



Early Dissemination of *qnrE1* in *Salmonella enterica* Serovar Typhimurium from Livestock in South America

 Daniel F. Monte,^{a,b}  Nilton Lincopan,^{c,d} Louise Cerdeira,^c Paula J. Fedorka-Cray,^b Mariza Landgraf^a

^aDepartment of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, Food Research Center, University of São Paulo, São Paulo, Brazil

^bDepartment of Population Health and Pathobiology, North Carolina State University, College of Veterinary Medicine, Raleigh, North Carolina, USA

^cDepartment of Clinical Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

^dDepartment of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

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The plasmid-mediated quinolone resistance (PMQR) gene *qnrE1* has been limited to South America to date; in 2007, it was identified for the first time in *Klebsiella pneumoniae* from a human infection in Argentina (1). A study in Brazil reported the identification of *qnrE1* in *K. pneumoniae* isolated from an infected parrot in 2016 (2). The presence of this gene was also confirmed in *Salmonella enterica* serovar Enteritidis, Newport, and Infantis strains isolated in 2011, 2013, and 2014, respectively, from humans in Brazil (3).

During a retrospective genomic surveillance study in our laboratory to examine the presence of the *qnrE1* gene, 43 *S. enterica* serovar Typhimurium strains obtained in 2000 to 2015 from the Brazilian agricultural food sector, including strains isolated from poultry, swine, and turkey, were directly sequenced using short-read whole-genome sequencing. Eight (18.6%) *S. Typhimurium* isolates from broiler chicken ($n = 6$) and swine ($n = 2$) samples, collected in São Paulo and Parana states, respectively, were positive for the *qnrE1* gene. These isolates were found to be genetically unrelated by pulsed-field gel electrophoresis (PFGE) using XbaI restriction (4), since isolates were clustered in two pulsotypes, designated A (A1, A2, and A3) and B (B1, B2, and B3) (see Fig. S1 in the supplemental material). Subcluster A1 included *S. Typhimurium* strains (STy2 and STy13) obtained from swine samples, whereas subclusters A2 (STy08) and A3 (STy011) were composed of *S. Typhimurium* strains obtained from broiler chicken samples. Additionally, isolates from cluster A were recovered from different geographical locations, in different periods (Fig. S1). Subclusters B1 (STy06 and STy07), B2 (STy013), and B3 (STy05) included *Salmonella* strains recovered from broiler chicken samples collected in São Paulo state in 2015 (Fig. S1).

All *qnrE1*-positive *S. Typhimurium* strains analyzed in this study shared a genetic environment with high levels of identity (>99%), composed of inverted repeat left (IRL)-*ISEcp1*-inverted repeat right (IRR)-*qnrE1-araJ-ahp* (alkyl hydroperoxidase-encoding gene) (4,659 bp in size) (Fig. 1). Among these isolates, we identified the presence of the *qnrE1* gene in a *S. Typhimurium* strain isolated from a pig liver sample in 2000, 7 and 11 years earlier than previously reported in Argentina (1) and Brazil (3), respectively.

Furthermore, the downstream region of the *qnrE1* genetic platform, as well as the upstream region, was found to be slightly different from that in previous surveys (1–3). First, as reported previously, pKp1130 (GenBank accession no. KY073238) possesses a 5'-truncated *ahp* gene that is 438 bp in size, while plasmid pKp41M (GenBank accession no. KY781949) and the 219/11 (GenBank accession no. QRCP01000000), 345/14 (GenBank accession no. GCA_003726045.1), and 291/13 (GenBank accession no. RDPW00000000.1) isolates have a different 5'-truncated *ahp* that is 327 bp in size. Therefore, while our isolates present a complete 1,035-bp *ahp* gene, compared to the reference sequence of the *ahp* gene from *S. Typhimurium* strain CFSAN033917

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Address correspondence to Daniel F. Monte, monte_dfm@usp.br, or Nilton Lincopan, lincopan@usp.br.

