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# Virulence genotype and nematode-killing properties of extra-intestinal *Escherichia coli* producing CTX-M β-lactamases

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## ABSTRACT

This study evaluated the virulence potential of *Escherichia coli* isolates producing CTX-M β-lactamases. During a 24-month period, 33 extended-spectrum β-lactamase (ESBL)-producing *E. coli*, including 14 CTX-M-producers, were isolated from urinary tract infections at Nîmes University Hospital, France. The prevalence of 14 major virulence factors (VFs) was investigated by PCR and compared with the prevalence in a group of 99 susceptible *E. coli* isolates. Ten VFs were less prevalent ($p < 0.05$) in the ESBL isolates than the susceptible *E. coli*, while *iutA* and *traT* were more prevalent in ESBL isolates ($p < 0.05$). Moreover, the CTX-M-producing isolates had significantly fewer VFs than TEM-producing isolates. A novel infection model using the nematode *Caenorhabditis elegans* was developed to assess the virulence properties of extra-intestinal pathogenic *E. coli* (ExPEC) strains *in vivo*. *C. elegans* infection assays, using 14 ESBL-producing *E. coli* and ten susceptible *E. coli* isolates, indicated that the ability to kill nematodes correlated with the presence of VFs, and that CTX-M-producing isolates had relatively low virulence *in vivo*. Overall, the results suggested that hospital-acquired CTX-M-producing *E. coli*, although adapted for survival in an antibiotic-rich environment such as the hospital milieu, have a relatively low intrinsic virulence potential.

**Keywords** *Caenorhabditis elegans*, CTX-M, *Escherichia coli*, pathogenicity, urinary tract infection, virulence factors

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## INTRODUCTION

The increase in the frequency of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* strains causing nosocomial infection is a major problem, exacerbated by the fact that ESBLs are generally encoded by plasmids that can be transmitted easily to other strains. The CTX-M group of ESBLs hydrolyse broad-spectrum cephalosporins, with higher levels of hydrolytic activity against cefotaxime than against ceftazidime, and are susceptible to suicide inhibitors [1]. Initially described during the second half of the 1980s in *E. coli* and *Salmonella*, the incidence of CTX-M-producing bacteria has increased dramatically since 1995, with spread of CTX-M genes to other enterobacteria being reported in most parts of the world [1]. CTX-M genes are now widespread in *E. coli* strains, which are the major cause of urinary tract infection (UTI), leading to serious problems in the management of these common infections [2,3]. This problem is exacerbated by the frequent association between CTX-M production and quinolone resistance.

As CTX-M-producing strains pose a significant therapeutic challenge, it is important to determine their intrinsic virulence potential. The main recognised virulence or fitness factors of extra-intestinal pathogenic *E. coli* (ExPEC) include adhesins, capsules, toxins and iron-acquisition

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systems, which are mainly encoded by chromosomal pathogenicity islands (PAIs) or by large plasmids [4–7]. Interestingly, several studies have suggested a correlation between resistance to quinolones or fluoroquinolones and a low level of virulence factors (VFs) [8–14]. In addition, two studies have shown an association between CTX-M-type ESBLs and fluoroquinolone resistance and a lack of VFs, based on the analysis of a small number of VFs [8,15], although another study has reported that CTX-M-producing strains from a community outbreak did not possess lower levels of VFs [16]. In all these studies, the relationship between resistance and virulence has been based on the presence/absence of VFs, and no animal studies have been conducted to monitor virulence *in vivo*.

The virulence of ExPEc *in vivo* is usually assessed using a model of ascending UTI in mice that is both technically and ethically unsuitable to evaluate the virulence of a large number of clinical isolates. A correlation between the virulence of *E. coli* in mice and the number of VFs has been demonstrated, based on the ability to kill mice [17]. The nematode *Caenorhabditis elegans* has been validated for use as a possible alternative model for studying the virulence properties of various pathogenic bacteria [18]. However, to date, this model has not been used to compare the lethality induced by different uropathogenic *E. coli* strains. The present study examined a collection of ESBL-producing uropathogenic *E. coli* isolates from a French University Hospital for the presence of genes encoding VFs and for virulence in the nematode model of infection.

