

Supplementary Information

Fig S1. Alignment of predicted amino acid sequences of the Orf35 protein of pCTX-M3 and YggA of the R64 plasmid

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Orf35 1  PAKTKAEVSESLGSKT  SLDLDFVWSELEKKT  LKSLKSLTKK  KDGRVYEDKELIK  LINDAGFS-DV-  TLKDKD  TAGRVGK  RFRSH  RKK  TGVD  EPCRTQ  N  STGC  PDASG  PQY  121
YggA  1  PASL  --  FVDD  D  I  Q  L  A  A  L  S  E  L  P  L  A  C  K  T  L  R  D  F  A  I  R  D  L  I  V  E  V  R  K  G  T  L  A  D  V  R  D  T  K  N  A  G  Y  E  V  G  E  K  A  L  R  O  I  --  R  E  A  S  S  -  K  A  F  S  R  E  S  S  K  T  A  S  K  K  T  S  A  R  K  K  D  S  A  R  K  K  D  I  D  I  N  N  -----  111

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■ identical aa
■ similar aa

Table S1. pCTX-M3 plasmids with deletions of individual genes in the *tra* and *trb* regions and their respective complementing plasmids

| Gene | pCTX-M3 derivative | Complementing plasmid | Construction details |
|--------------|--|-----------------------|---|
| <i>excA</i> | pCTX-M3 <i>excA::cat</i> | pM <i>TexA</i> | pMT5 with PCR-amplified <i>excA</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>nikA</i> | pCTX-M3 <i>nikA::cat</i> | pM <i>TnikA</i> | pMT5 with PCR amplified <i>nikA</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>nikB</i> | pCTX-M3 <i>nikB::cat</i> pCTX-M3Δ <i>nikB</i> | pM <i>TnikB</i> | pMT5 with PCR amplified <i>nikB</i> cloned into <i>SacI-SmaI</i> sites |
| <i>orf35</i> | pCTX-M3 <i>orf35::cat</i> | pA <i>Lorf35</i> | pAL3 with PCR amplified <i>orf35</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>orf36</i> | pCTX-M3 <i>orf36::cat</i> | pA <i>Lorf36</i> | pAL3 with PCR amplified <i>orf36</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>orf38</i> | pCTX-M3 <i>orf38::cat</i> | pM <i>Torf38</i> | pMT5 with PCR amplified <i>orf38</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>orf46</i> | pCTX-M3 <i>orf46::cat</i> | none | |
| <i>pri</i> | pCTX-M3 <i>pri::cat</i> pCTX-M3Δ <i>pri</i> | pA <i>Lpri</i> | pAL3 with <i>pri</i> from pHS11 (<i>KpnI-HincII</i>) cloned into <i>KpnI</i> -blunted <i>PstI</i> sites |
