

Salmonella

Annual Summary

2003



Department of Health and Human Services
Centers for Disease Control and Prevention
National Center for Infectious Diseases
Division of Bacterial and Mycotic Diseases
Foodborne and Diarrheal Diseases Branch
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Recommended Reference Citation:

CDC. *Salmonella* Surveillance: Annual Summary, 2003. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2004.

Single copies of *Salmonella* Surveillance: Annual Summary 2003 are available from:

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National *Salmonella* Surveillance System Annual Summary, 2003

This issue of the Annual Summary of the National *Salmonella* Surveillance System contains surveillance data on reported laboratory-confirmed *Salmonella* isolates in the United States for the year 2003. The National *Salmonella* Surveillance System collects reports of isolates of *Salmonella* from human sources from the United States. This information is reported through the Public Health Laboratory Information System (PHLIS), an electronic reporting system, by the State Public Health Laboratory Directors and State and Territorial Epidemiologists to the Foodborne and Diarrheal Diseases Branch (FDDB) and the Biostatistics and Information Management Branch (BIMB) of the Division of Bacterial and Mycotic Diseases in the National Center for Infectious Diseases.

The National *Salmonella* Surveillance System is based on data collected by state and territorial public health laboratories. *Salmonella* isolates are submitted to the state public health laboratory by clinical diagnostic laboratories. The state and territorial laboratories confirm the isolates as *Salmonella*, perform serotyping according to the Kauffmann-White scheme, and submit the data for reporting through PHLIS. Unusual or difficult isolates are forwarded to the National *Salmonella* Reference Laboratory at the Centers for Disease Control and Prevention for further characterization or confirmation. These results are reported back to the state laboratory, where they are reported through PHLIS.

The capture of isolates in the National *Salmonella* Surveillance System is considered to be fairly complete. However, some *Salmonella* isolates may not be forwarded to public health laboratories, and therefore are not reported. In addition, irrespective of the surveillance system, many cases of *Salmonella* illness are not reported because the ill person does not seek medical care, the health-care provider does not obtain a specimen for diagnosis, or the laboratory does not perform the necessary diagnostics tests. The results of surveillance reported herein should be considered underestimates. In addition, not every state submitted data in 2003.

The National *Salmonella* Surveillance System database is dynamic; the number of isolates reported for previous years may change according to the addition or correction of isolate reports. For example, the number of human *Salmonella* isolates published in the 2000 Annual Summary for 2000 was 32,022, whereas the number of isolates reported for 2000 in this Annual Summary is 33,556.

The number of isolates reported by geographical area (e.g. state) represents the area where laboratory confirmation and serotyping was performed. In some instances, the reporting area is not the same as the area of residence of the person from whom the isolate was obtained. For *Salmonella* serotype Typhi, only the first isolation in a year for each person is counted. For non-Typhi serotypes, only the first isolation within a thirty day period for each person is counted, given that the serotype and clinical source (e.g. stool or blood) are the same.

The data presented for *Salmonella* isolates from animals and related sources (i.e. environment and feeds) are gathered from isolates submitted to the U.S. Department of Agriculture, Animal and Plant Health Inspection Services, National Veterinary Services Laboratories (USDA/APHIS/NVSL) for serotyping. These isolates are submitted by animal disease diagnostic laboratories and the USDA, Food Safety and Inspection Service (FSIS) laboratories throughout the United States. Data from other United States laboratories that serotype *Salmonella* from animals and related sources and submit isolates to the NVSL are also included in this report. *Salmonella* serotyping results from clinical cases of animal disease are designated as "clinical" and shown in Table 6. Serotyping results from herd and flock monitoring and surveillance, feed sample testing, environmental testing, research projects, and isolates from USDA, FSIS food testing programs are designated as "nonclinical" (Table 7). Samples from non-human sources are tested for *Salmonella* for a variety of purposes and are obtained in a variety of ways. The sampling is therefore neither complete nor random and undoubtedly has sampling biases. Any interpretation of data should consider this limitation.

