Genetic diversity of Streptococcus suis clinical isolates from pigs and humans in Italy (2003-2007)

M S Princivalli[1], C Palmieri[1], G Magi[1], C Vignaroli[1], A Manzin[2], A Camporese[3], S Barocci[4], C Magistrali[4], B Facinelli (b.facinelli@univpm.it)[1]

1. Department of Biomedical Sciences, Polytechnic University of Marche Medical School, Ancona, Italy
2. Department of Biomedical Sciences and Technologies, Section of Medical Microbiology, University of Cagliari Medical School, Italy
3. Microbiology and Virology Department, S. Maria degli Angeli Regional Hospital, Pordenone, Italy
4. Experimental Zooprophylactic Institute of Umbria and Marche, Perugia, Italy

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Streptococcus suis, a major porcine pathogen, is emerging as a zoonotic agent capable of causing severe invasive disease in humans exposed to pigs or pork products. *S. suis* infection is rare in industrialised countries and usually arises as sporadic cases, with meningitis the most common clinical presentation in humans. Recent reports of two cases of meningitis in Sardinia and north-eastern Italy prompted this first characterisation of Italian *S. suis* isolates. Fifty-nine *S. suis* strains, the two recent human strains and 57 swine clinical isolates collected between 2003 and 2007 from different Italian herds and regions, were tested for antimicrobial susceptibility, PCR-screened for virulence and antibiotic resistance genes, and subjected to molecular typing. Phenotypic and genotypic analysis demonstrated an overall high genetic diversity among isolates, the majority of which were resistant to macrolides (78%) and tetracyclines (90%). The *erm*(B), *tet*(O), mosaic *tet*(O/W/32/O), *tet*(W), and *tet*(M) genes were detected. The *tet*(O/W/32/O) gene, the most frequent *tet* gene after *tet*(O), had never been described in the genus *Streptococcus* before. In addition, a virulent *cps*2, *erm*(B) *tet*(O) clone, belonging to sequence type 1 (ST1) of the ST1 complex, was found to be prevalent and persistent in Italian swine herds. Finally, the two human isolates (both ST1) carrying *cps*2, *erm*(B) and *tet*(W) were seen to be closely related to each other.

Introduction

Streptococcus suis, a major porcine pathogen endemic in nearly all countries with a developed swine industry, causes meningitis, pneumonia, arthritis, endocarditis, and septicaemia in pigs [1]. *S. suis* is also emerging as a zoonotic agent capable of causing severe invasive disease in humans exposed to pigs or to pork products [2,3]. A carriage state has been documented in pigs, healthy carriers being a source of *S. suis* transmission in herds, mainly through the respiratory route [1]. As discussed in recent reports, the possibility cannot be excluded that humans may also be healthy carriers [1,3,4] and that *S. suis* may become an opportunistic pathogen under particular circumstances such as stress, immunodeficiency or cancer [1,5]. Meningitis with possible residual deafness is the most frequent clinical presentation of the infection in humans; septicaemia, pneumonia, endocarditis, arthritis and toxic shock syndrome have also been described. In industrialised countries, *S. suis* disease is rare, albeit probably underdiagnosed, and usually occurs as sporadic cases [2,3]. Most human cases reported so far originated from Southeast Asia, where the disease can be considered endemic and where some outbreaks have occurred [3]. Three major sequence type (ST) clonal complexes (ST1, ST27 and ST87) dominate the population [6]. The virulent ST1 complex, frequently associated with invasive infections, includes sequence type ST1, spread worldwide and recently detected for the first time in Italy [5], and ST7, responsible for several cases of toxic shock syndrome during a recent outbreak in China [7].

