| Gene | Primer      | Sequence (5' - 3')   | Amplicon size (bp) | Gene length (bp) | Amino acid length (aa) |  |
|------|-------------|----------------------|--------------------|------------------|------------------------|--|
| basR | Eco-basR_F  | CGCAAACGCAACACTATTCA | 975                | 669              | 223                    |  |
|      | Eco-basR_R  | ATGATGTGGCGATCGTTGTT |                    |                  |                        |  |
| basS | Eco-basS_F  | GACAATGAACCCTCGACCAA | 1,605              | 1,092            | 364                    |  |
|      | Eco-basS_F1 | GTGCGTTTGCATCTGGAACT |                    |                  |                        |  |
|      | Eco-basS_R  | CGGCGTAACCATGAAAGAGA |                    |                  |                        |  |
| phoP | Eco-phoP_F  | CCCCCATAACCACATAATCG | 794                | 672              | 224                    |  |
|      | Eco-phoP_R  | CGTTGCCAACAGAAAACGTA |                    |                  |                        |  |
| phoQ | Eco-phoQ_F  | CGCGTTCGAATACACCATTA | 1,774              | 1,461            | 487                    |  |
|      | Eco-phoQ_F1 | TGCTGAGTGGAGATCATTCG |                    |                  |                        |  |
|      | Eco-phoQ_R  | CAAGAAAATCGGGCCAGTTA |                    |                  |                        |  |
| eptA | Eco-eptA_F  | CCAGAATCAGTCCCTGCAAT | 1,797              | 1,644            | 548                    |  |
|      | Eco-eptA_R  | TCCCCTTAATCCAGCAAACA |                    |                  |                        |  |

Supplementary Table S1. Primers used for amplifying and sequencing *basRS*, *phoPQ* and *eptA* 

## **Supplementary Table S2.** Primers for qRT-PCR

| Gene | Primer | Sequence (5' - 3')    | Amplicon size (bp) |  |  |
|------|--------|-----------------------|--------------------|--|--|
| rrsH | rrsH_F | GAATGCCACGGTGAATACGTT | 64                 |  |  |
|      | rrsH_R | ACCCACTCCCATGGTGTGA   |                    |  |  |
| basR | basR_F | ATTGCAGGGACTGATTCTGG  | 134                |  |  |
|      | basR_R | CGTCGGGTAACCCTAAATCC  |                    |  |  |
| phoP | phoP_F | GCAGAAGATGCCAAAGAAGC  | 143                |  |  |
|      | phoP_R | ACCAGAATCGGCAGTGAAAC  |                    |  |  |
| eptA | eptA_F | TCCCTAATACCGCATCTTGC  | 124                |  |  |
|      | eptA_R | ATGATATCCAGCACGCCTTC  |                    |  |  |
|      |        |                       |                    |  |  |