The Statistical Outbreak Detection Algorithm (SODA), developed by BIMB and FDDB, is a statistical algorithm based on the National *Salmonella* Surveillance System. It is designed to detect unusual clusters of isolates of *Salmonella* infection. SODA compares current *Salmonella* isolates reported through PHLIS by serotype to a 5-year historical baseline for that serotype and week to detect unusual increases from the baseline. Analyses can be conducted at state, regional, or national levels. Since 1996, SODA has been implemented at CDC and selected state health departments. If you would like more information on SODA, please call the PHLIS Helpdesk at telephone number (404) 639-3365.

Changes to the National *Salmonella* Surveillance System

In 2002, the National *Salmonella* Surveillance System implemented several changes in nomenclature and in surveillance practices. i) In order to improve the comparability of United States surveillance data with data from other countries, serotypes are now designated according to the Kauffmann-White Scheme (see below). ii) Reporting of *Salmonella* serotype I 4,[5],12:i:- (see discussion of this serotype below) was inconsistent in the past due to variability in the nomenclature used to report this serotype. This resulted in many

isolates of this serotype being reported as "Group B" or "Subspecies I". Beginning with the 2002 data, the submitted designation for this serotype was converted to the standard formula whenever possible. iii) Many non-subspecies I serotypes were not listed in the surveillance summaries in the past; instead, these isolates were reported by O group or subspecies only. Beginning with the 2002 surveillance data, all serotype formulas that were submitted to the national surveillance system, regardless of subspecies, were incorporated into the surveillance database. iv) Similarly, most "variants" of serotypes (monophasic, nonmotile or rough isolates) were not listed by their variant formulas in the past; instead, these isolates were reported by O group or subspecies only. Beginning with the 2002 surveillance data, all serotype variants that were submitted to the national surveillance system were converted to standard serotype formulas whenever possible and incorporated into the surveillance database. We hope that the changes in our surveillance practices will improve the accuracy of the surveillance data and enhance the detection of newly emerging serotypes. These changes should be kept in mind when comparing 2002 and 2003 data to previous years. The increased numbers of *Salmonella* serotype I 4,[5],12:i:-, of some non-subspecies I serotypes, and of serotype variants since 2002 may be due at least in part to improved surveillance.

In order to improve the utility of partial serotype data, we are changing the way that isolates that are not fully serotyped are designated and reported in PHLIS. In the past, these isolates were reported primarily by serogroup. While serogroups A through E are composed mainly of subspecies I serotypes, many of the other O serogroups are represented in several different subspecies. Most of the serogroups higher than E include serotypes from more than one subspecies, and nearly half (15 of 37) include serotypes from five different subspecies. Reporting isolates by serogroup alone combines unrelated isolates of different subspecies in the same serogroup category. Thus, we would like to move away from the "serogroup" categories. When full serotype information is not available, isolates are identified first by subspecies, then O serogroup and any additional serotype antigens. All available serotype information should be submitted to PHLIS (subspecies, O serogroup, O antigens, H antigens, whether one or two H antigens are detected, rough or mucoid status if appropriate). Partially serotyped isolates are listed in Table 3a.

Annual Summary Highlights for 2003

Human Sources

A total of 33,589 *Salmonella* isolates were reported from participating public health laboratories in 2003. Forty-nine states and the District of Columbia reported isolates; Florida, Montana and the District of Columbia reported partial serotype information. No isolates were reported from Texas. This represents a 9% decrease compared with 1993 and an increase over 2002 (3%). The national rate of reported *Salmonella* isolates in 2003 was 11.6 per 100,000 population based on 2003 census population figures for the United States.

Similar to other years, *Salmonella* was isolated most frequently from children under 5 years of age, accounting for 25% of isolates (Table 2). About 10% of isolates came from persons in each of the second through fifth decades of life, with declining numbers thereafter. The distribution of isolates between the sexes was different, with a greater number of isolates from male infants and children and fewer isolates from male adults and older persons (Table 2).

The twenty most common serotypes of *Salmonella* in 2003 are listed in Table 1. These represent 78% of all *Salmonella* isolates. Of the top twenty serotypes, the two most common serotypes, *S. Typhimurium* and *S. Enteritidis*, had substantial decreases in numbers from 1993-2003; the largest percent decrease in numbers compared with 1993 were *S. Hadar* and *S. Enteritidis* (Table 8). A dramatic increase in *S. Mississippi* (181% from 1993 to 2003) mainly occurred before 1998. *S. Newport* and *S. Javiana* had important increases in numbers from 1993 to 2003 (160% and 159% respectively). In 2003, serotypes *S. Bareilly* and *S. Stanley* increased in rank to be included in the top twenty serotypes, whereas *S. Berta* and *S. Poona* dropped from the top twenty serotypes compared with 2002.