## MATERIALS AND METHODS

### Data collection and bacterial strains

A surveillance programme for ESBL-producing *E. coli* isolates from UTI was introduced at the Nîmes University Hospital, Nîmes, France, between 1 April 2002 and 31 March 2004. Positive urine cultures were defined by leukocyturia of $>10^4$/mL and a bacterial concentration of $>10^5$ CFU/mL [2]. The genus and species were determined biochemically using the Vitek 2 GNS-F7 identification card (bioMérieux, Marcy-l'Etoile, France). For each ESBL-producing *E. coli* isolate identified during the study, three susceptible (i.e., to all antimicrobial agents tested) *E. coli* isolates from UTI were also selected. Information concerning the patients' age, gender, hospital admission, immunocompetence and McCabe scores, as well as the date of isolation of the bacteria, was collected. Patients were deemed to have community-acquired disease if the first culture positive for ESBL-producing *E. coli* was obtained within 48 h of admission. Duplicate isolates from the same patient were excluded, and only the first positive isolate from each urine specimen per patient was retained.

### Characterisation of β-lactamase-encoding and *qnr* genes

Isoelectric focusing was performed using polyacrylamide gels as described previously [19]. The $bla_{TEM}$, $bla_{SHV}$ and $bla_{CTX-M}$ genes were detected by PCR using specific primers [19–22], followed by sequencing of the PCR products. All quinolone-resistant isolates were screened by PCR for the *qnrA* gene [23].

### Susceptibility testing

Antibiotic susceptibility testing was performed using the Vitek 2 AST-N017 card (bioMérieux) and by disk-diffusion on Muller-Hinton agar with antibiotic disks (Pasteur Diagnostics, Marne-la-Coquette, France). Production of ESBLs was tested using the double-disk synergy test [24]. Isolates were studied in more detail whenever the synergy test for ESBL-production was positive. The isolates were classified as sensitive, intermediately-resistant, or resistant to the other antibiotics tested, according to the recommendations of the Antibiotic Susceptibility Testing Committee of the French Society for Microbiology (http://www.sfm.asso.fr). The susceptible *E. coli* strains used as controls were selected on the basis of their susceptibility to all 22 antibiotics tested.

### Pulsed-field gel electrophoresis (PFGE) analysis

Macrorestriction analysis of *Xba*I-digested chromosomal DNA was performed by PFGE with the CHEF DRII system (Bio-Rad, Ivry-sur-Seine, France) [25]. Electrophoresis was at 6 V/cm at 12°C for 30 h, with pulse times ranging from 40 s to 5 s, and 180 V. The PFGE patterns were analysed with Gel Compar v.3.5 (Applied Maths, Sint-Martens-Latem, Belgium) and compared by the unweighted-pair group method using arithmetic averages (UPGMA) with the Dice similarity coefficient. Isolates were considered to belong to a cluster if the similarity coefficient was >80%.

### Phylogenetic grouping

Phylogenetic grouping of the *E. coli* isolates was determined with a PCR-based method developed by Clermont *et al.* [26] using a combination of three DNA markers (*chuA*, *yjaA*, TspE4.C2).

### Virulence genotyping

The *E. coli* isolates were tested by PCR for the presence of a panel of genes encoding known VFs. PCR amplification of the *papG* alleles (encoding P fimbriae) was as described by Johnson [27]. Methods used to amplify *sfaS*, *focG* (S fimbriae and F1C fimbriae), *afa/draBC* (Dr family adhesin), *fimH* (mannose-specific adhesin subunit of type 1 fimbriae), *hlyA* (haemolysin), *cnf1* (cytotoxic necrotising factor-1), *iutA* (aerobactin), *kpsMT*K1 and *kpsMT*II (capsule synthesis), and *traT* (serum resistance) were as described by Johnson *et al.* [12]. Primers to amplify *iroN* (iron acquisition), *malX* (a marker for a PAI from archetypal uropathogenic strain CFT073) and *irp2* (yersiniabactin) were as described previously [28–30]. Southern blotting with a digoxigenin-dUTP-labelled *fimH* probe was used to confirm the low prevalence of *fimH* among CTX-M isolates.

