Genetic Diversity and Antimicrobial Resistance Profiles of Salmonella enterica Serotype Derby Isolated from Pigs, Pork, and Humans in France

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Genetic diversity and antimicrobial resistance profiles of *Salmonella enterica* serotype Derby isolated from pigs, pork and humans in France

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Abstract

In France, *Salmonella enterica* serotypes Typhimurium and Derby are the most often isolated serotypes in pigs. Moreover, serotype Derby usually ranks between 3rd and 4th in prevalence among human isolates in France. The aim of this study was to evaluate the genetic relationships between human and pig *S.* Derby isolates based on their Pulsed Field Gel Electrophoresis (PFGE) patterns after *Xba*I, *Bln*I and *Spe*I restriction and on their antimicrobial resistance profiles. The 196 studied isolates were isolated in 2006 and 2007: 73 from fattening pigs, 27 from pork and 96 from humans. Forty-four PFGE *Xba*I patterns were identified. A major pattern (SDX01) was identified for 96 isolates (49%). This pattern was common to pig, pork and human isolates. Among the 146 isolates tested for their antimicrobial resistance, 84.2% (n=123) showed resistance to at least one antibiotic and 69.2% (n=101) were simultaneously resistant to at least streptomycin, sulfonamides and tetracycline. Most of the isolates which resist to these 3 antibiotics also displayed the major SDX01 pattern. The use of two other restriction enzymes on a part of the panel (155 isolates) brought a significant increase in the discriminatory index, in particular for SDX01 strains. As *S.* Derby is essentially isolated from pigs, and major resistance and PFGE patterns of isolates from pigs and pork were very similar to human isolates, human salmonellosis due to *S. enterica* serotype Derby may be related to pigs.

INTRODUCTION

Salmonella enterica subsp. *enterica* is the leading cause of bacterial food-borne disease in the world. *Salmonella* typically causes acute gastroenteritis and may cause more serious septicemic disease, most often in very young, elderly and immuno-compromised subjects. In France, *Salmonella* is responsible for 65% of all recorded outbreaks of foodborne diseases (Haeghebaert, 2002). Between 5700 and 12000 hospitalizations a year and, 92 and 535 deaths a year, were attributed to *Salmonella* (Vaillant *et al.*, 2005). *Salmonella* can be isolated from various food items: animal products such as eggs, meat, milk and dairy products, seafood, fruits and vegetables. Pork has often been described as a source of human salmonellosis and pigs have been identified in a number of countries as major carriers of *Salmonella* spp (Hald, 2003, Letellier, 1999). *Salmonella* has the ability to colonize the guts of healthy pigs who then can serve as carriers. These asymptomatic pigs, when admitted to slaughterhouses, become a potential risk for *Salmonella* contamination. *S. enterica* serotypes Typhimurium and Derby (hereafter referred to as *S.* Derby) are both major *Salmonella* serotypes found in pigs in France and Europe (Beloeil *et al.*, 2004, EFSA, 2008a, Fablet, 2003). We focused in this study on *S.* Derby as this serotype is known to be especially linked to pigs (Giovannacci *et al.*, 2001, Valdezate *et al.*, 2005). Furthermore, over the last few years in France, it has been ranked 3rd to 6th in prevalence among human serotyped isolates and is responsible each year for one or two family and/or hospital-based outbreaks (http://www.pasteur.fr/ip/easysite/pasteur/fr/sante/centres-nationaux-de-reference-et-centres-collaborateurs-de-l-oms/cnr-et-ccoms/cnr-des-salmonella/actualites-rapports#rapports).

This serotype may also be implicated in larger outbreaks (Ebuchi *et al.*, 2006) and has been among the twenty most frequently serotyped human *Salmonella* isolates in several European and Asian countries over the past decade (Hendriksen *et al.*, 2011). Therefore, this serotype is a major public health concern.

