**Supplementary Information For**

**Fourth generation cephalosporin resistance among *Salmonella* enterica serovar Enteritidis isolates in Shanghai, China conferred by *bla*CTX-M-55 harboring plasmid**

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**Supplementary Table 1.** PCR primers used in this study

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene | Prime name | Sequence（5’—3’） | Size | Annealing temperatures | References |
| CTX-M | CTX-M-F | GAGTTTCCCCATTCCGTTTC | 909 bp | 50℃ | (Kiratisin et al., 2008; Wu et al., 2015) |
| CTX-M-R | CAGAATAAGGAATCCCATGGTT |  |
| TEM | TEM-F | ATGAGTATTCAACATTTCCG | 964 bp | 50℃ | (Archambault et al., 2006; Wu et al., 2015) |
| TEM-R | ACCAATGCTTAATCAGTGAG |  |
| SHV | SHV-F | TTCGCCTGTGTATTATCTCCCTG | 854 bp | 50℃ | (Archambault et al., 2006) |
| SHV-R | TTAGCGTTGCCAGTGCTCG |  |
| ACC | ACC-F | AGCCTCAGCAGCCGGTTAC | 818 bp | 55℃ | (Archambault et al., 2006) |
|  | ACC-R | GAAGCCGTTAGTTGATCCGG |  |  |  |
| OXA | OXA-F | ACCAGATTCAACTTTCAA | 590 bp | 55℃ | (Usha et al., 2008) |
|  | OXA-R | TCTTGGCTTTTATGCTTG |  |  |  |
| PSE | PSE-F | AATGGCAATCAGCGCTTCCC | 598 bp | 55℃ | (Shahada et al., 2006) |
|  | PSE-R | GGGGCTTGATGCTCACTACA |  |  |  |
| VEB | VEB-F | GATAGGAGTACAGACATATG | 914 bp | 60℃ | (Kiratisin et al., 2008) |
|  | VEB-R | TTTATTCAAATAGTAATTCCACG |  |  |  |
| PER | PER-F | ATGAATGTCATCACAAAATG | 927 bp | 56℃ | (Kiratisin et al., 2008) |
|  | PER-R | TCAATCCGGACTCACT |  |  |  |
| GES | GES-F | ATGCGCTTCATTCACGCAC | 864 bp | 57℃ | (Kiratisin et al., 2008) |
|  | GES-R | CTATTTGTCCGTGCTCAGG |  |  |  |