#### Nematode killing assay

The *C. elegans* infection assay was performed as described by Kurz *et al.* [31], except that the Fer15 mutant line, which has a temperature-sensitive fertility defect, was used rather than wild-type N2 worms. The nematodes and *E. coli* strain OP50 (an avirulent control strain) were provided by J. Ewbank (CIML, Marseille, France). To synchronise the growth of nematodes, eggs were collected using the hypochlorite method [31]. NGM agar plates [31] were inoculated with a drop of an overnight *E. coli* culture and incubated at 37°C for 8–10 h. The plates were allowed to cool to room temperature and were seeded with L4 stage nematodes (20–30 nematodes/plate). The plates were then incubated at 25°C and scored each day for live nematodes under a MS5 stereomicroscope (Leica, Wetzlar, Germany). At least three replica experiments, repeated three times, were performed for each selected clone. A nematode was considered dead when it no longer responded to touch. Worms that died as a result of becoming stuck to the wall of the plate were excluded from the analysis.

#### Statistical analysis

For each VF, comparisons between the CTX-M and TEM-ESBL groups, between the CTX-M and susceptible groups, and between the resistant (TEM and CTX-M) and susceptible groups were evaluated using Fisher's exact test (SAS/ETS software release v.8.1; SAS Institute Inc, Cary, NC, USA), with $p < 0.05$ considered to be statistically significant. To compare the entire survival curves in nematode killing assays, a Cox regression model was calculated using SPSS v.6.1.1 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Epidemiological background

During the 24-month period of the study, 33 ESBL-producing *E. coli* isolates were obtained from urine. All isolates were of nosocomial origin. The median age of the patients (66.7% female) was 76 years; 90.9% of the patients were immunocompromised, and all had at least one underlying co-morbid illness. The calculated vital prognostic was a McCabe score ≥1 in 57.1% of patients infected by CTX-M-producing *E. coli* and in 47.4% of patients infected by TEM-producing *E. coli*. The resistant bacteria were isolated from patients in the following units: medicine (36.3%), geriatrics (21.2%), recovery (15.2%), surgical (15.2%) and intensive care (12.1%).

A control group of 99 antibiotic-susceptible *E. coli* isolates from UTI patients was also included; 48.5% of these isolates were of nosocomial origin. The isolates were obtained from 99 patients (88.9% female, median age 54 years) in different medical units during the study period. The patients were mostly (94.9%) immunocompetent; 10.1% of the patients had at least one underlying co-morbid illness, and only 2.0% had a McCabe score ≥1.

### Antibiotic resistance and phylogenetic characterisation of *E. coli* isolates

PCR analysis showed that the ESBL isolates produced either CTX-M or TEM β-lactamases (Table 1). The CTX-M group (42.5% of the isolates) included *E. coli* producing CTX-M-15 (27.3%), CTX-M-14 (6.1%), CTX-M-3 (6.1%) and CTX-M-1 (3.0%). The TEM-group (57.5% of the isolates) included *E. coli* producing TEM-24 (48.5%), TEM-3 (3.0%), TEM-19 (3.0%) and TEM-129 (3.0%). Of the CTX-M-type ESBLs, 21.4% were associated with both OXA-1 and TEM-1, 50% with TEM-1 only, and 21.4% with OXA-1 only.

