| Strain       | Species          | Assembly no. | BioSample no. | Year | Country* | Host | ST                      | Plasmid replicon types                                      |
|-------------|------------------|--------------|---------------|------|----------|------|-------------------------|-------------------------------------------------------------|
| ECN1H2      | *E. hormaechei*  | GCA_000724505 | SAMN02713682  | 2012 | USA      | Environment | 93 | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncA/C2, IncN |
| GN02616     | *E. cloacae*     | GCA_001022695 | SAMN03732719  | 2007 | USA      | Human | 608 | IncH2, IncH2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II |
| SMART_1493  | *E. cloacae*     | GCA_001525175 | SAMN04431024  | 2014 | Viet Nam | Human | 462 | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncFII, IncN, Col440I, ColRNAI, Col440II |
| 20ES        | *E. hormaechei*  | GCA_002740835 | SAMN07452577  | 2013 | Romania  | Human | 254 | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncL/M, IncFII, IncFIB, IncR, IncFIA, Col440I, ColRNAI, Col440II |
|             |                  |              |               |      |          |      |                          |                                                             |
| 174         | *E. hormaechei*  | GCA_003031755 | SAMN04456586  | 2015 | USA      | Human | 231 | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncL/M, IncFIA, IncR |
| ENT1        | *E. asburiae*    | GCA_003046225 | SAMN08849070  | 2016 | Brazil   | Environment | New | IncFIB, IncFII, IncH2, IncH2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI |
| TUM9991     | *E. hormaechei*  | GCA_003176615 | SAMD00115713  | 2010 | NA       | Human | 93  | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncN, IncFII |
| 99B3        | *E. asburiae*    | GCA_003289825 | SAMN09435809  | 2016 | France   | Human | 1065 | IncH1A, IncH1B, IncFIB, Col440I, ColRNAI, Col440II |
| GEN00188    | *E. hormaechei*  | GCA_004164255 | SAMN10847478  | 2017 | France   | Human | 873  | IncH2, IncH2A, RepA_1_pKPC-CAV1321 |
| 3849        | *E. hormaechei*  | GCA_012974405 | SAMN14734641  | 2017 | Italy    | Human | 382  | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncN, Col440I, ColRNAI, Col440II |
| WP5-S18-CRE-02 | *E. kobei*     | GCA_014169295 | SAMD00194526 | 2018 | Japan    | Environment | 32  | IncL/M, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II |
| WP5-S18-ESBL-01 | *E. asburiae*  | GCA_014169315 | SAMD00194528 | 2018 | Japan    | Environment | New | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncFIA, IncFIB, Col440I, ColRNAI |
| STN0717-73  | *E. asburiae*    | GCA_015138375 | SAMD00195991  | 2018 | Japan    | Environment | 484 | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncFII, Col440I, ColRNAI, Col440II |
| KP0785      | *E. cloacae*     | GCA_015682695 | SAMN06330157  | 2014 | USA      | Human | 452  | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncFIB, IncFII, IncFIA |
| MS14406     | *E. cloacae*     | GCA_015683115 | SAMN14867396  | 2016 | Australia | Human | 167  | IncH2, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II |
| NR1247      | *E. cloacae*     | GCA_015683195 | SAMN06330201  | 2015 | USA      | Human | 454  | IncFIB, IncFII, IncN, Col440I, ColRNAI, Col440II |
| CQ545       | *E. cloacae*     | GCA_015683495 | SAMN11239595  | 2016 | Australia | Human | 167  | IncH2, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II |
| Ec_10_6     | *E. cloacae*     | GCA_015685455 | SAMEA3886779  | NA   | NA       | Human | 167  | IncH2, IncH2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II |
| AZ_872      | *E. cloacae*     | GCA_015959265 | SAMN07501554  | 2014 | Nigeria  | Human | 836  | IncFIB, IncH1B, Col440I, ColRNAI, Col440II |
| 176B9       | *E. hormaechei*  | GCA_016427675 | SAMN17141544  | 2018 | France   | Human | 873  | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncFIB |
| 189H4       | *E. hormaechei*  | GCA_016427965 | SAMN17141574  | 2018 | France   | Human | 873  | IncH2, IncH2A, RepA_1_pKPC-CAV1321, IncN, Col440I, ColRNAI |

*Table S1.* The detailed information of genomes of 81 MCR-1-producing ECC isolates collected in this study and downloaded from public database for comparison of genetic features.
