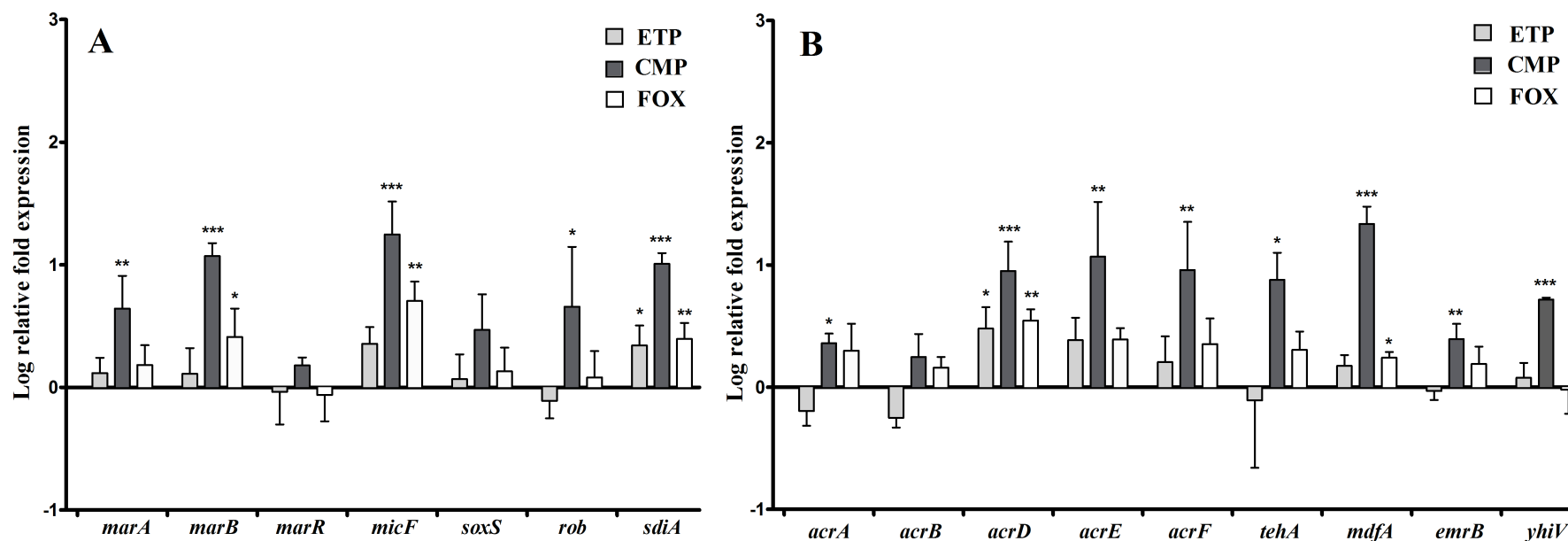


Figure S1. Relative mRNA expression level of genes implicated in MDR regulation (A), membrane efflux (B), permeability (C) for the ST131 H22 *E. coli* S250 and its mutants *in vitro*-selected by ertapenem (ETP), chloramphenicol (CMP) and cefoxitin (FOX).

The log-transformed averages of relative fold change of mutants compared to the parent strain for each antibiotic are presented. The error bars represent the standard deviation from three different RNA preparations. Significant differences from parent S250 using Dunnett's test are indicated by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).