**Supplementary Table 2.** Drug sensitivity of 38 CRSE to 17 antibiotics

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Salmonella* isolates | Zone diameters and Breakpoints (mm) | | | | | | | | | | | | | | | | | |
| AMC | AMP | CTX | CAZ | FEP | IPM | AMK | GEN | STR | SIZ | SXT | PMB | CHL | TET | NAL | CIP | OFX |
| 18-24 | 16-22 | 29-35 | 25-32 | 31-37 | 26-32 | 19-26 | 19-26 | 12-20 | 15-23 | 23-29 | 13-19 | 21-27 | 18-25 | 22-28 | 30-40 | 29-33 |
| SH10G391 | 18 | 0 | 0 | 13 | 13 | 27 | 22 | 22 | 0 | 0 | 20 | 15 | 24 | 23 | 0 | 27 | 20 |
| SH11G394 | 18 | 0 | 0 | 14 | 15 | 28 | 23 | 24 | 0 | 0 | 25 | 14 | 27 | 0 | 0 | 30 | 23 |
| SH11G405 | 18 | 0 | 0 | 15 | 15 | 30 | 22 | 24 | 0 | 0 | 20 | 14 | 25 | 22 | 0 | 28 | 23 |
| SH11G461 | 18 | 0 | 0 | 17 | 15 | 2 | 23 | 24 | 0 | 0 | 0 | 15 | 26 | 0 | 0 | 21 | 15 |
| SH11G1338 | 19 | 0 | 0 | 16 | 17 | 28 | 24 | 23 | 0 | 0 | 23 | 15 | 26 | 0 | 0 | 27 | 22 |
| SH11G1355 | 20 | 0 | 0 | 19 | 18 | 27 | 23 | 20 | 0 | 0 | 22 | 16 | 29 | 0 | 0 | 26 | 22 |
| SH11G1371 | 19 | 0 | 0 | 15 | 14 | 28 | 24 | 23 | 0 | 0 | 24 | 16 | 28 | 0 | 0 | 28 | 21 |
| SH12G402 | 17 | 0 | 0 | 20 | 9 | 28 | 23 | 22 | 18 | 16 | 0 | 15 | 27 | 0 | 17 | 27 | 21 |
| SH12G460 | 18 | 0 | 0 | 13 | 10 | 29 | 25 | 25 | 0 | 0 | 24 | 16 | 15 | 22 | 0 | 28 | 23 |
| SH12G465 | 20 | 0 | 0 | 11 | 11 | 28 | 22 | 20 | 17 | 15 | 24 | 14 | 25 | 22 | 0 | 26 | 20 |
| SH12G477 | 18 | 0 | 0 | 15 | 13 | 27 | 20 | 21 | 0 | 0 | 17 | 15 | 22 | 0 | 0 | 23 | 20 |
| SH12G514 | 21 | 0 | 0 | 17 | 17 | 27 | 23 | 23 | 0 | 0 | 23 | 15 | 0 | 0 | 0 | 27 | 22 |
| SH12G565 | 20 | 0 | 0 | 15 | 17 | 28 | 23 | 23 | 0 | 0 | 23 | 14 | 26 | 0 | 0 | 30 | 23 |
| SH12G706 | 18 | 0 | 0 | 15 | 15 | 27 | 21 | 22 | 0 | 0 | 21 | 15 | 27 | 21 | 0 | 27 | 20 |
| SH12G729 | 18 | 0 | 0 | 13 | 14 | 30 | 22 | 25 | 23 | 18 | 25 | 15 | 24 | 24 | 0 | 30 | 25 |
| SH12G937 | 21 | 0 | 0 | 17 | 12 | 29 | 23 | 28 | 0 | 0 | 20 | 16 | 28 | 0 | 0 | 32 | 27 |
| SH12G1019 | 19 | 0 | 0 | 14 | 15 | 27 | 22 | 23 | 0 | 0 | 23 | 14 | 26 | 0 | 0 | 29 | 23 |
| SH12G1079 | 23 | 0 | 7 | 18 | 20 | 30 | 22 | 25 | 0 | 0 | 19 | 15 | 0 | 0 | 0 | 30 | 23 |
| SH12G1166 | 21 | 0 | 0 | 18 | 16 | 28 | 20 | 21 | 17 | 15 | 21 | 15 | 24 | 19 | 0 | 20 | 18 |
| SH12G1178 | 16 | 0 | 0 | 11 | 11 | 29 | 23 | 25 | 0 | 0 | 23 | 15 | 27 | 22 | 0 | 29 | 24 |
| SH12G1276 | 17 | 0 | 8 | 21 | 17 | 28 | 23 | 0 | 0 | 0 | 21 | 14 | 26 | 23 | 0 | 31 | 23 |
| SH13G474 | 21 | 0 | 0 | 15 | 17 | 30 | 21 | 11 | 20 | 13 | 18 | 15 | 17 | 21 | 0 | 24 | 20 |
| SH13G961 | 17 | 0 | 0 | 14 | 15 | 27 | 23 | 22 | 0 | 0 | 23 | 15 | 27 | 22 | 0 | 27 | 22 |
| SH13G990 | 21 | 0 | 0 | 17 | 17 | 28 | 22 | 23 | 10 | 16 | 24 | 14 | 0 | 0 | 0 | 27 | 23 |
| SH13G1032 | 20 | 0 | 0 | 17 | 14 | 25 | 23 | 23 | 16 | 15 | 24 | 16 | 26 | 21 | 0 | 24 | 22 |
| SH13G1838 | 20 | 0 | 0 | 17 | 19 | 29 | 24 | 0 | 21 | 18 | 26 | 16 | 28 | 22 | 24 | 28 | 22 |
| SH13G1868 | 19 | 0 | 0 | 16 | 14 | 28 | 18 | 19 | 14 | 14 | 24 | 15 | 27 | 20 | 0 | 23 | 20 |
| SH13G1882 | 20 | 0 | 0 | 17 | 17 | 26 | 19 | 18 | 15 | 14 | 18 | 15 | 19 | 21 | 0 | 21 | 17 |
| SH13G1958 | 21 | 0 | 0 | 15 | 16 | 28 | 22 | 22 | 0 | 0 | 15 | 15 | 0 | 0 | 0 | 27 | 20 |
| SH14G065 | 22 | 0 | 0 | 17 | 17 | 26 | 22 | 20 | 14 | 16 | 23 | 19 | 25 | 20 | 0 | 27 | 19 |
| SH14G169 | 21 | 0 | 0 | 17 | 18 | 27 | 20 | 19 | 14 | 0 | 17 | 15 | 19 | 21 | 0 | 22 | 17 |
| SH14G548 | 22 | 0 | 0 | 17 | 17 | 28 | 23 | 23 | 19 | 15 | 24 | 14 | 26 | 22 | 0 | 29 | 22 |
| SH14G1579 | 21 | 0 | 0 | 16 | 17 | 28 | 23 | 21 | 0 | 0 | 22 | 14 | 7 | 0 | 0 | 28 | 21 |
| SH14G1588 | 25 | 0 | 0 | 20 | 21 | 31 | 25 | 25 | 0 | 0 | 23 | 16 | 27 | 21 | 0 | 30 | 22 |
| SH14G1019 | 0 | 0 | 0 | 0 | 7 | 15 | 22 | 22 | 13 | 0 | 0 | 16 | 28 | 21 | 0 | 24 | 23 |
| SH14G1041 | 0 | 0 | 0 | 0 | 15 | 16 | 25 | 0 | 15 | 0 | 0 | 17 | 28 | 22 | 0 | 28 | 22 |
| SH13SF278 | 22 | 0 | 8 | 17 | 17 | 26 | 22 | 20 | 0 | 0 | 17 | 16 | 0 | 0 | 0 | 23 | 20 |
| SH14SF008 | 20 | 0 | 0 | 15 | 15 | 25 | 23 | 21 | 0 | 0 | 14 | 16 | 0 | 0 | 0 | 26 | 18 |

