

Table S1. Primers used for DNA amplification

| Gene | Primer | Sequence (5'- 3') | [Primer] | Product (bp) | Annealing Temp. | References |
|--|------------------------------------|---|------------------|--------------|-----------------|----------------------|
| CTX-M-1 | MultiCTXMGP1_F MultiCTXMGP1-2_R | TTAGGAARTGTGCCGCTGYA CGATATCGTTGGTGGTRCCAT | 0.4 μM | 688 | 60°C | Dallenne et al, 2010 |
| CTX-M-2 | MultiCTXMGP2_F MultiCTXMGP1-2_R | CGTTAACGGCACGATGAC CGATATCGTTGGTGGTRCCAT | 0.2 μM 0.4 μM | 404 | 60°C | Dallenne et al, 2010 |
| CTX-M-9 | MultiCTXMGP_F MultiCTXMGP_R | TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG | 0.4 μM | 561 | 60°C | Dallenne et al, 2010 |
| CTX-M-8/25 | CTX-Mg8/25_F CTX-Mg8/25_R | AACRRCAGACGCTCTAC TCGAGCCGGAASGTGYAT | 0.4 μM | 326 | 60°C | Dallenne et al, 2010 |
| TEM | Primer TEM_F Primer TEM_R | CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC | 0.4 μM | 800 | 60°C | Dallenne et al, 2010 |
| SHV | Primer SHV_F Primer SHV_R | AGCCGCTTGAGCAAATTAAC ATCCCGCAGATAAATCACCAC | 0.4 μM | 713 | 60°C | Dallenne et al, 2010 |
| OXA | Primer OXA_F Primer OXA_R | GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCTCTGTAAGTG | 0.4 μM | 564 | 60°C | Dallenne et al, 2010 |
| ACC | MultiCaseACC_F MultiCaseACC_R | CACCTCCAGCGACTTGTTAC GTTAGCCAGCATCACGATCC | 0.2 μM | 346 | 60°C | Dallenne et al, 2010 |
| FOX | MultiCaseFOX_F MultiCaseFOX_R | CTACAGTGCGGGTGGTTT CTATTTGCGGCCAGGTGA | 0.5 μM | 162 | 60°C | Dallenne et al, 2010 |
| MOX; CMY-1, -8, -11; -19 | MultiCaseMOX_F MultiCaseMOX_R | GCAACAACGACAATCCATCCT GGGATAGGCGTAACTCTCCCAA | 0.2 μM | 895 | 60°C | Dallenne et al, 2010 |
| DHA | MultiCaseDHA_F MultiCaseDHA_R | TGATGGCACAGCAGGATATTC GCTTTGACTCTTTCGGTATTCG | 0.5 μM | 997 | 60°C | Dallenne et al, 2010 |
| LAT-1; -3, BIL-1, CMY-2 to -7, -12 to - 18, -21 to -23 | MultiCaseCIT_F MultiCaseCIT_R | CGAAGAGGCAATGACCAGAC ACGGACAGGGTTAGGATAGY | 0.2 μM | 538 | 60°C | Dallenne et al, 2010 |
| ACT-1; MIR-1 | MultiCaseEBC_F MultiCaseEBC_R | CGGTAAAGCCGATGTTGCG AGCCTAACCCCTGATACA | 0.2 μM | 683 | 60°C | Dallenne et al, 2010 |