| <i>traH</i> | pCTX-M3 <i>traH::cat</i> | pM <i>TtraH</i> | pMT5 with PCR amplified <i>traH</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>traI</i> | pCTX-M3 <i>traI::cat</i> | pM <i>TtraI</i> | pMT5 with PCR amplified <i>traI</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>traJ</i> | pCTX-M3 <i>traJ::cat</i> | pM <i>TtraJ</i> | pMT5 with PCR amplified <i>traJ</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>traK</i> | pCTX-M3 <i>traK::cat</i> | pM <i>TtraK</i> | pMT5 with PCR amplified <i>traK</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>traL</i> | pCTX-M3 <i>traL::cat</i> | pM <i>TtraL</i> | pMT5 with PCR amplified <i>traL</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>traM</i> | pCTX-M3 <i>traM::cat</i> | pM <i>TtraM</i> | pMT5 with PCR amplified <i>traM</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>traN</i> | pCTX-M3 <i>traN::cat</i> | pM <i>TtraN</i> | pMT5 with PCR amplified <i>traN</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>traO</i> | pCTX-M3 <i>traO::cat</i> | pM <i>TtraO</i> | pMT5 with PCR amplified <i>traO</i> cloned into <i>SacI-SmaI</i> sites |
| <i>traP</i> | pCTX-M3 <i>traP::cat</i> | pM <i>TtraP</i> | pMT5 with PCR amplified <i>traP</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>traQ</i> | pCTX-M3 <i>traQ::cat</i> | pM <i>TtraQ</i> | pMT5 with PCR amplified <i>traQ</i> cloned into <i>SacI-SmaI</i> sites |
| <i>traR</i> | pCTX-M3 <i>traR::cat</i> | pM <i>TtraR</i> | pMT5 with PCR amplified <i>traR</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>traU</i> | pCTX-M3 <i>traU::cat</i> pCTX-M3Δ <i>traU</i> | pA <i>LtraU</i> | pAL3 with PCR amplified <i>traU</i> cloned into <i>SacI-SmaI</i> sites |
| <i>traW</i> | pCTX-M3 <i>traW::cat</i> | pA <i>LtraW</i> | pAL3 with PCR amplified <i>traW</i> cloned into <i>SmaI</i> site |
| <i>traX</i> | pCTX-M3 <i>traX::cat</i> | pA <i>LtraX</i> | pAL3 with PCR amplified <i>traX</i> cloned into <i>EcoRI-PstI</i> sites |
| <i>traY</i> | pCTX-M3 <i>traY::cat</i> pCTX-M3Δ <i>traY</i> | pA <i>LtraY</i> | pAL3 with PCR amplified <i>traY</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>trbA</i> | pCTX-M3 <i>trbA::cat</i> | pA <i>LtrbA</i> | pAL3 with PCR amplified <i>trbA</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>trbB</i> | pCTX-M3 <i>trbB::cat</i> | pM <i>TtrbB</i> | pMT5 with PCR amplified <i>trbB</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>trbC</i> | pCTX-M3 <i>trbC::cat</i> | pA <i>LtrbC</i> | pAL3 with PCR amplified <i>trbC</i> cloned into <i>EcoRI-SmaI</i> sites |
| <i>trbN</i> | pCTX-M3 <i>trbN::cat</i> | pM <i>TtrbN</i> | pMT5 with PCR amplified <i>trbN</i> cloned into <i>EcoRI-SmaI</i> sites |

