Plasmid-mediated AmpC beta-lactamase-producing 
*Escherichia coli* causing urinary tract infection in the 
Auckland community likely to be resistant to commonly 
prescribed antimicrobials

Dragana Drinković, Arthur James Morris, Kristin Dyet, Sarah Bakker, Helen Heffernan

Abstract

**Aim** To estimate the prevalence and characterise plasmid-mediated AmpC beta-lactamase (PMACBL)-producing *Escherichia coli* in the Auckland community.

**Method** All cefoxitin non-susceptible (NS) *E. coli* identified at the two Auckland community laboratories between 1 January and 31 August 2011 were referred to ESR for boronic acid double-disc synergy testing, to detect the production of AmpC beta-lactamase, and polymerase chain reaction (PCR) to identify the presence of PMACBL genes. PMACBL-producing isolates were typed using pulsed-field gel electrophoresis (PFGE), and PCR was used to determine their phylogenetic group and to identify multilocus sequence type (ST)131. Antimicrobial susceptibility testing and detection of extended-spectrum beta-lactamases (ESBLs) were performed according to the Clinical and Laboratory Standards Institute recommendations.

**Results** 101 (51%) and 74 (37%) of 200 non-duplicate cefoxitin-NS *E. coli* were PMACBL producers or assumed hyper-producers of chromosomal AmpC beta-lactamase, respectively. The prevalence of PMACBL-producing *E. coli* was 0.4%. PMACBL-producing *E. coli* were significantly less susceptible to norfloxacin, trimethoprim and nitrofurantoin than *E. coli* that produced neither a PMACBL nor an ESBL. Very few (4%) PMACBL-producing *E. coli* co-produced an ESBL. Most (88%) of the PMACBL-producing isolates had a CMY-2-like PMACBL. The PMACBL-producing *E. coli* isolates were diverse based on their PFGE profiles, 44% belonged to phylogenetic group D, and only four were ST131.

100 of the 101 PMACBL-producing *E. coli* were cultured from urine, and were causing urinary tract infection (UTI) in the majority of patients. The median patient age was 56 years and most (94%) of the patients were women. A greater proportion of patients with community-acquired UTI caused by PMACBL-producing *E. coli* received a beta-lactam antimicrobial than patients with community-acquired UTI caused by other non-AmpC, non-ESBL-producing *E. coli*. Thirty-six (43%) patients with community-acquired UTI due to PMACBL-producing *E. coli* were neither hospitalised nor had any antimicrobial treatment in the previous 6 months.

**Conclusion** The prevalence of PMACBL-producing *E. coli* was relatively low in the Auckland community, but has increased in recent years. Typing revealed that the majority of the PMACBL-producing *E. coli* in the Auckland region were genetically unrelated meaning that a point source or direct person to person transmission are not drivers of local community spread currently. The isolates were more resistant to non-beta-lactam antimicrobials than other non-AmpC, non-ESBL-producing *E. coli*, leaving few treatment options. The majority of the PMACBL-producing *E. coli* isolates seemed to be acquired in the community and were most frequently isolated from women with UTI. A large proportion of patients with community-acquired UTI had not been hospitalised nor had any antimicrobial treatment in the previous 6 months.

AmpC beta-lactamases (ACBLs) are enzymes encoded on the chromosome of many *Enterobacteriaceae*, such as *Enterobacter* spp., *Citrobacter freundii*, *Morganella morganii*, *Aeromonas* spp. and *Hafnia alvei*. They are clinically significant because they confer resistance to most beta-lactam antimicrobials except fourth-generation cephalosporins and carbapenems. They
characteristically confer resistance to cephamycins, such as cefoxitin, a feature that can be used in the laboratory to distinguish them from extended-spectrum beta-lactamases (ESBLs).

*Escherichia coli* usually produce only low levels of ACBLs because their chromosomal *ampC* gene is typically down-regulated. However, *E. coli* can hyper-produce ACBLs following alterations in the regulation of the *ampC* gene. *E. coli* can also acquire plasmids containing genes for ACBLs. Acquisition of these plasmids enables very effective spread of extended resistance among bacteria and ultimately the spread among people.

