Quantifying the contribution of four resistance mechanisms to
ciprofloxacin minimum inhibitory concentration in *Escherichia coli*: a
systematic review

Authors

Boas C.L. van der Putten(1)(2)*
Daniel Remondini(3)
Giovanni Pasquini(3)
Victoria A. Janes(2)
Sébastien Matamoros(2)
Constance Schultsz(1)(2)

(1) Amsterdam UMC, University of Amsterdam, Department of Global Health, Amsterdam
Institute for Global Health and Development, Meibergdreef 9, Amsterdam, Netherlands
(2) Amsterdam UMC, University of Amsterdam, Department of Medical Microbiology,
Meibergdreef 9, Amsterdam, Netherlands
(3) Department of Physics and Astronomy (DIFA), University of Bologna, Viale Berti Pichat
6/2, 40127 Bologna, Bologna, Italy.

*Corresponding author. Telephone (+31)20 56 64860, E-mail
boas.vanderputten@amc.uva.nl

Running title: A systematic review of genetic determinants of ciprofloxacin MIC in *Escherichia coli*
Synopsis

Introduction

Ciprofloxacin resistance in *Escherichia coli* is widespread and adds to the burden of *E. coli* infections. Reviews assessing the genetic basis of ciprofloxacin resistance have mostly been qualitative. However, to allow for the prediction of a resistance phenotype of clinical relevance based on genotypic characteristics, it is essential to quantify the contribution of prevalent genotypic determinants to resistance. We carried out a systematic review to assess the relative contribution of currently known genomic resistance determinants to the minimum inhibitory concentration (MIC) of ciprofloxacin in *E. coli*.

Methods

PubMed and Web of Science were searched for English language studies that assessed both ciprofloxacin MIC and the presence or introduction of genetic determinants of ciprofloxacin resistance in *E. coli*. We included experimental and observational studies without time restrictions. Medians and ranges of MIC fold changes were calculated for each resistance determinant and for combinations of determinants.

Results

We included 66 studies, describing 604 *E. coli* isolates that carried at least one genetic resistance determinant. Genes coding for targets of ciprofloxacin (*gyrA* and *parC*) are strongest contributors to ciprofloxacin resistance, with median MIC fold increases ranging from 24 (range 4-133) for single Ser83Leu (*gyrA*) mutants to 1533 (range 256-8533) for triple Ser83Leu, Asp87Asn/Gly (*gyrA*) and Ser80Ile/Arg (*parC*) mutants. Other resistance mechanisms, including efflux, physical blocking or enzymatic modification, conferred smaller increases in ciprofloxacin MIC (median MIC fold increases typically around 15, range 1-125). However, the (combined) presence of these other resistance mechanisms further increases resistance with median MIC fold increases of up to 4000, and even in the absence of *gyrA* and *parC* mutations up to 250.
Conclusion

This report provides a comprehensive and quantitative overview of the contribution of different genomic determinants to ciprofloxacin resistance in *E. coli*. Additionally, the data demonstrate the complexity of resistance phenotype prediction from genomic data and could serve as a reference point for studies aiming to address ciprofloxacin resistance prediction using genomics, in *E. coli*.

Introduction

*Escherichia coli* is a Gram-negative bacterium able to adopt a commensal or pathogenic lifestyle in humans and animals.\(^1\) Adding to the danger of pathogenic *E. coli* is the rise of antimicrobial resistance. *Escherichia coli* has acquired resistance to some of our most important antimicrobials, including aminopenicillins, cephalosporins, aminoglycosides, carbapenems and fluoroquinolones.\(^2\)

Ciprofloxacin is an antimicrobial of the fluoroquinolone class, commonly prescribed for a wide variety of infections including infections caused by *E. coli*.\(^3\) As is the case for other fluoroquinolones, the substrate of ciprofloxacin is the complex formed by the DNA of the bacterium and either the DNA gyrase enzyme or the topoisomerase IV enzyme.\(^4\)–\(^6\) DNA gyrase creates single-stranded breaks in the DNA to negatively supercoil the DNA during replication or transcription.\(^7\) If ciprofloxacin binds DNA gyrase in complex with DNA, the single stranded DNA breaks cannot be religated and thus accumulate, leading to double stranded DNA breaks.\(^8\) A similar mechanism is hypothesized for topoisomerase IV.\(^9\)

The mechanisms of ciprofloxacin resistance in *E. coli* have been investigated intensively in the past 30 years. Mutations in genes coding for DNA gyrase and topoisomerase IV contribute to ciprofloxacin resistance in *E. coli*.\(^10\)\(^,^11\) In addition, efflux pumps may decrease drug accumulation
whilst peptides and enzymes may block drug targets or may modify the drug, respectively (Figure 1). Numerous reviews have covered the topic of ciprofloxacin resistance in *E. coli*, but these reviews have been overwhelmingly qualitative in nature.\textsuperscript{12–19}

With the rapidly increasing availability of next generation sequencing technologies, research aimed at the prediction of a resistance phenotype from genomic data is increasing. However, these efforts typically correlate genotypic data to a categorical measure of resistance, while a quantitative resistance phenotype prediction is of clinical relevance. Therefore, we carried out a systematic review, summarizing observational and experimental studies that assessed genetic ciprofloxacin resistance determinants and the ciprofloxacin minimum inhibitory concentration (MIC) conferred by these determinants in *E. coli*, to elucidate how the presence of genomic resistance determinants, either alone or in combination, affects ciprofloxacin MIC in *E. coli*. In addition, we performed an *E. coli* protein network analysis to detect potential additional determinants of ciprofloxacin resistance on the basis of the findings of the systematic review.

**Methods**

**Systematic search**

The PRISMA 2009 checklist was used as a guide for this systematic review.\textsuperscript{20} PubMed and Web of Science were searched using a defined set of keywords, selecting original research articles in English language reporting on susceptibility test results of *Escherichia coli* isolates measured as Minimum Inhibitory Concentration (MIC) due to genetic modifications identified in clinical, carriage or environmental isolates (observational) or introduced in *E. coli* strains *in vitro* (experimental) (Supplementary methods). No time limits were applied. In addition to the defined
search strategy, forward and backward citation searches of reviews and included articles was carried out. The final search was conducted on July 5th, 2018.

**Inclusion and exclusion criteria for experimental and observational studies**

Articles were not considered eligible for inclusion if they failed to mention any keyword (listed in the supplementary methods) describing ciprofloxacin resistance determinants in title or abstract. Eligible articles were screened by title, abstract and/or full text for inclusion based on the following inclusion and exclusion criteria (Figure 2). Studies could be included as experimental or as observational studies. For inclusion as an experimental study, the study needed to report a ciprofloxacin MIC before and after the introduction of a genetic modification in a single *Escherichia coli* strain. Studies were eligible to be included as observational studies if the ciprofloxacin MIC of at least one *Escherichia coli* isolate was reported, together with the observed genetic determinants of ciprofloxacin resistance. *In vitro* evolution studies where *E. coli* were exposed to ciprofloxacin resulting in decreased susceptibility to ciprofloxacin, were considered observational studies, since mutations are not actively introduced in these studies. Observational studies were excluded if they failed to test for the presence of all of the following resistance determinants: mutations in Ser83 and Asp87 of *gyrA*, mutations in Ser80 and Glu84 of *parC*, mutations in *acrR* and *marR*, presence of *oqxAB*, *qepA*, *qnrA*, *qnrB*, *qnrS* and *aac(6')Ib-cr*. If studies failed to indicate unambiguously which resistance determinants were tested, the study was excluded.

**Definitions**

For this systematic review, the conventional definition of MIC was used, meaning the lowest concentration of ciprofloxacin that inhibits the visible growth of a bacterial culture during overnight incubation. Clinical breakpoints (≤0.25 mg/L susceptible; 0.5 mg/L intermediately
resistant, ≥1 mg/L resistant) and epidemiological cutoffs (0.064 mg/L) were used as defined by EUCAST.\textsuperscript{22,23}

A genomic resistance determinant was defined as a mutation in a gene or the presence of a plasmid-mediated gene that decreases ciprofloxacin susceptibility. Since currently four mechanisms of ciprofloxacin resistance in \textit{E. coli} are known, an isolate can possess multiple resistance determinants encoding for multiple resistance mechanisms. In addition, a single resistance mechanism can be encoded by multiple resistance determinants.