The antibiotypes of the ESBL-producing *E. coli* are shown in Table 1. A large proportion of isolates were resistant to the aminoglycosides, notably to amikacin (45.5%), and to co-trimoxazole (60.6%). Gentamicin remained active against 72.7% of isolates, while imipenem was active against 100% of isolates. Production of TEM and CTX-M ESBLs was associated frequently with quinolone resistance; of 33 ESBL-positive isolates, 25 (75.7%) were resistant to nalidixic acid, of which 23 were also resistant to fluoroquinolones. The association with quinolone resistance was particularly high among the isolates producing CTX-M ESBLs (92.8%). The *qnrA* gene was detected in 12.1% of the ESBL-producing *E. coli* isolates belonging to the CTX-M group.

Analysis of the ESBL-producing isolates by PFGE showed that neither the CTX-M nor TEM groups, nor the susceptible isolates, had a clonal origin (data not shown). Phylogenetic grouping revealed that the CTX-M isolates belonged predominantly to phylogenetic group D (50%), while group B2 predominated (47.4%) among the TEM isolates (Table 1). The sensitive isolates belonged predominantly to group B2 (73.7%), and only 11.1% belonged to group D.

### Analysis of virulence genotypes

Table 2 shows the distribution of genes encoding VFs, while Table 3 summarises the number of VFs found in the different groups of isolates. One

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**Table 1.** Characteristics of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae isolated from urinary tract infections in a French university hospital, 2002-2004

| Isolate | Unit or ward | Phylogenetic group | *qnrA* | β-lactamase content | Antibiotype |
|---|---|---|---|---|---|
| CTX-M group | | | | | |
| NEC 3 | Medicine | A | – | CTX-M-15/OXA-1/TEM-1 | KTGNtA - NAL,OFX,NOR,CIP,PEF – TET |
| NEC 11 | Medicine | D | + | CTX-M-15/OXA-1/TEM-1 | KTNtA - NAL,OFX,NOR,CIP,PEF – TET, CHL |
| NEC 21 | Recovery unit | B2 | – | CTX-M-15/OXA-1/TEM-1 | KTNtA - NAL,OFX,NOR,CIP,PEF – TET, CHL |
| NEC 5 | Medicine | D | + | CTX-M-15/OXA-1 | TGNt - NAL,OFX,NOR,CIP,PEF – TET |
| NEC 8 | Medicine | D | + | CTX-M-15/OXA-1 | TGNt - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 18 | ICU | B2 | – | CTX-M-15/OXA-1 | TGNt - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 6 | ICU | D | + | CTX-M-15/TEM-1 | KTNtA - NAL,OFX,NOR,CIP,PEF – TET |
| NEC 24 | Medicine | B2 | – | CTX-M-15/TEM-1 | TGNt - NAL,OFX,NOR,CIP,PEF – TET |
| NEC 26 | ICU | B2 | – | CTX-M-15 | KTGNtA - NAL,OFX,CIP,NOR,PEF |
| NEC 9 | Surgery | D | – | CTX-M-14/TEM-1 | KTNtA - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 10 | Geriatric | D | – | CTX-M-14/TEM-1 | NAL,NOR – SXT, TET, CHL |
| NEC 7 | Surgery | A | – | CTX-M-3/TEM-1 | KTGNtA – NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 30[a] | Geriatric | A | – | CTX-M-3/TEM-1 | SXT, TET, CHL |
| NEC 22 | Medicine | D | – | CTX-M-1/TEM-1 | TGNt - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| TEM group | | | | | |
| NEC 17 | Recovery unit | B2 | – | TEM-24, TEM-1/OXA-1 | NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 1 | Geriatric | A | – | TEM-24, TEM-1 | KTNtA – SXT, TET, CHL |
| NEC 15 | Surgery | A | – | TEM-24, TEM-1 | KNtA – SXT, TET, CHL |
| NEC 25 | ICU | A | – | TEM-24, TEM-1 | NAL,OFX,NOR,CIP,PEF – TET, CHL |
| NEC 27 | Recovery unit | B2 | – | TEM-24, TEM-1 | TGNt - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 31 | Recovery unit | D | – | TEM-24, TEM-1 | KTNtA - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 20 | Geriatric | B2 | – | TEM-24, OXA-1 | TNt - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 2 | Geriatric | B2 | – | TEM-24 | T - SXT, TET, CHL |
| NEC 4 | Medicine | B2 | – | TEM-24 | KTNtA - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 12 | Geriatric | B1 | – | TEM-24 | KTNtA - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 16 | Recovery unit | D | – | TEM-24 | KTNtA - SXT, TET, CHL |
| NEC 19 | Surgery | A | – | TEM-24 | KTNtA - NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 28 | Medicine | B2 | – | TEM-24 | NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 29 | Medicine | A | – | TEM-24 | KTNtA - TET, CHL |
| NEC 32 | Medicine | A | – | TEM-24 | NAL,OFX,NOR,CIP,PEF – SXT, TET, CHL |
| NEC 33 | Medicine | B2 | – | TEM-24 | TET, CHL |
| NEC 13 | Surgery | B1 | – | TEM-3 | KTNtA – NAL – TET, CHL |
| NEC 23 | Geriatric | B2 | – | TEM-19 | NAL - SXT, TET, CHL |
| NEC 14 | Medicine | B2 | – | TEM-129 | TET |

