National Prevalence of Resistance to Third-Generation Cephalosporins in Escherichia coli Isolates from Layer Flocks in France

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Resistance of Escherichia coli to third-generation cephalosporin (3GC) in fecal samples representative of French egg production was studied. The susceptibility to cefotaxime of E. coli isolates obtained by culture on nonselective media was determined. Twenty-two nonsusceptible isolates were obtained (7.51%; 95% confidence interval, 4.49 to 10.54%), the majority of which came from young birds. Most isolates carried a blaCTX-M-1 group gene, and a few carried a blaCMY-2-like gene. Control of 3GC resistance in laying hens is needed.

Several studies have reported the high prevalence of resistance to third-generation cephalosporin (3GC) in broilers in different countries (1–4), but little is known about the prevalence in the egg production sector. Thus, fecal samples collected from French pullets and laying hens, in the framework of the official national plan for the control of Salmonella infection, were used to evaluate the prevalence of Escherichia coli not susceptible to 3GC.

From February to May 2011, 300 pools of fresh fecal samples were collected by veterinary services from 300 different production flocks (283 different farms) in 71 French departments, according to official instructions.

For each sample, one E. coli isolate, obtained on MacConkey medium, was randomly collected and identified (5). A standardized inoculum of each isolate was deposited on Mueller-Hinton agar containing cefotaxime (1 mg/liter) (6). The isolates with a MIC of cefotaxime higher than 1 mg/liter were further analyzed. Their susceptibility to other antimicrobials was tested by the disk diffusion method (7) and determination of MIC by using Sensititre plates (Biocentric, Bandol, France). Phylogenetic groups were determined (8), and pulsed-field gel electrophoresis (PFGE) was performed after restriction with XbaI (9). The presence of conjugative plasmids was checked by conjugation. Plasmids were prepared (10) and used to transform E. coli DH5α. 3GC resistance genes of the isolates were detected by use of microarrays (Check-MDR-CT101; Biocentric) (11) and sequencing (12, 13). The replicon types were identified (14).

Among 293 viable E. coli isolates, 22 (7.51%; 95% confidence interval [CI], 4.49 to 10.54%) could grow on cefotaxime-supplemented agar. Nonsusceptible isolates were more frequently obtained from young birds, and isolation probability decreased with age of infection flocks (283 different farms) in 71 French departments, according to official instructions. The hatchery for 180 of the flocks was unknown. Six different hatcheries were recorded for the other flocks, and the proportion of nonsusceptible isolates differed significantly among them (Fisher’s exact test, P = 0.002).

Disk diffusion assay showed that 17/22 nonsusceptible isolates showed synergy between amoxicillin-clavulanic acid and 3GC. Characteristics of extended-spectrum beta-lactamase (ESBL)-producing E. coli. Their characteristics are given in Table 1. The MIC of ceftriaxone for all these isolates was >64 mg/liter. The PFGE profiles of the 14 typeable strains all appeared different from each other. Conjugation and transformation experiments revealed that resistance to cephalosporins, and most often to tetracycline or trimethoprim-sulfamethoxazole, could be transferred to recipient cells. The replicons identified in the transformants were IncI1. The 3GC resistance genes belonged to the blaCTX-M-1 group for all strains. Profiles obtained after EcoRI digestion of plasmid DNA revealed that similar profiles could be obtained for plasmids obtained from strains 2 and 110, from strains 40, 96, and 112, and from strains 273 and 294 (data not shown).

For 5/22 isolates, all obtained from pullets, no synergy between amoxicillin-clavulanic acid and 3GC could be observed; these five isolates were resistant to cefoxitin. They also exhibited additional resistance to tetracycline, and four of them exhibited resistance to aminoglycosides. All were highly resistant to ceftriaxone (MIC, >8 mg/liter), except strain 260, for which the ceftriaxone MIC was 2 mg/liter. The four typeable strains showed profiles that were different from each other. Conjugation and transformation experiments indicated that resistance to cefoxitin could be transferred from the four strains that were highly resistant to ceftriaxone, one with resistance to both tetracycline and amikacin, and to amoxicillin-clavulanic acid (Table 1). The replicons associated with transfer of cefoxitin resistance were IncI1, B/O, A/C, or F. Four strains and their transformants contained a blaCMY-2-like gene. The electrophoresis profiles of the EcoRI-digested plasmids obtained from the transformants of isolates 121 and 134 were similar to each other (data not shown).

Very limited data concerning 3GC resistance in the egg production sector are available (15), except for one study involving a few organic production farms in Denmark (16). Wasyl et al. revealed that 42.3% of 163 samples from layers in Poland and other European countries contained 3GC-resistant E. coli when inocu-
lated on cefotaxime-supplemented medium, a sensitive procedure that enables the detection of rare resistant isolates among many nonresistant E. coli strains (15). When isolates from nonsupplemented media were tested, the proportion of resistant isolates was only 0.6%. The higher percentage obtained in our study probably reflects the fact that we sampled pullets and layers of all ages and not only birds arriving at the slaughterhouse, which, as discussed below, less frequently carry 3GC-resistant E. coli.

Our results showed that resistant E. coli strains were more often isolated from pullets than from laying hens and that the proportion of resistant isolates varied in samples obtained from birds from different hatcheries. It is tempting to speculate that this could be related to antimicrobial treatments of young birds or off-label use of cefotiofur in hatcheries (e.g., in ovo or subcutaneous injection) (17, 18). Moreover, vertical transmission and circulation of 3GC-resistant isolates in farms and hatcheries are probable in meat poultry (19) and egg (20) productions. It is also plausible that administration of frequently prescribed molecules (tetracycline or trimethoprim-sulfonamides) results in coselection of 3GC resistance.

As in broilers in France (1, 21), the most prevalent resistance gene was bla\(_\text{CTX-M-1/61}\) borne on an IncI1 plasmid. The dissemination of bla\(_\text{CTX-M-1}\) on IncI1 plasmids has also been observed in E. coli and in Salmonella spp. in a number of countries, in poultry and in other animal species (4), but characterization by plasmid multilocus sequence typing (pMLST) revealed that different plasmids may be present (22–25). The other gene detected in pullets was bla\(_\text{CMY-2/22/61}\), which is also frequent in poultry in other countries (26–30).

It is feared that ESBL genes might spread from poultry to humans either through professional exposure (23) or via food. In this context, it is interesting to underline that bla\(_\text{CTX-M-1}\) was, along with bla\(_\text{CTX-M-15}\), one of the two most frequent ESBL genes.
harbored by *E. coli* in fecal samples obtained from healthy subjects in a Parisian checkup center (31). Moreover, the transfer, in the hen gut, of plasmids encoding resistance to 3GC from *E. coli* to *Salmonella* spp. (32) could lead to problematic human infections with 3GC-resistant *Salmonella*-infected eggs. Therefore, the monitoring and control of the presence of 3GC-resistant *E. coli* and *Salmonella* in egg production are essential.

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**REFERENCES**


