Comparison of PFGE and antibioresistance profiles of *Salmonella enterica* serotype Derby isolated from pig and human salmonellosis in France

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Abstract

In France, *Salmonella enterica* serotypes Typhimurium and Derby are the most often isolated serotypes in pigs. On the other hand, serotype Derby ranks usually between 3rd and 4th in prevalence among human serotyped isolates in France. In 2006 and 2007, 150 and 127 human isolates were respectively collected by the French National Reference Center for *Salmonella*. The aim of this study was to evaluate the similarity between human and pig serotype Derby isolates on both their Pulsed Field Gel Electrophoresis (PFGE) patterns after *XbaI* restriction and on their antimicrobial resistance profiles. Seventy four *S. enterica* serotype Derby isolated from lymph nodes of pigs in 2006-2007 were compared to 96 Derby isolates collected in humans during the same period. Seventeen *S. enterica* serotype Derby isolated from turkey were also considered. Forty-three PFGE *XbaI* patterns were identified. Out of these 43 patterns, 12 were specifically attributed to pig isolates, 2 to turkey isolates and 21 to human isolates. Thirty-one PFGE patterns (72.1%) were encountered for only one isolate. Among the 8 remaining patterns, a major pattern (DXB01) was identified for 96 isolates (56.3%) (42, 43 and 11 of pig, human and turkey origins, respectively). Ten PFGE patterns presented at least 90% of similarity with DXB01 pattern, they gathered 23 isolates. Furthermore, 6 other patterns were common to pig and human isolates and one to turkey and human isolates. Resistance to at least one antibiotic is shown by 85.5% of the 134 tested isolates; 70% of them resist to at least streptomycine, sulfonamides and tetracycline simultaneously. The majority of these isolates presented the DXB01 pattern.

Finally human salmonellosis due to *S. enterica* serotype Derby could be related to pigs but genetic clonality of that serotype and antimicrobial resistance profile highlighted in this study could not always allow to ensure this link at least for DXB01 patterns.

Introduction

Nontyphoid salmonellosis is a foodborne disease of major importance throughout the world. In France, *Salmonella* is the main cause of bacterial gastroenteritis and is responsible for 65% of all recorded outbreaks of foodborne disease (Haeghebaert et al., 2002). Outbreaks of foodborne disease due to *Salmonella* are continually reported. *Salmonella* Typhimurium and S. Derby are both major *Salmonella* serotypes found in pigs in France (Fablet et al., 2003; Beloel et al., 2004; EFSA, 2008). S. Derby is known to be often associated to pig (Valdezate, et al. 2005). In this study, we focused on S. Derby as this serotype ranks usually between 3rd and 4th in prevalence among human serotyped isolates in France. In 2006 and 2007, 150 and 127 human isolates were respectively collected by the French National Reference Center for *Salmonella* (http://www.pasteur.fr/sante/cle/cadre/en/salmenr/salmenr-actualites.html). Our aim was to evaluate the link between pigs and human isolates. This comparison was performed on both their Pulsed Field Gel Electrophoresis (PFGE) patterns after Xba1 restriction and on their antimicrobial resistance profiles.

Material and methods

Strains

Animal strains were isolated during the European baseline studies carried out in 2006-2007. Seventy-four S. Derby were isolated from lymph nodes of pigs (EFSA, 2008) and, 17 S. Derby isolated from turkey.
during the same period were also considered in this study. The French National Reference Center for *Salmonella* provided us 96 *S. Derby* isolates collected from human salmonellosis cases during the same period (47 strains in 2006 and 49 in 2007).

**PFGE typing**

The genetic typing of the isolates was carried out by RFLP-PFGE according to the PulseNet protocol (Ribot et al., 2006), and a genetic profile *XbaI* was obtained for all the isolates. *Salmonella enterica* serotype Braenderup H9812 was used as molecular marker of size (Hunter et al., 2005).