[a]Correspond to isolates susceptible to quinolones and/or fluoroquinolones.
ICU, intensive care unit; K, kanamycin; T, tobramycin; G, gentamicin; Nt, netilmicin; A, amikacin; NAL, nalidixic acid; OFX, ofloxacin; NOR, norfloxacin; CIP, ciprofloxacin; PEF, pefloxacin; SXT, trimethoprim-sulphamethoxazole; TET, tetracycline; CHL, chloramphenicol.

**Table 2.** Virulence factors associated with resistant and susceptible *Escherichia coli* isolates causing pyelonephritis and cystitis

| Virulence factors | | Resistant UTI isolates | | | | p[a] | | |
|---|---|---|---|---|---|---|---|---|
| | | CTX-M *n* (%) | TEM *n* (%) | Total R *n* (%) | Susceptible UTI isolates *n* (%) | CTX vs. TEM | CTX vs. S | R vs. S |
| Number of isolates | | 14 | 19 | 33 | 99 | | | |
| Adhesins | *papG* | | | | | | | |
| | Class I | 0 (0) | 0 (0) | 0 (0) | 0 (0) | | | |
| | Class II | 2 (14.3) | 4 (21.1) | 6 (18.1) | 48 (48.5) | | 0.03 | <0.0001 |
| | Class III | 5 (35.7) | 13 (69) | 18 (54.6) | 34 (34.3) | 0.029 | | 0.004 |
| | Class II+III | 0 (0) | 1 (5.3) | 1 (3) | 12 (12.1) | | | |
| | None | 7 (50) | 1 (5.3) | 8 (24.2) | 25 (25.3) | 0.019 | | |
| | *sfaS* | 0 (0) | 2 (10.5) | 2 (6.1) | 19 (19.2) | | | |
| | *focG* | 0 (0) | 1 (5.3) | 1 (3.0) | 26 (26.3) | | 0.006 | <0.0001 |
| | *afa/draBC* | 0 (0) | 3 (15.8) | 3 (9.1) | 14 (14.1) | | | |
| | *fimH* | 3 (21.4) | 13 (68.4) | 16 (48.5) | 91 (91.9) | 0.013 | <0.0001 | <0.0001 |
| Toxins | *hlyA* | 1 (7.1) | 1 (5.3) | 2 (6.1) | 46 (46.5) | | 0.011 | <0.0001 |
| | haemolysin | 1 (7.1) | 1 (5.3) | 2 (6.1) | 46 (46.5) | | 0.011 | <0.0001 |
| | *cnf1* | 0 (0) | 1 (5.3) | 1 (3) | 49 (49.5) | | 0.001 | <0.0001 |
| Siderophores | *iutA* | 9 (64.3) | 11 (58) | 20 (60.6) | 36 (36.4) | | 0.07 | 0.042 |
| | *irp2* | 6 (42.9) | 5 (26.3) | 11 (33.3) | 77 (77.8) | | 0.03 | <0.0001 |
| | *iroN* | 2 (14.3) | 11 (58) | 13 (39.4) | 64 (64.6) | 0.015 | 0.002 | 0.042 |
| Capsules | *kpsMTII* | 1 (7.1) | 5 (26.3) | 6 (18.2) | 75 (75.8) | | <0.0001 | <0.0001 |
| | *kpsMTK1* | 1 (7.1) | 4 (21.1) | 5 (15.2) | 48 (48.5) | | 0.011 | 0.002 |
| Miscellaneous | *traT* | 7 (50) | 15 (79) | 22 (66.7) | 34 (34.3) | | | 0.007 |
| | *malX* | 5 (35.7) | 9 (47.4) | 14 (42.4) | 69 (69.7) | | 0.042 | 0.039 |

