

## Capsular Vi Polysaccharide Antigen in *Salmonella enterica* Serovar Typhi Isolates

Characterization of *Salmonella enterica* Serovar Typhi isolates for their Vi capsular polysaccharide antigen-related genetic sequences is desirable to define the role of isolates lacking such sequences in differential replication at unusual sites, including those coinfecting with human immunodeficiency virus (HIV). Infection with Vi-negative strains might produce typhoid fever among those immunized with various Vi vaccines.

*S. enterica* serovar Typhi isolates lacking Vi capsular polysaccharide antigen, evident during slide agglutination with Vi typing antisera, have been known for several decades. High molarity turns off expression of Vi antigen with a false label of Vi-negative *S. enterica* serovar Typhi. Genotypic sequences linked with an expression of *S. enterica* serovar Typhi Vi capsular polysaccharide antigen are located at three widely separated chromosomal loci, *viaA*, *viaB*, and *ompB*. The *ViaB* locus, an operon that contains two genes that encode enzymes for the biogenesis and translocation of Vi, is present in all Vi-positive strains. Enzymes encoded by genes *tviB*, *tviC*, *tviD*, and *tviE* catalyze intracellular Vi synthesis. The translocation and anchoring of Vi to bacterial surface is through export genes, *vexA*, *vexB*, *vexC*, *vexD*, and *vexE*. Two other chromosomal regions, the *ompB* operon and *viaA*, which is allelic to *resB-resC*, have a role in expression of Vi capsular polysaccharide antigen in high molarity and other environmental signals (9). Recently, even *S. enterica* serovar Typhi isolates that lacked *ViaB* sequences were responsible for an epidemic of multidrug-resistant typhoid fever in Calcutta, India (10).

*S. enterica* serovar Typhi strains devoid of capsular Vi antigen are frequently found at the National Salmonella Phage Typing Centre in the Department of Microbiology at the Lady Hardinge Medical College, New Delhi, India. During the year 2000, the facility received 1,289 isolates of *Salmonella enterica* serovar Typhi from different collaborating centers in India and Nepal. Upon receipt, each individual isolate was cultured in nutrient broth for a subculture on MacConkey agar. Biochemical, serological, and antibiotic sensitivity profiles were worked out. The presence or otherwise of Vi antigen was confirmed at room temperature by slide agglutination employing Vi typing antiserum (Murex Biotech Ltd., Dartford, England). The antibiotic sensitivity was carried out by disk diffusion method.

There were 113 Vi-negative *Salmonella enterica* serovar Typhi isolates drawn, including 88 isolates from blood, 3 isolates from bone marrow, and 1 isolate from a stool specimen. Such Vi-negative *Salmonella enterica* serovar Typhi were drawn from the patients presenting at the collaborating centers located in the northern, central, eastern, and southern parts of India and Nepal. Among the 113 isolates, 34 were resistant to ampicillin, tetracycline, chloramphenicol, and cotrimoxazole. None of the *Salmonella enterica* serovar Typhi isolates was resistant to ciprofloxacin or cephalexin. There was no information regarding the HIV serological status of any of the patients.

Vi-negative *Salmonella enterica* serovar Typhi isolates, though less infective than Vi-positive isolates, can cause a disease indistinguishable from the one caused by Vi-positive isolates (3). They could replicate selectively at abnormal sites in immunocompromised individuals. *S. enterica* serovar Typhi and HIV coinfection is frequent (8). Vi-negative isolates are frequently associated with glomerulonephritis, hepatic dysfunction (5), common iliac artery occlusion, and perforation of

terminal ileum and appendix (1). *Salmonella enterica* serovar Typhi even caused thyroiditis in a 62-year-old woman (4), multiple brain abscesses in a 2-month-old girl (2), and gluteal hematoma (7).

Vi-negative *S. enterica* serovar Typhi, on its entry to an intracellular niche, is protected from Vi antibody (3). Any prophylactic vaccine that does not elicit cellular immunity would be unlikely to confer protection against an intracellular replication of Vi-negative *S. enterica* serovar Typhi. That might have occurred during the trial with conjugated polysaccharide *S. enterica* serovar Typhi vaccine bound to nontoxic recombinant *Pseudomonas aeruginosa* endotoxin A (rEPA) in the Dong Thap Province in Vietnam. There were four isolations of *S. enterica* serovar Typhi from vaccinated children (6). Vi characterization, both phenotypic and genotypic, of *S. enterica* serovar Typhi isolates in prospective trials with any typhoid vaccine should be mandatory. That would reveal the role of Vi-negative *S. enterica* serovar Typhi in rare and unexpected failures with the vaccines presently in use, typhoid prophylactic vaccines.

The pathogenicity of typhoid fever is being evaluated intensively in Vietnam by Wain et al. (11). Among the 120 Vietnamese patients with enteric fever, there was a 30-fold higher concentration of *S. enterica* serovar Typhi in bone marrow. Furthermore, bacterial counts were elevated further with an increasing duration of illness. There were no details about a presence or absence of Vi antigen in the Vietnamese *S. enterica* serovar Typhi isolates. Such an investigation, retrospective and prospective, on *S. enterica* serovar Typhi isolates in Vietnam and elsewhere would be essential to evaluate any intracellular accumulation and dissemination of Vi-negative *S. enterica* serovar Typhi isolates. A deficient cellular immunity in the host could encourage even prolonged persistence of *S. enterica* serovar Typhi at aberrant locations.

To conclude, fiscal input for a microbiological surveillance for genotypic characterization of *S. enterica* serovar Typhi would be cost-effective. Apart from gaining fundamental information regarding pathogenesis of typhoid fever, with such support it would be possible to assay the efficacy of the newer conjugated typhoid vaccines. Moreover, Vi genotypic characterization of *S. enterica* serovar Typhi would be an asset to reference centers associated with any in-depth investigations performed with *Salmonella enterica* serovar Typhi.

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