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|-------|------------------|---|-------------|------|------|--------------------|
| MCR-1 | MCR1_F MCR1_R | AGTCCGTTTGTCTTGTGGC AGATCCTTGGTCTCGGCTTG | 0.5 μ M | 320 | 58°C | Rebelo et al, 2018 |
| MCR-2 | MCR2_F MCR2_R | CAAGTGTGTTGGTCGCAGTT TCTAGCCCGACAAGCATACC | 0.5 μ M | 715 | 58°C | Rebelo et al, 2019 |
| MCR-3 | MCR3_F MCR3_R | AAATAAAAATTGTTCCGCTTATG AATGGAGATCCCCGTTTTT | 0.5 μ M | 929 | 58°C | Rebelo et al, 2020 |
| MCR-4 | MCR4_F MCR4_R | TCACTTTCATCACTGCGTTG TTGGTCCATGACTACCAATG | 0.5 μ M | 1116 | 58°C | Rebelo et al, 2021 |
| MCR-5 | MCR5_F MCR5_R | ATGCGGTTGTCTGCATTTATC TCATTGTGGTTGTCCTTTTCTG | 0.5 μ M | 1644 | 58°C | Lu X et al, 2017 |
| Int1 | Int1_F Int1_R | GGTCAAGGATCTGGATTTCG ACATGCGTGTAATCATCGTC | 0.4 μ M | 436 | 60°C | Kargar et al, 2014 |
| Int2 | Int2_F Int2_R | CACGGATATGCGACAAAAGG TGTAGCAAACGAGTGACGAAATG | 0.4 μ M | 788 | 60°C | Kargar et al, 2014 |
| Int3 | Int3_F Int3_R | AGTGGGTGGCGAATGAGTG AGTGGGTGGCGAATGAGTG | 0.4 μ M | 600 | 60°C | Kargar et al, 2014 |

Table S2. Primers for Sanger sequencing.

| Gene | Primer | Sequence | [Primer] | Product (bp) | Annealing Temp. | Reference |
|------------|------------------------|--|-------------|--------------|-----------------|----------------------|
| TEM | FIN DEB | ATTCTTGAAGACGAAAGGGC ATGAGTAAACTTGGTCTGAC | 0.4 μ M | 1091 | 54°C | Caniça et al, 1997 |
| SHV | SHVF149P SHVR1059 | CGCTTCTTACTCGCCITTA TTAGCGTTGCCAGTGCTC | 0.5 μ M | 911 | 56°C | Rasheed et al, 1997 |
| CMY | CMYG2F CMYG2R | TTACGGAAGTATTTCATG TCGTCAGTTATTGCAGC | 0.5 μ M | 1143 | 56°C | Manageiro, 2011 |
| AmpC | Int-B2 Int-HN | TTCCTGATGATCGTTCTGCC AAAAGCGGAGAAAAGGTCCG | 0.5 μ M | 1315 | 57°C | Mammeri et al, 2006 |
| CTX-M-1 | CTXM15F CTXM1R | AGAATAAGGAATCCCATGGTT CCGTTCCGCTATTACAA | 0.5 μ M | 903 | 53°C | Mendonça et al, 2007 |
| CTX-M-9 | CTXM9F CTXMG9R2 | ATGGTGACAAAGAGAGTGCAAC AGTTACAGCCCTTCGGCGAT | 0.5 μ M | 878 | 55°C | Cottel et al, 2012 |
| CTX-M-8/25 | CTXM8/25F CTXM8/25R | GATRYTAATGACRACRGCCT TGGGTGAAGTAAGTSACCAG | 0.5 μ M | 777 | 57°C | This study |

^a **Y**=T or C; **R**=A or G; **S**=G or C; **D**=A or G or T
F, forward; **R**, reverse

Table S3. Prevalence of multidrug resistance patterns observed in *E. coli* isolates from broilers raised under intensive (n=77) and extensive production systems (n=42).

