

Precise Species Identification by Whole-Genome Sequencing of *Enterobacter* Bloodstream Infection, China

Appendix 1

Appendix 1 Table 1. Classification and nomenclature of the genus *Enterobacter* as of December 2018*

| Species | Hoffman cluster | Reference | Type strain | GenBank accession no. or current species name |
|--|-----------------|-----------|---------------------|---|
| Species name for validation (n = 14) | | | | |
| <i>Enterobacter asburiae</i> | I | (1) | JCM 6051 | CP011863 |
| <i>E. cancerogenus</i> | - | (2) | ATCC 35316 | ERR1854846 |
| <i>E. chuandaensis</i> | - | (3) | 090028 ^T | QZCS00000000 |
| <i>E. cloacae</i> | XI | | ATCC 13047 | ERR1854846 |
| <i>E. bugandensis</i> | IX | (4) | EB-247 | FYBI00000000 |
| <i>E. hormaechei</i> | VII | (5) | ATCC 49162 | MKEQ00000000 |
| <i>E. huaxiensis</i> | - | (3) | 090008 ^T | QZCT00000000 |
| <i>E. kobei</i> | II | (6) | ATCC BAA-260 | CP017181 |
| <i>E. ludwigii</i> | V | (7) | EN-119 | CP017279 |
| <i>E. mori</i> | - | (8) | LMG 25706 | AEXB00000000 |
| <i>E. soli</i> | - | (9) | ATCC BAA-2102 | LXES00000000 |
| <i>E. tabaci</i> | - | (10) | YIM Hb-3 | N/A |
| <i>E. xiangfangensis</i> [†] | VI | (11) | LMG 27195 | CP017183 |
| <i>E. sichuanensis</i> | - | (12) | WCHECI1597 | POVL00000000 |
| Species in doubt (n = 2) | | | | |
| <i>E. muelleri</i> [‡] | - | (13) | JM-458 | FXLQ00000000 |
| <i>E. siamensis</i> [§] | - | (14) | C2361 | N/A |
| Species name awaiting validation (n = 3) | | | | |
| <i>E. timonensis</i> | - | (15) | mt20 | FCOP00000000 |
| <i>E. chengduensis</i> | - | (16) | WCHECI-C4 | MTSO00000000 |
| <i>E. roggenkampii</i> | IV | (17) | DSM16690 | CP017184 |
| Species listed in LPSN but moved out of <i>E.</i> (n = 20) | | | | |

| Species | Hoffman | | | GenBank accession no. |
|----------------------------|---------|-----------|---------------|-----------------------------------|
| | cluster | Reference | Type strain | or current species name |
| <i>E. aerogenes</i> | | (18) | ATCC 13048 | <i>Klebsiella aerogenes</i> |
| <i>E. agglomerans</i> | | (19) | ATCC 27155 | <i>Pantoea agglomerans</i> |
| <i>E. amnigenus</i> | | (20) | ATCC 33072 | <i>Lelliottia amnigena</i> |
| <i>E. arachidis</i> | | (20) | KCTC 22375 | <i>Kosakonia arachidis</i> |
| <i>E. cowanii</i> | | (20) | CCUG 45998 | <i>Kosakonia cowanii</i> |
| <i>E. gergoviae</i> | | (20) | ATCC 33028 | <i>Pluralibacter gergoviae</i> |
| <i>E. helveticus</i> | | (20) | JCM 16470 | <i>Cronobacter helveticus</i> |
| <i>E. intermedius</i> | | (21) | ATCC 33110 | <i>Kluyvera intermedia</i> |
| <i>E. massiliensis</i> | | (22) | JC163 | <i>Metakosakonia massiliensis</i> |
| <i>E. nimipressuralis</i> | X | (20) | CIP 104980 | <i>Lelliottia nimipressuralis</i> |
| <i>E. oryzae</i> | | (20) | LMG 24251 | <i>Kosakonia oryzae</i> |
| <i>E. oryzendophyticus</i> | | (23) | LMG 26432 | <i>Kosakonia oryzendophytica</i> |
| <i>E. oryziphilus</i> | | (23) | LMG 26429 | <i>Kosakonia oryziphila</i> |
| <i>E. pulveris</i> | | (20) | DSM 19144 | <i>Cronobacter pulveris</i> |
| <i>E. pyrinus</i> | | (20) | ATCC 49851 | <i>Pluralibacter pyrinus</i> |
| <i>E. radicincitans</i> | | (20) | CIP 108468 | <i>Kosakonia radicincitans</i> |
| <i>E. sacchari</i> | | (11) | CGMCC 1.12102 | <i>Kosakonia sacchari</i> |
| <i>E. sakazakii</i> | | (24) | ATCC 29544 | <i>Cronobacter sakazakii</i> |
| <i>E. taylorae</i> | | (25) | ATCC 35317 | <i>Enterobacter cancerogenus</i> |
| <i>E. turicensis</i> | | (20) | DSM 18397 | <i>Cronobacter zurichensis</i> |