This study aimed at evaluating the link between pigs, pork and human isolates, in order to assess the farm-to-fork continuum. To demonstrate the spread of isolates from pigs *via* pork to humans, highly discriminatory typing methods are necessary. Traditional isolate typing methods such as serotyping or phage typing have been used for surveillance of *Salmonella* and outbreak investigations but they are supplemented nowadays with the development of molecular methods (Gebreyes *et al.*, 2006, Wonderling *et al.*, 2003). Pulsed field gel electrophoresis (PFGE), which is currently the gold standard, has generally good discriminatory power and has been proven to be highly useful and reliable especially for tracking contamination sources (Botteldoorn *et al.*, 2004, Vieira-Pinto *et al.*, 2006) and for outbreak investigations (Dominguez *et al.*, 2009, Noel *et al.*, 2006). As the PFGE method with *Xba*I has shown low discriminatory power for *S.* Derby (Kerouanton *et al.*, 2007), we combined it with cluster analysis of multiple restriction enzymes (*Xba*I, *Bln*I and *Spe*I) as recommended by Zheng *et al.* (Zheng *et al.*, 2011). Because resistance of *Salmonella* to antimicrobial agents is a worldwide problem, and antimicrobial resistance has already been described in human (Ling *et al.*, 2001) and non-human (Akiba *et al.*, 2006) *S.* Derby isolates, antibiotic drug susceptibility was also determined. The study was conducted over a two-year period on 196 isolates isolated in 2006 and 2007, 73 of which were from fattening pigs, 27 from pork and 96 from humans.

MATERIAL AND METHODS

S. Derby Isolates

A total of 196 *S.* Derby isolates from various origins were considered in this study. The animal isolates (n=73) were collected from pig lymph nodes during the European baseline studies carried out in 2006-2007 (EFSA, 2008) by the National Reference Laboratory (NRL) for Salmonella (HQPAP unit ANSES Ploufragan). Isolates from pork (n=27), collected by the French Salmonella network (Associated NRL for Salmonella, ANSES Maisons-Alfort) in 2006 and 2007 were also studied. Moreover, the French National Reference Center for Salmonella (FNRC-Salm, Institut Pasteur, Paris) provided 96 *S.* Derby isolates collected from human salmonellosis over the same period of time (47 isolates in 2006 and 49 in 2007). The set of isolates represented 34.3%, and 41.2% of all the *S.* Derby isolates for 2006 and 2007, respectively. In terms of representativity, it has been estimated that the FNRC-Salm network detected 66% of confirmed human *Salmonella* infections in France at the time of the study (Jourdan-Da Silva and Le Hello, 2012). In each lab, isolates were serotyped, according to the White-Kauffmann-Le Minor scheme, on the basis of somatic O and phase 1 and phase 2 flagellar antigens by agglutination tests with antisera (Bio-Rad, Marnes la Coquette, France; Eurobio, Les Ulis, France; World Health Organization Collaborative Center for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France).

PFGE typing

The genetic typing of the isolates was carried out by RFLP-PFGE with a CHEF-DR III system (Bio-Rad) according to the PulseNet protocol (Ribot *et al.*, 2006). *S. enterica* serotype Braenderup H9812 strain was used as a molecular size marker (Hunter *et al.*, 2005). After staining with Ethidium Bromide, DNA patterns were visualized under UV light and images were taken with the Biovision+ 1500/36M X PRESS system (Fisher Bioblock). *Bln*I and *Spe*I

enzymes were also used on a panel of 155 isolates. This panel is representative of isolates that did not give a unique pattern after *Xba*I restriction. For *Bln*I-digested DNA, migration conditions were the same as for *Xba*I: fragments were resolved on 1% agarose gel using electrophoresis conditions of 6.0V/cm at 14°C for 20h. Pulse times were ramped from 2 s (initial switch) to 64 s (final switch). For *Spe*I-digested DNA, migration was performed in two steps, a 11.5h step with an initial switch time of 20 s and a final switch time of 40 s, followed by a 9.5h step with an initial switch time of 7 s and a final switch time of 13 s, at a gradient of 6V/cm and an included angle of 120°. For cases of DNA lysis, we used an adapted DNA extraction protocol with HEPES buffer instead of Tris buffer and TBE migration was performed with 100 µM thiourea (Liesegang and Tschape, 2002, Silbert *et al.*, 2003)

Genetic profile analysis

Fragment size estimations and analysis of the similarities between genotypes were carried out using the BioNumerics® software (version 4.1, Applied Maths, Kortrijk, Belgium). The similarities were calculated using Dice's coefficient with a maximum tolerance of 1% and dendrograms were built according to the Unweighted Pair Group Method (UPGMA) (Struelens, 1996).