**Analyzes of the genetic profiles**

The estimates of the size of the fragments and the analysis of the similarities between genotypes were carried out by using the BioNumerics software (V 5.1, Applied Maths, Kortrijk, Belgium). The similarities between profiles, based on the position of the restricted fragments, were calculated using the coefficient of Dice with a maximum tolerance of 1% (Struelens, 1996). Dendrograms were built according to the Unweight Pair Group Method (UPGMA) using an arithmetic mean (Struelens, 1996). The Simpson’s index (Hunter, 1990) was calculated to estimate the diversity of the sample.

**Antimicrobial susceptibility tests**

For non-human strains, resistance phenotypes have been determined by disc diffusion method as recommended by the Antibiotic Comity of the French Society for Microbiology (http://www.sfm.asso.fr). 16 antimicrobials have been tested: Ampicillin, Amoxicillin + clavulanic acid, cefotaxime, ceftazidime, chloramphenicol, tetracycline, streptomycin, kanamycin, gentamicin, sulfonamides, cotrimoxazole, nalidixic acid, ofloxacin, enrofloxacin, colistin. Automatic readings have been performed by OSIRIS system (BioRad). For Human isolates, antibiotic susceptibility was determined by disc diffusion method with 32 antimicrobial drugs (Bio-Rad), as previously described (Weill et al., 2004).

**Results**

**PFGE types**

Forty-three PFGE *XbaI* patterns were identified (figure 1). The discriminatory ability (*D* value) of the method was 0.73 for the entire panel. Thirty-one PFGE patterns (72.1%) were encountered for only one isolate and then, specifically associated to one origin: 1 was attributed to turkey isolates, 10 to pig isolates and 20 to human isolates. In the other hand, a very frequent pattern (DXB01) was identified for 56.3% of isolates (42 isolates, 43 isolates and 11 isolates of pig, human and turkey origins, respectively). Ten PFGE patterns presented at least 90% of similarity with this major DXB01 pattern, they gathered 23 isolates. Six others patterns were common to pig and human isolates and one pattern common to turkey and human isolates.

**Antimicrobial susceptibility**

All pigs and 13 of the 17 turkey isolates have been tested for their antimicrobial susceptibility. For human isolates, only those isolated in 2006 have been studied (n=47). Resistance to at least one antibiotic was shown by 115 (85.5%) of the 134 tested isolates; 94 isolates (70%) resisted simultaneously to streptomycin, sulfonamides and tetracycline. Four isolates were particularly resistant with a resistance to 5 up to 7 antibiotics.

**Discussion**

Molecular characterization of *S. Derby* isolates by PFGE showed that the genome of this serotype is much conserved. The discriminatory ability (*D* value) of the method was 0.73 for the entire panel. This result is in agreement with the one obtained in the study of Kerouanton et al. (2007) where the *D* value was 0.75, with 90 typed strains of more various origins. In their study, the major PFGE pattern was also the same than in our work. In our study, this major pattern was highlighted in 37 of the 52 French studied areas. This profile was also associated with the major antimicrobial resistance pattern: 67 (76%) of the 88 isolates which were streptomycin, sulfonamides and tetracycline resistant were of DXB01 pattern. All
DXB01 isolates were resistant to at least these 3 antibiotics. Nevertheless, those three antimicrobial are not first line treatment of complicated human salmonellosis.

Conclusion

Human and pigs isolates showed a very high similarity in PFGE and antimicrobial susceptibility patterns. Finley et al. (2008) concluded that human salmonellosis due to S. enterica serotype Derby could be sometimes related to pigs. Here, the frequent molecular (DXB001) and phenotypic (resistance to streptomycin, sulfonamides and tetracycline) patterns, are also highlighting the possibilities of other sources.

Figure 1: Dendrogram representing the 43 PFGE-XbaI patterns and the number of isolates per origin with these patterns

References


STREULENS, M.J., MEMBERS OF THE EUROPEAN STUDY GROUP ON EPIDEMIOLOGICAL MARKERS (ESGEM), 1996. Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems. Clinical Microbiology and Infection, 2 : 2-11