[a] p values (Fisher's exact test) are shown where $p < 0.05$.
R, total resistant isolates; S, total susceptible isolates.

trend was clearly visible, namely, a striking difference in both the number and distribution of VFs between the ESBL-producing and susceptible isolates, with susceptible isolates possessing more VFs than the ESBL isolates ($p < 0.05$). ESBL-producing isolates had fewer urovirulence factors

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**Table 3.** Aggregated virulence factors associated with resistant and susceptible *Escherichia coli* isolates

| Virulence factors[a] | Resistant UTI isolates CTX-M (%) | TEM (%) | Total R (%) | Susceptible UTI isolates | p[b] CTX vs. TEM | CTX vs. S | R vs. S |
|---|---|---|---|---|---|---|---|
| Number of isolates | 14 | 19 | 33 | 99 | | | |
| <5 factors | 13 (87.5) | 9 (47.4) | 22 (66.7) | 27 (27.3) | 0.001 | 0.001 | <0.001 |
| 6–9 factors | 1 (12.5) | 10 (52.6) | 11 (33.3) | 53 (53.5) | 0.001 | 0.001 | NS |
| 10–14 factors | 0 (0) | 0 (0) | 0 (0) | 19 (19.2) | NS | 0.019 | 0.019 |

[a]Fourteen virulence factors (*sfaS, focG, afa/draBC, fimH, type I adhesin, hlyA, cnf1, iutA, iroN, traT, malX, irp2, kpsMTII, kpsMTK1*) were determined by PCR in each group.
[b]p values (Fisher's exact test) are shown where p <0.05.
R, resistant isolates; S, susceptible isolates; NS, not significant.

(*hlyA*, *cnf1*, *sfa/foc*, *kps*), but two VFs (*traT* and *iutA*), classically carried by plasmids, were numerically more prevalent among the ESBL isolates ($p < 0.05$). Interestingly, a comparison of the CTX-M-producing isolates with the other ESBL-producing isolates revealed that the CTX-M-producing isolates carried an even smaller quota of VFs. The CTX-M isolates appeared to lack genes encoding adhesins; 50% of these isolates did not possess a detectable *papG* allele, and *fimH* was present in only 21.4% of isolates, whereas this gene was present in 91.9% of susceptible isolates and in 68.4% of TEM isolates. The low prevalence of *fimH* among CTX-M isolates was confirmed by Southern blotting (data not shown).

