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Research Article

Characterization by PFGE of the Emerging *Salmonella* serotypes in Piedmont (Italy)

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Abstract

To detect the genetic relatedness of emerging *Salmonella* serotypes of human origin circulating in Piedmont, Italy, 42 strains of S. Derby (N = 21), S. Napoli (N = 14), and S. Infantis (N = 7), collected in 2016, were characterized by pulsed-field gel electrophoresis (PFGE). Cluster analysis indicated high genetic similarity among the three strains: S. Derby (> 83%), S. Napoli (>83%), and S. Infantis (> 88%). Most of the isolates had the same or similar fingerprints in each cluster, strongly suggesting foods of animal origin as a source of infection in humans. PFGE proved a powerful, discriminatory tool for revealing the genetic relationships among these emerging S. serotypes and for monitoring and preventing the spread of *Salmonella*.

Keywords: *Salmonella Derby; Salmonella Napoli; Salmonella Infantis; Emerging Serotypes; PFGE*

Abbreviations

S: *Salmonella*; *PFGE: Pulsed-field Gel Electrophoresis;* CeRTiS: Centro di Riferimento Regionale per la Tipizzazione Delle Salmonelle.

Introduction

Salmonella is a leading pathogen in foodborne illness worldwide *[1]*. *Salmonella* infection ranks first as the cause of confirmed foodborne outbreaks and second as the cause of zoonosis. *Salmonella enterica* consists of six subspecies (*S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica)* and more than 2500 serovars responsible for the majority of human salmonellosis cases *[1]*. In the European Union, *S. enteritidis* and *S. typhimurium* are among the serovars most frequently associated with human illness *[2]* transmitted by contaminated chicken meat, eggs, and pork.

Recent data from the Centro di Riferimento Regionale per la tipizzazione delle Salmonelle (CeRTiS) showed that six serovars of the 458 *Salmonella* isolates of human origin most often obtained in Piedmont in 2016 were the monophasic variants of Typhimurium (48%), Enteritidis (18%), Typhimurium (8%), Derby (5%), Napoli (3%), and Infantis (2%) *[3]* and that the prevalence of *S*. Derby, *S*. Infantis, and *S.* Napoli increased in the region between 2015 and 2017.

Prevention, control, and monitoring programs, which are powerful tools to reduce the presence of *Salmonella* serotypes in the food production chain, coupled with better knowledge of the regional features of the circulation of *Salmonella* serotypes and contamination sources, can help to inform food safety intervention priorities and implement appropriate control measures *[4]*. Molecular typing methods have proved effective in determining clonal and strain distribution in various environments and sources. In addition, several molecular typing methods have been developed to investigate the molecular epidemiology of bacterial pathogens *[5]*. Pulsed-field gel electro-

phoresis (PFGE) is the most widely used molecular method to investigate widespread outbreaks of bacterial foodborne illness. By virtue of its specificity, PFGE can be employed to detect outbreaks and control them at an earlier stage, as well as enhance the detection of geographically dispersed outbreaks *[6]*. In laboratories, owing to the stability of generated profiles, the method's discriminatory power and reproducibility of results, PFGE is considered the gold standard for genotyping *Salmonella* and it is the only universal molecular method appropriate for all *Salmonella* serotypes.

For this study we used PFGE to evaluate the genetic relatedness among three emerging *Salmonella* serotypes - *S.* Derby, *S.* Napoli, and *S.* Infantis - isolated from human sources in Piedmont.

Materials and Methods

A total of 42 strains of *S.* Derby (N = 21), *S.* Napoli (N = 14), and *S*. Infantis (N = 7), collected in 2016, were analyzed. The strains were obtained from the faeces of 42 patients (*mean age 50* years, range 1 - 89) with acute infections.

All isolates had been previously identified as *Salmonella* according to ISO 6579-1 *[7]*; serotyping was performed according to ISO 6579-3 *[8]* and the Kaufmann-White scheme using O and H antisera (Statens Serum Institut) *[9]*.