Genetic modifications were defined as an experimentally acquired mutation, insertion or deletion of a nucleotide or a sequence of nucleotides in the chromosome. The introduction of plasmid-mediated genes was also considered a genetic modification. Dominance tests as described by Heisig \textit{et al.} were considered experimental evidence.\textsuperscript{24} In short, a dominance test relies on increasing the susceptibility of a bacterium to an antimicrobial, by introducing a plasmid containing the wild type gene that codes for the antimicrobial’s target. In the studies included in this report, the MICs of bacteria with mutations in \textit{gyrA} or \textit{parC} were lowered by introducing a plasmid containing wild type \textit{gyrA} or wild type \textit{parC}.

\textbf{Data extraction and analysis}

The management of the literature search was performed using Pubreminer (\url{http://hgserver2.amc.nl/cgi-bin/miner/miner2.cgi}).

All data on genetic modifications were extracted from the articles or supplementary material, together with MIC data. For experimental data, the MICs of the isolates before and after a targeted genetic modification were extracted to calculate a fold change of ciprofloxacin MIC for each of the \textit{E. coli} isolates.
We calculated how frequently resistance determinants were tested in the experimental data. This frequency is expressed as the number of isolates in which the genetic modification was introduced, divided by the total number of isolates included from experimental studies. The frequency can be used to estimate the strength of evidence per resistance determinant (Table S1). Furthermore, the sample sources, country of origin and isolation date of included *E. coli* isolates were extracted from the observational studies.

The MIC fold change data plot and the correlation matrix were generated using the ggplot2 package RStudio version 1.1.383, running R version 3.4.2. Pearson correlation coefficients were calculated using the stats package and prepared for plotting using the reshape2 package.

**Network construction**

To investigate interactions between resistance determinants and to search for potential resistance determinants, a protein-protein interaction network was constructed. The *Escherichia coli* K-12 MG1655 interactome was extracted from the STRING-v10 database. STRING-v10 aims to be more complete in terms of coverage of proteins for each organism in comparison to the other meta-interactomes available. The functional association is the basic interaction unit of String in order to link proteins with a functional relation that are likely to contribute to a common biological purpose. Each interaction is derived from multiple sources, and we identify three groups of interactions (Table S3): PI interactions (where at least one physical protein interaction has been tested, imported from primary databases), FP interactions (determined by at least one functional prediction of an algorithm employed by String, genomic information, pathway knowledge, orthology relations) and TM interactions (supported only by automated text-mining of MedLine abstracts and full-text articles). Based on the sources, for each interaction in String a score is calculated, ranging from 0 to 1. In our analysis, only interactions with a score higher than 0.7 were retained (defined as high quality interactions by String), resulting in 3,890 nodes and 32,854 edges (with only 0.06% of the links supported only by TM interactions).
resulted by the systematic search were mapped to the EcoGene-3.0 database to obtain *E. coli* K-12 MG1655 identifiers (bnumber)\textsuperscript{28}, that were subsequently mapped to the MG1655 interactome.

**Results**

**Systematic search**

The systematic search yielded 5055 PubMed entries and 5873 Web of Science entries. After removal of duplicates, 1718 unique articles were screened on content by title, abstract and, if necessary, full text. This approach identified 50 articles that were included as experimental studies. Additionally, 10 experimental studies were identified through backward/forward searches in citations of included articles and known reviews. Three articles fulfilled inclusion criteria for observational studies, of which two articles were also included as experimental studies because they provided experimental data as well (figure 2).

The number of *E. coli* isolates which were confirmed to harbour at least one resistance determinant and for which MICs were reported, amounted to a total of 366 isolates from experimental studies (Table S1) and 238 isolates from observational studies (Table S2). A total of 43 different genomic determinants were described in the collected experimental data, of which 21 were shown to have an effect on ciprofloxacin MIC (Table 1).
Experimental studies focused primarily on mutations in Ser83 (28% of included isolates) and Asp87 (18%) of \textit{gyrA}, S80 (15%) of \textit{parC} and mutations in \textit{marR} (20%). Of all plasmid-mediated resistance genes, \textit{qnrA} (17%), \textit{qnrS} (12%) and \textit{aac(6')Ib-cr} (13%) were described most often. The other resistance determinants were tested in less than 10% of the experimentally modified isolates.

Target alteration mutations in \textit{gyrA}, \textit{gyrB}, \textit{parC} and \textit{parE}

Mutations in \textit{gyrA} were the first ciprofloxacin resistance determinants to be discovered (Hooper 1987). Mutations in \textit{parC}, \textit{gyrB} and \textit{parE} were later also proven or implied to decrease ciprofloxacin susceptibility.\textsuperscript{11,29,44} \textit{gyrA} and \textit{parC} mutations that reduce ciprofloxacin susceptibility cluster in regions termed the quinolone resistance-determining regions (QRDRs). Generally, the QRDR of \textit{gyrA} ranges from amino acid Ala67 to Gln106,\textsuperscript{45} and the QRDR of \textit{parC} from Ala64 to Gln103.\textsuperscript{11} \textit{gyrA} and \textit{parC} mutations accumulate stepwise in \textit{E. coli} when exposed to ciprofloxacin, increasing ciprofloxacin MIC concurrently.\textsuperscript{11,46–48} The most common initial mutation is Ser83Leu in \textit{gyrA}.\textsuperscript{46–48} In the collected experimental data, this mutation confers a median fold increase in MIC of 24 (range: 4-133x fold increase).\textsuperscript{11,49–55} This mutation is most often followed by Ser80Ile in \textit{parC}\textsuperscript{11,46,48} and finally by Asp87Asn or Asp87Gly in \textit{gyrA}.\textsuperscript{46–48} As mutations in \textit{gyrA} and \textit{parC} accumulate, ciprofloxacin MIC increases steeply. The ciprofloxacin MIC fold increase for a mutant of Ser83Leu (\textit{gyrA}) and Ser80Ile (\textit{parC}) is 62.5.\textsuperscript{51} A similar double mutant of Ser83Leu (\textit{gyrA}) and Ser80Arg (\textit{parC}) showed a ciprofloxacin MIC fold increase of 125.\textsuperscript{53} For a triple mutant of Ser83Leu, Asp87Asn (\textit{gyrA}) and Ser80Ile (\textit{parC}) the median ciprofloxacin MIC fold increase is 2000.\textsuperscript{11,51,54} A quadruple mutant of Ser83Leu, Asp87Asn (\textit{gyrA}) and Ser80Ile, Glu84Lys (\textit{parC}) has been tested, but this mutant did not show a higher ciprofloxacin MIC than triple mutants within the same study.\textsuperscript{11} In addition, Gly81Asp and Asp82Gly mutations in \textit{gyrA}
have been tested. These mutations caused low to no decrease in ciprofloxacin susceptibility (MIC fold changes: 2.6x and 1x, respectively, Table 2).\(^{49,56}\)

Only one \textit{gyrB} mutation (Asp426Asn) was shown to slightly increase ciprofloxacin resistance (Table 2).\(^{29}\) We did not find studies that showed a decreased ciprofloxacin susceptibility due to mutations in \textit{parE}. However, a Leu445His mutation in \textit{parE} of \textit{E. coli} caused a 2x fold increase in the MIC of norfloxacin, another fluoroquinolone.\(^{44}\)

**Efflux pump genes (\textit{acrAB}, \textit{tolC}) and their transcriptional regulators (\textit{marR}, \textit{acrR} and \textit{soxS})**

As with many other antimicrobials, bacterial efflux pumps also play a role in resistance against ciprofloxacin. Deletion of \textit{acrAB} or \textit{tolC} confers a clear increase in the ciprofloxacin susceptibility of \textit{E. coli} (4-8 fold decrease in MIC).\(^{30,31,57}\) Deletions of 14 other genes or operons coding for efflux pumps in \textit{E. coli} did not affect the ciprofloxacin MIC.\(^{31}\) The deletion of transcriptional repressors of expression of efflux pumps like \textit{marR} and \textit{acrR} has been shown to affect ciprofloxacin MIC. The only study in our collected experimental data to investigate deletion of \textit{acrR} showed that the MIC tripled after the repressor was deleted.\(^{51}\) Nine studies investigated the effects of \textit{marR} deletion or mutation, which reported a median fold increase in ciprofloxacin MIC of 4 (range 1.5-218x fold increase).\(^{30,51,52,54,58-60}\) A recent study by Pietsch et al. detected mutations in \textit{rpoB} in an \textit{in vitro} evolution experiment.\(^{33}\) These mutations arose after accumulation of other mutations, and were shown to increase the ciprofloxacin MIC of a wild type \textit{E. coli} by 1.5-3 fold change (Table 2). The mutations in \textit{rpoB} were shown to increase ciprofloxacin MIC by upregulating the expression of \textit{mdtK} (also known as \textit{ydhE}).