| Accession       | Strain              | Host    | Country   | Length | Function(s)                                                                 |
|-----------------|---------------------|---------|-----------|--------|-----------------------------------------------------------------------------|
| GCA_016427975   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016428155   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321                                       |
| GCA_016428465   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016428495   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016428505   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016632625   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016632955   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016633045   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016633055   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016633425   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016633475   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016633505   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016633515   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016633605   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016633615   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016633995   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016634305   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_016634365   | E. hormaechei       | Human   | France    | 873    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB                                |
| GCA_020673785   | E. cloacae          | Human   | Nigeria   | 836    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI                     |
| GCA_020673822   | E. cloacae          | Human   | Nigeria   | 836    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI                     |
| GCA_020675995   | E. cloacae          | Human   | Nigeria   | 836    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI                     |
| GCA_090075545   | E. asburiae         | Human   | UK        | 519    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II           |
| GCA_090077545   | E. asburiae         | Human   | UK        | 610    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, IncFIA, IncFII     |
| SAMEA2273206    | E. asburiae         | Human   | UK        | 267    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB, Col440I, ColRNAI, IncH1B     |
| SAMEA5852007    | E. asburiae         | Human   | NA        | 1065   | IncH1A, IncH1B, IncFIB, Col440I, ColRNAI, Col440II                        |
| Accession | Species       | Accession | Date  | Country | Host   | Length | Description |
|-----------|---------------|-----------|-------|---------|--------|--------|-------------|
| KR2727    | *E. hormaechei* | GCA_902713455 | 2011  | China   | Human  | 93     | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, IncR, IncFII |
| KR2728    | *E. hormaechei* | GCA_902713475 | 2011  | China   | Human  | 93     | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, IncR, IncFII, IncX4 |
| AI2613    | *E. hormaechei* | GCA_903993075 | 2017  | Spain   | Human  | 93     | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncR, IncFII, Col440I, ColRNAI |
| AN2360    | *E. hormaechei* | GCA_903993235 | 2017  | Spain   | Human  | 93     | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncR, IncFII, Col440I, ColRNAI |
| AI2705    | *E. asburiae*  | GCA_905219205 | 2016  | Spain   | Human  | 515    | IncFIB, IncFII, IncP, Col440I, ColRNAI, Col440II |
| AI2719    | *E. asburiae*  | GCA_905219325 | 2016  | Spain   | Human  | 515    | IncFIB, IncFII, IncFIA, Col440I, ColRNAI |
| AI2688    | *E. hormaechei* | GCA_905231985 | 2018  | Spain   | Human  | 78     | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB, IncFII, IncFIA, IncL/M, Col440I, ColRNAI |
| AI2689    | *E. hormaechei* | GCA_905232005 | 2018  | Spain   | Human  | 171    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncL/M, ColRNAI, Col440I |
| AI2656    | *E. hormaechei* | GCA_905232475 | 2018  | Spain   | Human  | 133    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Inc440I, ColRNAI, Col440II |
| AI2657    | *E. hormaechei* | GCA_905232485 | 2018  | Spain   | Human  | 133    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI |
