Supplementary Material

CRE isolates. Only the first CRE isolate was included from each patient except for one patient with two strains on presentation. Isolates were identified by biochemical testing and matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF) (Bruker Daltonics, Bremen, Germany).

Genotypic β -lactamase testing. Isolates were retrospectively screened for plasmid-encoded ESBL and AmpC cephalosporinases using the Check-Points CT 103 XL Check-MDR assay (Wageningen, The Netherlands) per the package instruction. The Check-Points assay detects the following ESBLs: bla$_{\text{CTX-M-1 group}}$, *bla*$_{\text{CTX-M-1-like}}$, *bla*$_{\text{CTX-M-15-like}}$, *bla*$_{\text{CTX-M-32-like}}$, *bla*$_{\text{CTX-M-2 group}}$, *bla*$_{\text{CTX-M-8, &-25 group}}$, *bla*$_{\text{CTX-M-9 group}}$, *bla*$_{\text{TEM-types}}$, *bla*$_{\text{SHV-types}}$, *bla*$_{\text{VEB}}$, *bla*$_{\text{PER}}$, *bla*$_{\text{BEL}}$, *bla*$_{\text{GES}}$; and the following AmpCs: *bla*$_{\text{CMY I/MOX}}$, *bla*$_{\text{ACC}}$, *bla*$_{\text{DHA}}$, *bla*$_{\text{ACT/MIR}}$, *bla*$_{\text{CMY II}}$, *bla*$_{\text{FOX}}$. Detection of carbapenemase genes was carried out using the Xpert Carba-R cartridge (Cepheid, Sunnyvale, CA), which detects *bla*$_{\text{KPC}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$ and *bla*$_{\text{OXA-48 like}}$; Check-Points assay which detects additionally *bla*$_{\text{OXA-23 like}}$, *bla*$_{\text{OXA-58 like}}$, *bla*$_{\text{SPM}}$, *bla*$_{\text{GES}}$, and *bla*$_{\text{GIM}}$; and three lab-developed multiplexed PCR assays which detect *bla*$_{\text{SME}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{SPM}}$, *bla*$_{\text{GES}}$, *bla*$_{\text{IMI}}$, *bla*$_{\text{NMC-A}}$, and *bla*$_{\text{GIM}}$ (Supplementary Table 1). DNA was extracted by boiling a bacterial colony in molecular-grade water for 10 min. PCR reactions consisted of 2 μL of forward and reverse primer to achieve 0.5 μM, 5 μL of 2× FastStart SYBR Green Master mix (Roche Applied Science, Indianapolis, IN), and 3 μL of DNA extract. The reactions were run on a Rotor-Gene 6000 real-time cycler (Qiagen, Germantown, MD) with following cycling parameters:

95°C for 5 min and 40 cycles of 95°C for 15 sec, 60°C for 30 sec, and 72°C for 30 sec, followed by melting with ramping from 60°C to 95°C in 0.2°C increments. Melting curve analysis was performed to identify the amplicons (Supplementary Table 1). Positive controls for each carbapenemase included *bla*$_{SME}$-positive *S. marcescen*s MBRL055 and *bla*$_{IMI}$-positive *Enterobacter cloacae* MBRL1077 provided by the Mayo Clinic (Rochester, MN); *bla*$_{SIM}$-positive *Acinetobacter baumannii* YMC 03/9/T104 provided by Yonsei University College of Medicine (Seoul, South Korea); *bla*$_{GIM}$-positive *E. cloacae* M15 provided by Heinrich Heine University Düsseldorf (Düsseldorf, Germany); *bla*$_{NMC-A}$-positive *E. cloacae* and *bla*$_{GES}$-positive *A. baumannii* provided by JMI Laboratories (North Liberty, IA); and 5 *bla*$_{GES}$-positive and 5 *bla*$_{SPM}$-positive *Pseudomonas aeruginosa* isolates provided by Merck (Schaumburg, IL).

Porin protein expression. Levels of OmpC and OmpF in *E. coli* and their analogs in other species were measured using mass spectrometry (MS). Isolates were cultured overnight in 20 mL of LB broth shaking at 250 revolutions per min at 37°C. Bacterial pellets were washed in sodium phosphate buffer (SPB) and resuspended in 0.5 mL of SPB and transferred to O-ring tubes containing 0.2 mL of 0.1-mm zirconia/silica beads. Bacteria were mechanically disrupted with three 0.5-min pulses at 2,500 oscillations per min in a Mini-BeadBeater-1 (BioSpec Products, Bartlesville, OK) with 1-min intervals on ice. The lysates were sedimented two times for 10 min at 1,500 × g to remove cellular debris. To enrich for membrane proteins, the supernatants were sedimented two times for 30 min each at 21,000 × g and the second pellet was resuspended in 45 µL of SPB. Protein concentrations were measured using the Quick Start™ Bradford Protein Assay

(Bio Rad, Hercules, CA) and 20 µg was separated on a 10% SDS-PAGE gel. Gels were stained with Coomassie Brilliant Blue R-250 and protein bands with molecular weight between 31 and 40 kDa were cut and digested with in-gel tryptic digestion kit (Thermo Scientific, Waltham, MA) per the package insert. Samples were concentrated in thermo savant iss110 speedvac system (Thermo Scientific) and resuspended in 20 µL of 0.1% formic acid in LC-MS grade water. Tryptic peptides (2 µL for each sample) were injected with a nanoAcquity sample manager (Waters, Milford, MA), trapped for 1 min at 15 µL/min on a Symmetry trap column (Waters), and separated on a 1.7 µm particle size BEH C18 column (Waters) by reversed phase LC using a nanoAcquity binary solvent manager (Waters). A 30 min linear acetonitrile gradient (3–35%) was applied. Peptides were ionized by nano-ESI using a pico-emitter tip (New Objective, Woburn, MA) and analyzed by an Impact HD UHR-QTOF mass spectrometer (Bruker Daltonics) in data-dependent acquisition mode. The acquisition parameters and batch processing conditions used for DDA have been previously reported (Kultz et al., 2015). Data was analyzed in PreView (Protein Metrics, San Carlos, CA) using the SwissProt FASTA database entries for *Enterobacteriaceae* (www.uniprot.org) to determine the dominant post-translational modifications and mass calibration parameters. A more specific search was carried out in Byonic (Protein Metrics, San Carlos, CA) using the TrEMBL database filtered for the taxonomy of the particular organism under study. MS and MS/MS tolerances were respectively set to 10 and 30 ppm. The main modifications considered were cysteine trioxidation, methionine oxidation and N-Term acetylation. The protein false detection rate was set to 1% and all matches with less than 2 unique peptides were discarded. The resulting protein lists were then compiled with an R script (http://www.R-project.org/) to