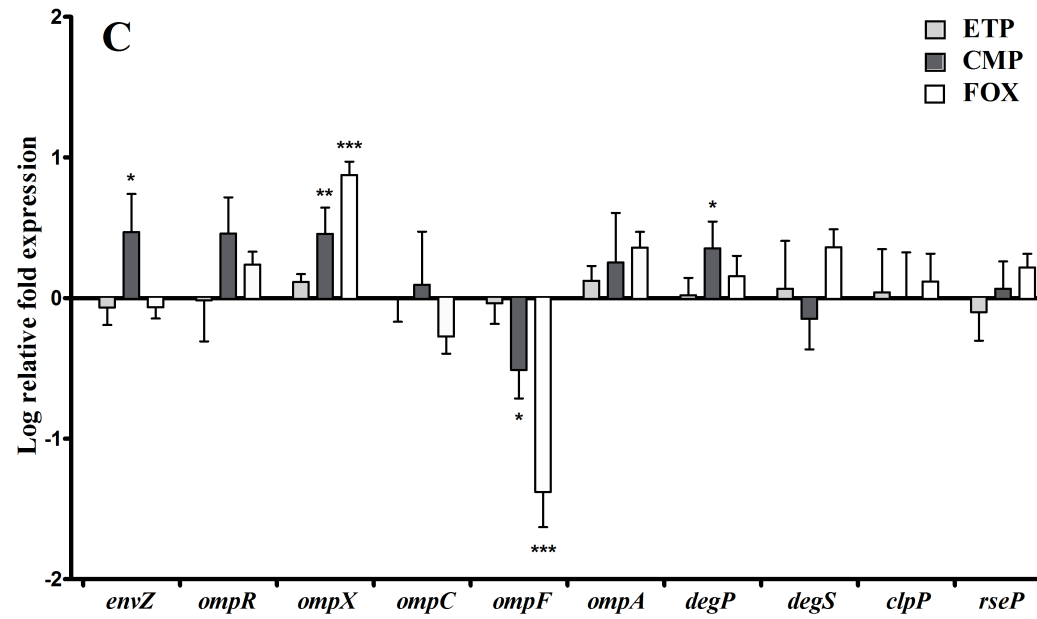
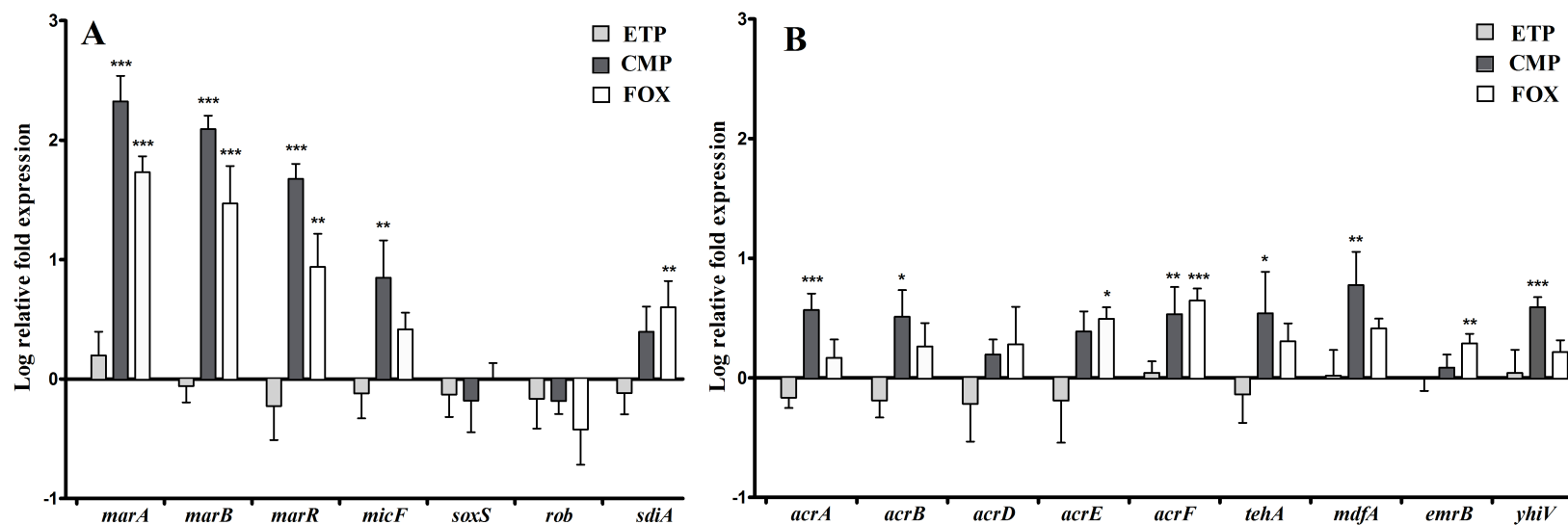


Figure S2. Relative mRNA expression level of genes implicated in MDR regulation (A), membrane efflux (B), permeability (C) for the reference strain *E. coli* CFT073 and its mutants *in vitro*-selected by ertapenem (ETP), chloramphenicol (CMP) and ceftiofur (FOX).

The log-transformed averages of relative fold change of mutants compared to the parent strain for each antibiotic are presented. The error bars represent the standard deviation from three different RNA preparations. Significant differences from parent CFT073 using Dunnett's test are indicated by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).