Two experimental studies reported mutations in efflux pump operons, influencing ciprofloxacin MIC. The first mutation was Ala12Ser in \textit{soxS}, leading to higher expression of \textit{acrB}, in turn leading to a ciprofloxacin MIC fold increase of 4.\(^{32}\) The second mutation was a Gly288Asp
mutation in acrB itself, conferring a 16.7 fold increase in ciprofloxacin MIC (Table 2). This acrB mutation however increased susceptibility to other antimicrobials.

**Plasmid-encoded efflux pump genes oqxAB and qepA**

In addition to chromosomally-encoded efflux pumps, the presence of plasmid-encoded efflux pump genes oqxAB and qepA has been shown to increase ciprofloxacin MIC in *E. coli*.34,35 oqxAB confers a median fold increase in MIC of 7.5 (range 2-16x fold increase)35,62–64, while qepA confers a median fold increase of 4.5 (range 2-31x fold increase, Table 2).34,52,65–68

**qnr genes**

qnrA was the first plasmid-mediated quinolone resistance (PMQR) determinant to be discovered.36 Qnr proteins are pentapeptide repeat proteins that decrease binding of fluoroquinolones to DNA gyrase by binding the DNA:DNA gyrase complex.69 Since 2002, many more qnr alleles have been discovered. Currently seven families of qnr genes are recognized: qnrA, qnrB, qnrC, qnrD, qnrE, qnrS and qnrVC.70 In the collected experimental data, all qnr families have been tested for their influence on ciprofloxacin MIC of *E. coli*, except for qnrVC. qnr genes confer ciprofloxacin MIC fold increases between 4 and 125. The median ciprofloxacin MIC fold increase differed per qnr allele (Table 2).

**aac(6')Ib-cr and crpP**

A plasmid mediated mutant aac(6')Ib gene that decreased fluoroquinolone susceptibility in *E. coli* was discovered in 2006.42 Until then, aac(6')Ib genes were only known to decrease *E. coli* susceptibility to aminoglycosides. A double mutation in the acetyltransferase-encoding gene enabled the resulting protein to acetylate both aminoglycosides and some fluoroquinolones, including ciprofloxacin. This novel variant, aac(6')Ib-cr, was shown to confer a median fold increase in ciprofloxacin MIC of 6.9 (range: 1-62.5x fold increase, Table 2).52,71–76
The most recently discovered ciprofloxacin resistance determinant in *E. coli* is *crpP*, a plasmid-mediated gene coding for a protein with the putative ability to phosphorylate certain fluoroquinolones such as ciprofloxacin.\(^4\) *crpP* was first detected in a clinical isolate of *Pseudomonas aeruginosa*, but was shown to confer a 7.5 fold-change increase in ciprofloxacin MIC when conjugated to *E. coli* J53.

**Effect of multiple modifications on MIC**

The fold change in MIC of each included experimental isolate was plotted, stratified for the resistance mechanism present (Figure 3). Target alteration resulted in the largest range of MIC fold changes which were on average higher than the fold changes observed as a result of the three other mechanisms. Whilst the presence of determinants representing different ciprofloxacin resistance mechanisms may result in a moderate fold change in MIC, the accumulation of multiple resistance determinants encoding multiple mechanisms of resistance is likely to increase the ciprofloxacin MIC significantly.

**Comparison of experimental and observational data**

We compared the findings from the experimental data with susceptibility test results and associated presence of mutations reported for isolates in observational studies. Because studies were excluded if isolates were not tested for the presence of all known resistance encoding determinants, only studies could be included that were published after *oqxAB* was linked to increased ciprofloxacin MIC in 2007.\(^3\) The description of *crpP* was only recently published and was therefore not used as an inclusion criterion. Only three observational studies reported on the presence of all currently known resistance determinants.\(^3\) Since mutations in both *acrR* and *marR* genes were shown to result in no to low fold changes in ciprofloxacin MIC, we added five observational studies that fulfilled all inclusion and exclusion criteria except testing for the presence of mutations in *acrR* and *marR* genes, in a secondary analysis. Thus, eight
observational studies published between 2012 and 2018 were included, contributing data on a total of 238 strains (Table S2). The studies reported data on 1 to 92 isolates, with a median of 13.5 isolates per study. Ciprofloxacin MICs of included isolates ranged from 0.015 to 1024 mg/L with a median MIC of 1 mg/L.

We analysed MIC distributions for combinations of resistance determinants that were reported at least five times in the experimental and observational data. These combinations of resistance determinants included the mutation Ser83Leu in gyrA, presence of qnrS1 and presence of aac(6’)Ib-cr. Although for most combinations of resistance determinants small numbers of isolates were reported, results of experimental and observational data appear comparable with the exception for the reported MICs for E. coli strains solely harbouring aac(6’)Ib-cr (Table 3).

We also examined if certain combinations of resistance mechanisms were more prevalent than others in the observational data. Calculating Pearson correlation coefficients between commonly observed resistance determinants showed that gyrA (Ser83, Asp87) and parC (Ser80) mutations were positively correlated with each other. Additionally, these three mutations were shown to inversely correlate with the presence of qnrB and qnrS genes in our observational data. This inverse correlation was not observed with other frequently reported plasmid-mediated resistance determinants such as aac(6’)Ib-cr (Figure 4).

Network visualization

In order to get a global picture of the mutation landscape associated with ciprofloxacin resistance, we mapped the selected chromosomal genes onto a Protein-Protein Interaction (PPI) network. The selected genes were evaluated in a wide range of E. coli strains, and we mapped them to the String-v10 database referring to the E. coli K-12 MG1655 model organism, since it
showed the highest number of matching edges and nodes among the strains available in String
database. We noted that plasmid-associated genes like \textit{oqxAB} and the \textit{qnr} gene family were not
described by interactomes in general, since interactomes mostly describe the core genome.
Moreover, some genes (such as \textit{yohG}) could not be mapped because they are not present in \textit{E. coli} K-12 MG1655.

Of the 43 selected genes, 31 (72\%) mapped to the PPI network, resulting in a fully connected
sub-module. The network highlighted the close relationship between gene connectivity and
ciprofloxacin resistance effects: the chosen visualization algorithm showed that genes with
similar effects tightly grouped in the interactome (Figure 5). Particularly, the genes that had an
increasing effect on ciprofloxacin resistance when mutated seemed to cluster, even if the genes
belonged to different resistance mechanisms. As expected, close relationships between
particular sets of genes were revealed. Transcriptional regulators such as \textit{marR}, \textit{acrR} and \textit{soxS}
were shown to interact with efflux pump genes such as \textit{acrA}, \textit{acrB}, \textit{acrD}, \textit{acrF} and \textit{tolC}. Also,
the physical interactions between \textit{gyrA}, \textit{gyrB} and \textit{parC} were depicted in the network.

**Discussion**

This report provides a comprehensive and systematic analysis of 66 papers linking genotype of
\textit{E. coli} to a quantitative ciprofloxacin resistance phenotype, spanning the years 1989-2018 and
amounting to a total of 604 isolates. Ciprofloxacin MIC in \textit{E. coli} is largely affected by target
mutations in specific residues in \textit{gyrA} (Ser83 and Asp87) and \textit{parC} (Ser80), conferring median
MIC fold increases ranging from 24 for single Ser83Leu (\textit{gyrA}) mutants to 1533 for triple
Ser83Leu, Asp87Asn/Gly (\textit{gyrA}) Ser80Ile/Arg (\textit{parC}) mutants. However, accumulation of
multiple resistance determinants, including those representing other resistance mechanisms,
can increase ciprofloxacin MIC even further, up to MIC fold increases of 4000.
Beside the MIC fold changes that are conferred by resistance determinants, it is important to consider how these genetic resistance determinants are acquired. The SOS response is an important driver of mutation after DNA damage is induced by quinolones such as ciprofloxacin. Two proteins that are central in the SOS response are LexA and RecA. In the absence of DNA damage, LexA dimers are bound to a SOS box (promoter region of SOS genes) and inhibit expression of SOS genes. If DNA damage is induced, for example through the presence of ciprofloxacin, RecA will bind ssDNA that is a result of the DNA damage. The activated RecA in turn mediates the self-cleavage of LexA, derepressing the SOS box, finally leading to expression of SOS genes and thus the SOS response. This SOS response induces mutations, among others, through DNA damage repair performed by error-prone DNA polymerases.

Currently, four ways are known in which the SOS response affects ciprofloxacin resistance in *E. coli*. First, the SOS response induces a higher mutation rate, making it more likely that ciprofloxacin resistance mutations will arise within a fixed population. Additionally, if the SOS response is knocked out in *E. coli*, ciprofloxacin MIC decreases. Clinically resistant *E. coli* that had recA knocked out showed MIC fold decreases of 4-8. Furthermore, the SOS response has been shown to induce expression of some qnr gene families, for example *qnrB* and *qnrD*. Finally, the SOS response has been shown to promote horizontal transfer of resistance genes when *E. coli* is grown in the presence of ciprofloxacin.

