Quinolone resistance in *Salmonella enterica serovar Typhi*: Mechanisms, factors driving the spread of resistance, current epidemiological trends and clinical significance

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Introduction

The human restricted bacteria, *Salmonella enterica serovar Typhi* is the major cause of typhoid fever (or enteric fever), a characteristic severe systemic illness [1]. In 2010, typhoid fever accounted for an estimated global burden of 27 million new cases and 200,000 deaths [2].

For over two decades, *S. enterica serovar Typhi* and other serovars have developed resistance to the first line antimicrobials (ampicillin, chloramphenicol, and co-trimoxazole). As a result of this multidrug resistance (MDR), quinolones became key antibiotics for treatment of *Salmonella Typhi* disease [3]. Quinolones are a group of antimicrobials with a 4-quinolone nucleus [4]. Quinolones target the bacterial enzymes DNA gyrase and topoisomerase IV, which are essential for DNA replication and transcription [4]. They are classified by differences in their in-vitro antimicrobial activity into:

- First-generation (nalidixic acid and cinoxacin),
- Second-generation (norfloxacin, ciprofloxacin, lomefloxacin, ofloxacin, and levofloxacin),
- Third-generation (sparfloxacin, gatifloxacin, and grepafloxacin), and
- Fourth-generation (trovafloxacin, moxifloxacin, and gemifloxacin)[5].

The second, third, and fourth-generation quinolones are also called fluoroquinolones, generated by addition of a fluorine atom and a cyclic diamine piperazine at C6 and C7 positions of the 4-quinolone nucleus respectively [4]. The second-generation quinolones have an expanded gram-negative and atypical coverage but limited gram-positive coverage, while the third-generation additionally have improved gram-positive coverage, and the fourth-generation have an additional anaerobic coverage [6].

Nalidixic acid was commonly used especially in developing countries but as a result of resistance and toxicity, ciprofloxacin became the most commonly used against *Salmonella Typhi*. However, in-vitro resistance to nalidixic acid in *Salmonella Typhi* also indicates resistance to ciprofloxacin [7].

Due to lack of availability and cost, other higher generation quinolones are not used in resource-limited countries. Ofloxacin, levofloxacin, moxifloxacin, and gatifloxacin are also used in the treatment of MDR tuberculosis; this could also be a reason for lack of their use in countries where tuberculosis is prevalent.

This review focuses on the mechanisms underlying susceptibility and resistances of quinolones in *Salmonella Typhi;* explaining the factors driving the spread of resistance, current epidemiological trends and clinical significance of the resistance.

Methodology

Articles for this review were identified by searches of PubMed, Web of Science, Science Direct, Scopus, Global Health Database, and the Liverpool School of Tropical Medicine electronic library 'DISCOVER' using the search terms "salmonella Typhi", "quinolones", and "resistance". Only articles published in English between 2002 and 2015 were used.

Mutations of gyrase and topoisomerase genes

The primary target of quinolones in gram-negative bacteria is the *gyr*A subunit of DNA gyrase, and point mutations usually occur within the quinolone resistance determining region (QRDR) of the DNA gyrase gene [4]. In *Salmonella Typhi,* single mutation of *gyr*A gene leads to resistance to nalidixic acid and reduced susceptibility to ciprofloxacin [minimum inhibitory concentration (MICs) of 0.125–0.25μg/mL], whereas complete resistance to ciprofloxacin (MIC > 4μg/mL) is caused by double mutation in the QRDR region [3]. A non-classical quinolone resistance in *Salmonella Typhi* exhibiting a *gyr*B gene mutation also exists [8]. In Gram-negative bacteria, point mutations in topoisomerase IV subunit genes *par*C and *par*E also occur, but are less common than *gyr*A mutations [4].

Efflux-based drug resistance

Bacteria have the ability to increase the expression of nonspecific energy-dependent efflux pumps that avoid the accumulation of effective intracellular concentrations of quinolones by actively pumping the drug across the cell membrane, thus leading to an efflux-based drug resistance [4]. Efflux-based drug resistance can also be caused by mutation in a drug transporter [9]. *Salmonella Typhi* expresses MDR transporters of the major facilitator super-family (MFS), which include STY4874 proton-dependent

efflux pump that transports several quinolones [9]. STY4874 pump is considered significant because it pumps quinolones out of the cells and is the most likely MDR transporter, as it confers resistance to quinolones, aminoglycosides and chloramphenicol. Efflux-mediated quinolone resistance becomes of clinical significance only when combined with other resistance mechanisms, such as mutations in target enzymes because they only produce low-level resistance [4].

Reduced outer membrane permeability

Gram-negative bacteria develop antimicrobial resistance (including quinolones) by reducing the levels of outer membrane porins that form the routes responsible for passive diffusion. This results in reduced outer membrane permeability to antimicrobials [4]. It has been shown that exposure of non-dividing cells to ciprofloxacin results in 'adaptive mutation' of the cells, thus resulting in decreased permeability of the bacterial outer membrane. This is considered to be a cause of gradual increase in mean MIC for ciprofloxacin in *Salmonella Typhi* [10].

