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Review

Salmonella source attribution based on microbial subtyping

Lisa Barco [a], Federica Barrucci [a], John Elmerdahl Olsen [b], Antonia Ricci [a,*]

[a] OIE, National Reference Laboratory for Salmonella, Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, Padova, 35020 Legnaro, Italy
[b] Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Stigboejlen 4, DK-1870 Frederiksberg C., Denmark

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ABSTRACT

Source attribution of cases of food-borne disease represents a valuable tool for identifying and prioritizing effective food-safety interventions. Microbial subtyping is one of the most common methods to infer potential sources of human food-borne infections. So far, *Salmonella* microbial subtyping source attribution models have been implemented by using serotyping and phage-typing data. Molecular-based methods may prove to be similarly valuable in the future, as already demonstrated for other food-borne pathogens like *Campylobacter*. This review assesses the state of the art concerning *Salmonella* source attribution through microbial subtyping approach. It summarizes the available microbial subtyping attribution models and discusses the use of conventional phenotypic typing methods, as well as of the most commonly applied molecular typing methods in the European Union (EU) laboratories in the context of their potential applicability for *Salmonella* source attribution studies.

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1. Introduction

Bacteria subtyping is defined as the characterization of bacteria beyond the species and/or subspecies level. Strains of food-borne pathogens responsible for human infections are expected to have indistinguishable or more similar subtypes to those of strains isolated from the originating source than those isolated from unrelated sources (Hyytiä-Trees et al., 2007).

Microbial subtyping is one of the major methodologies to attribute food-borne infectious diseases to their sources (EFSA, 2008). The principle behind the source attribution is the comparison of subtypes of isolates causing human disease with the distribution of these subtypes in their putative sources (e.g. animals, food, environment) (Pires et al., 2009). Microbial subtyping source attribution relies on subtyping laboratory methods to identify overlaps between subtypes

* Corresponding author at: OIE, National Reference Laboratory for Salmonella, Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro (Padova), Italy. Tel.: +39 0498084296; fax: +39 0498830268.
E-mail address: aricci@izsvenezie.it (A. Ricci).

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identified from cases of human disease and those from their potential sources.

Human infections caused by subtypes which have been exclusively or almost exclusively isolated from one single source can be attributed to that specific source. On the other hand, when human infections are caused by subtypes isolated in several sources, they can be attributed to those sources proportionally to the reported occurrence of subtypes in the sources (Hald et al., 2004).

The application of this methodology to *Salmonella* requires i) a collection of isolates from humans and from different sources; non-human isolates should be as representative as possible of the real exposure of humans to the potential vehicles of the pathogen; ii) appropriate subtyping methods that can provide detailed knowledge of *Salmonella* types in all relevant sources, and the correlation of isolates obtained from humans with those from the possible sources, and iii) a model to infer the sources of human infections based on the subtype distributions obtained within each investigated source.

The application of microbial subtyping approach for source attribution assumes that the distribution of subtypes in the collection of isolates in each source used in the attribution exercise is representative of the true distribution of subtypes in each source (Pires et al., 2009). Hence, it is essential to use a representative sample of isolates from each source investigated obtained through integrated surveillance programs including the major sources (e.g. food animals and food) and humans (EFSA, 2008). For this purpose, a stratified sampling scheme could be used to allocate the adequate sampling fraction in each of the strata considered (sources).

These collections of isolates should be subtyped by using appropriate and harmonized discriminatory typing methods and the profiles obtained should be evaluated through shared interpretative guidelines. Biases related to the lack of standardization of the methods used to subtype the isolates or to establish their clonality should be avoided since it is another essential requirement to guarantee the soundness of the results provided by the models.

Finally, it is equally important to have an in-depth understanding of the epidemiological data that are used as input to the model (EFSA, 2008).

This review aims to assess the state of the art concerning *Salmonella* source attribution through microbial subtyping approaches. The major attribution models used so far are summarized. Moreover, the usefulness of the most commonly applied typing methods in the European (EU) laboratories are discussed in the context of their potential application in *Salmonella* source attribution studies.