The Simpson index (Hunter and Gaston, 1988) was calculated to estimate the diversity of the sample and the 95% confidence intervals were also calculated based on the variance as suggested by Grundmann (2001).

Antimicrobial susceptibility tests

All pig and pork isolates were tested for their antimicrobial susceptibility. Resistance phenotypes were determined using the disc diffusion method as recommended by the Antibiogram Committee of the French Society for Microbiology (http://www.sfm.asso.fr/).

Sixteen antimicrobials (Bio-Rad) were tested: Ampicillin (10µg), amoxicillin + clavulanic acid (20/10 µg), cephalothin (30µg), cefotaxime (30µg), ceftazidime (30µg), chloramphenicol (30µg), tetracycline (30UI), streptomycin (10UI), kanamycin (30UI), gentamicin (15µg), sulfonamides (200µg), cotrimoxazole (1.25/23.75 µg), nalidixic acid (30µg), ofloxacin (5µg), enrofloxacin (5µg) and colistin (50µg). Automatic readings were performed using the OSIRIS system (Bio-Rad). For human isolates, only those isolated in 2006 were studied (n=47) by the FNRC-Salm for their resistance to the following 32 antimicrobial (Bio-Rad) as previously described (Weill et al., 2004).

RESULTS

PFGE types after XbaI restriction

The 196 isolates generated 44 different PFGE *Xba*I patterns (figure 1). The discriminatory ability (D-value) of the method for the entire panel was $0.75_{95\%CI}$ [0.68-0.81].

Thirty-three PFGE patterns (75%) were encountered for a single isolate and then specifically associated to a single origin: 4 PFGE patterns were attributed to a single pork isolate, 9 to pig isolates and 20 to human isolates.

One other pattern was also related to a single origin but grouped together 3 pig isolates.

Conversely, ten patterns grouped together isolates of different origins. Six PFGE patterns were common to pig, pork and human isolates (SDX01, 02, 12, 14, 30 and 32), 2 were common to human and pig isolates (SDX05 and 17), 1 to human and pork isolates (SDX42) and 1 to pig and pork (SDX39). Among these 10 patterns, SDX01 pattern was identified for 96 isolates among the 196 studied with 44.8% of those of human origin (43/96), 56.2% of those of pig origin (41/73), 44.4% of those of pork origin (12/27). Actually, there was no significant difference (χ^2, p=0.30) allowing to assign this major pattern to one of the origins.

Fourteen PFGE patterns –SDX02, SDX03, SDX04, SDX05, SDX06, SDX07, SDX09, SDX10, SDX11, SDX12, SDX18, SDX37, SDX43 and SDX46 patterns – were very similar to the major SDX01 pattern (86% similarity), and comprised 39 isolates. Thus, a total of 135 isolates among the 196 studied (68.9%) were grouped into a single major cluster. This cluster included 65.6% of the human isolates, 74% of the pig isolates and 66.7% of the pork isolates.

PFGE types after BlnI and SpeI restriction

Isolates (n=155) of various origins grouped into 8 of the 10 PFGE *Xba*I patterns gathering several isolates (isolates that did not give a unique pattern after *Xba*I restriction) were also analyzed after *Bln*I and *Spe*I restriction. A total of 18 *Bln*I PFGE profiles and 27 *Spe*I PFGE profiles have been highlighted. Eight isolates were non-typable with *Bln*I enzyme despite the addition of thiourea. A combination of the results obtained with the 3 enzymes gave 42 combined PFGE patterns (figure 2). The discriminatory index for this panel of 155 isolates was of 0.53, 0.60 and 0.68 when using respectively *Bln*I, *Xba*I, and *Spe*I, and increase to 0.72 when considering the results obtained with the three enzymes.

Most of the time, the use of the two others enzymes increased the discriminatory power of the PFGE method. For SDX05, SDX30 and SDX32 patterns, the use of *Bln*I and *Spe*I separated the human isolates from the others sources.

Sometimes, the use of *Bln*I and *Spe*I gave exactly the same discrimination than that obtained with *Xba*I. For example, for the 4 isolates carrying SDX12, the combined pattern SDX12/SDB01/SDS15 always correspond to 2 human, 1 pig and 1 pork isolates, as observed after only *Xba*I restriction.