The antiphagocytic polysaccharide capsule (encoded by the cps gene) is the major virulence factor of *S. suis*. Thirty-three serotypes based on capsular antigens are currently recognised [8,9]. Serotype 2 is responsible for severe infections in swine [1] and is the most common serotype affecting humans worldwide [2]. The small number of human *S. suis* infections in North America has been linked to the low prevalence of serotype 2 among swine [1]. Serotypes 4, 14 and 16 have also been described in humans [1]. Proposed *S. suis* virulence factors [1], the significance of which is still unknown, include the muramidase released protein MRP (encoded by *mrp*), a peptidoglycan-associated protein probably acting as an adhesin and the extracellular protein factor EF (*epf*), both of which are suitable virulence markers of serotype 2 strains [10] and are also detected in other serotypes [11], a serum opacity factor OFS (*ofs*), proposed as a virulence trait of *cps*2 isolates [12,13], suilysin (*sly*), a haemolysin with a cytotoxic effect on various cell types [1], and arginine deiminase (*arc*A), a factor linked to survival in stress conditions [14]. Despite the lack of evidence for a critical role of one or more of these putative virulence factors in virulence, they may nonetheless serve as virulence markers, since MRP, EF, and suilysin are typical of Eurasian strains of the ST1 complex, while they are almost absent in less virulent North American strains [1]. An immune evasion strategy has recently been proposed to account for the allelic variability observed in *mrp*, *epf*, and *ofs* genes [11,13].

A trend toward mounting *S. suis* resistance to macrolides and tetracyclines has been reported worldwide [15-17]. Studies of genetic resistance traits have demonstrated *erm*(B) (ribosomal methylation) and *mef*(A) (active efflux) for macrolide resistance, and *tet*(M) and *tet*(O) (both ribosomal protection) for tetracycline

resistance [18-21]. The *tet*(W) gene, an emerging determinant commonly found in species inhabiting human and animal intestinal tracts [22], was first detected by our group in a human isolate of *S. suis* from a case of meningitis in Italy [5].

Overall, three human cases of *S. suis* meningitis have been reported in Italy, one in the 1990s [23] and two quite recently, in the course of little more than a year. The short interval between the last two cases and their arising in distant geographic areas, i.e. north-eastern Italy [24] and Sardinia [5], prompted this first characterisation of Italian *S. suis* isolates.

Methods

S. suis strains

A total of 59 *S. suis* isolates were studied, two of human and 57 of porcine origin (Table 1). The human isolates, one from Sardinia (SsCA-1: *cps*2 ST1 *erm*(B) *tet*(W)) [5] and the other from north-east Italy [24], here designated as SsUD, were from cerebrospinal fluid (CSF) of two patients with *S. suis* meningitis. All pig isolates were from clinical samples (23 brain, 22 lung and 12 spleen samples) collected in 24 herds in northern and central Italy from 2003 to 2007. They were divided into invasive (brain and spleen isolates: 35 strains) and non-invasive (lung isolates: 22 strains) according to the source of isolation. All strains were isolated on 5% sheep blood agar (Oxoid Ltd) and identified with ID 32 STREP kit (bioMérieux). Serotyping was performed by slide agglutination using specific antisera (Statens Serum Institute).

Susceptibility testing

Antimicrobial susceptibility testing by agar disk diffusion and minimal inhibitory concentration (MIC) was carried out according to standard procedures [25,26] (erythromycin and tetracycline antibiotics: Sigma Chemical Co, disks: Oxoid). *S. pneumoniae* ATCC 49619 was used for quality control. The erythromycin resistance phenotype was determined on the basis of the triple disk test (erythromycin plus clindamycin and josamycin) [27].

Genotyping

PCR amplification was carried out under published conditions using the oligonucleotide primer pairs and target genes listed in Table 2 [28-33].

Pulsed-Field Gel Electrophoresis (PFGE) was applied to study the genetic diversity of *S. suis* [19,34-36]. Macrorestriction with *Sma*I endonuclease (Roche) and PFGE analysis were performed essentially as described previously [35]. PFGE data were analysed considering each band as a separate putative locus and scoring it as present (1) or absent (0) in each accession. The dendrogram was constructed by use of the Dice coefficient and the unweighted pair group method with arithmetic averages. Genetic relatedness was interpreted according to the criteria of Tenover *et al.* [37].