Salmonella serotype I 4,[5],12:i:- was introduced as the 18th most common serotype in 2002 and has increased in rank to 14th in 2003. The serotype has been tracked in the National Surveillance system since 1998, though many isolates were classified as only "Subspecies I" or "Group B" in the past. Recent efforts to correctly classify this serotype may be responsible for some of the increase in numbers identified since 2002. It is unknown how many of the 417 isolates reported as Subspecies I, Group B in 2003 could be this serotype (Table 3a). In 1998, this serotype was the fourth most commonly identified in Spain; genetic analysis of the Spanish isolates revealed a close relationship to *S. Typhimurium* (1). Many U.S. isolates of this serotype were characterized by pulsed field gel electrophoresis (PFGE) and the patterns submitted to PulseNet, the National Molecular Subtyping Network for Foodborne Disease Surveillance. The PFGE patterns for most *S. I 4,[5],12:i:-* isolates were closely related to *S. Typhimurium* PFGE patterns, indicating that they are most likely variants of *S. Typhimurium*.

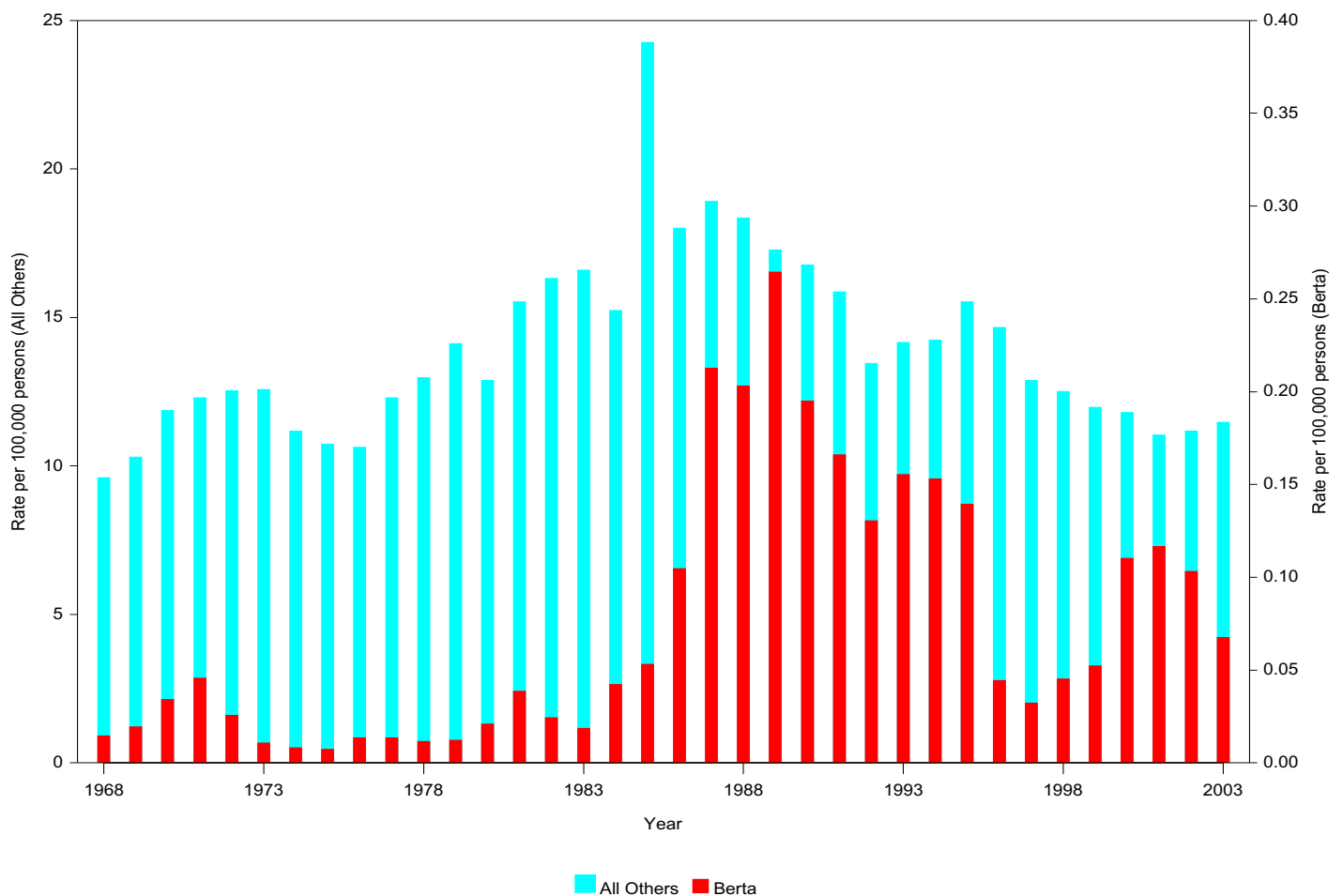
The three most common serotypes of *Salmonella* in 2003 (*Typhimurium*, *Enteritidis*, and *Newport*) accounted for 46% of isolates. Compared with 1993, the frequency rank of *S. Typhimurium* and *S. Enteritidis* in 2003 remained first and second respectively, though in 1994-1996 their rank was temporarily reversed (Figure 4). A large proportion of *S. Typhimurium* isolates were resistant to multiple antimicrobial drugs; in a 2002 national survey, 21% were resistant