After mutagenesis through mechanisms such as the SOS response, the fitness of the mutant indicates how likely the bacterium is to survive. In absence of ciprofloxacin, *gyrA* mutations and *parC* mutations have been shown to confer limited fitness costs compared to other resistance determinants. Additionally, mutations in *gyrA* and *parC* show positive epistasis, as the MIC fold change of the triple Ser83Leu, Asp87Asn (*gyrA*) and Ser80Ile (*parC*) mutant is higher (2000x fold increase) than would be expected based on the MIC fold changes conferred by the individual mutations (24x, 16x and 1x fold increases, respectively). This epistatic effect thus
raises ciprofloxacin MIC very efficiently. This, in combination with the low fitness costs in absence of ciprofloxacin might explain why ciprofloxacin resistance mutations in \textit{gyrA} and \textit{parC} are the most common ciprofloxacin resistance determinants observed in \textit{E. coli}.

Notably, other combinations of resistance determinants also show positive epistatic effects, although the observed effects are weaker. A similar positive epistatic effect was observed for chromosomal \textit{gyrA}/\textit{parC} mutations together with plasmid-mediated resistance determinants \textit{qepA} and \textit{aac(6')Ib-cr}. However, experimental studies of combinations of \textit{gyrA} and \textit{parC} mutations with \textit{qnr} genes showed discordant results. One study reported a negative epistatic effect on ciprofloxacin MIC of target alteration mutations with all \textit{qnr} genes tested (\textit{qnrA}, \textit{qnrB}, \textit{qnrC}, \textit{qnrD}, \textit{qnrS}) and another study observed a similar effect of target alteration mutations with \textit{qnrB}, but the opposite effect for target alteration mutations with \textit{qnrS} in terms of conferred MIC.

The complex relation between \textit{gyrA}/\textit{parC} mutations and \textit{qnr} genes is further illustrated by our findings from the observational data. We observed a clear negative correlation between presence of \textit{gyrA} or \textit{parC} mutations and presence of \textit{qnrB} and \textit{qnrS} genes. This finding is in line with an earlier study that reported an \textit{E. coli} population fixating \textit{gyrA}/\textit{parC} mutations at a reduced rate when the \textit{E. coli} population harboured a \textit{qnr} gene as opposed to when the \textit{E. coli} strain did not harbour a \textit{qnr} gene. However, no additional fitness costs are usually reported for \textit{E. coli} harbouring both \textit{gyrA}/\textit{parC} mutations and \textit{qnr} genes. One possible explanation was suggested by the study of Garoff et al., who reported an enhanced fitness cost conferred by \textit{qnr} genes when Lon protease was absent from an \textit{E. coli} genome. This finding shows that the fitness cost conferred by an antimicrobial resistance gene to an \textit{E. coli} strain can be influenced by genes that do not directly play a role in antimicrobial resistance.

By mapping the selected genes onto a known \textit{E. coli} interactome, we found a clear association between their role in ciprofloxacin resistance and their position in the network, with a significant
proximity of genes that produce a similar response in terms of resistance (i.e. increase or decrease). This global picture highlights the presence of common biological functions (mostly associated with the efflux pumps and their regulation), and it suggests that system biology approaches in the future will likely be helpful to identify new targets or specific pathways related to ciprofloxacin resistance or antimicrobial resistance in general. As an example, the position in the network of acrD and acrF genes, which were not identified as resistance-associated genes in the experiments reported so far, and their biological function as efflux pump protein complexes, suggest that their role in resistance should be more deeply investigated.

Despite its comprehensiveness our study has certain limitations. First, gene expression data are not included in this review because our study aims at prediction of MIC on the basis of a DNA sequence. It has been shown that increased expression of efflux pumps such as acrAB or transcriptional regulators of efflux pumps such as marA is significantly correlated with increased fluoroquinolone MIC in *E. coli*. Secondly, complex combinations of resistance determinants such as combinations of gyrA/parC mutations with plasmid-mediated resistance determinants have been reported sparsely in the experimental data. Therefore, the comparison of experimental and observational data for these combinations of resistance determinants is impossible using this dataset. Finally, only currently known ciprofloxacin resistance determinants could be included in this report. The very recent discovery of crpP suggests that more resistance determinants or resistance mechanisms are still waiting to be discovered. Additionally, complex mutation patterns influencing ciprofloxacin resistance through unknown pathways may exist, but current research methods do not usually detect these kinds of effects.

One possible solution for the issues described above would be the use of advanced machine learning algorithms to predict ciprofloxacin resistance. These algorithms should be able to associate large quantities of sequence data with phenotypic metadata in an unbiased manner. One such attempt has been made for ciprofloxacin resistance already. It was reported that
Ser83Phe, Ser83Thr \((gyrA)\), Ser80Arg \((parC)\) and presence of any \(qnr\) gene were the most important resistance determinants according to the algorithm used. However, this study used categorical (susceptible or resistant) and not quantitative phenotype data, and included various Enterobacteriaceae species and the results can thus not be directly compared with the data presented here for \(E. coli\) alone. This is exemplified by the fact that neither Ser83Phe nor Ser83Thr \((gyrA)\) were reported in our observational data. For future studies, the data collected for this review could serve as a benchmark, as this review presents a comprehensive set of quantitative data on the contribution of various resistance determinants to ciprofloxacin MIC in \(E. coli\).

Acknowledgments

We wish to thank the COMPARE consortium for support and helpful discussions.

Funding

No specific funding has been received for this work. BP was funded through an internal grant from the Academic Medical Center Amsterdam (‘Flexible OiO’ grant).

Transparency

None to declare.
References

1. Tenaillon O, Skurnik D, Picard B et al. The population genetics of commensal Escherichia coli. *Nat Rev Microbiol*. 2010;8(1740-1534):207-217. doi:10.1038/nrmicro2298

2. ECDC. *Annual Report of the European Antimicrobial Resistance Surveillance Network* (EARS-Net); 2014. doi:10.2900/93403

3. Johns Hopkins Medicine. *Antibiotic Guidelines 2015-2016*; 2016.

4. LeBel M. Ciprofloxacin: Chemistry, Mechanism of Action, Resistance, Antimicrobial Spectrum, Pharmacokinetics, Clinical Trials, and Adverse Reactions. *Pharmacother J Hum Pharmacol Drug Ther*. 1988;8(1):3-30.

5. Khodursky A, Zechiedrich E, Cozzarelli N. Topoisomerase IV is a target of quinolones in Escherichia coli. 1995;92(December):11801-11805.

6. Drlica K. Mechanism of fluoroquinolone action. *Curr Opin Microbiol*. 1999;2(5):504-508. doi:10.1016/S1369-5274(99)00008-9

7. Cozzarelli NR. DNA gyrase and the supercoiling of DNA. *Science (80-)*. 1980;207(4434):953-960. doi:10.1126/science.6243420

8. Kampranis SC, Maxwell A. The DNA Gyrase-Quinolone Complex. *J Biol Chem*. 1998;273(35):22615-22626.

9. Anderson VE, Gootz TD, Osheroff N. Topoisomerase IV catalysis and the mechanism of quinolone action. *J Biol Chem*. 1998;273(28):17879-17885. doi:10.1074/jbc.273.28.17879

10. Cullen ME, Wyke AW, Kuroda R et al. Cloning and characterization of a DNA gyrase A gene from Escherichia coli that confers clinical resistance to 4-quinolones. *Antimicrob*
11. Heisig P. Genetic Evidence for a Role of parC Mutations in Development of High-Level Fluoroquinolone Resistance in Escherichia coli. 1996;40(4):879-885.

12. Hooper DC, Wolfson JS, Ng EY et al. Mechanisms of action of and resistance to ciprofloxacin. Am J Med. 1987;82(4A):12-20.

