Molecular Epidemiology of Quinolone Resistant *Salmonella* Typhi and *Salmonella* Paratyphi A from India

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Background: *S.* Typhi and *S.* Paratyphi A are human adapted serovars, and emergence of antibiotic resistance is directly related to antibiotic use in humans. It is thus important to study whether this resistance to fluoroquinolone is emerging during treatment in different hosts (mutations occurring in different bacterial strains) or clonal expansion of a successful strain by person-to-person spread (identical mutations associated with a single strain). Typing is an important tool for surveillance and monitoring spread of resistance strains. In this study, we used Multi-locus variable number tandem repeats (MLVA-VNTR) typing method to study genetic diversity and epidemiology in the *S.* Typhi and *S.* Paratyphi A.

Methods: A total of 664 isolates of *S.* Typhi and *S.* Paratyphi were isolated from 2006-2011. Isolates were identified by standard biochemical tests and serotyped using specific antisera. The antimicrobial susceptibility was performed by disk diffusion method according to the CLSI (2012). MIC (minimum inhibitory concentration) for nalidixic acid and ciprofloxacin were determined by E-Test (AB Biodisk, Solna, Sweden). Multidrug resistance (MDR) was defined as simultaneous resistance to ampicillin, chloramphenicol and co-trimoxazole. 104 isolates of *S.* Typhi (80 isolates) and *S.* Paratyphi A (24 isolates) were studied. Isolates were selected on the basis of different MIC, fluoroquinolone resistance patterns, mutation in topoisomerase genes (*gyr*A,*gyr*B, *par*C and *par*E) and plasmid-mediated resistance gene (*qnr*A,B,S genes, *aac*(6')-*Ib*-cr and *qep*A). CT18 and Ty2 for *S.* Typhi and ATCC9150 for *S.* Paratyphi A were used as a reference isolates. Five VNTRs marker TR1, TR2, TR4699, Sal02 and Sal16 were used to establish the genetic diversity among fluoroquinolone resistant *S.* Typhi and *S.* Paratyphi A. MLVA typing was done by fluorescent PCR amplification of each VNTR locus separately. The fragment length analysis was done by capillary electrophoresis an automated ABI3130 Genetic analyzer (Applied Biosystems) and data analysed using GeneMapper (version 4.0) software (Applied Biosystems). The copy number was done by sequencing. A cluster dendrogram was constructed by the R-software (R version 2.15.2 (2012-10-26), to represent the genetic relationships of the MLVA profile on the basis of copy number. The diversity index (DI) was generated using two methods: Simpson's diversity and Hunter-Gaston diversity.

Results: Total 80 *S.* Typhi isolates differentiated into 73 MLVA profiles and 24 *S.* Paratyphi A isolates differentiated into 11 different MLVA profile with reference isolates. Our data showed that TR2, Sal02, TR4699 and Sal02 marker have more allelic distribution for *S.* Typhi and *S.* Paratyphi A, respectively. The discriminatory power was 0.999 for both *Salmonella.* Phylogenetic analysis

showed that the MLVA profiles can be divided into five and four clusters for *S.* Typhi and *S.* Paratyphi A, respectively.

Conclusions: Epidemiological studies of pathogens are of great importance in controlling their dissemination. The cluster analysis findings supported heterogeneous clone dispersion for *S.* Typhi i.e. all resistance phenotypes were distributed simultaneously in the environment while *S.* Paratyphi A isolates were homogeneous clone with limited diversity i.e. all phenotype are clonally spreading in the community. To our knowledge this is the first report of MLVA subtyping applied to *S.* Typhi and *S.* Paratyphi A from India. Rapid and high-level discriminatory power of MLVA may be useful for tracking and controlling the transmission of *S.* Typhi isolates during epidemiological investigations.