Supplemental Material

Occurrence of Clinically Important Lineages, Including the ST131 C1-M27 Subclone, among Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in Wastewater

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Sampling procedure.

The municipal WWTP from which samples were collected is in Shiga prefecture in the Kansai region of Japan (the final effluents from this WWTP flow into a lake). In October 2015, ten samples were collected from the WWTP on four different days. If samples were collected on the same day, each sampling was performed at least four hours apart. Samples from the WWTP were collected from effluent from the final settling tanks after biological (activated sludge) treatment. These samples consisted of treated wastewater prior to chlorination (some samples might have contained trace amounts of chemical agents used for chlorination. This is because the sampling points were near those where chemical agents were added to the wastewater). Samples were collected in sterile 50-ml centrifuge tubes at a depth of 0.1 m, transported to the laboratory, and processed within 24 hours of collection.

The hospital from which wastewater samples were collected is in Kyoto prefecture in the Kansai region of Japan. In October 2015, ten wastewater samples were collected from the hospital on four different days. If samples were collected on the same day, each sampling was performed at least three hours apart. Samples from the hospital consisted of untreated wastewater. Samples were collected by immersing a sterile bucket into wastewater running through sewer pipes, transferred to sterile 50-ml centrifuge tubes, transported to the laboratory, and processed within 24 hours of collection.

Table S1. Concentration of *E. coli* and ESBLEC in water samples.

	E. coli (CFU/100 ml)	ESBLEC (CFU/100 ml)
WWTP effluent (n = 10)		
Sample no. 1	15000	1160
Sample no. 2	39000	960
Sample no. 3	18000	600
Sample no. 4	22000	600
Sample no. 5	16000	800
Sample no. 6	12000	280
Sample no. 7	12000	1120
Sample no. 8	23000	520
Sample no. 9	17000	520
Sample no. 10	32000	1160
Hospital wastewater (n = 10)		
Sample no. 1	1400000	43600
Sample no. 2	800000	90000
Sample no. 3	1700000	55000
Sample no. 4	2800000	30000
Sample no. 5[a]	19600000	225000
Sample no. 6[a]	12000000	50000
Sample no. 7[a]	10900000	9500
Sample no. 8	18000000	300000
Sample no. 9	7400000	16000
Sample no. 10	600000	25000

[a] Strains isolated from these three samples contained *Citrobacter freundii*. Proportions of ESBLEC among total *E. coli* were not calculated for these samples.