Table S2. Primers used for generation of *cat* integration cassettes into genes from *tra* and *trb* regions of pCTX-M3

| Gene | Primer | Sequence 5'-3' |
|--------------|----------|---|
| <i>excA</i> | excAupP1 | AGATAAGAGCAAGGACGGCATCAAAGGAGCTAAAT GTGTAGGCTGGAGCTGCTTCG |
| | excAdnP2 | CATATTCGGCGGGTTAAACTCAATAATTGCTGTTACATATGAATATCCTCCTTA |
| <i>nikA</i> | nikAupP1 | CAAGCCCCGACCCCCCTAACGAGGTTAGCTATCTGGTGTAGGCTGGAGCTGCTTCG |
| | nikAdnP2 | CTTTTTGTCCCTTCGGCCCTCAATGATTTTAGGTATCATATGAATATCCTCCTTA |
| <i>nikB</i> | nikBupP1 | CAGAACTGCAACAGGAGATGGATGGTGATACCTAAGTGTAGGCTGGAGCTGCTTCG |
| | nikBdnP2 | AACGTTTGCATTTTGATTTTCATTTCTACTCCATTTATATGAATATCCTCCTTA |
| <i>orf35</i> | orf35uP1 | ATGGCATGGTCGATTTTGACATTATTGGGAGGGCAT GTGTAGGCTGGAGCTGCTTCG |
| | orf35dP2 | ATAATGTGGGATGCGATATGCGAAAGGTGAAGGTTAATATATGAATATCCTCCTTA |
| <i>orf36</i> | orf36uP1 | ATGCAAACAGTGATGCATTCCCGTTCCATTTGTAACGTGTAGGCTGGAGCTGCTTCG |
| | orf36dP2 | GAACAATGAGGTATACATGAGCGAACATAATGATTATATGAATATCCTCCTTA |
| <i>orf38</i> | orf37uP1 | GGCCCCGCTTGGCGGGCCAAATCGGAGTTCCTCGTGTAGGCTGGAGCTGCTTCG |
| | orf37dP2 | ATCACATCCTCAAAGAAAGAAATCATTTAAAACCTCTATATGAATATCCTCCTTA |
| <i>orf46</i> | orf46uP1 | GGCGTTTGC CGGCGTTGTTAATCAGGAGGCCAAACGGTGTAGGCTGGAGCTGCTTCG |
| | orf46dP2 | GGGGGACCACTCCCCGCTTTTAACACAAACCTTCATATATGAATATCCTCCTTA |
| <i>pri</i> | priUpP1 | TCTCAGCGATAACACATAACTACAGGGGTAGACAGTGTGTAGGCTGGAGCTGCTTCG |
| | priDnP2 | AGTCTTTGCGACTGGTGCATGAGTCTGTCTCTCCTTATATGAATATCCTCCTTA |
| <i>traH</i> | traHupP1 | ACGACAAATTC AATCCGAAGTAAATGGAGTAAGAAGTGTAGGCTGGAGCTGCTTCG |
| | traHdnP2 | CGGTAACGTCAGAAAGCAGCATCAGTTTAAAGTTTCATCATATGAATATCCTCCTTA |
| <i>traI</i> | traIupP1 | AAGAAAGGTTCAATTCCTCAGAGGGACCCGCCCATGAGTGTAGGCTGGAGCTGCTTCG |
| | traIdnP2 | TTGGACCAAAATGAATGCTTCCATCTACTGCCCTTATATGAATATCCTCCTTA |
| <i>traJ</i> | traJupP1 | GCAGCTGAAACCCGACCATACGTTAAGGGCAGTAGGTGTAGGCTGGAGCTGCTTCG |
| | traJdnP2 | GCATAACGCCATTTGTAAATGTCCATACTTGCCCTTATATGAATATCCTCCTTA |
| <i>traK</i> | traKupP1 | TATCTGGCGTTAAAAGAGGGCAAGTATGGACATTAAGTGTAGGCTGGAGCTGCTTCG |
| | traKdnP2 | AGGTTTCGCCATACTGTCTACCCCTGTAGTTATGTGTATATGAATATCCTCCTTA |
| <i>traL</i> | traLupP1 | ATGCACCAGTCGAAAGTCAGCGCCTCGGCCATAAAAGTGTAGGCTGGAGCTGCTTCG |
| | traLdnP2 | CTGTTTGCATCCTCACCCTCCACTCGCAGCTGCCTATATGAATATCCTCCTTA |