Plasmid-mediated AmpC beta-lactamase (PMACBL) genes are derived from bacteria with chromosomally encoded ACBLs. PMACBLs are most commonly detected in *E. coli, Klebsiella pneumoniae, Salmonella* and *Proteus mirabilis*. Since the first reports in the 1980s, PMACBLs have been increasingly detected throughout the world. In the 1980s and 1990s nosocomial outbreaks of PMACBL-producing organisms were reported. In recent years, PMACBL-producing organisms have also been isolated from patients in the community, outpatient clinics and long-term care facilities.

A national survey of urinary *E. coli* conducted in New Zealand in 2006 found that six (0.07%) of 8707 *E. coli* isolates were PMACBL producers.

Our aim was to estimate the prevalence and characterize PMACBL-producing *E. coli* in the Auckland community.

**Methods**

*E. coli* isolates and antimicrobial susceptibility testing—From 1 January to 31 August 2011, a total of 26,007 consecutive, non-duplicate clinical isolates of *E. coli* were isolated at the two Auckland community laboratories, Labtests (LTA) and Diagnostic Medlab (DML).

Identification and susceptibility testing of *E. coli* isolates was performed at the time of initial isolation. *E. coli* isolates were identified using standard laboratory methods. Antimicrobial susceptibility testing (AST) was performed by disc diffusion at LTA and by microbroth dilution using the Vitek system (bioMerieux Vitek Inc.) at DML. The susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. The presence of ESBLs was detected by the combination disc test, according to CLSI guidelines.

Testing for the presence of PMACBLs—All cefoxitin non-susceptible (NS) *E. coli* isolates were referred to the Antibiotic Reference Laboratory, ESR, for ACBL testing. A phenotypic boronic acid double-disc synergy test (BADDST) was used to detect ACBL production. Isolates testing positive for ACBL in the BADDST were tested by multiplex polymerase chain reaction (PCR) for PMACBL genes.

Molecular typing of PMACBL-producing *E. coli* isolates—Pulsed-field gel electrophoresis typing (PFGE) is a technique used to determine genetic relatedness of bacteria. It was performed using XbaI-digested genomic DNA as previously described.

PFGE banding patterns were analysed using BioNumerics software version 6.6 (Applied Maths, St-Martens-Latem, Belgium), with the Dice coefficient and unweighted-pair group method with arithmetic averages, at settings of 0.5% optimisation and 1.5% position tolerance. Phylogenetic groups of *E. coli* (A, B1, B2 and D) were determined using a multiplex PCR-based method.

A PCR-based method was used to determine whether an isolate belonged to multilocus sequence type (ST) 131. Isolates were screened for the presence of a 347 bp fragment of the *pabB* gene found in isolates belonging to the O25-ST131 clone, as well as ST131-associated single nucleotide polymorphisms in the *mdh* and *gyrB* genes. Isolates that generated amplicons for all three loci (O25b *pabB, mdh* and *gyrB*) were considered to be ST131.

Patient information and definitions—Data on patient age, gender, ethnicity, and antimicrobial treatment in the community and hospitalisation in the previous 6 months were collected from the regional electronic records. These electronic records do not capture antibiotics administered in hospitals.
A patient was considered to have a community-acquired (CA) infection if they were not a resident of a long-term care facility and their infection did not develop >48 hours after admission or <48 hours after discharge from a healthcare facility.

A patient was considered to have a urinary tract (UTI) infection if *E. coli* grew in the presence of pyuria (≥10×10^6 white blood cells/L).

For analysis of risk factors for CA UTI caused by PMACBL-producing *E. coli*, three controls were selected for each case. Patients in the control group had a CA UTI caused by *E. coli* that produced neither PMACBL nor ESBL during the study period.

Differences in antimicrobial susceptibility between PMACBL-producing *E. coli* and other *E. coli* were tested using the Chi-square test with Yates’ correction for sample size.

### Results

#### Prevalence of PMACBL-producing *E. coli*
— 200 (0.8%) of 26 007 *E. coli* isolates were cefoxitin NS. Among these 200 cefoxitin-NS isolates, 175 tested positive for ACBL production in the BADDST. PMACBL genes were detected in 101 of the 175 ACBL producers, and the remaining 74 ACBL producers were assumed to be hyper-producers of chromosomal ACBL. Therefore, 51% (101) and 37% (74) of the 200 cefoxitin-NS isolates were PMACBL producers and assumed hyper-producers of chromosomal ACBL, respectively. The overall prevalence of PMACBL-producing *E. coli* was 0.4% (101/26 007).