classify the identified porin variants based on homology into OmpC (OmpK36 used for *K. pneumoniae*) and OmpF (OmpK35 for *K. pneumoniae*) categories. The total intensity of all the MS/MS spectra contributing to peptide identification for each category was summed. Fold change in relative porin expression was determined by calculating the ratio of each porin in CRE isolates to averaged expression in four pan-sensitive strains of the same species.

Porin RNA expression. Porin RNA expression was performed on the 39 CRE isolates recovered between 2013 and 2015 excluding *S. marcescens* isolates and one *E. cloacae* complex. CRE isolates were cultured in Mueller Hinton broth in the presence of a carbapenem (either meropenem 2 μg/mL or imipenem 2 μg/mL and if necessary ertapenem 1μg/mL) at a starting density 1×10^5 CFU/mL and harvested at 1×10^8 CFU/mL. RNAprotect Bacteria Reagent (Qiagen) was added to cultures at a ratio 3:1 and incubated at ambient temperature for 5 min. RNA was extracted from bacterial pellets and DNase-treated using RNA Extraction Kit and RNase-free DNase Kit (Qiagen), respectively. cDNA was constructed using the QuantiTect Reverse Transcription Kit (Qiagen). An identical reaction not treated with reverse transcriptase was included to control for genomic DNA carryover. Quantitative reverse transcription-PCR (qRT-PCR) was performed for porin genes (*ompC* and *ompF* in *E. coli* and their analogs in other species) and the housekeeping gene *rpoB*. qRT-PCR primers are shown in Supplementary Table 2. Expression profiling of *ompF* analog in *E. cloacae* and *Citrobacter freundii* was not performed due to lack of PCR primers. PCR reactions were carried out in 10 μL containing of 0.5 μM of each primer, 1× FastStart SYBR Green Master mix, and 3 μL of

cDNA. Amplification conditions were as described above. The specificity of PCR products was confirmed by melting point analysis. The cDNA copy number of each gene was extrapolated from a standard curve prepared using serial 10-fold dilution of genomic DNA from the respective species. Expression of porin genes was normalized to *rpoB* in the same sample. Fold change in porin expression was determined by calculating the ratio of normalized porin expression in CRE isolates to a pan-sensitive control strain of the same species. Each experiment was performed in triplicate, and results were presented as mean value of three experiments. *E. coli* ATCC 25922, *E. cloacae* ATCC 13047, *E. aerogenes* ATCC 13048, *K. pneumoniae* ATCC13883, and *C. freundii* ATCC 8454 were used as negative controls and *K. pneumoniae* isolate #404 (Hong et al., 2013) was used as a positive control for porin down-regulation.

Whole genome sequencing. Genomic DNA was extracted from bacterial cultures with the Gentra Puregene Yeast/Bact. Kit (Qiagen) per the manufacturer's instructions. Dual-indexed sequencing libraries were prepared using the Nextera XT Sample Prep Kit (Illumina, San Diego CA). Libraries were subjected to 101bp paired-end sequencing on the Illumina HiSeq 4000 platform, to a median coverage of 200X per strain (range 115-278X) (Supplementary Table 6). Sequencing data were demultiplexed by unique barcodes. Reads were deduplicated using SuperDeduper v1.4 with the start location in the read at 5 bp (-s 5) and length 50 (-l 50) (Petersen et al., 2015). Deduplicated reads were then trimmed using TrimGalore v0.4.4, a wrapper for CutAdapt, with a minimum quality score of 30 for trimming (-q 30), minimum read length of 50 (--length 50) and the "--nextera" flag. Preprocessed reads for each isolate were aligned to the RefSeq reference

genome for the respective species using the Burrows-Wheeler Aligner (BWA) v0.7.10 with default parameters. Reference genomes are show in in Supplementary Table 3. Pileup files were generated using Samtools v1.5 (Li et al., 2009), and Varscan v2.3.9 was used to identify single nucleotide variants (SNVs) with at least 40× coverage (--min-coverage 40), 90% frequency (--min-var-freq 0.9), and base quality of at least 20 to support a base call (--min-avg-qual 20), with the strand filter parameter turned off (--strand-filter 0). (Koboldt et al., 2012). Varscan output was parsed with custom scripts to generate a consensus sequence for each sample, requiring at least 0.9 frequency to support a SNP or reference base call. SNVs between strain pairs were counted using custom scripts. To build phylogenetic trees, core genome positions were identified between all strains of a given species. Core genome positions are defined as genome positions where a base call can be made for each input genome. Core SNVs were concatenated into a FASTA file for each sample using custom scripts. Multiple sequence alignments were performed with MAFFT v7.31 with the "--auto" flag, and approximate maximum likelihood phylogenetic trees were computed from the resulting alignments using FastTree v2.1.7 (Katoh et al., 2002; Price et al., 2010). Trees were midpoint rooted and visualized with FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). An isolate of *E. coli* that was sequenced in two separate runs was analyzed with this pipeline and shown to yield zero SNVs, as one would expect for an identical strain. Genome sequences for CRE isolates were deposited in the NCBI BioSample database (accession numbers SAMN08623777- SAMN08623838) (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP133707).

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Supplementary Table 1. Carbapenemase gene primers used in this study.

