

Correspondence

Epidemiol. Infect. (2011).

doi:10.1017/S0950268810002487

First published online 5 November 2010

***Salmonella enterica* serovar Typhi and *S. Paratyphi A*: need to expand the QRDR region?**

To the Editor:

In the article entitled ‘Molecular analysis of high-level ciprofloxacin resistance in *Salmonella enterica* serovar Typhi and *S. Paratyphi A*: need to expand the QRDR region?’ by Capoor and co-workers [1], the authors have described new substitutions in DNA gyrase subunit, GyrA. DNA gyrase (topoisomerase II) is a multimeric enzyme composed of two pairs of subunits, Gyr A (878 residues) and GyrB, and the enzyme is inhibited by quinolones, like nalidixic acid, and fluoroquinolones, such as ciprofloxacin [2]. Resistance to nalidixic acid is often mediated by substitutions in position Ser⁸³ or Asp⁸⁷ of GyrA while resistance to both nalidixic acid and ciprofloxacin frequently is the result of substitutions in both positions [3, 4]. The positions 83 and 87 are parts of the so-called quinolone resistance-determining region (QRDR) which has been defined by Yoshida and co-workers to cover the region between position 67 and 106 of GyrA [5], but Capoor and colleagues have proposed a highly relevant expansion of QRDR [1].

They have also described new amino-acid replacements in GyrA which are Phe³¹→Tyr, Leu⁴⁴→Ile, Phe⁷²→Tyr, Asp⁷⁶→Asn, Glu¹³³→Gly [1] as well as previously described substitutions, i.e. Met⁵²→Leu [6], Ser⁸³→Phe, and Asp⁸⁷→Asn [7, 8]. The Leu⁴⁴→Ile, Phe⁷²→Tyr, Asp⁷⁶→Asn substitutions have previously been described by Capoor and colleagues [6]. Consensus is that e.g. Phe³¹→Tyr denotes a replacement of the predominant Phe residue in position 31 by a Tyr residue.

With a nucleotide sequence encompassing nt 76–480 from the *gyrA* gene of *Salmonella* Typhimurium strain 14028S [GenBank acc. no. CP001363] as query sequence we searched the nr/nt, refseq_

genomic, chromosome, and wgs databases of the NCBI GenBank (<http://www.ncbi.nlm.nih.gov/>) for hits by Megablast with an expected threshold of 10^{-4} . We also used *Salmonella* (taxid: 590) as the optional organism. Subsequently, we searched the nucleotide database by using the words ‘gyrA AND Salmonella’ and got additional hits.

In total, we achieved 358 entries (last accessed on 8 June 2010) all from different strains of divergent serovars of *Salmonella*. Owing to very low identity to GyrA or too short sequences only covering 11 positions including positions 83 and 87, 26 of the entries were discarded.

The resulting 332 sequences were analysed to determine the nature of the residues in positions 31, 44, 72, and 76 of GyrA. All 332 sequences contained the positions 72 and 76, but owing to paucity of sequence, 237 sequences also included position 44, whereas only 94 sequences also covered position 31. Ultimo 2007, 162 partial or complete sequences of GyrA from *Salmonella* have been released in the GenBank databases.

Each of the 332 sequences was carefully analysed, and our results showed that position 31 was inevitably occupied by Gly, residue 44 by Val, residue 72 by Asp, and position 76 by Lys (Fig. 1). These results are different from those reported by Capoor and co-workers [1] as seen in Table 1. Amino-acid replacements were not found in any of these four positions among all the analysed *Salmonella* sequences.

Amino-acid substitutions in GyrA from *Salmonella* are occasionally related to the sequence of GyrA from *Escherichia coli*, therefore we also examined the equivalent sequences of GyrA from this species. Of the 163 *E. coli* available sequences in the GenBank databases, we found in all instances Gly³¹, Val⁴⁴, Asp⁷², and Lys⁷⁶.

The discrepancies between our results and those reported by Capoor and colleagues [1, 6] could be caused by different numbering of the residues because the precursor GyrA protein may be processed into a signal