13. Hooper DC. Mechanisms of Action and Resistance of Older and Newer Fluoroquinolones. Clin Infect Dis. 2000;31:S24-S28. doi:10.1086/314056

14. Hooper DC. Emerging mechanisms of fluoroquinolone resistance. Emerg Infect Dis. 2001;7(2):337-341. doi:10.3201/eid0702.010239

15. Hooper DC. Mechanisms of fluoroquinolone resistance. Drug Resist Updat. 1999;2(1):38-55. doi:http://dx.doi.org/10.1054/drup.1998.0068

16. Webber M, Piddock LJ. Quinolone resistance in Escherichia coli. Vet Res. 2001;32(3-4):275-284. doi:10.1051/vetres:2001124

17. Strahilevitz J, Jacoby GA, Hooper DC et al. Plasmid-mediated quinolone resistance: A multifaceted threat. Clin Microbiol Rev. 2009;22(4):664-689. doi:10.1128/CMR.00016-09

18. Hawkey PM. Mechanisms of quinolone action and microbial response. J Antimicrob Chemother. 2003;51:29-35. doi:10.1093/jac/dkg207

19. Hopkins KL, Davies RH, Threlfall EJ. Mechanisms of quinolone resistance in Escherichia coli and Salmonella: Recent developments. Int J Antimicrob Agents. 2005;25(5):358-373. doi:10.1016/j.ijantimicag.2005.02.006

20. Moher D, Liberati A, Tetzlaff J et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009;6(7).
doi:10.1371/journal.pmed.1000097

21. Andrews JM, Andrews JM. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother.* 2001;48:5-16. doi:10.1093/jac/48.suppl_1.5

22. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. version 8.1. http://www.eucast.org/clinical_breakpoints/. Published 2018.

23. The European Committee on Antimicrobial Susceptibility Testing. MIC and zone diameter distributions and ECOFFs. http://www.eucast.org/mic_distributions_and_ecoffs/. Published 2018.

24. Heisig P, Wiedemann B. Use of a broad-host-range gyrA plasmid for genetic characterization of fluoroquinolone-resistant gram-negative bacteria. *Antimicrob Agents Chemother.* 1991;35(10):2031-2036. doi:10.1128/aac.

25. Szklarczyk D, Franceschini A, Wyder S *et al.* STRING v10: Protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* 2015;43(D1):D447-D452. doi:10.1093/nar/gkx1003

26. Hu P, Janga SC, Babu M *et al.* Global functional atlas of Escherichia coli encompassing previously uncharacterized proteins. *PLoS Biol.* 2009;7(4):0929-0947. doi:10.1371/journal.pbio.1000096

27. Ogris C, Guala D, Kaduk M *et al.* FunCoup 4: new species, data, and visualization. *Nucleic Acids Res.* 2018;46(D1):D601-D607. doi:10.1093/nar/gkx1138

28. Zhou J, Rudd KE. EcoGene 3.0. *Nucleic Acids Res.* 2013;41(D1):613-624. doi:10.1093/nar/gks1235
29. Yoshida H, Bogaki M, Nakamura M et al. Quinolone Resistance-Determining Region in the DNA Gyrase gyrB Gene of Escherichia coli. *Antimicrob Agents Chemother.* 1991;35(8):1647-1650.

30. Linde HJ, Notka F, Metz M et al. In vivo increase in resistance to ciprofloxacin in Escherichia coli associated with deletion of the C-terminal part of marR. *Antimicrob Agents Chemother.* 2000;44(7):1865-1868. doi:10.1128/AAC.44.7.1865-1868.2000

31. Sulavik MC, Houseweart C, Cramer C et al. Antibiotic Susceptibility Profiles of Escherichia coli Strains Lacking Multidrug Efflux Pump Genes. *Antimicrob Agents Chemother.* 2001;45(5):1126-1136. doi:10.1128/AAC.45.4.1126

32. Aly SA, Boothe DM, Suh SJ. A novel alanine to serine substitution mutation in SoxS induces overexpression of efflux pumps and contributes to multidrug resistance in clinical Escherichia coli isolates. *J Antimicrob Chemother.* 2015;70(8):2228-2233. doi:10.1093/jac/dkv105

33. Pietsch F, Bergman JM, Brandis G et al. Ciprofloxacin selects for RNA polymerase mutations with pleiotropic antibiotic resistance effects. *J Antimicrob Chemother.* 2017;72(1):75-84. doi:10.1093/jac/dkw364

34. Yamane K, Wachino JI, Suzuki S et al. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an Escherichia coli clinical isolate. *Antimicrob Agents Chemother.* 2007;51(9):3354-3360. doi:10.1128/AAC.00339-07

35. Hansen LH, Jensen LB, Sørensen HI et al. Substrate specificity of the OqxAB multidrug resistance pump in Escherichia coli and selected enteric bacteria. *J Antimicrob Chemother.* 2007;60(1):145-147. doi:10.1093/jac/dkm167

36. Tran JH, Jacoby GA. Mechanism of plasmid-mediated quinolone resistance. *Proc Natl...*
37. Jacoby GA, Walsh KE, Mills DM et al. qnrB, Another Plasmid-Mediated Gene for Quinolone Resistance. *Antimicrob Agents Chemother.* 2006;50(4):1178-1182. doi:10.1128/AAC.50.4.1178

38. Wang M, Guo Q, Xu X et al. New plasmid-mediated quinolone resistance gene, qnrC, found in a clinical isolate of Proteus mirabilis. *Antimicrob Agents Chemother.* 2009;53(5):1892-1897. doi:10.1128/AAC.01400-08

39. Cavaco LM, Hasman H, Xia S et al. qnrD, a novel gene conferring transferable quinolone resistance in Salmonella enterica serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob Agents Chemother.* 2009;53(2):603-608. doi:10.1128/AAC.00997-08

40. Albornoz E, Tijet N, De Belder D et al. QnrE1, a member of a new family of plasmid-located quinolone resistance genes, originated from the chromosome of enterobacter species. *Antimicrob Agents Chemother.* 2017;61(5):1-8. doi:10.1128/AAC.02555-16

41. Hata M, Suzuki M, Matsumoto M et al. Cloning of a Novel Gene for Quinolone Resistance from a Transferable Plasmid in Shigella flexneri 2b. *Antimicrob Agents Chemother.* 2005;49(2):801-803. doi:10.1128/AAC.49.2.801

42. Robicsek A, Strahilevitz J, Jacoby GA et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med.* 2006;12(1):83-88. doi:10.1038/nm1347

43. Chávez-Jacobo V, Hernández-Ramírez K, Romo-Rodríguez P et al. CrpP Is a Novel Ciprofloxacin-Modifying Enzyme Encoded by the Pseudomonas aeruginosa pUM505 Plasmid. *Antimicrob Agents Chemother.* 2018;62(6):1-11.
44. Breines DM, Ouabdesselam S, Ng EY et al. Quinolone resistance locus nfxD of Escherichia coli is a mutant allele of the parE gene encoding a subunit of topoisomerase IV. *Antimicrob Agents Chemother.* 1997;41(1):175-179.

45. Weigel LM, Steward CD, Tenover FC. gyrA mutations associated with fluoroquinolone resistance in eight species of Enterobacteriaceae. *Antimicrob Agents Chemother.* 1998;42(10):2661-2667.

46. Liu X, Lazzaroni C, Aly SA et al. In vitro selection of resistance to pradofloxacin and ciprofloxacin in canine uropathogenic Escherichia coli isolates. *Vet Microbiol.* 2014;174(3-4):514-522. doi:10.1016/j.vetmic.2014.10.011

47. Heisig P, Tschorny R. Characterization of fluoroquinolone-resistant mutants of Escherichia coli selected in vitro. 1994;38(6):1284-1291. doi:10.1128/AAC.

48. Huseby DL, Pietsch F, Brandis G et al. Mutation Supply and Relative Fitness Shape the Genotypes of Ciprofloxacin-Resistant Escherichia coli. *Mol Biol Evol.* 2017;34(5):1029-1039. doi:10.1093/molbev/msx052

49. Truong QC, Van Nguyen JC, Shlaes D et al. A novel, double mutation in DNA gyrase A of Escherichia coli conferring resistance to quinolone antibiotics. *Antimicrob Agents Chemother.* 1997;41(1):85-90.

