genes

# Vertical and horizontal transmission of ESBL plasmid from *Escherichia coli* O104:H4

**Supplementary Materials:**

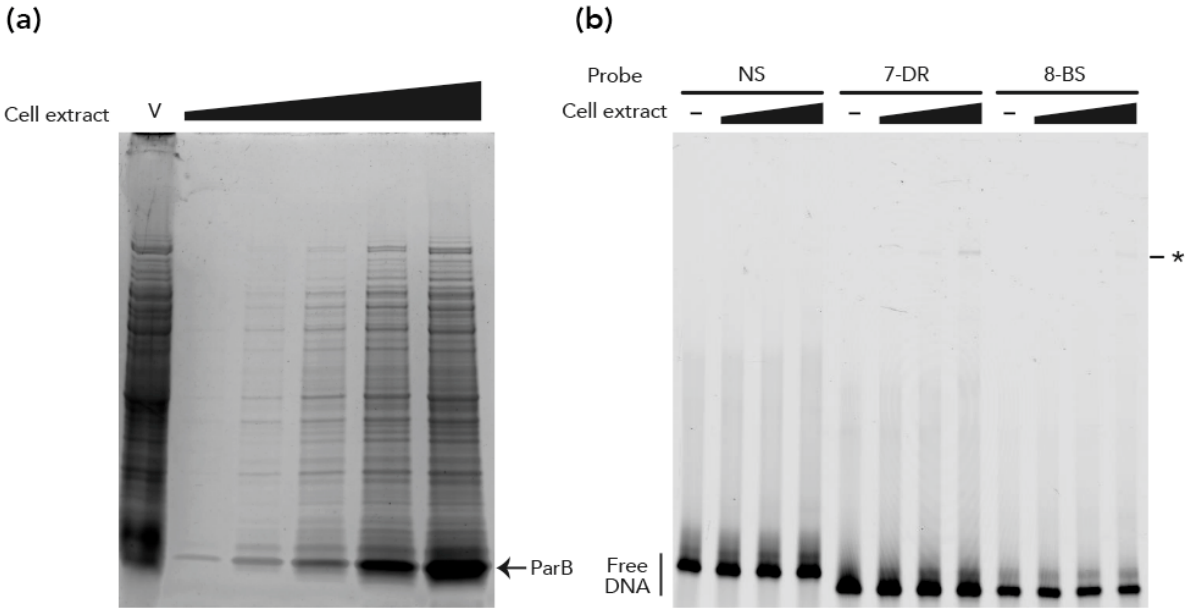


**Figure S1.** Crude cellular extracts and EMSA in the absence of ParB. (**a**) SDS-PAGE followed by Coomassie blue staining of an increasing range (1, 3, 10, 30 and 100; black triangle) of total extracts from cells expressing $ParB_{pESBL}$. The cell extract from the vector control (V) is loaded at the corresponding range 100. The position of ParB is indicated by the arrow. (**b**) Cy3-labeled dsDNA probes were incubated without (–) or with increasing amounts of cell extract from the vector control (range 10, 30, 100; black triangle). Free DNA fragments of the NS, 7-DR and 8-BS probes were indicated on the left. A faint non-specific complex, labeled with an asterisk (*) is visible at high cell extracts with the 7-DR and 8-BS but not with the NS probes.

