

## Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains

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**Objectives:** Recently, a CTX-M-15 extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli* O25b-ST131 clone, belonging to the B2 phylogenetic group and with a high virulence potential, has been reported all over the world, representing a major public health problem. The present study was carried out to develop a rapid and simple detection assay that identifies members of this clone.

**Methods:** A total of 627 *E. coli* isolates of which 373 produced an ESBL, collected across four continents, were screened using a O25b-ST131 clone allele-specific PCR for the *pabB* gene.

**Results:** One hundred and forty-three ESBL isolates were found positive with the assay. These isolates were all of O25b type and, when studied by multilocus sequence typing (25 cases), were all of ST131. The O25b-ST131 clone was found to produce ESBLs other than CTX-M-15, specifically CTX-M-2, -3, -14, -27, -32 and -61 as well as TEM-24. This clone represents 3% of non-ESBL B2 isolates originating from urinary tract infections in Paris.

**Conclusions:** We have developed a PCR-based assay that easily identifies a clone with high likelihood of producing ESBLs, including CTX-M-15.

Keywords: B2 phylogenetic subgroup I, ESBL, O25, ST 131

### Introduction

In recent years, the epidemiology of extended-spectrum  $\beta$ -lactamases (ESBLs) has radically changed, with the emergence of *Escherichia coli* producing CTX-M enzymes, both in hospitals

and in the community. Recently, an *E. coli* clone producing ESBL CTX-M-15 with a high virulence potential has been reported all over the world, representing a major public health problem.<sup>1–5</sup> This clone belongs to the B2 phylogenetic subgroup I, to the multilocus sequence type (MLST) 131 and

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exhibits a specific O25 type (O25b).<sup>1-5</sup> The *E. coli* species can be divided into four main phylogenetic groups (A, B1, D and B2), with commensal strains belonging mainly to A and B1 phylogenetic groups whereas extraintestinal pathogenic strains are essentially from the B2 and D groups.<sup>6</sup> The fact that this O25b-ST131 clone includes diverse PFGE patterns (all related at  $\geq 60\%$  similarity) complicates recognition of its members.<sup>2,4,5</sup>

As a consequence, it is of major concern to be able to easily and rapidly identify this clone, and for this purpose we have developed a PCR assay. With this simple assay, we aimed to identify the O25b-ST131 clone in a worldwide collection of ESBL- and ESBL-non-producing *E. coli* isolates.

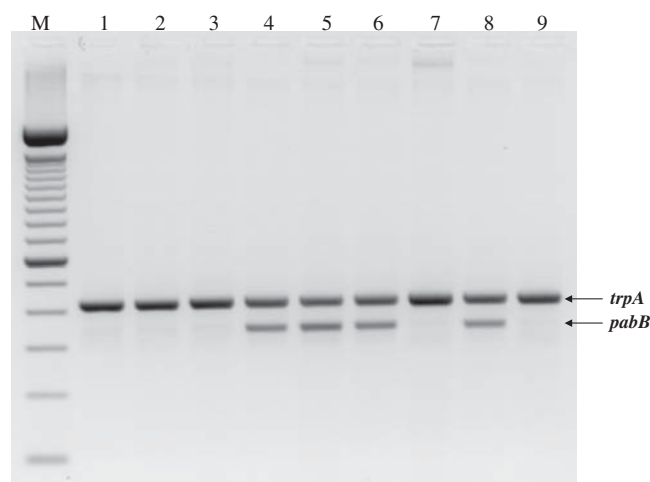
## Materials and methods

### Bacterial isolates

A total of 627 *E. coli* were screened, representing three sets of isolates: (i) 373 were ESBL producers (60 with TEM types, 28 with SHV, 280 with CTX-M enzymes including 16 CTX-M-1, 10 CTX-M-2, 60 CTX-M-3, 1 CTX-M-8, 11 CTX-M-9, 35 CTX-M-14, 133 CTX-M-15, 12 CTX-M-27, 1 CTX-M-32 and 1 CTX-M-61, and 5 with VEB-1). These isolates were identified between 1994 and 2007 and belong to several published and unpublished collections originating from Europe (France, UK), Asia (Turkey, Cambodia, Thailand), Africa (Central African Republic) and America (Brazil, Canada). The majority of isolates originated from hospital settings (75%) whereas the remainder were from the community. The UK isolates have been typed by MLST using the Achtman schema (<http://mlst.ucc.ie>), and 25 belong to ST131; (ii) 129 phylogenetic group B2 ESBL-non-producing *E. coli* isolated in Tenon hospital (Paris, France) from patients with urinary tract infections (UTIs); (iii) 125 ESBL-non-producing reference isolates belonging to the four main *E. coli* phylogenetic groups and B2 subgroups.<sup>7,8</sup>

