**Supporting Information, Plasmid-Mediated Quinolone Resistance in Human Nontyphoidal *Salmonella* Infections: An Emerging Public Health Problem in the United States**

The presence of PMQR genes *aac(6’)-Ib-cr*, *oqxA*, *oqxB*, *qepA, qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS* and mutations in *gyrA* and *parC* was determined for a subset of isolates with the study phenotype using polymerase chain reaction (PCR) and/or whole genome sequencing (WGS). DNA templates for PCR were prepared by lysing the bacteria at 95°C for 10 minutes and collecting the supernatant following centrifugation for 10 minutes at 20,000xg. PCR was conducted using 2x HotStarTaq PCR Master Mix (Qiagen Inc., Valencia, CA), 0.4 µM of each primer, 5 µL of lysate, and sterile PCR water to a final volume of 50 µL. PCR primers used in this study are presented in Supplemental Table 1. Direct sequencing of the PCR product confirmed the presence of the *aac(6’)-Ib-cr* variant. The *gyrA* and *parC* PCR products were sequenced as previously described (Gay et al., 2006). Genomic DNA for WGS was purified using the DNeasy Blood and Tissue Kit (Qiagen Inc.) or the Agencourt Genfind V2 kit (Beckman Coulter Life Sciences, Indianapolis, IN) according to the manufacturer’s protocols. To improve DNA yield from the Agencourt Genfind V2 kit, a heating step at 85°C for 7 minutes in molecular grade water was added and the shake step was extended to 45 minutes. Libraries were constructed for the genome inclusive of plasmid using NexteraXT library kits and paired end, base pair reads were generated on a MiSeq or HiSeq (Ilumina, San Diego, CA). *gyrA* and *parC* were examined for previously described mutations conferring resistance (Gay et al., 2006) by mapping the raw reads to a reference genome (AE014613) in CLC Genomics Workbench 8.5 (Qiagen Inc.). The raw reads were assembled *de novo* using CLC Genomics Workbench 8.5 and assemblies were analyzed using tools developed by the Center of Genomic Epidemiology (<http://cge.cbs.dtu.dk/services/>). ResFinder was used with a 90% threshold for percent identity and 60% gene coverage. Sequence reads were submitted to the Sequence Read Archive of the National Center for Biotechnology Information (NCBI) and corresponding BioSample numbers are provided in Supplemental Table 2.

**SUPPLEMENTAL TABLE 1.** PCR primers used for detection of quinolone resistance mechanisms

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Primer name | Sequence (5’-3’) | Reference |
| *aac(6’)-Ib-cr* | *aac(6’)-Ib-cr* Forward | 5’-TTGCGATGCTCTATGAGTGGCTA-3’ | Park et al., 2006 |
| *aac(6’)-Ib-cr* Reverse | 5’-CTCGAATGCCTGGCGTGTTT-3’ |
| *gyrA* | *gyrA Forward* | 5’-CGTTGGTGACGTAATCGGTA-3’ | Eaves et al., 2002 |
| *gyrA Reverse* | 5’-CCGTACCGTCATAGTTATCC-3’ |
| *oqxA* | *oqxA Forward* | 5’-CCGGACAAAGGAAGTGGC-3’ | Yuan et al., 2012 |
| *oqxA Reverse* | 5’-GGAGACGACGTTGGTATGGA-3’ |
| *oqxB* | *oqxB Forward* | 5’-ACCAACACGCCGAATACC-3’ | Yuan et al., 2012 |
| *oqxB Reverse* | 5’-CATCAGGACCACCAGACCC-3’ |
| *parC* | *parC Forward* | 5’-CTATGCGATGTCAGAGCTGG-3’ | Eaves et al., 2004 |
| *parC Reverse* | 5’-TAACAGCAGCTCGGCGTATT-3’ |
| *qepA* | *qepA Forward* | 5’-AACTGCTTGAGCCCGTAGAT-3’ | Kim et al., 2009 |
| *qepA Reverse* | 5’-GTCTACGCCATGGACCTCAC-3’ |
| *qnrA* | *qnrA* Forward | 5’-ATTTCTCACGCCAGGATTTG-3’ | Robicsek et al., 2006 |
| *qnrA* Reverse | 5’-GATCGGCAAAGGTTAGGTCA-3’ |
| *qnrB* | *qnrB* Forward | 5’-GATCGTGAAAGCCAGAAAGG-3’ | Robicsek et al., 2006 |
| *qnrB* Reverse | 5’-ACGATGCCTGGTAGTTGTCC-3’ |
| *qnrC* | *qnrC* Forward | 5’-GGGTTGTACATTTATTGAATC-3’ | Wang et al., 2009 |
| *qnrC* Reverse | 5’-TCCACTTTACGAGGTTCT-3’ |
| *qnrD* | *qnrD* Forward | 5’-CGAGATCAATTTACGGGGAATA-3’ | Cavaco et al., 2009 |
| *qnrD* Reverse | 5’-AACAAGCTGAAGCGCCTG-3’ |
| *qnrS* | *qnrS* Forward | 5’-ACGACATTCGTCAACTGCAA-3’ | Robicsek et al., 2006 |
| *qnrS* Reverse | 5’-TAAATTGGCACCCTGTAGGC-3’ |

