Salmonellosis

Salmonella are Gram-negative rod-shaped bacteria. Salmonella serovar Typhi, which infects only humans, is generally considered separately from non-typhoidal Salmonella, which have multiple reservoirs. In this issue of EpiTREND, we focus on the various laboratory methodologies that are currently performed and new testing methods utilizing advanced molecular technology that are used to characterize Salmonella.

The Organism

The genus Salmonella is separated into two species, Salmonella enterica and Salmonella bongori. The species S. enterica is further classified into six subspecies, of which the subspecies S. enterica subsp. enterica contains almost all the serotypes (such as Typhimurium) pathogenic for humans; the organism commonly referred to as Salmonella Typhimurium is formally called Salmonella enterica enterica Typhimurium.

Salmonellosis is typically characterized by the acute onset of fever, diarrhea, nausea and abdominal pain, with or without vomiting. While illness is usually mild, resolving after several days in immunocompetent individuals, infection with Salmonella may be severe in the very young, elderly, or those with chronic illnesses or untreated HIV infection. Bloodstream or organ infections can occur.

Epidemiology of Salmonellosis

Salmonella are among the top four pathogens associated with foodborne illness, with a relatively stable incidence over time. Over 1.2 million cases of salmonellosis are estimated to occur each year in the United States, associated with more than 23,000 hospitalizations and 450 deaths. In Washington, 600-800 cases are reported annually (around 10 to 12 cases per 100,000 population), and the incidence of reported salmonellosis has remained relatively stable during the past two decades. Salmonellosis is most frequently diagnosed in the late summer and early fall (July-October), with highest reported incidence among children <5 years of age.
About 95% of exposures for salmonellosis are food contaminated by common reservoirs such as birds, rodents, and cattle; other risk exposures are live animals including poultry, reptiles, frogs, and hedgehogs. In the United States from 2011-2012, Centers for Disease Control and Prevention (CDC) reported multiple sources of exposure for Salmonella outbreaks including ground beef, turkey burgers, chicken livers, fish, cantaloupe, mangoes, papayas, pine nuts, alfalfa sprouts, dry dog food, and peanut butter. CDC’s PulseNet system has improved recognition of such outbreaks through national surveillance.

Laboratory Methodologies

Multiple laboratory methodologies provide varying phenotypic and genetic identification of strains for epidemiological surveillance and investigation of Salmonella cases.

I. Serotyping. Established in 1929, serotyping is based on determining the biochemical composition of bacterial cell surface. The genus Salmonella is comprised of over 2,500 serotypes (or serovars), typically named for the geographic location of first identification. Serotyping is the “first-generation” subtyping method and PulseNet’s first tier methodology. Performed routinely at the Washington State Public Health Laboratories (PHL), serotyping is an essential component of epidemiological surveillance and outbreak investigation. Two serotyping methods – traditional and molecular – are employed.

Traditional serotyping is based on the interaction between the isolate’s cell surface and antiserum containing antibodies that recognize specific serotypes. While the antisera approach has been the standard method, it is labor intensive (requiring several days), subject to the quality of antisera, and limited to phenotypically determinable isolates.

Molecular serotyping is based on the makeup of genes that determine the composition of the isolate’s cell surface. Fully implemented at PHL since 2012, this approach employs a DNA sequence-specific amplification process called polymerase chain reaction (PCR). The makeup of the target genes is identified by mixing the products from PCR with tiny beads that bind only to specific DNA products. This PCR-based assay is rapid, cost-effective, high-throughput, and able
identify isolates that are difficult to distinguish with traditional biochemical approaches. The drawback is that only the top 100 *Salmonella* serotypes are currently detectable, and conventional serotyping must be used for the less common serotypes.

**II. Molecular subtyping** Pulsed-field gel electrophoresis and multiple locus variable-number tandem repeat analysis are the two main subtyping (or “fingerprinting”) tools used for PulseNet.

**Pulsed-field gel electrophoresis (PFGE)** is the current gold standard for PulseNet. PFGE distinguishes strains based on fragments produced when enzymes cut specific DNA sequences (restriction sites). PFGE has been applied widely for the past two decades and at PHL is routinely conducted on all isolates of *Salmonella*. PFGE analysis provides rapid detection of outbreak-related strains; allows longitudinal and temporal tracking of epidemiologically relevant strains; can be applied to a wide variety of bacteria; and is a stable and reproducible method. Some drawbacks of PFGE are the time and training requirements; failure to distinguish among all unrelated isolates; lack of information at the DNA sequence level beyond the presence of certain restriction sites; and inability to type some strains due to their inherent genetic makeup.

**Multiple locus variable-number tandem repeat analysis (MLVA)** is a newer method based on detecting naturally occurring repeat sequences throughout the genome. Since the copy number of tandem repeat DNA sequences can vary greatly among different bacterial strains, this variation can be used for more in-depth discrimination of strains. MLVA employs the PCR technique and
determines the size of the amplified DNA products using high resolution capillary electrophoresis. MLVA analysis can detect variation in copy number of short DNA sequences and thus provides more power in differentiating bacterial strains that may be indistinguishable by PFGE patterns. Moreover, because MLVA is PCR-based, the technique can be used by many laboratories where other microbiological tools may not be readily available.

**III. Next Generation Sequencing (NGS).** Target genes or an entire genome can be sequenced.

Multiple locus sequence testing (MLST) functions at the DNA sequence level. Similar to MLVA, MLST uses PCR to amplify targeted regions or genes of the bacterial genome, typically seven housekeeping genes involved in basic cell maintenance. In MLVA, the PCR products are sequenced to provide information at the nucleotide sequence level, including differences at a single nucleotide site (called a single nucleotide polymorphism, SNP). The main advantage of MLST is that sequence data provide highly discriminating, precise, and unambiguous characterization of bacterial isolates.

Whole genome sequencing goes beyond the scope of the above phenotypic and molecular methodologies by providing DNA sequence information of the entire bacterial (or viral) genome. It is currently the most powerful DNA analysis method as a single test yields information about the species, serotype, subtype, virulence, phylogenetic relationships, and if applicable genetic profile of antimicrobial resistance.

In addition to the advantage of providing unmatched in-depth information, next generation sequencing methods have the potential to be automated with more rapid results; however, the costs are 10–30 fold more than PFGE and the methods require advanced instruments and computational platforms, as well as expertise in molecular biological skills and bioinformatics.

Advances in molecular and genomic technological tools have provided public health agencies with accurate and rapid information for epidemiological surveillance and investigation. While PFGE continues to be the gold standard and primary subtyping technique for investigating salmonellosis outbreaks, newer molecular and sequence-level approaches are likely to become more widely used and accepted in the near future.

**Resources**


CDC Salmonella page: http://www.cdc.gov/salmonella/index.html

CDC PulseNet page: http://www.cdc.gov/pulsenet/

**For Advanced Reading**

Tsolis R et al. “From bench to bedside: stealth of enteroinvasive pathogens” Nature Reviews Microbiology (Dec 2008)


APHL “Pulsenet on the Front Lines of Foodborne Disease Surveillance” (April 2013).