# High Prevalence of the Animal-Associated bla CTX-M-1 IncI1/ST3 Plasmid in Human Escherichia coli Isolates

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# High Prevalence of the Animal-Associated $bla_{CTX-M-1}$ IncI1/ST3 Plasmid in Human *Escherichia coli* Isolates

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In humans and animals, extended-spectrum beta-lactamases (ESBLs) are widespread enzymes conferring resistance to broad-spectrum cephalosporins. Consumption of ESBL-contaminated foodstuffs or contacts with ESBL-colonized/infected animals enhance the risk of human spread of ESBL genes from nonhuman sources. Moreover, ESBL genes are mostly located on plasmids of various incompatibility (Inc) groups and subtypes, which play a key role in their horizontal transfer (1).

In animals, $bla_{CTX-M-1}$ is the most common ESBL gene reported so far. It is broadly disseminated on several plasmids, among which IncI1/ST3 was repeatedly identified from companion and food animals and foodstuffs (2–6). In humans, the dominant ESBL genes are $bla_{CTX-M-15}$ and $bla_{CTX-M-14}$. However, recent studies showed that the prevalence of CTX-M-1 producers in humans in France was high. Indeed, CTX-M-1 *Escherichia coli* accounted for 26% of all ESBL producers colonizing children (7) and 24% of clinical ESBL producers in hospitals (8). The prevalence of CTX-M-1 was also equal to that of CTX-M-15 among ESBL *E. coli* isolates from healthy adults in Paris (9). In parallel, whole-genome sequencing recently suggested the efficient spread of highly similar $bla_{CTX-M-1}$ IncI1/ST3 plasmids in humans and animals (10). Accordingly, our aim was to characterize $bla_{CTX-M-1}$-carrying plasmids from various human sources to investigate their potential commonalities with those of animal origin.

Our study focused on 48 unrelated human CTX-M-1 *E. coli* isolates obtained from three independent published studies. This collection included (i) clinical CTX-M-1 *E. coli* isolates ($n = 35$) from hospitalized patients (network of 38 hospitals of Assistance Publique/Hôpitaux Paris, 23,000 beds, November 2008 to June 2009) (8) and (ii) nonclinical (carriage) CTX-M-1 *E. coli* isolates ($n = 13$), either from children attending day care centers (DCCs) in southern France ($n = 7$, January to April 2012) (7) or from adults living in the Paris area ($n = 6$, mid-February to mid-March 2011) (9).

Plasmids carrying the $bla_{CTX-M-1}$ gene were transferred by conjugation to *E. coli* rifampin-resistant K-12 J53 recipient strains selected on Mueller-Hinton agar with rifampin (250 μg/ml) and cefotaxime (4 μg/ml). Plasmids were further characterized using S1 nuclease-treated DNA followed by pulsed-field gel electrophoresis (S1-PFGE), PCR-based replicon typing, restriction fragment length polymorphism (RFLP) analysis, plasmid multilocus sequence typing (pMLST), and Southern blot hybridizations with the $bla_{CTX-M-1}$ gene and IncI1-specific digoxigenin (DIG)-labeled probes (Roche Applied Science, Mannheim, Germany).

Among the 48 *E. coli* isolates, 40 (83.3%) carried the $bla_{CTX-M-1}$ gene on an IncI1 plasmid; among the latter, 32/40 (80%) were IncI1/ST3 (Table 1). IncI1/ST3 was dominant in both diseased (21/35, 60%) and healthy (11/13, 85%) humans and was similarly distributed among children (6/7, 85%) and adults (5/6, 83%). Closely related restriction profiles were obtained for those $bla_{CTX-M-1}$ IncI1/ST3 plasmids, whatever their human origin (Fig. 1). For comparison purposes, $bla_{CTX-M-1}$ IncI1/ST3 plasmids isolated from dogs originating in Paris (different animals and *E. coli* clones) were included (4) and also showed related RFLP patterns (Fig. 1). Besides, five IncI1 plasmids belonged to ST7, one to ST35, and one to ST157 (2/18/4/1/2, closely related to ST3), and one was untypeable.