| Multidrug resistance patterns | Extensive system | | Intensive system | | Total | |
|---------------------------------|------------------|------|------------------|------|-------|------|
| | N | % | N | % | N | % |
| AMP-CIP-NAL-TET | 7 | 16.6 | 2 | 2.6 | 9 | 7.6 |
| AMP-CIP-SMX-TMP | 1 | 2.4 | 0 | 0 | 1 | 0.8 |
| AMP-CIP-NAL-SMX | 1 | 2.4 | 1 | 1.3 | 2 | 1.7 |
| AMP-SMX-TET-TMP | 2 | 4.8 | 2 | 2.6 | 4 | 3.4 |
| AMP-CIP-GEN-NAL | 1 | 2.4 | 0 | 0 | 1 | 0.8 |
| AMP-CHL-CIP-NAL | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| CIP-GEN-NAL-SMX | 1 | 2.4 | 0 | 0 | 1 | 0.8 |
| CIP-NAL-SMX-TET | 2 | 4.8 | 1 | 1.3 | 3 | 2.5 |
| GEN-SMX-TET-TMP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-CIP-NAL-SMX-TET | 2 | 4.8 | 1 | 1.3 | 3 | 2.5 |
| AMP-CIP-NAL-SMX-TMP | 1 | 2.4 | 1 | 1.3 | 2 | 1.7 |
| AMP-CHL-CIP-NAL-SMX | 0 | 0 | 2 | 2.6 | 2 | 1.7 |
| AMP-CIP-NAL TET-TMP | 1 | 2.4 | 3 | 3.9 | 4 | 3.4 |
| AMP-CIP-SMX-TET-TMP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-CHL-CIP-NAL-TET | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-AZI-CIP-NAL-TMP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-CIP-NAL-SMX-TET-TMP | 8 | 19 | 7 | 9.1 | 15 | 12.6 |
| AMP-CHL-CIP-NAL-SMX-TMP | 1 | 2.4 | 0 | 0 | 1 | 0.8 |
| AMP-CHL-CIP-GEN-NAL-SMX | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-CHL-CIP-NAL-SMX-TET | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-AZI-CHL-NAL-SMX-TMP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-AZI-CIP-NAL-SMX-TET-TMP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-CHL-CIP-NAL-SMX-TET-TMP | 2 | 4.8 | 9 | 11.7 | 11 | 9.2 |
| AMP-AZI-CIP-GEN-NAL-SMX-TET-TMP | 1 | 2.4 | 4 | 5.2 | 5 | 4.2 |
| AMP-FOT-TAZ-CIP-SMX-FEP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CHL-GEN-SMX-FEP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CHL-CIP-NAL-SMX-FEP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CHL-CIP-SMX-TET-FEP | 0 | 0 | 2 | 2.6 | 2 | 1.7 |
| AMP-FOT-TAZ-CIP-SMX-TMP-FEP-FOX | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CHL-SMX-TET-FEP-FOX | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CIP-NAL-SMX-TMP-FEP | 1 | 2.4 | 1 | 1.3 | 2 | 1.7 |

| | | | | | | |
|---|---|-----|----|-----|----|-----|
| AMP-FOT-TAZ-CIP-NAL-SMX-TET-FEP | 2 | 4.8 | 2 | 2.6 | 4 | 3.4 |
| AMP-FOT-TAZ-CIP-NAL-SMX-TET-FEP-FOX | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CIP-NAL-SMX-TMP-FEP-FOX | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CIP-NAL-SMX-TET-TMP-FEP | 3 | 7.1 | 1 | 1.3 | 4 | 3.4 |
| AMP-FOT-TAZ-CHL-CIP-NAL-SMX-TET-FEP | 1 | 2.4 | 10 | 13 | 11 | 9.2 |
| AMP-AZI-FOT-TAZ-CHL-CIP-SMX-TET-TMP-FEP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CHL-CIP-NAL-SMX-TET-FEP-FOX | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CIP-NAL-SMX-TMP-FEP-FOX-ETP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-FOT-TAZ-CHL-CIP-NAL-SMX-TET-TMP-FEP | 0 | 0 | 2 | 2.6 | 2 | 1.7 |
| AMP-FOT-TAZ-CHL-CIP-COL-NAL-SMX-TET-FEP | 0 | 0 | 1 | 1.3 | 1 | 0.8 |
| AMP-AZI-FOT-TAZ-CIP-NAL-SMX-TET-TMP-FEP | 1 | 2.4 | 0 | 0 | 1 | 0.8 |
| AMP-AZI-FOT-TAZ-CHL-CIP-NAL-SMX-TET-TMP-FEP | 2 | 4.8 | 0 | 0 | 2 | 1.7 |
| AMP-AZI-FOT-TAZ-CHL-CIP-GEN-NAL-SMX-TET-TMP-FEP | 1 | 2.4 | 5 | 6.5 | 6 | 5 |
| AMP-FOT-TAZ-CHL-CIP-GEN-NAL-SMX-TET-TMP-FEP-FOX | 0 | 0 | 1 | 1.3 | 1 | 0.8 |

AMP, Ampicilin; AZI, Azithromycin; FOT, Cefotaxime; TAZ, Ceftazidime; CHL, Chloramphenicol; CIP, Ciprofloxacin; GEN, Gentamicin; NAL, Nalidixic Acid; SMX, Sulphamethoxazole; TET, Tetracycline; TMP, Trimethoprim; FEP, Cefepime; FOX, Cefoxitin