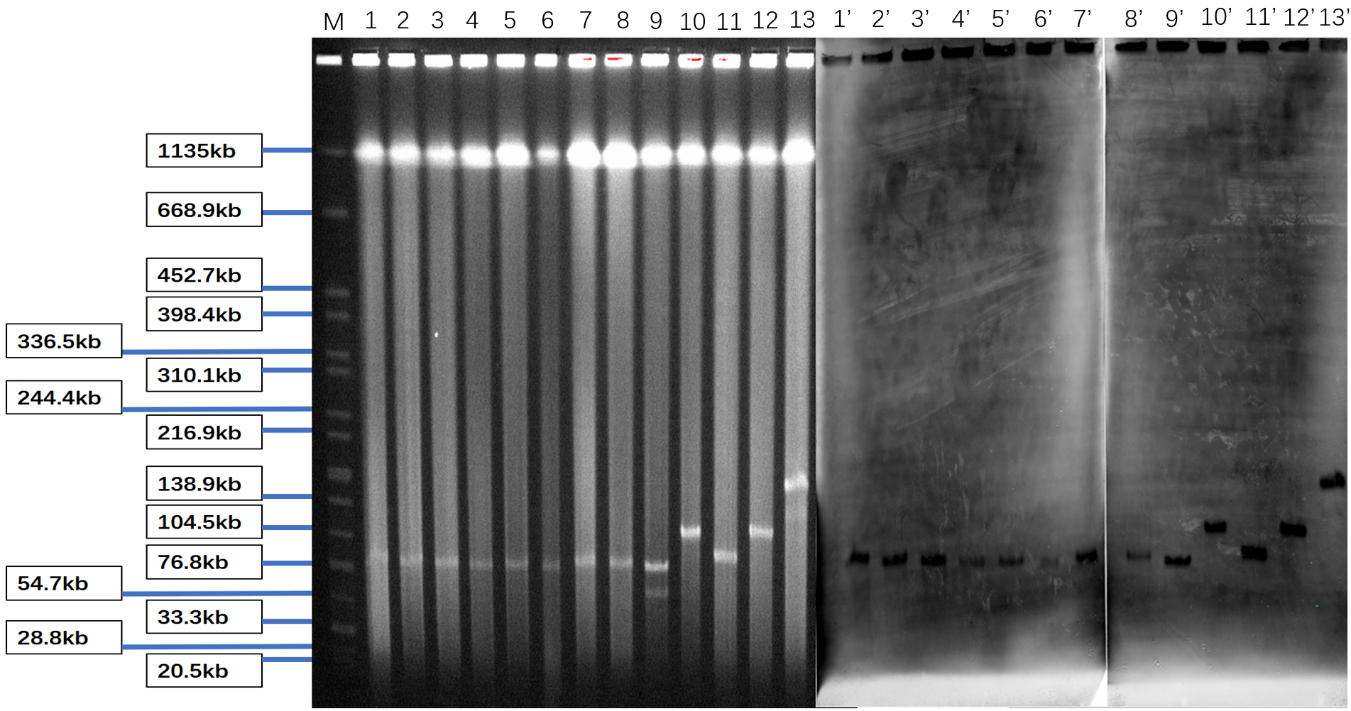
**Supplementary Table 3.** The resistance pattern of the 38 cefepime-resistant *Salmonella* Enteritidisstrains