Primer Set	Name	Target Gene	Sequence	Amplicon Length (bp)	Tm (°C)	Concentration (µM)
Reaction 1	16S univ 806-F	16S rRNA	ATTAGATACCCTGGTAGTCC	319	82	0.25
	16S univ 1104-R	16S rRNA	TCGTTGCGGGACTTAACC			
	IMI FWD-2	*blaIMI/ blaNMC-A*	GCTTTACAATATAGCGACAATGG	133	73.9	0.5
	IMI REV-2	*blaIMI/ blaNMC-A*	GATCTAACTCCCAACGATCGAG			
	SME FWD-2	*blaSME*	ATTTCTTGGCGGTCCTGAG	180	76.9	0.5
	SME REV-2	*blaSME*	TTGAGAACATTCCCCAAAGC			
Reaction 2	GIM FWD-3	*blaGIM*	GATCGCACTGCTGGTATCAA	186	78.9	0.5
	GIM REV-3	*blaGIM*	ATCCTCTGTATGCCCAGCAC			
	SIM FWD-2	*blaSIM*	CAACACATTTCCACGACGAC	249	75.3	0.5
	SIM REV-2	*blaSIM*	AGCCCCCGAATAGGATTTTT			
Reaction 3	GES FWD-3	*blaGES*	TCTAGCATCGGGACACATGA	164	81.5	0.5
	GES REV-3	*blaGES*	CTTTCCGGTCTAGCCGACTC			
	SPM FWD-1	*blaSPM*	AAAATCTGGGTACGCAAACG	271	79.0	0.5
	SPM REV-1	*blaSPM*	ACATTATCCGCTGGAACAGG			

16S rRNA was used as internal control for extraction and amplification

Supplementary Table 2. Quantitative reverse transcription-PCR primers used in this study.

Organism	Name	Target Gene	Sequence	Amplicon Length (bp)
E. coli, K. pneumoniae, C. freundii complex	ompC-FWD-02	*OmpC/ OmpK36*	AAGTCGAACTGGTACTGAGCAA	98
	ompC-REV-02		CAGTACACCCAGACCTACAACG	
E. aerogenes	ompC-FWD-02	*OmpC* analog	AAGTCGAACTGGTACTGAGCAA	98
	ompC-REV-02-Eaer		CAGTACACCCAGACYTACAACG	
E. cloacae complex	ompC-FWD-01-Eclo	*OmpC* analog	GCRCCMACRTCAACATAYTTCAG	123
	ompC-REV-01-Eclo		GGTTGCKCAGTATCAGTTCGAC	
E. coli, C. freundii complex	ompF-FWD-06	*OmpF*	TCTGGCAGCATATCGGTGTA	182
	ompF-REV-06		CGGTTATGGTCAGTGGGAAT	
K. pneumoniae	ompF-FWD-07	*OmpK35*	GAARCCGTCGCCATTCTG	179
	ompF-REV-07		ATGACCGGYCGTACCAAC	
E. aerogenes	ompF-FWD-07	*OmpF* analog	GAARCCGTCGCCATTCTG	140
	ompF-REV-07-Eaer		ATGACCGGMCGTACCAAC	
E. coli, K. pneumoniae, E. aerogenes, C. freundii, E. cloacae complex	rpoB-FWD-01	*rpoB*	AAGGCGAATCCAGCTTGTTCAGC*	148
	rpoB-REV-01		TGACGTTGCATGTTCGCACCCATCA*	

*Obtained from Hong et al., 2013

Supplementary Table 3. Reference genomes used for phylogenetic analysis.

Species	Reference genome	NCBI Nucleotide accession
Citrobacter freundii complex	*Citrobacter freundii CFNIH1*	NZ_CP007557.1
Enterobacter aerogenes	*Enterobacter aerogenes KCTC 2190*	NC_015663.1
Enterobacter cloacae complex	*Enterobacter cloacae subsp. cloacae ATCC 13047*	NC_014121.1
Escherichia coli	*Escherichia coli str. K-12 substr. MG1655*	NC_000913.3
Klebsiella pneumoniae	*Klebsiella pneumoniae subsp. pneumoniae HS11286*	NC_016845.1
Serratia marcescens	*Serratia marcescens subsp. marcescens Db11*	NZ_HG326223.1

Supplementary Table 4. Annual CRE rates at Stanford Health Care.

Species	No. of CRE/CRE + non-CRE isolates (%)				
	2013	2014	2015	2016	2013-16
Citrobacter freundii complex	0/120 (0)	2/81 (2.5)	0/93 (0)	1/108 (0.9)	3/402 (0.7)
Enterobacter aerogenes	2/122 (1.6)	1/92 (1.1)	4/104 (3.8)	1/115 (0.9)	8/433 (1.8)
Enterobacter cloacae complex	3/240 (1.3)	0/226 (0)	3/239 (1.3)	8/289 (2.8)	14/994 (1.4)
Escherichia coli	1/3117 (0)	4/2080 (0.2)	2/3008 (0.1)	4/3834 (0.1)	11/12039 (0.1)
Klebsiella pneumoniae	4/631 (0.6)	7/503 (1.4)	7/565 (1.2)	1/824 (0.1)	19/2523 (0.8)
Serratia marcescens	1/161 (0.6)	0/113 (0)	2/157 (1.3)	4/143 (2.8)	7/574 (1.2)
All species	11/5001 (0.2)	14/3550 (0.4)	18/4752 (0.4)	19/5968 (0.3)	62/19271 (0.3)

No CRE isolates were found for *Citrobacter koseri*, *Klebsiella oxytoca*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, or *Salmonella enterica*. Per the CLSI, imipenem MIC for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be in the non-susceptible range due to mechanisms other than carbapenemases. Therefore, imipenem non-susceptiblity was not used in CRE rate calculation for these organisms.

Supplementary Table 5. Genotypic and phenotypic antibiotic susceptibility testing results and relative porin expression of CRE isolates in this study.

