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Vertical transmission of *Escherichia coli* carrying plasmid-mediated AmpC (pAmpC) through the broiler production pyramid

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Objectives: In Sweden the prevalence of Enterobacteriaceae with transferable resistance to extended-spectrum cephalosporins (ESCs) is low. However, in broilers ESC-resistant *Escherichia coli* is common, with a clear dominance of bla_{CMY-2}. Antimicrobials are rarely used in broiler production in Sweden and cephalosporins are never used. Introduction through imported breeding stock and subsequent vertical transmission of the bacteria through the production pyramid could be one explanation for this high prevalence.

Methods: To test this hypothesis, paper linings from imported flocks of grandparent animals were screened for the presence of ESC-resistant *E. coli* and a positive flock, together with its progeny, was followed longitudinally through the production pyramid using boot swabs. The relationship of isolated ESC-resistant *E. coli* was investigated using multiple-locus variable number tandem repeat analysis (MLVA).

Results: ESC-resistant *E. coli* carrying bla_{CMY-2} was isolated from six out of eight imported flocks of grandparent animals. One clone of *E. coli* carrying bla_{CMY-2} occurred in all levels of the production pyramid and in flocks of imported grandparent animals.

Conclusions: *E. coli* carrying bla_{CMY-2} is frequently present among grandparent animals imported to Sweden for breeding purposes. The occurrence of one clone in all levels of the production pyramid indicates that its introduction through imported breeding stock and vertical transmission through the production pyramid could be one explanation for the high proportion of Swedish broilers colonized with ESC-resistant *E. coli*.

Keywords: ESBL, bla_{CMY-2}, resistance

Introduction

It has been concluded that the occurrence of Enterobacteriaceae with transferable resistance to extended-spectrum cephalosporins (ESCs) due to extended-spectrum β-lactamases (ESBLs) or plasmid-mediated AmpC (pAmpC) in farm animals, especially broilers, can have an impact on public health.[1] For example, in the Netherlands *Escherichia coli* of the same type and with the same ESBL-encoding gene on identical plasmids has been isolated from broilers and humans.[2] In Sweden, there are only sporadic findings of ESC-resistant Enterobacteriaceae in animals except in broilers, where they are found frequently (∼50%).[3] However, the situation in broilers differs from that in most other European countries in that there is dominance of one gene, bla_{CMY-2}, and in 2012 this was the only gene identified.[3,4] The high prevalence was unexpected because the use of antimicrobials for broilers or breeding stock in Sweden is very limited and cephalosporins are never used.[3,5] A possible explanation might be transfer through the production pyramid from birds imported for breeding purposes. Such top-down transmission has been suggested previously.[6] Also, in a pilot study in Sweden ESC-resistant *E. coli* was identified within the pyramid and from breeding animals.[5]

To investigate the hypothesis that the high proportion of Swedish broilers colonized with ESC-resistant *E. coli* is due to introduction through imported breeding stock and subsequent vertical transmission, samples from imported flocks of grandparent animals were selectively cultured and a positive flock, together with its progeny, was followed longitudinally through the production pyramid.

Methods

From July 2010 to August 2011, paper linings from the boxes in which imported flocks of grandparent animals arrived in Sweden were screened for ESC-resistant *E. coli*. In addition, one positive flock was sampled longitudinally through the production pyramid (Figure 1). Boot swabs ('Sterisocks humid'; Sodibox®, Névez, France) were taken in compartments where the grandparent animals were kept when they were 17, 21 and 28 weeks old ($n = 14$, 8 and 8). In the same way, one flock of parent

