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MECHANISMS OF RESISTANCE

Phylogenomic Investigation of IncI1-Iγ Plasmids Harboring bla_{CMY-2} and bla_{SHV-12} in *Salmonella enterica* and *Escherichia coli* in Multiple Countries

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ABSTRACT The objective of this study was to elucidate the genetic and evolutionary relatedness of bla_{CMY-2}- and bla_{SHV-12}-carrying IncI1-Iγ plasmids. Phylogenomic analysis based on core genome alignments and gene presence/absence was performed for different IncI1-Iγ sequence types (STs). Most IncI1-Iγ/ST12 and IncI1-Iγ/ST231 plasmids had near-identical core genomes. The data suggest that widely occurring bla_{CMY-2}-carrying IncI1-Iγ/ST12 plasmids originate from a common ancestor. In contrast, bla_{SHV-12} was inserted independently into different IncI1-Iγ/ST231-related plasmids.

KEYWORDS IS*Ecp1*, IS*1294*, IS*26*, Tn*1721*, *sugE*, *blc*, *deoR*, *Salmonella* Paratyphi B var. Java, *S.* Heidelberg, broiler, chicken

Plasmid-encoded extended-spectrum and AmpC β-lactamases (ESBL/pAmpC) are the dominant causes of resistance to extended-spectrum cephalosporins in *Enterobacteriaceae* (1–3). Poultry and poultry products have been considered reservoirs of ESBL/pAmpC-producing *Salmonella enterica* and *Escherichia coli* (2–9). ESBL/pAmpC-carrying plasmids can be classified in different incompatibility groups, including IncI1-Iγ (10, 11). IncI1-Iγ plasmids harboring ESBL/pAmpC are dominant in *S. enterica* and *E. coli* originating from poultry in multiple countries (4, 12–17). Using plasmid multilocus sequence typing (pMLST) (18, 19), specific ESBL/pAmpC variants were found to be associated with particular IncI1-Iγ STs (12, 13, 16, 17). bla_{CMY-2} carriage has been associated with IncI1-Iγ/ST12 in isolates from poultry (12–14, 16, 17, 20). In contrast, bla_{SHV-12} has been described in multiple IncI1/STs in isolates originating from humans, animals (mainly poultry), and the environment (20–23). However, a resolution higher than the nucleotide sequences of the five housekeeping genes in the pMLST scheme is required to identify the evolutionary relatedness of plasmids belonging to the same ST (4, 17, 24). The objective of the present study was to elucidate the genetic and evolutionary relatedness of bla_{CMY-2}- and bla_{SHV-12}-carrying IncI1-Iγ plasmids within the same pMLSTs using whole-genome sequence (WGS)-based phylogenetic analysis.

Sequences of IncI1-Iγ plasmids originating from previous characterization of ESBL/pAmpC-carrying strains from Colombian baseline studies in poultry were selected. All sequences of bla_{CMY-2}-carrying ($n = 20$) and bla_{SHV-12}-carrying ($n = 4$) IncI1-Iγ plasmids from *Salmonella* (17) and all available bla_{CMY-2}-carrying ($n = 15$) and bla_{SHV-12}-carrying IncI1-Iγ plasmids ($n = 4$) from *E. coli* (16) were included. Plasmid sequences from *Salmonella* were characterized using Illumina WGS and electroporation of reference plasmids as previously described (17). For *E. coli*,

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TABLE 1 Inventory of sequenced IncI1-Iγ plasmids from Colombian poultry and previous reports used for detailed phylogenetic comparisons and analysis of the genetic environment

	n and pMLST of sequenced IncI1-Iγ plasmids from:	
ESBL/pAmpC	**Colombian poultry**	**GenBank or previously published[d]**
bla_{CMY-2}	32 ST12, 2 SLV ST12,[c] 1 ST231	29 ST12, 8 ST2,[e] 5 SLV ST12,[c] 3 ST23,[e] 1 ST20,[e] 1 ST265[e]
bla_{SHV-12}	4 ST231, 1 ST12, 1 ST26, 1 SLV ST26,[c] 1 ST230[f]	1 ST95,[e] 1 ST178,[g] 1 ST231
Other *bla* genes[a]		5 ST12, 1 ST107,[f] 1 ST131,[f] 1 ST270[f]
No *bla* genes[b]		1 ST12, 1 SLV ST12,[c] 1 ST230[f]
Total	43	61