Similar *Bln*I and/or *Spe*I patterns were also observed for different *Xba*I patterns. For example, the SDB01 pattern was highlighted for isolates carrying SDX01, SDX05 and SDX12 patterns; and SDS10 for isolates carrying SDX30, SDX32 and SDX39 patterns. Moreover we also noted that all animal isolates from these 3 *Xba*I patterns were non typable by *Bln*I enzyme.

Antimicrobial susceptibility

No more than 15.5% (23/147) of the tested isolates were susceptible to all antimicrobials. For the remaining isolates, 11 different patterns of resistance were observed, with from one to 6 resistances (Table 1). 79% of the isolates showed at least tetracycline (TE) resistance. TE

resistance was often associated with streptomycin (S) and sulfonamides (SSS) resistance. Hence, 98 isolates (66.7%) displayed the S SSS TE pattern of resistance. Four isolates were resistant to more than 3 antimicrobials. However, no resistance to fluoroquinolone or third generation cephalosporins was detected. For each resistance pattern, Table 1 shows the corresponding PFGE patterns. The 102 isolates harboring at least resistance to tetracycline, streptomycin and sulfonamides were subdivided into 18 PFGE patterns while 75 isolates (73.5%) belonged to the major PFGE pattern SDX01.

DISCUSSION

The genetic diversity of *S.* Derby isolates has rarely been investigated. In this study, a large panel of 196 isolates was studied and molecular characterization by PFGE showed that the genome of this serotype seems to have a highly homogeneous genetic composition. The discriminatory ability (*D* value) of the method was 0.75 [0.68-0.81] for the entire panel, confirming a previous study (Kerouanton *et al.*, 2007). A major *Xba*I pattern, SDX01, was found for 49% (96/196) of the isolates. This PFGE pattern was also observed in a German study by Hauser et al. (2011) but only for one isolate, while their main PFGE pattern, corresponding to our SDX42 pattern, was only found for two of our isolates (one pork and one human). This suggests that each country could have its own prevalent *S.* Derby pattern.

In our study, 9 patterns presented more than 90% similarity with the major PFGE pattern and 14 patterns more than 86% similarity.

Among the 96 tested isolates carrying the pattern SDX01, 4 *Bln*I and 9 *Spe*I PFGE profiles were identified. However, among these 96 isolates, 81 isolates (32, 37 and 12 of human, pig and pork origin, respectively) remain identical after the use of *Bln*I and *Spe*I enzymes. Isolates with identical genetic profiles came from all tested sources. This result could suggest either that these isolates are closely related and can be carried by different hosts or that PFGE is not an appropriate method for *S.* Derby. Several other typing methods based on PCR amplification and capillary electrophoresis or sequencing such as MLVA (Bergamini *et al.*, 2011, Boxrud *et al.*, 2007, Lindstedt *et al.*, 2003) and MLST (Achtman *et al.*, 2012, Ben-Darif *et al.*, 2010, Torpdahl *et al.*, 2005), CRISPR (Fabre *et al.*, 2012) are now used for *Salmonella* discrimination but they have rarely been described for *S.* Derby isolates. Genetic diversity of *S.* Derby evidenced after use numerous methods was recently discussed (Hauser *et al.*, 2011) but the conclusion was that PFGE remained the method with the highest index of diversity followed by VNTR sequence typing (STTR5 primer only) and *sop* genes (*sop*A, *sop*B and

*sop*D) sequence typing (sop-ST). MLST or DNA microarray on 275 genes provided lower discrimination of the 82 isolates studied. Consequently, according to the observations of Hauser *et al*. (2011) with regard to PFGE, our results seem to indicate that our isolates, with similar profiles after 3 different digestions, are genetically similar and have the ability to colonize different hosts.

The antimicrobial susceptibility tests revealed a low percentage (15.5%) of wild type S. Derby isolates (i.e., with no acquired mechanisms of resistance). This rate of wild type S. Derby isolates is very different depending on studies: from 0% (Ellerboek et al., 2010) to 82% (Piras et al., 2011). In our study, the majority of isolates (70.7%) were resistant to 3 antimicrobials, including the S SSS TE pattern that accounted for 66.7% of the isolates. Resistance to these 3 antimicrobials was already highlighted for pigs isolates (Michael et al., 2006b) and pork meat (Anjum et al., 2011, Mürmann et al., 2009). Antimicrobial resistance has been commonly associated with extended use in farm animals (Wegener et al., 2003). While it is true that, in France, tetracycline is the most commonly-used antimicrobial for pigs (Chevance and Moulin, 2011), consumption of antibiotics in pigs and resistance in S. Derby cannot be systematically linked. Although penicillins are used as often as sulfonamides, no resistance to beta-lactam antibiotics has been detected in any isolate from pigs or pork, whereas resistance to sulfonamides was detected in most isolates.