## *C. elegans* killing model

A panel of isolates, including 14 ESBL-producing *E. coli* (seven CTX-M-15, six TEM-24, one TEM-3) and ten susceptible *E. coli*, was tested in the *C. elegans* infection assay. These groups of isolates produced 1–4, 5–8, 6 and 10–15 VFs, respectively. All clinical isolates showed virulence in the *C. elegans* model; however, the susceptible isolates were more virulent than the resistant isolates. The mean survival time for nematodes fed on clinical isolates was 3.01 (± 0.13) days for susceptible isolates, 4.66 (± 0.36) days for TEM-producing isolates, and 6.28 (± 1.24) days for CTX-M-producing isolates (Fig. 1). The mean survival times for nematodes fed with the avirulent OP50 control

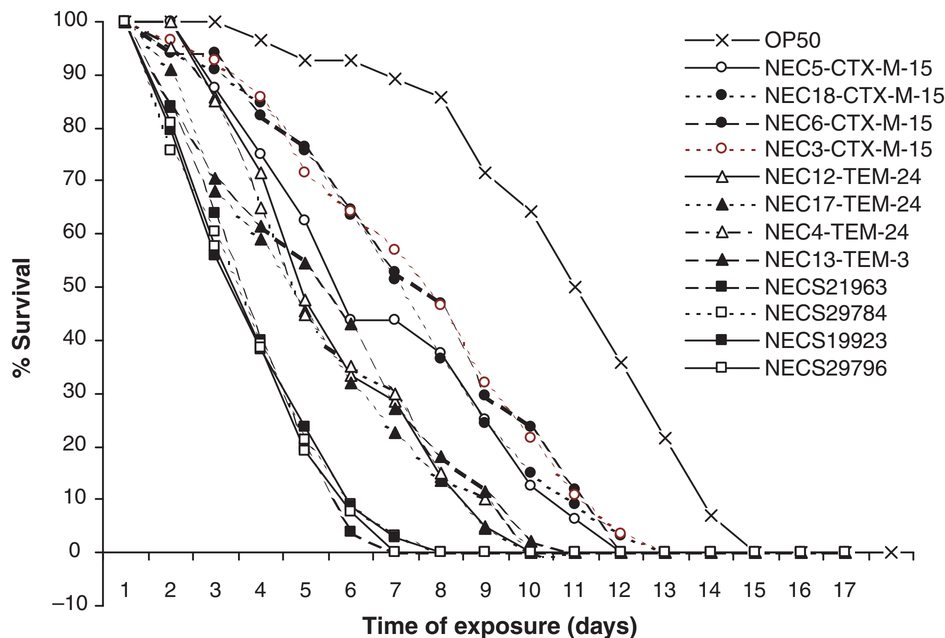


**Fig. 1.** Kinetics of killing of *Caenorhabditis elegans* infected by CTX-M-producing *Escherichia coli* (circles), TEM-producing *E. coli* (triangles), and susceptible *E. coli* (squares). For each group of isolates (CTX-M, TEM and susceptible), four isolates representative of the results are shown. The line with crosses shows the survival curve for *C. elegans* fed with the non-pathogenic *E. coli* OP50 strain. In all cases, *C. elegans* was grown on NGM agar plates [31] at 25°C, with 20–30 N2 hermaphrodites used in each test. The curves are representative of at least three independent trials for each group of isolates.

strain was 10.33 (± 0.98) days. All the nematodes infected with susceptible *E. coli* isolates were killed within 8 days (7.5 ± 0.5 days). This time was shorter than that for nematodes infected with *E. coli* producing TEM (10.25 ± 0.7 days) or CTX-M-15 (12.5 ± 0.8 days) enzymes. The experiment was repeated three times with similar results. Cox regression analysis revealed that an infection with a susceptible *E. coli* isolate reduced the survival of nematodes by a factor of 4.29 (OR (Wald statistic) 64.82, d.f. = 1, $p < 0.00001$) compared with nematodes infected with CTX-M-producing *E. coli* isolates. This result was highly reproducible, with no significant difference among three repeated experiments (Wald statistic 0.56, d.f. 3, p 0.91). Thus, there was a clear correlation between the ability to kill *C. elegans* and the number of VFs present in the genome of each *E. coli* isolate.