50. Allou N, Cambau E, Massias L et al. Impact of low-level resistance to fluoroquinolones due to qnrA1 and qnrS1 genes or a gyrA mutation on ciprofloxacin bactericidal activity in a murine model of Escherichia coli urinary tract infection. *Antimicrob Agents Chemother.* 2009;53(10):4292-4297. doi:10.1128/AAC.01664-08

51. Marcusson LL, Frimodt-Møller N, Hughes D. Interplay in the selection of fluoroquinolone resistance and bacterial fitness. *PLoS Pathog.* 2009;5(8).
558  doi:10.1371/journal.ppat.1000541

559  52. Emrich NC, Heisig A, Stubbings W et al. Antibacterial activity of finafloxacin under different pH conditions against isogenic strains of Escherichia coli expressing combinations of defined mechanisms of fluoroquinolone resistance. *J Antimicrob Chemother*. 2010;65(12):2530-2533. doi:10.1093/jac/dkq375

563  53. Briales A, Rodríguez-Martínez JM, Velasco C et al. In vitro effect of qnrA1, qnrB1, and qnrS1 genes on fluoroquinolone activity against isogenic Escherichia coli isolates with mutations in gyrA and parC. *Antimicrob Agents Chemother*. 2011;55(3):1266-1269. doi:10.1128/AAC.00927-10

567  54. Khan DD, Lagerbäck P, Cao S et al. A mechanism-based pharmacokinetic/pharmacodynamic model allows prediction of antibiotic killing from MIC values for WT and mutants. *J Antimicrob Chemother*. 2015;70(11):3051-3060. doi:10.1093/jac/dkv233

571  55. Webber MA, Buckner MMC, Redgrave LS et al. Quinolone-resistant gyrase mutants demonstrate decreased susceptibility to triclosan. *J Antimicrob Chemother*. 2017;72(10):2755-2763. doi:10.1093/jac/dkx201

574  56. Cambau E, Bordon F, Collatz E. Novel gyrA point mutation in a strain of Escherichia coli resistant to fluoroquinolones but not to nalidixic acid. *Antimicrob Agents Chemother*. 1993;37(6):1247-1252. doi:10.1128/AAC.37.6.1247.Updated

577  57. Oethinger M, Kern W V., Jellen-Ritter AS et al. Ineffectiveness of topoisomerase mutations in mediating clinically significant fluoroquinolone resistance in Escherichia coli in the absence of the AcrAB efflux pump. *Antimicrob Agents Chemother*. 2000;44(1):10-13. doi:10.1128/AAC.44.1.10-13.2000
58. Yaron S, White DG, Matthews KR. Characterization of an Escherichia coli O157:H7 marR mutant. *Int J Food Microbiol.* 2003;85(3):281-291. doi:10.1016/S0168-1605(02)00547-0

59. Machuca J, Briales A, Labrador G *et al.* Interplay between plasmid-mediated and chromosomal-mediated fluoroquinolone resistance and bacterial fitness in Escherichia coli. *J Antimicrob Chemother.* 2014;69(12):3203-3215. doi:10.1093/jac/dku308

60. Praski Alzrigat L, Huseby DL, Brandis G *et al.* Fitness cost constrains the spectrum of marR mutations in ciprofloxacin-resistant Escherichia coli. *J Antimicrob Chemother.* 2017;72(11):3016-3024. doi:10.1093/jac/dkx270

61. Blair JMA, Bavro VN, Ricci V *et al.* AcrB drug-binding pocket substitution confers clinically relevant resistance and altered substrate specificity. *Proc Natl Acad Sci U S A.* 2015;112(11):3511-3516. doi:10.1073/pnas.1419939112

62. Zhao J, Chen Z, Chen S *et al.* Prevalence and dissemination of oqxAB in Escherichia coli isolates from animals, farmworkers, and the environment. *Antimicrob Agents Chemother.* 2010;54(10):4219-4224. doi:10.1128/AAC.00139-10

63. Sato T, Yokota SI, Uchida I *et al.* Fluoroquinolone resistance mechanisms in an Escherichia coli isolate, HUE1, without quinolone resistance-determining region mutations. *Front Microbiol.* 2013;4(May):1-12. doi:10.3389/fmicb.2013.00125

64. Wang J, Guo Z-W, Zhi C-P *et al.* Impact of plasmid-borne oqxAB on the development of fluoroquinolone resistance and bacterial fitness in Escherichia coli. *J Antimicrob Chemother.* 2017;72(5):1293-1302. doi:10.1093/jac/dkw576

65. Yamane K, Wachino JI, Suzuki S *et al.* Plasmid-mediated qepA gene among Escherichia coli clinical isolates from Japan. *Antimicrob Agents Chemother.* 2008;52(4):1564-1566. doi:10.1128/AAC.01137-07
66. Périchon B, Courvalin P, Galimand M. Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic fluoroquinolones by QepA-mediated efflux in Escherichia coli. *Antimicrob Agents Chemother.* 2007;51(7):2464-2469. doi:10.1128/AAC.00143-07

67. Machuca J, Briales A, Díaz-de-Alba P et al. Effect of the efflux pump QepA2 combined with chromosomally mediated mechanisms on quinolone resistance and bacterial fitness in Escherichia coli. *J Antimicrob Chemother.* 2015;70(9):2524-2527. doi:10.1093/jac/dkv144

68. Manageiro V, Félix D, Jones-Dias D et al. Genetic background and expression of the new qepa4 gene variant recovered in clinical TEM-1- and CMY-2-producing Escherichia coli. *Front Microbiol.* 2017;8(Oct):1-6. doi:10.3389/fmicb.2017.01899

69. Tran JH, Jacoby GA, Hooper DC. Interaction of the Plasmid-Encoded Quinolone Resistance Protein QnrA with Escherichia coli Topoisomerase IV Interaction of the Plasmid-Encoded Quinolone Resistance Protein QnrA with Escherichia coli Topoisomerase IV. *Antimicrob Agents Chemother.* 2005;49(7):4-7. doi:10.1128/AAC.49.7.3050

70. Jacoby GA. qnr Numbering and Sequences. http://www.lahey.org/qnrstudies/. Accessed January 26, 2018.

71. Chowdhury G, Pazhani GP, Nair GB et al. Transferable plasmid-mediated quinolone resistance in association with extended-spectrum β-lactamases and fluoroquinolone-acetylating aminoglycoside-6′-N-acetyltransferase in clinical isolates of Vibrio fluvialis. *Int J Antimicrob Agents.* 2011;38(2):169-173. doi:10.1016/j.ijantimicag.2011.04.013

72. Silva-Sánchez J, Cruz-Trujillo E, Barrios H et al. Characterization of Plasmid-Mediated
Quinolone Resistance (PMQR) Genes in Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae Pediatric Clinical Isolates in Mexico. *PLoS One.* 2013;8(10). doi:10.1371/journal.pone.0077968

Shaheen BW, Nayak R, Foley SL *et al.* Chromosomal and plasmid-mediated fluoroquinolone resistance mechanisms among broad-spectrum-cephalosporin-resistant Escherichia coli isolates recovered from companion animals in the USA. *J Antimicrob Chemother.* 2013;68(5):1019-1024. doi:10.1093/jac/dks514

Varela AR, Macedo GN, Nunes OC *et al.* Genetic characterization of fluoroquinolone resistant Escherichia coli from urban streams and municipal and hospital effluents. *FEMS Microbiol Ecol.* 2015;91(5):1-12. doi:10.1093/femsec/fiv015

Machuca J, Ortiz M, Recacha E *et al.* Impact of AAC(6')-Ib-cr in combination with chromosomal-mediated mechanisms on clinical quinolone resistance in Escherichia coli. *J Antimicrob Chemother.* 2016;(July):6-11. doi:10.1093/jac/dkw258

Yanat B, Machuca J, Díaz-De-Alba P *et al.* Characterization of Plasmid-Mediated Quinolone Resistance Determinants in High-Level Quinolone-Resistant *Enterobacteriaceae* Isolates from the Community: First Report of *qnrD* Gene in Algeria. *Microb Drug Resist.* 2017;23(1):90-97. doi:10.1089/mdr.2016.0031

Heisig P, Schedletzky H, Falkenstein-Paul H. Mutations in the gyrA gene of a highly fluoroquinolone-resistant clinical isolate of Escherichia coli. *Antimicrob Agents Chemother.* 1993;37(4):696-701. doi:10.1128/AAC.37.4.696

Webber MA, Talukder A, Piddock LJ V. Contribution of mutation at amino acid 45 of AcrR to acrB expression and ciprofloxacin resistance in clinical and veterinary Escherichia coli isolates. *Antimicrob Agents Chemother.* 2005;49(10):4390-4392.
Prevalence and Characterization of Plasmid-Mediated Quinolone Resistance Genes in Extended-Spectrum β-Lactamase–Producing Enterobacteriaceae Isolates in Mexico. *Microb Drug Resist*. 2011;17(4):497-505. doi:10.1089/mdr.2011.0086

Contribution of QnrA, a plasmid-mediated quinolone resistance peptide, to survival of *Escherichia coli* exposed to a lethal ciprofloxacin concentration. *Jpn J Infect Dis*. 2015;68(3):196-202. doi:10.7883/yoken.JJID.2014.153

Low selection of topoisomerase mutants from strains of *Escherichia coli* harbouring plasmid-borne qnr genes. *J Antimicrob Chemother*. 2008;61(5):1007-1015. doi:10.1093/jac/dkn077

Impact of low-level fluoroquinolone resistance genes qnrA1, qnrB19 and qnrS1 on ciprofloxacin treatment of isogenic *Escherichia coli* strains in a murine urinary tract infection model. *J Antimicrob Chemother*. 2012;67(10):2438-2444. doi:10.1093/jac/dks224

