Immediate after birth transmission of epidemic Salmonella enterica typhimurium monophasic strains in pigs is a likely event

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ST3 plasmids were probably due to additional resistance (i.e. bla_{TEM}) genes and not to major variations of the plasmid scaffold (Figure S2, available as Supplementary data at *JAC* Online).

In this study, we report indistinguishable or highly similar $bla_{CTX\text{-}M\text{-}1}$/IncI1/ST3 plasmids in different *E. coli* isolates from a wide range of animal species in France. All animals were unrelated, had different owners and originated from highly distant areas. They were also sampled at various periods of time, from 2006 to 2010. Consequently, we demonstrate the spread of the $bla_{CTX\text{-}M\text{-}1}$/IncI1/ST3 plasmid in the animal population in France, irrespective of the *E. coli* backgrounds and animal species. To our best knowledge, this is also the very first report of an ESBL in a goat.

In a previous work, we suggested that $bla_{CTX\text{-}M\text{-}1}$/IncI1/ST3 plasmids could have transferred to cattle from poultry, a recognized reservoir of IncI1 plasmids carrying ESBL genes.[5] In fact, this ESBL plasmid may have spread more extensively than previously thought into the animal population. Alternately, IncI1 plasmids, which are highly prevalent in animals, may have acquired the $bla_{CTX\text{-}M\text{-}1}$ gene independently within different hosts. Equally worrying is the detection of an ESBL producer in small ruminants, farming of which is relatively spared from excessive antibiotic usage. Interestingly, $bla_{CTX\text{-}M\text{-}1}$/IncI1/ST3 plasmids successfully expanded in animals in France, whereas most $bla_{CTX\text{-}M\text{-}1}$/IncI1 plasmids reported from food-producing animals in the Netherlands were of the ST7 subgroup.[8] Taken together, the differential expansion among countries of different ESBL plasmid subtypes with regard to animal or human sources of ESBL genes is of interest for a better understanding, and subsequent control, of the ESBL epidemiology in both populations.

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Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Naseer U, Sundsfjord A. The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. *Microb Drug Resist* 2011; **17**: 83–97.

2 Carattoli A. Resistance plasmid families in *Enterobacteriaceae*. *Antimicrob Agents Chemother* 2009; **53**: 2227–38.

3 Cloeckaert A, Praud K, Lefevre M *et al*. IncI1 plasmid carrying extended-spectrum-β-lactamase gene $bla_{CTX\text{-}M\text{-}1}$ in *Salmonella enterica* isolates from poultry and humans in France, 2003 to 2008. *Antimicrob Agents Chemother* 2010; **54**: 4484–6.

4 Garcia-Fernandez A, Chiaretto G, Bertini A *et al*. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum β-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. *J Antimicrob Chemother* 2008; **61**: 1229–33.

5 Madec J-Y, Doublet B, Ponsin C *et al*. Extended-spectrum β-lactamase $bla_{CTX\text{-}M\text{-}1}$ gene carried on an IncI1 plasmid in multidrug-resistant *Salmonella enterica* serovar Typhimurium DT104 in cattle in France. *J Antimicrob Chemother* 2011; **66**: 942–4.

6 Madec J-Y, Poirel L, Saras E *et al*. Non-ST131 *Escherichia coli* from cattle harbouring human-like $bla_{CTX\text{-}M\text{-}15}$-carrying plasmids. *J Antimicrob Chemother* 2012; **67**: 578–81.

7 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

8 Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J *et al*. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin Microbiol Infect* 2011; **17**: 873–80.

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Immediate after birth transmission of epidemic *Salmonella enterica* Typhimurium monophasic strains in pigs is a likely event

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Sir,

Salmonellosis remains an important foodborne disease to date and *Salmonella enterica* subsp. *enterica* serovar 4,[5],12:i:−, also known as monophasic *Salmonella* Typhimurium, has been rising as a cause of infection in humans and is widely spread among certain animal populations, namely in the pig reservoir.[1–3] Little is known on the dynamics of transmission of this serovar and, as such, our aim in this study was to determine the likelihood of immediate after birth transmission of monophasic *Salmonella* Typhimurium from sow to piglet.