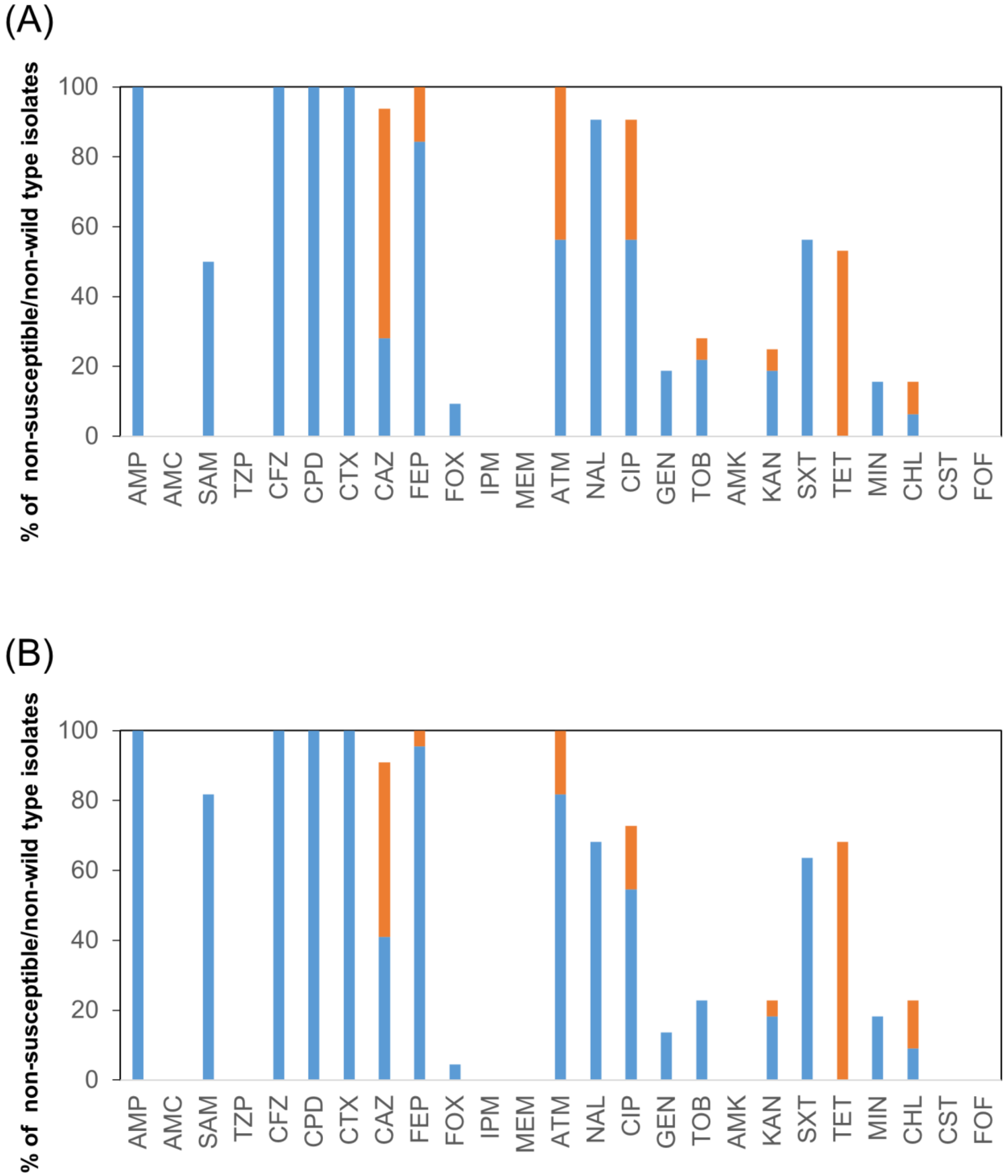

FIG S1 Antimicrobial susceptibilities of (A) 32 ESBLEC strains from the WWTP and (B) 22 ESBLEC strains from hospital wastewater. The orange bar indicates the ratio of strains susceptible to the antibiotic according to the clinical breakpoint but non-wild type according to ECOFF criteria. The blue bar indicates the ratio of strains non-susceptible to the antibiotic according to the clinical breakpoint. Note that ECOFF values are higher than clinical breakpoints for chloramphenicol and tetracycline. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; CFZ, cefazolin; CPD, cefpodoxime; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; IPM, imipenem; MEM, meropenem; ATM, aztreonam; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; KAN, kanamycin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; MIN, minocycline; CHL, chloramphenicol; CST, colistin; FOF, fosfomycin.