A multilocus sequence typing (MLST) scheme for *S. suis* was developed in 2002 [6]. Primers for PCR amplification and sequencing of the housekeeping gene fragments of *aro*A (EPSP synthase), *cpn60* (60-kDa chaperonin), *dpr* (peroxide resistance), *gki* (glucose kinase), *mutS* (DNA mismatch repair enzyme), *recA* (homologous recombination) and *thrA* (aspartokinase) were

Table 1

***Streptococcus suis* isolates, Italy, 2003-2007 (n=59)**

Origin (no. of isolates)	Strain (herd*)	Area in Italy	Year
Pig (57)			
Brain (23)	v3 (PG/5), v20 (PG/1), v24 (PG/2)	Centre	2003
	v27 (PG/4), v28 (PG/2), v29 (MC/1), v31 (PG/1), v32 (PG/1), v34 (AR/1), v35 (AR/1), v40 (TR), v42 (PG/1), v36 (PG/1)	Centre	2004
	v54 (MC/2), v75 (PG/1), v76 (PG/1)	Centre	2005
	v96 (PG/1), v97 (PG/3)	Centre	2006
	170167 (RE), 188509 (RE), 219624 (RE), 202707 (RE)	North	2007
	v123 (PG/1)	Centre	2007
Spleen (12)	v73 (LT)	Centre	2005
	45445 (AP/1)	Centre	2006
	240370 (RE), 205206 (RE), 210671 (RE), 167757 (RE)	North	2007
	20801 (LI), 1303 (AP/1), 22583 (AP/1), 11683 (AP/1), 11707 (PG/7), 13469 (AP/1)	Centre	2007
Lung (22)	v21 (PU), v23 (IS), v25 (CH), v26 (PG/2)	Centre	2003
	3721 (AP/5), v38 (PG/8)	Centre	2004
	v92 (PG/3), (AP/1) 27894, (AP/1) 33421, (AP/3) 18237, 30676 (AP/1)	Centre	2006
	227794 (RE), 176414 (RE)	North	2007
	9649 (PG/6), 22919 (AP/2), 10432 (PG/6), 36774 (AP/1), 30203 (AP/4), 18315 (AN), 1227 (AP/4), 10584 (AP/1), 32457 (AP/1)	Centre	2007
Human (2)			
CSF (2)	SsUD	North	2006
	SsCA-1	Sardinia	2007

* AN: Ancona, AP: Ascoli Piceno (5 herds), AR: Arezzo, CH: Chieti, IS: Isernia, LI: Livorno, LT: Latina, MC: Macerata (2 herds), PG: Perugia (8 herds), PU: Pesaro/Urbino, RE: Reggio Emilia, TR: Terni.
CSF: cerebrospinal fluid.

synthesised according to the primer sequences on the *S. suis* MLST database website (http://ssuis.mlst.net). Sequences were compared with previously observed allelic sequences in the *S. suis* MLST database for identification of ST.

The nucleotide sequences reported here have been submitted to the GenBank/EMBL sequence database and assigned accession numbers FM201280 (*ofs*$^{type\ 1S}$), FN357200 (*epf*^{915}), FN356743 (*tet*(W)) and FM164392 (*tet*(O/W/32/O)). Sequence similarity searches were carried out using BLAST, available online from the National Center for Biotechnology Information of the National Library of Medicine (http://www.ncbi.nlm.nih.gov).

Results

Capsular (cps) and virulence-associated genes

The 59 *S. suis* isolates were investigated by PCR using primer pairs specific for *cps*1, *cps*2, *cps*7, and *cps*9, and for virulence-associated genes *mrp*, *epf*, *ofs*, *sly*, and *arc*A. Size variants were detected by restriction analysis (*epf*: *Hin*dIII; *ofs*: *Mbo*I) and sequencing (*ofs*) of PCR products (Table 3). The distributions of *cps* and virulence-associated genes are reported in the Figure, and virulence profiles among invasive and non-invasive isolates are shown in Table 4.