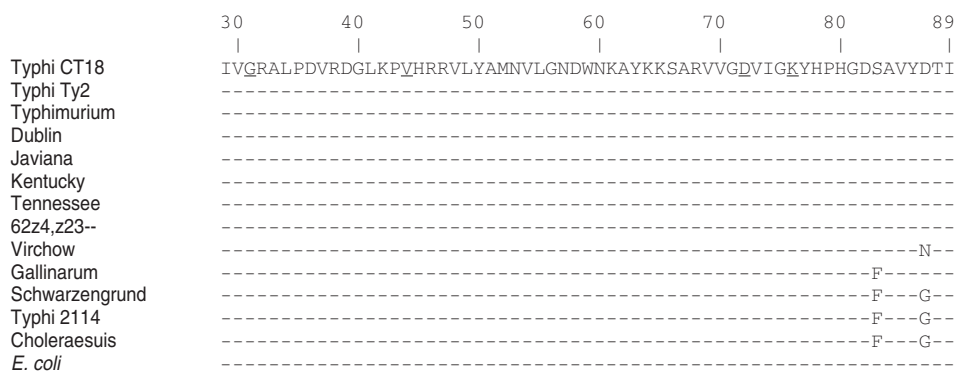


Fig. 1. ClustalW alignment of a section including residues 29–89 of non-redundant genomic GyrA sequences from different serovars of *S. enterica* subsp. *enterica* and *S. enterica* subsp. *arizonae* serovar 62z4,z23 – in comparison with GyrA from *Escherichia coli* K-12 substrain MG1655. The residues discussed in the text are underlined. The GenBank accession nos. are: Typhi CT18, NC_003198; Typhi Ty2, NC_004631; Typhimurium, NC_003197; Dublin, NC_011205; Javiana, NZ_ABEH02000005; Kentucky, NZ_ABEI01000001; Tennessee, ACBF01000027; 62z4,z23–, NC_010067; Virchow, NZ_ABFH02000001; Gallinarum, NC_011274; Schwarzengrund, NC_011094; Typhi 2114, AM283471; Choleraesuis, NC_006905; *E. coli* K-12, NC_000913.

Table 1. Comparison of the amino-acid replacements in different positions of GyrA reported by Capoor *et al.* [1] and the predominant residues in the same positions found by analyses of 332 GyrA sequences

Position	Capoor <i>et al.</i> [1]	Our results
31	Phe ³¹ →Tyr	Gly
44	Leu ⁴⁴ →Ile	Val
52	Met ⁵² →Leu	Met
72	Phe ⁷² →Tyr	Asp
76	Asp ⁷⁶ →Asn	Lys
83	Ser ⁸³ →Phe	Ser
87	Asp ⁸⁷ →Asn	Asp

peptide and a functional enzyme. However, this is contradicted by the numbering of the residues, Met⁵², Ser⁸³, and Asp⁸⁷, which is in accordance with an unprocessed precursor GyrA protein. That precursor GyrA remains unmodified has also been corroborated by the absence of predicted signal peptides and the prediction of the subcellular localization of GyrA. The web-based applications, SignalP v. 3.0 (<http://www.cbs.dtu.dk/services/SignalP/>) [9] and PSORTb v. 3.0 (<http://www.psort.org/>) [10] were used for prediction of putative signal peptide sequences and the subcellular localization of GyrA, respectively. Signal peptides were not predicted by either Neural Networks or Hidden Markov models, and the score for a predicted cytoplasmic location was 9.26 whereas the next highest score was 0.48 for a predicted periplasmic location. These predictions are consistent with the known function of GyrA which takes place in the cytoplasm. The precursor GyrA thus remains unprocessed.

Based on our analyses, it is our opinion that the positions of the amino-acid replacements, Phe³¹→Tyr, Leu⁴⁴→Ile, Phe⁷²→Tyr, and Asp⁷⁶→Asn, described by Capoor and co-workers [1] are debatable; to our knowledge, GenBank entries documenting the replacements characterized by Capoor *et al.* have not been released.

The positions of the described Met⁵²→Leu, Ser⁸³→Phe, and Asp⁸⁷→Asn substitutions are compatible with the *Salmonella* GyrA sequences (see Fig. 1). The Ser⁸³→Phe and Asp⁸⁷→Asn substitutions have also been reported in several other publications [8, 9].

We do not dispute the nature of described substitutions by Capoor *et al.* [1], but we have justifiable reservations about their correct positions in the GyrA sequence. Our analyses of the available 332 GyrA sequences have unequivocally shown that Phe³¹, Leu⁴⁴, Phe⁷², and Asp⁷⁶ are not found in any of the GenBank sequences. If the positions are maintained it consequently means that Phe³¹, Leu⁴⁴, Phe⁷², and Asp⁷⁶ themselves are substitutions. Therefore, we consider the reported Phe³¹→Tyr, Leu⁴⁴→Ile, Phe⁷²→Tyr, and Asp⁷⁶→Asn substitutions in GyrA as controversial and possibly incorrect.

In their article, Capoor *et al.* [1] have corroborated the Phe⁷²→Tyr substitution by referring to an Asp⁷²→Gly substitution in *Salmonella* Senftenberg described by Eaves *et al.* [8]. The latter substitution is consistent with a dominant Asp residue in position 72 of GyrA (Fig. 1).

