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ORIGINAL ARTICLE

A foodborne outbreak of *Salmonella enterica* serotype Brandenburg as a hint to compare human, animal and food isolates identified in the years 2005-2009 in Italy

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Key words

Salmonella Brandenburg • Molecular epidemiology • Foodborne outbreak

Summary

Introduction. *There are only a few reported cases of Salmonella enterica serotype Brandenburg foodborne outbreaks in the literature. In Italy Brandenburg is consistently present among the top-ten serotypes from human source, but at low prevalences.*
Materials and methods. *Fifty-five S. Brandenburg isolates from human, animal, environmental and food sources, including twelve isolates from a foodborne outbreak, were genotyped by Pulsed-Field Gel Electrophoresis (PFGE).*
Results and discussion. *Eight pulsogroups and 19 pulsotypes were detected, with a unique pulsotype being attributed to the outbreak strains. Molecular subtyping can reliably complement the epidemiological investigations. Moreover, mapping molecular types of Salmonella isolates from human and non-human source may greatly contribute to risk assessment, by tracking possible animal sources, so improving cost-effectiveness of the prevention and control strategies.*

Introduction

Salmonella enterica serotype Brandenburg (*S*. Brandenburg) is one of the more than 2500 different serotypes of *Salmonella*. Kauffmann and Mitsui reported the first case of *S*. Brandenburg infection in 1930 following a case in Germany [1]. There have also been reports of outbreaks of *S*. Brandenburg gastroenteritis in England in 1963, Switzerland in 1992 and in Japan in 2001 [1, 2]. There are only a few reported cases of invasive *S*. Brandenburg infection in the literature [4-7]. In Europe, according to the WHO Global Foodborne Infections Network, in 2006 serotype Brandenburg ranked 15th among the isolates from human source [8]. In Italy, in the years 2000-2006, Brandenburg is present among the top-ten serotypes from human source, except for 2004 and 2005, but at low prevalences ranging between 0,9 and 1,7% [9]. The most recent report of the Enter-vet network places Brandenburg among the 15 *Salmonella* serotypes most frequently identified in 2007 from turkeys (3.2%) and in 2008 from pigs (1.1%), respectively [10].
On May 25th 2008, a large restaurant outbreak caused by *S*. Brandenburg occurred in Sesto Fiorentino (Florence), Tuscany. The event involved approximately two hundred individuals attending a banquet that had been organized for the participants to a volley tournament and their families. The outbreak prompted us to perform a molecular epidemiological study to support the standard epidemiological investigation and to elucidate the recent epidemiology of serotype Brandenburg in Italy.

Materials and methods

The outbreak

Twenty-seven attendees aged 12 to 76 years got advice from their general practitioners (GPs) or were hospitalized because of enteric symptoms and fever. A higher, but undefined, number of individuals suffered a less serious illness. A case-control study was conducted to evaluate specific food items from banquet. Meal companions of cases and attendees who reported no gastrointestinal symptoms since their restaurant meal served as controls. The second course, a meat dishes combination including lamb, pork, beef, rabbit and sausages, was identified as the outbreak source (data not shown). No food leftover was available for microbiological analysis. The environmental investigation revealed some pitfalls in structural features of kitchen and food handling practices. In particular, the kitchen appeared to be undersized to provide for the banquet under investigation. Twelve human *S*. Brandenburg isolates were available for the study.

Bacterial strains

Forty-one *S*. Brandenburg strains isolated from the outbreak and from apparently sporadic human cases occurring in the years 2005-2009 in different regions of Italy were analyzed. Fourteen isolates from foodstuffs, animals and environment were also available (Tab. I). The strains were identified by conventional methods and serotyped with respect to cell wall (O) and flagellar (H) antigens.

Pulsed-field gel electrophoresis

Preparation of cells and restriction of the bacterial DNA by digestion with the restriction endonucleases *Xba*I (5'-T/CTAGA-3') were done according to the Enternet proposed standard-protocol for Pulsed-Field Gel Electrophoresis (PFGE). DNA fragments were subjected to PFGE in agarose 1.2% w/v in 0.5X Tris Borate EDTA (TBE) (0.5M Tris, 0.1M boric acid, 0.2 mM EDTA, pH 8.0) buffer on a CHEF MAPPER system (Bio-Rad, Hemel Hempstead, United Kingdom) according to instructions of the manufacturer. Run times and pulse times were 2.20 to 54.0s for 22 h with linear ramping. *Xba*I-cleaved DNA of *S*. Braendenburg H9812 DNA was used as molecular size marker. DNA fragment patterns were visually assessed and the criteria for assigning of PFGE types and subtypes was according to published guidelines [11]. Four or more band differences in the electrophoretic profile defined a distinct PFGE type. Each major PFGE type was designated by a capital letter and subtype profiles indicated by a numerical suffix.