to one or more drugs and 30% had a five-drug resistance pattern characteristic of a single phage type, DT104 (2). Similarly, *S. Newport* has emerged as a major multidrug-resistant pathogen. In 2002, 53 (23%) of 239 *S. Newport* isolates submitted to the National Antimicrobial Resistance Monitoring System were resistant to at least nine of 17 antimicrobial agents tested, including extended-spectrum cephalosporins (3). Similar to other years, there were marked regional differences in the frequency of *Salmonella* isolates among serotypes. The rate of isolations by region has been followed closely for *S. Enteritidis* as a means of assessing the impact of egg safety regulations and industry improvements. As indicated in Figure 2, *S. Enteritidis* rates of isolation had been relatively high in New England, Mid Atlantic and Pacific regions, but have shown significant decreases since 1995. Though New England had an increase in *S. Enteritidis* in 2000 and 2001 compared to 1999, the isolation rate decreased in 2002 and 2003.

Beginning in 1984, *S. Berta* isolates increased from a baseline rate of 0.023/100,000 persons to a peak of 0.27 in 1989 (Figure 1). From 1995 and forward the isolation of *S. Berta* decreased to rates prior to 1985. Again in 1999 we observed a new increase, this time peaking in 2001 with a rate of 0.12. In 2003 the isolation rate of *S. Berta* has decreased by 42% to 0.07 and *S. Berta* has dropped from the 20 most common serotypes. Through the years 1968 to 2003 *S. Berta* has most commonly been identified from non-clinical samples from turkey and chicken sources. There are currently no good explanations to the fluctuations in the isolation rate of *S. Berta*; although *S. Berta* has decreased it has still been implicated in recent foodborne outbreaks.

Non-human Sources

Data on *Salmonella* isolates obtained from non-human sources can help identify possible sources of human illness. *S. Typhimurium*, the most common serotype in humans, is identified most commonly from clinical samples from bovine sources, and from non-clinical samples from chicken sources. *S. Enteritidis* and *S. Heidelberg*, the second and fourth most common serotype in humans, respectively, are identified most commonly from clinical and non-clinical chicken sources (Table 6 and 7).



Adoption of the Kauffmann-White Scheme for designation of *Salmonella* serotypes

Salmonella serotyping has been the cornerstone for epidemiological surveillance and outbreak investigations for this important pathogen. The National *Salmonella* Surveillance system has tracked *Salmonella* isolates by serotype since 1968. New subtyping methods have come and gone, but serotyping continues to provide essential subtype information for *Salmonella*. For example, PulseNet, our state-of-the-art genotyping system for *Salmonella*, relies on accurate serotype information as the "first-tier" subtype information. PFGE pattern determination, by itself, does not replace serotyping, but rather subdivides within serotype.

