

Antimicrobial resistance profiles and genetic typing of *Salmonella* serovars from chicken embryos in China

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Supplementary Figures

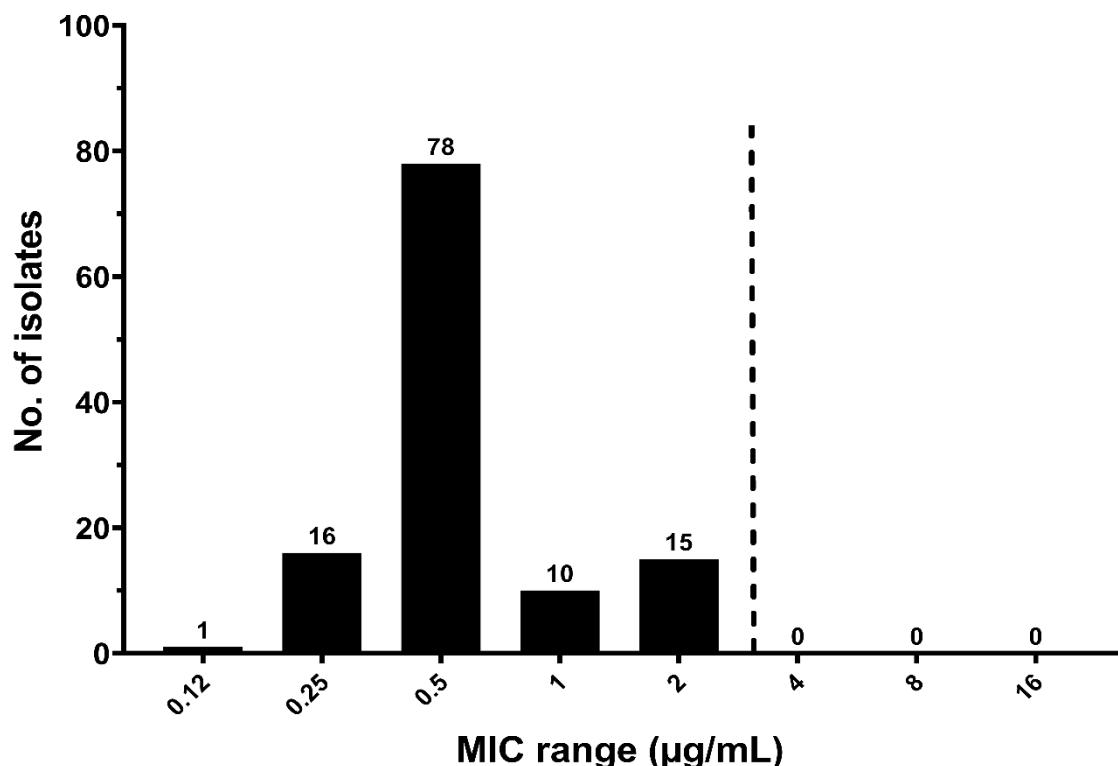


Figure S1. The distribution of minimum inhibitory concentration (MIC) values among the examined *Salmonella* isolates (n=120) against Colistin (CST). The dashed line indicates the cutoff level of the MIC, in which the lowest values correspond to susceptibility and the highest values correspond to the resistance.

Supplementary Tables

Table S1: Primers used for the detection of antimicrobial resistance genes.