**SUPPLEMENTAL TABLE 2.** BioSample numbers and isolate information for sequenced nontyphoidal *Salmonella*

isolates with the study phenotype (ciprofloxacin MIC ≥0.25 µg/mL and nalidixic acid susceptibility)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| BioSample no.† | Serotype | Year | Quinolone resistance mechanism(s) identified | Manuscript table no. |
| SAMN08339881 | Apapa | 2014 | *qnrB19* | 1, 3 |
| SAMN05596266 | Bareilly | 2014 | *qnrS1* | 1 |
| SAMN05907729 | Berta | 2014 | none | 1 |
| SAMN08339809 | Enteritidis | 2012 | *gyrA* (Gly81Asp) | 1 |
| SAMN08340035 | Enteritidis | 2014 | *qnrB19* | 1 |
| SAMN08339827 | Guinea | 2014 | *qnrB19* | 1, 3 |
| SAMN08340040 | Guinea | 2014 | *qnrB19* | 1 |
| SAMN08342306 | Isangi | 2014 | *qnrB19* | 1 |
| SAMN08340045 | Ituri | 2010 | *gyrA* (Gly81Asp), *parC* (Ser80Arg) | 1 |
| SAMN08339814 | Ituri | 2014 | *qnrB19* | 1 |
| SAMN02911999 | Litchfield | 2012 | *qnrS1* | 1, 3 |
| SAMN02911952 | Muenchen | 2011 | *qnrB19* | 1 |
| SAMN08339820 | Muenchen | 2014 | *qnrB19* | 1 |
| SAMN08339876 | Muenster | 2010 | *qnrB19* | 1, 2 |
| SAMN08340051 | Muenster | 2011 | *qnrB19* | 1 |
| SAMN02368948 | Newport | 2010 | *qnrB2* | 1 |
| SAMN08340022 | Newport | 2014 | *qnrB19* | 1 |
| SAMN02911986 | Reading | 2012 | *qnrB19* | 1 |
| SAMN02911932 | Saintpaul | 2012 | *qnrS1* | 1 |
| SAMN05941621 | Saintpaul | 2014 | *qnrS1* | 1 |
| SAMN02911939 | Stanley | 2012 | *qnrA1* | 1, 2 |
| SAMN05907757 | Stanley | 2014 | *qnrS1* | 1, 2 |
| SAMN08339836 | Telelkebir | 2014 | *qnrB19* | 1, 3 |
| SAMN05596278 | Typhimurium | 2014 | *qnrB19* | 1 |
| SAMN08340029 | Urbana | 2014 | *qnrB19* | 1, 3 |
| SAMN05596649 | I 4,[5],12:i:- | 2014 | *qnrS1* | 1, 2 |
| SAMN05907740 | I 4,[5],12:i:- | 2014 | none | 1 |
| SAMN08342519 | IV 44:z4,z23:- | 2014 | *qnrB19* | 1, 3 |
| †BioSample accession numbers in the National Center for Biotechnology Information (NCBI) Sequence Read Archive, <https://www.ncbi.nlm.nih.gov/sra/>.  |

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