

Epidemiological characterization and phylogenetic analysis of the emerging *Salmonella enterica* serovar Napoli: the first cause of invasive nontyphoidal salmonellosis in Lombardy, northern Italy

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Introduction and purpose

Salmonella enterica serovar Napoli (*S. Napoli*) is a nontyphoidal serotype that is currently emerging in Europe, mainly affecting Switzerland, France and Italy (1). In the latter, *S. Napoli* incidence has increased by 28.2% during the period 2000-2011, most cases occurring in Lombardy region (2). Furthermore, a recent large outbreak occurred in Milan, northern Italy, associated to elevated rates of hospitalization and bacteraemia, has evidenced that *S. Napoli* infections may become invasive. By considering the elevated invasive rate observed for that recent outbreak and the knowledge gaps, we aimed to investigate the role of *S. Napoli* in invasive salmonellosis in Lombardy, during the period 2010-2014, and infer the phylogeny of this emerging serotype.

Methods

Data of human salmonellosis cases in Lombardy during the period 2010-2014 (10,858 records) were obtained from IT-ENTER-NET (<http://www.iss.it/salm/?lang=1&id=1&tipo=4>), a network of diagnostic laboratories. General nontyphoidal salmonellosis (NTS) case definition was 'an isolate of *Salmonella enterica* with identified serovar from a human sample'. Invasive nontyphoidal salmonellosis (iNTS) case definition was 'an isolate of *Salmonella enterica* with identified nontyphoidal serovar from blood human sample'. Comparative genomics analysis based on sequence data for 93 core genome loci, as described by den Bakker et al. (3), was performed to define phylogenetic relationships of 14 *Salmonella enterica* subsp. *enterica* serotypes, including *S. Napoli* outbreak strain [4], and one *S. enterica* subsp. *arizonae* strain. Phylogenetic analysis was done using Maximum Likelihood method based on the Tamura-Nei model [5] using MEGA 6.0 [6].

Results

S. Napoli incidence in Lombardy continued to increase during the period 2010-2014. Moreover, this serotype was the first cause of invasive nontyphoidal salmonellosis during this period, with 35 invasive cases out of 691 and an invasive rate of 5.1% (Table 1). Phylogenetic analysis revealed that *S. Napoli* belongs to the Typhi subclade of clade A, and the most related serotype was found to be Paratyphi A. Additionally, *S. Napoli* genome carries the invasive determinant gene *cdtB* and the pathogenicity island SPI-18 (Figure 1).

Table 1. Total cases, invasive cases (iNTS) and invasive rate of the top five serotypes causing nontyphoidal salmonellosis (NTS) in Lombardy (northern Italy) during 2010-2014.

Serotype	Total cases	Invasive cases (iNTS)	Invasive rate*
S. Typhimurium	2646	23	0.87%
Monophasic variant of S. Typhimurium	3294	32	0.97%
S. Napoli	691	35	5.1%
S. Enteritidis	884	19	2.15 %
S. Derby	314	2	0.63%
TOTALS	10858	272	2.5%