Target	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing Temperature (°C)	Product size (bp)	References
β-Lactamases and ESBLs:					
<i>blaTEM</i>	GTATTCAACATTTCCGTGTCG	CCAATGCTTAATCAGTGAGGC	55	854	[1]
<i>blasHV</i>	CCCTGTTAGCCACCCCTGCCG	CGTTGCCAGTGTGATCAGC	68	829	[1]
<i>blaCTX-M-1</i>	GCTGTTGTAGGAAGTGTGCCG	GCCGCCGACGCTAATACATC	63	798	[1]
<i>blaCTX-M-2</i>	AAATGTGCTGCTCCTTCGTGAGC	AGGGTTCGTTGCAAGACAAGACTG	55	1122	[2]
<i>blaCTX-M-3</i>	GTTACAATGTGTGAGAACGAG	CCGTTCCGCTATTACAAAC	55	1018	[3]
<i>blaCTX-M-8</i>	TAACGCACAGACGGCTTAC	TGGCTGGGTGAAGTAAGTGAC	55	637	[4]
<i>blaCTX-M-9</i>	GATCAGTCAGTGGGATAGTT	TACTCGGCGTTAACTGATTA	47	671	[5]
<i>blaCTX-M-25</i>	GCGATGTTAATGACGACAGC	AACCGTCGGTGACAATTCTG	55	847	[4]
<i>blaOXA-1</i>	ACACAATACATATCAACTTCGC	AGTGTGTTAGAATGGTGATC	55	814	[3]
<i>blaOXA-2</i>	TTCAAGCCAAAGGCACGATAG	TCCGAGTTGACTGCCGGTTG	60	703	[3]
<i>blaOXA-10</i>	CGTGCTTGTAAAAGTAGCAG	CATGATTTGGTGGGAATGG	55	389	[3]
<i>blapSE</i>	TGCTTCGCAACTATGACTAC	AGCCTGTGTTGAGCTAGAT	55	438	[3]
Cephalosporins:					
<i>blamox</i>	GCTGCTCAAGGAGCACAGGAT	CACATTGACATAGGTGTTGC	55	520	[6]
<i>blacIT</i>	TGGCCAGAACTGACAGGCAA	TTTCTCCTGAACGTGGCTGGC	55	462	[6]
<i>bladHHA</i>	AACTTTCACAGGTGTGCTGGT	CCGTACGCATACTGGCTTGC	55	405	[6]
<i>blaACC</i>	AACAGCCTCAGCAGCCGGTTA	TTCGCCGCAATCATCCCTAGC	55	346	[6]
<i>blaEBC</i>	TCGGTAAAGCCGATGTTGCGG	CTTCCACTGCGGCTGCCAGTT	55	302	[6]
<i>blaFOX</i>	AACATGGGTATCAGGGAGATG	CAAAGCGCGTAACCGGATTGG	55	190	[6]
Aminoglycosides:					
<i>armA</i>	CAAATGGATAAGAATGATGTT	TTATTTCTGAAATCCACT	55	777	[7]
<i>rmtA</i>	ATGAGCTTGACGATGCCCTA	TCACTTATTCCCTTTATCATG	55	756	[7]
<i>rmtB</i>	ATGAACATCAACGATGCCCT	CCTTCTGATTGGCTTATCCA	55	769	[7]
<i>rmtC</i>	CGAAGAAAGTAACAGCCAAAG	ATCCCAACATCTCTCCACT	55	711	[7]
<i>rmtD</i>	CGGCACCGGATTGGGAAGC	CGGAAACGATGCGACGAT	55	401	[7]
<i>rmtE</i>	ATGAATATTGATGAAATGGTTGC	TGATTGATTCCCTCCGTTTG	55	819	[7]
<i>npmA</i>	CTCAAAGGAACAAAGACGG	GAAACATGCCAGAAACTC	55	641	[8]
<i>aac(3)-IV</i>	GTTACACCGGACCTTGGA	AACGGCATTGAGCGTCAG	55	674	[9]

<i>aac6'-Ib</i>	TTGCGATGCTCTATGAGTGGCTA	CTCGAATGCCTGGCGTGT	55	482	[10]
<i>aadA1-like</i>	GTGGATGGCGGCCCTGAAGCC	ATTGCCAGTCGGCAGCG	55	527/1916	[9]
Sulphonamides:					
<i>sul1</i>	TGGCGTCGCGACTGCGAAAT	TGGTGACGGTGGCATTCT	55	813	[11]
<i>sul2</i>	GTTTCTCCGATGGAGGCCGGT	AGCGAGGTTTGGGAGGCAGC	55	517	[11]
<i>sul3</i>	GATAGTTTCCGATGGAGG	GAAGCCCATAACCGGATCAA	55	495	[11]
Tetracyclines:					
<i>tetA</i>	TGGTCCGGAGGCCAGACGTG	TTCCGAGCATGAGTGCCCGC	55	867	[11]
<i>tetG</i>	TCTTGCAAGGAGCCGCAGTCGAT	GGCCGGCATGCCAACACCC	55	721	[11]
<i>tetX</i>	CAATAATTGGTGGTGGACCC	TTCTTACCTTGGACATCCCG	55	468	[11]
<i>tetB</i>	AAATAACAGCAAACAGTAATGG	AAGTAGGGTTGAGACGCAGCTA	55	493	[12]
<i>tetC</i>	TCTAACAAATCGCCTCATCGT	GGTTGAAGGCTCTCAAGGGC	55	589	[12]
<i>tetR</i>	AGGACCGACGGTGTGCT	ATGAGGACTGGCGGGTGT	55	295	[12]

