***Supplementary Material***

**Supplemental figure S1**

**Figure 1** Antibiotic resistance of EPEC strains isolates from bovine mastitis in five-major dairy product regions of China.

**Supplemental table S1** PCR primers and conditions for multilocus sequence analysis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Target gene | Primer sequence | PCR conditions  | PCR-product length（bp） | Reference |
| **Molecular idetification***16S* | GTTAATACCTTTGCTCATTGAACCAGGGTATCTAATCCTGTT | 94°C 1min，94°C 60s，55°C 30 s，72°C 90s，72°C 3.5min（37cycles） | 340 | (Malinen et al., 2003) |
| *rpoB*1698f*rpoB*2041r | AACATCGGTTTGATCAACCGTTGCATGTTGGTACCCAT | 94°C 5min，94°C 30 s，50°C 90 s，72°C 90 s，72°C 10min（25cycles） | 343 | (Dahllöf et al., 2000) |
| **Resistance genes***tet*A | GTGAAACCCAACATACCCCGAAGGCAAGCAGGATGTAG | 95°C 5min，94°C 30 s，63°C 90 s，72°C 90s，72°C 7min（30cycles） | 888 | (Harel et al., 1991) |
| *tet*B | AGTGGAGCGATTACAGAACATATGTCCTGGCGTGTCTA | 95°C 5min，94°C 30 s，63°C 90 s，72°C 90s，72°C 7min（30cycles） | 158 | (Harel et al., 1991) |
| *bla*TEM | TTGCTCACCCAGAAACGCTGGTGTACGATACGGGAGGGCTTACC | 95°C 5min，94°C 30 s，63°C 90 s，72°C 90s，72°C 7min（30cycles） | 708 | (Ribeiro et al., 2016) |
| *bla*CTX-M | ATGTGCAGYACCAGTAARGTKATGGMTGGGTRAARTARGTSACCAGAAYCAGCGG | 95°C 5min，94°C 30 s，63°C 90 s，72°C 90s，72°C 7min（30cycles） | 593 | (Ribeiro et al., 2016) |
| *flo*R | ATGACCACCACACGCCCCGCCGAAGGAGCACCAGCC | 95°C 3min，94°C 60 s，50°C 90 s，72°C 60s，72°C 10min（35cycles） | 1177 | (Yaqoob et al., 2018) |
| *gyr*A | AAATCTGCCCGTGTCGTTGGTGCCATACCTACGGCGATACC | 94°C 4min，94°C 30s，55°C 45 s，72°C 60 s，72°C 10min（30cycles） | 344 | (Rodriguez-Martinez et al., 2006) |
| *aac*3 | GGCTCAAGTATGGGCATCATTCACCGTAATCTGCTTGCAC | 94°C 5min，94°C 30s，55°C 30s，72°C 60 s，72°C 10min（30cycles） | 389 | (Ojdana et al., 2018) |
| *bla*OXA48 | GCGTGGTTAAGGATGAACACCATCAAGTTCAACCCAACCG | 94°C 3min，94°C 30s，56°C 35s，72°C 45s，72°C 5min（35cycles） | 400 | (Doi and Paterson) |
| *NDM* | GGTTTGGCGATCTGGTTTTCCGGAATGGCTCATCACGATC | 94°C 3min，94°C 30s，56°C 35s，72°C 45s，72°C 5min（35cycles） | 600 | (Doi and Paterson) |
| *aac*6 | ATGSCCTTGCGATGCTCTATGACGAATGCCTGGCGTGTTT  | 94°C 10min，94°C 50s，54°C 45s，72°C 50s，72°C 10min（32cycles） | 486 | (Fernandez-Martinez et al., 2015) |
| *bla*KPC | CGTCTAGTTCTGCTGTCTTGCTTGTCATCCTTGTTAGGCG | 94°C 3min，94°C 30s，56°C 35s，72°C 45s，72°C 15min（35cycles） | 700 | (Doi and Paterson) |
| *bla*FOX | CTACAGTGCGGGTGGTTTCTATTTGCGGCCAGGTGA  | 94°C 3min，94°C 40s，60°C 40s，72°C 60s，72°C 7min（30cycles） | 162 | (Perez-Perez and Hanson, 2002) |