|  |  |  |  |
| --- | --- | --- | --- |
| The resistance pattern | No. of antibiotics | No. of  resistant strains | Proportion |
| AMP-CTX-CAZ-FEP-STR-SIZ-SXT-CHL-TET-NAL | 10 | 5 | 13.16% |
| AMC-AMP-CTX-CAZ-FEP-GEN-STR-SIZ-SXT-NAL | 10 | 1 | 2.63% |
| AMP-CTX-CAZ-FEP-STR-SIZ-SXT-TET-NAL | 9 | 4 | 10.53% |
| AMP-CTX-CAZ-FEP-GEN-SIZ-SXT-CHL-NAL | 9 | 2 | 5.26% |
| AMC-AMP-CTX-CAZ-FEP-GEN-SIZ-SXT-NAL | 9 | 1 | 2.63% |
| AMP-CTX-CAZ-FEP-STR-SIZ-CHL-TET-NAL | 9 | 1 | 2.63% |
| AMP-CTX-CAZ-FEP-STR-SIZ-TET-NAL | 8 | 5 | 13.16% |
| AMP-CTX-CAZ-FEP-STR-SIZ-SXT-NAL | 8 | 3 | 7.89% |
| AMC-AMP-CTX-CAZ-FEP-STR-SIZ-NAL | 8 | 2 | 5.26% |
| AMP-CTX-CAZ-FEP-SIZ-SXT-CHL-NAL | 8 | 1 | 2.63% |
| AMC-AMP-CTX-CAZ-FEP-SXT-TET-NAL | 8 | 1 | 2.63% |
| AMC-AMP-CTX-CAZ-FEP-SIZ-SXT-NAL | 8 | 1 | 2.63% |
| AMP-CTX-CAZ-FEP-STR-SIZ-CHL-NAL | 8 | 1 | 2.63% |
| AMP-CTX-CAZ-FEP-STR-CHL-TET-NAL | 8 | 1 | 2.63% |
| AMP-CTX-CAZ-FEP-AMK-SIZ-NAL | 7 | 1 | 2.63% |
| AMP-CTX-CAZ-FEP-STR-SIZ-NAL | 7 | 1 | 2.63% |
| AMP-CTX-CAZ-FEP-SXT-NAL | 6 | 1 | 2.63% |
| AMP-CTX-CAZ-FEP-GEN | 5 | 5 | 13.16% |
| AMP-CTX-CAZ-FEP-NAL | 5 | 1 | 2.63% |