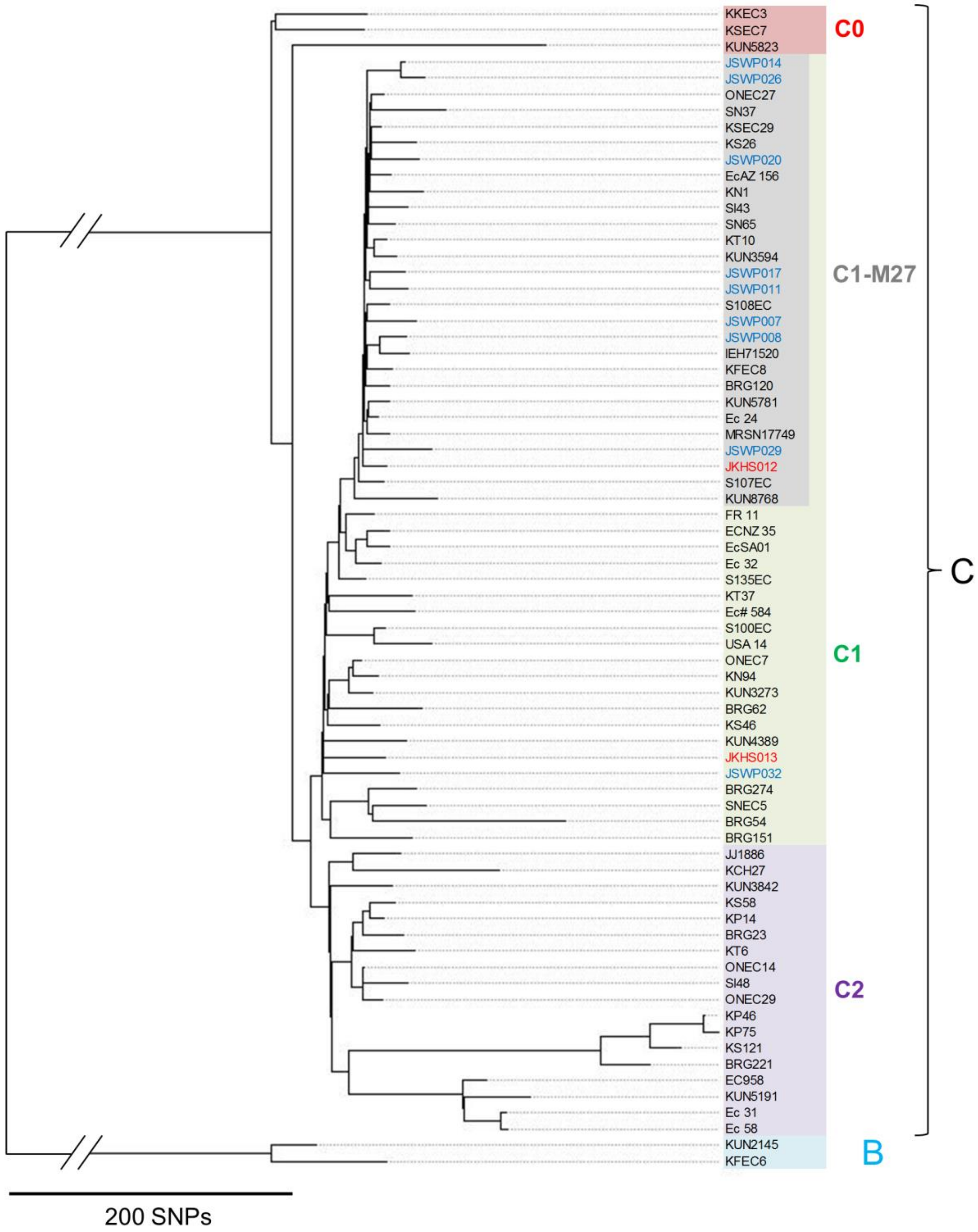

FIG S2 Core SNP-based phylogenetic tree of ST131 clade B and clade C strains. The tree was visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Colors of the strain names reflect the isolation sources, i.e., blue for the WWTP, red for hospital wastewater, and black for those analyzed in our previous study.

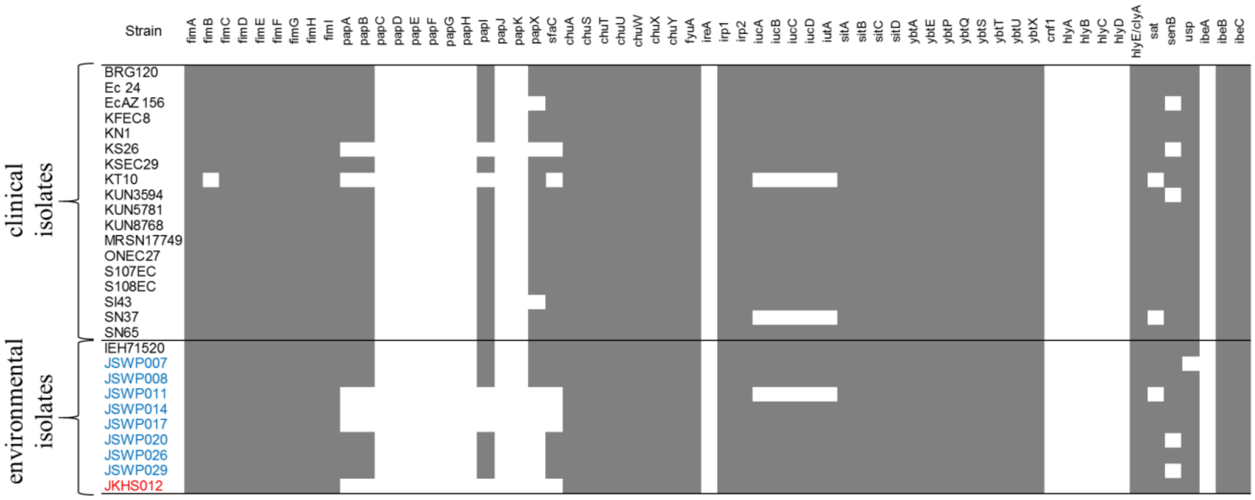

FIG S3 Virulence gene profiles of clinical and environmental C1-M27 isolates.