*LPSN, The list of Prokaryotic Names with Standing in Nomenclature.

†The species status of *E. xiangfangensis* has been doubted previously and it has been proposed as a subspecies of *E. hormaechei* rather than a valid species (17,26). However, its type strain has only 94.48% ANI and 60.0% isDDH with *E. hormaechei* type strain ATCC 49162^T (GenBank accession no. MKEQ00000000). Therefore, *E. xiangfangensis* and *E. hormaechei* are clearly 2 different species.

‡*E. muelleri* is a later synonym of *E. asburiae*.

§It has been proposed to reject *E. siamensis* because the 16S rRNA sequence of its type strain available in collections does not match its record in GenBank (27).

Appendix 1 Table 2. The 6 unnamed *Enterobacter* spp. identified in genomic study of *Enterobacter* bloodstream infection, China*

| Species | | | | |
|------------|-----------------------|----------------------|--------------------------|------------|
| assignment | Representative strain | Genome accession no. | Closest species | isDDH (%)† |
| Taxon 1 | DSM 14563‡ | CP017186 | <i>E. xiangfangensis</i> | 66.6 |
| Taxon 2 | e362 | FKDT00000000 | <i>E. roggenkampii</i> | 65.4 |
| Taxon 3 | e773 | FKGE00000000 | <i>E. asburiae</i> | 65.3 |
| Taxon 4 | e2032 | FKBK00000000 | <i>E. asburiae</i> | 52.0 |
| Taxon 5 | e483 | FKEG01000000 | <i>E. asburiae</i> | 49.6 |
| Taxon 6 | 153C2 | QMCQ01000000 | <i>E. xiangfangensis</i> | 52.8 |

*isDDH, in silico DNA–DNA hybridization.

†isDDH values between the representative strain and the type strain of closest species.

‡Strain DSM 14563 has been proposed as the type strain of *E. hormaechei* subspecies *Hoffmannii*. However, the strain has only 94.13% ANI and 58.0% isDDH with *E. hormaechei* type strain ATCC 49162T (GenBank accession no. MKEQ00000000). It is clear that the *E. hormaechei* subspecies *hoffmannii* is actually not a subspecies of *E. hormaechei* but rather represents a new, unnamed *Enterobacter* species. In this study, we temporarily designated the species taxon 1 for simplicity.

Appendix 1 Table 3. Profiles of sequence types in genomic study of *Enterobacter* bloodstream infection, China*

