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Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011

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Carbapenem-resistant *Enterobacteriaceae* (CRE) are emerging as a public health problem in various settings. In Italy, a rapid and remarkable increase of carbapenem-non-susceptible *Klebsiella pneumoniae* has been reported since 2010. Here we report on the results of a countrywide cross-sectional survey, carried out from 15 May to 30June 2011 to investigate the diffusion of CRE in Italy and to characterise the most prevalent resistance mechanisms and their dissemination patterns. CRE were reported from most (23 of 25) participating laboratories, with an overall proportion of 3.5% and 0.3% among consecutive non-duplicate clinical isolates of *Enterobacteriaceae* from inpatients (n=7,154) and outpatients (n=6,595), respectively. *K. pneumoniae* was the most frequent species (proportion of carbapenem-non-susceptible isolates: 11.9%), while a minority of CRE of other species were detected. Carbapenemase production was detected in the majority (85%) of CRE. KPC-type enzymes were by far the most common (89.5% of carbapenemase producers), followed by VIM-1 (9.2%) and OXA-48 (1.3%). KPC-producing *K. pneumoniae* (KPC-KP) were detected in most centres and contributed majorly to the epidemic dissemination of CRE recently observed in our country. Dissemination of KPC-KP was mostly sustained by strains of clonal complex 258 (ST-258 producing KPC-2 or KPC-3, and ST-512 producing KPC-3), while a minority belonged to ST-101.

Introduction

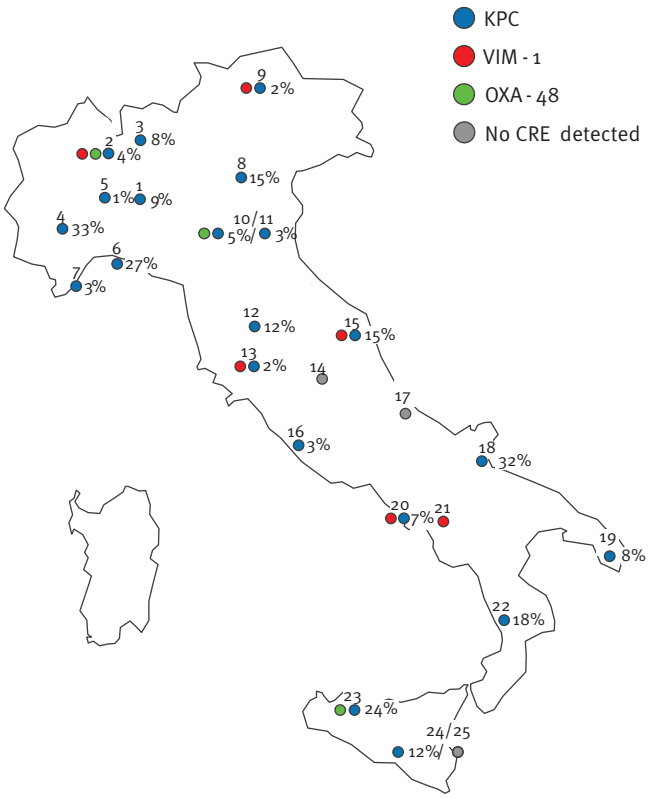
The increasing resistance to carbapenems among *Enterobacteriaceae* has become a public health problem of major concern [1,2]. Carbapenem-resistant *Enterobacteriaceae* (CRE) usually exhibit complex multidrug resistance phenotypes that leave very few therapeutic options [3,4], and infections caused by CRE are associated with increased morbidity and mortality in comparison with those caused by carbapenem-susceptible strains (72% versus 22%) [5].

At least two mechanisms can be responsible for acquired carbapenem resistance in *Enterobacteriaceae*: (i) reduced outer membrane permeability by porin loss in combination with the production of an extended-spectrum beta-lactamase (ESBL) or of AmpC-type beta-lactamase; and (ii) production of beta-lactamases capable of hydrolysing carbapenems (carbapenemases) [2]. While the former mechanism is a result of mutation and has a low overall propensity to disseminate, acquired carbapenemases are encoded by transferable genes that can disseminate among different strains and different species, and carbapenemase production is the leading carbapenem resistance mechanism in *Enterobacteriaceae* [2,6,7]. Several types of acquired carbapenemases have been detected in CRE, with KPC-, VIM-, NDM- and OXA-48-type enzymes being the most prevalent, although with a notable geographical variability [2,6-9].

In Europe, individual cases or outbreaks of CRE have been reported in several countries [6] but data from the EARS-NET database show that, until 2009, the proportion of CRE has remained overall low in most countries except Greece and Cyprus, where high-level endemicity of carbapenem-nonsuscetible *Klebsiella pneumoniae* has been reported since the mid-2000s [10]. In Italy, sporadic cases or outbreaks caused by CRE of various species and with different resistance mechanisms have been reported since the early 2000s [11-24], but only since 2010 an abrupt and notable increase in the proportion of carbapenem-non-susceptible *K. pneumoniae* has ben reported by the EARS-NET surveillance system

Figure 1

Location of the laboratories participating in the survey on carbapenem-resistant *Enterobacteriaceae*, Italy, 15 May–30 June 2011 (n=25)



1: Milan; 2: Varese; 3: Lecco; 4: Turin; 5: Novara; 6: Genoa; 7: Sanremo; 8: Verona; 9: Bolzano; 10-11: Modena; 12: Florence; 13: Siena; 14: Perugia; 15: Ancona; 16: Rome; 17: Pescara; 18: San Giovanni Rotondo; 19: Lecce; 20: Naples; 21: Avellino; 22: Cosenza; 23: Palermo; 24-25: Catania. The types of carbapenemases detected in different laboratories, and the proportion of KPC-producing *Klebsiella pneumoniae* versus the total number of *K. pneumoniae* isolates are indicated.

[10]. This trend has recently been confirmed by data from the Micronet sentinel surveillance network [25]. However, the resistance mechanisms responsible for this increase have not been investigated.

In this work we report the results of a countrywide cross-sectional survey promoted by the Italian Society of Clinical Microbiologists (AMCLI) and carried out in mid-2011, to investigate the diffusion of CRE in Italy and to characterise the most prevalent resistance mechanisms and their dissemination patterns. Results confirmed that CRE have reached epidemic dissemination in Italy, and revealed that this condition was mostly related with the clonal diffusion of *K. pneumoniae* producing KPC-type carbapenemases (KPC-KP) of clonal complex (CC) 258.

Methods

Study design

Twenty-five large clinical microbiology laboratories from 23 Italian cities, distributed across the national territory and covering most Italian regions, participated in the study (Figure 1). During the period from 15 May to 30 June 2011, each laboratory collected consecutive non-replicate clinical isolates of *Enterobacteriaceae*, from any site of infection, that exhibited minimum inhibitory concentrations (MICs) for imipenem and/or meropenem and/or ertapenem higher than 1 mg/L (for isolates of *Proteeae*, i. e. *Morganella morganii*, *Proteus* spp. and *Providencia* spp., only meropenem and ertapenem MICs were considered). The collected isolates were transferred to reference laboratories for confirmation of species identification and carbapenem MICs, and for characterisation of the carbapenem resistance mechanisms and analysis of clonal relatedness. For each isolate, information on the clinical specimen and type of ward (in case of isolates from inpatients) were provided. Moreover, each participating laboratory provided information on the total number of consecutive non- duplicate clinical isolates of *Enterobacteriaceae* observed during the collection period.

Characterisation of bacterial isolates and of resistance determinants

Bacterial identification and antimicrobial susceptibility testing were carried out by the collecting laboratories using either the Phoenix Automated Microbiology System (Becton Dickinson Diagnostic Systems, Sparks, United States) or the Vitek-2 System (bioMérieux, Marcy l'Etoile, France). Confirmatory identification was carried out by Matrix-assisted laser desorption ionisation – time of the flight (MALDI-TOF) mass spectrometry (Vitek-MS, bioMérieux). Confirmatory MIC testing for imipenem, meropenem and ertapenem was carried out by Etest (bioMérieux). All collected isolates confirmed to be non-susceptible to imipenem (not considered for *Proteeae*) and/or meropenem and/or ertapenem according to the EUCAST breakpoints [26] were considered as CRE for the purposes of this study. For KPC-KP, MICs of colistin, tigecycline and gentamicin were determined by the reference broth microdilution method [27], and results were interpreted according to the EUCAST breakpoints [26].

CRE were evaluated for carbapenemase production by meropenem plus EDTA and meropenem plus phenylboronic acid using the disk diffusion method [28,29], and for the presence of the most common carbapenemase genes (bla_{KPC}-, bla_{VIM}-, bla_{NDM}-, bla_{OXA-48}-type) by dot-blot hybridisation [30]. PCR amplification [13,31-33] and sequencing of PCR amplicons were used to identify the carbapenemase genes detected by hybridisation. All CRE isolates testing negative in disk diffusion test and/or in hybridisation assays were further investigated for production of carbapenemase activity by modified Hodge test [34] and by spectrophotometric assay with crude extracts [35].