A correlation between macrorestriction patterns and resistance patterns was previously reported (Botteldoorn *et al*., 2004, Michael *et al*., 2006a, Michael *et al*., 2006b). This correlation was also clearly observed in our study as the major PFGE pattern SDX01 was linked to the major antimicrobial resistance pattern: 75 (76.5%) of the 98 isolates resistant to streptomycin, sulfonamides and tetracycline were from the SDX01 pattern. All SDX01 isolates tested (n=75) were resistant to these 3 antibiotics. A recent study (Cardoso *et al.*, 2011) showed that each *S.* Derby isolate presenting a major PFGE pattern also exhibited the S

SSS TE pattern and carried a class 1 integron. Indeed, class 1 integrons carry the *sul1* gene encoding sulphonamide resistance and frequently contain the *aadA* cassette gene, which is associated with streptomycin/spectinomycin resistance (Michael *et al.*, 2006a). Presence of a class 1 integron and *aad*A gene in *S.* Derby from pigs was also reported by Gebreyes *et al.* (2004). Less frequently *S.* Derby isolates from Asia have been found to contain the *Salmonella* genomic island 1 (SGI1) variant, which carries *aadA2*, *sul1*, *floR* and *tet*(A) resistance genes (Chiu *et al.*, 2007).

As reported by Huang *et al.* (2009), in pig *S.* Derby isolates showed a low resistance rate to beta-lactams. Ampicillin resistance was found only in one human isolate. In our study, only four isolates showed resistance to more than three antibiotics; two were isolated from humans, one from pigs and one from pork. Pigs and pork isolates only showed resistance to streptomycin, sulfonamides, trimethoprim and tetracycline. Nevertheless, these antimicrobials are not the first used as a treatment for human salmonellosis.

A statistical attribution study based on outbreak data (Pires *et al.*, 2010) estimated that less than 1% of outbreak-associated salmonellosis cases were attributed to pork in Europe in 2005 and 2006. However, in a report based on community observations, the European Food Safety Authority (EFSA) estimated than 10-20% of human *Salmonella* infections in the EU may be attributable to the pig reservoir (EFSA, 2010) and a more recent study (Pires *et al.*, 2011) found the pig reservoir to be the second largest contributor to human salmonellosis in the EU, responsible for 29.6% (95% CI: 28.9-30.3%) of reported cases. Pires *et al.* (2011) also showed that pigs were the main source of human salmonellosis in eight countries.

In this epidemiological study, isolates of human and pig origin showed a very high similarity in PFGE and in antimicrobial susceptibility. As serotype Derby is mostly associated with pigs and is the most prevalent, along with serotype Typhimurium, in pig production in France, we can conclude, as did Finley *et al*. (2008), that human salmonellosis due to this serotype is in

all likelihood linked to pigs. Implementation of another molecular method which is more discriminatory than PFGE, as a complete MLVA scheme, and additional studies on isolates from diverse origins could help us to confirm or infirm this hypothesis.