The Kauffmann-White Scheme for designation of *Salmonella* serotypes is maintained by the WHO Collaborating Centre for Reference and Research on *Salmonella* at the Institut Pasteur and is used by most of the world. Up until 2002, the CDC used a slightly different version of the scheme, the "Modified Kauffmann-White Scheme". A unified format for serotype designation is essential for accurate surveillance via PulseNet, Global SalmSurv, and other international networks. Therefore, to improve the accuracy of our surveillance data and to make us in-step with the rest of world with respect to *Salmonella* serotype designation, the Centers for Disease Control and Prevention adopted the Kauffmann-White Scheme on January 1, 2003.

The adoption of the Kauffmann-White Scheme affected only a few of the more common serotypes. The primary differences between the two schemes are:

i) *Salmonella* are divided into six subspecies that can be differentiated by biochemical and genetic tests. Under the Kauffmann-White Scheme, subspecies I serotypes are named; subspecies II through VI serotypes are identified by formula. The Modified Kauffmann-White Scheme used names for those subspecies II through VI serotypes that were designated before 1968 and formulas for those serotypes identified after 1968. With the adoption of the Kauffmann-White scheme, all named serotypes are subspecies I; serotypes from all other subspecies are designated by formula. In 2002, there were four named serotypes among the top 100 serotypes that did not belong to subspecies I and were effected by this change.

- *S. Marina* is now designated as *S. IV 48:g,z₅₁*:-
- *S. Flint* is now designated as *S. IV 50:z₄,z₂₃*:-
- *S. Kralendyk* is now designated as *S. IV 6,7:z4,z24*:-
- *S. Chameleon* is now designated as *S. IV 16:z4,z32*:-

ii) Under the Kauffmann-White Scheme, serogroups E2 and E3 were combined with serogroup E1. This reflects the fact that the antigenic changes in serogroups E2 and E3 are the result of lysogenic conversion by bacteriophages and thus represent minor variants of serogroup E1 serotypes. The Modified Kauffmann-White Scheme used separate serotype names for these variants. Two serotypes in the top 100 in 2002 that were affected by the merging of serogroups E2 and E3 with serogroup E1.

- *S. Newington* is now *S. Anatum* variety (var.) 15+
- *S. Newbrunswick* is now *S. Give* var. 15+.

iii) Under the Kauffmann-White Scheme, two biotypes of *S. Paratyphi B* are recognized; they are differentiated primarily by the ability to ferment tartrate. *S. Paratyphi B* is tartrate negative and is associated with more severe, typhoid fever-like disease. *S. Paratyphi B* var. L-tartrate + (also referred to as "*S. Paratyphi* var. Java") is tartrate positive and commonly associated with gastroenteritis. *S. Paratyphi B* var. L-tartrate + was known as "*S. Java*" in the Modified Kauffmann-White Scheme. The two biovars of *S. Paratyphi B* have been a source of confusion in the past because they have the same antigenic formula (I 1,4,[5],12:b:1,2), and are differentiated only by biotype. It is essential that the tartrate test be performed to accurately identify and report the two biotypes.

Overview of *Salmonella* Serotype Designation

1) *Salmonella* Taxonomy¹

The **genus *Salmonella*** divided into two species, *Salmonella enterica* and *Salmonella bongori*.

Salmonella enterica is further subdivided into 6 subspecies that are designated by names or Roman numerals. The Roman numerals are simpler and more commonly used. Subspecies IIIa and IIIb were historically considered a separate genus, ***Arizonae***, and are still sometimes referred to by this name.

<i>Salmonella enterica</i> subspecies	
I	<i>enterica</i>
II	<i>salamae</i>
IIIa	<i>arizonae</i>
IIIb	<i>diarizonae</i>
IV	<i>houtenae</i>
VI	<i>indica</i>

Salmonella bongori was originally designated *S. enterica* **subspecies V**. It has since been determined to be a separate species of *Salmonella*. However, for simplicity and convenience, these strains are commonly referred to as "subspecies V" for the purpose of serotype designation.

2) *Salmonella* Serotype Antigens

Salmonella serotype is based on the immunoreactivity of two surface structures, **O** and **H antigen**.