## DISCUSSION

CTX-M-producing bacteria have now been isolated worldwide, both in hospitals and in the community [1,32–37]. CTX-M-producing isolates now represent up to 50% of ESBL-producing *E. coli* isolates from French hospitals [1,15,19]. In particular, the number of CTX-M-15 producers has increased dramatically [33,38–43]. The aim of the present study was to determine the virulence potential of hospital-acquired CTX-M-producing *E. coli* isolates, which are often associated with other antibiotic resistances, in order to assess the risk they present in the hospital and the community.

The present study clearly revealed that ESBL-producing *E. coli* isolates from UTI lack extra-intestinal VFs, and that this phenomenon was more marked among CTX-M-producing isolates compared with TEM-producing isolates. Notably absent from CTX-M-producing isolates were classical extra-intestinal VFs, such as haemolysin production and fimbriae. The two exceptions were *iutA* and *traT*, both of which are usually plasmid-encoded. These results corroborate and extend recent studies on ESBL-producing isolates in which the presence of a more limited panel of VFs was investigated [8,15]. CTX-M isolates are generally also fluoroquinolone-resistant, and there appears to be a link between phylogeny and virulence, as the CTX-M isolates belonged mostly to non-B2 phylogenetic groups (predominantly the D group), while the sensitive and TEM isolates belonged predominantly to the B2 group. Previous studies have reported that fluoroquinolone-resistant isolates, with low levels of VFs, occur predominantly in non-B2 phylogenic backgrounds, while susceptible isolates were predominantly from group B2 [13,17,29,30,44]. Nevertheless, members of the B2 group were significantly represented among the CTX-M-15-producing isolates in the present study, which is consistent with other recent reports [15,16]. However, one study failed to observe a lower prevalence of VFs, which might be linked to the fact that the majority of isolates were obtained from outbreaks [16].

The low levels of VFs seen in the CTX-M-producing isolates could be linked to the loss of unstable PAIs carrying VFs from a previously virulent strain following the acquisition of ESBL resistance. Alternatively, the CTX-M-producing isolates could be derived from strains with low pathogenicity that have acquired a CTX-M-encoding plasmid. Such an event might be favoured by a specific genetic background, as these isolates are predominantly from the phylogenetic group D background, whereas ExPEC generally belong to the B2 group. Another plasmid-associated gene, *iutA*, encoding the aerobactin system, was often observed among CTX-M-producing isolates. Interestingly, the gene responsible for plasmid-mediated quinolone resistance, *qnrA* [45], was detected in some CTX-M isolates, but not in other isolates. Overall, these results suggest that CTX-M isolates might be derived from strains that are highly receptive to plasmid acquisition.

There is a growing interest in using the invertebrate *C. elegans* as a model host system for investigating virulence mechanisms and defence responses against human pathogens [18], and the *C. elegans* model has been used to study EPEC virulence [46]. The present study demonstrated, for the first time, that the ability to kill nematodes can be used to evaluate ExPEC virulence. The ability of ExPEC strains to kill nematodes was found to correlate with the presence of VFs, as antibiotic-susceptible isolates killed nematodes faster than the TEM- or CTX-M-producing isolates. The epidemiological features of the patients, the molecular virulence profiles and the in-vivo behaviour all suggested that the CTX-M group genotype, although adapted for survival in an antibiotic-rich environment, such as the hospital milieu or the microflora of hosts exposed to antibiotics, has a limited intrinsic virulence

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potential. However, the CTX-M-producing isolates remained more virulent than the avirulent *E. coli* OP50 control strain, indicating that CTX-M-producing isolates retain an intrinsic virulence potential, despite the absence of major VFs such as fimbriae. These results suggest that nosocomial CTX-M-producing isolates may be opportunistic pathogens of low virulence whose ability to cause disease is limited to compromised hosts, as was the case in the present study. Community outbreaks of CTX-M-producing bacteria are likely to be caused by strains that have a higher virulence potential [16].

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