Quinolone resistance from a transferable plasmid. *Lancet*. 1998;351(9105):797-799. doi:10.1016/S0140-6736(97)07322-4

Interaction of plasmid and host quinolone resistance. *J Antimicrob Chemother*. 2003;51(4):1037-1039. doi:10.1093/jac/dkg157

Plasmidic qnr Genes Confer Clinical Resistance to Ciprofloxacin under Urinary Tract Physiological Conditions. *Antimicrob Agents Chemother*. 2017;61(4):61-64.
86. Rodríguez-Martínez JM, Velasco C, Pascual A et al. Correlation of quinolone resistance levels and differences in basal and quinolone-induced expression from three qnrA-containing plasmids. *Clin Microbiol Infect.* 2006;12(5):440-445. doi:10.1111/j.1469-0691.2006.01389.x

87. Rodríguez-Martínez JM, Velasco C, Briales A et al. Qnr-like pentapeptide repeat proteins in Gram-positive bacteria. *J Antimicrob Chemother.* 2008;61(6):1240-1243. doi:10.1093/jac/dkn115

88. Wang M, Tran J, Jacoby G. Plasmid-mediated quinolone resistance in clinical isolates of Escherichia coli from Shanghai, China. *Antimicrob Agents Chemother.* 2003;47(7):2242-2248. doi:10.1128/AAC.47.7.2242

89. Xu X, Wu S, Ye X et al. Prevalence and expression of the plasmid-mediated quinolone resistance determinant qnrA1. *Antimicrob Agents Chemother.* 2007;51(11):4105-4110. doi:10.1128/AAC.00616-07

90. Jones-Dias D, Manageiro V, Francisco AP et al. Assessing the molecular basis of transferable quinolone resistance in Escherichia coli and Salmonella spp. from food-producing animals and food products. *Vet Microbiol.* 2013;167(3-4):523-531. doi:10.1016/j.vetmic.2013.08.010

91. Shin JH, Jung H, Lee J et al. High rates of plasmid-mediated quinolone resistance QnrB variants among ciprofloxacin-resistant Escherichia coli and Klebsiella pneumoniae from urinary tract infections in Korea. *Microb Drug Resist.* 2008;14(3):221-226. doi:10.1089/mdr.2008.0834

92. Cerquetti M, García-Fernández A, Giufrè M et al. First report of plasmid-mediated quinolone resistance determinant qnrS1 in an Escherichia coli strain of animal origin in
Italy. Antimicrob Agents Chemother. 2009;53(7):3112-3114. doi:10.1128/AAC.00239-09

Okumura R, Liao CH, Gavin M et al. Quinolone induction of qnrVS1 in Vibrio splendidus and plasmid-carried qnrS1 in Escherichia coli, a mechanism independent of the SOS system. Antimicrob Agents Chemother. 2011;55(12):5942-5945. doi:10.1128/AAC.05142-11

Xue G, Li J, Feng Y et al. High Prevalence of Plasmid-Mediated Quinolone Resistance Determinants in Escherichia coli and Klebsiella pneumoniae Isolates from Pediatric Patients in China. Microb Drug Resist. 2017;23(1):107-114. doi:10.1089/mdr.2016.0004

Bönemann G, Stiens M, Pühler A et al. Mobilizable IncQ-related plasmid carrying a new quinolone resistance gene, qnrS2, isolated from the bacterial community of a wastewater treatment plant. Antimicrob Agents Chemother. 2006;50(9):3075-3080. doi:10.1128/AAC.00378-06

Ruiz E, Sáenz Y, Zarazaga M et al. Qnr, aac(6')-Ib-cr and qepA genes in Escherichia coli and Klebsiella spp.: Genetic environments and plasmid and chromosomal location. J Antimicrob Chemother. 2012;67(4):886-897. doi:10.1093/jac/dkr548

Sekyere JO, Amoako DG. Genomic and phenotypic characterisation of fluoroquinolone resistance mechanisms in Enterobacteriaceae in Durban, South Africa. PLoS One. 2017;12(6):1-14. doi:10.1371/journal.pone.0178888

Vinué L, Hooper DC, Jacoby GA. Chromosomal mutations that accompany qnr in clinical isolates of escherichia coli. Int J Antimicrob Agents. 2018. doi:10.1016/j.ijantimicag.2018.01.012

Walker G. Mutagenesis and inducible responses to deoxyribonucleic acid damage in Escherichia coli. Microbial Rev. 1984;48(1):60-93.
100. Erill I, Campoy S, Barbé J. Aeons of distress: An evolutionary perspective on the bacterial SOS response. *FEMS Microbiol Rev*. 2007;31(6):637-656. doi:10.1111/j.1574-6976.2007.00082.x

101. Recacha E, Machuca J, Díaz de Alba P *et al.* Quinolone resistance reversion by targeting the SOS response. *MBio*. 2017;8(5). doi:10.1128/mBio.00971-17

102. Da Re S, Garnier F, Guérin E *et al.* The SOS response promotes qnrB quinolone-resistance determinant expression. *EMBO Rep*. 2009;10(8):929-933. doi:10.1038/embor.2009.99

103. Wang M, Jacoby GA, Mills DM *et al.* SOS regulation of qnrB expression. *Antimicrob Agents Chemother*. 2009;53(2):821-823. doi:10.1128/AAC.00132-08

104. Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature*. 2004;427(January):72-74. doi:10.1038/nature02241

105. Hughes D, Andersson DI. Evolutionary Trajectories to Antibiotic Resistance. *Annu Rev Microbiol*. 2017;71(1):579-596. doi:10.1146/annurev-micro-090816-093813

106. Garoff L, Yadav K, Hughes D. Increased expression of Qnr is sufficient to confer clinical resistance to ciprofloxacin in *Escherichia coli*. *J Antimicrob Chemother*. 2017;(January):348-352. doi:10.1093/jac/dkx375

107. Shigemura K, Tanaka K, Yamamichi F *et al.* Does mutation in *gyrA* and/or *parC* or efflux pump expression play the main role in fluoroquinolone resistance in *Escherichia coli* urinary tract infections?: A statistical analysis study. *Int J Antimicrob Agents*. 2012;40(6):516-520. doi:10.1016/j.ijantimicag.2012.07.019

108. Swick MC, Morgan-Linnell SK, Carlson KM *et al.* Expression of multidrug efflux pump genes *acrAB-tolC*, *mdfA*, and *norE* in *Escherichia coli* clinical isolates as a function of
fluoroquinolone and multidrug resistance. *Antimicrob Agents Chemother.* 2011;55(2):921-924. doi:10.1128/AAC.00996-10

109. Pesesky MW, Hussain T, Wallace M *et al.* Evaluation of machine learning and rules-based approaches for predicting antimicrobial resistance profiles in gram-negative bacilli from whole genome sequence data. *Front Microbiol.* 2016;7(NOV):1-17. doi:10.3389/fmicb.2016.01887
Figure 1. Schematic representation of four mechanisms of ciprofloxacin resistance in *E. coli*. A) Target alteration. B) Decreased ciprofloxacin accumulation. C) Physical blocking of ciprofloxacin target. D) Enzymatic modification of ciprofloxacin.
Figure 2. Flow chart adapted from the PRISMA guidelines (Moher 2009), showing the process of including articles starting from a systematic search of PubMed and Web of Science. *2 Studies contributed experimental and observational data, and were thus included for both types of articles.
Figure 3. Median fold change (interquartile range) in ciprofloxacin MIC for each resistance mechanism or combination of resistance mechanisms experimentally tested in 366 isolates. Fold changes were calculated by dividing the MIC after modification by the MIC before modification for each isolate. Data points represent single *E. coli* isolates. Darker fill of data points indicates the presence of multiple resistance mutations or resistance genes in the isolate. Isolates that showed a decreased ciprofloxacin MIC after modification (such as deletion of *acrAB* or *tolC*) are not shown but are listed in table S1. TA = target alteration (mutations in *gyrA*, *gyrB* or *parC*), EP = efflux pump (mutations in *acrB*, *marR*, *acrR*, *rpoB* or presence of *qepA* or *oqxAB*), PB = physical blocking (presence of *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrE* or *qnrS*), EM = enzymatic modification (presence of *aac(6')Ib-cr* or *crpP*).
Figure 4. Matrix displaying Pearson correlation coefficients calculated between resistance determinants in a pairwise manner. All 238 strains used for this analysis were screened for all displayed resistance determinants. The reported frequencies of resistance determinants in our dataset are displayed on the y-axis. Full data is provided in table S2.
Figure 5. Network of *E. coli* ciprofloxacin resistance-associated chromosomal genes. 31 genes that were examined for their influence on ciprofloxacin and were present in the *E. coli* K-12 MG1655 genome were mapped to the String-v10 PPI database. Genes were coloured green if a mutation conferring increased ciprofloxacin resistance was observed; genes were coloured red when a mutation decreased ciprofloxacin resistance; genes were coloured blue when a mutation showed no effect on ciprofloxacin resistance. The network is displayed by R package iGraph employing the force-directed layout algorithm by Fruchterman and Reingold. The list of edges with corresponding data categories (PI, FP or TM) is available as supplementary table 3.
Table 1. Ciprofloxacin resistance mechanisms in *Escherichia coli* and genes involved in these mechanisms. Note that in this overview, only genes are displayed that were shown to have any effect on ciprofloxacin susceptibility when mutations are present (chromosomal genes) or if the resistance gene is present (plasmid-encoded genes).