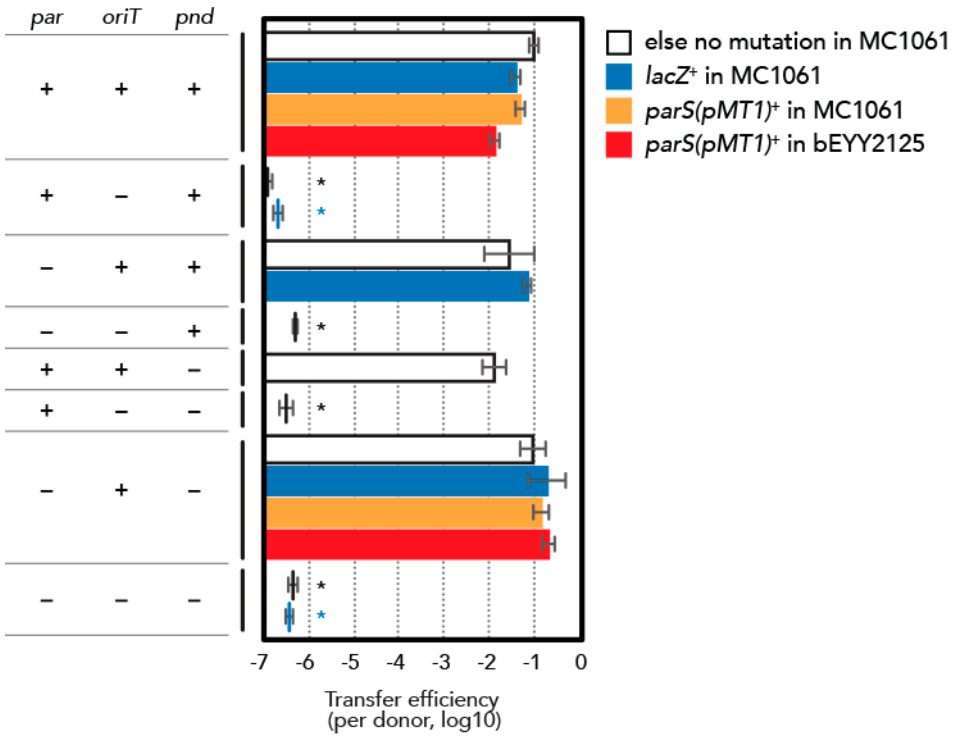


**Figure S2.** Transfer efficiency of different pESBL mutants. The presence (+) or absence (–) of *par*, *oriT* and *pnd* genes are indicated on the left, and additional characteristics in pESBL and donor *E. coli* are indicated with colored bars. Exconjugants were never obtained in all *oriT* mutants (*), thus the limit of detection was presented with vertical lines. Average and standard deviations for more than three independent experiments are displayed.