TABLE 1

Proportions of carbapenem-non-susceptible *Enterobacteriaceae* detected in the first countrywide survey, Italy, 15 May–30 June 2011 (n=13,749)

Species	Isolates from inpatients		Isolates from outpatients		All isolates	
	Total	CRE (%)	Total	CRE (%)	Total	CRE (%)
Escherichia coli	3,844	4 (0.10)	4,765	1 (0.02)	8,609	5 (0.06)
Klebsiella pneumoniae	1,346	219 (16.3)	618	15 (2.4)	1,964	234 (11.9)
Klebsiella oxytoca	203	0	116	1 (0.86)	319	1 (0.31)
Enterobacter cloacae	361	15 (4.2)	144	0	505	15 (3.0)
Enterobacter aerogenes	147	4 (2.7)	68	2 (2.9)	215	6 (2.8)
Serratia marcescens	117	4 (3.4)	39	1 (2.6)	156	5 (3.2)
Proteus mirabilis	624	1 (0.16)	491	0	1,115	1 (0.09)
Citrobacter freundii	79	0	56	1 (1.8)	135	1 (0.74)
Hafnia alvei	24	2 (8.3)	8	0	32	2 (6.2)
Other species	409	0	290	0	699	0
Total	**7,154**	**249 (3.5)**	**6,595**	**21 (0.32)**	**13,749**	**270 (2.0)**

CRE: carbapenem-resistant *Enterobacteriaceae*.

All collected isolates confirmed to be non-susceptible to imipenem (not considered for *Proteeae*) and/or meropenem and/or ertapenem according to the EUCAST breakpoints [26] were considered as CRE for the purposes of this study.

Analysis of clonal relatedness

Genotyping of *K. pneumoniae* isolates by pulsed-field gel electrophoresis (PFGE) profiling of genomic DNA was carried out after digestion with XbaI with a CHEF-DRIII apparatus (Bio-Rad, Hemel Hempstead, United Kingdom) [36], and results interpreted as recommended by Van Belkum et al. [37]. Multi-locus sequence typing (MLST) of *K. pneumoniae* isolates was performed as previously described [38], and sequence types (STs) were assigned using the MLST web site [39].

Results

Proportions of carbapenem-resistant *Enterobacteriaceae* from inpatients and outpatients in Italy

During the study period (15 May–30 June 2011), a total of 13,749 consecutive non-replicate clinical isolates of *Enterobacteriaceae* were isolated at the 25 Italian laboratories participating in the survey. Overall, 270 isolates (2.0%) were confirmed as CRE. The proportion of CRE was approximately 10-fold higher among isolates from inpatients (3.5%) than among those from outpatients (0.3%) (Table 1).

Proportions of CRE in different species are shown in Table 1. *K. pneumoniae* was the most affected species (proportion: 11.9%) and contributed to the majority of CRE (234 of 270, 86.7%). Lower CRE proportions, but still higher than 2%, were observed among *Enterobacter* spp., *Serratia marcescens*, and *Hafnia alvei*. In *Escherichia coli* the proportion of CRE was very low (0.06%).

CRE were reported from 23 of the 25 participating laboratories (Figure 1). Carbapenem-nonsusceptible isolates of *K. pneumoniae* were detected at any of these 23 laboratories, with proportions ranging from 1.2 to 32.7% (mean: 11.5%) (Figure 1).

Carbapenem resistance mechanisms in carbapenem-resistant *Enterobacteriaceae*

Carbapenemase production was detected in the majority of CRE (85%) (Table 2). KPC-type enzymes were by far the most common (89.5% of carbapenemase producers). Other types of carbapenemases included VIM-1 and OXA-48 (9.2% and 1.3% of carbapenemase producers, respectively). KPC-type enzymes were only detected in *K. pneumoniae* and in one *E. coli*. VIM-1, although much less prevalent, was detected in a wider variety of bacteria: *K. pneumoniae*, *Klebsiella oxytoca*, *E. coli* and *Enterobacter cloacae*. OXA-48 was only detected in three *K. pneumoniae* isolates (Table 2). KPC-producers were detected in 21 of 25 centres, showing a countrywide distribution. VIM-1-producers were detected in six centres, while the three OXA-48-producers were from three different centres (Figure 1). Other types of carbapenemases, including NDM, were not detected.

TABLE 2

Mechanisms of resistance in carbapenem-nonsusceptible isolates of *Enterobacteriaceae*, Italy, 15 May–30 June 2011 (n=270)

Species	Isolates	Carbapenemase				Non-carbapenemase
		Total (%)	KPC	VIM-1	OXA-48	Total (%)
Escherichia coli	5	2 (40.0)	1	1	0	3 (60.0)
Klebsiella pneumoniae	234	223 (95.3)	204	16	3	11 (4.7)
Klebsiella oxytoca	1	1 (100.0)	0	1	0	0
Enterobacter cloacae	15	3 (20.0)	0	3	0	12 (80.0)
Others[a]	15	0	0	0	0	15 (100.0)
Total	**270**	**229 (84.8)**	**205**	**21**	**3**	**41 (15.2)**

[a] Including *Enterobacter aerogenes* (n=6), *Serratia marcescens* (n=5), *Proteus mirabilis* (n=1), *Citrobacter freundii* (n=1), and *Hafnia alvei* (n=2).

Carbapenem-non-susceptible *Klebsiella pneumoniae*: proportion in clinical specimens, distribution in hospital wards, and carbapenem MICs

The overall proportion of carbapenem non-susceptibility was approximately seven-fold higher in *K. pneumoniae* isolates from inpatients than in those from outpatients (16.3% versus 2.4%, Table 1). Considering isolates from inpatients, the proportion of carbapenem non-susceptibility was higher among bloodstream isolates than among isolates from other specimens (Table 3), revealing that carbapenem-non-susceptible *K. pneumoniae* strains circulating in Italy retained a remarkable potential for causing invasive infections. In the case of outpatients, carbapenem-non-susceptible isolates of *K. pneumoniae* were obtained only from urine, which was by far the most common specimen, and most of them were KPC-producers (Table 3).

Concerning the in-hospital distribution, 42.5% of the 219 carbapenem-nonsusceptible *K. pneumoniae* from inpatients were from intensive care units (ICUs), while 32.4% were from medical wards, 21.5% from surgical wards, and 3.6% from other areas.

Carbapenem MICs of the 234 carbapenem-nonsusceptible *K. pneumoniae* are reported in Table 4. Virtually all isolates were resistant to ertapenem, while some were intermediate or susceptible to the other carbapenems. A susceptible or intermediate phenotype to imipenem and/or meropenem was observed with most of the VIM producers, with the OXA-48 producers and with the non-carbapenemase producers, while most of the KPC producers were resistant also to these drugs.

Molecular epidemiology and susceptibility to non-beta-lactam agents of KPC-producing *Klebsiella pneumoniae*

To gather information on the molecular epidemiology of KPC-KP circulating in Italy, the 204 KPC-KP isolates were characterised by MLST and by PFGE genotyping, and their KPC allelic variants were determined by sequencing.

TABLE 3

Proportions of carbapenem-non-susceptible *Klebsiella pneumoniae* from different clinical sources, Italy, 15 May–30 June 2011 (n=1,346)

Source	Isolates from inpatients[a]		Isolates from outpatients[b]	
	Total	CNS-KP (%)	Total	CNS-KP (%)
Blood	179	40 (22.3)	7	0
Lower respiratory tract	219	40 (18.3)	38	0
Urine	647	93 (14.4)	503	15 (3.0)
Other	301	46 (15.3)	70	0
Total	**1,346**	**219 (16.3)**	**618**	**15 (2.4)**

CNS-KP: carbapenem-non-susceptible *Klebsiella pneumoniae*.

[a] Mechanisms of resistance: KPC (n=190; 86%);VIM (n=15; 7%); OXA-48 (n=3; 2%); non-carbapenemase producer (n=11; 5%).
[b] Mechanisms of resistance: KPC (n=14; 93%); VIM (n=1; 7%).

TABLE 4

Susceptibility of carbapenem-non-susceptible *Klebsiella pneumoniae* to various carbapenems, Italy, 15 May–30 June 2011 (n=234)

Resistance mechanism	Meropenem					Imipenem					Ertapenem				
	Range	MIC50	MIC90	%S	%R	Range	MIC50	MIC90	%S	%R	Range	MIC50	MIC90	%S	%R
KPC (n=204)	4 to >32	>32	>32	0	94	4 to >32	32	>32	0	96	16 to >32	>32	>32	0	100
VIM (n=16)	2 to >32	2	32	62	19	1 to >32	2	8	50	6	4 to >32	8	>32	0	100
OXA-48 (n=3)	1 to 8	1	1	66	0	0.5 to 8	1	1	66	0	>32	>32	>32	0	100
Non-carbapenemase (n=11)	0.25 to 4	0.5	1	90	0	0.25 to 1	0.5	1	100	0	1 to >32	8	32	0	90
Total CPE (n=223)	1 to >32	>32	>32	6	87	0.5 to >32	>32	>32	5	87	4 to >32	>32	>32	0	100
TOTAL (n=234)	0.25 to >32	>32	>32	10	82	0.25 to >32	32	>32	10	83	1 to >32	>32	>32	0	99

CPE: carbapenemase-producing *Enterobacteriaceae*; MIC: minimum inhibitory concentration; R: resistant; S: susceptible.

