Studies on Antimicrobial Resistance and Molecular Subtyping of *Salmonella* Typhi Isolates from Kolkata During 2014-2015

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Background: Typhoid fever is an acute, invasive and potentially fatal systemic infection caused by *Salmonella enterica* subspecies *enterica* serotype Typhi (*S*. Typhi). Antimicrobial therapy is the main mode of treatment but drug resistance to antimicrobials has become a problem in developing countries like India. Molecular subtyping is essential for discriminating *Salmonella enterica* serovar Typhi (*S*. Typhi) isolates leading to improved molecular epidemiological analysis for prevention and control of typhoid fever. Pulsed field gel electrophoresis (PFGE) is considered the gold standard for *Salmonella* molecular typing, while sequence-based multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA) provides high-level discrimination.

Methods: A total of 176 *S*. Typhi isolates were collected from clinically suspected enteric fever patients attending various hospitals in Kolkata, India, from January 2014 to December 2015 and were tested for antimicrobial resistance following standard protocol. To assess genetic diversity, 50 representative strains of different resistance profile were analyzed by PFGE and MLVA.

Results: A majority of the isolates were resistant to nalidixic acid (97.7%) followed by ciprofloxacin (29.5%). Only 3.4% MDR (resistance to ampicillin, chloramphenicol, cotrimoxazole) isolates and 1.1% of tetracycline and cotrimoxazole resistant isolates were found during this period. A single non conjugative plasmid of 180 kb was found in 83.3% (5/6) of MDR *S*. Typhi and one 50kb plasmid was found in tetracycline and cotrimoxazole resistant *S*. Typhi. Various AMR markers (blaTEM-1, *cat*A, *sul*1, *sul*2, *dfrA*15, *str*A-*str*B) and class 1 integron with *dfr*A7 gene were detected in MDR *S*. Typhi isolates by PCR and sequencing. PFGE subtyping divided the isolates into three clusters including 16 pulsotypes whereas MLVA subtyping divided the isolates into four clusters including 48 MLVA types.

Conclusions: The results of the present study suggests MLVA provides high level discrimination among clonal MDR, NALR and NALR-CIPR *S.* Typhi isolates in PFGE. The study reiterated the importance of continuous monitoring of AMR and molecular subtypes of Salmonella isolates from endemic regions for better understanding of the disease epidemiology.