To characterize the strains, PFGE analysis was conducted on all 42 *Salmonella* strains isolated according to a standardized PulseNet protocol *[10]*. Briefly, equal volumes of 1% agarose (SeaKem Gold Agarose, Lonza, Rockland, ME, USA) were mixed with 100 µL cell standardized suspension to form plugs. The bacteria within the plugs were lysed with cell lysis buffer and incubated at 55°C for 2h. The plugs were washed with sterile deionised water and TE buffer. Plug slices (1.5 mm thick) were then digested with 10 units of restriction enzyme *Xba*I (Thermo Scientific, Waltham, MA, USA) at 37°C for 2 h and loaded onto a 1% agarose gel (SeaKem Gold Agarose, Lonza, Rockland, ME, USA). PFGE was performed on a CHEF Mapper system (Bio-Rad, CA, USA) under the following conditions: initial switch time 6s - final switch time 24s, gradient 6V/cm, included angle 120, running time 20h at 14°C. The agarose gel was then stained with GelRed (Biotium, USA) and photographed under ultraviolet transillumination (Gel-Doc, Bio-Rad Laboratories s.r.l., Milano, Italy). *Xba*I restricted-*Salmonella* Braenderup, strain H9812 was used as the DNA size marker and positive control. The PFGE patterns obtained from the 42 strains were compared using Bionumerics version 7.6 software (Applied Maths, Belgium) with the Dice coefficient *[11]* with 2% band tolerance and 2% optimization and the unweighted pair group method with arithmetic averages (UPGMA) *[12]*. *PFGE* types were assigned using an in-house *nomenclature. Salmonella* strains sharing 100% similarity were considered identical and were assigned to the same PFGE type.

Results and Discussion

PFGE analysis with *XBa*I restriction enzyme was performed to determine the genetic relatedness of 42 emerging *Salmonella* strains. All the isolates had appreciable restricted digestion patterns ranging from approximately 40 to 1100 kb.

Analysis of the dendrogram highlighted a similarity coefficient of 78% for the 21 S. Derby strains (Figure 1). The strains grouped into two main clusters (A and B): cluster A consisted of 13 strains that shared 83% genetic homology, 9 different PFGE profiles, and 5 strains with 100% genetic similarity (PFGE type 0.001); cluster B consisted of 6 strains with 100% pattern homology (PFGE type 0.002). The S. Derby strains were genetically closely related and prevalently matched three areas in the region (Biella, Cuneo, Verbania). Among the 14 S. Napoli strains, there were 14 different PFGE patterns that had 76% homology (Figure 2). The dendrogram showed the presence of two main clusters: 3 strains with a similarity of 83% belonging to cluster A and 6 strains with a similarity of 90% belonging to cluster B, isolated in three areas of eastern Piedmont (Verbania, Alessandria, Novara). Finally, 5 strains of 7 S. Infantis isolated from western Piedmont (Turin and Cuneo) had 88% homology (Figure 2).

Like its prevalence elsewhere in Europe, *S*. Infantis accounted for 2.8% of all *Salmonella* serotypes, while the prevalence of *S*. Napoli and *S*. Derby (4% and 3.3%, respectively) was somewhat higher *[1]*. Between 2011 and 2015, the *Salmonella* serovars most frequently identified in humans in Italy were the monophasic variant of Typhimurium (37.3%), Typhimurium (21.6%), Enteritidis (9.3%), Napoli (4.7%), Derby (3.1%),

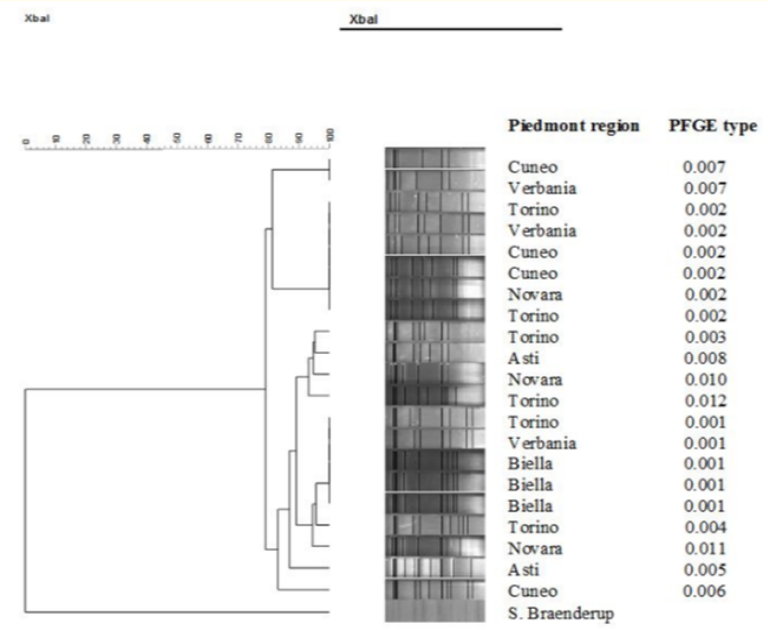

Figure 1: *Dendrogram of 21 pulsed-field gel electrophoresis–based profiles of Salmonella enterica serovar Derby strains isolated from human, in Piemonte, 2016.*