2. *Salmonella* source attribution models based on microbial subtyping

Several applications of the microbial subtyping approach for *Salmonella* source attribution have been described. In 1999, Van Pelt and colleagues developed the so called “Dutch model”, based on the principle of comparing the number of reported human cases, caused by a particular bacterial subtype (*Salmonella* serovar), with the relative occurrence of that subtype in each source. The number of reported cases per subtype *i* attributed by the model to source *j* was estimated by the equation:

$$\lambda_{ij} = \frac{p_{ij}}{\sum_j p_{ij}} x_i$$

where λ_{ij} was the expected number of cases per year of subtype *i* from source *j* estimated from p_{ij}, the proportion of *Salmonella* subtype *i* in source *j* among all the sources sampled, $\sum_j p_{ij}$, the proportion of *Salmonella* subtype *i* among all the sources sampled, and x_i, the estimated number of human cases of subtype *i* per year (Van Pelt et al., 1999).

The Dutch model had the advantage of being easy to calculate, but it relied on the false assumptions that all *Salmonella* subtypes have the same virulence and all sources an equal probability of causing human disease. Conversely, it is widely recognized that different *Salmonella* subtypes have different probabilities of causing disease in humans and with different levels of severity (EFSA, 2012). Moreover, food types have different abilities to act as vehicles for food-borne disease agents, due to their physical properties, such as pH and water activity, or due to the applied processing and preparation procedures.

In 2004, Hald and colleagues developed a model that, similar to the Dutch model, compared the number of human cases caused by different *Salmonella* subtypes with their prevalence in different food sources, but this model also incorporated bacterial and food-source dependent factors, using a Bayesian approach. In the Hald model, the number of human cases per subtype *i* was assumed to follow a Poisson distribution with expected number of cases Λ_i (unknown parameter) given by the equations:

$$\Lambda_i = \lambda_i + t_i - o_i$$
$$\lambda_i = \sum_j \lambda_{i,j}$$
$$\lambda_{ij} = M_j \cdot p_{ij} \cdot q_i \cdot a_j$$

where λ_i was the expected number of domestic and sporadic cases, t_i the number of travel-related cases, o_i the number of outbreak-related cases minus one for each outbreak, λ_{ij} the expected number of sporadic and domestic cases per year per *Salmonella* subtype *i* from source *j*, p_{ij} the prevalence of *Salmonella* subtype *i* in source *j*, q_i the bacterial-dependent factor for subtype *i*, a_j the food-source-dependent factor for source *j* and M_i the amount of source *j* available for consumption per year. Bacterial-dependent factors were represented by survivability during food processing and ability to cause human disease (virulence/pathogenicity), while food-source-dependent factors were represented by likelihood of the investigated sources to cause food-borne infections (e.g. differences in bacterial load, food characteristics influencing growth behavior or preparation procedures). This model, known as “Hald model” or “Danish *Salmonella* source account model”, also took into consideration the origin of human cases (domestic or travel-related) and whether the cases were sporadic or from an outbreak. For the unknown parameters q_i, and a_j, uninformative uniform distributions were chosen as prior distribution, except for most frequent serotype, and all the subtypes within that serotype, for which q_i was set to 1. Posterior distributions were calculated through Markov Chain Monte Carlo (MCMC) simulation. This implemented model was used to estimate the contribution to human salmonellosis in Denmark in 1999 due to pork, beef, eggs, broilers, turkeys, ducks, as animal-food sources and included the *Salmonella* serovars: Enteritidis, Typhimurium, Hadar, Agona, Virchow, Newport, Infantis and Dublin. For *Salmonella* Enteritidis and *Salmonella* Typhimurium phage-types were also considered. Moreover, the model estimated the bacterial-dependent factors for each considered serovar and the food-source-dependent factors for each considered source (Hald et al., 2004). In 2007, the Hald model was used to estimate the source of human *Salmonella* infections in Denmark over the period 2000–2001 by including *Salmonella* subtypes based on the results of serotyping, antimicrobial resistance profiles and phage-types of *S.* Enteritidis and *S.* Typhimurium (Hald et al., 2007).

Wahlström et al. (2011) adapted the Hald model to estimate the number of reported human cases of sporadic *Salmonella* infections in Sweden during 2004–2006 attributable to each of nine different sources (Wahlström et al., 2011) and similarly, Guo et al. (2011) adapted the Hald model to estimate the burden of food-borne salmonellosis in the United States from 1998 through 2003, attributed to chicken, turkey, egg products, ground beef, intact beef and pork. For the first model serotyping and phage-type data were used, whereas the second model considered only *Salmonella* serotyping data. Pires and Hald (2010) have further developed the Hald model and presented a three-dimensional version, accommodating data for multiple years in order to attribute human cases of *Salmonella* in Denmark