| ST | Species | <i>dnaA</i> | <i>fusA</i> | <i>gyrB</i> | <i>leuS</i> | <i>pyrG</i> | <i>rplB</i> | <i>rpoB</i> | Closest ST† |
|-----|--------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|----------------------------|
| | | | | | | | | | (no. of allele difference) |
| 1 | <i>E. cloacae</i> | 1 | 1 | 1 | 1 | 1 | 1 | 1 | |
| 12 | <i>E. ludwigii</i> | 13 | 2 | 45 | 24 | 52 | 2 | 14 | |
| 50 | <i>E. xiangfangensis</i> | 4 | 4 | 4 | 6 | 37 | 4 | 25 | |
| 78 | Taxon 1 | 8 | 9 | 6 | 9 | 9 | 6 | 8 | |
| 97 | Taxon 1 | 59 | 9 | 62 | 9 | 62 | 25 | 6 | |
| 104 | Taxon 1 | 59 | 40 | 76 | 9 | 70 | 6 | 6 | |
| 127 | <i>E. xiangfangensis</i> | 46 | 20 | 74 | 44 | 45 | 24 | 6 | |
| 171 | <i>E. xiangfangensis</i> | 49 | 21 | 19 | 44 | 45 | 12 | 32 | |
| 316 | Taxon 1 | 59 | 88 | 82 | 9 | 67 | 6 | 6 | |
| 337 | <i>E. xiangfangensis</i> | 67 | 21 | 9 | 129 | 45 | 12 | 32 | |
| 418 | <i>E. xiangfangensis</i> | 53 | 35 | 154 | 44 | 45 | 4 | 6 | |
| 499 | <i>E. bugandensis</i> | 164 | 18 | 183 | 200 | 120 | 8 | 29 | |
| 519 | <i>E. cloacae</i> | 1 | 107 | 158 | 1 | 168 | 36 | 1 | |
| 528 | <i>E. hormaechei</i> | 95 | 56 | 112 | 116 | 104 | 4 | 63 | |
| 550 | <i>E. xiangfangensis</i> | 179 | 4 | 4 | 6 | 112 | 4 | 6 | |
| 568 | Taxon 1 | 189 | 9 | 12 | 9 | 67 | 6 | 6 | |
| 696 | <i>E. hormaechei</i> | 225 | 140 | 93 | 268 | 224 | 109 | 141 | |
| 718 | <i>E. bugandensis</i> | 140 | 18 | 248 | 31 | 230 | 8 | 29 | |
| 828 | <i>E. xiangfangensis</i> | 9 | 4 | 14 | 61 | 257 | 4 | 9 | |
| 879 | <i>E. asburiae</i> | 152 | 15 | 102 | 15 | 101 | 11 | 133 | |

| ST | Species | <i>dnaA</i> | <i>fusA</i> | <i>gyrB</i> | <i>leuS</i> | <i>pyrG</i> | <i>rplB</i> | <i>rpoB</i> | Closest ST† (no. of allele difference) |
|-----|--------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---|
| 922 | <i>E. cloacae</i> | 169 | 107 | 61 | 168 | 36 | 77 | 1 | |
| 984 | <i>E. roggenkampii</i> | 65 | 57 | 49 | 94 | 49 | 12 | 47 | |
| N1 | <i>E. huaxiensis</i> | n1 | n1 | n1 | n1 | n1 | n1 | n1 | None |
| N2 | <i>E. roggenkampii</i> | 191 | n2 | 254 | 193 | 49 | 12 | 26 | 613 (3) |
| N3 | <i>E. xiangfangensis</i> | n2 | 20 | 148 | 44 | 45 | 4 | 6 | 886/916/986 (1) |
| N4 | <i>E. xiangfangensis</i> | 4 | 4 | 15 | 4 | 11 | 30 | 6 | 111/981 (2) |
| N5 | <i>E. xiangfangensis</i> | 58 | 22 | 14 | 6 | 39 | 4 | 9 | 79 (1) |
| N6 | <i>E. xiangfangensis</i> | 58 | 41 | 14 | 6 | 69 | 4 | n2 | 106 (1) |
| N7 | <i>E. xiangfangensis</i> | 178 | 4 | 4 | 6 | 92 | 4 | 6 | 542 (1) |
| N8 | <i>E. xiangfangensis</i> | 4 | 37 | 4 | 6 | 42 | 4 | 6 | 329 (1) |
| N9 | <i>E. chuandaensis</i> | n3 | n3 | n2 | n2 | n2 | n2 | 120 | 573/944 (6) |
| N10 | <i>E. bugandensis</i> | 309 | 18 | n3 | n3 | 34 | 8 | n3 | 1084 (3) |
| N11 | <i>E. sichuanensis</i> | n4 | 98 | 170 | n4 | n3 | 68 | n4 | 472/607/738/847 (4) |
| N12 | <i>E. asburiae</i> | n5 | 15 | n4 | 124 | n4 | 11 | 68 | 319 (3) |
| N13 | Taxon 1 | 59 | 9 | n5 | n5 | 79 | 37 | n5 | 157/419/792 (4) |
| N14 | Taxon 2 | 151 | 108 | n6 | n6 | n5 | 14 | 93 | 474 (3) |
| N15 | <i>E. xiangfangensis</i> | n6 | 69 | 19 | 44 | 64 | 4 | 32 | 270 (2) |
| N16 | <i>E. bugandensis</i> | 140 | 18 | n7 | 31 | 230 | 8 | 29 | 718 (1) |