**Supplemental table S2** PCR primers and conditions for amplification of virulence genes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Target gene | Primer sequence | PCR conditions | PCR product length（bp） | Reference |
| *bfp*EP | AATGGTGCTTGCGCTTGCTGCGGCGCTTTATCCAACCTGGTA | 94°C 5min，94°C 30 s，61°C 60 s，72°C 120 s，72°C 10min（25cycles） | 397 | Gunzburg, S. T. et al.(1995) |
| *eae* | GAGAATGAAATAGAAGTCGT GCGGTATCTTTCGCGTAATCGCC | 94°C 2min，94°C 60s，55°C 60 s，72°C 60 s，72°C 10min（35cycles） | 775 | (Rey et al., 2006) |
| *esp* | AAACAGCAGGCACTTGAACGGGAGTCGTCAGTCAGTAGAT | 94°C, 30 s; 56°C, 60 s;72°C, 150 s（40cycles） | 1,830 | (Brunder et al., 1999) |
| *agg*R | GTATACACAAAAGAAGGAAGCACAGAATCGTCAGCATCAGC | 94°C 4min，94°C 60s，55°C 60 s，72°C 90 s，72°C 7min（30cycles） | 254 | (Rúgeles et al., 2010) |
| *elt* | TCTCTATGTGCATACGGAGCCCATACTGATTGCCGCAAT | 96°C 4min，94°C 30s，58°C 30 s，72°C 60 s，72°C 7min（35cycles） | 322 | (Tamanai-Shacoori et al., 1994) |
| *ipa*H | GGTTCCTTGACCGCCTTTCCGATACCGTCGCCGGTCAGCCACCCTCTGAGAGTAC | 94°C 2min，92°C 30s，60°C 30 s，72°C 30 s，72°C 5min（40cycles） | 619 | (Sethabutr et al., 1993) |
| *stx*1 | TTAGACTTCTCGACTGCAAAGTGTTGTACGAAATCCCCTCTG | 94°C 2min，94°C 60s，55°C 60 s，72°C 60 s，72°C 10min（35cycles） | 302 | (Rey et al., 2006) |
| *stx*2 | TTATATCTGCGCCGGGTCTGAGACGAAGATGGTCAAAACG | 94°C 4min，94°C 60s，55°C 60 s，72°C 90 s，72°C 7min（30cycles） | 326 | (Woodward et al., 1992) |

**Supplemental table S3** Sequences of oligonucleotide primers used for typing of eae intimin gene

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene | Oligonucleotide sequence (5'-3')  | PCR product length（bp） | Annealing temperature | Reference |
| eae-α1 | AAAACCGCGGAGATGACTTC | 820 | 60 | (Blanco et al., 2004c)　 |
| 　 | CACTCTTCGCATCTTGAGCT | 　 |  | 　 |
| eae-α2 | AGACCTTAGGTACATTAAGTAAGC | 517 | 60 | (Blanco et al., 2004c) |
| 　 | TCCTGAGAAGAGGGTAATC | 　 |  | 　 |
| eae-β1 | ACTTCGCCACTTAATGCCAGC | 730 | 66 | (Blanco et al., 2004d)　 |
| 　 | TTGCAGCACCCCATGTTGAAT | 　 |  | 　 |
| eae-ξR/β2 | AAGGGGGGAACCCCTGTGTCA | 604 | 66 | (Blanco et al., 2004d)　 |
| 　 | ATTTATTCGCAGCCCCCCACG | 　 |  | 　 |
| eae-δ/κ/β2O | AAAACCGCGGAGATGACTTC | 833 | 60 | (Blanco et al., 2004c)　 |
| 　 | CTTGATACACCCGATGGTAAC | 　 |  | 　 |
| eae-γ1 | AAAACCGCGGAGATGACTTC | 804 | 60 | (Blanco et al., 2004c)　 |
| 　 | AGAACGCTGCTCACTAGATGTC | 　 |  | 　 |
| eae-θ/γ2 | AAAACCGCGGAGATGACTTC | 808 | 58 | (Blanco et al., 2004c)　 |
| 　 | CTGATATTTTATCAGCTTCA | 　 |  | 　 |
| eae-ε1 | AAAACCGCGGAGATGACTTC | 722 | 66 | (Blanco et al., 2004c)　 |
| 　 | AGCTCACTCGTAGATGACGGCAAGCG | 　 |  | 　 |
| eae-νR/ε2 | AATACAGAAGTTAAGGCAT | 378 | 58 | (Blanco et al., 2004d) |
| 　 | ACGACCACTATTCATTTC | 　 |  | 　 |
| eae-ζ | GGTAAGCCGTTATCTGCC | 206 | 62 | (Blanco et al., 2004c)　 |
| 　 | ATAGCAAGTGGGGTGAAG | 　 |  | 　 |
| eae-η | AAAACCGCGGAGATGACTTC | 702 | 60 | (Blanco et al., 2004d)　 |
| 　 | TAAGCGACCACTATTCGTG | 　 |  | 　 |
| eae-ι1 | AAAACCGCGGAGATGACTTC | 651 | 55 | (Blanco et al., 2004d)　 |
| 　 | GTCATATTTAACTTTTACACTA | 　 |  | 　 |
| eae-μR/ι2 | CTGGTAAAGCGATAGTCAAAC | 936 | 58 | (Blanco et al., 2004d)　 |
| 　 | GCGTTTTTGAAGAAACATTTTGC | 　 |  | 　 |
| eae-λ | CGGTCAGCCTGTGAAGGGC | 466 | 643 | (Blanco et al., 2004c)　 |
| 　 | ATAGATGCCTCTTCCGGTATT | 　 |  | 　 |
| eae-μB | AAAACCGCGGAGATGACTTC | 665 | 60 | (Blanco et al., 2004d)　 |
| 　 | ACTCATCATAATAAGCTTTTTGG | 　 |  | 　 |
| eae-νB | CACAGCTTACAATTGATAACA | 311 | 60 | (Blanco et al., 2004c)　 |
| 　 | CTCACTATAAGTCATACGACT | 　 |  | 　 |
| eae-ξB | AAAACCGCGGAGATGACTTC | 468 | 66 | (Blanco et al., 2004c)　 |
| 　 | ACCACCTTTAGCAGTCAATTTG | 　 |  | 　 |