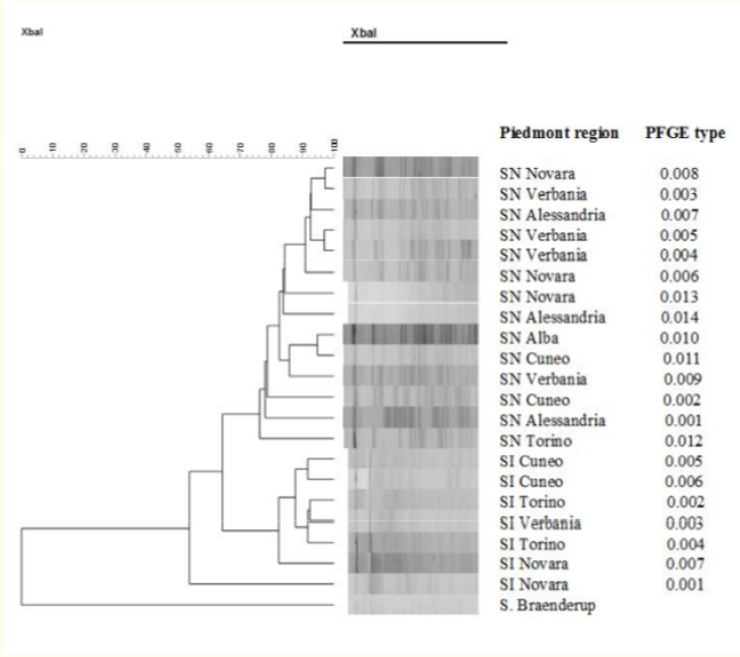

Figure 2: *Dendrogram of 21 pulsed-field gel electrophoresis–based profiles of Salmonella enterica serovar Napoli (SN) (N = 14) and Infantis (SI) (N = 7) strains isolated from human, in Piemonte, 2016.*

and Infantis (1.9%) *[13]*. Similar data were reported by the CeRTiS in 2016 for Piedmont. Outbreaks of *S.* Derby, *S*. Infantis, and *S*. Napoli infections of human origin occurred in Piedmont between 2015 and 2017, with an increase in the prevalence of these emerging serotypes from 18 to 27 cases for *S*. Derby (+ 50%), from 10 to 15 for *S*. Infantis (+ 50%), and from 9 to 27 for *S*. Napoli (+ 200%) (CeRTiS data not published).

PFGE has been successfully employed for molecular typing of *S*. infantis, *S*. Derby, and *S*. Napoli strains isolated from different sources worldwide. As observed in our study, epidemiological evidence supports the clonal distribution of these emerging *Salmonella* serotypes. Recent studies showed high similarity among *S*. infantis, *S*. Derby, and *S*. Napoli strains isolated from different sources in Italy. *S*. Infantis isolated from humans, animals, and the environment had a homology > 90% *[14]*. The relationship between porcine and human cases of salmonellosis caused by *S.* Derby has also been demonstrated *[15]*. Moreover, the human and environmental strains of *S.* Napoli (mainly cluster A in northern Italy and cluster B in central Italy from 2011 to 2015) suggest direct transmission of infection *[13]*.

The present study underlines the high similarity shared by the *Salmonell*a strains isolated from humans in Piedmont. Between 1 and 3 similar or identical PFGE profiles were found for each *Salmonel*la serotype in several areas of the region, highlighting the presence of genetically related *Salmonella* strains. The isolates in some clusters possessed the same or similar fingerprints, which strongly suggests the presence of related clones circulating in these environments and that foods of animal origin (poultry meat, eggs, and pork) are the probable source of human infection.PFGE proved a powerful, discriminatory tool for revealing genetic relationships among emerging *Salmonella* serotypes and for monitoring the incidence and dissemination of S. Derby, S. Infantis, and S. Napoli throughout the region.

Conclusion

This study extends our knowledge of the clones of *S*. Derby, *S*. Napoli, and *S*. Infantis circulating in Piedmont. As regards the other *Salmonella* serotypes, the uncontrolled spread of these emerging *Salmonella* clones in the environment and via associated foodborne illnesses poses a serious public health concern in the areas where they circulate.

Conflict of Interest

Declare if any financial interest or any conflict of interest exists.

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