Such a dominance of a single IncI1 plasmid subtype (IncI1/ST3) spreading $bla_{CTX-M-1}$ in humans was never previously reported and is surprising with regard to the diversity of contexts studied (children/adults, infection/colonization, different geographical origins and time periods). As determined in the original studies (7, 8, 9), the vast majority of the human *E. coli* isolates were of different lineages and/or PFGE types, which indicates that these results do not reflect the clonal spread of a particular strain. Considering the abundance of

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**TABLE 1** Plasmid types and subtypes carried by the 48 *E. coli* isolates included in the study

| Isolate source | Study reference | No. of $bla_{CTX-M-1}$-carrying plasmids ($n = 48$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | IncI1 | | | | | | |
| | | ST3 | ST7 | ST35 | ST157 | NT[a] | IncF | NT |
| Clinical cases ($n = 35$) | 8 | 21 | 5 | 0 | 0 | 1 | 1 | 7 |
| Healthy day care center children ($n = 7$) | 7 | 6 | 0 | 1 | 0 | 0 | 0 | 0 |
| Healthy adults ($n = 6$) | 9 | 5 | 0 | 0 | 1 | 0 | 0 | 0 |
| Total ($n = 48$) | | 32 | 5 | 1 | 1 | 1 | 1 | 7 |

[a] NT, not typeable.

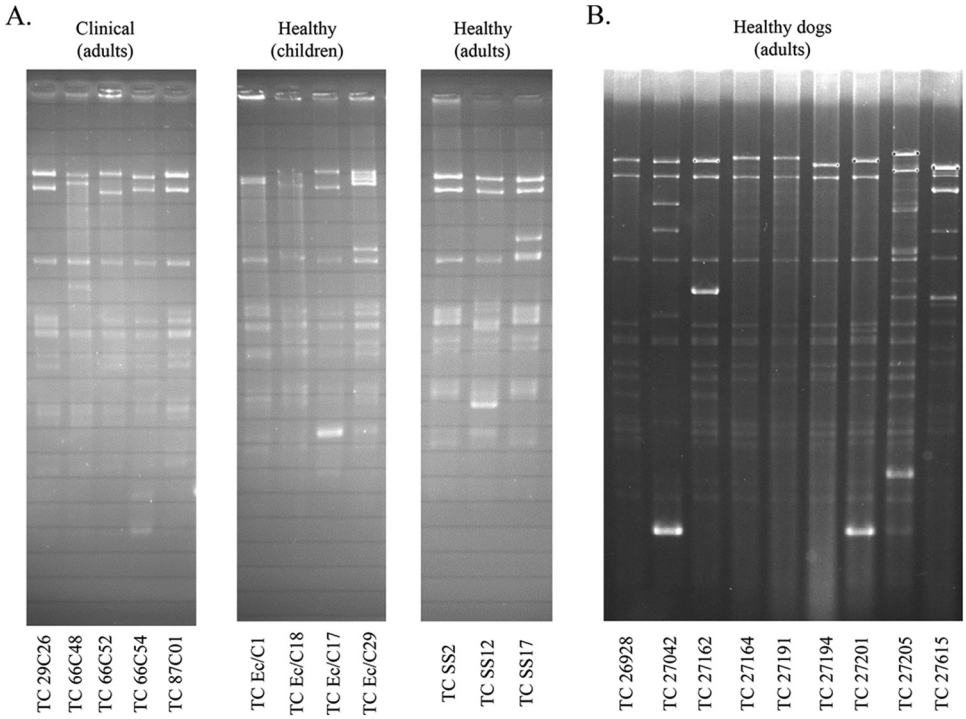


**FIG 1** Restriction fragment length polymorphism (RFLP) analysis of transconjugants of *E. coli* (TC-*E. coli*) plasmid DNA digested with EcoRI. TC-*E. coli* plasmid originated from humans (A) or dogs (B). Isolate numbers correspond to the original numbers given in references 4, 7, 8, and 9.

*bla*$_{CTX-M-1}$ IncI1/ST3 in animals, together with that of the new *bla*$_{CTX-M-1}$ IncI1/ST157 plasmid (close to *bla*$_{CTX-M-1}$ IncI1/ST3) reported here and of the 5 *bla*$_{CTX-M-1}$ IncI1/ST7 plasmids also previously recognized in humans and poultry (11), these data might suggest an animal contribution to the CTX-M-1 reservoir in humans through the spread of specific plasmids, such as IncI1/ST3. Beside food animals, which are often proposed as a possible source for such transfers (5, 10), our results also raise questions with respect to the contribution of companion animals to this burden.

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We declare that we have no conflicts of interest.

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