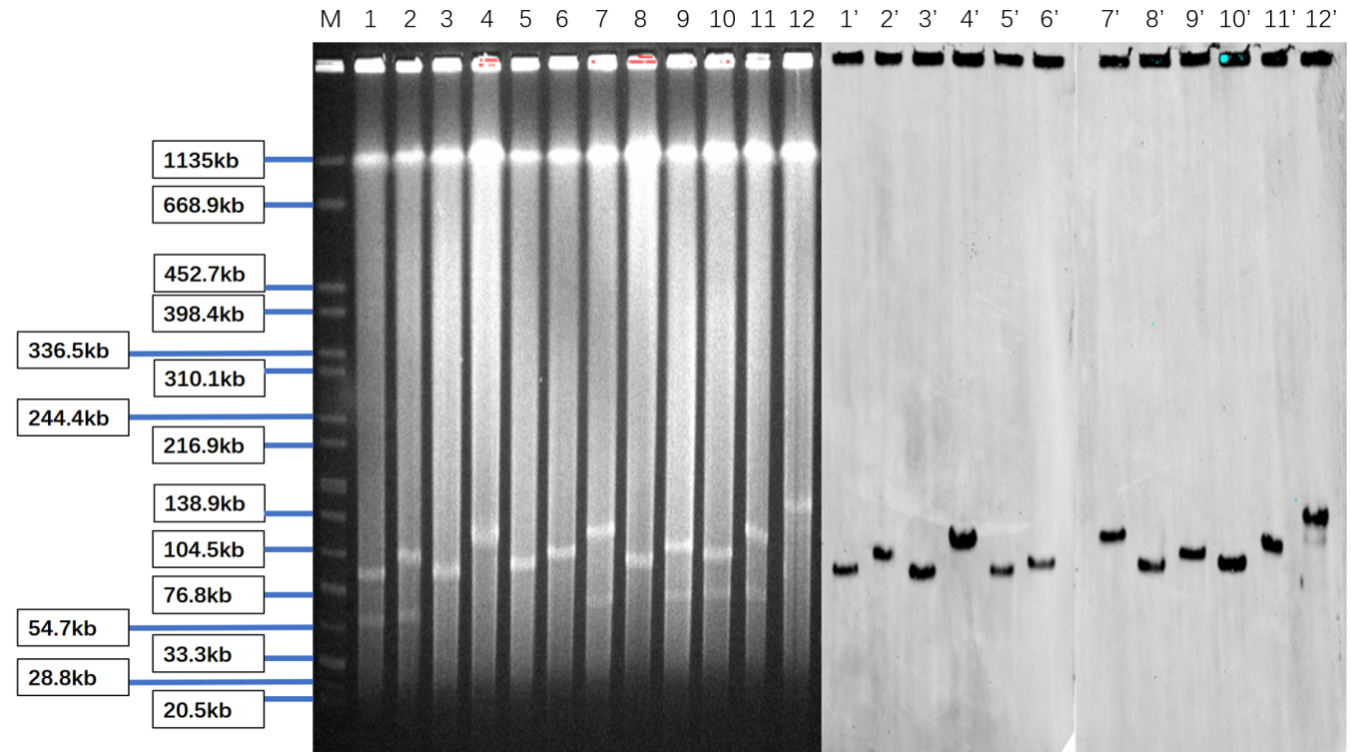
\* AMP: ampicillin, AMC: amoxicillin-clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, FEP: cefepime, GEN: gentamicin, STR: streptomycin, AMK: [amikacin](C:/Users/rena/AppData/Local/youdao/dict/Application/7.1.0.0421/resultui/dict/?keyword=amikacin), SXT: trimethoprim/sulfamethoxazole, SIZ: sulfisoxazole, NAL: nalidixic acid, OFX: ofloxacin, CIP: ciprofloxacin, CHL: chloramphenicol, IPM: [imipenem](C:/Users/rena/AppData/Local/youdao/dict/Application/7.1.0.0421/resultui/dict/?keyword=imipenem), PMB: polymyxin B, TET: tetracycline

**Supplementary Table 4.** Commonly used drugs susceptibility test for *Salmonella* Enteritidis isolated from Shanghai from 2005-2014

