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A comprehensive review of non-*enterica* subspecies of *Salmonella enterica*

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ABSTRACT

Salmonella is a major foodborne pathogen with a complex nomenclature. This genus is composed of two species, *S. enterica* and *S. bongori*. *S. enterica* is divided into six subspecies. *S. enterica* subspecies *enterica* is composed of more than 1500 serotypes with some of great importance, such as *S.* Typhimurium and *S.* Enteritidis. *S. enterica* subsp. *enterica* is responsible of more than 99% of human salmonellosis and therefore it is widely studied. However, the non-*enterica* subspecies of *S. enterica* have been little studied. These subspecies are considered to be related to cold-blooded animals and their pathogenicity is very limited. Phenotype and genotype information generated from different studies of non-*enterica* subspecies reveal poor ability to invade host cells and the absence or modification of important virulence factors. Also, the great majority of human infections due to non-*enterica* subspecies are related to a previous depressed immune system. Therefore, we propose to treat these subspecies only as opportunistic pathogens. For establish this premise, the present review evaluated, among other things, the genomic characteristics, prevalence, antimicrobial resistance and reported human cases of the non-*enterica* subspecies.

1. Introduction

Salmonella is a genus of Gram-negative, facultative anaerobe bacillus with flagella and mobility, composed of 2579 different serotypes (Grimont and Weill. 2007). Since *Salmonella* was discovered by Daniel Elmer Salmon and Theobald Smith in 1885 (Salmon and Smith, 1886) it has become, along with *Escherichia coli*, among the most studied microorganisms. The main reason for this attention is that *Salmonella* spp. is a worldwide foodborne pathogen and the second most responsible for gastrointestinal human infections after *Campylobacter* spp. In 2015, there were 94,625 confirmed cases of salmonellosis in humans and 126 deaths in the EU (EFSA-ECDC 2016). The *Salmonella* nomenclature is somewhat complex and has been an issue of debate for many years. Thus, it has been difficult to reach agreement concerning the number of species and subspecies that form the *Salmonella* genus (Agbaje et al., 2011). However, finally, in 2005 the international community gave official approval for the designation (Tindall et al., 2005). Since that point, the *Salmonella* genus has been divided into two different species, *S. bongori* and *S. enterica*. The latter is then divided into six different subspecies, each designated with Roman numeral: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI). The other species, *S. bongori* (V) is composed of 22 serotypes which have been little studied as they are mainly associated with cold-blooded animals and human infections are very uncommon, mainly affecting mainly children aged 1 month to 3 years (Giammanco et al., 2002).

S. enterica has received more attention by researchers. However, this attention has not been equally distributed and has been focused mainly on *S. enterica* subsp. *enterica*. This subspecies is composed of 1531 serotypes, among which *S.* Typhimurium and *S.* Enteritidis stand out as they are principally responsible for human infections (Grimont and Weill, 2007EFSA-ECDC 2016). A great number of studies have evaluated the prevalence, antimicrobial resistance, virulence, infection ability and biofilm formation of *S. enterica* subsp. *enterica*. Indeed, it is possible find a large number of genome sequences of these serotypes in the National Center for Biotechnology Information (NCBI) database. However, there is still only very limited genomic information concerning *S. salamae*, *S. arizonae*, *S. diarizonae*, *S. houtenae* and *S. indica* (Desai et al., 2013; Wang et al., 2015; Sathyabama et al., 2014). Therefore, this review evaluates the existing knowledge of these subspecies, where future research should be focused to demonstrate without a doubt that these subspecies are only opportunistic pathogens and what position legislation should adopt in relation to these subspecies.

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Table 1
Subspecies of *S. enterica* and main biochemical characteristics.

Subspecies	Number	Serotypes	Biochemical characteristics								
			Dulcitol	Gelatinase	Sorbitol	Lactose	Growth with KCN	L(+)-tartato	Malonate	Galacturonate	Lysed by phage O1
S. enterica subsp. *enterica*	I	1531	+	–	+	–	–	+	–	–	+
S. enterica subsp. *salamae*	II	505	+	+	+	–	–	–	+	+	+
S. enterica subsp. *arizonae*	IIIa	99	–	+	+	–	–	–	+	–	–
S. enterica subsp. *diarizonae*	IIIb	336	–	+	+	+	–	–	+	+	+
S. enterica subsp. *houtenae*	IV	73	–	+	+	–	+	–	–	+	–
S. enterica subsp. *indica*	VI	13	-/+	+	–	-/+	–	–	–	+	+

2. Classification and biochemical characteristics of non-*enterica* subspecies of *Salmonella enterica*

More than 2500 serotypes of *Salmonella* have been identified to date (Grimont and Weill, 2007). Table 1 shows the distribution of the 2557 different serotypes of *S. enterica*. As already mentioned, a great number of these serotypes (1531) are found in subspecies *enterica*. The rest of the serotypes are unevenly distributed among the other subspecies. While *S. enterica* subsp. *salamae* has 505 different serotypes, subspecies *indica* only has 13 different serotypes (Grimont and Weill, 2007).