[a]*bla* genes other than bla_{CMY-2} and bla_{SHV-12}.
[b]No *bla* genes detected with ResFinder.
[c]SLV due to incomplete match or missing 1 allele from the pMLST scheme.
[d]Sequences of plasmids listed in this column were obtained based on the allele sequences of the highly prevalent IncI1-Iγ/ST12 and ST231 from GenBank and publications from Europe.
[e]Selected for analysis of the genetic environment of bla_{CMY-2}/bla_{SHV-12}.
[f]SLV of ST231.
[g]SLV of ST12.

previously transformed *E. coli* DH10B cells harboring bla_{CMY-2} and bla_{SHV-12} on IncI1-Iγ plasmids were subjected to Illumina WGS for the present study (16). Chromosomal contigs were detected and removed using BLAST as previously described for *Salmonella* (17). In addition, the allele sequences of IncI1-Iγ STs (https://pubmlst.org/plasmid/) encountered more than once in the selection of plasmids described above were concatenated as separate sequences for each allele in a single FASTA file and used as a query for the nucleotide database using BLAST (last accessed 29 May 2018). *E. coli*-derived plasmid sequences of two publications were used to include additional IncI1-Iγ/ST12 plasmids ($n = 12$) from Europe (see Table S1C in the supplemental material) (12, 13). Overall, ESBL/pAmpC gene variants and plasmids were characterized *in silico* with ResFinder 2.1 (25), PlasmidFinder 1.3, and pMLST 1.4 (19). A summary of all included plasmids is given in Table 1. The plasmid STs that were found repeatedly in *Salmonella* and *E. coli* from Colombian poultry were IncI1-Iγ/ST12 and ST231. From GenBank, 28 plasmids belonged to IncI1-Iγ/ST12 or ST12 single-locus variants (SLVs), and 5 plasmids belonged to IncI1-Iγ/ST231 or ST231 SLVs. Plasmids from GenBank originated from different *S. enterica* serovars and *E. coli*. Information regarding the source, isolation year, and *in silico* characterization of all plasmids (19, 25) and strains (26, 27) is shown in Table S1. The genome sequences of transformed *E. coli* DH10B strains harboring plasmids from *Salmonella* and *E. coli* from Colombia, which were used for reference, were submitted to the European Nucleotide Archive (ENA) under project numbers PRJEB23610 and PRJEB29690, respectively.

Phylogenomic reconstruction was based on core plasmid genome alignments using Parsnp v1.2 (28). Phylogenetic maximum-likelihood (midpoint-rooted) trees were constructed using FastTree2 v2.1.8 (29). Gene presence/absence maximum-likelihood trees were built by annotating the plasmid genomes using Prokka v.1.13 (30) followed by orthology predictions using Roary (31). The resulting gene presence/absence data were encoded as binary values, and trees were constructed using RAxML v.8.2.4 (32) with the BINCAT model. Genome annotations were used to describe the genetic environment of *bla* genes. Visualization of the trees was made with FigTree (http://tree.bio.ed.ac.uk/software/figtree/). The core genome of the resulting tree based on IncI1-Iγ/ST12-related plasmids was 40,056 bp (~40% of the plasmid genome) (see Fig. S1 in the supplemental material). The sublineage of IncI1-Iγ/ST12 and ST12 SLVs is shown in Fig. 1A. Most IncI1-Iγ/ST12 plasmids carried bla_{CMY-2} and originated from samples from poultry (Fig. 1A). Although frequently reported (12–14, 24, 33, 34), detailed genomic relatedness of bla_{CMY-2}-carrying IncI1-Iγ/ST12