Table 2

Streptococcus suis **PCR primers and target genes**

Primers	Gene target	Primer sequence (5'-3')	Product length (bp)	Reference
Macrolide resistance genotype				
ERMB 1 ERMB 2	*erm*(B)	GAAAAGGTACTCAACCAAATA AGTAACGGTACTTAAATTGTTTAC	639	[28]
III_{10} III_8	*erm*(TR)	AGGTTATAATGAAACAGA GCATGACATAAACCTTCA	208	[29]
MEFA 1 MEFA 2	*mef*(A)	AGTATCATTAATCACTAGTGC TTCTTCTGGTACTAAAAGTGG	346	[28]
Tetracycline resistance genotype				
TETK-up TETK-rev	*tet*(K)	TATTTTGGCTTTGTATTCTTTCAT GCTATACCTGTTCCCTCTGATAA	1,159	[30]
TETL-up TETL-rev	*tet*(L)	ATAAATTGTTTCGGGTCGGTAAT AACCAGCCAACTAATGACAATGAT	1,077	[30]
TETM F TETM R	*tet*(M)	GAACTCGAACAAGAGGAAAGC ATGGAAGCCCAGAAAGGAT	740	[31]
TETO 1 TETO 2	*tet*(O)	AACTTAGGCATTCTGGCTCAC TCCCACTGTTCCATATCGTCA	519	[31]
TETOFF2 TETOFR3	*tet*(O)	TTGTTTTGGGGCTATTGGAG TATATGACTTTTGCAAGCTG	2,038	[32]
TETQ F TETQ R	*tet*(Q)	AGAATCTGCTGTTTGCCAGTG CGGAGTGTCAATGATATTGCA	167	[33]
TETS F TETS R	*tet*(S)	GAAAGCTTACTATACAGTAGC AGGAGTATCTACAATATTTAC	168	[33]
TETT F TETT R	*tet*(T)	AAGGTTTATTATATAAAAGTG AGGTGTATCTATGATATTTAC	167	[33]
TETWF F TETWF R	*tet*(W)	TTGGGGCTGTAAAGGGAGGAC CTTTACATTACCTTCTGA	1948	[32]
Virulence-associated factors				
CPS1F CPS1R	*cps*1J	TGGCTCTGTAGATGATTCTGCT TGATACGTCAAAATCCTCACCA	637	[11]
CPS2F CPS2R	*cps*2J	TTTGTCGGGAGGGTTACTTG TTTGGAAGCGATTCATCTCC	498	[11]
CPS7F CPS7R	*cps*7H	AATGCCCTCGTGGAATACAG TCCTGACACCAGGACACGTA	379	[11]
CPS9F CPS9R	*cps*9H	GGGATGATTGCTCGACAGAT CCGAAGTATCTGGGCTACTGA	303	[11]
MRP1 MRP2	*mrp*	ATTGCTCCACAAGAGGATGG TGAGCTTTACCTGAAGCGGT	188 [a]	[11]
EPF1 EPF2	*epf*	CGCAGACAACGAAAGATTGA AAGAATGTCTTTGGCGATGG	744[a]	[11]
OFS-F OFS-R2	*ofs*	GATGTGACTGTCCGCAGAGC AAAGTACCTGAGCTCCTACA	1,960 [b]	[13]
SLY1 SLY2	*sly*	GCTTGACTTACGAGCCACAA CCGCGCAATACTGATAAGC	248	[11]
ARC-A1 ARC-A2	*arc*A	TGATATGGTTGCTGCTGGTC GGACTCGAGGATAGCATTGG	118	[11]

[a] Reference strain D282; [b] Reference strain NIAH11433.

Table 3

The *mrp*, *epf*, and *ofs* gene size variants observed in *Streptococcus suis* isolates, Italy, 2003-2007

Target gene	Size variant	Amplicon size (bp)	References
mrp			
	mrp	1,148	[11]
	*mrp**	1,556	[11]
	mrp^{S}	747	[11]
epf			
	epf	744	[11]
	$epf^{class\ I}$	3,112	[40]
	epf^{915}	915	This study
ofs			
	$ofs^{type\ 1}$	1,960	[13]
	$ofs^{type\ 1S}$	1,636	This study
	$ofs^{type\ 2}$	2,113	[13]
	$ofs^{type\ 3a}$	1,627	[13]
	$ofs^{type\ 3b}$	1,786	[13]

Three *cps* genes were detected in 43 of the 59 isolates: *cps*1 (n=3 isolates, one invasive), *cps*2 (n=30, 23 invasive, including the two human CSF isolates) and *cps*9 (n=10, eight invasive). In agglutination tests, all *cps*2 strains showed agglutination with sera specific for serotype 2. The remaining 16 isolates, of which five were invasive, were negative and are referred to as non-typeable (NT).