MICs in mg/L, interpreted according to EUCAST breakpoints [26]. Percentage of isolates with intermediate susceptibility not shown in the table.

MLST revealed that most isolates belonged in CC 258, either ST-258 or ST-512 (a single locus variant of ST-258), while a minority were ST-101. ST-258 and ST-512 isolates were detected in many centres, with an overall countrywide distribution, while ST-101 isolates were detected in only two centres, where ST-512 was also present (Figure 2). Isolates of ST-258 carried either bla_{KPC-2} or bla_{KPC-3} alleles, while those of ST-512 and of ST-101 carried bla_{KPC-3} and bla_{KPC-2}, respectively (Figure 2).

PFGE genotyping revealed an oligoclonal population of KPC-KP, with a prevalent PFGE profile, named A, consisting of several variants (A0 to A6) and including the isolates of CC-258. In particular, the A0, A4 and A5 variants included isolates of ST-512 producing KPC-3, while the A1 and A2 variants included isolates of ST-258 producing KPC-3, and the A3 and A6 variants included isolates of ST-258 producing KPC-2 (Figure 2). Each variant was detected in multiple centres (Figure 2), confirming the epidemic propensity of this lineage. An additional PFGE profile, named B and consisting of only a few variants (B0 to B2), included the isolates of ST-101 producing KPC-2 and was detected in two centres (Figure 2).

Susceptibility testing of the 204 KPC-KP against non-beta-lactam agents which often retain activity against these strains, revealed that 22.4% were resistant to colistin, 20.9% were non-susceptible to tigecycline, and 15.8% were non-susceptible to gentamicin. Concerning co-resistances, 1.5% of the KPC-KP were non-susceptible to all three drugs, while 6.4% were non-susceptible to tigecycline and colistin, 1% to gentamicin and colistin, and 2.7% to tigecycline and gentamicin. No association was detected between ST and non-susceptibility to colistin or tigecycline, while non-susceptibility to gentamicin was more frequent in isolates of ST-101 (85.8% versus 12.6% in ST-258 or 10% in ST-512, $p<0.05$).

Discussion

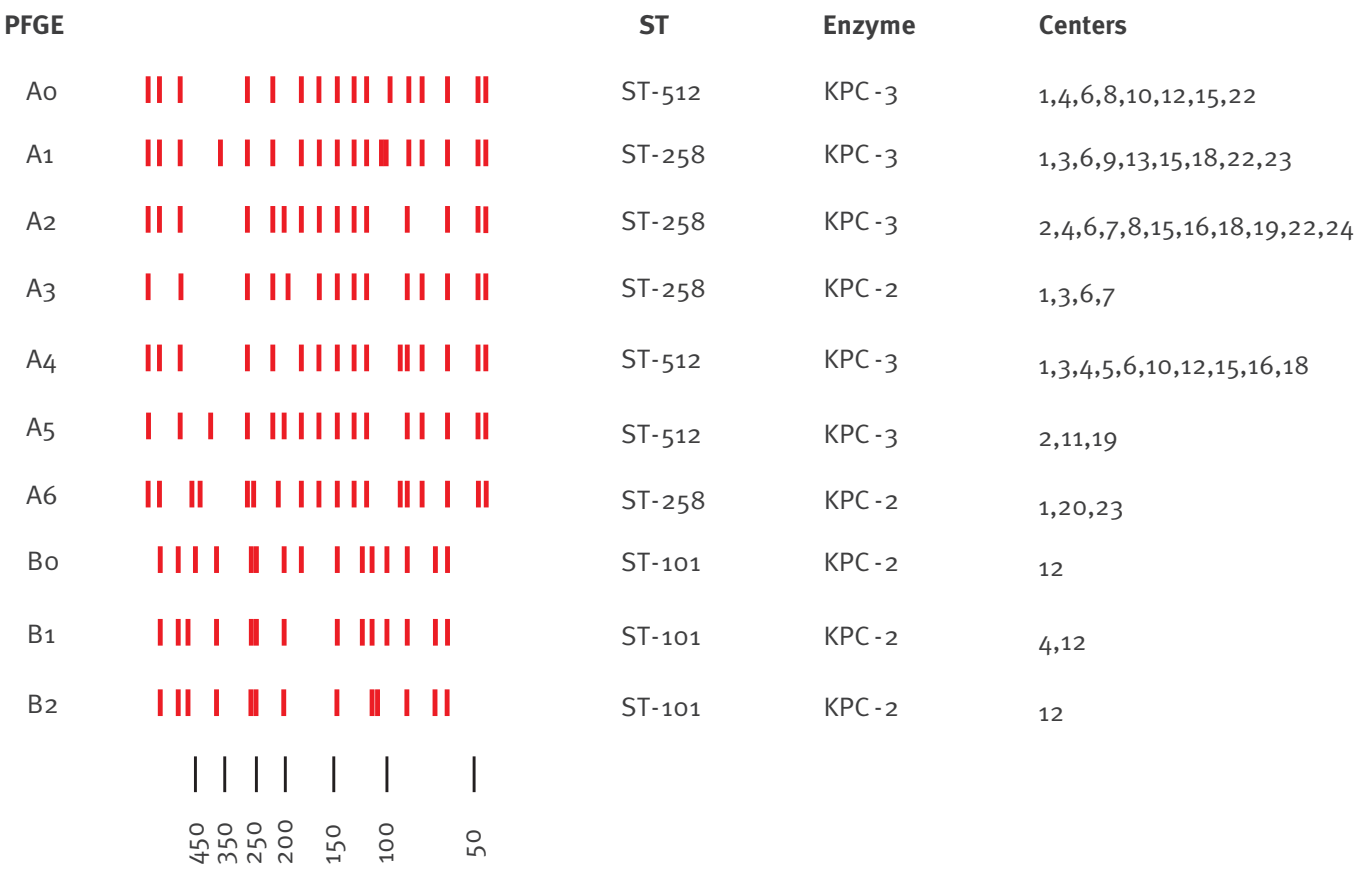
A rapid dissemination of carbapenem-non-susceptible *K. pneumoniae* has been reported in Italy since 2010 [10,24]. Results of this cross-sectional survey, carried out in mid-2011, confirmed that CRE have undergone an epidemic diffusion in Italy, with a widespread distribution across the national territory (although with variable proportions in different areas), and that the phenomenon was mostly related with rapid dissemination of carbapenem-non-susceptible *K. pneumoniae*, while the contribution by CRE of other enterobacterial species was much more limited.

Production of KPC-type carbapenemases was the most prevalent carbapenem resistance mechanism. Production of other carbapenemases and non-carbapenemase-mediated mechanisms were also detected, but remained in the background. However, continuous surveillance should be enforced, considering a recent outbreak caused by NDM-1-producing *K. pneumoniae* [18].

Expansion of KPC-KP strains belonging to variants (ST-258 and ST-512) of the hyperepidemic CC-258, detected for the first time in Italy in late 2008 [13], was responsible for a major part of the CRE epidemic in Italy. A similar

Figure 2

XbaI PFGE profiles of KPC-KP in combination with MLST results and KPC-type alleles, Italy, 15 May–30 June 2011 (n=204)



PFGE: pulsed-field gel electrophoresis; KPC-KP: KPC-type carbapenemase-producing *Klebsiella pneumoniae*; MLST: multi-locus sequence typing.

DNA size standards for PFGE profiles are indicated at the bottom. Distribution by centres of different PFGE-types is also indicated: 1: Milan; 2: Varese; 3: Lecco; 4: Turin; 5: Novara; 6: Genoa; 7: Sanremo; 8: Verona; 9: Bolzano; 10-11: Modena; 12: Florence; 13: Siena; 14: Perugia; 15: Ancona; 16: Rome; 17: Pescara; 18: San Giovanni Rotondo; 19: Lecce; 20: Naples; 21: Avellino; 22: Cosenza; 23: Palermo; 24-25: Catania.

phenomenon was also observed in other countries and further underscores the propensity for dissemination of multi-resistant *K. pneumoniae* strains belonging to ST-258 and ST-512 [40-47]. Emergence of KPC-KP belonging to ST-101 was also observed, although only in two of the participating centres. This finding, along with recent reports of ST-101 isolates of KPC-KP from Italy, Brazil and the United States [21,42,48,49], emphasises the emerging role in dissemination of KPC of this clonal lineage which is also involved in the dissemination of other carbapenemases, such as OXA-48 and OXA-181 [50,51] as well as extended-spectrum beta-lactamases [52,53].