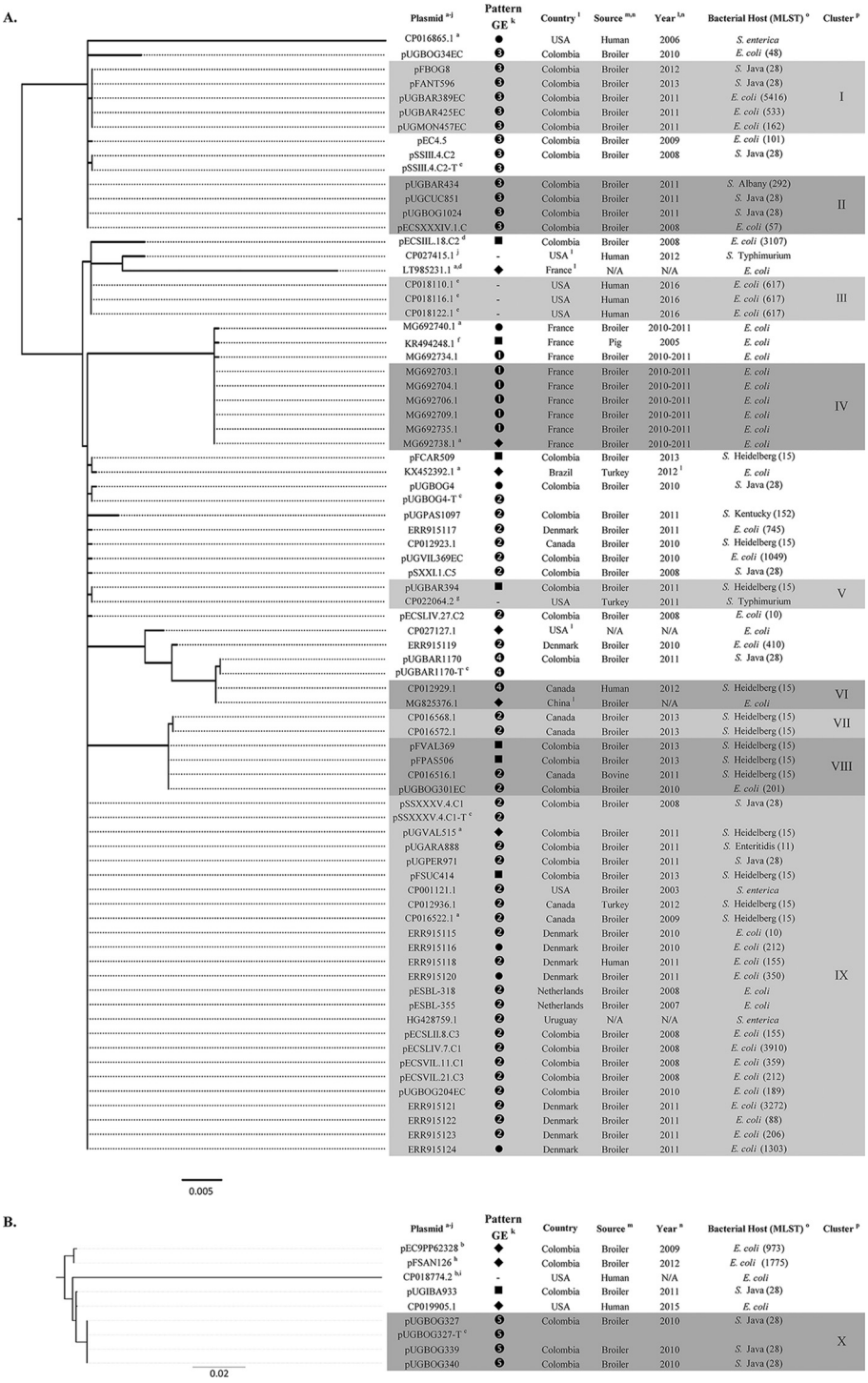

FIG 1 Phylogenetic tree based on core genome of closely related bla_{CMY-2}-carrying IncI1-Iγ/ST12 plasmids and its SLVs (A) and bla_{SHV-12}-carrying IncI1-Iγ/ST231 and its SLVs (B). [a]SLVs of IncI1-Iγ/ST12. [b]SLVs of IncI1-Iγ/ST231. [c]Transformed plasmids from Colombian *Salmonella* included as reference. [d]Carrying bla_{SHV-12}. [e]Carrying $bla_{TEM1B-like}$. [f]Carrying bla_{CMY-2} together with $bla_{CTX-M-1}$. [g]Carrying $bla_{CMY-2-like}$. [h]Carrying bla_{CMY-2}. [i]Carrying bla_{TEM1A}. [j]Carrying no *bla* genes. [k]The patterns of the genetic environment (GE) with their designated numbers can be found in Fig. S3 in the supplemental material. ◆, unique pattern; ●, genetic environment of CMY-2 was characterized by bla_{CMY-2}-*blc-sugE* and for SHV-12 by bla_{SHV-12}-*deoR* (Fig. S3); -, carrying no bla_{CMY-2} or bla_{SHV-12} genes. [l]Data from information accompanying the sequence submission in GenBank but not specifically found in the metadata fields. [m]Details of the source listed in this column are available in Table S1 in the supplemental material. [n]N/A, data not available. [o]Strain MLST was added when information or complete sequence of strains was available. [p]Clusters I to X of plasmids referred to in the manuscript are grouped in shaded boxes. Scale bars at the bottom of the phylogenetic trees represent nucleotide substitutions per site.

plasmids originating from multiple countries and sources has not been assessed. In this study, several plasmids with an identical core genome were identified (clusters I to IX, Fig. 1A). Cluster IX included plasmids from European and American countries, which showed high similarity between *Salmonella*- and *E. coli*-derived plasmids. The gene presence/absence phylogeny grouped most of the plasmids from ST12 and SLVs in a sublineage within the tree (MG825376.1 to ERR915116) (see Fig. S2 in the supplemental material). bla_{CMY-2}-carrying IncI1-Iγ/ST12 plasmids from nonpoultry sources, such as other livestock species and humans, were also found (Fig. 1A). These findings underscore the potential of IncI1-Iγ plasmids to be transferred in strains from *Salmonella* and *E. coli* outside the poultry environment (13, 35–38). The