

Supplementary Information for Microbiota-based Antimicrobial resistance Risk Screening (MARS): a novel PCR method for dynamic assessment of AMR colonization risk and ESBL carrier identification

Kawasuji *et al.* **The PDF file includes** Figs. S1–S4 and Table S1

A



B

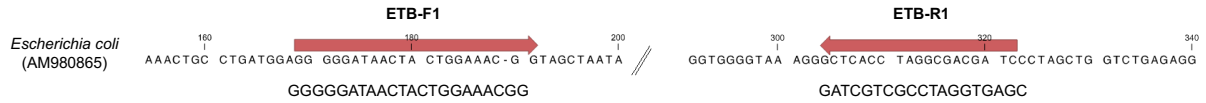
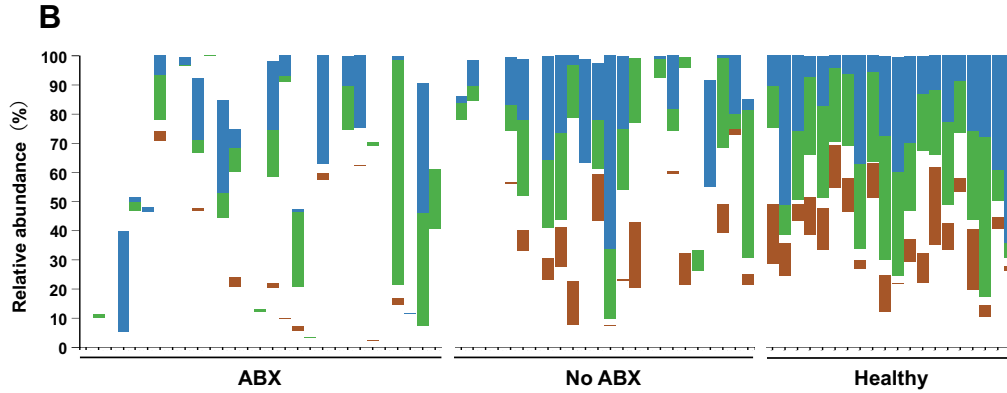
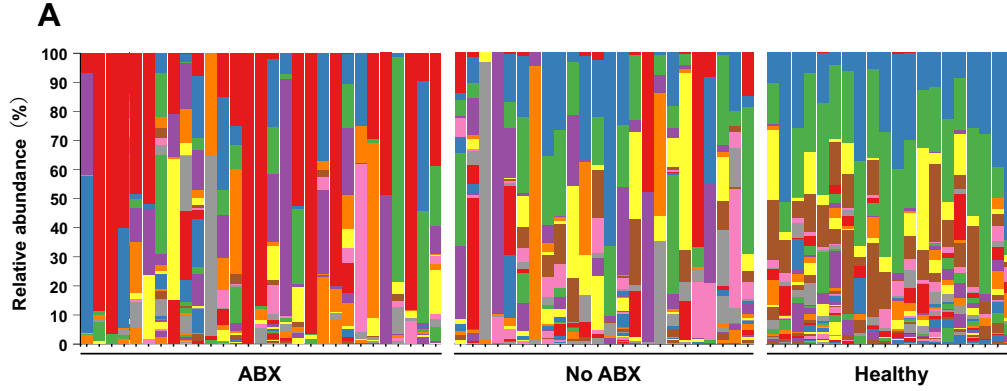


Fig. S1: Target regions within the 16S rRNA gene amplified by the MARS method.

(A) Primer binding sites for the forward and reverse primers used in the LRB assay. (B) Primer binding sites for the forward and reverse primers used in the ETB assay.

MARS, microbiota-based antimicrobial resistance risk screening; LRB, Lachnospiraceae,

Ruminococcaceae, and Bacteroidaceae; ETB, Enterobacteriaceae.