O antigen is a carbohydrate (also called a polysaccharide) that is the outermost component of lipopolysaccharide. It is a polymer of **O subunits**; each O subunit is typically composed of four to six sugars depending on the O antigen. Variation in O antigen results from variation in the sugar components of the O subunit, from variation in the nature of the covalent bond between the sugars of the subunit, and from variation in the nature of the linkage between the O subunits that form the O antigen polymer.

O antigens are designated by numbers and are divided into **O serogroups**, also called **O groups**. O groups are designated by the primary **O factor(s)** that are associated with the group. Many of the common O groups were originally designated by letter and are still commonly referred to by letter (e.g., *S. Typhimurium* belongs to Group O:4 or Group B, *S. Enteritidis* belongs to group O:9 or Group D1; *S. Paratyphi A* belongs to Group O:2 or Group A).

Additional O factors are associated with some O groups and are often variably present or variably expressed. The *Salmonella* O groups and the additional O antigens that may be present in serotypes of that group are listed below. When multiple O factors are present, they are listed sequentially and separated by commas.

H antigen is the filamentous portion of the bacterial flagella; H antigen is made up of protein subunits called flagellin. The ends of flagellin are conserved and give the filament its characteristic structure. The antigenically variable portion of flagellin is the middle region of the protein, which is surface-exposed. ***Salmonella*** is unique among the enteric bacteria in that it can express two different H antigens, which are encoded by two different genes. Typically, expression of the two genes is coordinated so that only one flagellar antigen is expressed at a time in a single bacterial cell. The two distinct flagellar antigens are referred as Phase 1 and Phase 2. "**Monophasic**" isolates are those that express only a single flagellin type. These occur naturally in some serotypes (e.g., *S. Enteritidis*, *S. Typhi*, most subspecies IIIa and IV serotypes), or can occur through the inactivation or loss of the gene encoding the Phase 1 or Phase 2 antigen.

The H antigens of *Salmonella* are listed below. Some antigens are composed of multiple factors, which are separated by commas; for example, the second phase antigen of *S. Typhimurium* is composed of factors 1 and 2. H antigens composed of multiple factors are grouped into complexes.

3) *Salmonella* Serotype Identification

Salmonella serotypes are typically identified in a cascade of tests. First, an isolate is identified and the subspecies is determined, typically by biochemical testing. O antigens and H antigens are detected in independent agglutination assays using antisera that react with groups of related antigens or a single antigen. Both H antigens can sometimes be detected in a single culture, particularly for older strains or for isolates that have been passed multiple times. When only one H antigen is detected, the isolate is inoculated onto the top of a tube of **phase reversal media**, a semisolid media containing antisera to the H antigen that has already been identified. Organisms expressing the previously detected H antigen are immobilized by the added antisera and grow only at the top of the tube. Organisms expressing the second H antigen are able to move away from the top of tube, evidenced by growth throughout the tube. The second H antigen is then determined using organisms recovered from the bottom of the phase reversal media.

4) *Salmonella* Serotype Designation

All *Salmonella* serotypes can be designated by a formula. Additionally, subspecies I serotypes are given a name (e.g., Typhimurium, Enteritidis, Typhi, etc).

The typical format for a serotype formula is:

Subspecies [space] O antigens [colon] Phase 1 H antigen [colon] Phase 2 H antigen

Examples:

I 4,5,12:i:1,2 (*S. Typhimurium*)

I 4,12:i:1,2 (*S. Typhimurium*)

I 9,12:g,m:- (*S. Enteritidis*)

II 47:b:1,5 (*S. II 47:b:1,5*)

IV 48:g,z₅₁:- (*S. IV 48:g,z₅₁:-*)

IIIb 65:(k):z (*S. IIIb 65:(k):z*)

Other conventions:

- * Some O and H factors are variably present. This is indicated in the generic serotype formula by underline when the factor is encoded on a bacteriophage (e.g., 1) or by square brackets (e.g., [5]) when the antigen is variably present. For an individual isolate, if the variable factor is detected it is included in the formula without additional notation. If the variable factor is not detected, it is not listed in the formula. Weakly recognized antigens are indicated by parentheses (e.g., (k)).
- * The absence of an H antigen is indicated by a minus sign (“-”) for the particular phase. For example, the “monophasic Group B” isolates that are becoming more common in the US are designated as “S. I 4,5,12:i:-” or “S. I 4,12:i:-”. Nonmotile isolates (express no H antigen) are indicated by minus signs in both phases, but can also be designated by “NM” or “nonmotile” in place of the H antigens.
- * Isolates that do not express O antigen (rough isolates) or express a capsule that prevents immunologic detection of the O antigen (mucoid isolates) are indicated by “O Rough” or “Mucoid” in place of the O antigen.
- * Rarely, isolates express a third H antigen that is noted by a colon followed by the antigen after the Phase 2 H antigen (e.g., *S. II 13,23:b:[1,5]:z42*, formerly *S. Acres*)