| <i>traM</i> | traMupP1 | TTATGTTGCTCATGTATACCTCATTGTTTCATTAACGTGTAGGCTGGAGCTGCTTCG |
| | traMdnP2 | AGTACAGTTAACAGTTTAACTTTCATGATTGACCTCTATATGAATATCCTCCTTA |
| <i>traN</i> | traNupP1 | GGCCATTCTCAAACCCCGAACTACTGAGGTCAATCGTGTAGGCTGGAGCTGCTTCG |
| | traNdnP2 | TTCTTTACCTTATCTGTAGCCAGCGTCTCCCTTATATGAATATCCTCCTTA |
| <i>traO</i> | traOupP1 | TGACGTTGAAGTTGAGCTGAATTAAGGGGAACGACGGTGTAGGCTGGAGCTGCTTCG |
| | traOdnP2 | ATTGTCATCTTCAAACAGATTCAATATCAATCTCCTTATATGAATATCCTCCTTA |
| <i>traP</i> | traPupP1 | CACCAATAACCCGTAATACCGTTAAGGAGATTGATAGTGTAGGCTGGAGCTGCTTCG |
| | traPdnP2 | ATTCTAAATCCATAGTTTCGCCTTTTAGGCTCTGTTATATGAATATCCTCCTTA |
| <i>traQ</i> | traQupP1 | CCTTATCCGCTAAACAGGACCTAAAAAGGGCGAACTGTGTAGGCTGGAGCTGCTTCG |
| | traQdnP2 | ATAAGCTTAAACAATTTTCAATAAAGTATCTTTTTTGATATATGAATATCCTCCTTA |
| <i>traR</i> | traRupP1 | CATTAACATTCACTGTAAATCAAAAAGGATACTTTGTGTAGGCTGGAGCTGCTTCG |
| | traRdnP2 | ATACCACTAACGATTTTGCACCGTTCGCGCAGCTCATATGAATATCCTCCTTA |
| <i>traU</i> | traUupP1 | TGAAATTTACAAGGGTAAGAGAGGTTTTAAATGATTGTGTAGGCTGGAGCTGCTTCG |
| | traUdnP2 | TTTTTGCTTTCCGATTTTATCATCTGAATCCCCTTATATGAATATCCTCCTTA |
| <i>traW</i> | traWupP1 | AATCATTTCTGATGTGAGAAGGGGATTCAGATGATAGTGTAGGCTGGAGCTGCTTCG |
| | traWdnP2 | CTGTTTTATTTTCGTCGGTCATTTTCTGGATACTCCTATATGAATATCCTCCTTA |
| <i>traX</i> | traXupP1 | GGAGAGTGTGAACGCGGGAGTATCCAGAAAATGACCGTGTAGGCTGGAGCTGCTTCG |
| | traXdnP2 | AAGCAACCTTTTTAATTTACTCATGTGGCCCCGTTATATGAATATCCTCCTTA |
| <i>traY</i> | traYupP1 | CGCAGGCTGGTCAAGAGCGGGGGCCACATGAGTAAAGTGTAGGCTGGAGCTGCTTCG |
| | traYdnP2 | GTCAGTGTGATAACGCATTCATATTTAAGCTCCTTATATGAATATCCTCCTTA |
| <i>trbA</i> | trbAupP1 | ATCGGGAAGGTTACCAAAAATTTCTGAGGTTCTGAGTGTAGGCTGGAGCTGCTTCG |
| | trbAdnP2 | CGATCGGTTTTTACGGTGAGTTTCATACTTCTCTCGCTATATGAATATCCTCCTTA |
| <i>trbB</i> | trbBupP1 | CCCGTCCCGATCACCTGGAACCTAAGCGAGGAAGTGTGTAGGCTGGAGCTGCTTCG |
| | trbBdnP2 | GCAGCGGGCATCGTCTTTGACAGCAACCGGAGCCGACATATGAATATCCTCCTTA |
| <i>trbC</i> | trbCupP1 | CTCAGCAGGCAATAGGAAATAACCCGAGCCGCTGTGTAGGCTGGAGCTGCTTCG |
| | trbCdnP2 | CTGATTAACAACCCGGCAAACGCCGGCTTCAGGGTATATGAATATCCTCCTTA |
| <i>trbN</i> | trbNupP1 | TTTTTTACTCGAAATAGAAATCATTAGGAGATTGTTGTGTAGGCTGGAGCTGCTTCG |
| | trbNdnP2 | CCCTTTCCGTTGTGGCTGGTTTTGCATCAGAACCCTCTATATGAATATCCTCCTTA |

