Salmonella Serotyping

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CDC

10th Annual PulseNet Update Meeting
April 5, 2006
What is *Salmonella* serotyping?

* The “first-generation” subtyping method
  - Established in 1929
  - Now the “first tier” subtyping for PulseNet

* Phenotypic characterization of strains based on the immunologic reactivity of two surface structures:
  - Lipopolysaccharide (O antigen)
  - Flagellin protein (H antigen)

* In *Salmonella*, includes species and subspecies identification
  - Isolates of different subspecies can have the same O and H antigens
Schematic Representation of *Salmonella* Serotype Antigens

Salmonella Typhimurium 4,5,12:i:1,2

O antigen → LPS

Flagella

H antigen

Salmonella Typhimurium 4,5,12:i:1,2

O antigen

LPS

Flagella

H antigen
Why is serotyping important?

- Basis for the US National *Salmonella* Surveillance System
  - About 35,000 isolates serotyped per year by SHDs
  - 50 years worth of data based on serotype

- Useful for epidemiologic classification of strains and for outbreak investigations
  - Strains of the same serotype, especially a rarer one, may be related
  - Serotype can correlates with disease or epidemiology
    - S. Typhi, other invasive serotypes
    - Subspecies IIIb serotypes common in reptiles

- An international “language”
**Salmonella taxonomy**

* Two species of *Salmonella*
  - *Salmonella enterica*
    - Its official!
    - Judicial Commission, Opinion 80. IJSEM 2005
  - *Salmonella bongori* (formerly subspecies V)

* *S. enterica* further divided into 7 subspecies
  - Approximately 99% of human isolates are subspecies I
  - Subspecies IV, IIIb, II, IIIa, VI (order of frequency in human isolates)
  - Subspecies VII recognized but not used for the purpose of serotype designation

* Species/subspecies typically determined by biochemical testing
**Salmonella enterica subspecies**

- Subspecies designated by taxonomic name or, more commonly for serotype designation, Roman numeral

<table>
<thead>
<tr>
<th>Roman Numeral</th>
<th>Subspecies Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td><em>S. enterica</em> subspecies <em>enterica</em></td>
</tr>
<tr>
<td>II</td>
<td><em>S. enterica</em> subspecies <em>salmonae</em></td>
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<tr>
<td>IIIa</td>
<td><em>S. enterica</em> subspecies <em>arizonae</em></td>
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<tr>
<td>IIIb</td>
<td><em>S. enterica</em> subspecies <em>diarizonae</em></td>
</tr>
<tr>
<td>IV</td>
<td><em>S. enterica</em> subspecies <em>houtenae</em></td>
</tr>
<tr>
<td>VI</td>
<td><em>S. enterica</em> subspecies <em>indica</em></td>
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</tbody>
</table>

- Subspecies IIIa and IIIb originally described as the genus *Arizonae*
  - Still identified as “Arizona” by some automated ID systems
Differentiating *Salmonella* subspecies

**Salmonella O antigen**

* Outermost portion of lipopolysaccharide (LPS)

* Carbohydrate antigen

* Different sugars and different linkages between sugars produce the different antigens
Salmonella O Antigens

- Two types
  - O Group antigens
  - “Ancillary” O antigens

- O Group antigens
  - Most important for determining serotype
  - *rfb* region contains genes responsible for O group
  - Found in all *Enterobacteriaceae*

- Ancillary O antigens
  - Typically encoded by extra-chromosomal elements (bacteriophages, plasmids)
  - Found in specific O groups
  - Most can vary within a given serotype, so are less important for serotype determination
Salmonella O Serogroups

- 46 O serogroups
- O groups initially designated by capital letters
  - Ran out of letters … started using numbers
  - Now, all O Groups are designated by numbers
  - Letter designations still commonly used

<table>
<thead>
<tr>
<th>O Group (number designation)</th>
<th>O Group (letter designation)</th>
<th>Typical O antigens</th>
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<tr>
<td>2</td>
<td>A</td>
<td>2,12</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>4,12</td>
</tr>
<tr>
<td>7</td>
<td>C1</td>
<td>6,7</td>
</tr>
<tr>
<td>8</td>
<td>C2</td>
<td>6,8</td>
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<tr>
<td>9</td>
<td>D1</td>
<td>9,12</td>
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<td>3,10</td>
<td>E1</td>
<td>3,10</td>
</tr>
<tr>
<td>13</td>
<td>G</td>
<td>13</td>
</tr>
</tbody>
</table>

These O groups represent about 97% of human isolates
**Distribution of Salmonella O Groups**

Subspecies determination is critical for serotype identification, particularly for “higher” O groups.