Since aggressive infection control was shown to be effective in controlling the dissemination of KPC-KP [54-56], present results mandate for strong and prompt intervention in Italy. Implementation of infection control measures on a countrywide scale appears now to be necessary, in addition to the actions that have already been taken at local and regional level (the Italian public healthcare system has a typically regional organisation) with positive results. In a hospital in Catania, Sicily, it was possible to control the spread of a KPC-3-producing *K. pneumoniae* clone without closing the ICU, by applying a multimodal infection control programme [16]. The Emilia-Romagna region issued in July 2011 guidelines and protocols to monitor and control the spread of carbapemenase-producing *Enterobacteriaceae* in all the healthcare structures of the region. The increasing trend of KPC-producing *K. pneumoniae* slowed down in the second half of 2011 and early 2012, and in hospitals that had been able to implement all control activities the number of cases had a remained stable or showed a sustained decrease [57]. Dissemination of carbapenem-non-susceptible *K. pneumoniae* was not restricted to ICU settings, but affected all major hospital sectors. Although at least some of these patients could have been transferred from ICU settings, this propensity to dissemination on

multiple wards should be considered when planning infection control strategies.

Although cases and outbreaks of CRE have been reported in many European countries [6], only Greece, Cyprus and Italy have experienced such an extensive CRE epidemic to date. However, the phenomenon observed in these countries deserves considerable attention by public health authorities in Europe, considering the high mobility of people (tourists, workers, patients) within Europe, and the difficulties in containing the epidemic diffusion of CRE.

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Participants of the AMCLI-CRE Survey (the first Italian survey on carbapenem-resistant *Enterobacteriaceae*, promoted by the Italian Society of Clinical Microbiologists)

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Phenotypic and molecular characterisation of multiresistant monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) in Greece, 2006 to 2011

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Recently, multiresistant *Salmonella enterica* serovar 1,4,[5],12:i:-, a monophasic variant of *S.* Typhimurium (1,4,[5],12:i:1,2) emerged, and is now among the most common serovars isolated from humans in many countries. In Greece, monophasic Typhimurium which was recorded for the first time in human isolates in 2007 (0.3% of total isolates), increased sharply thereafter, and since 2009 is the third most frequent serovar. In the present study, 119 *S. enterica* 1,4,[5],12:i:- strains of human, animal and food origin, isolated during the period between 2006 and 2011, were examined. Strains verified as monophasic Typhimurium variants by polymerase chain reaction (PCR) (97 strains), were further characterised by phenotypic (antibiotic resistance and phage typing) and molecular (pulsed-field gel electrophoresis – PFGE) methods. The results indicate that multiple clones of multiresistant monophasic Typhimurium are circulating in Greece. The most frequently encountered clone in humans and pigs was that of phage type DT120, R-type ASSuTSpTm and PFGE profile STYMXB.0010, while in poultry other clones were detected. The data indicate that pigs may be a reservoir of this clone in Greece.

Introduction

Salmonella enterica serovars Enteritidis and Typhimurium have been reported to be the most common causes of human salmonellosis worldwide [1,2]. *S. enterica* serovar 1,4,[5],12:i:- is considered to be a monophasic variant of Typhimurium due to antigenic and genotypic similarities between the two serovars [3,4]. Recently, the number of cases infected with multiresistant *S. enterica* serovar 1,4,[5],12:i:- increased and, although it is difficult to monitor trends of monophasic strains because of the inconsistent way reported by different countries and organisations internationally, it is now considered to be among the ten most common serovars isolated from humans in many countries in Europe [5] and the United States of America (US) [2].

The European Food and Safety Authority (EFSA) proposes the use of polymerase chain reaction (PCR) to confirm *S.* Typhimurium monophasic variants, and also to discriminate from the other serovars that share the same somatic and flagellar (phase 1) antigens [5].

It is not as yet clear if infections caused by *S.* Typhimurium and *S. enterica* serovar 1,4,[5],12:i:- strains differ in severity, even though studies so far indicate that both serovars present similar virulence mechanisms [6-9].

Several studies [9,10] indicate that certain *S. enterica* monophasic Typhimurium isolates belong to multiple clones or clonal lines, which have emerged through independent deletion events, and can be further differentiated only by highly sensitive molecular methods (e.g. multilocus variable-number tandem repeat analysis (MLVA) and DNA microarray analysis).

Many studies support the hypothesis that pigs may be the reservoir of this serovar [6,11]. In the European Union (EU) wide baseline survey of slaughter pigs carried out in the period between 2006 and 2007 [12], serovar 1,4,[5],12:i:- ranked fourth in frequency in the pig lymph nodes, with an isolation rate of 4.9% in the EU.

Several foodborne outbreaks caused by this monophasic serovar have been reported, mainly due to contamination of pig products, e.g. in Luxembourg in 2006 [13] and France in 2010 [14], where pork meat and dried pork sausage were the suspected vehicles, respectively. Recently, other food vehicles were involved in outbreaks; a multistate outbreak was recorded in the US in 2011 linked to the consumption of contaminated alfalfa sprouts [15]. In Denmark an outbreak was reported in August 2012 due to beef [16].

In Greece *S. enterica* serovar 1,4,[5],12:i:- was first recorded from humans in 2007, accounting for only

0.3% of human cases of salmonellosis; its rate of isolation increased sharply thereafter, ranking third in frequency since 2009 [17]. In an EFSA report for the EU-wide baseline survey on the prevalence of *Salmonella* in slaughter pigs, the 'top five' serovars in Greece were *S.* Typhimurium, *S.* Derby, *S.* Thompson, *S.* Bredeney, *S.* Enteritidis, with *S.* serovar 1,4,[5],12:i:- accounting for the 2.7% of the isolates [12]. However, if all isolates gathered during the study are considered (including those that did not meet certain criteria as assessed by EFSA) the 'top five' serovars were *S.* Typhimurium, *S.* serovar 1,4,[5],12:i:-, *S.* Derby, *S.* Kottbus, and *S.* Bredeney [18].

In this study, we present the results of the phenotypic and molecular characterisation of *S.* serovar 1,4,[5],12:i:- strains, of human, food and animal origin isolated in Greece since 2006, with respect to their antimicrobial susceptibility profile, phage type and DNA fingerprinting using the pulsed-field gel electrophoresis (PFGE) method.

Methods

Human isolates

Human salmonellosis is a mandatory notifiable disease in Greece. All cases diagnosed by clinical and diagnostic laboratories must be notified to the Hellenic Centre for Disease Control and Prevention (HCDCP), which manages the surveillance of infectious diseases in Greece; *Salmonella* isolates are forwarded on voluntary basis to the National Reference Centre for *Salmonella* (NRCS) for serotyping and antimicrobial susceptibility testing. NRCS has been accredited by the National Accreditation Board (ESYD). Molecular typing is performed in outbreak investigations and epidemiological studies. During the period from 2006 to 2011, NRCS received 2,995 clinical isolates from 77 clinical microbiology laboratories (64 hospitals and 13 diagnostic laboratories). Surveillance data (patient information, specimen source, travel history, sporadic case/outbreak) were reported. During this period, 70 isolates were serotyped as *Salmonella enterica* serovar 1,4,[5]:i:- and were included in the study (Table 1).

Table 1

Origin of *Salmonella enterica* serovar 1,4,[5],12:i:- strains included in the present study, Greece, 2006–2011 (n=119)

Year	Source of *S. enterica* serovar 1,4,[5],12:i:- isolates		
	Human N	Animal N (type)	Food N (type)
2006	0	1 (pig)[a]	0
2007	2	15 (pig)[a]	2 (chicken meat)
2008	5	0	4 (beef, chicken, pork meat)
2009	18	2 (poultry)	2 (pork cold meat)
2010	20	2 (poultry, cattle)	8 (pork cold meat, pork souvlaki, beef ground meat, bivalve mollusc – shellfish)
2011	25	9 (poultry)	4 (beef, lamb ground meat)
Total	**70**	**29**	**20**

[a] Porcine lymph nodes, isolated and serotyped during the European Union monitoring study in 2006–2007 on the prevalence of Salmonella in slaughter pigs, from various regions in Greece [12,18].

Food and veterinary isolates

The National Reference Laboratory for *Salmonella* in Animals (NRL-Vet) receives strains isolated during official monitoring programmes carried out by the Hellenic Food Authority [19], food and feed business operators' control programmes, EU baseline studies, national control programmes and also veterinary isolates. NRL-Vet has been accredited by the National Accreditation Board (ESYD). Between 2006 and 2011 a total of 1,660 isolates, 577 from foodstuff and 1,083 from livestock, were submitted to NRL-Vet for serotyping; *Salmonella enterica* serovar 1,4,[5]:i:- accounted for 20 isolates from food of Greek origin and 29 isolates from animals, all included in the study (Table 1).

Strain characterisation

Serotyping of *Salmonella* spp. isolates was performed for the identification of somatic antigen O and flagellar antigens H (phase 1 and 2) by the slide agglutination method according to the White-Kaufmann–Le Minor Scheme [20]. To confirm that strains serotyped as *S.* serovar 1,4,[5]:i:- were *S.* Typhimurium monophasic variants, one multiplex PCR assay was applied to detect the presence of a specific for *S.* Typhimurium IS200 fragment, and the phase 2 (*fljB*) flagellar antigen gene, as described by Tennant et al. [21].