genetic environment of bla_{CMY-2} in most IncI1-Iγ/ST12 plasmids and ST12 SLVs was similar and characterized upstream by insertion sequence IS*Ecp1* and downstream by *blc* and *sugE* (Fig. 1A and Fig. S3A in the supplemental material). IS*1294* (39) and IS*26* were found upstream of bla_{CMY-2} in non-ST12 plasmids (see Fig. S4 in the supplemental material).

The core genome of the tree based on IncI1-Iγ/ST231-related plasmids was 32,789 bp (~32% of the plasmid genome) (see Fig. S5 in the supplemental material). The sublineage of IncI1-Iγ/ST231 and related ST231 SLVs is shown in Fig. 1B. The phylogeny based on gene presence/absence of ST231-related plasmids confirmed phylogenetic distance between the plasmids from Colombian *Salmonella* and *E. coli*. Thus, no evidence of the exchange of bla_{SHV-12}-carrying plasmids between these bacterial species was observed (see Fig. S6 in the supplemental material). In contrast, the plasmids from Colombian *Salmonella* and one from *E. coli* from a human in the United States were found to be closely related, at both the core genome and gene content levels. In this case, these plasmids may be derived from a common ancestor. Despite differences in core genome and gene presence/absence, the genetic environment of bla_{SHV-12} in all IncI1-Iγ/ST231 and SLVs was characterized upstream by IS*26* and downstream by *deoR* (see Fig. S7 in the supplemental material). This pattern of genetic environment was found repeatedly (Fig. 1B and Fig. S3B). However, the results of ST231-related plasmids have to be interpreted with care, given the limited number of plasmids available for phylogenetic analysis.

In conclusion, WGS-based analysis supports the hypothesis that bla_{CMY-2}-carrying IncI1-Iγ/ST12 plasmids in *Salmonella* and *E. coli* likely originated from a common ancestor. As previously suggested, the source of the contamination with these plasmids may be related to similar practices in poultry trade and farming (40, 41). bla_{SHV-12} in association with IS*26* was likely introduced independently in different lineages within IncI1-Iγ/ST231. More observations are needed to better understand the transmission of bla_{SHV-12} in ST231 plasmids.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02546-18.