* Dendrogram taken from Whittam and Bumbaugh, Curr Opin Gen Dev 12:719-725 (2002)
Salmonella H antigen

- Flagellin, the flagellar filament
  - A protein antigen
  - Variation in the middle surface-exposed portion of the protein

- *Salmonella* is unique in having 2 different H antigens:
  - Phase 1/Phase 2
    - Phase 1 has a homolog in other enterics
    - Phase 2 gene is in a *Salmonella*-specific region of the genome
  - The 2 flagellins are coordinately expressed—one is off when other is on
**H Antigens Designations**

- 119 H antigens (Phase 1 & Phase 2)
  - Typically designated by lower case letters
    - a; b; c; d; e,h; e,n,x; etc
    - 1,2; 1,5; 1,7; *et al* are the notable exceptions
  - Ran out of letters ... started using numbered z’s
    - Z₄, Z₆, Z₁₀, Z₁₅, ... Z₈₉
    - Typically, *no* antigenic relationships between “z” antigens

- Some H antigens are antigenically related
  - Related antigens referred to as “complexes”
  - Typically, have one antigen in common plus secondary antigens
    - 1 complex: 1,2; 1,5; 1,6; 1,7, etc.
    - G complex: g,m; g,m,s; f,g,t; f,g,s; etc.
Designation of *Salmonella* Serotypes

- Designated according to the conventions of the Kauffmann-White Scheme
  - 2,541 serotypes in 2002
  - 10-20 new recognized serotypes each year
    - Confirmed at CDC and IP
    - Subspecies I serotypes: submitting lab gets to name the serotype

- Kauffmann-White Scheme maintained by Institut Pasteur
    - Published every five years
    - Updated annually (last updated 2002 …)
  - .pdf and MS Access versions available from the CDC
Subspecies I serotypes are designated by a name and a formula

*Salmonella* Typhimurium

“Group O:4” or “Group B”

<table>
<thead>
<tr>
<th>Subspecies</th>
<th>O antigen</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4, [5], 12</td>
<td>i : 1,2</td>
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</table>

“[5]” means O antigen 5 may or may not be present.

<table>
<thead>
<tr>
<th>Subspecies</th>
<th>O antigen</th>
<th>Phase 1</th>
<th>Phase 2</th>
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<tr>
<td>I</td>
<td>4,5,12:i:1,2</td>
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<tr>
<td>I</td>
<td>4,12:i:1,2</td>
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</tbody>
</table>

*Salmonella* Typhimurium var. O 5 -
or
*Salmonella* Typhimurium var. Copenhagen
Subspecies II through VI serotypes are designated by formula only

“Group O:48” or “Group Y”

IV 48 : g,z51 : - *

Subspecies O Phase 1 “monophasic” antigen

* Salmonella IV 48:g,z51:- was formerly known as Salmonella Marina.
Examples of Serotype Designations

- *Salmonella enterica* subspecies *enterica*
  - serotype Typhimurium
  - *Salmonella enterica* serotype Typhimurium
  - *Salmonella* ser. Typhimurium
  - *Salmonella* Typhimurium

- *Salmonella enterica* subspecies *enterica*
  - serotype Typhi
  - *Salmonella enterica* serotype Typhi
  - *Salmonella* ser. Typhi
  - *Salmonella* Typhi

- *Salmonella enterica* subspecies *houtenae*
  - serotype 48:g,z51:-
  - *Salmonella enterica* serotype IV 48:g,z51:-
  - *Salmonella* IV ser. 48:g,z51:-
  - *Salmonella* IV 48:g,z51:-
Monophasic Serotypes and Monophasic Variants

**Monophasic**: the state of having or expressing only one flagellar antigen when two flagellar antigens might be expected