#### Antimicrobial susceptibility
— The susceptibility of PMACBL-producing *E. coli* to carbapenems and non-beta-lactam antimicrobials is presented in the Table 1. The susceptibility of the PMACBL-producing *E. coli* is compared with the susceptibility of *E. coli* that produced neither PMACBL nor ESBL and that were isolated during the study period from patients with CA infections, 99% of which were UTIs.

PMACBL-producing isolates were significantly more resistant to norfloxacin, trimethoprim and nitrofurantoin. Co-resistance to norfloxacin and trimethoprim was present in 20% (20/100) of PMACBL-producing *E. coli* isolates. All PMACBL-producing isolates tested were susceptible to carbapenems and amikacin. Four (4%) isolates co-produced ESBL.

### Table 1. Antimicrobial susceptibility of PMACBL-producing *E. coli* compared with other *E. coli*

<table>
<thead>
<tr>
<th>Antimicrobial*</th>
<th>PMACBL-producing <em>E. coli</em> % susceptible (No. S/No. tested)</th>
<th>Other <em>E. coli</em> † % susceptible (No. S/No. tested)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norfloxacin</td>
<td>68 (68/100)</td>
<td>95 (24694/25985)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>62 (62/100)</td>
<td>75 (19439/25982)</td>
<td>0.005</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>97 (97/100)</td>
<td>99 (25804/25984)</td>
<td>0.005</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>98 (63/64)</td>
<td>99 (137/138)</td>
<td>0.57</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>88 (77/88)</td>
<td>91 (411/452)</td>
<td>0.32</td>
</tr>
<tr>
<td>Amikacin</td>
<td>100 (10/10)</td>
<td>99 (445/446)</td>
<td>0.88</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>100 (85/85)</td>
<td>100 (151/151)</td>
<td>1.0</td>
</tr>
<tr>
<td>ESBL</td>
<td>4 (4/101)</td>
<td>NA‡</td>
<td></td>
</tr>
</tbody>
</table>

*The isolates were tested for susceptibility to fosfomycin, gentamicin, amikacin and carbapenems only if multiresistant or if the patient had an allergy; † Other *E. coli* = *E. coli* that produced neither PMACBL nor ESBL isolated from patients with community-acquired infections (99% of which were urinary tract infections) during the study period; ‡ NA = not applicable.
Molecular epidemiology of PMACBL-producing *E. coli*—CMY-2-like PMACBLs were the predominant type of PMACBL accounting for 89% (90/101) of isolates, followed by DHA accounting for 12% (12/101). One isolate co-produced both a CMY-2-like and DHA PMACBL.

Seven isolates were untypable by PFGE. The remaining 94 isolates were diverse based on their PFGE profiles. There were, however, several small clusters at the 85% similarity level, as well as two sets of two isolates that were indistinguishable (Figure 1).

The majority of the isolates were in phylogenetic group D, 44% (44/101); followed by group B2, 23% (23/101); group A, 19% (19/101); and group B1, 15% (15/101).

Four (4%) of the 101 PMACBL-producing *E. coli* isolates were positive in the ST131 PCR assay. One of these isolates was untypable by PFGE and the other three had distinct PFGE profiles. One of the ST131 *E. coli* co-produced a CTX-M group 1 type ESBL.

**Patients with PMACBL-producing *E. coli*—**The median age of the patients was 56 (range, 1–97) years. When the seven children <16 years of age were excluded, the median age was 59 (range, 17–97) years. Most of the patients (95; 94%) were female. Ethnicity data was available for 85 (84%) patients. Most (45, 53%) patients were New Zealand Europeans, followed by other European 11 (13%), Indian 7 (8%), other Asian 6 (7%), NZ Maori 5 (6%), Chinese 4 (5%), Fijian, Tongan and Samoan 2 (2%) each, and Middle Eastern 1 (1%).

Twenty-one (21%) patients were hospitalised in the previous six months and 61 (60%) had antimicrobial treatment in the community in the previous 6 months. In all but one of the patients, the organism was isolated from urine, and 95% of these patients had a UTI. Eighty-three (82%) patients had a CA UTI, 36 (43%) of whom had neither antimicrobial treatment in the community nor were hospitalised in the previous six months.