Isolate ID No.	Species	Carbapenemase gene	Carbapenemase activity by mCIM	ESBL/pAmpC	Porin Protein Quantitation*		RNA Porin Expression*		MIC (μg/ml)						
					OmpC or analog	OmpF or analog	*ompC* or analog	*ompF* or analog	Imipenem (MicroScan)	Meropenem (MicroScan)	Ertapenem (MicroScan)	Imipenem-Relabactam (BMD)	Meropenem-Vaborbactam (test strip)	Ceftazidime-Avibactam (Etest)	Ceftolozane-Tazobactam (Etest)
CRE01	*E. coli*	Negative	Negative	CTX-M group (1,type-15 like)	0↓	0↓	0.22↓	0.05↓	4-R	8-R	>4-R	0.5-S	2-S	1-S	128-R
CRE02	*E. cloacae* complex	*bla* $_{IMP}$	Positive	SHV (238S+240K)	1.82=	0.1↓	4.11↑	ND	≤1-S	4-R	>4-R	0.5-S	8-I	32-R	≥256-R
CRE03	*E. aerogenes*	Negative	Negative	Negative	0↓	0.7=	6.52↑	0.76=	2-I	≤1-S	2-R	0.25-S	0.12-S	1-S	2-S
CRE04	*K. pneumoniae*	Negative	Negative	CTX-M group (1, type-15 like)	0.09↓	0↓	8.94↑	0.01↓	4-R	8-R	>4-R	0.5-S	4-S	4-S	≥256-R
CRE05	*S. marcescens*	Negative	Negative	Negative	1.68=	0.5=	ND	ND	>8-R	≤1-S	≤0.5-S	4-R	0.25-S	2-S	4-I
CRE06	*E. cloacae* complex	Negative	Negative	ACT/MIR	0↓	2.02=	ND	ND	≤1-S	≤1-S	4-R	0.25-S	0.5-S	2-S	32-R
CRE07	*K. pneumoniae*	*bla* $_{OXA-48-like}$	Positive	CTX-M group (1, type-15 like)	0.33↓	0.23↓	0.67=	1.36=	2-I	≤1-S	4-R	1-S	4-S	0.25-S	4-I
CRE08	*K. pneumoniae*	*bla* $_{OXA-48-like}$	Positive	CTX-M group (1, type-15 like)	0.49↓	0↓	2.07↑	1.27=	≤1-S	≤1-S	>4-R	1-S	1-S	1-S	32-R
CRE09	*E. aerogenes*	Negative	Negative	Negative	0↓	0.01↓	0.14↓	0.04↓	>8-R	>8-R	>4-R	8-R	16-R	4-S	16-R
CRE10	*K. pneumoniae*	*bla* $_{OXA-48-like}$	Positive	CTX-M group (1, type-15 like)	0↓	0.04↓	0.27↓	1.71=	4-R	2-I	>4-R	8-R	16-R	1-S	32-R
CRE11	*E. cloacae* complex	Negative	Negative	ACT/MIR	0.26↓	3↑	0.38↓	ND	2-I	4-R	>4-R	0.25-S	1-S	2-S	32-R
CRE12	*K. pneumoniae*	*bla* $_{IMP}$	Positive	DHA; CTX-M group (1,type-15 like)	0.03↓	0↓	7.95↑	1.45=	≤1-S	>8-R	>4-R	0.5-S	16-R	≥256-R	≥256-R
CRE13	*K. pneumoniae*	*bla* $_{KPC}$	Positive	SHV(238S+240k)	1.38=	0↓	23.28↑	1.53=	>8-R	>8-R	>4-R	0.5-S	0.5-S	4-S	64-R
CRE14	*E. coli*	*bla* $_{OXA-48-like}$	Positive	CMY-II	1.35=	0.34↓	7.56↑	50.61↑	≤1-S	≤1-S	≤1-S	0.5-S	1-S	0.5-S	32-R
CRE15	*E. coli*	Negative	Negative	CTX-M group (1,type-15 like)	0↓	0↓	0.03↓	162.56↑	>8-R	>8-R	>4-R	8-R	8-I	2-S	64-R
CRE16	*E. coli*	*bla* $_{OXA-48-like}$	Positive	CTX-M9	0.36↓	0.03↓	4.28↑	0.06↓	≤1-S	≤1-S	4-R	0.5-S	4-S	1-S	4-I
CRE17	*E. coli*	*bla* $_{NDM}$	Positive	CTX-M group (1,type-15 like)	0.88=	0↓	1.64=	0.11↓	>8-R	>8-R	>4-R	64-R	≥256-R	≥256-R	≥256-R
CRE18	*K. pneumoniae*	*bla* $_{NDM}$	Positive	Negative	0.17↓	0↓	0.28↓	0.33↓	8-R	>8-R	>4-R	8-R	32-R	≥256-R	≥256-R
CRE20†	*C. freundii* complex	Negative	Negative	Negative	1.43=	0.45↓	18.10↑	ND	≤1-S	≤1-S	≤0.5-S	0.5-S	0.5-S	0.5-S	64-R
CRE21†	*C. freundii* complex	Negative	Negative	Negative	4.37↑	1.45=	59↑	ND	2-I	≤1-S	4-R	0.5-S	2-S	1-S	8-R
CRE22	*K. pneumoniae*	Negative	Negative	CTX-M group (1, type-15 like)	0.02↓	0↓	0↓	0.64=	≤1-S	4-R	>4-R	0.5-S	2-S	1-S	128-R
CRE23	*E. aerogenes*	Negative	Negative#	Negative	0↓	0.01↓	0.31↓	0.2↓	>8-R	>8-R	>4-R	0.5-S	2-S	1-S	2-S
CRE24	*K. pneumoniae*	Negative	Negative	CTX-M group (1, type-15 like)	0.05↓	0↓	3.02↑	0.37↓	≤1-S	2-I	>4-R	0.5-S	1-S	1-S	2-S
CRE25	*K. pneumoniae*	Negative	Negative	CTX-M group (1, type-15 like)	0.03↓	0↓	1.35=	1.51=	4-R	>8-R	>4-R	4-R	8-I	8-S	≥256-R
CRE26	*K. pneumoniae*	*bla* $_{OXA-48-like}$	Positive	CTX-M group (1, type-15 like)	0.08↓	0↓	11.25↑	5.88↑	2-I	>8-R	>4-R	4-R	16-R	2-S	≥256-R
