

## Prevalence of Fecal Carriage of Acquired Expanded-Spectrum Cephalosporin Resistance in *Enterobacteriaceae* Strains from Cattle in France<sup>∇</sup>

In gram-negative pathogens, extended-spectrum beta-lactamases (ESBLs) confer resistance to penicillins, cephalosporins (including extended-spectrum cephalosporins), and aztreonam, but not to cephamycins and carbapenems, and are inhibited by beta-lactamase inhibitors (8). Recently, cefotaximases have become the most prevalent ESBLs worldwide (7) and have also been detected in animals (see, for instance, references 2 and 9).

Contrary to the human field (see, for instance, references 10, 11, and 13), little has been reported on the prevalence of fecal carriage of ESBL producers by animals (1, 3, 4, 12), and this issue was examined here by testing 1,264 nonduplicate specimens collected from cattle at farms and slaughterhouses in France.

From March to October 2006, fecal samples from 657 sick cattle (farm) were plated on agar supplemented with ceftazidime (1 µg/ml) or cefotaxime (1 µg/ml), 117 of which allowed colonies to grow. After determination of MICs with ESBL Etest strips, 52 ESBL or cephalosporinase producers were identified. Species identification revealed 41 *Escherichia coli* and 11 non-*E. coli* isolates, i.e., 7 of *Acinetobacter* sp., 2 of *Pseudomonas aeruginosa*, 1 of *Citrobacter freundii*, and 1 of *Hafnia alvei*. Antibiotic susceptibility testing, including a double-disk synergy test, confirmed an acquired *bla*-encoded phenotype in all of the *E. coli* isolates, whereas a natural phenotype was identified in all 11 non-*E. coli* isolates. The 41 *E. coli* isolates were tested by PCR for the *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, *bla*<sub>CMY</sub>, and *bla*<sub>CTX-M</sub> groups as described previously (9), revealing 17 ESBL producers (12 *bla*<sub>CTX-M-1</sub> isolates, 1 *bla*<sub>CTX-M-15</sub>

isolate, 3 *bla*<sub>CTX-M-14</sub> isolates, and 1 *bla*<sub>TEM-126</sub> isolate), either alone (15 isolates) or with a *bla*<sub>TEM</sub> gene (2 isolates).

Similarly, of the fecal samples collected from 607 healthy cattle at abattoirs from March 2005 to June 2006, 61 allowed colonies to grow, 46 of which were ESBL or cephalosporinase producers (35 *E. coli* and 11 non-*E. coli* isolates, i.e., 8 *Acinetobacter* sp. isolates, 1 *Pseudomonas aeruginosa* isolate, 1 *Enterobacter cloacae* isolate, and 1 *Hafnia alvei* isolate). Only the 35 *E. coli* isolates harbored an acquired *bla*-encoded phenotype, 25 of which produced ESBLs (21 had *bla*<sub>CTX-M-1</sub>, 2 had *bla*<sub>CTX-M-14</sub>, and 2 had *bla*<sub>SHV-12</sub>), either alone (19 isolates) or with a *bla*<sub>TEM</sub> gene (6 isolates).

Thus, the prevalence of *E. coli* producing acquired expanded-spectrum cephalosporinases was 6.2% and 5.8% in sick and healthy cattle, respectively (Table 1). All 42 ESBL-producing *E. coli* isolates were *qnrA*, *-B*, and *-S* negative by PCR, and most of them (39/42) showed unrelated pulsed-field gel electrophoresis patterns (not shown).

The use of expanded-spectrum cephalosporins (such as ceftiofur) in veterinary medicine may select ESBL producers in animals. Most genes were of the CTX-M-1 group, so that the epidemiology in farm animals seems to mirror the trend observed in humans in France (5, 6). The absence of the *qnr* gene was reassuring, and the predominant absence of clonality may argue for multiple transferable genetic elements supporting ESBL-encoding genes.

Overall, our study indicates a worrisome prevalence of fecal carriage of cephalosporin resistance in cattle in France, with a higher prevalence of ESBL-producing *E. coli* at slaughterhouses compared to farms (Table 1).

TABLE 1. Cephalosporin resistance in *E. coli* isolates from sick and healthy cattle

| Cattle group (no.) and cephalosporin resistance mechanism | No. of <i>E. coli</i> isolates | % in cattle |
|---|--------------------------------|-------------|
| <b>Sick (657)</b>   |                                |             |
| CTX-M alone   | 14                             | 2.1         |
| CTX-M + TEM   | 2                              | 0.3         |
| Other ESBL alone  | 1                              | 0.2         |
| All ESBLs   | 17                             | 2.6         |
| CMY   | 2                              | 0.3         |
| Non-CMY <sup>a</sup>                                      | 22                             | 3.4         |
| All non-ESBLs   | 24                             | 3.7         |
| All acquired cephalosporin resistance mechanisms          | 41                             | 6.2         |
| <b>Healthy (607)</b>                                      |                                |             |
| CTX-M alone   | 17                             | 2.8         |
| CTX-M + TEM   | 6                              | 1.0         |
| Other ESBL alone  | 2                              | 0.3         |
| All ESBLs   | 25                             | 4.1         |
| Non-CMY <sup>a</sup>                                      | 10                             | 1.6         |
| All non-ESBLs   | 10                             | 1.6         |
| All acquired cephalosporin resistance mechanisms          | 35                             | 5.8         |

<sup>a</sup> Another chromosomal or plasmidic AmpC mechanism, as deduced from resistance to cefoxitin, cefotaxime, and ceftazidime, but not cefepime, cefpirome, and imipenem, and without cephalosporin-clavulanate synergy.

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