Sequences homologous to the *cat* gene are shown in bold

Table S3. Primers used for verification of *cat* integration and *cat* elimination

| Gene | Primer | Sequence 5'-3' | Expected product size (bp) | | |
|--------------|----------|-------------------------------|----------------------------|--|----------------------------|
| | | | WT plasmid template | mutated plasmid template with <i>cat</i> insertion | with <i>cat</i> eliminated |
| <i>excA</i> | excAsU | CAGTGTGGGTGATATGCAG | 1191 | 1567 | |
| | excAsD | CCGTAAGGTTATGTAAACGC | | | |
| <i>nikA</i> | nikAsD | TCAGGAGTAGGCTGAACTGT | 542 | 1246 | |
| | oriTminU | ATAGGATCCAGTACGGGACAAGATGTGTT | | | |
| <i>nikB</i> | nikBsU | CGGAGTTCATAGCAAGGAGT | 2149 | 1197 | 267 |
| | nikBsD | CCTTTTCGCTTTGAGAGGCA | | | |
| <i>orf35</i> | orf35sU | GCTATATGCGAAAGGTGAAGG | 670 | 1320 | |
| | oriTminD | TAAGTGCAGAGATAGCTAACCTCGTTAGG | | | |
| <i>orf36</i> | orf36sU | GGATGAGGTATGCAATACGG | 928 | 1368 | |
| | pCTX96 | CCGAGTCAGTTTGATCCATA | | | |
| <i>orf38</i> | orf38sU | CTGACCGCATGGATCTCTTC | 702 | 1235 | |
| | orf38sD | CCTGCGACGTAAGCCCTACA | | | |
| <i>orf46</i> | orf46sU | ACGACGTGCTTTACCACAG | 980 | 1487 | |
| | pCTX101 | ACCCGGAATAGGGTATACTG | | | |
| <i>pri</i> | priSu | GCCATGATGGGCTACACGTT | 3461 | 1313 | 389 |
| | CTX13200 | TATAGGCAGCATCAGCACCAGC | | | |
| <i>traH</i> | traHsU | GAAACATCGTTTGATTCGTG | 706 | 1223 | |
| | traHsD | TGGCGGGTCCATGTAGTAAT | | | |
| <i>traI</i> | traIsU | CAACTGGCTAAACAGCGTGG | 1117 | 1359 | |
| | traIsD | CGCCCTGCAACAGAATATCG | | | |
| <i>traJ</i> | traJsU | TTAAGGCTCCAGTCATCACA | 1482 | 1353 | |
| | traJsD | GGTAACGTGTAGCCAATCAT | | | |
| <i>traK</i> | traKsU | TCGACGACCTGTTGATCGCT | 599 | 1363 | |
| | traKsD | TCTTTCAGCCGCTCAGGTT | | | |
| <i>traL</i> | traLsU | CACGGATACGGCCATGTTGA | 765 | 1363 | |
| | traLsD | ACCGCCGCGTTGAAATACGA | | | |
| <i>traM</i> | traMsU | TGGCTTTTCGTCGTTCCGCAC | 1028 | 1285 | |
| | traMsD | GTGACTGCTGATTACGGGC | | | |
| <i>traN</i> | traNsU | ACTTTGCCAACCCTACAATG | 1828 | 1691 | |
| | traNsD | TGAAGCTATCCCCTGTGAGA | | | |
| <i>traO</i> | traOsU | GCCAGGGAGTACCAAAGTCT | 1717 | 1384 | |
| | traOsD | CCACCAATCCATGCATAGTC | | | |
| <i>traP</i> | CTX17550 | TGCAACCTCAGCAGATGCCTCAGC | 970 | 1281 | 301 |
| | traPsD | GCAGGCTCATCAAGTATCCA | | | |
| <i>traQ</i> | traQsU | AGTCAACTTGGCGGAGTCAC | 753 | 1238 | |
| | traQsD | CAGCGGCAATACCACTAACG | | | |
| <i>traR</i> | CTX18700 | AAAGACTCTTACCTTGAGGGCC | 750 | 1706 | |
| | CTX19450 | ATTTTCTCCGGATGTTCCGAGCG | | | |
| <i>traU</i> | traUsU | TGTCGCTTCTGTTGCAGAGT | 3246 | 1233 | |
| | traUsD | GCGTTGAGATGGGAATGCTG | | | |
| <i>traW</i> | traWsU | CCCTATGGGAAGTGCATCAA | 1534 | 1365 | |
| | traWsD | AAGAGCAGGCTGAATGTTTG | | | |
| <i>traX</i> | traXsU | CAAACAGCAGCTGGAGAAAC | 892 | 1281 | |
| | traXsD | CATTTGACGCGATAGGTCAC | | | |
| <i>traY</i> | traYsU | CGCCGAAGTTATGAAAACGGA | 2777 | 1629 | 699 |
| | traYsD | GCACTGCATCGATCACAAG | | | |
| <i>trbA</i> | 5c25 | TTCAAAGCGGAACAGCTGCACCGC | 1667 | 1455 | |
| | 3c56n | ACTTTTGCCTCTGCAATGGC | | | |
| <i>trbB</i> | trbBsU | GACACCCCGAACAACAGTCT | 1829 | 1948 | |
| | pCTX103 | AGGAACTGAGCAAACGCTAT | | | |
| <i>trbC</i> | trbCsU | GTTGCCGATTCGGGTGATGT | 2299 | 1225 | |
| | trbCsD | GGAGCCAGGAACGAGAAGTA | | | |
| <i>trbN</i> | trbNsU | CATTGTCTCTGTCGAAGGCA | 648 | 1274 | |
| | trbNsD | GACAGGCTCCGTAATCACA | | | |