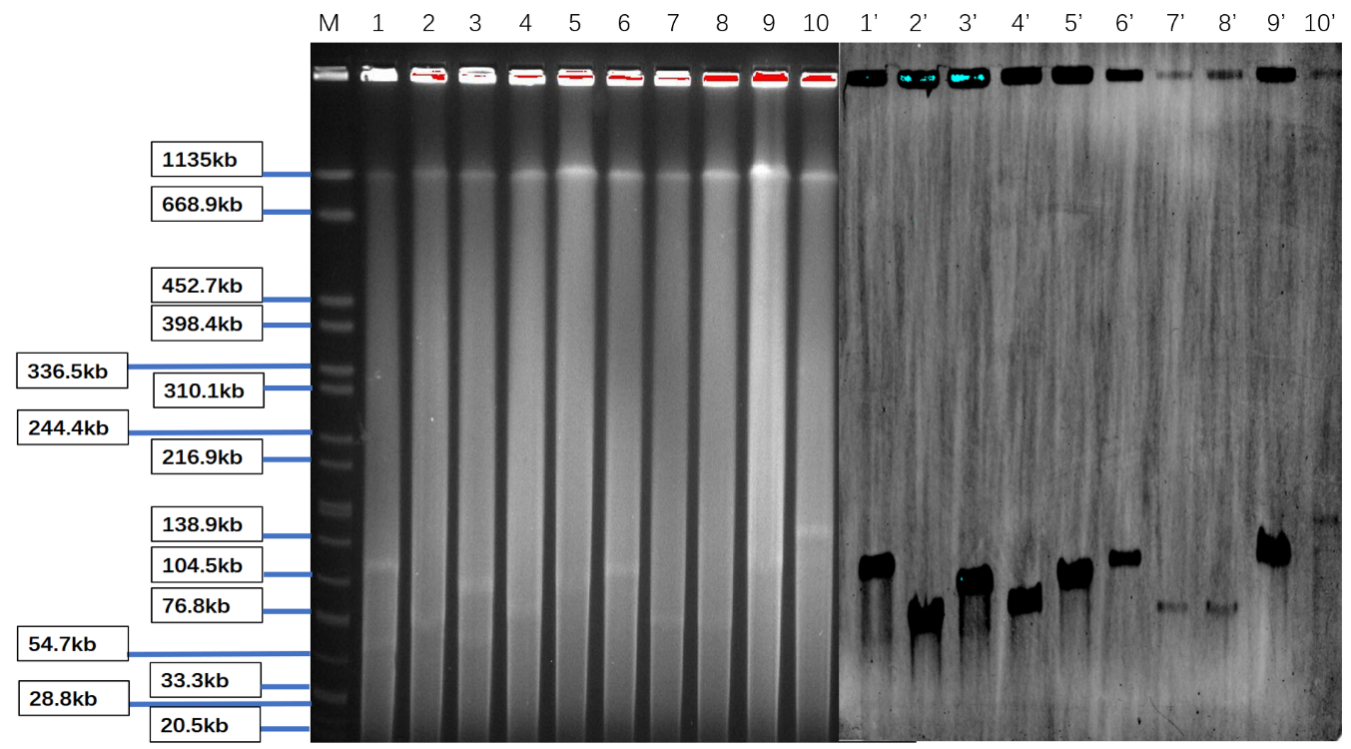
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Antibiotics | Years (n=the number of *S.* Enteritidis isolates) | | | | | | | | | | | |
| 2005  (n=8) | | 2006  (n=62) | 2007  (n=57) | 2008  (n=125) | 2009  (n=123) | 2010  (n=222) | 2011  (n=679) | 2012  (n=547) | 2013  (n=629) | 2014  (n=462) | Sum  (n=2914) |
| Nalidixic Acid | | 100.0% | 93.5% | 86.0% | 94.4% | 96.7% | 95.0% | 97.9% | 95.8% | 95.7% | 95.7% | 95.1% |
| Sulfisoxazole | | 87.5% | 43.5% | 49.1% | 40.8% | 53.7% | 52.3% | 69.1% | 56.3% | 53.4% | 63.0% | 56.9% |
| Ampicillin | | 25.0% | 25.8% | 35.1% | 38.4% | 56.9% | 58.1% | 64.8% | 62.0% | 65.5% | 70.1% | 50.2% |
| Streptomycin | | 12.5% | 29.0% | 38.6% | 34.4% | 46.3% | 44.6% | 53.0% | 47.5% | 49.6% | 61.3% | 41.7% |
| Tetracycline | | 12.5% | 32.3% | 19.3% | 22.4% | 32.5% | 28.8% | 30.9% | 19.0% | 28.3% | 21.9% | 24.8% |
| Gentamicin | | 0.0% | 9.7% | 5.3% | 5.6% | 11.4% | 16.7% | 10.5% | 10.4% | 4.5% | 2.6% | 7.7% |
| Cefotaxime | | 0.0% | 3.2% | 0.0% | 2.4% | 16.3% | 2.7% | 4.6% | 8.8% | 22.4% | 9.3% | 7.0% |
| Trimethoprim-Sulfamethoxazole | | 0.0% | 6.5% | 1.8% | 5.6% | 11.4% | 12.2% | 10.0% | 7.5% | 5.2% | 4.8% | 6.5% |
| Ceftazidime | | 0.0% | 4.8% | 0.0% | 0.0% | 4.9% | 1.4% | 4.1% | 6.0% | 16.4% | 6.3% | 4.4% |
| Chloramphenicol | | 0.0% | 4.8% | 1.8% | 0.8% | 9.8% | 1.8% | 4.9% | 2.9% | 11.8% | 2.4% | 4.1% |
| Amoxicillin-Clavulanic Acid | | 12.5% | 1.6% | 0.0% | 1.6% | 4.1% | 0.9% | 4.0% | 1.5% | 2.2% | 2.6% | 3.1% |
| Ciprofloxacin | | 0.0% | 1.6% | 0.0% | 0.8% | 4.9% | 1.8% | 2.8% | 0.5% | 1.1% | 0.0% | 1.4% |
| Ofloxacin | | 0.0% | 1.6% | 0.0% | 0.8% | 1.6% | 0.0% | 0.7% | 0.0% | 1.1% | 0.0% | 0.6% |