CRE27	*E. aerogenes*	Negative	Negative#	Negative	0↓	0.01↓	0.49↓	0.08↓	2-I	4-R	>4-R	1-S	0.25-S	2-S	4-I
CRE28	*K. pneumoniae*	*bla* $_{NDM}$	Positive	CTX-M group (1, type-15 like)	0.87=	0↓	1.85=	0.02↓	>8-R	>8-R	>4-R	16-R	16-R	≥256-R	≥256-R
CRE30	*K. pneumoniae*	Negative	Negative	SHV(238S+240k)	0.01↓	0↓	0.06↓	0.01↓	4-R	8-R	>4-R	1-S	4-S	1-S	1-S
CRE31	*K. pneumoniae*	*bla* $_{VIM}$	Positive	Negative	1.3=	0↓	20.92↑	1.5=	8-R	8-R	4-R	8-R	8-I	≥256-R	≥256-R
CRE32	*K. pneumoniae*	Negative	Negative	CTX-M group (1, type-15 like)	0.18↓	0↓	0.37↓	0.2↓	≤1-S	4-R	4-R	0.5-S	1-S	2-S	32-R
CRE33	*E. cloacae* complex	Negative	Negative	ACT/MIR	0.12↓	0↓	3.16↑	ND	4-R	4-R	>4-R	0.5-S	4-S	2-S	16-R
CRE34	*E. cloacae* complex	Negative	Negative#	ACT/MIR	5.7↑	0.31↓	0.71=	ND	2-I	≤1-S	≤0.5-S	0.5-S	0.12-S	1-S	4-I
CRE35	*S. marcescens*	*bla* $_{SME}$	Positive	Negative	1.3=	1.53=	ND	ND	>8-R	>8-R	>4-R	4-R	0.06-S	0.25-S	1-S
CRE36	*E. cloacae* complex	*bla* $_{KPC}$	Positive	ACT/MIR; SHV (238S+240K)	5.36↑	0.12↓	3.19↑	ND	8-R	8-R	>4-R	0.5-S	0.12-S	1-S	8-R
CRE37	*K. pneumoniae*	*bla* $_{KPC}$	Positive	CTX-M group (CTX-M9); SHV(238S+240k)	0.09↓	0↓	3.41↑	6.93↑	>8-R	>8-R	>4-R	0.5-S	4-S	2-S	32-R
CRE38	*E. aerogenes*	Negative	Negative	Negative	9.33↑	0.01↓	6.96↑	0.27↓	>8-R	8-R	>4-R	0.5-S	2-S	2-S	4-I
CRE39	*E. aerogenes*	Negative	Negative	Negative	2.2↑	0↓	5.30↑	0.6=	2-I	4-R	>4-R	0.5-S	2-S	2-S	8-R
CRE40	*K. pneumoniae*	Negative	Negative	CTX-M group (1, type-1 like)	0.02↓	0↓	0.12↓	0.05↓	≤1-S	4-R	>4-R	0.5-S	1-S	0.5-S	8-R
CRE41	*E. aerogenes*	Negative	Negative	Negative	0↓	0↓	1.14=	0.18↓	4-R	≤1-S	1-I	1-S	0.25-S	0.5-S	1-S
CRE42	*E. coli*	Negative	Negative	Negative	0.19↓	0↓	9.50↑	9.15↑	≤1-S	4-R	>4-R	0.25-S	4-S	2-S	≥256-R
CRE43	*K. pneumoniae*	*bla* $_{KPC}$	Positive	CTX-M group (1, type-15 like)	0.31↓	2.1↑	14.04↑	4.82↑	2-I	2-I	1-I	0.25-S	0.06-S	0.25-S	4-I
CRE44	*S. marcescens*	Negative	Negative	Negative	1.33=	0.07↓	ND	ND	>8-R	2-I	>4-R	1-S	2-S	2-S	2-S
CRE45	*E. coli*	*bla* $_{NDM}$	Positive	CTX-M group (1,type-1 like)	0.86=	0↓	5.29↑	13.01↑	>8-R	>8-R	>4-R	4-R	32-R	≥256-R	≥256-R
CRE49	*S. marcescens*	*bla* $_{SME}$	Positive	Negative	0.88=	0.99=	ND	ND	>8-R	>8-R	>4-R	4-R	0.06-S	0.12-S	0.5-S
CRE50	*E. coli*	Negative	Negative	CTX-M1, type 15-like	0.11↓	0↓	ND	ND	≤1-S	2-I	>4-R	0.25-S	1-S	1-S	2-S
CRE54	*E. cloacae* complex	Negative	Negative	Negative	0.28↓	0.3↓	ND	ND	2-I	≤1-S	4-R	0.5-S	1-S	2-S	1-S
CRE60	*E. coli*	Negative	Negative	CMY II	0.11↓	0↓	ND	ND	≤1-S	≤1-S	1-I	0.25-S	0.12-S	1-S	16-R
CRE65	*E. cloacae* complex	Negative	Negative	ACT/MIR	0.14↓	0↓	ND	ND	≤1-S	4-R	>4-R	0.5-S	1-S	2-S	32-R
CRE71	*E. cloacae* complex	Negative	Negative#	Negative	4.69↑	4.22↑	ND	ND	2-I	≤1-S	1-I	0.5-S	0.25-S	1-S	1-S
CRE72	*S. marcescens*	*bla* $_{SME}$	Positive	Negative	0.15↓	0.01↓	ND	ND	>8-R	>8-R	>4-R	2-I	0.06-S	0.12-S	0.25-S
CRE73	*E. cloacae* complex	*bla* $_{KPC}$	Positive	ACT/MIR	0.06↓	2.22↑	ND	ND	8-R	8-R	>4-R	0.5-S	0.06-S	1-S	4-I
CRE74	*E. cloacae* complex	Negative	Negative	ACT/MIR	0.07↓	0.91=	ND	ND	≤1-S	≤1-S	2-R	0.5-S	0.25-S	2-S	32-R
CRE75	*E. coli*	Negative	Negative	CTX-M1, type 15-like	0.04↓	0↓	ND	ND	≤1-S	≤1-S	>4-R	0.25-S	1-S	1-S	16-R
CRE77	*E. aerogenes*	Negative	Negative#	Negative	0.5=	0.02↓	ND	ND	2-I	≤1-S	1-I	0.5-S	0.12-S	2-S	4-I
CRE78	*S. marcescens*	*bla* $_{SME}$	Positive	Negative	0.27↓	0.22↓	ND	ND	>8-R	>8-R	>4-R	2-I	0.12-S	0.12-S	0.25-S
CRE81	*E. cloacae* complex	Negative	Negative	ACT/MIR	2.58↑	0.63=	ND	ND	2-I	≤1-S	2-R	1-S	0.25-S	1-S	1-S