Table S4. Primers used for verification of the presence of helper plasmids (pKD46 and pCP20) and for sequencing

| Primer | Sequence 5'-3' | Application |
|----------|----------------------|--|
| repKD46F | TTTGCGTGAGCCATGAGAAC | pKD46 and pCP20 <i>repA101</i> detection - 774 bp |
| repKD46R | GGAAAGAACGGACGGTATCG | |
| TEMfor | CTGGATCTCAACAGCGGT | detection of <i>bla</i> _{TEM-1} in pKD46 and pCTX-M3 - 730 bp (1) |
| catU142 | GTCGGCAGATGCTTAATG | sequencing primer |
| traKsta | ATGGACATTAACAAATGGCG | sequencing primer |
| TnTEMrev | CTGACAGTTACCAATGCT | (1) |
| pri1F | TGAATTTACGGCGTGGCTTG | pAL <i>pri</i> sequencing primer |
| pri2F | AGCAGCCAGTATCAGCGAAG | |
| pri3F | AGCTGCCGAACCGGAATACG | |
| pri4F | GAATGGAATGCGCTGGGAA | |
| pri5F | ACAGCCAACAGCAGGGAATG | |

Table S5. Primers used for additional multiplex PCR verification of *cat* elimination

| Gene | Primer | Sequence 5'-3' | Expected product size (bp) | |
|-------------|----------|----------------------------|----------------------------|----------------------------|
| | | | with <i>cat</i> insertion | with <i>cat</i> eliminated |
| <i>nikB</i> | nikBDVer | GCTTACGGGTCAGGTAATTG | 402 | 732 |
| | nikAF | CCATGAAGCAGGGCTTAGTC | | |
| | catReVer | GCTGGTGATATGGGATAGTG | | |
| <i>pri</i> | priDVer | GCTTTACCGTCCACTGTATC | 476 | 638 |
| | priUVer | CTGCTGGCCATGATGGGCTACAC | | |
| | catReVer | GCTGGTGATATGGGATAGTG | | |
| <i>traU</i> | traUsU | TGTCGCTTCTGTTGCAGAGT | 286 | 589 |
| | traUDVer | ATGATTTAGCGCCCTGAACCTC | | |
| | catReVer | GCTGGTGATATGGGATAGTG | | |
| <i>traY</i> | traYsU | CGCCGAAGTTATGAAACGGA | 424 | 545 |
| | traYDVer | GGCGACGCCACAACGAAAAGAGATAG | | |
| | catReVer | GCTGGTGATATGGGATAGTG | | |