5) *Salmonella* Serotype Statistics

There were 2541 *Salmonella* serotypes as of 2002; approximately 60% belong to subspecies I. In the US, approximately 99% of reported human isolates belong to subspecies I. The “top 10” serotypes account for approximately 74% of all isolates reported in the US; the “top 100” serotypes account for about 98% of all isolates. Among the top 100 serotypes, only *S. IV 48:g,z₅₁:-* (formerly *S. Marina*), *S. IV 50:z4,z23:-* (formerly *S. Flint*), *S. IV 6,7:4,z24:-* (formerly *S. Kralendyk*), and *S. IV 16:z4,z32:-* (formerly *S. Chameleon*) are not subspecies I. Among the non-subspecies I isolates, subspecies IV isolates are the most common, followed by subspecies IIB, IIIa, and II. Subspecies VI and *S. bongori* isolates are very rare.

- ¹ According to the Bacteriological Code, the legitimate species name for *S. enterica* is *S. choleraesuis*, and there are a few other differences from the nomenclature described. The official taxonomic designations are confusing and proposals to change them are currently under consideration. The taxonomy described here is used by most laboratories worldwide, including the CDC.

Salmonella O serogroups and associated O antigens

O Group (number designation)	O Group (letter designation)	Antigens present in all serotypes	Additional antigens that may be present in some serotypes
2	A	2,12	1
4	B	4,12	1; 5; 27
7	C1	6,7	14; (Vi)
8	C2	8	6; 20
9	D1	9,12	1; (Vi)
9,46	D2	9,46	none
9,46,27	D3	9,12,46,27	1
3,10	E1	3,10	15; 15,34
1,3,19	E4	1,3,19	10; 15
11	F	11	none
13	G	13	1; 22; 23
6,14	H	6,14	1; 24; 25
16	I	16	none
17	J	17	none
18	K	18	6; 14
21	L	21	none
28	M	28	none
30	N	30	none
35	O	35	none
38	P	38	none
39	Q	39	none
40	R	40	1
41	S	41	none
42	T	42	1
43	U	43	none
44	V	44	1
45	W	45	none
47	X	47	1
48	Y	48	none
50	Z	50	none
51		51	1
52		52	none
53		53	1
54 (provisional)		54	21; 3; 3,15; 4,12; 8,20; 6,7
55		55	none
56		56	none
57		57	none
58		58	none
59		59	1
60		60	none
61		61	none
62		62	none
63		63	none
65		65	none
66		66	none
67		67	none

H (flagellar) antigens of *Salmonella*

1 complex:	1,2 1,5 1,6 1,7 1,2,5 1,2,7 1,5,7 1,6,7	Other antigens (not part of a complex):	a b c d e,h i k (k) r r,i y z z6 z10 z29 z35 z36 z36,z38 z38 z39 z41 z42 z44 z47 z50 z52 z53 z54 z55 z56 z57 z60 z61 z64 z65 z67 z68 z69 z71 z81 z83 z87 z88
EN complex:	e,n,x e,n,x,z15 e,n,z15		
G complex:	f,g f,g,m,t f,g,s f,g,t g,m g,m,p,s g,m,q g,m,s g,m,s,t g,m,t g,p g,p,s g,p,u g,q g,s,q g,s,t g,t g,z51 g,z62 g,z63 g,z85 m,p,t,u m,t		
L complex:	l,v l,w l,z13 l,z13,z28 l,z28		
Z4 complex:	z4,z23 z4,z23,z32 z4,z24 z4,z32		

Acknowledgements

Thanks to Richard Bishop for assembling this summary and to all the State Public Health laboratories and epidemiologists who participate in this surveillance.

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