- d_Bacteria.p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Enterococcaceae
- d_Bacteria.p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Lachnospirales;f_Lachnospiraceae
- d_Bacteria.p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae
- d_Bacteria.p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae
- d_Bacteria.p_Actinobacteriota;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Oscillospirales;f_Ruminococcaceae
- d_Bacteria.p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Tannerellaceae
- d_Bacteria.p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae
- d_Bacteria.p_Firmicutes;c_Negativicutes;o_Veillonellales-Selenomonadales;f_Veillonellaceae
- d_Bacteria.p_Fusobacteriota;c_Fusobacteria;o_Fusobacteriales;f_Fusobacteriaceae
- d_Bacteria.p_Verrucomicrobiota;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Akkermansiaceae
- d_Bacteria.p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae
- d_Bacteria.p_Firmicutes;c_Negativicutes;o_Acidaminococcales;f_Acidaminococcaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Oscillospirales;f_Oscillospiraceae
- d_Bacteria.p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae
- d_Bacteria.p_Actinobacteriota;c_Coribacteriia;o_Coribacteriales;f_Coribacteriaceae
- d_Bacteria.p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Aeromonadaceae
- d_Bacteria.p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Marinifilaceae
- d_Bacteria.p_Proteobacteria;c_Gammaproteobacteria;o_Burkholderiales;f_Sutterellaceae
- d_Bacteria.p_Firmicutes;c_Negativicutes;o_Veillonellales-Selenomonadales;f_Selenomonadaceae
- d_Bacteria.p_Firmicutes;c_Bacilli;o_Erysipelotrichales;f_Erysipelatoclostridiaceae
- d_Bacteria.p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Dysgonomonadaceae
- d_Bacteria.p_Desulfobacterota;c_Desulfovibrionia;o_Desulfovibrionales;f_Desulfovibrionaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Clostridia_vadinBB60_group;f_Clostridia_vadinBB60_group
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Peptostreptococcales-Tissierellales;f_Peptostreptococcaceae
- d_Bacteria.p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Barnesiellaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Oscillospirales;f_[Eubacterium]_coprostanoligenes_group
- d_Bacteria.p_Actinobacteriota;c_Actinobacteria;o_Corynebacteriales;f_Corynebacteriaceae
- d_Bacteria.p_Firmicutes;c_Bacilli;o_Erysipelotrichales;f_Erysipelotrichaceae
- d_Bacteria.p_Firmicutes;c_Bacilli;o_Staphylococcales;f_Staphylococcaceae
- d_Bacteria.p_Actinobacteriota;c_Coribacteriia;o_Coribacteriales;f_Eggerthellaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Oscillospirales;f_Butyricococcaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Christensenellales;f_Christensenellaceae
- d_Bacteria.p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Pasteurellaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Monoglobales;f_Monoglobaceae
- d_Bacteria.p_Firmicutes;c_Bacilli;o_Bacillales;f_Bacillaceae
- d_Bacteria.p_Proteobacteria;c_Alphaproteobacteria;o_Rhodospirillales;f_uncultured
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Peptostreptococcales-Tissierellales;f_Family_XI
- d_Bacteria.p_Actinobacteriota;c_Actinobacteria;o_Actinomycetales;f_Actinomycetaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Clostridia_UCG-014;f_Clostridia_UCG-014
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Eubacteriales;f_Eubacteriaceae
- d_Bacteria.p_Firmicutes;c_Clostridia;f_
- d_Bacteria.p_Firmicutes;c_Clostridia;o_Peptostreptococcales-Tissierellales;f_Anaerovoracaceae
- d_Bacteria.p_Synergistota;c_Synergistia;o_Synergistales;f_Synergistaceae
- d_Bacteria.p_Actinobacteriota;c_Coribacteriia;o_Coribacteriales;f_Coribacteriales_Incertae_Sedis
- d_Bacteria.p_Actinobacteriota;c_Coribacteriia;o_Coribacteriales;f_Atopobiaceae
- d_Bacteria.p_Proteobacteria;c_Gammaproteobacteria;o_Burkholderiales;f_Neisseriaceae
- d_Bacteria.p_Firmicutes;c_Bacilli;o_Lactobacillales;f_P5D1-392
- d_Bacteria.p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Muribaculaceae

Fig. S2: Family-level relative abundance in patients with or without antibiotic exposure and in healthy individuals.

(A) Family-level relative abundance in patients who received antibiotics (ABX, n = 29), patients who did not receive antibiotics (No ABX, n = 24), and healthy individuals (Healthy, n = 20). (B) Relative abundance of Lachnospiraceae (green), Ruminococcaceae (brown), and Bacteroidaceae (blue) in each sample is shown.