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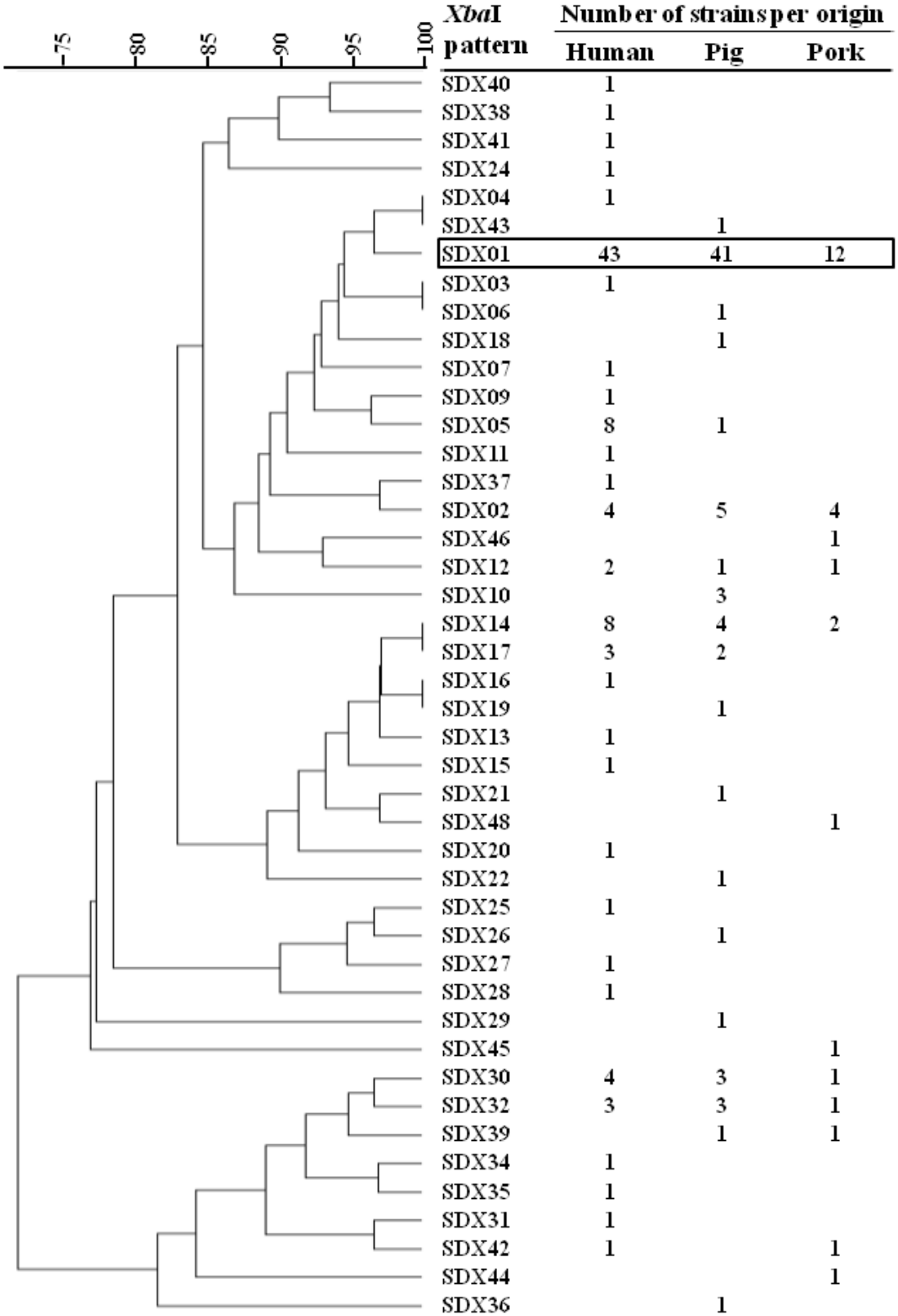
Table 1: Antimicrobial resistance distribution according to isolate origins

Antimicrobials*	**Sample Origin**			**Associated *Xba*I patterns (No)**
	Human No	Pig No	Pork No	
Wild type strains (no acquired resistance)	8	10	5	SDX02 (5), SDX14 (3), SDX30 (4), SDX31 (1), SDX32 (4), SDX36 (1), SDX37 (1), SDX39 (2), SDX41 (1), SDX42 (1)
TE	2		1	SDX013 (1), SDX25 (1), SDX48 (1)
S		4	1	SDX02 (1), SDX14 (1), SDX17 (1), SDX19 (1), SDX30 (1)
SSS, TE		2		SDX02 (2)
S, SSS		2		SDX22 (1), SDX43 (1)
S, TE		4		SDX14 (3), SDX26 (1)
SSS, TMP, TE	3		2	SDX09 (1), SDX17 (2), SDX42 (1), SDX45 (1)
S, SSS, TMP		1		SDX21 (1)
S, SSS, TE	32	49	17	SDX01 (75), SDX02 (1), SDX05 (7), SDX07 (1), SDX10 (3), SDXB11 (1), SDXB12 (2), SDXB14 (2), SDXB18 (1), SDXB29 (1), SDXB34 (1), SDXB40 (1), SDXB44 (1), SDXB46 (1)
S, SSS, TMP, TE		1	1	SDX02 (1), SDX06 (1)
A, S, SSS, TE	1			SDX35 (1)
S, K, TOB, GM, SSS, TE	1			SDX02 (1)