CRE83	*K. pneumoniae*	Negative	Negative	CTX-M9	0.31↓	0↓	ND	ND	≤1-S	2-I	>4-R	0.12-S	0.06-S	1-S	4-I
CRE86	*E. cloacae* complex	Negative	Negative	ACT/MIR	3.19↑	0↓	ND	ND	≤1-S	≤1-S	2-R	0.5-S	0.5-S	1-S	0.5-S
CRE87	*E. coli*	bla_{NDM}	Positive	CTX-M-1, SHV(type-15 like)	0.93≈	0↓	ND	ND	>8-R	>8-R	>4-R	8-R	32-R	≥256-R	≥256-R
CRE88	*E. cloacae* complex	Negative	Negative	ACT/MIR	0.02↓	0.02↓	ND	ND	≤1-S	≤1-S	4-R	1-S	1-S	1-S	2-S
CRE89	*C. freundii* complex	Negative	Negative	CMY II	1.29≈	0.12↓	ND	ND	≤1-S	≤1-S	2-R	0.25-S	0.12-S	2-S	16-R
CRE94	S. marcescens	bla_{SME}	Positive	Negative	0.17↓	0.09↓	ND	ND	>8-R	8-R	4-R	8-R	0.06-S	0.12-S	0.25-S

pAmpC, plasmid-encoded AmpC; BMD, broth microdilution; ND, not done; S, susceptible; I, intermediate; R, resistant. † Isolates obtained from same patient.

* Relative to susceptible control strains; RNA porin expresssion normalized to rpoB gene. Arrows show direction of change by 2-fold; ≈ indicates no change**.** # mCIM indeterminate but negative with MALDI-TOF-based assay**.** Grey shading identifies isolates that were called CRE by Vitek2 and/or disk diffusion but did not meet CRE definition with MicroScan testing; 8/10 isolates with grey shading tested had Microscan and Vitek2 results within 1 doubling dilution.

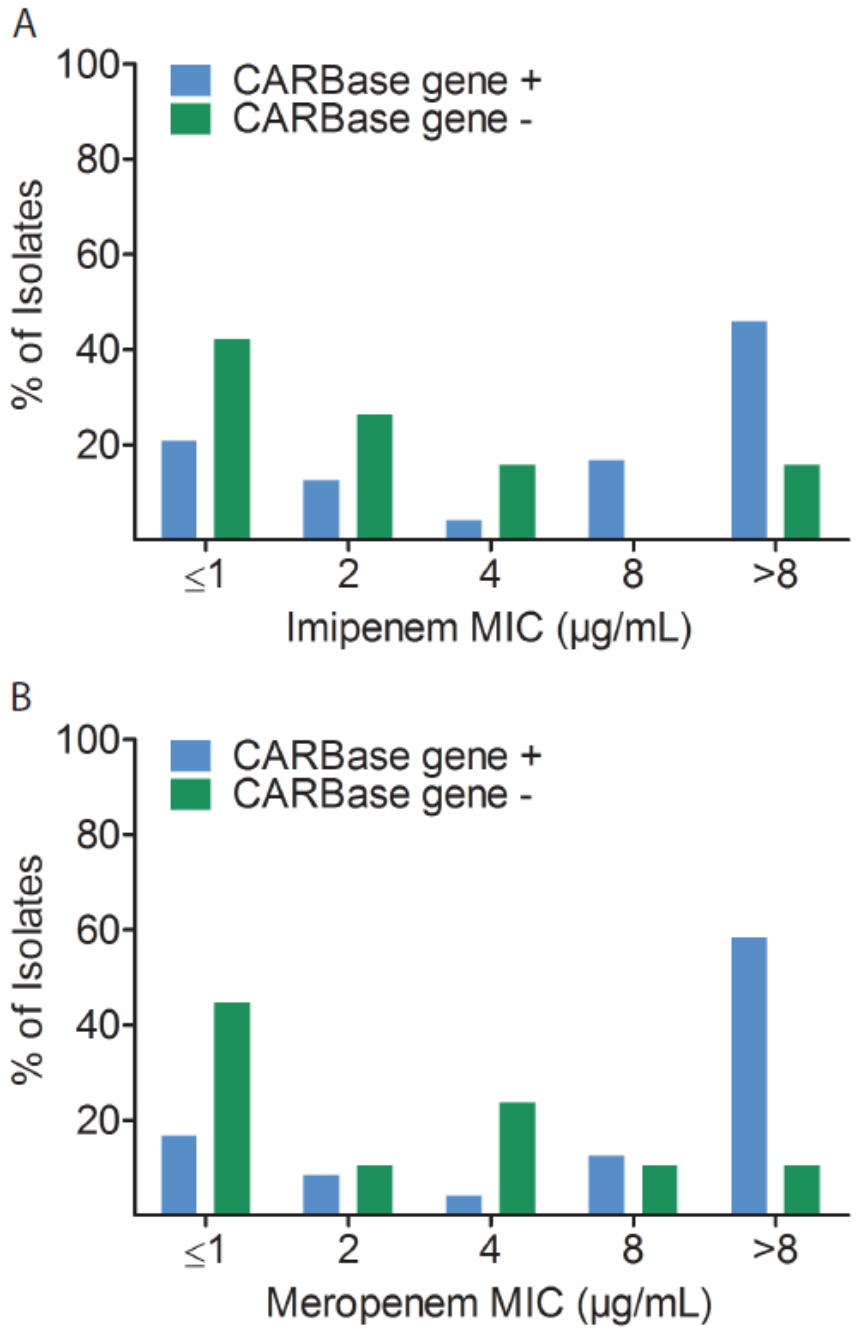
Supplementary Table 6. Whole-genome sequencing statistics