Table 1. Characterization of monophasic *Salmonella* Typhimurium isolates obtained from families A, B and D with a positive sow and at least one positive piglet

Sample	Resistance pattern	Antimicrobial resistance genes	Plasmid size (kbp)	Pulsed-field type cluster (XbaI restriction)	Pulsed-field subtype[a]
Sow FMV A	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A1
Piglet FMV A1	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Piglet FMV A2	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A5
Piglet FMV A3	AMC AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Piglet FMV A4	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B)	5.6	A	A
Piglet FMV A5	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Piglet FMV A6	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A3
Piglet FMV A7	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A2
Sow FMV B	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Piglet FMV B2	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Piglet FMV B5	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Piglet FMV B7	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Sow FMV D	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A3
Piglet FMV D2	AMC AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Piglet FMV D3	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A1
Piglet FMV D4	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Piglet FMV D5	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A
Piglet FMV D6	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A6
Piglet FMV D7	AMP NAL NEO STR SUL TET	bla_{TEM}, *tet*(B), *sul2*	5.6	A	A4

AMC, amoxicillin/clavulanate; AMP, ampicillin; NAL, nalidixic acid; NEO, neomycin; STR, streptomycin; SUL, sulphonamide compound; TET, tetracycline.
[a]The first pattern identified for a subtype in a type was assigned only a capital letter and the remaining subtypes were named with capital letters and numbers, according to Carriço *et al*.[4]

At one large industrial pig herd in Portugal, 10 sows and 7 piglets from each sow's litter were randomly chosen and sampled at birth. *Salmonella* was isolated according to the protocol described in ISO 6579:2002 Annex D and serotyped based on the Kauffmann–White–Le Minor scheme.[2] The genus and the absence of the second-phase flagellar antigen *fljB* were confirmed by PCR, as recommended by the European Food Safety Authority (EFSA) Panel on Biological Hazards.[2] To determine the likelihood of a sow carrying monophasic *Salmonella* Typhimurium transmitting *Salmonella* to her offspring, Fisher's exact test was employed using R software (http://www.r-project.org). Susceptibility to 17 antimicrobials was determined using the disc diffusion and broth microdilution methods (VetMIC Stördjur, National Veterinary Institute, Uppsala, Sweden), and interpreted according to CLSI guideline M31-A3. CLSI M100-S21 susceptibility criteria were used for nalidixic acid. For neomycin, recommendations from the veterinary working party of the Antibiogram Committee of the French Society for Microbiology were followed. All isolates were screened for bla_{TEM}, bla_{SHV}, bla_{OXA-1}, bla_{CTX-M}, *aadA*, *tet*(A), *tet*(B), *floR*, *sul1*, *sul2*, *dfrA1*, *qnrA*, *qnrB* and *qnrS* using PCR. Plasmid extraction was performed using standard protocols and the size was determined after linearization with S1 exonuclease. Clonality was assessed by PFGE with XbaI restriction, according to the Pulsenet protocol. The patterns were analysed by BioNumerics software using the unweighted pair group method with arithmetic mean and the Dice similarity coefficient. The definition of a pulsed-field type cluster was based on a similarity cut-off value of ≥80% and a subtype on a value of ≥97%.[4]