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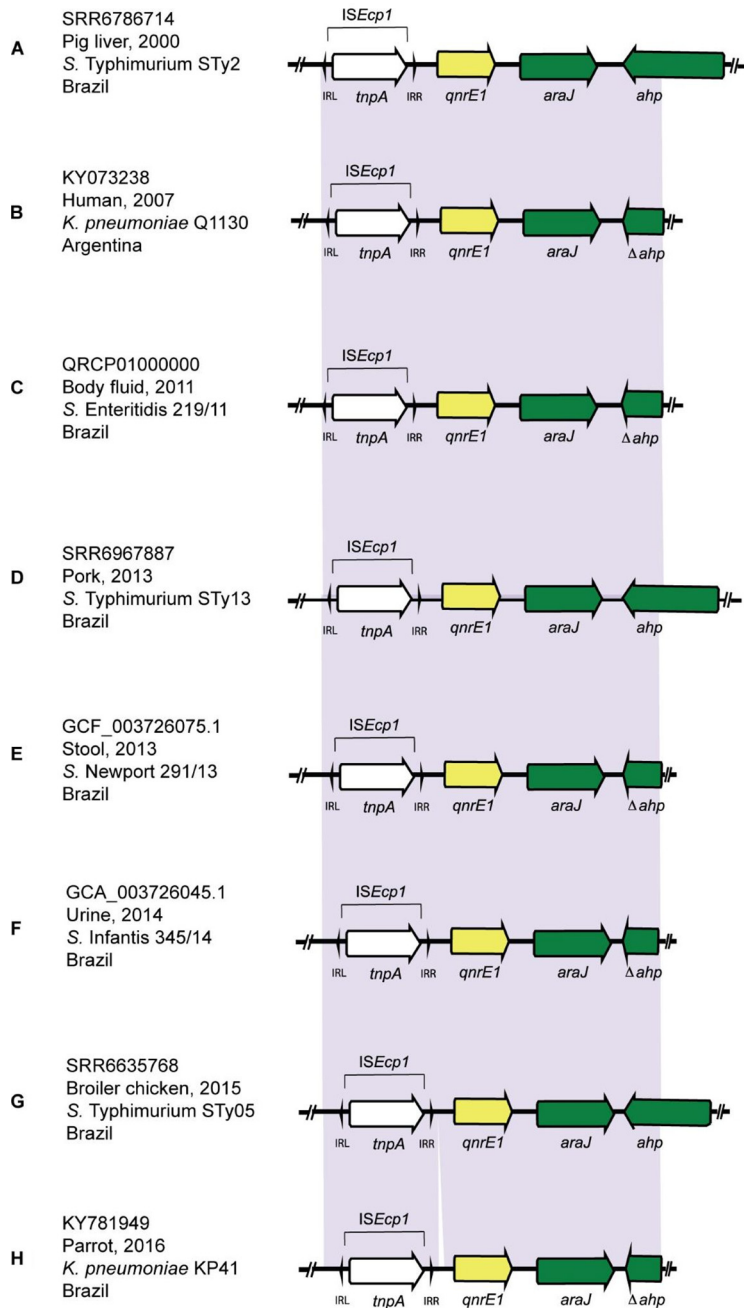


FIG 1 Chronological comparison between genetic contexts of *qnrE1* genes carried by *S. enterica* serovars (A, C, D, E, F, and G) and *K. pneumoniae* (B and H) strains isolated in South America. Genes and shotgun sequences were extracted from the GenBank database. Arrows indicate the positions and directions of the genes; Δ indicates truncated genes. Regions with $>99\%$ identity are indicated with purple shading. (A, D, and G) Strains contain *ahp* genes of 1,035 bp. (B) Strain contains an *ahp* gene of 438 bp. (C, E, F, and H) Strains contain *ahp* genes of 327 bp.

(GenBank accession no. [KYE07389.1](#)), deletions of 597 bp and 708 bp in the *ahp* gene could be observed, as reported previously for *K. pneumoniae* and *Salmonella* isolates, respectively (1, 3). Another difference within this genetic platform was that, although an IRR sequence was identified downstream of *ISEcp1* in our isolates, an alternative IRR could not be found at the right edge, in contrast to previous observations for *K. pneumoniae* (1). Moreover, pKp41M (GenBank accession no. [KY781949](#)) contained an additional 152 bp in the upstream region of the *qnrE1* gene (Fig. 1H). These findings

provide new insights into this genetic environment and suggest that the emergence of *qnrE1* was much earlier than previously thought.