Sample	Species	Raw reads	Preprocessed reads	Reference genome size (Mb)	X coverage (raw reads)
CRE20	*Citrobacter freundii* complex	13621438	10894438	5.37	254
CRE21	*Citrobacter freundii* complex	9564406	7713852	5.37	178
CRE89	*Citrobacter freundii* complex	13308770	8630022	5.37	248
CRE03	*Enterobacter aerogenes*	9541404	7508428	5.28	181
CRE09	*Enterobacter aerogenes*	6462500	5028208	5.28	122
CRE23	*Enterobacter aerogenes*	9118030	7400354	5.28	173
CRE27	*Enterobacter aerogenes*	11193950	8940376	5.28	212
CRE38	*Enterobacter aerogenes*	9115142	7316246	5.28	173
CRE39	*Enterobacter aerogenes*	8060022	6031210	5.28	153
CRE41	*Enterobacter aerogenes*	12977462	8201800	5.28	246
CRE77	*Enterobacter aerogenes*	8545212	6796522	5.28	162
CRE02	*Enterobacter cloacae* complex	13531886	8761830	5.60	242
CRE06	*Enterobacter cloacae* complex	7377564	5602404	5.60	132
CRE11	*Enterobacter cloacae* complex	8613630	6452940	5.60	154
CRE33	*Enterobacter cloacae* complex	8360194	6470158	5.60	149
CRE34	*Enterobacter cloacae* complex	6420570	5113068	5.60	115
CRE36	*Enterobacter cloacae* complex	13144454	8457494	5.60	235
CRE54	*Enterobacter cloacae* complex	7455674	5970668	5.60	133
CRE65	*Enterobacter cloacae* complex	6499668	5309170	5.60	116
CRE71	*Enterobacter cloacae* complex	9122156	7348270	5.60	163
CRE73	*Enterobacter cloacae* complex	9670596	7753642	5.60	173
CRE74	*Enterobacter cloacae* complex	8312990	6673682	5.60	148
CRE81	*Enterobacter cloacae* complex	10786676	6973542	5.60	193
CRE86	*Enterobacter cloacae* complex	9809582	6329290	5.60	175
CRE88	*Enterobacter cloacae* complex	13698842	8538698	5.60	245
CRE01	*Escherichia coli*	10950698	8549600	5.59	196
CRE14	*Escherichia coli*	12461068	8080870	5.59	223
CRE15	*Escherichia coli*	8607294	6926228	5.59	154
CRE16	*Escherichia coli*	8904536	7173848	5.59	159
CRE17	*Escherichia coli*	12166272	7735208	5.59	218
CRE42	*Escherichia coli*	11185524	8868436	5.59	200
CRE45	*Escherichia coli*	15567734	9865222	5.59	278
CRE50	*Escherichia coli*	13599548	8634854	5.59	243
CRE60	*Escherichia coli*	7044844	5638744	5.59	126
CRE75	*Escherichia coli*	9623464	7529836	5.59	172