Susceptibility testing was performed by the agar disk diffusion method (Kirby-Bauer) according to the protocols and guidelines of the Clinical and Laboratory Standard Institute (CLSI) [22]. The following antibiotics (Biorad) were tested: ampicillin (A), amoxicillin-clavulanic acid, ceftazidime, ciprofloxacin, chloramphenicol (C), ceftriaxone, kanamycin, tobramycin, netilmicin, nalidixic acid (Na), streptomycin (S), spectinomycin (Sp), sulfonamides (Su), tetracycline (T), trimethoprim (Tm), sulfamethoxazole-trimethoprim.

Phage typing was performed on 50 isolates, 29 of human, 16 of animal and five of food origin, according to the protocol of the former Health Protection Agency (HPA).

PFGE was performed after digestion of genomic DNA with *Xba*I according to the Pulse-Net protocol [23]. Fingerprints were analysed using GelCompar II v.4.1 software (Applied Maths) and submitted to the PulseNet Europe database for assigning profile names. Dendrograms were constructed using the Dice

Table 2

Salmonella Enteritidis, Typhimurium and monophasic Typimurium human isolates, Greece, 2006–2011 (n=2,995)

S. enterica serovars	2006		2007		2008		2009		2010		2011		Total n (%)
	n (%)	Rank	n (%)	Rank	n (%)	Rank	n (%)	Rank	n (%)	Rank	n (%)	Rank	
Enteritidis	407 (58)	1	353 (56)	1	378 (63)	1	208 (51)	1	73 (30)	1	130 (31)	1	**1,549 (52)**
Typhimurium[a]	87 (12)	2	68 (11)	2	40 (7)	2	39 (10)	2	55 (23)	2	86 (21)	2	**375 (13)**
Monophasic Typhimurium[b]	0	NA	2 (0)	16	5 (1)	10	18 (4)	3	11 (5)	3	17 (4)	3	**53 (2)**
All others	203 (29)	NA	210 (33)	NA	176 (29)	NA	144 (35)	NA	102 (42)	NA	183 (44)	NA	**1,018 (34)**
Total n (%)[c]	697 (99)[c]		633 (100)[c]		599 (100)[c]		409 (100)[c]		241 (100)[c]		416 (100)[c]		**2,995 (101)[c]**

NA: not applicable.

The rank represents the classification of a serovar in terms of its relative frequency compared to all other serovars identified in human isolates in Greece.

[a] Excluding monophasic variants.

[b] After confirmation by polymerase chain reaction.

[c] Due to rounding, the sum of the percentages in a given category does not always equal to 100.

Figure 1

Differentiation of monophasic and biphasic *Salmonella* Typhimurium 1,4,5,[12]:i from other H:i serovars by polymerase chain reaction

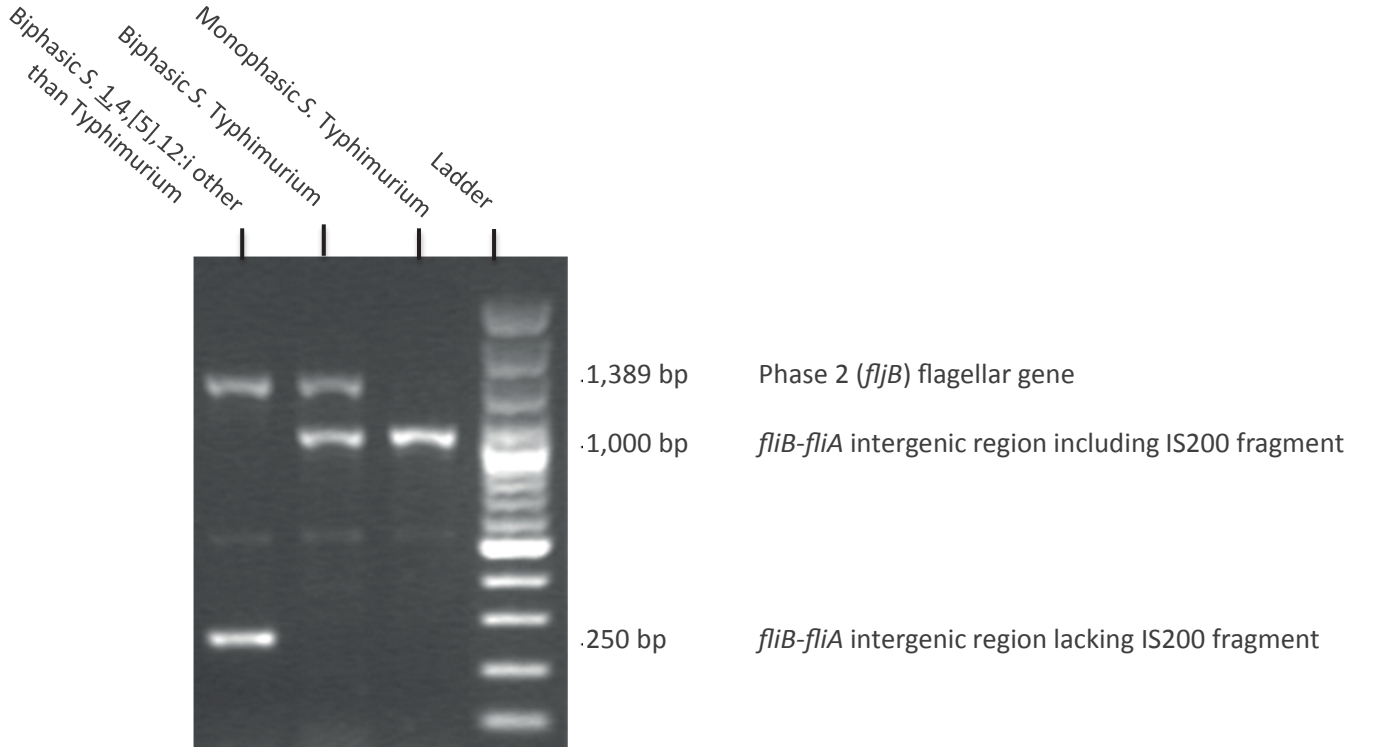


Table 3

Salmonella monophasic Typhimurium resistance-types and corresponding pulsed-field gel electrophoresis profiles and phage types, Greece, 2006–2011 (n=97)

Resistance types			Pulsed-field gel electrophoresis profiles			Phage types[a]		
Resistance-type	n (%)	Origin	STYMXB	n (%)	Origin	Phage type	n (%)[a]	Origin
ASSuTSpTm	54 (56)	1C, 5F, 30H, 11PG, 7PL	0010	39 (40)	4F, 23H, 11PG, 1PL	DT120	24 (48)	1F, 13H, 10 PG
						DT193	2 (4)	1H, 1PG
						DT97	1 (2)	1F
			0079	9 (9)	1F, 3H, 5PL	NT	NA	NA
			0131	3 (3)	2H, 1PL	DT120	2 (4)	2H
			Other	3 (3)	1C, 2H,	UT[b]	2 (4)	2H
ASSuT	22 (23)	7F, 10H, 3PG, 2PL	0010	4 (4)	2F, 2PG	DT 193	3 (6)	1F, 2PG
			0079	3 (3)	1F, 1H, 1PL	NT	NA	NA
			0131	7 (7)	5H, 1PG, 1PL	DT193	3 (6)	2H, 1PG
			Other	8 (8)	4F, 4H	DT193	2 (4)	1F, 1H
Other[c]	21 (22)	6F, 13H, 2PG	0010	3 (3)	1F, 1H, 1PG	DT120	2 (4)	1F, 1H
						DT193	1 (2)	1PG
			0079	6 (6)	4F, 1H, 1PG	DT120	1 (2)	1PG
						DT195	1 (2)	1H
						UT[b]	1 (2)	1F
			0131	5 (5)	5H	DT193	1 (2)	1H
			Other	7 (7)	1F, 6H	DT120	2 (4)	2H
						DT7	2 (4)	2H

C: cattle; F: food; H: human; NA: not applicable; NT: not typed; PG: pig; PL: poultry; UT: untypable.
Due to rounding, the sum of the percentages in a given category does not always equal to 100.

[a] Of the 97 isolates considered, only 50 were phage typed, so for the phage types the percentage is relative to a total of 50.
[b] Isolates that do not react with any of the typing phages.
[c] T (n=6), ACSSuSpTTm (n=4), SuSSpTm (n=4), ASSuSpTm (n=3), AT (n=1), S (n=1), AS (n=1), ASSuTTm (n=1).

similarity coefficient and the unweighted pair group method with arithmetic averages (UPGMA), with optimisation and position tolerance set at 0.5% and 1.5%, respectively.