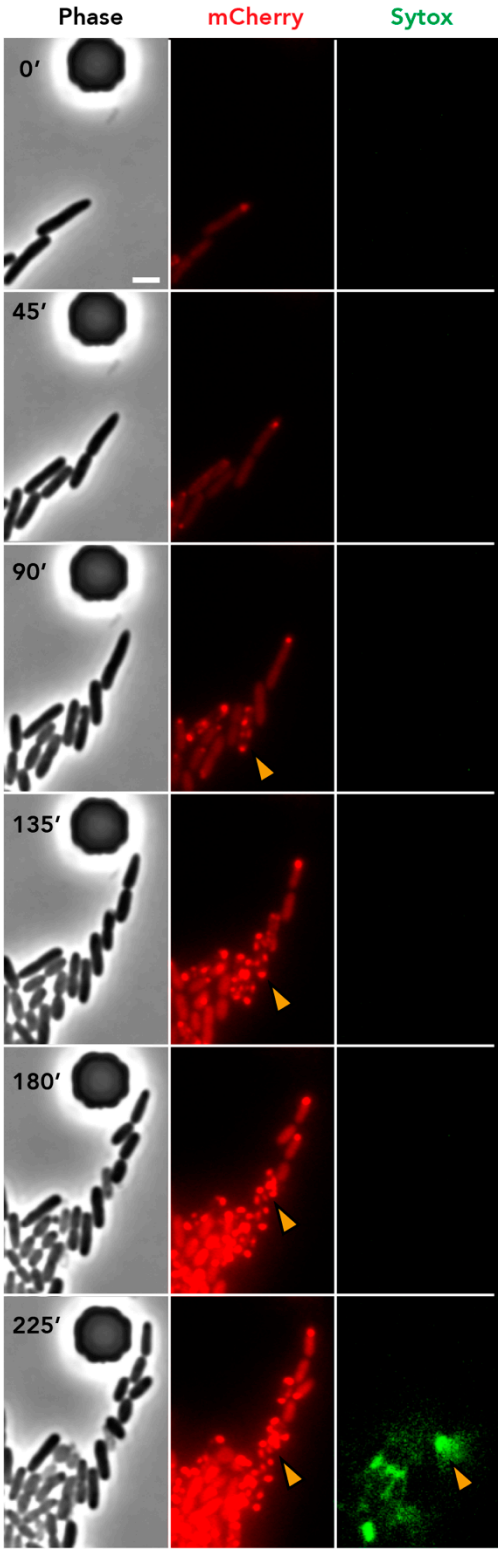


**Figure S3.** Representative time-lapse images of the fate of cells that have lost Δ*par* Δ*pnd par*$S_{pMT1}^{+}$ pESBL. The cell culture was loaded in a microfluidic chamber, and Amp and Sytox green nucleic acid stains were added in the flow. Arrowheads represent a cell that loses the plasmid, resulting in the aberrant accumulation of mCherry and sensitivity to Amp, which is reflected by the lysis of the cell and release of the chromosome DNA in the media stained by sytox. Bars = 2 μm.

**Table S1: Strains used in this study**

| Strain | Description | Reference |
|---|---|---|
| *Host* E. coli *strains* | | |
| BL21 (DE3) | Host cell for T7 promoter-based protein expression | Laboratory stock |
| BW25113 | F- DE*(araD-araB)567 lacZ4787(del)::rrnB-3* LAM- *rph-1* DE*(rhaD-rhaB)568 hsdR514* | [20] |
| MC1061 | *hsdR2 hsdM+ hsdS+ araD139 Δ(ara-leu)7697 Δ(lac)X74 galE15 galK16 rpsL mcrA mcrB1* | Laboratory stock |
| SM10 λ*pir* | *thi thr leu tonA lacY supE recA::RP4-2-Tc::Mu_kan λpir* | Laboratory stock |
| β2163 | *F- RP4-2-Tc::Mu_kan ΔdapA::(erm-pir)* | [62] |
| YBA268 | MC1061 / pXX705 | [63] |
| bEYY2082 | MC1061 / pEYY367 | This study |
| bEYY2092 | MC1061 / pEYY378 | This study |
| bEYY2118 | BW25113 *ΔgalK::mcherry-parB$_{pMT1}$_kan* | This study |
| bEYY2125 | MC1061 *ΔgalK::mcherry-parB$_{pMT1}$_kan* | This study |
| *Strains for allelic exchange* | | |
| bEYY1101 | SM10 λ *pir* / pEYY40 | This study |
| bEYY1204 | β2163 / pEYY84 | This study |
| bEYY2009 | SM10 λ *pir* / pEYY352 | This study |
| bEYY2060 | SM10 λ *pir* / pEYY371 | This study |
| bEYY2091 | SM10 λ *pir* / pEYY377 | This study |
| *Strains harboring pESBL* | | |
| YBB1195 | MC1061 / pESBL | [14] |
| bEYY1116 | MC1061 / pESBL Δ*oriT* | This study |
| bEYY1230 | MC1061 / pESBL *hp4::lacZ_spc* | This study |
| bEYY1438 | MC1061 / pESBL *ΔoriT hp4::lacZ_spc* | This study |
| bEYY2026 | MC1061 / pESBL *Δpar* | This study |
| bEYY2051 | MC1061 / pESBL *ΔoriT Δpar* | This study |
| bEYY2072 | MC1061 / pESBL *IG_hp23::parS$_{pMT1}$* | This study |
| bEYY2093 | MC1061 / pESBL *Δpnd* | This study |
| bEYY2094 | MC1061 / pESBL *ΔoriT Δpnd* | This study |
| bEYY2095 | MC1061 / pESBL *Δpar Δpnd* | This study |
| bEYY2096 | MC1061 / pESBL *ΔoriT Δpar Δpnd* | This study |
| bEYY2115 | MC1061 / pESBL *Δpar Δpnd IG_hp23::parS$_{pMT1}$* | This study |
| bEYY2127 | bEYY2125 / pESBL *IG_hp23::parS$_{pMT1}$* | This study |
| bEYY2166 | bEYY2125 / pESBL *Δpar Δpnd IG_hp23::parS$_{pMT1}$* | This study |
| bEYY2167 | bEYY2125 / pESBL *ΔoriT Δpar Δpnd IG_hp23::parS$_{pMT1}$* | This study |
| bEYY2202 | MC1061 / pESBL *Δpar Δpnd hp4::lacZ_spc* | This study |
| bEYY2203 | MC1061 / pESBL *ΔoriT Δpar Δpnd hp4::lacZ_spc* | This study |
| bEYY2242 | MC1061 / pESBL *Δpar hp4::lacZ_spc* | This study |