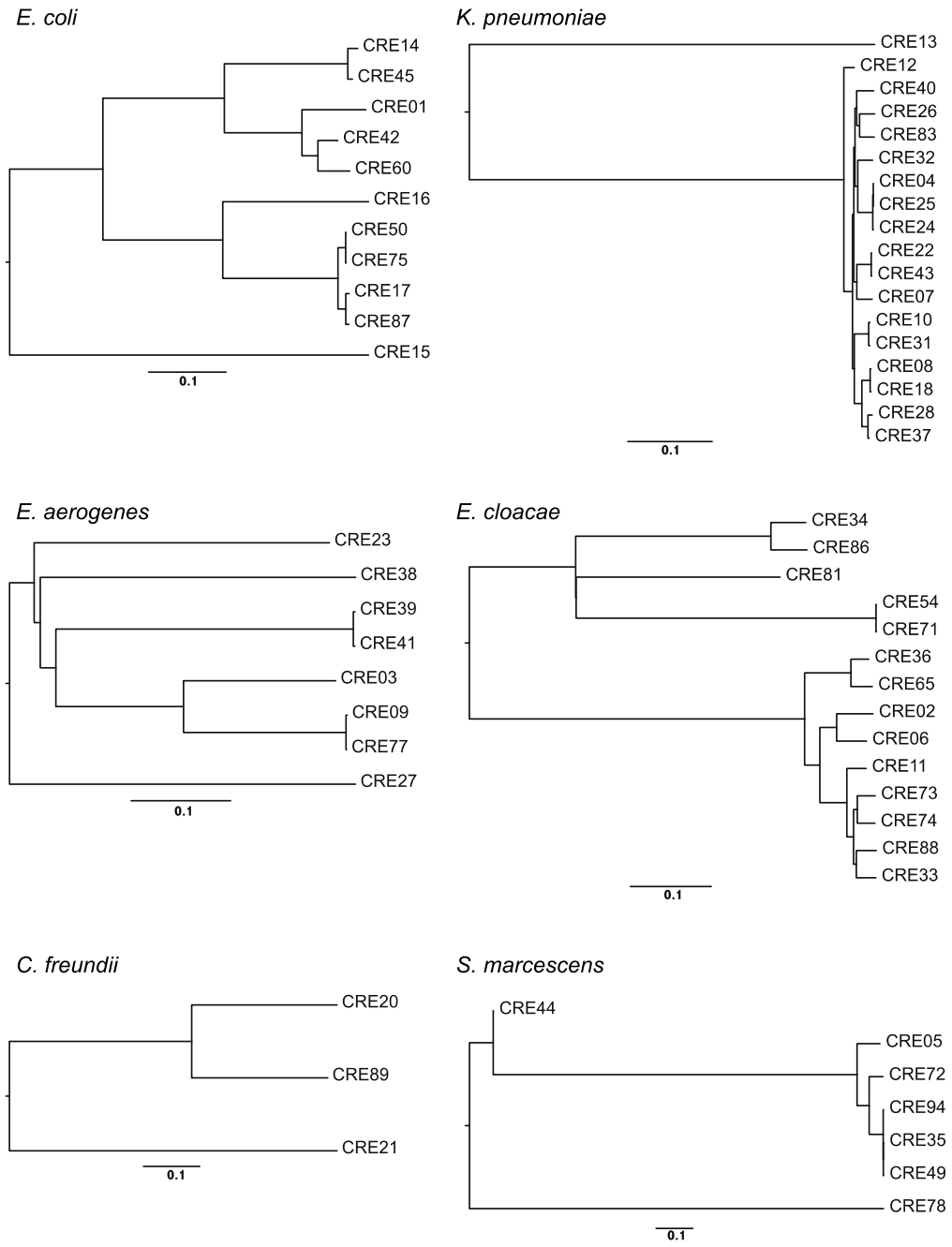
CRE87	*Escherichia coli*	14558562	9381560	5.59	260
CRE04	*Klebsiella pneumoniae*	6765882	5326958	5.68	119
CRE07	*Klebsiella pneumoniae*	12140446	7919442	5.68	214
CRE08	*Klebsiella pneumoniae*	12406860	8005792	5.68	218
CRE10	*Klebsiella pneumoniae*	15118352	9446164	5.68	266
CRE12	*Klebsiella pneumoniae*	12280676	8010072	5.68	216
CRE13	*Klebsiella pneumoniae*	12545194	7958630	5.68	221
CRE18	*Klebsiella pneumoniae*	11050234	7131952	5.68	195
CRE22	*Klebsiella pneumoniae*	14653398	11638104	5.68	258
CRE24	*Klebsiella pneumoniae*	9173260	5992434	5.68	162
CRE25	*Klebsiella pneumoniae*	9606814	7768046	5.68	169
CRE26	*Klebsiella pneumoniae*	11895904	7704752	5.68	209
CRE28	*Klebsiella pneumoniae*	11307766	7251890	5.68	199
CRE30	*Klebsiella pneumoniae*	10572724	6785704	5.68	186
CRE31	*Klebsiella pneumoniae*	12827720	8271230	5.68	226
CRE32	*Klebsiella pneumoniae*	9863376	7210062	5.68	174
CRE37	*Klebsiella pneumoniae*	12438958	7990386	5.68	219
CRE40	*Klebsiella pneumoniae*	10061548	8201864	5.68	177
CRE43	*Klebsiella pneumoniae*	11872312	7534866	5.68	209
CRE83	*Klebsiella pneumoniae*	12139628	7782548	5.68	214
CRE05	*Serratia marcescens*	10587920	6785166	5.11	207
CRE35	*Serratia marcescens*	11824004	7577772	5.11	231
CRE44	*Serratia marcescens*	10640256	6829166	5.11	208
CRE49	*Serratia marcescens*	11317462	7249150	5.11	221
CRE72	*Serratia marcescens*	12050524	7664790	5.11	236
CRE78	*Serratia marcescens*	11661294	7549384	5.11	228
CRE94	*Serratia marcescens*	12125614	7619854	5.11	237

Supplementary Table 7. Single nucleotide variants (SNVs) of related CRE strains.

Isolate ID No.	Species	Collection Date	Carbapenemase gene	SNVs
CRE50	*E. coli*	2/1/2016	Negative	136
CRE75		8/16/2016	Negative	
CRE17	*E. coli*	10/15/2014	bla_{NDM}	1527
CRE87		11/13/2016	bla_{NDM}	
CRE04	*K. pneumoniae*	4/6/2013	Negative	5
CRE24		12/28/2014	Negative	
CRE04	*K. pneumoniae*	4/6/2013	Negative	7
CRE25		12/31/2014	Negative	
CRE24	*K. pneumoniae*	12/28/2014	Negative	10
CRE25		12/31/2014	Negative	
CRE08	*K. pneumoniae*	8/2/2013	$bla_{OXA\text{-}48\ like}$	1907
CRE18		10/19/2014	bla_{NDM}	
CRE22	*K. pneumoniae*	11/19/2014	Negative	163
CRE43		12/24/2015	bla_{KPC}	
CRE35	*S. marcescens*	9/2/2015	bla_{SME}	30
CRE49		1/27/2016	bla_{SME}	
CRE35	*S. marcescens*	9/2/2015	bla_{SME}	40
CRE94		12/5/2016	bla_{SME}	
CRE49	*S. marcescens*	1/27/2016	bla_{SME}	40
CRE94		12/5/2016	bla_{SME}	
CRE39	*E. aerogenes*	10/30/2015	Negative	1258
CRE41		11/18/2015	Negative	
CRE09	*E. aerogenes*	8/23/2013	Negative	176
CRE77		8/30/2016	Negative	
CRE54	*E. cloacae*	3/1/2016	Negative	55
CRE71		8/25/2016	Negative	



Supplementary Figure 1. Distribution of imipenem and meropenem MICs in CRE isolates with and without a carbapenemase gene. Bars show percentage of imipenem (A) and meropenem (B) MICs for carbapenemase gene-negative (CARBase gene -; green bars) and carbapenemase gene-positive (CARBase gene +; blue bars) CRE isolates. MICs were obtained with MicroScan.



Supplementary Figure 2. Phylogenetic trees for CRE isolates based on whole genome sequencing. Midpoint-rooted approximate maximum likelihood phylogenetic

trees were computed from multiple sequence alignments of concatenated SNVs on a per-species basis. Scale bars show evolutionary distances.