Results

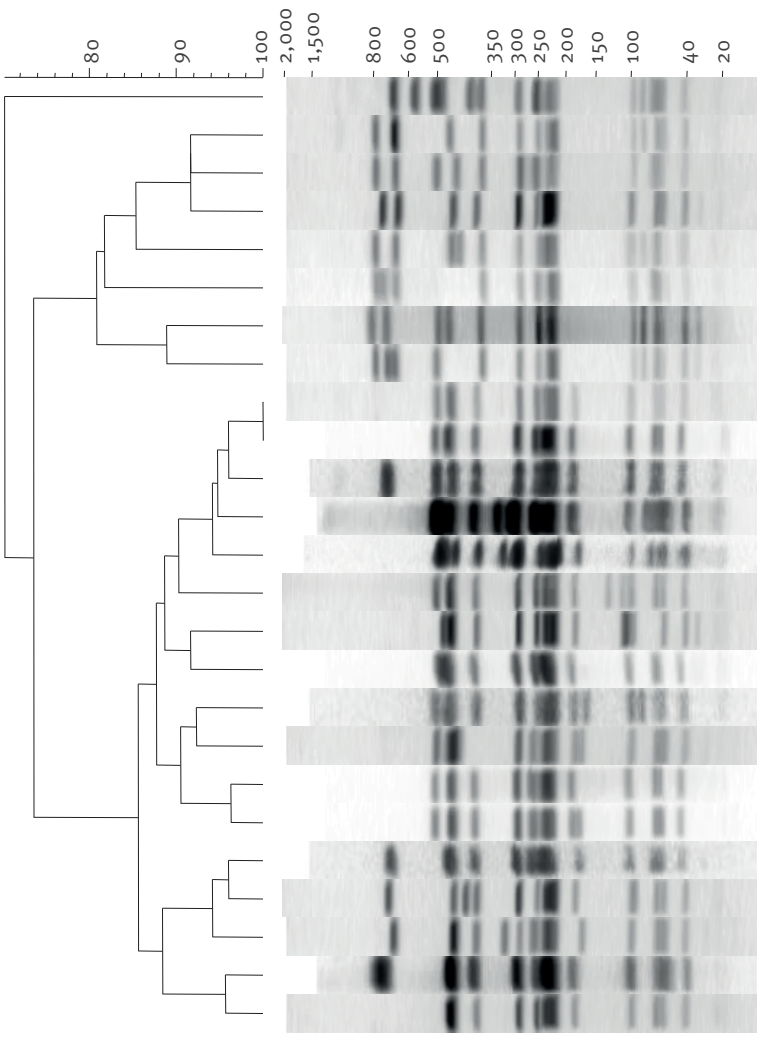
Total *Salmonella* isolates and percentages of the most frequent serovars, *S.* Typhimurium and *S.* Enteritidis, along with monophasic Typhimurium variants, from 2006 to 2011, are presented in Table 2. Total *Salmonella* isolates decreased by 40% (281/697) from 2006 (697 isolates) to 2011 (416 isolates). The frequency of *S.* Enteritidis decreased by 47% during the six-year period studied, from 58% (407/697) of all isolates in 2006 to 31% (130/416) in 2011. On the contrary, *S.* Typhimurium frequency (excluding monophasic variants) increased by 9%, from 12% (87/697) in 2006 to 21% (86/416) in 2011. Since 2007, when monophasic Typhimurium variants were first isolated from humans in Greece (2 isolates), their number increased almost ten times in 2011 (17 isolates), ranking third in frequency since 2009 (>4% of total isolates).

From a total of 119 isolates (70 of human, 29 of animal and 20 of food origin) (Table 1) serotyped as monophasic *S.* 1,4,[5],12:i:-, 97 (53 of human, 26 of animal and 18 of food origin), or 82%, were confirmed by the PCR assays as *S.* Typhimurium monophasic variants. The remaining of the 119 isolates included 18 (16 of human, 1 of food and 1 of animal origin) classified as *S.* Typhimurium and four (1 of human, 1 of food and 2 of animal origin) characterised as biphasic serovars but not Typhimurium (positive for the phase 2 (*fljB*) flagellar gene, but negative for the 1,000 bp IS200 fragment) (Figure 1).

Concerning the resistance of the 97 monophasic Typhimurium isolates to selected antibiotics, although several multiresistant patterns were observed (Table 3), the ASSuTSpTm pattern predominated (54/97, 56%), when all sources were considered together. The ASSuT

Figure 2

Pulsed-field gel electrophoresis profiles identified in isolates determined as *Salmonella* monophasic Typhimurium 1,4,5,[12]:i:- (n=97) and *S.* biphasic Typhimurium (n=18) by polymerase chain reaction, Greece, 2006–2011



Antigenic Type	PFGE profile	Number of isolates (n) Source
1,4,[5],12:i:1,2	Unnamed[a]	(1) H
1,4,[5],12:i:1,2	STYMXB0151	(8) 1F 6H 1PG
1,4,[5],12:i:1,2	STYMXB0217	(1) H
1,4,[5],12:i:-	STYMXB0028	(1) H
1,4,[5],12:i:1,2	STYMXB0013	(4) H
1,4,[5],12:i:1,2	STYMXB0202	(1) H
1,4,[5],12:i:1,2	STYMXB0087	(1) H
1,4,[5],12:i:1,2	STYMXB0161	(1) H
1,4,[5],12:i:1,2	STYMXB0010	(1) H
1,4,[5],12:i:-	STYMXB0010	(46) 7F 24H 14PG 1PL
1,4,[5],12:i:-	STYMXB0079	(18) 6F 5H 1PG 6PL
1,4,[5],12:i:-	Unnamed[a]	(2) H
1,4,[5],12:i:-	Unnamed[a]	(2) H
1,4,[5],12:i:-	Unnamed[a]	(1) C
1,4,[5],12:i:-	Unnamed[a]	(1) F
1,4,[5],12:i:-	STYMXB0132	(1) H
1,4,[5],12:i:-	STYMXB0054	(1) F
1,4,[5],12:i:-	Unnamed[a]	(1) H
1,4,[5],12:i:-	STYMXB0083	(2) H
1,4,[5],12:i:-	STYMXB0131	(15) 12H 1PG 2PL
1,4,[5],12:i:-	STYMXB0080	(2) F
1,4,[5],12:i:-	Unnamed[a]	(1) H
1,4,[5],12:i:-	STYMXB0206	(1) F
1,4,[5],12:i:-	Unnamed	(1) H
1,4,[5],12:i:	Unnamed	(1) H

C: cattle; F: food; H: human; PFGE: Pulsed-field gel electrophoresis; PG: pig; PL: poultry.

[a] No match to PulseNet PFGE profile names.

phenotype was observed in 23% (22/97) of the isolates; a remaining 21% (21/97) displayed resistance to one to four antibiotics.

PFGE analysis identified 17 unique profiles (Figure 2). Seventy-nine of 97 isolates (81%) were represented by the three predominant profiles (STYMXB.0010, STYMXB.0079 and STYMXB.0131) that shared more than 85% similarity (Figure 2). The remaining 14 profiles corresponded to one to two isolates each. The most frequent PFGE profile was STYMXB.0010 (46/97, 47%) (Table 4).

Phage typing of the 50 strains examined using the *S.* Typhimurium typing phages identified five different phage types (PTs), DT120, DT193, DT7, DT195, DT97 (Table 3). When all sources were considered together, the most frequently identified PTs were DT120 (31/50, 62%) and DT193 (12/50, 24%).

Combining PFGE profile and resistance-type (R-type) of all isolates, the STYMXB.0010 and ASSuTSpTm cluster was predominant (39/97, 40%); most of the isolates of this cluster belonged to DT120 (Table 3). This clone, represented in humans (13 of 53 human isolates) and pigs (10 of 16 pig isolates), is the most frequently

Table 4

Origin and proportion of pulsed-field gel electrophoresis profiles in *Salmonella* monophasic Typhimurium isolates, Greece, 2006–2011 (n=97)

Pulsed-field gel electrophoresis profile	n (%)	Origin
STYMXB.0010	46 (47)	7F, 24H, 14PG, 1 PL
STYMXB.0079	18 (19)	6F, 5H, 1PG, 6PL
STYMXB.0131	15 (15)	12H, 1PG, 2 PL
Other	18 (19)	1C, 5F, 12 H

C: cattle; F: food; H: human; PG: pig; PL: poultry.

occurring clone in Greece. Many other combinations were observed, though at very low frequencies (2–6%). Fifty-six percent (5/9) of the poultry isolates belonged to the cluster of ASSuTSpTm and STYMXB.0079.

Strains identified after PCR as biphasic Typhimurium presented different R-type (11 susceptible, five ACST(Na), and 1 T) and grouped in a PFGE cluster of 74% similarity with that of monophasic (Figure 2); only one strain exhibited similar R-type and PFGE profile as monophasic Typhimurium (ASSuTSpTm, STYMXB.0010).