| AI2658    | *E. hormaechei* | GCA_905232495 | 2018  | Spain   | Human  | 133    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II |
| AI2662    | *E. hormaechei* | GCA_905232535 | 2018  | Spain   | Human  | 171    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncL/M, Col440I, ColRNAI |
| AI2760    | *E. hormaechei* | GCA_905232815 | 2018  | Spain   | Human  | 182    | IncFIB, IncFII, IncL/M, Col440I, ColRNAI |
| AI2797    | *E. asburiae*  | GCA_905233045 | 2018  | Spain   | Human  | 24     | IncFIB, IncFII, IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II |
| AI2798    | *E. hormaechei* | GCA_905233055 | 2018  | Spain   | Human  | 78     | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB |
| AI2799    | *E. hormaechei* | GCA_905233065 | 2018  | Spain   | Human  | 1015   | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB, IncL/M |
| AI2802    | *E. asburiae*  | GCA_905233075 | 2018  | Spain   | Human  | 24     | IncFIB, IncFII, IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II |
| AI2747    | *E. hormaechei* | GCA_905329565 | 2018  | Spain   | Human  | 182    | IncFIB, IncFII, IncHI2, IncHI2A, RepA_1_pKPC-CAV1321 |
| AI2804    | *E. hormaechei* | GCA_905329655 | 2018  | Spain   | Human  | 78     | IncFIB, IncFII, IncHI2, IncHI2A, RepA_1_pKPC-CAV1321 |
| AI2936    | *E. hormaechei* | GCA_905330775 | 2018  | Spain   | Human  | 78     | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB, Col440I, ColRNAI, Col440II |
| AI2939    | *E. hormaechei* | GCA_905330795 | 2018  | Spain   | Human  | 133    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II |
| AI2984    | *E. asburiae*  | GCA_905331215 | 2016  | Spain   | Human  | 515    | IncFIB, IncFII, Col440I, ColRNAI, Col440II |
| AJ3013    | *E. hormaechei* | GCA_905331365 | 2018  | Spain   | Human  | 764    | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIB, IncFII, IncFIA, Col440I, ColRNAI, Col440II |
|   | Species        | Accession       | Year | Country | Host   | IS Elements                                                                 |
|---|----------------|-----------------|------|---------|--------|-----------------------------------------------------------------------------|
| 51118 | E. hormaechei  | GCA_021285885   | 2014 | China   | Human  | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI                    |
| 52272 | E. hormaechei  | GCA_021285525   | 2014 | China   | Human  | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321                                      |
| 52319 | E. hormaechei  | GCA_021285505   | 2014 | China   | Human  | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321                                      |
| 52744 | E. kobei       | GCA_021285945   | 2014 | China   | Human  | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321                                      |
| 53287 | E. hormaechei  | GCA_021285905   | 2015 | China   | Human  | IncFIB, IncFII, IncX3, Col440I, ColRNAI, Col440II                           |
| 54401 | E. hormaechei  | GCA_021285655   | 2015 | China   | Human  | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II           |
| 54570 | E. hormaechei  | GCA_021285645   | 2015 | China   | Human  | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, Col440I, ColRNAI, Col440II           |
| 58918 | E. kobei       | GCA_021285445   | 2016 | China   | Human  | IncX3, IncFIB, IncFII, Col440I, ColRNAI, Col440II                          |
| 58941 | E. hormaechei  | GCA_021285385   | 2016 | China   | Human  | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncFIA, IncFII                      |
| 61363 | E. hormaechei  | GCA_021285405   | 2017 | China   | Human  | IncHI2, IncHI2A, RepA_1_pKPC-CAV1321, IncN                                |
| 60403 | E. hormaechei  |                 | 2010 | China   | Human  | IncFIB, IncHI2, IncHI2A, RepA_1_pKPC-CAV1321                               |

* NA, not available.
**Table S2.** Susceptibility profiles and MICs for 11 clinical isolates and their transconjugants.

| Isolate/ transconjugant* | AMC | TZP | CTX | CAZ | CPO | ATM | IMP | MEM | GEN | AMK | LVX | CIP | SXT | FLR | TCY | FOS | CHL | COL | TGC | Conjugation temperature (°C) |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------------------------------|
| 51118                     | 64  | 2   | 8   | 128 | 4   | >128| 0.25| 0.03| >128| 2   | 4   | 4   | >152| 32  | >128| 8   | >64 | 0.5 | 0.5 |                             |