References

1. González-Sanz, R.; Herrera-León, S.; de la Fuente, M.; Arroyo, M.; Echeita, M.A. Emergence of extended-spectrum β -lactamases and AmpC-type β -lactamases in human *Salmonella* isolated in Spain from 2001 to 2005. *J. Antimicrob. Chemother.* **2009**, *64*, 1181–1186, doi:10.1093/jac/dkp361.
2. Garcia, D.D.O.; Doi, Y.; Szabo, D.; Adams-Haduch, J.M.; Vaz, T.M.I.; Leite, D.; Padoveze, M.C.; Freire, M.P.; Silveira, F.P.; Paterson, D.L. Multiclonal outbreak of *Klebsiella pneumoniae* producing extended-spectrum β -lactamase CTX-M-2 and novel variant CTX-M-59 in a neonatal intensive care unit in Brazil. *Antimicrob. Agents Chemother.* **2008**, *52*, 1790–1793, doi:10.1128/AAC.01440-07.
3. Riaño, I.; Moreno, M.A.; Teshager, T.; Sáenz, Y.; Domínguez, L.; Torres, C. Detection and characterization of extended-spectrum β -lactamases in *Salmonella enterica* strains of healthy food animals in Spain. *J. Antimicrob. Chemother.* **2006**, *58*, 844–847, doi:10.1093/jac/dkl337.
4. Eller, C.; Simon, S.; Miller, T.; Frick, J.S.; Prager, R.; Rabsch, W.; Guerra, B.; Werner, G.; Pfeifer, Y. Presence of β -lactamases in extended-spectrum-cephalosporinresistant *salmonella enterica* of 30 different serovars in germany 2005-11. *J. Antimicrob. Chemother.* **2013**, *68*, 1978–1981, doi:10.1093/jac/dkt163.
5. Coque, T.M.; Oliver, A.; Pérez-Díaz, J.C.; Baquero, F.; Cantón, R. Genes encoding TEM-4, SHV-2, and CTX-M-10 extended-spectrum β -lactamases are carried by multiple *Klebsiella pneumoniae* clones in a single hospital (Madrid, 1989 to 2000). *Antimicrob. Agents Chemother.* **2002**, *46*, 500–510, doi:10.1128/AAC.46.2.500-510.2002.
6. Pérez-Pérez, F.J.; Hanson, N.D. Detection of plasmid-mediated AmpC β -lactamase genes in clinical isolates by using multiplex PCR. *J. Clin. Microbiol.* **2002**, *40*, 2153–2162, doi:10.1128/JCM.40.6.2153-2162.2002.
7. Granier, S.A.; Hidalgo, L.; Millan, A.S.; Escudero, J.A.; Gutierrez, B.; Brisabois, A.; Gonzalez-Zorn, B. ArmA methyltransferase in a monophasic *Salmonella enterica* isolate from food. *Antimicrob. Agents Chemother.* **2011**, *55*, 5262–5266, doi:10.1128/AAC.00308-11.
8. Fritsche, T.R.; Castanheira, M.; Miller, G.H.; Jones, R.N.; Armstrong, E.S. Detection of methyltransferases conferring high-level resistance to aminoglycosides in enterobacteriaceae from Europe, North America, and Latin America. *Antimicrob. Agents Chemother.* **2008**, *52*, 1843–1845, doi:10.1128/AAC.01477-07.
9. Guerra, B.; Junker, E.; Miko, A.; Helmuth, R.; Mendoza, M.C. Characterization and localization of drug resistance determinants in multidrug-resistant, integron-carrying *Salmonella enterica* serotype typhimurium strains. *Microb. Drug Resist.* **2004**, *10*, 83–91, doi:10.1089/1076629041310136.
10. de Toro, M.; Rojo-Bezares, B.; Vinué, L.; Undabeitia, E.; Torres, C.; Sáenz, Y. In vivo selection of aac(6')-Ib-cr and mutations in the gyrA gene in a clinical qnrS1-positive *Salmonella enterica* serovar Typhimurium DT104B strain recovered after fluoroquinolone treatment. *J. Antimicrob. Chemother.* **2010**, *65*, 1945–1949, doi:10.1093/jac/dkq262.
11. Lu, S.; Juan, W.; XiuMei, H.; ZhiNa, Q.; JunWei, W.; RuiMei, Y.; ZengRen, Z. Detection of *Salmonella* resistance genes and correlation analysis of drug resistance in broiler slaughter production chain. *China Anim. Heal. Insp.* **2017**, *34*, 35–42.
12. YaoHui, X.; TongWei, D.; YaRu, Q.; WenLong, H.; JianZhou, L.; ZengHai, J. Isolation, identification and antimicrobial resistance of *Salmonella* from dead chicken embryos in Sanhuang breeder farms. *Acta Vet. Zootech. Sin.* **2018**, *49*, 1081–1088.