Table S6. Primers used for PCR-amplified gene cloning intopAL3 and pMT5 vectors

| Gene | Primer | Sequence 5'-3' |
|--------------|----------|---|
| <i>excA</i> | excAUEc | GATGAATTC <u>AAGGACGG</u> CATCAAAGGAGC |
| | excAsD | CCGTAAGGTTATGTAAACGC |
| <i>nikA</i> | nikAUEc | CCCCGAATTC <u>CCCTA</u> ACGAGGTTAGC |
| | nikAsD | TCAGGAGTAGGCTGAACTGT |
| <i>nikB</i> | nikBUSa | AGGAGCTCCAACAGGAGATGGATGG |
| | nikBsD | CCTTTTCGCTTTGAGAGGCA |
| <i>orf35</i> | orf35UEc | TCGAATTCGACATTATTGGGAGGGC |
| | orf35sU | GCTATATGCGAAAAGGTGAAGG |
| <i>orf36</i> | orf36UEc | TGGAATTC <u>ACA</u> ATCCGGCAGCTGCGAGTGG |
| | pCTX96 | CCGAGTCAGTTTGATCCATA |
| <i>orf38</i> | orf38UEc | CCGAATTC <u>CGGGG</u> CCAATATCGGAGTTC |
| | orf38sD | CCTGCGACGTAAGCCCTACA |
| <i>traH</i> | traHUEc | TTGAATTCGAAGTAAAATGGAGTAAG |
| | traHDKp | GCGGTACCGTCAGAAACACGATCAG |
| <i>traI</i> | traIUEc | AAGAATTCATTCCTCAGAGGGACCC |
| | traIsD | CGCCCTGCAACAGAATATCG |
| <i>traJ</i> | traJUEc | GAGAATTCGTTAAGGGGCAGTAGATG |
| | traJDKp | ATGGTACCATTGTGTTAATGTCCATACTTGC |
| <i>traK</i> | traKUEc | CTGGAATTC <u>AAAGAGGG</u> CAAGTATGGAC |
| | traKsD | TCTTTCAGCCGGCTCAGGTT |
| <i>traL</i> | traLUEc | AAGAATTCGAAAGGAGAGAACAGACTC |
| | traLsD | ACCGCCGCGTTGAAATACGA |
| <i>traM</i> | traMUEc | CAGAATTCCTGCACAGGATTCGTAATC |
| | traMDKp | GCGGTACCATAAGTACAGTTAACAG |
| <i>traN</i> | traNUEc | GGGAATTC <u>TCAA</u> ACCCCGGAECTACTG |
| | traNDKp | TCGGTACCTTTATCTGTAGCCATCG |
| <i>traO</i> | traOUSa | TTGAGCTCAATTAAGGGGAACGAC |
| | traOsD | CCACCAATCCATGCATAGTC |
| <i>traP</i> | traPUEc | CCGAATTC <u>CCGTTA</u> AGGAGATTGATAATG |
| | traPDKp | CTGGTACCATAAGTTTCGCTTTTTTAGG |
| <i>traQ</i> | traQUSa | TATGAGCTCAACAGGACCTAAAAAGGC |
| | traQsD | CAGCGGCAATACCACTAACG |
| <i>traR</i> | traRUEc | TAAGAATTC <u>ACTGG</u> TAAATCAAAAAGGATAC |
| | CTX19450 | ATTTTCTCCGGATGTTGAGCG |
| <i>traU</i> | traUUSa | GGGAGCTCATTACAAGGGTAAGAGAG |
| | traUsD | GCGTTGAGATGGGAATGCTG |
| <i>traW</i> | traWUMu | ATCAATTGTGATGTGAGAAGGGGATTCAGATG |
| | traWDKp | TTGGTACCGGTCATTTTCTGGATACTCCC |
| <i>traX</i> | traXUEc | CTGAATTC <u>TGTGA</u> ACGCGGGAGTATCCAG |
| | traXsD | CATTTGACGCGATAGGTCAC |
| <i>traY</i> | traYUEc | TTAAGAATTCGAGGCTGGTCAAGAGCGG |
| | traYsD | GCACTGCATCGATCACAAAG |
| <i>trbA</i> | trbAUEc | CCAGAATTC <u>TCTG</u> AGGTTCTGATG |
| | 5c25 | TTCAAAGCGGAACAGCTGCACCGC |
| <i>trbB</i> | trbBuEc2 | CCGAATTC <u>CCAGT</u> CTGCGACTGACAGACGATG |
| | trbBDKp | GCGGTACCGGGTTATTCTCCTATTGC |
| <i>trbC</i> | trbCUEc | AGAATTC <u>CAATAG</u> GAGAATAACCCG |
| | trbCsD | GGAGCCAGGAACGAGAAGTA |
| <i>trbN</i> | trbNUEc | TAGAATTCATTAGGAGATTGTTATGGTTG |
| | trbNDKp | CCGGGTACCGTTGTGGCTGGTTTTG |