*** TE: tetracycline, S: streptomycin, SSS: sulfonamides, TMP: trimethoprim, A: ampicillin, K: kanamycin, TOB: tobramycin, GM: gentamicin. No: Number of isolates.**

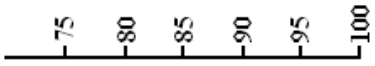
Figure 1: Dendrogram representing the 44 PFGE-XbaI Patterns designated SDX01 to SDX48. The number of isolates of each source (Human, Pig and Pork) is shown on the right side. The major PFGE profile SDX01 is circled.

NB: Some patterns appear to be 100% identical with BioNumercis® analysis (1% tolerance), but they have been visually identified as different from each other.



*Xba*I pattern	Number of strains per origin		
	Human	Pig	Pork
SDX40	1		
SDX38	1		
SDX41	1		
SDX24	1		
SDX04	1		
SDX43		1	
SDX01	43	41	12
SDX03	1		
SDX06		1	
SDX18		1	
SDX07	1		
SDX09	1		
SDX05	8	1	
SDX11	1		
SDX37	1		
SDX02	4	5	4
SDX46			1
SDX12	2	1	1
SDX10		3	
SDX14	8	4	2
SDX17	3	2	
SDX16	1		
SDX19		1	
SDX13	1		
SDX15	1		
SDX21		1	
SDX48			1
SDX20	1		
SDX22		1	
SDX25	1		
SDX26		1	
SDX27	1		
SDX28	1		
SDX29		1	
SDX45			1
SDX30	4	3	1
SDX32	3	3	1
SDX39		1	1
SDX34	1		
SDX35	1		
SDX31	1		
SDX42	1		1
SDX44			1
SDX36		1	

Figure 2: Dendrogram representing the 42 XbaI-BlnI-SpeI combined patterns obtained on 170 strains (nt = non typable strains). The number of strains of each origin is shown on the right side.



PFGE Patterns			Number of strains per origin		
*Xba*I	*Bln*I	*Spe*I	Human	Pig	Pork
SDX02	SDB21	SDS29	1		
SDX02	SDB21	SDS27		2	1
SDX02	SDB21	SDS05	1	2	2
SDX02	SDB21	SDS28	1		
SDX02	SDB22	SDS26			1
SDX02	SDB23	SDS05	1		
SDX02	SDB24	SDS25		1	
SDX05	SDB01	SDS34	3		
SDX05	SDB01	SDS01	2		
SDX05	SDB01	SDS31	1		
SDX05	SDB01	SDS30	1		
SDX05	SDB01	SDS08		1	
SDX05	SDB01	SDS32	1		
SDX01	SDB02	SDS01		1	
SDX01	SDB03	SDS01		1	
SDX01	SDB01	SDS07	2		
SDX01	SDB01	SDS06	1		
SDX01	SDB01	SDS18	1		
SDX01	SDB01	SDS01	32	37	12
SDX01	SDB01	SDS16		2	
SDX01	SDB01	SDS17	2		
SDX01	SDB01	SDS04	1		
SDX01	SDB01	SDS21	1		
SDX01	SDB01	SDS09	1		
SDX01	SDB06	SDS04	1		
SDX12	SDB01	SDS15	2	1	1
SDX14	SDB14	SDS11	1		
SDX14	SDB08	SDS11			1
SDX14	SDB16	SDS11	4	3	
SDX14	SDB16	SDS22		1	
SDX14	SDB18	SDS11	2		1
SDX14	SDB17	SDS11	1		
SDX17	SDB20	SDS13		1	
SDX17	SDB15	SDS24	2	1	
SDX17	SDB15	SDS14	1		
SDX30	SDB11	SDS10	1		
SDX30	nt	SDS10		1	1
SDX39	nt	SDS10		1	1
SDX30	SDB10	SDS10	3		
SDX32	SDB09	SDS10	3		
SDX32	nt	SDS10		3	1