**Table S2: Plasmids used in this study**

| Plasmid | Description | Construction/reference |
|---|---|---|
| *Vectors* | | |
| pBAD33 | p15A*ori cat* $P_{ara}$*_X* | [64] |
| pDM4 | R6K*ori cat sacB* $mob_{RP4}$ | [65] |
| pKD46 | *oriR101 repA101ts bla* $P_{ara}$*_exo_bet_gam* | [20] |
| pXX705 | F*ori amp-R ΔsopABC* | [29] |
| pEYY240 | pBAD33 *X-m.sf.gfp-mut3* | *m.sf.gfp.mut3* amplified with oYo578 x oYo579 followed by SacI + XbaI digestion cloned into pBAD33 / SacI + XbaI |
| *Allelic exchange plasmids* | | |
| pEYY10 | pDM4 for *hp4::X* | 3 pieces Gibson assembly: left arm amplified with oYo17 x oYo24, right arm amplified with oYo25 x oYo20 and pDM4 / XbaI + XhoI |
| pEYY40 | pDM4 for *ΔoriT* | 3 pieces Gibson assembly: left arm amplified with oYo107 x oYo108, right arm amplified with oYo110 x oYo111 and pDM4 / XbaI + XhoI |
| pEYY84 | pDM4 for *hp4::lacZ_spc* | 3 pieces Gibson assembly: *spc* from pVI36 amplified with oYo226 x oYo227, *lacZ* amplified with oYo228 x oYo229 and pEYY10 / XbaI |
| pEYY352 | pDM4 for *Δpar* | 3 pieces Gibson assembly: left arm amplified with oYo881 x oYo873, right arm amplified with oYo880 x oYo879, and pDM4 / XbaI + XhoI |
| pEYY371 | pDM4 for *IG_hp23::*$parS_{pMT1}$ | 4 pieces Gibson assembly: $parS_{pMT1}$ amplified with oYo946 x oYo947, left arm amplified with oYo944 x oYo945, right arm amplified with oYo948 x oYo949 and pDM4 / XbaI + XhoI |
| pEYY377 | pDM4 for *Δpnd* | 3 pieces Gibson assembly: left arm amplified with oYo1026 x oYo1027, right arm amplified with oYo1028 x oYo1029, and pDM4 / XbaI + XhoI |
| *Other plasmids* | | |
| pEE18 | R6K*ori* $mob_{RP4}$ *cat magellan5-MmeI (kan)* | pEE22 [22] but without SC2 reporter |
| pSN70 | pUC*ori bla* $lacI^{q}$ $P_{lac}$*_mcherry-*$ParB_{pMT1}$[1] | [39] |
| pR6K biofab-sf.gfp | R6K*ori FRT_kan_FRT* $PB_{iofab}$*_sf.gfp* | Gibson Assembly of annealed oligo nucleotides ol307 + ol308 and PCR fragment from pR6KplaciQ1-sfGFP [39] with ol305 x ol306 |
| pVI36 | R6K*ori bla FRT_spc_FRT* | [63] |
| pEYY367 | pBAD33 *m.sf.gfp-mut3-*$parB_{pESBL}$ | Gibson assembly of $parB_{pESBL}$ *(hp7)* amplified with oYo960 x oYo961 and pEYY240 /NotI + XbaI |
| pEYY373 | R6K*ori FRT_kan_FRT* $PB_{iofab}$*_ mcherry-*$ParB_{pMT1}$[1] | Gibson assembly of vector from pR6K biofab-sf.gfp amplified with oYo998 x oYo999 and *mCherry-parBpMT1* amplified from pSN70 with oYo1000 x oYo1001 |
| pEYY378 | pXX705 $par_{pESBL}$ | Gibson assembly of *par* locus amplified with oYo1032 x oYo1033 and pXX705 / BamHI |
| pEYY395 | pET28b $parB_{pESBL}$ | Gibson assembly of $parB_{pESBL}$ *(hp7)* amplified with oYo1092 x oYo1094 and pET28b / HindIII + NcoI |