The different subspecies of *S. enterica* present different biochemical characteristics (Grimont and Weill, 2007). While subspecies *enterica* and *salamae* are dulcitol positive, the rest of the subspecies are negative (Table 1). Subspecies *arizonae* and *diarizonae* are beta galactosidase and malonate positive, *S. enterica* subsp. *salamae* also being positive for this reaction. All the subspecies of *S. enterica*, with the exception of subspecies *enterica*, are gelatinase positive and all the subspecies are sorbitol positive, with the exception of subspecies *indica.* Only *S. enterica* subsp. *enterica* is L(+)-tartrate positive and only subspecies *houtenae* is able to grow in medium containing potassium cyanide and is salicin positive. Unlike the others, subspecies *diarizonae* and *houtenae* are mucate negative. An important biochemical characteristic that differentiates the subspecies of *S. enterica* is their capacity to use lactose. Subspecies *diarizonae* is lactose positive and some serotypes of subspecies *indica* are lactose positive.

3. Genomics

3.1. Phylogenetic relationship

The High-throughput methods for DNA sequencing along with bioinformatics tools developed in the last years have revolutionized the microbiological research because they made whole genome sequencing of microorganisms very nearly to be a routine exercise. In the last decade the availability of next-generation sequencing (NGS) has increased as cost has decreased. As a result of this, nowadays it is possible to apply NGS to investigate a broad range of non-model organisms and stablish phylogenetic relationships between them. The application of NGS technology has allowed studying the phylogenetic diversity of *Salmonella* genus by sequencing the genomes of strains from the two *Salmonella* species and the six subspecies of S. enterica (Desai et al., 2013; Fookes et al., 2011). In this sense, the application of High-Throughput technologies have allowed to deep into the genome of non-*enterica* subspecies (Table 2).

The ability of *Salmonella* to colonize the host and cause virulence is found in virulence plasmids (pSLT) and different gene clusters located in chromosomes called *Salmonella* pathogenicity islands (SPIs) (Fig. 1). These SPIs have been acquired by *Salmonella* during their evolution. There are five principal SPIs (1–5) in *Salmonella* related to their pathogenicity, of which SPI-1 and SPI-2 are the most studied. SPI-1 encodes several proteins related to the invasion of epithelial cells and SPI-2 contributes to survival and replication inside host cells such as epithelial cells and macrophages (Fabrega and Vila, 2013). Phylogenetic studies have demonstrated that *Salmonella* and *E. coli* diverged from a common ancestor 120–150 million years ago (Doolittle et al., 1996; Bäumler et al., 1998). A key factor in the divergence between these microorganisms was the acquisition of SPI-1 by the genus *Salmonella* (Ochman and Groisman, 1996). Phylogenetic studies of *Salmonella* species based in the use of NGS have demonstrated differences between *S. bongori* and *S. enterica*, placing the first in evolutionary terms in a particular position between *E. coli* and *S. enterica* (Fookes et al., 2011). The two species of *Salmonella* diverged 40–63 million years ago and their evolutionary history has given rise to important differences between the two *Salmonella* species (McQuiston et al., 2008). The separation of these two species is represented by the acquisition of the SPI-2 by *S. enterica*, used for bacterial replication and the systemic spreading of infection once the bacteria invade the nonphagocytic cell epithelium and M-cells (Wallis and Galyov, 2000). Also, *S. bongori* has a lower content of G + C than *S. enterica*, it has a basic virulence kit, only carrying three of the 22 reported SPIs, it has the capacity to degrade but not ferment lactose and it carries the same genes for L-tartrate and citrate fermentation as *E. coli*. This different evolution could be due to the adaptation of *S. bongori* to cold-blooded animals as hosts (Fookes et al., 2011). WGS and microarray studies have demonstrated that *S. enterica* subsp. *arizonae* is located somewhere between *S. bongori* and the other subspecies of *S. enterica* (Chan et al., 2003; Desai et al., 2013; Porwollik et al., 2002). Microarray data also showed that subspecies *arizonae* only shared the 77% of genes with *S.* Typhimurium and a great divergence in pSLT and SPI 2 genes between subspecies *arizonae* and *S enterica* subsp. *enterica* serotypes. These data support the fact that the adquisicion of virulence genes was key in the divergence of the different *Salmonella* subspecies (Chan et al., 2003). Subspecies *diarizonae* and *houtenae* diverged from subspecies *enterica*, *indica* and *salamae* 30 million years ago. The divergence between subspecies *arizonae* and *diarizonae* is represented by the gain of SPI-18, some *Salmonella* fimbriae or the β-glucuronide utilization by subspecies *diarizonae*. Finally, *S. enterica* subsp. *salamae* and *S. enterica* subsp. *indica* were the last to diverge from subspecies *enterica* 20 million years ago. In the divergence of subspecies *salamae* from the others *Salmonella* subspecies is remarkable the gain of the locus of enterocyte effacement (LEE), a pathogenicity island also inserted in the genome of other pathogens as *E. coli*, and the loss in SPI-5 by subspecies *salamae* (Desai et al., 2013; Franzin and Sircili 2015). The divergence of subspecies *indica* is also characterized by loss in SPI-5 and D-sorbitol and L-ascorbate utilization (Desai et al., 2013). The divergence of subspecies *enterica* from the other *Salmonella* subspecies is clearly characterized by gain in SPI-6, 2-aminoethylphosphonate metabolism, Island STM3779-STM3785, Island STM4065-STM4080 and Autoinducer 2 (AI-2) transport and processing. AI-2 is a signalling molecule related to quorum sensing (Surette et al., 1999). Although its role in *Salmonella* signalling and its metabolism is still unclear, it is believed that it plays a role in communication with the microbiota of the gut, regulating the virulence and facilitating genetic information exchange (Gart et al., 2016). The acquisition of this mechanism by subspecies *enterica* is clearly a differentiator factor that contributes to a more efficient colonization of host by *Salmonella*.