**Fig. S1**. S1-PFGE of cefepime resistant, CTX-M-producing *S.* Enteritidis isolates’ respective transconjugants (left) and Southern blot hybridization with the *bla*CTX-M probe (right). M: H9812, Lanes 1–13: The transconjugants SH11G394-C, SH11G405-C, SH11G1338-C, SH11G1355-C, SH11G1371-C, SH12G565-C, SH12G706-C, SH13G961-C, SH12G460-C, SH12G514-C, SH12G729-C, SH12G1079-C, SH12G1166-C; Lanes 1’–13’: The location of *bla*CTX-M gene by southern blot hybridization with the *bla*CTX-M probe.



**Fig. S2**. S1-PFGE of cefepime resistant, CTX-M-producing *S.* Enteritidis isolates’ respective transconjugants (left) and Southern blot hybridization with the *bla*CTX-M probe (right). M: H9812, Lanes 1–13: The transconjugants SH13G474-C, SH13G990-C, SH13G1032-C, SH13G1838-C, SH13G1868-C, SH13G1882-C, SH13G1958-C, SH14G065-C, SH14G169-C, SH14G548-C, SH14G1579-C, SH13SF278-C; Lanes 1’–13’: The location of *bla*CTX-M gene by southern blot hybridization with the *bla*CTX-M probe.



**Fig. S3**. S1-PFGE of cefepime resistant, CTX-M-producing *S.* Enteritidis isolates’ respective transconjugants (left) and Southern blot hybridization with the *bla*CTX-M probe (right). M: H9812, Lanes 1–13: The transconjugants SH14SF008-C, SH12G402-C, SH14G1019-C, SH14G1041-C, SH12G477-C, SH12G1178-C, SH12G465-C, SH12G1019-C, SH10G391-C, SH12G1276-C; Lanes 1’–13’: The location of *bla*CTX-M gene by southern blot hybridization with the *bla*CTX-M probe.

**References**

Archambault, M., Petrov, P., Hendriksen, R.S., Asseva, G., Bangtrakulnonth, A., Hasman, H., et al. (2006). Molecular characterization and occurrence of extended-spectrum beta-lactamase resistance genes among Salmonella enterica serovar Corvallis from Thailand, Bulgaria, and Denmark. Microb Drug Resist 12(3), 192-198. doi: 10.1089/mdr.2006.12.192.

Kiratisin, P., Apisarnthanarak, A., Laesripa, C., and Saifon, P. (2008). Molecular characterization and epidemiology of extended-spectrum-beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. Antimicrob Agents Chemother 52(8), 2818-2824. doi: 10.1128/AAC.00171-08.

Shahada, F., Chuma, T., Tobata, T., Okamoto, K., Sueyoshi, M., and Takase, K. (2006). Molecular epidemiology of antimicrobial resistance among Salmonella enterica serovar Infantis from poultry in Kagoshima, Japan. Int J Antimicrob Agents 28(4), 302-307. doi: 10.1016/j.ijantimicag.2006.07.003.

Usha, G., Chunderika, M., Prashini, M., Willem, S.A., and Yusuf, E.S. (2008). Characterization of extended-spectrum beta-lactamases in Salmonella spp. at a tertiary hospital in Durban, South Africa. Diagn Microbiol Infect Dis 62(1), 86-91. doi: 10.1016/j.diagmicrobio.2008.04.014.

Wu, H., Wang, Y., Wu, Y., Qiao, J., Li, H., Zheng, S., et al. (2015). Emergence of beta-lactamases and extended-spectrum beta-lactamases (ESBLs) producing Salmonella in retail raw chicken in China. Foodborne Pathog Dis 12(3), 228-234. doi: 10.1089/fpd.2014.1859.