SUPPLEMENTAL FILE 1, PDF file, 11.2 MB.

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REFERENCES

1. Jacoby GA. 2009. AmpC β-lactamases. Clin Microbiol Rev 22:161–182. https://doi.org/10.1128/CMR.00036-08.
2. Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. 2012. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 18:646–655. https://doi.org/10.1111/j.1469-0691.2012.03850.x.
3. Dorado-García A, Smid JH, van Pelt W, Bonten MJM, Fluit AC, van den

Bunt G, Wagenaar JA, Hordijk J, Dierikx CM, Veldman KT, de Koeijer A, Dohmen W, Schmitt H, Liakopoulos A, Pacholewicz E, Lam T, Velthuis AG, Heuvelink A, Gonggrijp MA, van Duijkeren E, van Hoek A, de Roda Husman AM, Blaak H, Havelaar AH, Mevius DJ, Heederik D. 2018. Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis. J Antimicrob Chemother 73:339–347. https://doi.org/10.1093/jac/dkx397.

4. de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y, Hu J, Lei Y, Li N, Tooming-Klunderud A, Heederik DJJ, Fluit AC, Bonten MJM, Willems RJL, de la Cruz F, van Schaik W. 2014. Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet 10:e1004776. https://doi.org/10.1371/journal.pgen.1004776.
5. Antunes P, Mourão J, Campos J, Peixe L. 2016. Salmonellosis: the role of poultry meat. Clin Microbiol Infect 22:110–121. https://doi.org/10.1016/j.cmi.2015.12.004.
6. Vincent C, Boerlin P, Daignault D, Dozois CM, Dutil L, Galanakis C, Reid-Smith RJ, Tellier PP, Tellis PA, Ziebell K, Manges AR. 2010. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerg Infect Dis 16:88–95. https://doi.org/10.3201/eid1601.091118.
7. Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. 2005. β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemother 56:115–121. https://doi.org/10.1093/jac/dki190.
8. Dierikx C, van der Goot J, Fabri T, van Essen-Zandbergen A, Smith H, Mevius D. 2013. Extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. J Antimicrob Chemother 68:60–67. https://doi.org/10.1093/jac/dks349.
9. EFSA Panel on Biological Hazards (BIOHAZ). 2011. Scientific opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. Efsa J 9:2322. https://doi.org/10.2903/j.efsa.2011.2322.
10. Carattoli A. 2009. Resistance plasmid families in *Enterobacteriaceae*. Antimicrob Agents Chemother 53:2227–2238. https://doi.org/10.1128/AAC.01707-08.
11. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, Mevius DJ, Hordijk J. 2018. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother 73:1121–1137. https://doi.org/10.1093/jac/dkx488.
12. Smith H, Bossers A, Harders F, Wu G, Woodford N, Schwarz S, Guerra B, Rodríguez I, Van Essen-Zandbergen A, Brouwer M, Mevius D. 2015. Characterization of epidemic IncI1 plasmids harboring ambler class A and C genes in *Escherichia coli* and *Salmonella enterica* from animals and humans. Antimicrob Agents Chemother 59:5357–5365. https://doi.org/10.1128/AAC.05006-14.
13. Hansen KH, Bortolaia V, Nielsen CA, Nielsen JB, Schonning K, Agerso Y, Guardabassi L. 2016. Host-specific patterns of genetic diversity among IncI1-Iγ and IncK plasmids encoding CMY-2 β-lactamase in *Escherichia coli* isolates from humans, poultry meat, poultry, and dogs in Denmark. Appl Environ Microbiol 82:4705–4714. https://doi.org/10.1128/AEM.00495-16.
14. Folster JPP, Pecic G, Singh A, Duval B, Rickert R, Ayers S, Abbott J, McGlinchey B, Bauer-Turpin J, Haro J, Hise K, Zhao S, Fedorka-Cray PJJ, Whichard J, McDermott P. 2012. Characterization of extended-spectrum cephalosporin–resistant *Salmonella enterica* serovar Heidelberg isolated from food animals, retail meat, and humans in the United States 2009. Foodborne Pathog Dis 9:638–645. https://doi.org/10.1089/fpd.2012.1130.
15. Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJM, Mevius DJ. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 17:873–880. https://doi.org/10.1111/j.1469-0691.2011.03497.x.
16. Castellanos LR, Donado-Godoy P, León M, Clavijo V, Arevalo A, Bernal JF, Timmerman AJ, Mevius DJ, Wagenaar JA, Hordijk J. 2017. High heterogeneity of *Escherichia coli* sequence types harbouring ESBL/AmpC genes on IncI1 plasmids in the Colombian poultry chain. PLoS One 12:e0170777. https://doi.org/10.1371/journal.pone.0170777.