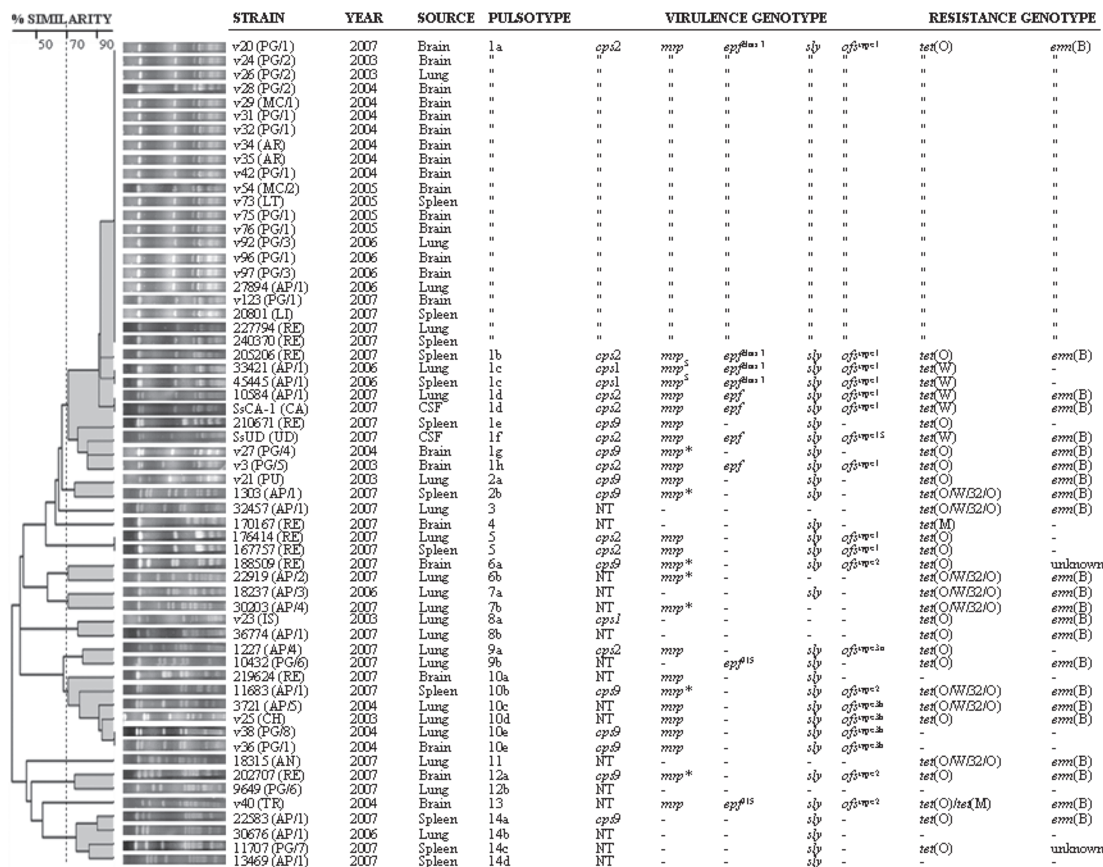
The *mrp* gene (three size variants: *mrp*; *mrp** and mrp^{S}) was detected in 47 strains (all 30 *cps*2 isolates, nine *cps*9, six NT, and two *cps*1 isolates); *epf* (three size variants: *epf*; $epf^{class\ I}$ and epf^{915}) was detected in 31 strains (27 *cps*2, two *cps*1 and two NT isolates); *ofs* (five size variants: $ofs^{type\ 1}$, $ofs^{type\ 1S}$, $ofs^{type\ 2}$, $ofs^{type\ 3a}$ and $ofs^{type\ 3b}$) was detected in 40 strains (all 30 *cps*2, five *cps*9, three NT and two *cps*1 isolates); sly was detected in 52 strains (all *cps*2 and *cps*9 isolates, two *cps*1 and 10 NT isolates), and *arc*A was found in all isolates.

Susceptibility testing and detection of resistance genes

The 59 strains were tested for susceptibility to tetracycline and erythromycin using phenotypic and genotypic methods. Fifty-three strains (90%) were resistant to tetracycline (MIC 8-64 mg/L) and

Figure

Similarity index of the 59 Streptococcus suis isolates, Italy, 2003-2007



For each isolate, the year and the source of isolation and the virulence and resistance genotypes are shown. Pulsed-field gel electrophoresis pulsotypes sharing >70% similarity were grouped into clusters (gray).
Unknown: neither *erm*(A) nor *erm*(B) nor *mef*(A).
ScCA-1 and SsUD are the two human isolates

46 (78%) were constitutively resistant to erythromycin (MIC >128 mg/L: n=44, including SsCA-1; MIC 4 mg/L: n=2, including SsUD). All erythromycin-resistant strains were also tetracycline-resistant. The *erm*(B) gene was the only erythromycin resistance determinant (Figure), found in 44 of 46 erythromycin-resistant strains. Neither *erm*(A) nor mef(A) were detected in the two erythromycin-resistant (MIC >128 mg/L) *erm*(B)-negative strains. Tetracycline resistance genes were distributed as follows: *tet*(O) (n=38), *tet*(O/W/32/O) (n=8), *tet*(W) (n=5); *tet*(M) (n=1), and *tet*(O)/*tet*(M) (n=1).