### PCR detection of the O25b-ST131 clone isolates

Primers O25pabBspe.F (5'-TCCAGCAGGTGCTGGATCGT-3') and O25pabBspe.R (5'-GCGAAATTTTCGCCGACTGT-3') were used to amplify a 347 bp fragment of the *pabB* gene specifically in isolates belonging to the O25b-ST131 clone. The *pabB* gene is one of the genes used in the Institut Pasteur MLST schema (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html>). The primer sequences were designed using the MLST datasets of Le Gall *et al.*<sup>7</sup> and Clermont *et al.*,<sup>3</sup> which include the *pabB* gene, in regions conserved within isolates of all phylogenetic groups except that the 3' nucleotides of both primers (underlined in the sequences above) are specific to B2 subgroup I isolates of O type 25b. These isolates belong to ST131 as defined by the Achtman MLST schema.<sup>2,3</sup> A positive PCR control was included in the assay to confirm that any amplification failure with the *pabB* allele-specific primers was not due to poor DNA quality or to failure of the PCR itself. This control PCR targeted a 427 bp fragment of the *trpA* gene, amplified with primers *trpA*.F (5'-GCTACGAATCTCTGTTTGCC-3') and *trpA*.R (5'-GCAACGCGCCTGGCGGAAG-3'). *trpA* is another gene used in the Institut Pasteur MLST schema. PCR was carried out in a 20  $\mu$ L volume containing 2  $\mu$ L of 10 $\times$  buffer (supplied with *Taq* polymerase), 1.5 mM MgCl<sub>2</sub>, 20 pmol of each of the *pabB*spe primers and 12 pmol of each of the *trpA* primers, 2  $\mu$ M each dNTP, 1 U of *Taq* polymerase (Invitrogen, Cergy Pontoise, France) and 3  $\mu$ L of bacterial lysate or 2  $\mu$ L of purified DNA (Wizard<sup>®</sup> Genomic DNA purification kit, Promega). PCR was performed with an



**Figure 1.** PCR detection of *Escherichia coli* O25b-ST131 clone. The *trpA* band (427 bp) corresponds to the positive control amplifying in all isolates whereas the *pabB* band (347 bp) corresponds to the allele-specific amplification obtained only for the O25b-ST131 clone strains. M, molecular weight marker, 100 bp DNA ladder; lane 1, *E. coli* CAR74 (CTX-M-15, phylogroup A, O25b negative); lane 2, *E. coli* N06-731 (CTX-M-15, phylogroup B1, O25b negative); lane 3, *E. coli* N04-423 (CTX-M-15, phylogroup D, O25b negative); lane 4, *E. coli* N06-192 (CTX-M-2, phylogroup B2, O25b positive); lane 5, *E. coli* TN32 (CTX-M-14, phylogroup B2, O25b positive); lane 6, *E. coli* CAMB27 (CTX-M-27, phylogroup B2, O25b positive); lane 7, *E. coli* CAMB2 (CTX-M-15, phylogroup B2, O25b negative); lane 8, *E. coli* TN03 (CTX-M-15, phylogroup B2, O25b positive); lane 9, *E. coli* ECOR15 (ESBL-non-producing strain, phylogroup A, O25b positive).

Eppendorf Mastercycler with MicroAm tubes in the following conditions: denaturation 4 min at 94°C, 30 cycles of 5 s at 94°C and 10 s at 65°C, and a final extension step of 5 min at 72°C. PCR products were loaded on 2% agarose gel with SYBR<sup>®</sup> Safe DNA gel stain (Invitrogen, Cergy Pontoise, France). After electrophoresis, gels were photographed under UV light. Two control DNAs were systematically included in each PCR: an O25b-ST131 strain (TN03) positive with both PCR products and an O25b-non-ST131 strain (ECOR15), positive only for the *trpA* gene product (Figure 1).

### PCR O typing

O25b typing was performed by PCR as previously described, using the *rfbO25b.r* and *rfb1bis.f* primers.<sup>3</sup>

## Results and discussion

### Validation of the PCR assay to detect isolates belonging to the O25b-ST131 clone

We have developed an allele-specific PCR assay based on nucleotide sequence polymorphisms in the *pabB* gene specific to the B2 subgroup I/O25b, which corresponds to the ST131 clone (Figure 1). We first validated this assay by testing 25 known representatives of this clone, all producing CTX-M-15 enzyme and belonging to ST131 as defined using the Achtman MLST schema. All these isolates were positive with our assay and were also O25b O type by PCR. Sixty-seven ESBL-non-producing isolates of phylogenetic group B2, representative of the genetic

diversity of this group but not belonging to the O25b-ST131 clone, as well as 58 ESBL-non-producing isolates representative of other phylogenetic groups, were also tested with our assay.<sup>7,8</sup> They were all negative for the *pabB* polymorphisms (data not shown).

We have verified that this assay could be used on both purified DNAs and fresh bacterial lysates (data not shown). The assay is robust, as it has been tested in two distinct laboratories (Denamur and Woodford) without any discrepancy. The only precaution with the assay is to use newly synthesized primers, i.e. as always in allele-specific PCR, 3' end base degradation of the primers can give false-positive results.