17. Castellanos LR, van der Graaf-van Bloois L, Donado-Godoy P, León M, Clavijo V, Arévalo A, Bernal JF, Mevius DJ, Wagenaar JA, Zomer A, Hordijk J. 2018. Genomic characterization of extended-spectrum cephalosporin-resistant *Salmonella enterica* in the Colombian poultry chain. Front Microbiol 9:2431. https://doi.org/10.3389/fmicb.2018.02431.
18. García-Fernández A, Chiaretto G, Bertini A, Villa L, Fortini D, Ricci A, Carattoli A. 2008. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum β-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. J Antimicrob Chemother 61:1229–1233. https://doi.org/10.1093/jac/dkn131.
19. Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, Aarestrup FM, Hasman H. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.
20. Accogli M, Fortini D, Giufrè M, Graziani C, Dolejska M, Carattoli A, Cerquetti M, Giufre M, Graziani C, Dolejska M, Carattoli A, Cerquetti M. 2013. IncI1 plasmids associated with the spread of CMY-2, CTX-M-1 and SHV-12 in *Escherichia coli* of animal and human origin. Clin Microbiol Infect 19:E238–E240. https://doi.org/10.1111/1469-0691.12128.
21. Liakopoulos A, Mevius D, Ceccarelli D. 2016. A review of SHV extended-spectrum β-lactamases: neglected yet ubiquitous. Front Microbiol 7:1374. https://doi.org/10.3389/fmicb.2016.01374.
22. Jones-Dias D, Manageiro V, Martins AP, Ferreira E, Caniça M. 2016. New class 2 integron In*2-4* among IncI1-positive *Escherichia coli* isolates carrying ESBL and PMAβ genes from food animals in Portugal. Foodborne Pathog Dis 13:36–39. https://doi.org/10.1089/fpd.2015.1972.
23. Jones-Dias D, Manageiro V, Caniça M. 2016. Influence of agricultural practice on mobile *bla* genes: IncI1-bearing CTX-M, SHV, CMY and TEM in *Escherichia coli* from intensive farming soils. Environ Microbiol 18:260–272. https://doi.org/10.1111/1462-2920.13021.
24. Carattoli A, Villa L, Fortini D, García-Fernández A. 2018. Contemporary IncI1 plasmids involved in the transmission and spread of antimicrobial resistance in Enterobacteriaceae. Plasmid https://doi.org/10.1016/j.plasmid.2018.12.001.
25. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.
26. Yoshida CE, Kruczkiewicz P, Laing CR, Lingohr EJ, Gannon VPJ, Nash JHE, Taboada EN. 2016. The *Salmonella in silico* typing resource (SISTR): an open web-accessible tool for rapidly typing and subtyping draft *Salmonella* genome assemblies. PLoS One 11:e0147101. https://doi.org/10.1371/journal.pone.0147101.
27. Achtman M, Wain J, Weill FX, Nair S, Zhou Z, Sangal V, Krauland MG, Hale JL, Harbottle H, Uesbeck A, Dougan G, Harrison LH, Brisse S. 2012. Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. PLoS Pathog 8:e1002776. https://doi.org/10.1371/journal.ppat.1002776.
28. Treangen TJ, Ondov BD, Koren S, Phillippy AM. 2014. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol 15:524. https://doi.org/10.1186/s13059-014-0524-x.
29. Price MN, Dehal PS, Arkin AP. 2010. FastTree 2 – approximately maximum-likelihood trees for large alignments. PLoS One 5:e9490. https://doi.org/10.1371/journal.pone.0009490.
30. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
31. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, Fookes M, Falush D, Keane JA, Parkhill J. 2015. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31:3691–3693. https://doi.org/10.1093/bioinformatics/btv421.
32. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
33. Edirmanasinghe R, Finley R, Parmley EJ, Avery BP, Carson C, Bekal S, Golding G, Mulvey MR. 2017. A whole-genome sequencing approach to study cefoxitin-resistant *Salmonella enterica* serovar Heidelberg isolates from various sources. Antimicrob Agents Chemother 61:e01919-16. https://doi.org/10.1128/AAC.01919-16.
34. Baron S, Le Devendec L, Touzain F, Jouy E, Lucas P, de Boisséson C, Larvor E, Kempf I. 2018. Longitudinal study of *Escherichia coli* plasmid resistance to extended-spectrum cephalosporins in free-range broilers. Vet Microbiol 216:20–24. https://doi.org/10.1016/j.vetmic.2018.01.012.
35. Roer L, Overballe-Petersen S, Hansen F, Johannesen TB, Stegger M, Bortolaia V, Leekitcharoenphon P, Korsgaard HB, Seyfarth AM, Mossong