The presence of the mosaic gene was suspected from incongruent findings in PCR experiments, where a 519 bp amplicon was obtained in 38 strains using primers internal to *tet*(O) (TETO1 and TETO2), and a 2,038 bp amplicon was obtained in 46 strains (of which eight were negative when internal primers were used) using full-length *tet*(O) primers (TETOFF2 and TETOFR3). In the latter strains the presence of the mosaic gene *tet*(O/W/32/O) was confirmed by *Alu*I and *Hin*fI restriction analysis and sequencing of PCR products. Sequence analysis (FM164392) revealed that this gene was 99% identical to the tetracycline resistance gene *tet*(O/W/32/O) (EF065523.1) of an uncultured bacterium isolated from pig faeces [32]. The *tet*(W) gene was detected in three pig isolates and in both human isolates by *Hin*fI restriction analysis of the amplicons obtained with the tetWFF and tetWFR primer pair and sequencing. Sequence analysis (FN356743) disclosed that it was 99% identical to the tetracycline resistance gene *tet*(W) (DQ519395.1) of a porcine isolate of *Arcanobacterium pyogenes* [38].

Table 4

Virulence-associated gene profiles in *Streptococcus suis* isolates, Italy, 2003-2007 (n=59)

Profile	Invasive	Non-invasive
*cps*2 isolates (*n* = 30)	23	7
mrp epf$^{\text{class I}}$ *ofs* $^{\text{type 1}}$ *sly arcA*	19	4
mrp epf ofs $^{\text{type 1}}$ *sly arcA*	2*	1
mrp epf ofs $^{\text{type 1S}}$ *sly arcA*	1	-
mrp ofs $^{\text{type 1}}$ *sly arcA*	1	1
mrp ofs $^{\text{type 3a}}$ *arcA*	-	1
*cps*1 isolates (*n* = 3)	1	2
mrp^{S} *epf*$^{\text{class I}}$ *ofs* $^{\text{type I}}$ *sly arcA*	1	1
arcA	-	1
*cps*9 isolates (*n* = 10)	8	2
*mrp** *ofs* $^{\text{type 2}}$ *sly arcA*	3	-
mrp ofs $^{\text{type 3b}}$ *sly arcA*	1	1
mrp sly arcA	1	1
*mrp** *sly arcA*	2	-
sly arcA	1	-
^{a}NT isolates (*n* = 16)	5	11
mrp epf^{915} *ofs* $^{\text{type 2}}$ *sly arcA*	1	-
mrp ofs $^{\text{type 3b}}$ *sly arcA*	-	2
epf^{915} *sly arcA*	-	1
mrp sly arcA	1	-
*mrp** *arcA*	-	2
sly arcA	3	2
arcA	-	4

^{a} NT: non-typeable (neither *cps*1, nor 2, 7 or 9).
* Human isolates

PFGE typing and MLST

All strains were PFGE-typed after *Sma*I digestion of total DNA. Thirty-four different pulsotypes were detected and grouped into 14 PFGE types (types 1 to 14) on the basis of a cut-off of 70% similarity (Figure). PFGE type 1 accounted for 52% of isolates and comprised eight pulsotypes (types a to h), of which pulsotype 1a was shared by 22 pig isolates collected from 10 different herds in northern and central Italy in the period from 2003 to 2007. Pulsotype 1d was shared by the human strain SsCA-1 (isolated in 2007) and the pig isolate 10584 (isolated in 2006), and pulsotype 1f was displayed by the human strain SsUD. Comparison of 1d with both pulsotypes 1a and 1f yielded a two-band difference, and comparison of 1a with 1f a three-band difference. MLST of strains v20 (chosen as representative of pulsotype 1a), SsCA-1 (1d), and SsUD (1f) identified the same allelic profile, corresponding to ST1.