*N1 to N16 are new sequence types. New alleles are temporarily assigned n1 to n6. ST, sequence type.

†For new sequence types only.

Appendix 1 Table 4. Single nucleotide polymorphisms between the 3 ST78 strains in genomic study of *Enterobacter* bloodstream infection, China*

| Strain | 090039 | 090030 | 090019 |
|--------|--------|--------|--------|
| 090039 | – | 1,052 | 814 |
| 090030 | 1,052 | - | 306 |
| 090019 | 814 | 306 | – |

*The 3 genomes were mapped against the complete chromosome

sequence of ST78 strain AR_0050 (GenBank accession no. CP021896)

by using Parsnp version 1.2 and alignment was obtained by using Harvest

(28).

Appendix 1 Table 5. Complete genome and antimicrobial resistance genes of strain 090011 and strain 045001 in genomic study of *Enterobacter* bloodstream infection, China*

| Strain | Size, bp | Replicon type, Inc | Genes mediating resistance to | | | | | | | |
|----------------|-----------|-----------------------|---|---|--|------------|-------------|-----------------|--------------|--------------------------------|
| | | | β -lactam | Aminoglycoside | Fluoroquinolone | Fosfomycin | Rifampin | Sulfonamid e | Tetracycline | Trimethoprim |
| 090011 | | | | | | | | | | |
| Chromosome | 4,639,926 | - | <i>bla</i> _{ACT-7} | | | | <i>fosA</i> | | | |
| pNDM5_090011 | 46,161 | X3 | <i>bla</i> _{NDM-5} | | | | | | | |
| pCTXM65_090011 | 102,543 | FIA, FIB, R | <i>bla</i> _{CTX-M-65} , <i>bla</i> _{TEM-1B} | <i>aadA16</i> , <i>aac(6')-Ib-cr</i> | <i>aac(6')-Ib-cr</i> | | | <i>arr-3</i> | <i>sul1</i> | <i>tet(A)</i> <i>dfrA27</i> |
| 045001 | | | | | | | | | | |
| Chromosome | 4,698,270 | - | <i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-1} , <i>bla</i> _{ACT-7} | <i>aac(3)-IIa</i> , <i>aac(6')-Ib-cr</i> | <i>aac(6')-Ib-cr</i> , <i>qnrB1</i> | | <i>fosA</i> | | | |
| pNDM1_045001 | 85,718 | FII | <i>bla</i> _{NDM-1} , <i>bla</i> _{TEM-1} | <i>rmtB</i> | | | | | | <i>tet(A)</i> <i>dfrA14</i> |
| p1_045001 | 78,247 | ND | | | | | | | | |
| p2_045001 | 2,496 | ND | | | | | | | | |

*ND, undetermined.

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- comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. Syst Appl Microbiol. 2013;36:309–19. [PubMed](#) <https://doi.org/10.1016/j.syapm.2013.03.005>
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