| Resistance mechanism                        | Chromosomal genes involved in ciprofloxacin resistance | Plasmid-encoded genes involved in ciprofloxacin resistance |
|--------------------------------------------|--------------------------------------------------------|----------------------------------------------------------|
| Target alteration                          | *gyrA*<sup>12</sup>, *gyrB*<sup>29</sup>, *parC*<sup>11</sup> | -                                                        |
| Decreased ciprofloxacin accumulation       | *marR*<sup>30</sup>, *acrRAB*<sup>31</sup>, *tolC*<sup>31</sup>, *soxS*<sup>32</sup>, *rpoB*<sup>33</sup> | *qepA*<sup>34</sup>, *qoxAB*<sup>35</sup>               |
| Physical blocking of ciprofloxacin target   | -                                                      | *qnrA*<sup>35</sup>, *qnrB*<sup>27</sup>, *qnrC*<sup>35</sup>, *qnrD*<sup>19</sup>, *qnrE*<sup>40</sup>, *qnrS*<sup>41</sup> |
| Enzymatic modification of ciprofloxacin     | -                                                      | *aac(6')-Ib-cr*<sup>42</sup>                             |
|                                            |                                                        | *crpP*<sup>43</sup>                                      |
**Table 2.** Medians and ranges of ciprofloxacin MIC fold changes stratified by resistance determinants. Only data from isolates harbouring resistance determinants from a single mechanism are shown.

| Resistance determinant | Median ciprofloxacin MIC fold change (range) | # of isolates | References |
|------------------------|---------------------------------------------|---------------|------------|
| Gly81Asp (*gyrA*)      | 2.6 (1-4.2)                                 | 2             | 49,56      |
| Asp82Gly (*gyrA*)      | 1                                           | 1             | 49         |
| Ser83Trp (*gyrA*)      | 6.3                                         | 1             | 10         |
| Ser83Leu (*gyrA*)      | 23.8 (4-133.3)                              | 9             | 11,49–51,53–55 |
| Asp87Asn (*gyrA*)      | 15.6 (7.5-15.6)                             | 3             | 51,54,55   |
| Gly81Asp, Asp82Gly (*gyrA*) | 2                                     | 1             | 49         |
| Ser83Leu, Asp87Asn (*gyrA*) | 23.8 (15-23.8)                             | 3             | 51,54,59   |
| Ser83Leu, Asp87Gly (*gyrA*) | 4266.7                                   | 1             | 77         |
| Asp426Asn (*gyrB*)     | 8                                           | 1             | 29         |
| Ser80Ile (*parC*)      | 1                                           | 1             | 51         |
| Ser83Trp (*gyrA*), Gly78Asp (*parC*) | 33.3                                  | 1             | 11         |
| Ser83Leu (*gyrA*), Ser80Ile (*parC*) | 62.55                                 | 1             | 51         |
| Ser83Leu (*gyrA*), Ser80Arg (*parC*) | 125                                             | 1             | 53         |
| Asp87Asn (*gyrA*), Ser80Ile (*parC*) | 23.8                                              | 1             | 51         |
| Ser83Leu, Asp87Asn (*gyrA*), Ser80Ile (*parC*) | 2000 (1066.7-2000)       | 3             | 11,51,54   |
| Ser83Leu, Asp87Gly (*gyrA*), Ser80Ile (*parC*) | 1024 (256-8533.3)                          | 3             | 11         |
| Mutations                                         | Frequency (%) | References       |
|--------------------------------------------------|---------------|------------------|
| Ser80Ile (parC)                                  |               |                  |
| Ser80Ile, Ser83Leu, Asp87Asn (gyrA)              | 2258.3 (250-4266.7) | 2, 11, 59        |
| Ser80Ile, D87Y (gyrA), Ser80Ile (parC)           | 256           | 11               |
| Ser80Ile, Ser83Leu, Asp87Asn (gyrA), Glu84Lys (parC) | 533.3         | 11               |
| Ser80Ile, Ser83Leu, Asp87Gly (gyrA), Glu84Lys (parC) | 4266.7        | 11               |
| Ser80Ile, Ser83Leu, Asp87Asn (gyrA), Ser80Ile, Glu84Gly (parC) | 1600 (1066.7-2133.3) | 11               |
| acrB: Gly228Asp                                   | 16.7          | 61               |
| ΔacrAB                                           | 0.1 (0-0.3)   | 10, 30, 31, 57   |
| ΔtolC                                            | 0.3           | 31               |
| marR (various mutations)                         | 3.5 (1.5-4)   | 14, 60           |
| ΔmarR                                            | 3.8 (2-218)   | 5, 30, 51, 54, 58,59 |
| acrR (various mutations)                         | 4 (2-16)      | 6, 78            |
| ΔacrR                                            | 2.9           | 51               |
| soxS: Ala12Ser                                   | 4             | 32               |
| rpoB (various mutations)                         | 3 (1.5-3)     | 3, 33            |
| oqxAB                                            | 7.5 (2-16)    | 17, 35, 62-64    |
| qepA                                             | 8.3 (1.9-64)  | 13, 34, 52, 65-68,79 |
| qepA, ΔmarR                                      | 15            | 1, 67            |
| qnrA (unspecified allele)                        | 31.3 (20.8-31.7) | 12, 60          |
| qnrA1                                            | 31 (4-66.7)   | 37, 39, 50, 52, 53, 81-89 |
| Gene        | Minimum (Maximum) | Frequency | Reference Numbers |
|------------|------------------|-----------|-------------------|
| qnrA3      | 31.3             | 1         | 81                |
| qnrB1      | 12.5 (4-62.5)    | 8         | 8, 52, 53, 85, 87 |
| qnrB2      | 15.6 (11.8-31.3) | 4         | 81, 90            |
| qnrB4      | 15.6 (15.6-15.6) | 3         | 91                |
| qnrB5      | 15.6 (15.6-15.6) | 2         | 72                |
| qnrB6      | 15.6             | 1         | 72                |
| qnrB19     | 11.9             | 1         | 92                |
| qnrC1      | 31.3 (15-62.5)   | 3         | 59, 38, 85        |
| qnrD1      | 15 (7.5-62.5)    | 3         | 59, 39, 85        |
| qnrE1      | 62.5             | 1         | 40                |
| qnrS (unspecified allele) | 12.3 (2-83.3) | 6         | 74, 76            |
| qnrS1      | 33.3 (4-125)     | 24        | 39, 50, 52, 55, 63, 79, 81, 82, 85, 87, 90, 92–94 |
| qnrS2      | 15               | 1         | 95                |
| aac(6')Ib-cr | 6.9 (1-62.5)    | 28        | 52, 42, 71, 73–76, 79, 94, 96 |
| crpP       | 7.5              | 1         | 43                |
Table 3. Median ciprofloxacin MICs for three resistance determinants that were reported at least five times in both experimental and observational data. The EUCAST epidemiological cut-off for ciprofloxacin resistance in *E. coli* is 0.064 mg/L.

| Resistance determinant(s) | Median and range of ciprofloxacin MIC in experimental data (mg/L) | Number of isolates in experimental data | Median and range of ciprofloxacin MIC in observational data (mg/L) | Number of isolates in observational data |
|---------------------------|---------------------------------------------------------------|----------------------------------------|---------------------------------------------------------------|-----------------------------------------|
| Ser83Leu (gyrA)           | 0.25 (0.06-0.38)                                              | 5                                      | 0.25 (0.125-64)                                               | 34                                      |
| *qnrS*1                   | 0.25 (0.032-1)                                                | 16                                     | 0.2 (0.1-4)                                                   | 19                                      |
| aac(6′)Ib-cr              | 0.06 (0.004-0.5)                                              | 22                                     | 0.25 (0.25-0.5)                                               | 5                                       |