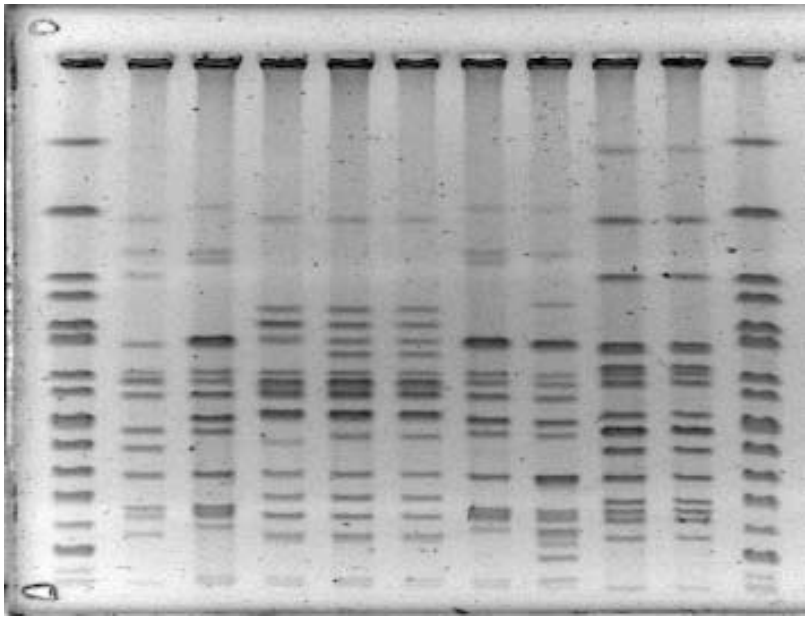
Results and discussion

Eight pulsogroups and 19 pulsotypes were detected (Tab. I). A unique pulsotype named A was shared by the outbreak isolates, by confirming their attribution to a point source and to a strain never identified in the same region from human cases. No food or animal isolates showed pulsotype A. Pulsogroup E, including nine pulsotypes, was the largest cluster and included human, animal, food and environmental isolates. This confirmed the prominent role of the swine reservoir in the transmission chain of such a serotype. Pulsogroups B and F also included isolates from pigs or pork and human source. Four pulsogroups, namely C, D, G and H, included isolates from human

Tab. I. Characteristics of the *S.* Brandenburg isolates under study.

Pulsogroup	Pulsotype	Source	Geographic area and year of isolation	Number of isolates
A	A	Human (outbreak)	Tuscany, 2008	12
B	B1	Human	Lazio, 2008	1
	B2	Human	Sicily, 2005	1
	B3	Animal (swine)	Lombardy, 2005	1
C	C1	Human	Tuscany, 2005 Lazio, 2008 Tuscany, 2008	4
	C2	Human	Tuscany, 2009	1
D	D	Human	Tuscany, 2005 Tuscany, 2008	2
E	E1	Human	Tuscany, 2006 Lombardy, 2007 Sicily, 2008	9
		Animal (swine)	Tuscany, 2009 Lombardy, 2006 Lombardy,2007	7
	E2	Human	Tuscany, 2008 Tuscany, 2009	3
	E3	Human food (pork)	Tuscany, 2006 Tuscany, 2008	1 1
	E4	Environment (urban sewage effluent)	Sicily, 2006	1
	E5	Food (pork)	Sicily, 2005	1
	E6	Human	Lombardy, 2005	1
	E7	Animal (swine)	Lombardy, 2005	1
	E8	Animal (swine)	Lombardy, 2007	1
	E9	Human	Tuscany, 2008	1
F	F	Human	Sicily, 2007 Sicily, 2008 Lombardy, 2007	3
		Food (pork)	Sicily, 2007	1
G	G	Human	Lombardy, 2008	1
H	H	Human	Tuscany, 2005	1

Fig. 1. Pulsotypes of representative isolates of *S.* Brandenburg.



Lanes: 1, pulsotype B1; 2 and 6, pulsotype C; 3, pulsotype E1; 4 and 5, pulsotype E2; 7, pulsotype D; 8, and 9, pulsotype A (oubreak type).

source only, thus suggesting a possible role for a non-swine source or reservoir.
S. Brandenburg is not a common *Salmonella* serotype and seems to have a relatively restricted host range. However, the isolates under study exhibited an unexpectedly wide range of pulsotypes.
In such conditions, mapping molecular types of *Salmonella* isolates from human and non-human source may greatly contribute to risk assessment, by tracking possible animal sources, so improving cost-effectiveness of the prevention and control strategies. Molecular subtyping can also reliably complement the epidemiological investigations, by confirming or withdrawing the role of suspected food exposures and allowing for more adequate corrective measures to be adopted [12, 13].

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