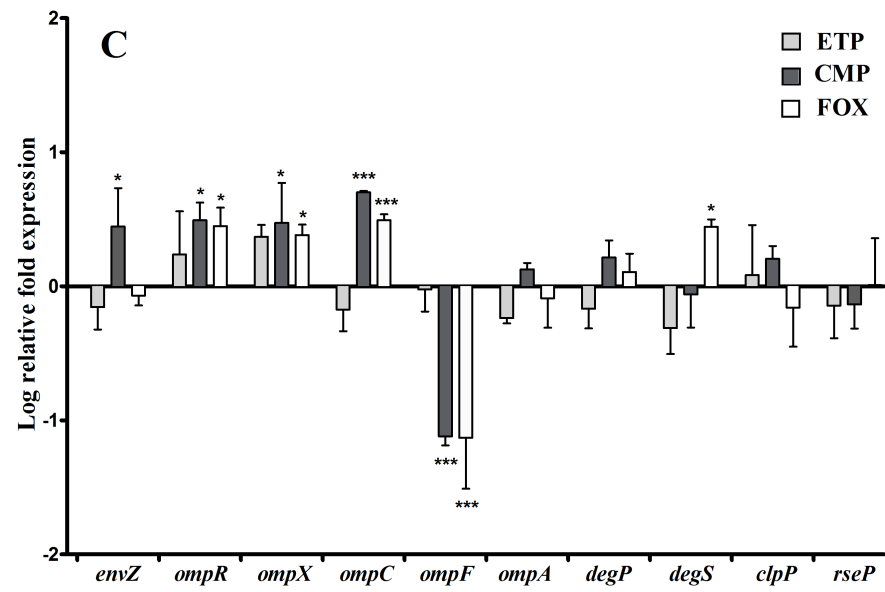


Table S1. Primers used in the study.

| Primer use and target function | Target region | Primer name | Oligonucleotide sequence | Tm (°C) | Reference | |
|-------------------------------------|--------------------|-----------------------------------|--|--|--|--------------|
| Sequencing | | | | | | |
| Global regulator of drug resistance | <i>marOR</i> | MarOR orab2 | 5'-GCTAGCCTTGCATCGCAT-3' | 56 | (1) | |
| | | MarOR or1R | 5'-GAATGGTAATAGCGTCAG-3' | 51.4 | | |
| | <i>acrR</i> | acrFR1 | 5'-CTAACGCCTGTAAATTCACG-3' | 55.3 | (1) | |
| | | acrRV1 | 5'-CCAGGAAAAATCCTGGAGTCAG-3' | 60.3 | | |
| | <i>soxRS</i> | SoxRS seq_F | 5'-GGCGAAGCTTCCGCAGGTGTTTATGC-3' | 68 | (2) | |
| SoxRS seq_R | | 5'-CGTCGGGGGAAGCTTTC CTGTGTACC-3' | 69.5 | | | |
| RT-PCR | | | | | | |
| Housekeeping gene | <i>gapA</i> | GAPDH_F GAPDH_R | 5'-ACTTACGAGCAGATCAAAGC-3' 5'-AGTTTCACGAAGTTGTCGTT-3' | 55.3 53.2 | (3) | |
| Global regulator of drug resistance | <i>marA</i> | marA_F marA_R | 5'-CATAGCATTTTGGACTGGAT-3' 5'-TACTTTCCTTCAGCTTTTGC-3' | 53.2 53.2 | (3) | |
| | | <i>marB</i> | marB_F marB_R | 5'-ATAGCAGCTGCGCTTATTC-3' 5'-ACTTATCACTGCCAGTACCC-3' | | 54.5 57.3 |
| | <i>marR</i> | | marR_F marR_R | 5'-AGCGATCTGTTCAATGAAAT-3' 5'-TTCAGTTCAACCGGAGTAAT-3' | 51.1 53.2 | (3) |
| | | <i>soxS</i> | soxS_F soxS_R | 5'-CCATTGCGATATCAAAAATC-3' 5'-ATCTTATCGCATGGATTGAC-3' | 51.1 53.2 | |
| | <i>rob</i> | | rob_F rob_R | 5'-GTCGTCTTTATCCTGACTCG-3' 5'-TTTGTACCCTGGAAGATAC-3' | 57.3 55.3 | (3) |
| | | <i>envZ</i> | envZ_F envZ_R | 5'-CGTTGAGGTCAACAAAAGTT-3' 5'-GTCGGTTCTGGATACGAATA-3' | 53.2 55.3 | |
| | <i>micF</i> | | micF_F micF_R | 5'-TCATCATTAACCTTTATTTATTACCG-3' 5'-GCATCCGGTTGAAATAGG-3' | 53.1 53.7 | (3) |
| | | <i>sdiA</i> | sdiA_F sdiA_R | 5'-CTTTCGCGCACCAGTAACTG-3' 5'-CGCAGAAGAGGTCTACCATG-3' | 59.4 59.4 | |
| | Efflux transporter | | <i>acrA</i> | acrA_F acrA_R | 5'-CTTAGCCCTAACAGGATGTG-3' 5'-TTGAAATTACGCTTCAGGAT-3' | 57.3 51.1 |
| | | <i>acrB</i> | | acrB_F acrB_R | 5'-CGTACACAGAAAGTGCTCAA-3' 5'-CGCTTCAACTTTGTTTTCTT-3' | 55.3 51.1 |
| | | | <i>acrD</i> | acrD_F acrD_R | 5'-GATTATCTTAGCCGCTTCAA-3' 5'-CAATGGAGGCTTTAACAAAC-3' | 53.2 53.2 |
| | | <i>acrE</i> | | acrE_F | 5'-GCCCTCCTTTATTCTGATCT-3' | 55.3 |