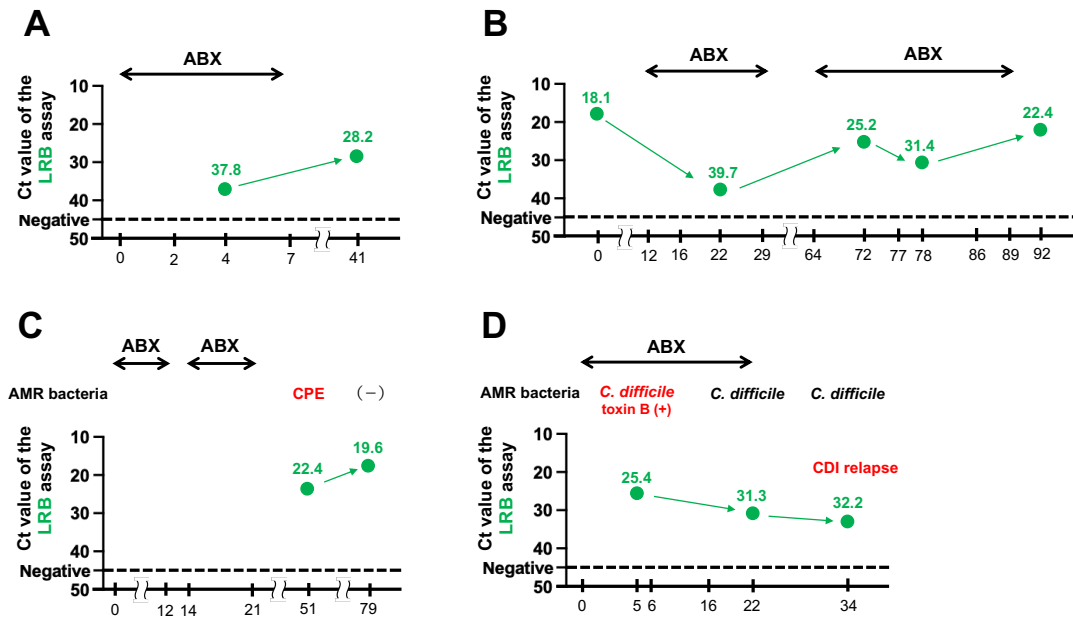


Fig. S3: Longitudinal changes in LRB assay Ct values in relation to antibiotic use and AMR colonization or infection in representative clinical cases.

(A) LRB assay Ct values decreased after discontinuation of antibiotics, indicating microbiota recovery. (B) LRB assay Ct values fluctuated in parallel with ongoing antibiotic administration. (C) Carbapenemase-producing Enterobacteriaceae (CPE) were detected on day 51; Ct values declined thereafter, and CPE became undetectable by day 79. (D) *Clostridioides difficile* infection (CDI) was diagnosed and treated with vancomycin for 10 days, alongside continued broad-spectrum antibiotics. Ct values remained elevated on day 34, and the patient experienced a CDI relapse.

Ct, cycle threshold; LRB, Lachnospiraceae, Ruminococcaceae, and Bacteroidaceae; AMR, antimicrobial resistance; CPE, carbapenemase-producing Enterobacteriaceae; CDI, *Clostridioides difficile* infection.