[1] $ParB_{pMT1}$ lacks N-terminal region to (pMT1 Δ23ParB, see [33]) to avoid dimerization and interaction to ParA.

**Table S3: Oligo DNAs used in this study**

| Oligo | Sequence (5′ -> 3′) |
|---|---|
| oYo17 | GCGGAGTGTATATCAAGCTTATCGAAGTGATTCGGGAGCTTAGC |
| oYo20 | TTGTGAGCGGATAACAATTTGTGGGAGCCCTTGCTGTGACAATG |
| oYo24 | GACCTTTCAGACATTCCAGGTCTAGAATGGCATCAAGACACTCACG |
| oYo25 | CGTGAGTGTCTTGATGCCATTCTAGACCTGGAATGTCTGAAAGGTC |
| oYo107 | GCGGAGTGTATATCAAGCTTATCGAAAGCCCTGGTATTTATGCC |
| oYo108 | CACTCACTTCAGGCTCCTTACTAGTCTTATGCAGACGGCAG |
| oYo110 | CTGCCGTCTGCATAAGACTAGTAAGGAGCCTGAAGTGAGTG |
| oYo111 | TTGTGAGCGGATAACAATTTGTGGTTGACCGCAACGTGAACATG |
| oYo226 | CGTGAGTGTCTTGATGCCATTTAGCTTGCAGTGGGCTTAC |
| oYo227 | GAGGTCGATATTGACCCAATTCTCATTGGCTGGCACCAAGC |
| oYo228 | GCTTGGTGCCAGCCAATGAGAATTGGGTCAATATCGACCTC |
| oYo229 | GACCTTTCAGACATTCCAGGTTGGCTTATTGTGGGGATGAC |
| oYo578 | AATTCGAGCTCAAGGAGGAAAACCATGAGTAAAGGTGAAGAACTGTTC |
| oYo579 | ACTCTAGATTAGGCGGCCGCCGCTTTGTAGAGTTCATCCATGC |
| oYo873 | TTGTGAGCGGATAACAATTTGTGGTGATGAAATCCAGCCCCG |
| oYo879 | GCGGAGTGTATATCAAGCTTATCGCCGGCTGGCTGGTTTATT |
| oYo880 | CGACGCAGATGACCAGAAAACAGAGGTATCACGATTGATAGCA |
| oYo881 | TGCTATCAATCGTGATACCTCTGTTTTCTGGTCATCTGCGTCG |
| oYo944 | GCGGATAACAATTTGTGGCGACACCTGTCCTGAA |
| oYo945 | ATTTCGCGCTCTGTTTAATCAGACTCGTGGC |
| oYo946 | TTAAACAGAGCGCGAAATTATGAGTCACG |
| oYo947 | CAGGGCTTTAGGATGCCGAAGAGC |
| oYo948 | CGGCATCCTAAAGCCCTGGTATTTATGCC |
| oYo949 | GGAGTGTATATCAAGCTTATCGTAGTCTTATGCAGACGGC |
| oYo960 | TCTACAAAGCGGCGGCCGCCAGAAATAGAGCAAACACTTCACC |
| oYo961 | TGCCTGCAGGTCGACTCTAGATTATCTTTGTTTTACTTCTGCTATCAA |
| oYo998 | GGTGAGTAACCCGGGTGTAGGCTG |
| oYo999 | GCTCACCATATTCACCACCCTGAATTGAC |
| oYo1000 | GTGGTGAATATGGTGAGCAAGGGCG |
| oYo1001 | TACACCCGGGTTACTCACCTGATTCTGGAAG |
| oYo1026 | TTGTGAGCGGATAACAATTTGTGGCATCACTGATAATGTCCTCGC |
| oYo1027 | GTCAGCCTTCGCAACAAAGCCCGCAGCTATTCTAACG |
| oYo1028 | CGTTAGAATAGCTGCGGGCTTTGTTGCGAAGGCTGAC |
| oYo1029 | GCGGAGTGTATATCAAGCTTATCGCCCACTCTGTAACGGAAC |
| oYo1032 | GACATCCAGCCTGCTGTTGGGTTCCACTGAGCGTCAGACCC |
| oYo1033 | CTCGTTTCTGACACTTGCAGACTGACAGATTTGCCAGTAGCC |
| oYo1092 | GGGCCATGGTGAGAAATAGAGCAAACACTTCACC |
| oYo1094 | CCCAAGCTTTTATCTTTGTTTTACTTCTGCTATCAA |
| ns-F | Cy3-CCGTTCTAGCTCATTCTGTTCTTGCTTGCTGCATCATCACTGCATCATCCCAATGCCG ATCTAGCTCATTACTGTTCTAT |
| ns-R | ATAGAACAGTAATGAGCTAGATCGGCATTGGGATGATGCAGTGATGATGCAGCAAGCA AGAACAGAATGAGCTAGAACGG |
| 7DR-F | Cy3-CCATAATATTGTTATCTTGTTATCTTGTTATCTTGTTATCTTGTTATCTTGTTGTCTTGT TATCTTAATTAA |
| 7DR-R | TTAATTAAGATAACAAGACAACAAGATAACAAGATAACAAGATAACAAGATAACAAG ATAACAATATTATGG |
| 8BS-F | Cy3-TTGAGATAACAAGATAACAAGATAACAAGATAACACCTTGTTATCTTGTTATCTTG TTATCTTGTCATCGAC |
| 8BS-R | GTCGATGACAAGATAACAAGATAACAAGATAACAAGGTGTTATCTTGTTATCTTGTTAT CTTGTTATCTCAA |
| ol305 | TATAATAGATTCATGGATGCAAAATAAGAGAGTCAATTCAGGGTGGT |
| ol306 | GCGAAGTCAATACTCTATCGATGCTTCCAGTCGGGAAACCTGTCGTG |
| ol307 | GCATCGATAGAGTATTGACTTCGCATCTTTTTGTACCTATAATAGATTCATGGATGCAAA AT |
| ol308 | ATTTTGCATCCATGAATCTATTATAGGTACAAAAAGATGCGAAGTCAATACTCTATCGA TGC |