J, Wattiau P, Boland C, Hansen DS, Hasman H, Hammerum AM, Hendriksen RS. 2019. ST131 fimH22 Escherichia coli isolate with a blaCMY-2/IncI1/ST12 plasmid obtained from a patient with bloodstream infection: highly similar to E. coli isolates of broiler origin. J Antimicrob Chemother 74:557–560. https://doi.org/10.1093/jac/dky484.

36. Sidjabat HE, Seah KY, Coleman L, Sartor A, Derrington P, Heney C, Faoagali J, Nimmo GR, Paterson DL. 2014. Expansive spread of IncI1 plasmids carrying bla_{CMY-2} amongst *Escherichia coli*. Int J Antimicrob Agents 44:203–208. https://doi.org/10.1016/j.ijantimicag.2014.04.016.
37. Tagg KA, Ginn AN, Jiang X, Ellem J, Partridge SR, Iredell JR. 2015. Distribution of acquired AmpC β-lactamase genes in Sydney, Australia. Diagn Microbiol Infect Dis 83:56–58. https://doi.org/10.1016/j.diagmicrobio.2015.06.001.
38. Harris PNA, Ben Zakour NL, Roberts LW, Wailan AM, Zowawi HM, Tambyah PA, Lye DC, Jureen R, Lee TH, Yin M, Izharuddin E, Looke D, Runnegar N, Rogers B, Bhally H, Crowe A, Schembri MA, Beatson SA, Paterson DL, Harris BT, Lorenc P, McNamara J, Underwood N, Eisenmann J, Stewart J, Henderson A, Ali J, Chiang D, Hwa SS, Kang Y, Pei OS, Ying D, Holland U, Korman T. 2018. Whole genome analysis of cephalosporin-resistant *Escherichia coli* from bloodstream infections in Australia, New Zealand and Singapore: high prevalence of CMY-2 producers and ST131 carrying $bla_{CTX-M-15}$ and $bla_{CTX-M-27}$. J Antimicrob Chemother 73:634–642. https://doi.org/10.1093/jac/dkx466.
39. Tagg KA, Iredell JR, Partridge SR. 2014. Complete sequencing of IncI1 sequence type 2 plasmid pJIE512b indicates mobilization of bla_{CMY-2} from an IncA/C plasmid. Antimicrob Agents Chemother 58:4949–4952. https://doi.org/10.1128/AAC.02773-14.
40. Dierikx CM, Van Der Goot JA, Smith HE, Kant A, Mevius DJ. 2013. Presence of ESBL/AmpC -producing *Escherichia coli* in the broiler production pyramid: a descriptive study. PLoS One 8:e79005. https://doi.org/10.1371/journal.pone.0079005.
41. Nilsson O, Börjesson S, Landén A, Bengtsson B. 2014. Vertical transmission of *Escherichia coli* carrying plasmid-mediated AmpC (pAmpC) through the broiler production pyramid. J Antimicrob Chemother 69:1497–1500. https://doi.org/10.1093/jac/dku030.