A total of 10 sows and 70 piglets were sampled, yielding 6 sows and 17 piglets positive for *Salmonella* 4,[5],12:i:−. Among the positive animals, three sows and one or more of the respective piglets carried monophasic *Salmonella* Typhimurium (named as families A, B and D) (Table 1). All offspring from the other three positive sows were negative and one positive piglet descended from a sow negative for monophasic *Salmonella* Typhimurium. All *Salmonella* 4,[5],12:i:− isolates from sows and their respective piglets were further characterized. Two resistance patterns were identified: 17 strains were resistant to ampicillin, neomycin, nalidixic acid, sulphonamides, streptomycin and tetracycline, and 2 strains were additionally resistant to amoxicillin/clavulanate. As for the resistance genes identified, all strains harboured bla_{TEM}, *tet*(B) and *sul2*, except for a single piglet isolate that was negative for *sul2*. The plasmid linearization yielded a single small plasmid of ∼5.6 kbp for all isolates. All isolates belonged to a unique pulsed-field type cluster. Furthermore, 11 strains shared a similar profile representing a subtype with >97% similarity when a 2.5% band tolerance setting was used (see Figure S1, available as Supplementary data at *JAC* Online).

There was a higher probability of a sow positive for monophasic *Salmonella* Typhimurium having a positive offspring with the same serovar (OR=16.10, P=0.001). Accordingly, the probability of a piglet having monophasic *Salmonella* Typhimurium was ∼16 times higher if it belonged to a positive sow.

Several researchers have reported a high *Salmonella* prevalence in sows and their respective piglets early in life.[1,5] Beyond the food safety risk when the sow ultimately enters the food chain, information on vertical transmission from the sow to her offspring is scarce. One study reported the contradictory fact that *Salmonella* serovars isolated from a sow often differed from those isolated from her piglets.[5] In this study, the similarity of the resistance patterns, the antimicrobial resistance genes detected, the plasmid profiles and the PFGE types support the transmission of monophasic *Salmonella* Typhimurium from sow to piglet during or immediately after birth as a means of *Salmonella* colonization of the newborn's gut. To the best of our knowledge, this constitutes the first report of mother-to-piglet horizontal transmission of monophasic *Salmonella* Typhimurium in pigs. The antimicrobial resistance core profile was similar to the chromosomally encoded European highly disseminated clone of monophasic *Salmonella* Typhimurium (ampicillin, streptomycin, sulphonamides and tetracyclines).[3,6] With the continuous rise of antimicrobial resistance in *Salmonella*[1,3,6] and the wide spread of the monophasic serovar, this route of transmission poses an increasingly significant threat to public health, allowing the bacteria to persist and further spread in the swine reservoir. It is also important to mention that the sows and piglets showed no clinical symptoms, increasing the odds of *Salmonella* carriage going undetected throughout the productive cycle. Further studies are needed to fully assess the dynamics of monophasic *Salmonella* Typhimurium during the entire productive pig life cycle, and its impact on food safety and human and animal health.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Soyer Y, Switt AM, Davis MA *et al*. *Salmonella enterica* serotype 4,5,12:i:−, an emerging *Salmonella* serotype that represents multiple distinct clones. *J Clin Microbiol* 2009; **47**: 3546–56.

2 EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on monitoring and assessment of the public health risk of "*Salmonella* Typhimurium-like" strains. *EFSA Journal* 2010; **8**: 1826.

3 Hopkins KL, Kirchner M, Guerra B *et al*. Multiresistant *Salmonella enterica* serovar 4, [5], 12:i:− in Europe: a new pandemic strain? *Euro Surveill* 2010; **15**: pii=19580.

4 Carriço A, Pinto FR, Simas C *et al*. Assessment of band-based similarity coefficients for automatic type and subtype classification of microbial isolates analyzed by pulsed-field gel electrophoresis. *J Clin Microbiol* 2005; **43**: 5483–90.

5 Funk JA, Davies PR, Nichols MA. Longitudinal study of *Salmonella enterica* in growing pigs reared in multiple-site swine production systems. *Vet Microbiol* 2001; **83**: 45–60.

6 Lucarelli C, Dionisi AM, Torpdahl M *et al*. Evidence for a second genomic island conferring multidrug resistance in a clonal group of strains of *Salmonella enterica* serovar Typhimurium and its monophasic variant circulating in Italy, Denmark, and the United Kingdom. *J Clin Microbiol* 2010; **48**: 2103–9.