**Supplemental table S4** Primer sequences and sizes of PCR products used in the phylotyping method.

|  |  |  |  |
| --- | --- | --- | --- |
| Target  | Primer sequences  | PCR product (bp) | Reference  |
| *chuA* | ATGGTACCGGACGAACCAAC | 288 | (Clermont et al., 2000) |
|  | TGCCGCCAGTACCAAAGACA |  |  |
| yjaA | CAAACGTGAAGTGTCAGGAG | 211 | (Clermont et al., 2000) |
|  | AATGCGTTCCTCAACCTGTG |  |  |
| TspE4.C2 | CACTATTCGTAAGGTCATCC | 152 | (Clermont et al., 2000) |
|  | AGTTTATCGCTGCGGGTCGC |  |  |
| arpA | AACGCTATTCGCCAGCTTGC | 400 | (Clermont et al., 2000) |
|  | TCTCCCCATACCGTACGCTA |  |  |
| arpA-1 | GATTCCATCTTGTCAAAATATGCC | 301 | (Lescat et al., 2013) |
|  | GAAAAGAAAAAGAATTCCCAAGAG |  |  |
| trpA | AGTTTTATGCCCAGTGCGAG | 219 | (Lescat et al., 2013) |
|  | TCTGCGCCGGTCACGCCC |  |  |
| trpA-1 | CGGCGATAAAGACATCTTCAC | 489 | (Clermont et al., 2008) |
|  | GCAACGCGGCCTGGCGGAAG |  |  |

PCR reactions were performed under the following conditions: denaturation 4 min at 94°C, 30 cycles of 5 s at 94°C and 20 s at 57°C (group E) or 59°C (quadruplex and group C), and a final extension step of 5 min at 72°C. The primers used for the allele-specific phylo-groups E and C PCRs were arpA-1 and trpA respectively (Lescat et al., 2012). In E- and C-specific PCR reactions, the primers trpA-1 are added to provide an internal control.

**Supplemental table S5** Oligonucleotide primers for PCR assays for *E. coli* O serotyping by multiplex PCR

|  |  |  |  |
| --- | --- | --- | --- |
| Target gene | Primer sequence | PCR product length（bp） | Reference |
| O121 | TCCAACAATTGGTCGTGAAA | 628 | (DebRoy et al., 2011) |
| 　 | AGAAAGTGTGAAATGCCCGT | 　 |  |
| O111 | TGTTTCTTCGATGTTGCGAG | 438 | (DebRoy et al., 2011) |
| 　 | GCAAGGGACATAAGAAGCCA | 　 |  |
| O128 | GCTTTCTGCCGATATTTGGC | 289 | (Ranjbar et al., 2017) |
| 　 | CCGACGGACTGATGCCGGTGATT | 　 |  |
| O91 | CGCATTTAAGGACTGGCTGT  | 277 | (Taghadosi et al., 2018) |
| 　 | GTAGCAGATATGCCGACCGT | 　 |  |
| O22 | TGTCGCCACTACTTTCCGCGTTTA | 458 | (Lee et al., 2019) |
| 　 | AGCCCATGACATTACTACGGCACT  | 　 |  |
| O26 | CAATGGGCGGAAATTTTAGA | 155 | (DebRoy et al., 2011) |
| 　 | ATAATTTTCTCTGCCGTCGC | 　 |  |
| O113 | TTGCTATAAATGGAAGCCATTCTTT | 107 | (Perelle et al., 2004) |
| 　 | TGCATGAAATGTTTAAATGCAGCGGGT | 　 |  |