*The O25b-ST131 clone encompasses isolates producing a wide diversity of ESBLs as well as ESBL-non-producing isolates*

In a second step, we used our assay to screen 348 ESBL-producing isolates, including 108 with CTX-M-15 ESBLs. As expected, we found 49/108 CTX-M-15-producing isolates (45% of the total number of CTX-M-15) belonging to the O25b-ST131 clone. But we also found members of this clone with CTX-M enzymes belonging to the three main CTX-M groups, group 1 [CTX-M-3, 53 isolates (88% of the total number of CTX-M-3); CTX-M-32, 1 isolate; CTX-M-61, 1 isolate], group 2 (CTX-M-2, 1 isolate) and group 9 [CTX-M-14, 11 isolates (31% of the total number of CTX-M-14); CTX-M-27, 1 isolate], as well as a TEM-24 enzyme (Table 1). The systematic screening of ESBL-producing isolates by our assay revealed a greater diversity of ESBLs than was recognized by others.<sup>4,5</sup> Of note, the majority of CTX-M-3-producing isolates are from Belfast. All the isolates positive with our assay were also O25b O type by PCR. The complete MLST schema of Achtman<sup>2</sup> was performed on the isolates producing non-CTX-M-15 ESBLs to confirm that they belonged to ST131 (data not shown). Of note, we found two O25b isolates from the UK that were negative with our assay, and belonged to the B2 and A phylogenetic groups (Table 1), indicating that this O type is not restricted to the ST131 clone.<sup>3</sup>

Except for the UK collection, which is biased toward the ST131 clone, the prevalence of the O25b-ST131 clone in the different collections can be considered (Table 1). A dramatic increase of this clone (6% to 46%) was observed in France in the 2000s, first appearing in October 2001 and reaching levels similar to those observed in Canada. A high prevalence was also observed in other countries studied.

As a clone lacking ESBLs was reported in the stools of 7% of healthy subjects in 2006 in Paris,<sup>9</sup> we wanted to know its prevalence in the more frequent extraintestinal *E. coli* infection, UTI. We therefore applied our test to 129 unrelated *E. coli* without ESBL, all belonging to the B2 phylogenetic group, recovered from patients suffering from a UTI. They were collected at Tenon hospital in Paris from December 2002 to March 2003 (S. Vimont and G. Arlet, personal data). Four isolates (3%) were positive with our assay and these isolates were also O25b O type by PCR. Based on the frequency of B2 strains in UTI, it can be estimated that 1.5% of UTI caused by *E. coli* belonged to the O25b-ST131 clone.

These data indicate a high potential to acquire and maintain ESBL plasmids for the O25b-ST131 clone chromosomal genetic

**Table 1.** Results of PCR based screening for the *E. coli* O25b-ST131 clone among 373 ESBL-producing isolates

Country (date of isolation) <sup>a</sup>	Number of isolates	O25b PCR positive, n (%)	pabBspe PCR positive (ST131) n (%)	ESBL types found in positive ST131 isolates (n)
UK (2004–07)	103	86 (83)	84 (81)	CTX-M-3 (52), CTX-M-15/28 (32)
France 1 (1994–2003) <sup>11</sup>	128	8 (6)	8 (6)	CTX-M-15 (5), CTX-M-14 (2), TEM-24 (1)
France 2 (2006–07)	41	19 (46)	19 (46)	CTX-M-14 (1), CTX-M-15 (17), CTX-M-32 (1)
Canada (2004–06)	41	17 (41)	17 (41)	CTX-M-2 (1), CTX-M-14 (2), CTX-M-15 (13), CTX-M-61 (1)
Brazil (2001–05)	5	0	0	
Central African Republic (2004–06) <sup>12</sup>	10	5 (50)	5 (50)	CTX-M-15 (5)
Turkey (2006)	10	2 (20)	2 (20)	CTX-M-3 (1), CTX-M-15 (1)
Cambodia (2004–05) <sup>13</sup>	30	8 (27)	8 (27)	CTX-M-14 (6), CTX-M-15 (1), CTX-M-27 (1)
Thailand (1999)	5	0	0	

<sup>a</sup>A reference citation for the collection is given where available.

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background, possibly reflecting fine tuning between the chromosome and the plasmid, as recently suggested for the specific genotype D<sub>2</sub> of *E. coli*.<sup>10</sup> The presence of this clone without ESBL in commensal and pathogenic conditions could represent a potential threat for further emergence of resistance.

In conclusion, we have developed a rapid, simple and inexpensive PCR test that allows the detection of the *E. coli* O25b-ST131 clone that often produces CTX-M-15 enzyme, which may also host other ESBLs. This assay will help to trace the highly resistant and virulent O25b-ST131 clone in the community and hospitals.

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### Transparency declarations

None to declare.

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