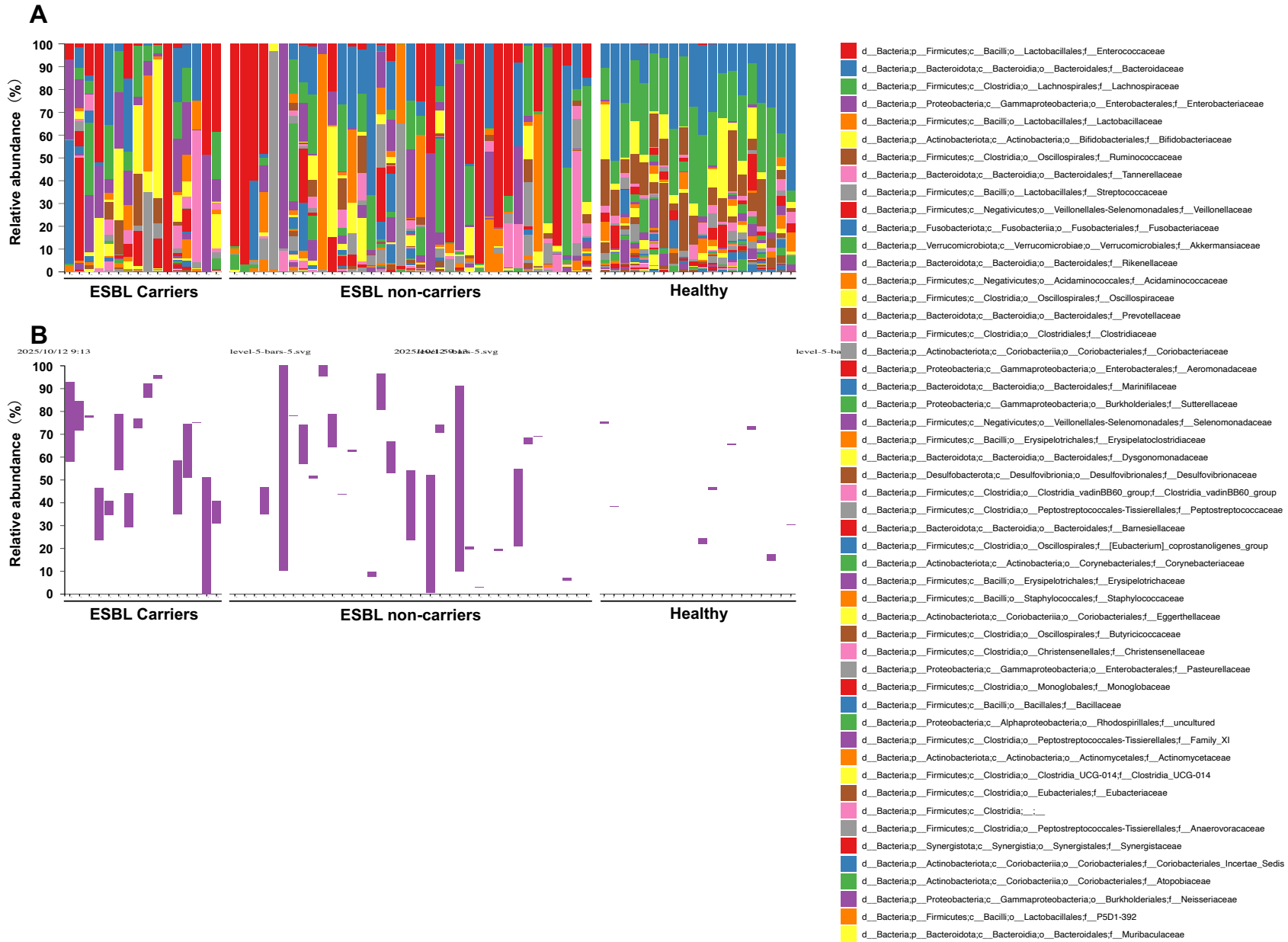


Fig. S4: Family-level relative abundance in patients with or without ESBL carriage and in healthy individuals.

(A) Family-level relative abundance in patients who were ESBL carriers (n = 16), ESBL non-carriers (n = 37), and healthy individuals (n = 20). (B) Relative abundance of Enterobacteriaceae (purple) in each sample is shown.

Table S1. Oligonucleotide sequences used in this study.

Transcript	Sequence (5'–3')
LRB-forward	
LRB-F1	GCGTTAAGTATTCCACCTGGGG
LRB-F2	ACAATAAGTATCCCACCTGGGG
LRB-F3	GCAATAAGTATACCACCTGGGG
LRB-reverse	
LRB-R1	GGTAAGGTTCTTCGCGTATC
LRB-R2	GGTAAGGTTCTTCGCGTTGC
ETB-forward	
ETB-F1	GGGGGATAACTACTGGAAACGG
ETB-reverse	
ETB-R1	GATCGTCGCCTAGGTGAGC
16s rRNA V3-V4 region	
341F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGG NGGCWGCAG (with Illumina adapters attached)
805R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACH VGGGTATCTAATCC (with Illumina adapters attached)

LRB, Lachnospiraceae, Ruminococcaceae, and Bacteroidaceae; ETB, Enterobacteriaceae