Discussion

The incidence of infections caused by different *Salmonella* serovars and subtypes associated with different animal sources appears to change considerably over time. In Greece, between 2006 and 2011, the number of human isolates submitted to NRCS decreased by 40% and the notification rate fell by 47% [24]. Annual differences observed in the number of isolates are almost comparable to reported cases, even in 2010, when a sharp decrease of isolates (58%) and notifications (57%) occurred [24]. This decrease resulted from the proportionally fewer isolates and cases reported by each one of the clinical laboratories (hospitals and diagnostic laboratories). A decreasing trend was also observed for the Enteritidis serovar; the number of *S.* Enteritidis isolates decreased by 68% between 2006 and 2011, whereas total number of *S.* Typhimurium, and other isolates were fairly consistent over time. Considering together *S.* Typhimurium and its monophasic variant, their total numbers in 2010 and 2011 approximate those of *S.* Enteritidis. The above data indicate that the reduction of the total number of isolates can be attributed to the reduction of Enteritidis serovar. In the EU, the numbers of human salmonellosis confirmed cases declined from 2006 to 2010, by almost 40% [1,5], attributed to the marked reduction of *S.* Enteritidis cases reported in several European countries [25-28] and the successful *Salmonella* control programmes in fowl populations.

In the first decade of 2000, an international increase of the monophasic 1,4,5,[12]:i:- serovar was observed. This serovar appears to be ecologically successful since it has spread rapidly causing numerous human infections in many countries [5]; so far it does not appear to carry such virulent mechanisms as *S.* Typhimurium DT104, a particularly virulent clonal subtype that emerged in the early 1990s [10,29]. In Greece, monophasic Typhimurium ranks third among all serovars in humans since 2009 and in the EU was the fourth most common serovar in humans in 2010 [1]. According to our results, 18% of isolates (22/119) serotyped as 1,4,5,[12]:i:- were not confirmed as monophasic Typhimurium variants by PCR assays. Hopkins et al. [10] who examined 116 *Salmonella* 1,4,5,[12]:i:- isolates by PCR from several European countries, concluded that 19% were biphasic *S.* Typhimurium. Given that *S.* Typhimurium is included in the Commission regulations concerning the reduction of the prevalence of certain *Salmonella* serovars in *Gallus gallus* [30,31], and that the monophasic variant is targeted by measures to control *Salmonella* serovars of public health concern in laying hens [32], standard serotyping needs to be combined with PCR for the correct reporting of strains as Typhimurium (monophasic or biphasic) [33]. Moreover, the recent increase observed in monophasic variants of various serovars (NRCS, data not shown), makes the use of PCR assays necessary for epidemiological surveillance of such strains. For consistency in reporting monophasic strains in Greece, since 2012, NRCS and NRL-Vet have combined serotyping with PCR (IS200 fragment and phase 2 (*fljB*) flagellar antigen gene detection). This will also allow the identification of variants of Typhimurium serovar.

In this study, multiresistant phenotype ASSuT(SpTm) in all sources combined, accounted for 79% (76/97) of monophasic isolates in Greece between 2006 and 2011. This phenotype, being characteristic for monophasic *Salmonella* Typhimurium (1,4,[5],12:i), represents only 3.7% of *S.* Typhimurium human isolates in Greece (2006–2011) [17]. From 2007, when a multiresistant monophasic Typhimurium variant was first reported in humans, to 2011, susceptible non-monophasic Typhimurium isolates increased almost three times and penta-resistant ACSSuT – related to phage type DT104 – and other resistant isolates decreased proportionally in Greece [17]. According to Hopkins et al. [10], multiresistant monophasic Typhimurium variant counteracted to some extent the overall decline in the level of resistance in serovar Typhimurium observed in several European countries.

According to Hopkins et al. [10] and Lucarelli et al. [34] a clonal group of this monophasic serovar with resistance pattern ASSuT has emerged in Denmark, France, Germany, Italy, Poland, Spain, the Netherlands and the United Kingdom. However, in our results the majority of the isolates (54/97, 56%) presented additional resistance to trimethroprim and spectinomycin, both used widely in the pig and poultry industry in Greece.

Resistance to spectinomycin or trimethoprim is quite frequent in human and pig *Salmonella* Typhimurium isolates in European countries [35-37]. Whether these additional resistances are located on the same genomic resistance regions as ASSuT resistances [38], needs to be further investigated.

Seventeen unique PFGE profiles were identified among the 97 isolates, supporting previous observations that serovar 1,4,5,[12]:i:- can demonstrate considerable diversity, even among isolates from a single country [4,9,11,39]. However the majority of the monophasic isolates were allocated in the three most common profiles (STYMXB.0010, STYMXB.0079 and STYMXB.0131) with profile STYMXB.0010 accounting for 47% (46/97). Phage type DT120 (31/50, 62%) and DT193 (12/50, 24%) were the most frequently observed phage types for human, animal and food isolates.

Hopkins et al. [10], who examined 116 monophasic strains, of human and pig origin, from seven European countries, found that the most common phage types included DT193 (44%) and DT120 (23%), also found in biphasic *S.* Typhimurium; 47% of strains were represented by one of the three PFGE profiles also observed in Greece, STYMXB.0010, STYMXB.0079 and STYMXB.0131, with STYMXB.0131 being the predominant (28%).

All strains (except one) classified after PCR as *S.* biphasic Typhimurium in this study were of different R-type and PFGE cluster compared to monophasic variants, an indication that they represent a different clone.

According to our results, the main clone of monophasic Typhimurium (1,4,5,[12]:i:-) circulating in humans in Greece is that of phage type DT120, R-type ASSuTSpTm and PFGE profile STYMXB.0010. This clone predominates among pig isolates as well, indicating that pigs may be a reservoir of this clone in Greece. Clone of DT193, R-type ASSuT and PFGE profile STYMXB.0131, is predominant within several European countries [10,40], but has a low frequency in Greece.

This is an ongoing study, given that preliminary NRCS data, (January–September 2012), reveal a further increase of *S.* monophasic Typhimurium isolates in Greece (›15% of total isolates).

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Letter to the editor: Distinguishing between hantavirus-induced haemorrhagic fever with renal syndrome and pregnancy-induced liver pathologies (AFLP and HELLP syndromes)

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To the editor:

In their recent article, Macé et al. pointed appropriately to the many disturbing clinical similarities between haemorrhagic fever with renal syndrome (HFRS) due to Seoul virus (SEOV) infection – an emerging zoonosis – and two pregnancy-related pathologies affecting mainly the liver, acute fatty liver of pregnancy (AFLP) syndrome and haemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome [1]. At present, HFRS is one of the most frequent, but still heavily underestimated, forms of acute infectious kidney injury, with up to 200,000 cases per year worldwide. Moreover, SEOV affects not only the kidneys, but also often the liver as well [2].

Whereas urgent delivery is the gold standard for preserving both mother and fetus when a pregnant women has AFLP or HELLP, such intervention is rarely if ever needed when the women have in fact HFRS, as spontaneous self-remittance within two to three weeks is the rule [2,3]. Thus, therapeutic decision-making should be early and quick, preferably without awaiting time-consuming hantavirus serology, but should also be guided by subtle clinical differences at presentation. Anomalies in blood levels (not specified here) of alkaline phosphatase, bilirubin, glucose, uric acid and fibrin degradation products are more suggestive of AFLP or HELLP, rather than of HFRS [4,5]. Moreover, the classic symptom triad of pre-eclampsia (hypertension, proteinuria and oedema) often seen in women with AFLP or HELLP [4,5] was apparently absent in the reported case before and after admission. Conversely, C-reactive protein levels greater than 100 mg/L (norm: 1-5 mg/L), hyponatraemia, hypokalaemia or the 'lipid paradox' (very low acute cholesterolaemia, contrasting with hypertriglyceridaemia) could have pointed to HFRS [2,3]. Sudden, massive and nonselective proteinuria, even before hospital admission, is distinctive for HFRS, and simple urine examination should not be delayed until day 7, as in this case. Moreover, nephrotic-range proteinuria of 3.35 g/24 h, diminishing within three days to less than a fifth of its value, is highly atypical for AFLP (or HELLP), but is commonly seen in HFRS, where all 'lesions' heal rapidly without sequelae. A sudden renal deterioration a few days after worsening thrombocytopenia is very typical for HFRS, but should not suggest the need for any form of surgery or biopsy, even less so at a time (day 7) when activated partial thromboplastin time and levels of platelets, lactate dehydrogenase, C-reactive protein and most liver enzymes were already clearly normalising.

The authors of the article call, justifiably, for large studies focusing on SEOV epidemiology. Such studies would add to the findings of an earlier (1994) large seroepidemiological hantavirus study carried out in Northern Ireland [6]. Similar clinical cases of SEOV-induced HFRS, with both kidney and liver involvement, were also seroconfirmed in Portugal (1993), Northern Ireland (1994) and Bosnia and Herzegovina (1994) [as described in 7].

The presence of SEOV sero- and/or antigen-positive rats was documented in 1994, using an immunofluorescence assay (IFA) and/or enzyme-linked immunosorbent assay (ELISA), in 34 countries in the New and Old World (including France) [8]. This study yielded several SEOV isolates and stressed the importance of SEOV – the only worldwide pathogenic hantavirus, which is transmitted by the omnipresent wild rat.

Conflict of interest

None declared.

Authors' contributions

J. Clement conceived the article, and wrote the text. V. Vergote and L. Laenen performed or controlled the laboratory work. M. Van Ranst coordinated and edited the text.

