MG1655. The scale bar under the tree represents the phylogenetic distance (in %). The red bars indicate the conservation of the MIR17-GI using the respective MGI of *E. coli* K12 strain MG1655 as the reference. For the MGI comparisons, an 80% DNA similarity cut-off was used in DNA plotter. The scale bar under the MGI alignment indicates the sequence position of the reference MGI. The color codes mark publicly available sequences of *E. coli* isolates, and *Salmonella* and *Shigella* species that belong to particular phylogenetic groups or clades. The MIR17-GI was represented in all 100 investigated genomes, most of which were derived from a study by Clermont *et al.* (*Microbiology* 2015, 161: 980–988). Of note, the *ampC* gene was absent from the MGI in all sequenced *Salmonella* genomes.



Supplemental Figure S2. Genetic map of the 5' end of the MIR17-GI mobile genomic island of study isolate 1 up to the high frequency lysogeny operon. MIR17-GI is integrated in the Phe-GAA tRNA gene at the 5'end, which has a P4 prophage-associated attachment site in the 3'-5'direction. The positions of genes for AmpR, AmpD and AmpE orthologues and other identified genes are indicated.



Supplemental Figure S3. Neighbor joining tree showing the diversity of *ampC* genes on MIR17-GI-like MGIs in *E. coli* and *Shigella*. The neighbor joining tree is based on genome sequences used to construct the phylogenetic tree in Supplemental Figure S1. The scale bar represents the % difference among 197 nucleotides of *ampC*, using the *ampC* gene of the *E. coli* K12 strain MG1655 as a reference.



Supplemental Figure S4. Numbers of expressed proteins in *E. cloacae* isolates 1 and 2.

The Venn diagram shows overlapping and uniquely expressed proteins of the *E. cloacae* isolates 1 and 2 in the exponential (Exp) and stationary (St) growth phases. The diagram was created using the Venn diagram web tool of the VIB and the University of Gent in Belgium (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).