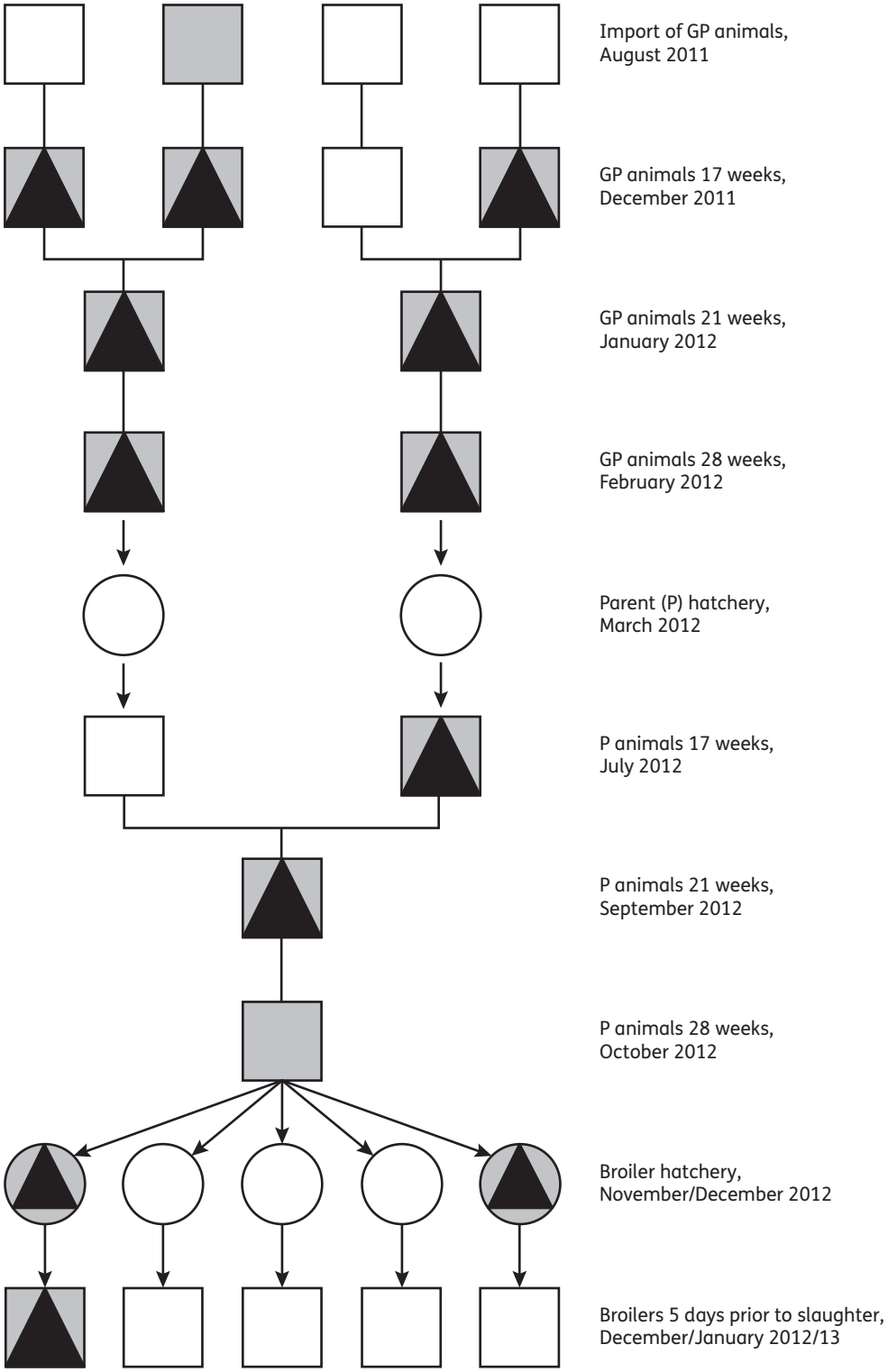

Figure 1. Illustration of the different sampling occasions and whether ESC-resistant *E. coli* was isolated (shaded) or not isolated (not shaded), and whether the resistant isolates belonged to the dominating MLVA CC (black triangles) or not. Squares indicate paper linings or boot swabs and circles indicate environmental samples at hatcheries. Note that the clonal lineage found the whole way through the production pyramid was found in three earlier imports of grandparent (GP) animals, including the two imports preceding that followed longitudinally.

animals originating from these grandparent animals was sampled 17, 21 and 28 weeks ($n=5$, 4 and 4) after hatching and five broiler flocks originating from the parent animals were sampled ~5 days prior to slaughter ($n=1$ per flock). Environmental samples from parent animal and broiler hatcheries were taken directly after the specific batch had been processed with sterile cloths (Sodibox®) that had been factory pre-impregnated with buffered peptone solution with 10% neutralizing agent ($n=2$ each time). Before placements of birds, environmental samples were taken with sterile cloths from the houses where the imported grandparent animals and the parent animals were first placed ($n=4$ each time), from the houses to which they were moved when they were 18–19 weeks old ($n=16$ and 8) and from the houses in which the broilers were raised ($n=2$ each time).

Samples were treated by stomaching (Stomacher 400 circulator, Seward, UK) in saline for 1 min, 230 revolutions. Then, 0.1 mL was streaked on MacConkey agar (Difco, Hampshire, UK) with cefotaxime (1 mg/L; Sigma Aldrich, China) and incubated overnight at 37°C. Furthermore, 10 mL was mixed 1:1 with 2× MacConkey broth (Lab M, Lancashire, UK) with cefotaxime (2 mg/L) and incubated at 37°C overnight before 0.1 mL was streaked on MacConkey agar with cefotaxime and incubated as above. However, paper linings from two of the imports were only cultured without enrichment and from three of the imports only with enrichment. Colonies with morphology typical of *E. coli* were sub-cultured on horse blood agar (Oxoid, Basingstoke, UK) and indole tested. From samples of imported grandparent flocks, 1 isolate of ESC-resistant *E. coli* was collected per sample and from all the other samplings ≤10 isolates from each branch (fathers' fathers, fathers' mothers etc.) of the production pyramid were selected.