Relevant restriction sites are underlined

Table S7. Primers for RT-qPCR

| Gene | Primer | Sequence 5'-3' |
|--------------|----------|---------------------------|
| <i>repA</i> | pRepAaF | TCACATGGGACCCGTTTAACC |
| | pRepAaR | GCGCGGCTTTAACAGAGATTTC |
| <i>nikA</i> | pCNikAaF | TCTTATCAAATCTGGGCTTGAAAAG |
| | pCNikAaR | ACCATCCATCTCCTGTTGCAG |
| <i>nikB</i> | pCNikBaF | GAAAGAATGCTGCTCAAGGGG |
| | pCNikBaR | CGTTTGAACTCAGCCAGGTTG |
| <i>traH</i> | pCTraHaF | CCCGTTACAAATTCCTACC |
| | pCTraHaR | GCAACTGTTCAAACGTAACC |
| <i>orf35</i> | pCOrf5aF | AAGCATTCTCGACGACTTG |
| | pCOrf5aR | CCGGCAGTAATGTCCTTG |
| <i>orf36</i> | pCOrf3aF | TAGCTTGCCGGTTCGTATTTTC |
| | pCOrf3aR | CCGAGATGAGCGACGATGAG |
| <i>traL</i> | pCtraLaF | GGGAGCCTGACTAATACAAC |
| | pCtraLaR | ATGGAACGGGAATGCATCAC |
| <i>traM</i> | pCtraMaF | TTCAGGGTFACTGGCTTCAC |
| | pCtraMaR | CCGCGGACGCTGGTTAATTG |
| <i>traN</i> | pCtraNaF | GCAGACACTGGAAGAGTTAC |
| | pCtraNaR | CCGTACCTGCGGAATGGATG |
| <i>traO</i> | pCtraOaF | AGATAAAGCGGCTCTCACAG |
| | pCtraOaR | ACGTAGCCACCGCTTTAGCC |
| <i>traP</i> | pCtraPaF | GGCAGGATTCTGGTGACTTC |
| | pCtraPaR | GCCCATAACCAGCGCTTTC |
| <i>traQ</i> | pCtraQaF | GCAGTCGTTGGGATCATTGG |
| | pCtraQaR | CTGACGGGTCCGAAGCTAAC |
| <i>traU</i> | pCtraUaF | GCCGTGAACGCGCTTACATC |
| | pCtraUaR | CAATCAGGCGCCACAGCATC |
| <i>traW</i> | pCtraWaF | GCAGGCACAGGATAAGTACC |
| | pCtraWaR | AACTTCGGCCGTTGAAGATG |
| <i>traX</i> | pCtraXaF | CCCGGAAGGGAAGGCTAAAC |
| | pCtraXaR | TTCATAACTTCGGCGAACGG |
| <i>traY</i> | pCtraYaF | TGGTGCGGTTCAATCATTGG |
| | pCtraYaR | TGGCCAGAAGCCTCCATTG |
| <i>trbN</i> | pCTrbNaF | GTGGATTCTCGCCTTGTGG |
| | pCTrbNaR | TGTTGTATTTGAGGATGTAGGCAC |
| <i>excA</i> | pCexcAaF | TGGACGGTTAGAGACGGTTC |
| | pCexcAaR | TTCTCCGGGCCAGGTCATAG |

References

1. Avison MB, von Heldreich CJ, Higgins CS, Bennett PM, Walsh TR. 2000. A TEM-2 β -lactamase encoded on an active TnI-like transposon in the genome of a clinical isolate of *Stenotrophomonas maltophilia*. J Antimicrob Chemother **46**: 879–884.