|                       | Minimum Inhibitory Concentration (mg/L) <sup>a, b, c</sup> |           |        |           |           |           |         |     |            |            |
|-----------------------|--|-----------|--------|-----------|-----------|-----------|---------|-----|------------|------------|
| E. coli strain        | CL   | IMI       | AMK    | СРМ       | CRO       | CIP       | TET     | RIF | P/T        | A/S        |
| E015                  | 0.25 (S)   | 0.125 (S) | 16 (I) | 64 (R)    | >64 (R)   | 16 (R)    | 64 (R)  | 32  | 2/4 (S)    | 32/16 (R)  |
| E139                  | 0.25 (S)   | 0.125 (S) | 8 (S)  | >64 (R)   | >64 (R)   | 64 (R)    | 4 (S)   | 16  | 64/4 (I)   | >64/32 (R) |
| E154                  | 0.25 (S)   | 0.125 (S) | 4 (S)  | >64 (R)   | >64 (R)   | 32 (R)    | 4 (S)   | 32  | 4/4 (S)    | >64/32 (R) |
| E015R                 | 64 (R)   | 0.125 (S) | 2 (S)  | >64 (R)   | >64 (R)   | 16 (R)    | >64 (R) | 32  | 4/4 (S)    | 64/32 (R)  |
| E139R                 | >64 (R)  | 0.125 (S) | 2 (S)  | 0.25 (S)  | 0.063 (S) | >64       | 2 (S)   | 32  | 4/4 (S)    | 64/32 (R)  |
| E154R                 | 64 (R)   | 0.125 (S) | 2 (S)  | 0.125 (S) | 0.063 (S) | 0.063 (S) | 64 (R)  | 8   | 2/4 (S)    | 64/32 (R)  |
| QIA18                 | 8 (R)  | 0.125 (S) | 2 (S)  | 0.125 (S) | 0.063 (S) | 64 (R)    | >64 (R) | 64  | 4/4 (S)    | 64/32 (R)  |
| QIA24                 | 8 (R)  | 0.063 (S) | 2 (S)  | 0.125 (S) | 0.063 (S) | 0.063 (S) | >64 (R) | 64  | 8/4 (S)    | 64/32 (R)  |
| QIA32                 | 8 (R)  | 0.125 (S) | 1 (S)  | 0.125 (S) | 0.063 (S) | 0.25 (S)  | 64 (R)  | 16  | 2/4 (S)    | 64/32 (R)  |
| QIA33                 | 16 (R)   | 0.063 (S) | 2 (S)  | 0.125 (S) | 0.063 (S) | 0.25 (S)  | 32 (R)  | 16  | 2/4 (S)    | 32/16 (R)  |
| EC006                 | 8 (R)  | 0.125 (S) | 2 (S)  | >64 (R)   | >64 (R)   | 16 (R)    | >64 (R) | 64  | 8/4 (S)    | 64/32 (R)  |
| EC019                 | 8 (R)  | 0.125 (S) | 4 (S)  | >64 (R)   | >64 (R)   | 64 (R)    | 2 (S)   | 32  | 8/4 (S)    | 64/32 (R)  |
| EC111                 | 16 (R)   | 0.125 (S) | 2 (S)  | >64 (R)   | >64 (R)   | 32 (R)    | 2 (S)   | 32  | >256/4 (R) | >64/32 (R) |
| J53 <sub>pEC006</sub> | 4 (R)  | 0.25 (S)  | 2 (S)  | 0.25 (S)  | 0.063 (S) | 0.063 (S) | 2 (S)   | 32  | 4/4 (S)    | 32/16 (R)  |
| J53 <sub>pEC019</sub> | 4 (R)  | 0.125 (S) | 2 (S)  | 0.125 (S) | 0.063 (S) | 0.063 (S) | 2 (S)   | 16  | 8/4 (S)    | 32/16 (R)  |
| J53 <sub>pEC111</sub> | 4 (R)  | 0.125 (S) | 2 (S)  | 0.125 (S) | 0.063 (S) | 0.063 (S) | 2 (S)   | 32  | 8/4 (S)    | 32/16 (R)  |
| MG1655                | 0.25 (S)   | -         | -      | -         | -         | -         | -       |     | -          | -          |
| J53                   | 0.25 (S)   | -         | -      | -         | -         | -         | -       |     | -          | -          |

Supplementary Table S3. Antimicrobial resistance profile of the *E. coli* strains.

<sup>a</sup> CL, colistin; PB, polymyxin B; IMI, imipenem; AMK, amikacin; CPM, cefepime; CRO, ceftriaxone; CIP, ciprofloxacin; TET, tetracycline; RIF, rifampicin; P/T, piperacillin-tazobactam; A/S, ampicillin-sulbactam <sup>b</sup> S, susceptible; I, intermediate-resistant; R, resistant <sup>c</sup> There is no CLSI breakpoint for rifampicin

Supplementary Figure S1. The results of MALDI-TOF mass spectrometry. (A) Isogenic strains E015 and E015R, (B) a parental *E. coli* J53 and a transconjugant J53<sub>pEC019</sub>, and (C) WT *mcr-1*-positive strains EC006, EC019, and EC111. Bis-phosphorylated and hexa-acylated lipid A peak (1797.2 EM) was identified in all isolates. An addition of PEtN to the hexa-acylated lipid A (1920 EM) was detected from strains displaying colistin resistance.