| TC-51118                  | 64  | 2   | 8   | 64  | 2   | >128| 0.25| 0.03| >128| 2   | 4   | 4   | >152| 16  | >128| 8   | >64 | 0.5 | 0.5 |                             |
| 52272                     | 128 | 2   | 16  | 64  | 8   | >128| 0.25| 0.06| 16  | 16  | 0.5 | 0.5 | >152| 16  | 64  | 4   | >64 | 1   | 0.25 |                             |
| TC-52272                  | 16  | 1   | 8   | 32  | 4   | 32  | 0.25| 0.03| 8   | 8   | 0.25| 0.5 | >152| 16  | 32  | 0.5 | >64 | 1   | ≤0.125|                             |
| 52319                     | 64  | 16  | 32  | 128 | 16  | >128| 0.25| 0.06| 32  | 16  | 4   | 2   | >152| >64 | >128| 4   | 32  | 2   | 2   |                             |
| TC-52319                  | 16  | 4   | 8   | 16  | 2   | 32  | 0.25| 0.03| 8   | 8   | 0.125| 0.25| >152| 8   | 32  | 0.5 | 8   | 0.5 | ≤0.125|                             |
| 52744                     | 128 | 4   | 32  | 128 | 8   | >128| 0.25| 0.06| 8   | 2   | 0.5 | 1   | >152| 16  | 4   | 16  | >64 | 64  | 0.25 |                             |
| TC-52744                  | 64  | 2   | 32  | 64  | 8   | 128 | 0.25| 0.06| 8   | 2   | 0.5 | 0.5 | >152| 16  | 4   | 8   | >64 | 16  | 0.25 |                             |
| 54401                     | 128 | 2   | 16  | 64  | 8   | >128| 0.5 | 0.03| 8   | 8   | 0.25| 0.5 | >152| 16  | 4   | 16  | >64 | 1   | ≤0.125|                             |
| TC-54401                  | 16  | 2   | 8   | 64  | 4   | 128 | 0.5 | 0.03| 8   | 2   | 0.25| 0.5 | >152| 16  | 4   | 8   | >64 | 0.5 | ≤0.125|                             |
| 54570                     | 64  | 2   | 8   | 128 | 2   | >128| 0.25| ≤0.015| 2   | 0.25| 0.5 | >152| 16  | >128| 64  | 8   | 1   | 0.25 |                             |
| TC-54570                  | 64  | 2   | 8   | 128 | 0.5 | 64  | 0.25| 0.03| >128| 2   | 0.25| 0.5 | >152| 8   | 128 | 0.5 | 8   | 0.5 | ≤0.125|                             |
| 58918                     | 64  | 1   | 4   | 32  | 64  | 16  | 128 | 0.25| 0.03| >128| 2   | 4   | >128| 2   | 0.25| 1   | ≤2.375| 16  | >128| >256| >64 | 128 | 0.25 | NA |
| 58941                     | 64  | 2   | 32  | 64  | 16  | 128 | 0.25| 0.03| >128| 2   | 16  | 16  | >152| 16  | >128| 16  | 16  | 2   | ≤0.125|                             |
| TC-58941                  | 64  | 1   | 4   | 32  | 0.5 | 128 | 0.25| 0.03| >128| 2   | 16  | 0.25| 0.5 | >152| 8   | >128| 0.5 | 8   | 1   | ≤0.125|                             |
| 61363                     | 128 | 128 | >128| >128| 64  | 4   | 8   | 4   | 8   | 2   | 1   | 1   | >152| 16  | 4   | 16  | 16  | 1   | 0.5 |                             |
| TC-61363                  | 128 | 128 | >128| >128| 64  | 4   | 8   | 4   | 4   | 2   | 0.5 | 1   | >152| 8   | 2   | 2   | 8   | 1   | ≤0.125|                             |
| 60403                     | 128 | >128| >128| >128| 64  | 128 | 0.5 | 0.5 | >128| >128| 16  | 64  | >152| 32  | >128| >256| >64 | 1   | 0.5 | NA |
| 53287                     | 128 | >128| >128| >128| 4   | 8   | 2   | 8   | 2   | 0.06| 0.06| ≤2.375| 16  | 4   | 8   | 16  | 1   | 0.25 | NA |
| J53                       | 16  | 1   | 0.06| 0.25| 0.125| 0.25| 0.25| 0.03| 1   | 1   | 0.015| 0.03| ≤2.375| 8   | 2   | 0.5 | 8   | 1   | ≤0.125|                             |
*TC, transconjugants; MIC values (mg/ml); AMC: Amoxicillin/clavulanic acid; TZP: Piperacillin/tazobactam; CTX: Cefotaxime; CAZ: Ceftazidime; CPO: Cefpirome; ATM: Aztreonam; IMP: Imipenem; MEM: Meropenem; GEN: Gentamicin; AMK: Amikacin; LVX: Levofloxacin; CIP: Ciprofloxacin; SXT: Trimethoprim/sulfamethoxazole; FLR: Florfenicol; TCY: Tetracycline; FOS: Fosfomycin; CHL: Chloramphenicol; COL: Colistin; TGC: Tigecycline; NA, not applicable.
Figure S1. ANIb analysis of MCR-9-producing ECC isolates.
**Figure S2.** Circular representation of the studied *bla*<sub>NDM-1</sub>-carrying plasmids. A) Sequence comparison of the IncX3 plasmid p53287-NDM-1 carried by the bacteremia associated with *E. hormaechei* strain 53287. B) Sequence comparison of the *bla*<sub>NDM-1</sub>-
carrying IncN plasmid p61363-NDM-1. GC content and GC Skew were represented on the inner map's distance scale (in kbp). Each plasmid was compared to its most closely-related plasmid. The red arc around the map indicated ORFs. Certain important genes were also indicated on the ring.