**Monophasic variant**: variants of serotypes that are typically expected to have two flagellar antigens

* Some serotypes are “naturally” monophasic
  - No second phase flagellar antigen
    - Specific subspecies I serotypes: S. Typhi, S. Enteritidis, S. Berta, others
    - Most subspecies IIIa and IV serotypes

* Monophasic variants lack either of the two flagellar antigens
  - I 4,5,12:i:- (likely variant of S. Typhimurium)
  - I 9,12:l,z28:- (likely variant of S. Javiana)
  - I 4,5,12:-:1,2 (could be a variant of Typhimurium, Heidelberg, Saintpaul, Paratyphi B, …)
Serotype Variants: Unable to detect all serotype antigens

* Subspecies I: unable to give a “name” when all antigens not detected, but can still identify by a formula

* Monophasic variants
  - $\textit{Salmonella I 4,5,12:i:-}$
  - $\textit{Salmonella I 4,12:i:-}$
  - $\textit{Salmonella I 4,5,12:b:-}$

* Nonmotile variants
  - $\textit{I 4,5,12:nonmotile}$ ($\textit{Salmonella I 4,5,12:-:-}$)

* Rough strains (no longer express O antigen)
  - $\textit{Salmonella I Rough:i:1,2}$
  - $\textit{Salmonella I Rough:nonmotile}$

* Mucoid strains (capsule blocks O antigen detection)
  - $\textit{Salmonella I O Mucoid:i:1,2}$

All of these strains are fully serotyped
PulseNet: Keeping the serotyping lab on its toes

PFGE pattern can be indicative of serotype

PulseNet: Keeping the serotyping lab on its toes

PFGE-XbaI

Salmonella Enteritidis
l 9,12:g,m:-

Salmonella Berta
l 9,12:[f],g,[t]:-
**PulseNet: Keeping the serotyping lab on its toes**

* PFGE pattern can be indicative of serotype

<table>
<thead>
<tr>
<th>Serotype</th>
<th># isolates 1999-2003</th>
<th>Ssp</th>
<th>O Group</th>
<th>Phase 1</th>
<th>Phase 2</th>
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<td>Lomalinda</td>
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<td>9</td>
<td>a</td>
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<td>Oranienburg</td>
<td>2922</td>
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<td>7</td>
<td>m,t</td>
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<tr>
<td>Othmarschen</td>
<td>95</td>
<td>I</td>
<td>7</td>
<td>g,m,t</td>
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</table>

* But not always ....

<table>
<thead>
<tr>
<th>Serotype</th>
<th># isolates 1999-2003</th>
<th>Ssp</th>
<th>O Group</th>
<th>Phase 1</th>
<th>Phase 2</th>
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<td>Sandiego</td>
<td>632</td>
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<td>4</td>
<td>e,h</td>
<td>e,n,z15</td>
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</table>
PulseNet: Helping solve old questions

Salmonella Paratyphi B

vs

Salmonella Paratyphi B var. L (+) tartrate+ (aka var. Java)

- Paratyphi B is invasive, typhoidal
- Paratyphi B var. L (+) tartrate + is less invasive, GI pathogen
- To date, tartrate+ vs tartrate- phenotype has been the only way to differentiate
- Likely that these two variants are not very accurately tracked in the national surveillance database
<table>
<thead>
<tr>
<th>Serotype</th>
<th>Tartrate Fermentation</th>
<th>Tartrate PCR</th>
<th>sopE PCR</th>
<th>avrA PCR</th>
<th>Isolate</th>
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<td>-</td>
<td>-</td>
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</tbody>
</table>

Disclaimer: PFGE done by *Salmonella* reference lab and NOT PulseNet!
The Future: NextGen Serotyping Methods

- Serotyping is easy
  - Preparing serotyping reagents in hard
    - Hundreds of antisera required
    - Typically use rabbit antisera—multiple absorptions to get desired specificity

- Solution: Use DNA-based methods to ID serotype
  - Base on genes responsible for serotype to make it compatible with serotypes determined by traditional methods

- To date, have probes for about 20 O groups and 25 H antigens
  - Currently adapting to the Luminex platform
The National *Salmonella* Reference Lab

* Linda Gheesling
* Lonnie Bryant
* Sarah Duda
* Matt Mikoleit
* Collette Fitzgerald
* John McQuistion
* Marcus Collins