Plasmid-mediated resistance

The plasmid-encoded *gnr* gene is a naturally occurring gene which can mediate reduced susceptibility to quinolones, but it can cause a higher level of resistance by potentiating the effect of other resistance mutations [4]. Plasmid gene *qnr*B and *qnr*S in *Salmonella Typhi* were shown to be responsible for plasmid-mediated quinolone resistance [11].

Biofilm

Biofilm does not affect drug resistance in *Salmonella Typhi* but results in delayed clearance from typhoid patients [12].

Factors contributing to the spread of resistance and epidemiological trends

The current global *Salmonella Typhi* resistance to quinolones emerged as a result of extensive quinolone use/misuse, including selective pressure from empirical use[13]. The use of substandard fluoroquinolones and their usage in animals also significantly contributed to the resistance [14, 15]. Three trends of antimicrobial resistance to *Salmonella Typhi* occurred as a result of ciprofloxacin use: (i) ongoing resistance to the first-line antimicrobials and low prevalence of quinolone-resistant *Salmonella Typhi,* (ii) equal prevalence of nalidixic acid-resistant *Salmonella Typhi,* and (iii) a slow increase of nalidixic acid-resistant Salmonella Typhi (NARST) and reduced prevalence of multidrug-resistant *Salmonella Typhi* (MDRST)[13]- see Figure 1.

Most of the resistant strains of *Salmonella Typhi* (especially the haplotype H58, a prevalent MDR clone that has spread over Asia and Africa) emerged from Southeast Asia and then spread to other regions of the world [13]. Travellers

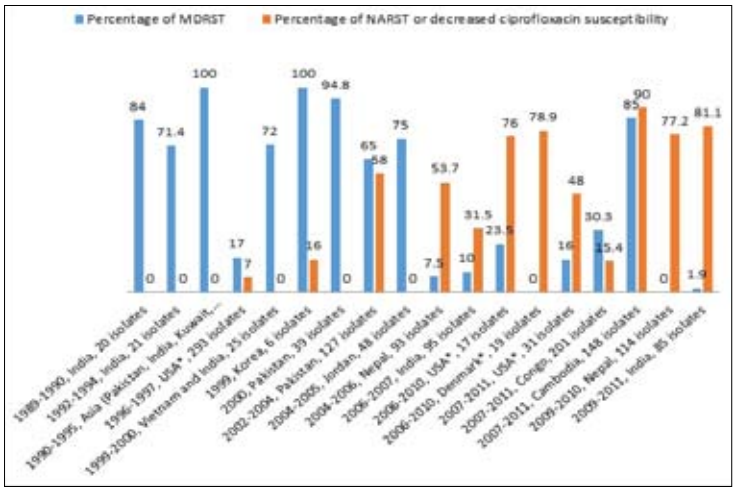

Figure 1. Emergence and global resistance trends of Salmonella Typhi to nalidixic acid and ciprofloxacin. Data from Tatavarthy et al. 2014. – see reference 13.
*Indicates foreign travel related.

also played a significant role in spreading the resistant *Salmonella Typhi,* especially to the developed world [16, 17]. The quinolone-resistant *Salmonella Typhi* is not only prevalent in hospital settings but also in the community [18].

Clinical significance and conclusion

Enteric fever caused by NARST isolates which also have reduced susceptibility to ciprofloxacin are associated with poor clinical outcomes including treatment failure [18]. These isolates are usually classified as being ciprofloxacin susceptible when using the previous susceptibility breakpoints for ciprofloxacin [18]. However in order to avoid such misclassifications, the clinical and laboratory standard institute (CLSI) approved a reduced susceptibility breakpoint for *Salmonella Typhi* (≤0.06 µg/mL) and also suggested that the nalidixic acid screen should be used to test for reduced quinolone susceptibility in *Salmonella Typhi,* although it may miss other quinolone resistant strains[19]. Therefore ciprofloxacin MIC is a significant determinant of clinical response to treatment and it should be considered in all *Salmonella Typhi* isolates [20].

Convalescent faecal carriage as a result of quinolone-resistant *Salmonella Typhi* plays a critical role in spreading the disease in the community [21]. This is difficult to control especially in countries where there is limited access to adequate sanitation and clean water facilities.

Although not available in many resource-limited settings, a community-based drug susceptibility data is critically needed to facilitate rational use of antimicrobials in general and improve antimicrobial choices made for enteric fever.

Third-generation cephalosporins and azithromycin remain the treatment of choice for typhoid fever in areas with quinolone resistance and for travellers returning from these areas [17]. There is some evidence supporting the effectiveness of newer fluoroquinolones (gatifloxacin) in areas where resistance to nalidixic acid and ciprofloxacin

exist [22]. However, cheaper oral quinolones such as ciprofloxacin can still be used where there is evidence of absence of *Salmonella Typhi* resistance. Prevention through access to clean and safe water, adequate sanitation, and education should be encouraged in all settings. A recent Cochrane review showed that both the licensed Ty21a and Vi polysaccharide vaccines and the unlicensed Vi-rEPA vaccine are efficacious [23]. However, their role-out on a large scale, especially in resource-limited settings where typhoid fever is endemic, would be a significant challenge, although travellers to those areas may benefit.

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