Clones

The distribution of *cps* genes, virulence-associated genes, and tetracycline and erythromycin resistance determinants among the 59 *S. suis* strains subdivided by PFGE types and pulsotypes is detailed in the Figure. *S. suis* isolates with a unique combination of a given PFGE pulsotype, a given *cps* gene, a given virulence profile, and a given resistance genotype and phenotype were considered to represent a clone. According to this criterion, 34 different clones, corresponding to the 34 different pulsotypes, were recognised, 32 of which were found among the 57 pig isolates (Figure). A major *cps*2 swine clone (clone 1a: *mrp*, *epf*$^{\text{class I}}$; *ofstype 1*, *sly*, *arc*A; *tet*(O) *erm*(B)) accounted for 37% of the 59 isolates. Moreover, clones 1d (*mrp, epf; ofs*$^{\text{type 1}}$, *sly*, *arc*A; *tet*(W) *erm*(B)) and 1f (*mrp, epf; ofs*$^{\text{type 1S}}$, *sly*, *arc*A; *tet*(W) *erm*(B)), containing the two human isolates (SsCA-1 and SsUD, respectively), were seen to be closely related.

Discussion and conclusion

This is the first study of virulence and resistance traits in swine and human strains of *S. suis* in Italy. The cps genes coding for the capsular polysaccharide as well as *mrp, epf, ofs*, and *sly* genes were investigated. The most prevalent capsular gene was cps2, followed by *cps*9 and *cps*1. The *cps*2 and *cps*9 genes were detected more frequently among invasive isolates; NT isolates were more frequent among non-invasive isolates.

In the present study, virulence-associated genes *mrp, epf, sly,* and *ofs* were found in a large proportion of isolates, including NT isolates. The *arc*A gene was seen in all strains, confirming previous studies [1]. The *epf* gene was not detected in *cps*9 strains, in line with a previous report [11], whereas the recently described ofs gene [12,13] was detected not only in all *cps*2 but also in some *cps*1, *cps*9, and NT strains. Human and pig *cps*2 isolates carrying *mrp* and *epf*, were detected. Interestingly, strains carrying mrp and *epf* have been previously proved to induce meningitis and septicaemia in experimentally infected pigs [39]. Moreover, *cps*2 strains carrying *mrp epf*$^{\text{class I}}$ and *ofs*$^{\text{type 1}}$ were detected in pig isolates. The size variants *mrp* and *epf*$^{\text{class I}}$ have been described in human isolates in Europe [40] and recently found in invasive *cps*2 swine clones from Europe and Brazil [11,41]. The size variant *ofs*$^{\text{type 1}}$ has been found to be associated with the ST1 complex [13]. Other profiles, such as *cps*1 *mrp*S- and *cps*9 *mrp**- have also been described in isolates from diseased pigs in European countries [10,11].

The finding that invasive and non-invasive isolates share identical virulence profiles seems to support the hypothesis that other, as yet unknown virulence factors are involved in *S. suis* pathogenesis [1,3]. The high allele variability of these genes was confirmed by detection of several size variants of *mrp, epf*, and *ofs*, of which some had previously been described [10,11,13,40] and some were new (*epf*^{915}and *ofs*$^{type\ 1S}$).

High rates of resistance to macrolides and tetracyclines suggested widespread resistance to these antibiotics in Italy. In Europe, rising rates of resistance have been attributed to intensive use by swine breeders of the macrolide-class antibiotic tylosin as a growth promoter and of tetracycline as a therapeutic agent [15]. Co-resistance to macrolides and tetracyclines can be explained by the fact that tetracycline and erythromycin resistance determinants are often linked on mobile genetic elements [42].

All strains were PCR screened for *erm*(A), *erm*(B), and *mef*(A). Neither *erm*(A) nor *mef*(A) were detected. The *erm*(B) gene was found in all but two erythromycin-resistant pig strains, confirming its prevalence in *S. suis* in Europe [18,19]. A possible explanation for the erythromycin-resistant, *erm*(A)-, *erm*(B)- and *mef*(A)-negative strains could be an erythromycin resistance determinant previously unreported in *S. suis* [21]. The presence of *erm*(B) in both human isolates is consistent with its dissemination in the Italian swine population. The genetic basis of erythromycin resistance in human *S. suis* isolates has barely been investigated [5,21]. The very recent paper by Chu *et al.* [21] describes the prevalence of *mef*(A) in isolates from Hong Kong. Interestingly, all *mef*(A) isolates belonged to ST7 (endemic in Asia) whereas the only *erm*(B) strain belonged to ST1 (spread worldwide, including in Europe) [21].