**Table S4: Summary of Tnseq**

| Experiment | | Illumina barcode[1] | P5 adaptor[2] | # of reads | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Raw reads | Bowtie-mapped | TA-mapped | | |
| | | | | | | Total | Chr[3] | pESBL |
| Input library | #1 | 41 | ATGCTA | 15,444,882 | 16,905,660 | 14,040,424 | 13,956,375 | 84,049 |
| | #2 | 1 | CAGT | 14,776,122 | 15,414,730 | 15,214,960 | 15,188,012 | 26,948 |
| | #3 | 8 | ACAGT | 12,687,763 | 12,876,419 | 12,699,636 | 12,654,016 | 45,620 |
| | Total | | | | | 41,955,020 | 41,798,403 | 156,617 |
| Output library | #1 | 42 | AGCATA | 14,389,584 | 14,691,470 | 14,516,201 | 14,480,809 | 35,392 |
| | #2 | 5 | CGTA | 18,531,553 | 20,124,007 | 19,864,167 | 19,765,407 | 98,760 |
| | #3 | 9 | TACTC | 14,609,016 | 15,189,471 | 14,970,843 | 14,902,612 | 68,231 |
| | Total | | | | | 49,351,211 | 49,148,828 | 202,383 |

[1] Index number (visit https://support.illumina.com for details). [2] See [22]. [3] Chromosome.