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Authors' reply: Distinguishing between hantavirus-induced haemorrhagic fever with renal syndrome and pregnancy-induced liver pathologies (AFLP and HELLP syndromes)

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To the editor:

We thank Clement et al. for their interest in our work [1] and for questioning the diagnostic sequence and management of such a complex clinical and biological situation. During pregnancy, diagnosis of haemorrhagic fever with renal syndrome (HFRS) caused by hantaviruses, including Seoul virus (SEOV), can easily be missed due to its rarity and its 'pseudo-vasculoplacental' clinical presentation. Distinguishing between hantavirus infection and certain liver pathologies of pregnancy, especially severe preeclampsia with haemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome and acute fatty liver of pregnancy (AFLP), can be problematic.

While hantavirus infection can biologically mimic HELLP syndrome – because of proteinuria and the biological triad anaemia-thrombocytopenia-elevated levels of liver enzymes – its presentation and natural history are different. In a hantavirus-induced syndrome, viral symptoms are most noticeable, while hypertension can appear only secondarily. Anaemia occurs in the later stages of the infection and renal failure (interstitial nephritis) dominates the biological picture. Regarding AFLP, there are clearly clinical and biological similarities with hantavirus infection, even if AFLP preferentially occurs in women in the third trimester of pregnancy whereas hantavirus infection can be observed throughout pregnancy. The initial symptoms are common digestive disorders. Jaundice is common in AFLP and absent in hantavirus infection. Hypertension and hyperthermia may be observed in both AFLP and hantavirus infection. Biologically, renal failure, elevated levels of liver enzymes, thrombocytopenia and coagulation abnormalities are also frequent in both pathologies [2-7]. The Table synthesises the principal similarities between the three pathologies.

As pointed out by Clement et al. [1], fetal and maternal prognosis is highly dependent on the therapeutics used and in such confusing cases during pregnancy, and aetiological distinction before decision-making can be very difficult.

In AFLP or HELLP syndrome, emergency delivery is the only treatment, whereas in HFRS, continuation of pregnancy and symptomatic treatment are possible, thus avoiding neonatal prematurity without compromising maternal renal function. However, a dramatic reduction in renal function, or particular conditions such as a single kidney, could lead to a more aggressive management, as described in our case [8].

We agree with Clement et al. that the knowledge of clinical and biological natural history can help, to some extent, to discriminate between the possible diagnoses and evoke SEOV infection (especially normalisation of coagulopathy before renal failure), but obstetric decision-making must also take into account the worst probable outcome when the mother's health is at stake. Firstly, contrary to what has been suggested by Clement et al., alkaline phosphatase and bilirubin rates cannot be interpreted during pregnancy and we consider that the 'lipid paradox' is not relevant in an urgent situation. Secondly, SEOV infection has a quite reproducible pattern – initially with thrombocytopenia, then elevated levels of liver enzymes and finally transitory renal failure at the end of the illness [9] – which is not the case for HELLP and AFLP. Indeed, renal function can deteriorate dramatically in persons with HELLP or AFLP condition, potentially leading to terminal failure. Thus, if there is a rapid increase in the level of serum creatinine, waiting for the renal function to recover is risky when fetal extraction might restore the mother to health.

Table

Clinical and biological profiles of Seoul hantavirus-induced HFRS, AFLP and HELLP syndrome

Profile	Prevalence of symptom or condition in affected patients (%)		
	Seoul hantavirus-induced HFRS	AFLP	HELLP syndrome
Physical symptoms			
Fever	100	25–32	0
Headaches	70–89	ND	50
Myalgia	56–73	ND	ND
Retro-orbital pain	43–68	ND	ND
Back pain	58–85	ND	ND
Digestive symptoms	100	ND	ND
Nausea/vomiting	88	70	40
Abdominal pain	62–68	35–50	60–80
Diarrhoea	33	ND	ND
Hot flushes	41–78	ND	ND
Neurological signs	17–23	30–40	40–60
Hypertension	ND	50	85
Biological findings			
Haemolysis	0	15–20	50–100
Proteinuria	78	30–50	90–95
Hyperleucocytosis	72	ND	ND
Thrombopenia	82	100	100
Hepatic cytolysis	70–88	100	100
Renal failure	57–77	90–100	50
Coagulopathy (DIC or abnormal prothrombin time)	100	73	<20

AFLP: acute fatty liver of pregnancy; DIC: disseminated intravascular coagulation; HELLP: haemolysis, elevated liver enzymes and low platelet count; HFRS: haemorrhagic fever with renal syndrome; ND: not described.

Source: Seoul hantavirus-induced HFRS [9-11], AFLP [6,7], HELLP syndrome [6,7].

On the other hand, if one is aware of the similarities of these aetiologies, patients could be asked about hantaviral risk factors and a hantavirus serological IgM rapid-test, if available, could be requested once inflammatory biological syndrome (elevated C-reactive protein, elevated levels of liver enzymes) with coagulopathy occurs, while renal function is still correct or only moderately altered. This means that the observation period leading to diagnosis is very limited. When renal failure with proteinuria occurs, unless it has been clearly demonstrated that there is a hantavirus infection in another person living in the same environment, or in case of positive hantavirus rapid test, we suggest not to delay the delivery.

Conflict of interest

None declared.

Authors' contributions

Guillaume Macé wrote the letter. All co-authors reviewed the letter.

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European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2013 - call for abstracts

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The seventh European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) will take place in Stockholm, Sweden from 5 to 7 November 2013.

As every year, ESCAIDE 2013 will draw together professionals from around the world to present and discuss developments in infectious disease prevention and control.

The call for abstracts for the conference is now open, and abstracts can be submitted via the dedicated 'call for abstracts' portal on the ESCAIDE website (http://www.escaide.eu/). The closing date for submissions is 5 July 2013.

Abstracts are welcomed in all areas related to infectious disease intervention, including epidemiology, public health microbiology, surveillance, vaccinology and the application of tools and methods to prevent and control communicable diseases.

The 2013 conference aims at emphasising the relevance of core public health disciplines such as epidemiology and microbiology, and their use in supporting outbreak containment, disease prevention and ultimately public health policy. Hence abstracts which focus on public health science in support of action and policy are particularly welcomed.

The final programme details and conference registration instructions will be posted soon on the ESCAIDE website.

For further information, contact:
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The European Monitoring Centre for Drugs and Drug Addiction publishes the 'European Drug Report 2013: Trends and developments'

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On 28 May 2013, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) published the European Drug Report 2013: Trends and developments (EDR). The EDR consists of four interlinked elements: (i) the report Trends and developments, (ii) the Statistical bulletin, (iii) Country overviews and (iv) Perspectives on drugs. It is based on information provided by European Union (EU) Member States, Croatia, Turkey and Norway in 2011 or the latest year available [1].

The EMCDDA reports positive developments with regard to more traditional drugs. There are fewer new users of heroin and fewer injecting drug users. However, there are concerns about new drugs as 73 new psychoactive substances were notified officially for the first time through the EU Early Warning System (EWS) in 2012. There are concerns about globalisation and internet technology driving changes in supply and demand, as the availability of drugs on the internet has increased.

Drug injection continues to be important for the transmission of infectious diseases, including HIV/AIDS and hepatitis C. The report states that the long-term decline in new HIV diagnoses related to drug injection in Europe might be halted following outbreaks among injecting drug users in Greece and Romania [1,2]. Hepatitis C virus (HCV) antibody prevalence among national samples of injecting drug users in 2010–11 varied from 18% to 80%, with eight of the 12 countries with national data reporting a level over 40%. Among countries with national trend data 2006–11, declining HCV prevalence in injecting drug users was reported in three (Italy, Portugal, Norway), while two others observed an increase (Greece, Cyprus) [1].

Providing the information necessary to assist the implementation of the EU strategy on drugs for 2013–2020, adopted in December 2012, is an important part of the EMCDDA mission, and the EDR plays an important part in this context.

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The European Union Agency for Fundamental Rights publishes the European Union lesbian, gay, bisexual and transgender survey

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On 17 May 2013, the European Union Agency for Fundamental Rights (FRA) presented the results of its online survey on fear, isolation and discrimination in the lesbian, gay, bisexual and transgender (LGBT) community [1].

In 2010 the European Commission requested the FRA to collect data on hate crime and discrimination among LGBT people in the European Union (EU) and Croatia. Over 93,000 people responded to the online survey 'European Union survey of discrimination and victimisation of lesbian, gay, bisexual and transgender persons'.

The results of the survey reveal that 47% of respondents had felt discriminated against or harassed during the preceding year. Of the respondents which had been attacked during that period, a majority (59%) reported that an attack or threat of violence were entirely or partly due to being perceived to be LGBT. However, respondents rarely report violence or discrimination as they do not believe that reporting incidents to authorities would make any difference.

The fear of disclosing sexual orientation can have effects also on the reporting of diseases, such as human immunodeficiency virus (HIV) infection. In countries where discrimination against gay men, for example, is high, the reporting of HIV may become misrepresented, as gay men with HIV may not dare disclose the nature of their sexuality. In such circumstances an HIV case may be reported as a heterosexually transmitted case rather than as a homosexually transmitted one.

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