Regarding the Glu¹³³→Gly substitution [1], it is correct that Glu is the prevalent residue in position

133 of GyrA among the different serovars of *Salmonella*, apart from *Salmonella* Typhi. Position 133 in GyrA is covered by 315 of the obtained GyrA sequences from GenBank, and 214 of these sequences are from serovars other than Typhi. However, only two sequences (GenBank acc. nos. AY302581 and AY302582) among the 214 sequences carry a Glu¹³³→Gly substitution indicating that Glu¹³³ is the dominant residue in GyrA from these serovars. In *E. coli*, Glu¹³³ is also found in all sequences.

This picture is reversed in GyrA from *S. Typhi* because only four of the 101 sequences that cover position 133 hold a Glu¹³³ residue (GenBank acc. nos. AY302585, AY302588, GU190905, EF176634) while the remaining 97 sequences contain Gly¹³³.

These findings make it reasonable to assume that the genuine substitution in position 133 of GyrA from *S. Typhi* is in fact Gly¹³³→Glu and not Glu¹³³→Gly. Among the 162 *Salmonella* GyrA sequences available in the GenBank database Ultimo 2007, 90 sequences were from *S. Typhi*, and 87 of these contained a Gly¹³³ residue while only three had a Glu¹³³ residue.

Some of the amino-acid replacements reported by Capoor and colleagues [1] form the basis of their conclusion on an expansion of QRDR, but owing to the dubious positions of some of the described substitutions as shown in this letter, this conclusion may need a reassessment.

DECLARATION OF INTEREST

None.

References

1. **Capoor MR, et al.** Molecular analysis of high-level ciprofloxacin resistance in *Salmonella enterica* serovar Typhi and *S. Paratyphi A*: need to expand the QRDR region? *Epidemiology and Infection* 2009; **137**: 871–878.
2. **Drlica K, et al.** Quinolones: action and resistance updated. *Current Topics in Medicinal Chemistry* 2009; **9**: 981–998.
3. **Hirose K, et al.** DNA sequence analysis of DNA gyrase and DNA topoisomerase IV quinolone resistance-determining regions of *Salmonella enterica* serovar Typhi and serovar Paratyphi A. *Antimicrobial Agents and Chemotherapy* 2002; **46**: 3249–3252.
4. **Izumiya H, et al.** Characterization of isolates of *Salmonella enterica* serovar Typhimurium displaying high-level fluoroquinolone resistance in Japan. *Journal of Clinical Microbiology* 2005; **43**: 5074–5079.
5. **Yoshida H, et al.** Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* 1990; **34**: 1271–1272.
6. **Capoor MR, et al.** *Salmonella enterica* serovar Typhi: molecular analysis of strains with decreased susceptibility and resistant to ciprofloxacin in India from 2001–2003. *Brazilian Journal of Infectious Diseases* 2007; **11**: 423–425.
7. **Ling JM, et al.** Mutations in topoisomerase genes of fluoroquinolone-resistant salmonellae in Hong Kong. *Antimicrobial Agents and Chemotherapy* 2003; **47**: 3567–3573.
8. **Eaves DJ, et al.** Prevalence of mutations within the quinolone resistance-determining region of *gyrA*, *gyrB*, *parC*, and *parE* and association with antibiotic resistance in quinolone-resistant *Salmonella enterica*. *Antimicrobial Agents and Chemotherapy* 2004; **48**: 4012–4015.
9. **Bendtsen JD, et al.** Improved prediction of signal peptides: SignalP 3.0. *Journal of Molecular Biology* 2004; **340**: 783–795.
10. **Gardy JL, et al.** PSORTb v.2.0: expanded prediction of bacterial protein subcellular localization and insights gained from comparative proteome analysis. *Bioinformatics* 2005; **21**: 617–623.

JAN WALTHER-RASMUSSEN* AND NIELS HØIBY

Department of Clinical Microbiology,
The National University Hospital,
Juliane Mariesvej, Copenhagen, Denmark
(Email: jawalras@live.dk)
(*author for correspondence)

The authors reply

We congratulate Drs Walther-Rasmussen and Høiby on their work. As regards our article, which was accepted for publication in 2008, our findings were the result of collaboration of a group from the National Institute of Communicable Diseases (NICD) in New Delhi and the Pasteur Institute in Lille.

It may be that the variation in our data may be due to regional differences in isolates. Our sequencing was undertaken during 2005 and 2006. We had hoped to be able to repeat the sequencing and retrieve the data. Unfortunately, this has not proved possible; the laboratory at the Pasteur Institute has closed and two co-authors from NICD have moved to other countries.

DEEPTHI NAIR AND MALINI R. CAPOOR
(on behalf of the authors)

Department of Medical Microbiology,
VM Medical College and Safdarjung Hospital,
New Delhi, India
(Email: deepthinair2@gmail.com)