| | | | | | |
|-----------------------------|--------------------|--------|------------------------------|------|-----|
| | | acrE_R | 5'-GGCTATACGATAAGCATTGG-3' | 55.3 | |
| | <i>acrF</i> | acrF_F | 5'-TAGCAATTTTCCTTTGTGGTT-3' | 51.1 | (3) |
| | | acrF_R | 5'-CCTTTACCCTCTTTCTCCAT-3' | 55.3 | |
| | <i>emrB</i> | emrB_F | 5'-ATTATGTATGCCGTCTGCTT-3' | 53.2 | (3) |
| | | emrB_R | 5'-TTCGCGTAAAGTTAGAGAGG-3' | 55.3 | |
| | <i>mdfA</i> | mdfA_F | 5'-TTTATGCTTTCGGTATTGGT-3' | 51.1 | (3) |
| | | mdfA_R | 5'-GAGATTA AACAGTCCGTTGC-3' | 55.3 | |
| | <i>tehA</i> | tehA_F | 5'-TGCTTCATTCTGGAGTTTCT-3' | 53.2 | (3) |
| | | tehA_R | 5'-TCATTCTTTGTCCTCTGCTT-3' | 53.2 | |
| | <i>yhiV (mdtF)</i> | yhiV_F | 5'-GCACTCTATGAGAGCTGGTC-3' | 59.4 | (3) |
| | | yhiV_R | 5'-CCTTCTTTCTGCATCATCTC-3' | 55.3 | |
| Outer membrane permeability | <i>ompF</i> | ompF_F | 5'-GAAC TTCGCTGTT CAGTACC-3' | 57.3 | (3) |
| | | ompF_R | 5'-CGTACTTCAGACCAGTAGCC-3' | 59.4 | |
| | <i>ompC</i> | ompC_F | 5'-CTTCAAAGGTGAAACTCAGG-3' | 55.3 | (3) |
| | | ompC_R | 5'-GTTGTCAGAACCGTAGGTGT-3' | 57.3 | |
| | <i>ompA</i> | ompA_F | 5'-ACGACTGGTTAGGTCGTATG-3' | 57.3 | (3) |
| | | ompA_R | 5'-ACGTTGGATTTAGTGTCTGC-3' | 55.3 | |
| | <i>ompX</i> | ompX_F | 5'-ACCTGAAAATACCGCTATGAA-3' | 53.2 | (3) |
| | | ompX_R | 5'-TCAGTGGTCTGGAATTTACC-3' | 55.3 | |
| | <i>ompR</i> | ompR_F | 5'-GACGTCTTCGTAGTCAGAGC-3' | 59.4 | (3) |
| | | ompR_R | 5'-TTGAACTTACCGAAAAGCAAT-3' | 51.1 | |
| | <i>degP</i> | degP_F | 5'-TGGTAGTGAACAACGTGAAA-3' | 53.2 | (3) |
| | | degP_R | 5'-AACAGGTAGATGGTGCTGTC-3' | 57.3 | |
| | <i>clpP</i> | clpP_F | 5'-CAAAAGGTAAACGTTTTTGC-3' | 51.1 | (3) |
| | | clpP_R | 5'-AATGATTGACCCGTATGAAG-3' | 53.2 | |
| | <i>rseP</i> | rseP_F | 5'-TTGTTTATTACCTGCCGTTT-3' | 51.1 | (3) |
| | | rseP_R | 5'-ATTAACAGCACCAGCAGAAT-3' | 53.2 | |
| | <i>degS</i> | degS_F | 5'-TTCCAGTTAGCAACCAAAAT-3' | 51.1 | (3) |
| | | degS_R | 5'-TGACACTTCATTAACCACGA-3' | 53.2 | |

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