Isolates from imported grandparent flocks were screened by PCR for genes encoding ESBL and pAmpC.[7–9] The isolates from the longitudinal part were screened only for pAmpC genes. Isolates from imported grandparent flocks that were PCR positive for the investigated genes were subjected to sequencing.[10] Clonal relationship was investigated by multiple-locus variable number tandem repeat analysis (MLVA).[11]

Results

Eight flocks of imported grandparent animals were investigated and ESC-resistant *E. coli* was isolated from six. In the longitudinal sampling, ESC-resistant *E. coli* was isolated on every occasion that samples were taken from grandparent and parent animals, as well as in one of the samples from broilers and two of the samples from broiler hatcheries (Figure 1). ESC-resistant *E. coli* was not isolated from empty houses. All isolates were positive for the bla_{CMY-2} group and the bla_{CMY-2} gene was identified in sequenced isolates. Therefore, and considering the previously described dominance of bla_{CMY-2} among isolates of the pAmpC genotype from broilers in Europe, it is here assumed that all the isolates carry this gene if positive for the bla_{CMY-2} group.[1,3,4] All isolates from imported grandparent flocks were negative for all other genes investigated.

In total, 33 MLVA profiles were identified among the isolates from breeding stock and broilers and five different clonal clusters (CCs), differing in no more than one allele, were identified. One CC included isolates from the grandparent animals, parent animals, broilers and the broiler hatchery (Figure 1). This CC differed only in allele CVN0016 with one or two repeats (Table 1). However, the *E. coli* carrying bla_{CMY-2} in the imported flock followed longitudinally differed substantially. Therefore, isolates from the earlier imported grandparent flocks were subjected to MLVA and the specific MLVA type was identified in three flocks. Two of these were those preceding the imported flock followed longitudinally and contained birds originating from the same great grandparents' farms.

Discussion

Escherichia coli carrying bla_{CMY-2} of highly related MLVA types was found in all levels of the production pyramid, including imported grandparent animals. This result supports the hypothesis that the high proportion of Swedish broilers colonized with *E. coli* carrying bla_{CMY-2} can be explained, at least partly, by introduction through imported breeding stock and vertical transmission through the production pyramid. The results of the study also show that *E. coli* carrying bla_{CMY-2} is frequently present in flocks of grandparent animals imported to Sweden.

Transmission through the production pyramid was corroborated by the presence of one highly related clonal lineage, differing only between one and two repeats in the CVN0016 locus (Table 1), in all levels of the production pyramid. It should, however, be noted that within the same level of the production pyramid CVN0016 had an identical number of repeats. It has been suggested that isolates differing in MLVA profiles in no more than one locus may share the same source, especially if that locus is highly variable.[12,13] It has also been demonstrated that the MLVA protocol used in the current study is a useful tool for epidemiological studies and a suitable method for tracing the spread of *E. coli*.[14] The identified clonal lineage found in this study can therefore be considered to represent one clone, transmitted from one source, likely the import of animals for breeding purposes. However, the clone was not identified in the samples taken upon arrival of the specific grandparent flock followed longitudinally. Isolates of the same MLVA type were, however, identified among isolates from imports directly preceding this flock. This proves that the clonal lineage was present in flocks of grandparent animals imported from the UK to Sweden. The reason the clone was not isolated in the samples taken upon arrival of the flock followed longitudinally could be because it was missed as not all boxes of birds were sampled. Another explanation is that the

Table 1. The three different highly related MLVA types identified among *E. coli* carrying bla_{CMY-2}

	Allele										
MLVA type	CVN001	CVN002	CVN003	CVN004	CVN007	CVN0014	CVN0015	CCR001	CVN0016	CVN0017	Detected in level:
A	5	2	−2	13	3	7	5	1	11	−2	H, B
B	5	2	−2	13	3	7	5	1	12	−2	I, GP, H
C	5	2	−2	13	3	7	5	1	13	−2	P

B, broilers; GP, grandparents; H, hatchery; I, import samples; P, parents.

bacteria persisted in the environment in empty houses, but no ESC-resistant *E. coli* were isolated from environmental samples from the empty houses. The current study is also supported by previous work suggesting vertical transmission of *E. coli* from grandparent animals via parent animals all the way to broilers.[6,15–17]

The epidemiology of ESC-resistant *E. coli* in Swedish broiler production is probably also influenced by factors other than vertical transmission. For example, recirculation on farms must be considered. It is also likely that the epidemiology is influenced by transfer between bacteria of plasmids carrying the ESC genes. The use of antimicrobials and other management factors has also been shown to be of importance in a Belgian study.[17]

In conclusion, this study suggests that *E. coli* carrying bla_{CMY-2} in imported breeding animals can transmit vertically in the broiler production pyramid despite the absence of a known selective pressure. That *E. coli* carrying bla_{CMY-2} was isolated from six of the eight imported flocks sampled demonstrates that there is a common influx of such bacteria via imported animals. To be able to minimize the occurrence of or completely remove ESC-resistant *E. coli* in broiler production, it is important that imported animals are free from such bacteria.

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Transparency declarations

None to declare.

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