**Supplementary file 1**

**Artesunate, EDTA and colistin work synergistically against MCR-negative and -positive colistin-resistant *Salmonella***

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Running title: Synergy of artesunate, EDTA and colistin

**Supplementary file 1a. Sequences of primers used in this study**

|  |  |  |
| --- | --- | --- |
| **Primer** | **Sequence (5’ → 3’)** | **References** |
| *cheA* – F | AATCTCGAGGTGAGCATGGATATTAGCGA | This study |
| *cheA* – R | AATGAATTCTCAGGCGGCTGTGATCGCCA |
| *cheY* – F | ACACTCGAGATGGCGGATAAAGAGCTTAA | This study |
| *cheY* – R | ACAGAATTCTCACATGCCCAGTTTCTCAA |
| *STMDT2\_34621* - F | GGCCTCGAGATGAAAAATATCAAAGTCATCAC | This study |
| *STMDT2\_34621* - R | GGCGAATTCTTAGAAGCTTTCCCAGTTCG |
| *aer* - F | AATCTCGAGATGTCTTCTCATCCCTACGT | This study |
| *aer* - R | GGCCTGCAGTTAATGCAGTACCGTGA |
| *fliD* - F | GGCCTCGAGATGGCTTCAATTTCATCATT | This study |
| *fliD* - R | AACCTGCAGTCAGGACTTGTTCATAGCT |
| *fliT* - F | AATCTCGAGATGACCTCAACCGTGGA | This study |
| *fliT* - R | AATGAATTCTTATGAGGCGCCAGGCG |
| *oppuBB* - F | GGCCTCGAGATGGATACGATACATTATATG | This study |
| *oppuBB* - R | AATGAATTCTTATCGTATCCCCTTCGGTG |
| *gltI* - F | GGCCTCGAGATGATAAAGGAGTTGGATAT | This study |
| *gltI* - R | GGCGAATTCTTAGTTAAGCGCTTTATCATTC |
| *dppB* - F | AATCTCGAGATGTTGCAGTTCATTCTCCG | This study |
| *dppB* - R | GGCGAATTCTTACTTCTTATGCCGAATACGC |
| *dppC* - F | AGGCTCGAGATGTCACAGGTTACTGAAAA | This study |
| *dppC* - R | AGGGAATTCTTACTGCTTCAGTTTGGGATC |
| *spvD* - F | AATCTCGAGATGAGAGTTTCTGGTAGTGC | This study |
| *spvD* - R | GGCGAATTCTCAATCGTGTTTTTCATCAT |
| *mcr*-*1* - qF | TGCTCCAAAATGCCCTACAGACC | Yi *et al*, 2022 |
| *mcr*-*1* - qR | TGCCCCAAGTCGGATAATCCAC |
| 16S rRNA - qF | TGTCGTCAGCTCGTGTTGTG | Yi *et al*, 2022 |
| 16S rRNA - qR | ATCCCCACCTTCCTCCAGTT |

**Supplementary file 1b. The antibacterial activities of COL, AS and EDTA against the tested strains after single and double combinations**

| Strains | *mcr-1* | MICs (mg/L) | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Alone | | | COL + AS | | | | COL + EDTA | | | |
| COL | AS | EDTA | 1/4  AS | 1/8  AS | 1/16  AS | **Fold**  **change** | 1/4  EDTA | 1/8  EDTA | 1/16  EDTA | **Fold**  **change** |
| S29 | + | 2 | 1250 | 125 | 0.015 | 0.125 | 0.5 | **4-133** | 1 | 1 | 2 | **0-2** |
| S31 | + | 4 | 1250 | 125 | 0.25 | 0.5 | 2 | **2-16** | 2 | 2 | 2 | **0** |
| S23 | + | 4 | 1250 | 125 | 0.25 | 0.5 | 1 | **4-16** | 2 | 2 | 2 | **0** |
| S93 | + | 4 | 1250 | 125 | 0.25 | 2 | 2 | **2-16** | 2 | 2 | 2 | **0** |

**Supplementary file 1c. The antibacterial activities of COL against the tested strains after single and triple combinations**

| Strains | MICs (mg/L) | | | | | | | | | **Fold change** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| COL  alone | COL + 1/4 AS +EDTA | | | | COL + 1/8 AS +EDTA | | | |
| 1/4  EDTA | 1/8  EDTA | 1/16  EDTA | 1/32  EDTA | 1/4  EDTA | 1/8  EDTA | 1/16  EDTA | 1/23  EDTA |
| S29 | 2 | 0.00003 | 0.001 | 0.03 | 0.0625 | 0.008 | 0.03 | 0.125 | 0.125 | **16-66667** |
| S31 | 4 | 0.03 | 0.25 | 0.25 | 0.5 | 0.125 | 0.25 | 0.5 | 0.5 | **8-133** |
| S23 | 4 | 0.0625 | 0.125 | 0.125 | 0.25 | 0.125 | 0.25 | 0.5 | 0.5 | **8-64** |
| S93 | 4 | 0.03 | 0.0625 | 0.125 | 0.25 | 0.25 | 0.5 | 0.5 | 2 | **2-133** |

**Supplementary file 1d. The MICs of different antimicrobial drugs against the S16 and S30**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Drug | FFC | ENR | DOX | AMP | AMK | FOS | CAZ | CRO | CTX | TGC |
| Breakpoint | ≥16 | 2 | ≥16 | ≥32 | ≥16 | ≥256 | ≥16 | ≥4 | ≥4 | ≥4 |
| S16 | 2 | ＜0.5 | 2 | 512 | ＜0.5 | 2 | ＜0.5 | ＜0.5 | ＜0.5 | 4 |
| S30 | 128 | 2 | 32 | ＞512 | 1 | 8 | ＜0.5 | ＜0.5 | ＜0.5 | 4 |

Note: FFC: Flufenicol; ENR: Enrofloxacin; DOX: Doxycycline; AMP: Ampicillin; AMK: Amikacin; FOX: Fosfomycin; CAZ: ceftazidime; CRO: Ceftriaxone; CTX:Cefotaxime; TGC: Tigecycline.