The *tet*(M) and *tet*(O) genes are common resistance determinants in *S. suis*, found worldwide both in pig and in human isolates [19,20]. In this study, four *tet* genes, all coding for ribosomal protection proteins (http://faculty.washington.edu/marilynr/), were found in the Italian *S. suis* population. While *tet*(O) was prevalent, *tet*(M) was, inexplicably, almost absent. In addition *tet*(W), and the mosaic *tet*(O/W/32/O), the *tet* gene found most frequently in pig isolates after *tet*(O), were detected. The *tet*(W) gene is associated with tetracycline resistance in a wide range of bacterial species, including obligate anaerobic rumen bacteria and isolates from human gut and oral mucosa. *tet*(W) was first detected in *S. suis* by our group in the human isolate SsCA-1 [5], and then here in the other human strain (SSUD) and in some pig isolates. These data suggest that *tet*(W) could be widespread in *S. suis.*

The mosaic gene *tet*(O/W/32/O) has not been described in the genus Streptococcus before. Mosaic tet genes, originating from *tet*(O) and *tet*(W), were first detected in 2003 in anaerobic Gram-negative *Megasphaera elsdenii* from swine intestine [43,44]. Other mosaic genes, also comprising *tet*(32), were later detected in *Clostridium difficile* [45]. Initially thought to be confined to a small group of anaerobic bacteria [22], mosaic tet genes have now been found to be abundant in human and animal faecal samples [32] and have also been detected in *Bifidobacterium thermophilum* and *Lactobacillus johnsonii* isolates [46]. Further studies on the genetic elements carrying tet genes are warranted to explain the atypical tet distribution observed in Italian *S. suis* isolates.

Overall, the *S. suis* pig isolates demonstrated a high genetic diversity that correlates with a wide distribution of *S. suis* in Italy. In a heterogeneous background population, an identical virulence and resistance profile (*cps*2 *mrp epf*$^{class\ I}$ *ofs*$^{type\ 1}$ *sly erm*(B) *tet*(O)) and pulsotype were shared by more than a third of swine isolates, collected between 2003 and 2007 from different Italian herds and regions, demonstrating the presence and persistence of a dominant clone, 1a.

The results further revealed that the two human isolates shared a number of common or related features, i.e. both were serotype 2 and harboured *cps*2, both were resistant to erythromycin (MIC 4 µg/ml and >128 µg/ml, respectively) and contained the erm(B) gene, and both were resistant to tetracycline (MIC 16 µg/ml) and contained the *tet*(W) gene. Moreover, while sharing the same *mrp* and *epf* variants as well as *sly*, the two human isolates SsUD and SsCA-1 bore two different *ofs* variants, respectively *ofs*$^{type\ 1}$ and *ofs*$^{type\ 1S}$, a new variant with a 324 bp deletion in the *ofs*$^{type\ 1}$ coding sequence.

According to Tenover's criteria [37], a close relatedness between SsUD and SsCA-1 and between each human isolates and the dominant swine clone was documented by PFGE analysis which yielded pulsotypes with a difference in only two or three bands. MLST analysis assigned clones 1a and 1f (SsUD) to ST1 of the highly virulent ST1 complex, as previously demonstrated also for SsCA-1 (clone 1d) [5]. Overall, our data show that typical Eurasian strains, i.e. strains carrying genes coding for MRP, EF, and suilysin and belonging to the ST1 complex [1], are widespread in Italy.

In conclusion, this study demonstrated a high genetic diversity of Italian *S. suis* isolates, with a prevalent *cps*2, *erm*(B), *tet*(O) ST1 clone persistent in the swine population. It also demonstrated a close relatedness between two recently isolated *cps*2 *erm*(B) and *tet*(W) ST1 human strains and between human isolates and the dominant swine clone. Finally, it is the first report to demonstrate *tet*(O/W/32/O) in *S. suis* and suggests that mosaic tet genes should be sought in *S. suis* and in other streptococci.

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