*Invasive rate = (invasive infections/total cases)X100

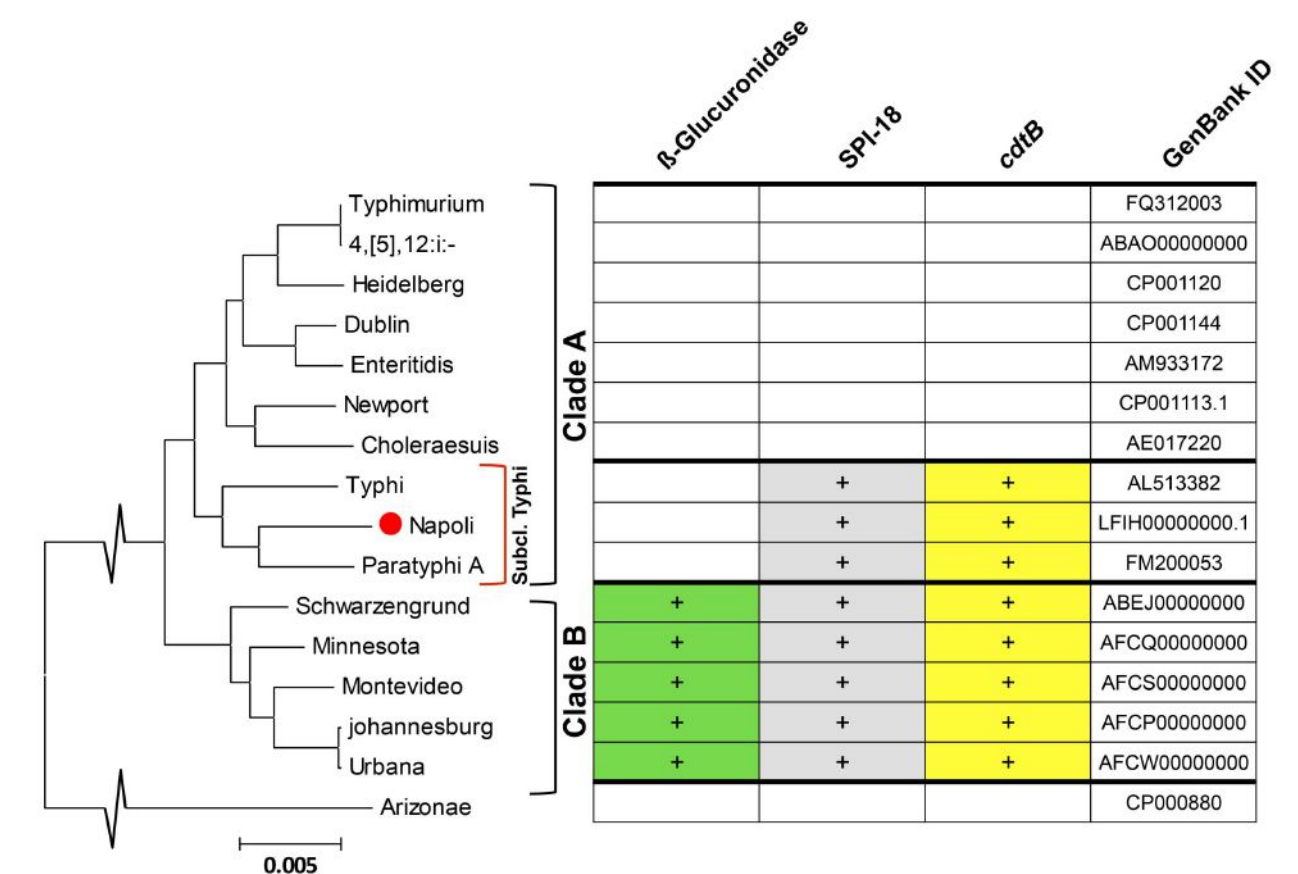


Figure 1. Phylogenetic relationships of serovars of 15 *Salmonella enterica* subsp. *enterica* and one *S. enterica* subsp. *arizonae* isolate based on sequence data for 93 core-genome genes [5]. The blocks to the right of the phylogenetic tree indicate the presence of the β -Glucuronidase operon (green), SPI-18 (grey) and the *CdtB*-islet (yellow) in their genomes.

Conclusions

S. Napoli is an emerging public health concern in Italy. Nevertheless, the role of nontyphoidal serotypes in invasive disease is yet poorly studied. A combination of SPI-18 island and *cdtB* gene was previously reported only in *S. Typhi*, *S. Paratyphi A* and clade B serotypes, all of them associated to elevated rates of invasive disease. Thus, to the best of our knowledge, this is the first time that a clade A nontyphoidal serotype presents the same virulence-genes pattern of *S. Typhi* and *S. Paratyphi A*. Our work suggests that *S. Napoli* potential virulence deserves attention and highlights a need to discuss its classification in nontyphoidal group.

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