Another interesting observation is that, for both human and parrot infected hosts reported in Brazil, the occurrence of *qnrE1* has been associated with carriage of *bla*_{CTX-M-8} and *bla*_{OXA-9} β -lactamase genes, conferring a broad spectrum and a narrow spectrum, respectively, of activity against cephalosporins (2, 3, 5). This finding may suggest an evolutionary mechanism of adaptation, most likely driven by selective pressure through the use of cephalosporins in infected hosts such as parrots (2) and humans (3), particularly in Brazil, since all *S. Typhimurium* strains analyzed in this study were isolated from healthy animals and did not carry these genes.

A limitation of this study was that plasmids harboring the *qnrE1* gene were not reconstructed, because of the limitations of short-read sequencing (6). However, the findings demonstrated in this study substantially aid in the ongoing development of this new genetic platform; it has not escaped our attention that further studies are necessary to fully comprehend the epidemiology of *S. Typhimurium* strains carrying the *qnrE1* gene and their potential to be spread throughout the food chain.

In summary, we present evidence that *qnrE1* genes have been circulating in South America since at least 2000, which is much earlier than reported previously. Therefore, since *qnrE1*-carrying *Enterobacteriaceae* strains are established in Latin America (1–3), we demonstrate the need for continuous surveillance programs to track this and other antimicrobial resistance genes important in therapeutic efficacy. Further, continuous surveillance can aid in the prevention of wide dissemination of this gene following the development of mitigation strategies. It is likely that, unless continuous surveillance is established, we will continue to underestimate the prevalence of these and other antimicrobial resistance genes at the environment-animal-human interface.

Data availability. Sequence data were deposited as part of the GenomeTrakr Project. The genome sequences of the *S. Typhimurium* strains have been deposited in DDBJ/ENA/GenBank with BioSample accession numbers [SAMN08606827](https://doi.org/10.6026/9781107050231.ch010), [SAMN08874445](https://doi.org/10.6026/9781107050231.ch010), [SAMN08386803](https://doi.org/10.6026/9781107050231.ch010), [SAMN07279711](https://doi.org/10.6026/9781107050231.ch010), [SAMN08606818](https://doi.org/10.6026/9781107050231.ch010), [SAMN08874450](https://doi.org/10.6026/9781107050231.ch010), [SAMN08386758](https://doi.org/10.6026/9781107050231.ch010), and [SAMN07283712](https://doi.org/10.6026/9781107050231.ch010).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00571-19>.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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We have no conflicts of interest to declare.

REFERENCES

1. Albornoz E, Tijet N, De Belder D, Gomez S, Martino F, Corso A, Melano RG, Petroni A. 2017. *qnrE1*, a member of a new family of plasmid-located quinolone resistance genes, originated from the chromosome of *Enterobacter* species. *Antimicrob Agents Chemother* 61:e02555-16. <https://doi.org/10.1128/10.1128/AAC.02555-16>.
2. Cunha MPV, Davies YM, Cerdeira L, Dropa M, Lincopan N, Knöbl T. 2017. Complete DNA sequence of an IncM1 plasmid bearing the novel *qnrE1* plasmid-mediated quinolone resistance variant and *bla*_{CTX-M-8} from *Klebsiella pneumoniae* sequence type 147. *Antimicrob Agents Chemother* 61:e00592-17. <https://doi.org/10.1128/10.1128/AAC.00592-17>.
3. Soares FB, Camargo CH, Cunha MPV, de Almeida EA, Bertani AMJ, Carvalho E, de Paiva JB, Fernandes SA, Tiba-Casas MR. 2019. Co-occurrence of *qnrE1* and *bla*_{CTX-M-8} in IncM1 transferable plasmids contributing to MDR in different *Salmonella* serotypes. *J Antimicrob Chemother* 74:1155–1156. <https://doi.org/10.1093/jac/dky516>.
4. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathog Dis* 3:59–67. <https://doi.org/10.1089/fpd.2006.3.59>.
5. Antunes NT, Fisher JF. 2014. Acquired class D β -lactamases. *Antibiotics (Basel)* 3:398–434. <https://doi.org/10.3390/antibiotics3030398>.
6. Arredondo-Alonso S, Willems RJ, van Schaik W, Schürch AC. 2017. On the (im)possibility of reconstructing plasmids from whole-genome short-read sequencing data. *Microb Genom* 3:e000128. <https://doi.org/10.1099/mgen.0.000128>.