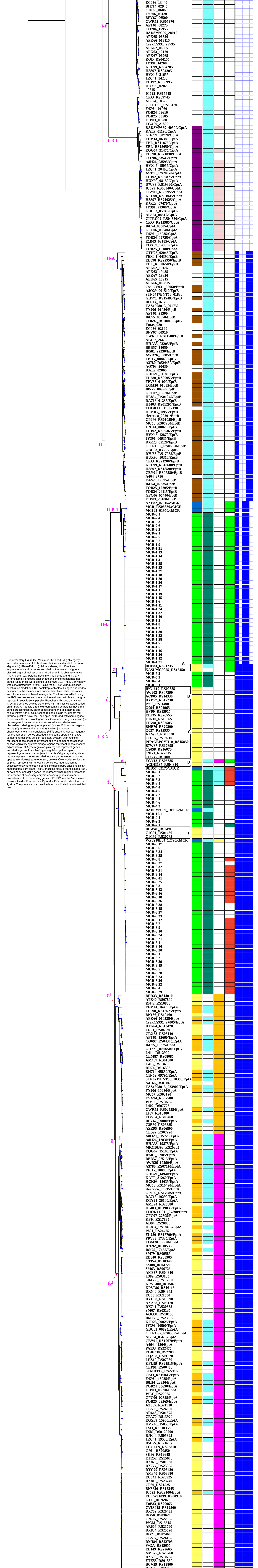


Tree scale: 1

- A-Gene family**
- CptA
 - EptA
 - EptB
 - Chromosomally encoded MCR
 - Known MCR
 - Putative novel MCR
 - NCBI annotated as PET
- B-Localization**
- Chromosome
 - Plasmid
 - Unplaced
- C-Regulator proximity**
- Response-regulator 2 component system
 - Response-regulator 2 component system (sig. strand)
 - TetR-family regulator
 - AraC-family regulator
 - Y6C-family
 - None
- D-Accessory enzyme**
- Pap2
 - DgkA
 - Pap2 DgkA
 - None



Supplementary Figure S2. Maximum likelihood (ML) phylogeny inferred from a nucleotide back-translation-based multiple sequence alignment (NTbin-MSA) of (i) 98 mcr alleles, (ii) 125 unique sequences of mcr-like genes encoded on the same contig as a λ 1 plasmid origin of replication and λ 1 other antimicrobial resistance (AMR) gene (i.e., "putative novel mcr-like genes"), and (iii) 237 chromosomally encoded phosphoethanolamine transferase (pet) genes. Sequences were aligned using MUSCLE. The ML phylogeny was constructed with RAxML, using the GTRGAMMA nucleotide substitution model and 100 bootstrap replicates. Linages and clades described in the main text are numbered in blue, while subspecies and clusters are numbered in magenta. The tree was edited using the iTOL web server and rooted at the midpoint, with branch lengths reported in substitutions per site. Branches with bootstrap values $\geq 70\%$ are denoted by blue stars. Five PET families clustered based on an 80% AA identity threshold representing 26 putative novel mcr genes are identified by black boxes around the taxa names and capital letters A to E. Color-coded regions in strip (A) denote mcr families, putative novel mcr, and eptA, eptB, and cptA homologues as shown in the left color legend key. Color-coded regions in strip (B) denote gene localization as chromosomally encoded (cyan), plasmid-encoded (green), or unplaced (white). Color-coded regions in strip (C) represent the regulatory system juxtaposing phosphoethanolamine transferase (PET)-encoding genes: magenta regions represent genes encoded in the same operon with a two-component response sensor-regulatory system; cyan regions represent genes encoded divergent of a two-component response sensor-regulatory system; orange regions represent genes encoded adjacent to a TetR-type regulator; pink regions represent genes encoded adjacent to an AraC-type regulator; yellow regions represent genes encoded adjacent to a Y6C-type regulator; white regions represent genes encoded as a single gene operon and no upstream or downstream regulatory protein. Color-coded regions in strip (D) represent PET-encoding genes localized adjacent to accessory enzyme-encoding genes, including pap2 encoding lipid A phosphatase (light green), dgkA encoding diacylglycerol kinase (red), or both pap2 and dgkA genes (dark green); white regions represent the absence of accessory enzyme-encoding genes upstream or downstream of PET-encoding genes. DS1-DS5 are the 5 conserved consecutive disulfide bonds in EptA (disulfide bond 1, disulfide bond 2, etc.). The presence of a disulfide bond is indicated by a blue-filled box.