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Topic of the Thesis: **"Molecular mechanisms of resistance to fluoroquinolones in enteric fever pathogens."**

Abstract

Salmonella enterica serovar Typhi (*S.* Typhi) and *Salmonella enterica* serovar Paratyphi A (*S.*Paratyphi A) cause clinically indistinguishable systemic diseases, collectively called enteric fever. Enteric fever continues to be endemic in India and antibiotic treatment remains a central pillar of control. With the emergence of Multi-Drug Resistance (MDR), Fluoroquinolones (FQ) are the drug of choice for management. However, of late the efficacy of this antibiotic too has been questioned due to reports of increasing defervescence time and poor patient response. This indicates that the organism has begun to develop resistance to FQs, and is corroborated by a steady increase in Minimum Inhibitory Concentration (MIC) of ciprofloxacin. Resistance to FQ usually arises due to mutations within the quinolone resistance determining region (QRDR) of topoisomerase genes *gyr*A, *gyr*B, and *par*C or *par*E, decreased expression of outer membrane porins, or overexpression of efflux pumps and recently plasmid-mediated quinolone resistance (PMQR) genes *qnr* (A, B and S), aac(6')-Ib–cr and *qep*A. Acquisition of high-level resistance appears to be a multifactorial process. Most studies have focused on role of *gyr*A and the role of mutations in *gyr*B , *par*C, *par*E and PMQR genes in FQ resistance is sparse.

As *S.*Typhi and *S.*Paratyphi A are host adapted serovars, epidemiological studies are important to investigate the spread of FQ resistance isolates.

The present study was the first attempt to describe the antibiotic resistance and molecular mechanism of FQ resistance among *S.*Typhi and *S.*Paratyphi A. Mutations at QRDR region of topoisomerase genes (*gyr*A, *gyr*B, and *par*C, *par*E) were screened by high throughput technique using denaturing high-performance liquid chromatography (DHPLC). Role of PMQR {*qnr* (A, B and S), aac(6')-Ib–cr and *qep*A }was investigated using PCR and the contribution of permeability changes was investigated using cyclohexane tolerance and PAβN inhibitor to study the efflux pump and alteration of OMP was studied by SDS-PAGE.

Genetic diversity among FQ resistance was studied using MLVA-VNTR typing method using five VNTR loci (TR1,TR2, TR4699, Sal02 and Sal16) by capillary electrophoresis for *S*.Typhi and *S*.ParatyphiA collected over a six year period (2006-2011).

The study confirmed that *S*.Typhi was the predominant cause of enteric fever (84%). There was a distinct difference in antibiograms of the *S*.Typhi and *S*.ParatyphiA. MDR was observed only among *S*.Typhi during six year period. The main mechanism of FQ resistance was attributed to mutations in target genes (*gyr*A, *gyr*B and *par*C). Mutations in different target sites contributed to distinct phenotypes and had varied affect on MIC of ciprofloxacin (CIP) and nalidixic acid (NAL). In *gyr*A gene we observed S83F/Y, D87N/G in *S*.Typhi and S83F/Y, D87N in *S*.ParatyphiA sequence variant and was associated with classical quinolone resistance ($NAL^{R}CIP^{DCS}$) and these isolates were effectively screened by nalidixic acid disc test. In *gyr*B gene three sequence variants (S→F/Y/T) were detected at codon 464, and a novel mutation S→T was also detected among *S*.Typhi only. *gyr*B mutation was associated with non classical quinolone resistance ($NAL^{S}CIP^{DCS}$) and was distinct from classical quinolone resistance associated with *gyr*A mutations and also not detected by previously CLSI proposed NAL disc test. High level FQ resistance was associated with multiple mutations at *gyr*A and *par*C. Mutations in *par*C in combination with *gyr*A appear to contribute to slight increase in ciprofloxacin MIC and do not give any survival benefit to isolates. No mutation was observed in *par*E in both *S*.Typhi and *S*.ParatyphiA. Only one isolate of *S*.Typhi with high level ciprofloxacin resistance showed positive for PMQR gene *qnr*S1 found in combination with *gyr*A and *par*C mutations. Active efflux pump and OMP alteration did not contribute to FQ resistance in *S*.Typhi and *S*.ParatyphiA. DHPLC was found as high throughput screening method to highly reliable, economic, less labour intensive and reduction of samples for final sequencing for screening mutations in topoisomerase genes.

MLVA-VNTR typing confirmed that FQ resistance has emerged in diverse background strains. 73 VNTR profile among *S*.Typhi showed heterogeneous clone dispersion and 11 VNTR profile in *S*.ParatyphiA showed homogeneous clone dispersion in the environment.

This study concluded that a new mechanism of FQ resistance due to mutation in *gyr*B can pose diagnostic challenge in clinical laboratories and was reported first time in India. There is an urgent need to develop rapid disc test for screening isolates with decreased ciprofloxacin susceptibility (DCS). The MLVA technique was found to be discriminatory and reproducible, relatively fast, easier to perform, inexpensive. In future this method of subtyping can be used for outbreak investigation.