When the 83 patients with CA UTI caused by PMACBL-producing *E. coli* (AmpC group) were compared with 249 patients who had CA UTI caused by non-AmpC, non-ESBL-producing *E. coli* during the study period (Control group), we found that age, gender, ethnicity, hospitalisation in the previous 6 months, antimicrobial treatment in the community in the previous 6 months and the number of antimicrobial courses were not significantly associated with infection with PMACBL-producing *E. coli* (Table 2).

Beta-lactams were the most commonly prescribed antimicrobials in both groups. A greater proportion of patients in the AmpC group received a beta-lactam antimicrobial (*p* value=0.02), but, when the analysis was confined to broad-spectrum beta-lactams (i.e. when penicillin and flucloxacillin were excluded), there was no difference between the groups (*p* value=0.1).
Figure 1. Dendogram of 94 PMACBL-producing *E. coli* isolates that were typable by pulsed-field gel electrophoresis (PFGE). The reference number, PMACBL genotype, phylogenetic group and the presence of ST131 are given for each strain.
Figure 1 notes:
1. The vertical line marks the 85% similarity level.
2. ST131 status determined using a PCR-based assay.15,17
3. Among the 7 isolates untypable by PFGE:
   • 6 had a CMY-2-like and 1 had a DHA type PMACBL;
   • 5 belonged to phylogenetic group B1, 1 to group B2 and 1 to group D;
   • 1 isolate was ST131.

Table 2. Factors associated with community-acquired urinary tract infections

<table>
<thead>
<tr>
<th>Variables</th>
<th>AmpC group* (n=83 patients)</th>
<th>Control group† (n=249 patients)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>51 (1–97)</td>
<td>52 (1–102)</td>
<td>0.77</td>
</tr>
<tr>
<td>Female</td>
<td>78 (94%)</td>
<td>223 (90%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Hospitalisation in the previous 6 months</td>
<td>13 (16%)</td>
<td>25 (10%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Antimicrobials in the community in the previous 6 months</td>
<td>45 (54%)</td>
<td>112 (45%)</td>
<td>0.16</td>
</tr>
<tr>
<td>No antimicrobials in the community or hospitalisation in the previous 6 months</td>
<td>36 (43%)</td>
<td>128 (51%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Treatment with any beta-lactam antimicrobials</td>
<td>37 (45%)</td>
<td>74 (30%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Treatment with broad spectrum beta-lactams (penicillin, fluoroquinolones excluded)</td>
<td>31 (37%)</td>
<td>69 (28%)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Data available for 68 (82%) patients</th>
<th>Data available for 217 (87%) patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NZ European</td>
<td>33 (49%)</td>
<td>124 (57%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Other European</td>
<td>8 (12%)</td>
<td>19 (9%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Maori</td>
<td>5 (7%)</td>
<td>20 (9%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Chinese</td>
<td>4 (6%)</td>
<td>8 (4%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Indian</td>
<td>6 (9%)</td>
<td>7 (3%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Other Asian</td>
<td>5 (7%)</td>
<td>6 (3%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Samoan</td>
<td>2 (3%)</td>
<td>9 (4%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Tongan</td>
<td>2 (3%)</td>
<td>8 (4%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Other ethnicity</td>
<td>3 (4%)</td>
<td>16 (7%)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* AmpC group=group of patients with CA UTI caused by PMACBL-producing E. coli
† Control group=group of patients with CA UTI caused by non-AmpC, non-ESBL-producing E. coli during the study period.

Discussion
Since first reported in 1989, PMACBLs have gained a worldwide distribution, although they are less common than ESBLs.16 Our study showed that the prevalence of PMACBL-producing E. coli was 0.4% in the Auckland community. This is similar to the prevalence rates reported elsewhere.19-22

The prevalence of PMACBLs was higher (p<0.001) than the rate of 0.07% found in a national survey in 2006.9,11 Increasing rates have been reported throughout the world. A significant increase in acquired ampC genes in Enterobacteriaceae from 0.06% in 1999 to 1.3% in 2007 was observed in a Spanish hospital.2

A survey from five children’s hospitals in China detected an overall increase in AmpC beta-lactamases from 2.6% in 2005 to 9.3% in 2006.7 The rate of DHA-1-producing K. pneumoniae significantly increased from 0.6% in 2002 to 4.3% in 2004 at a Korean university hospital.4

In this study, PMACBL-producing E. coli had elevated resistance rates to non-beta-lactam antimicrobials and were significantly more resistant to norfloxacin, trimethoprim and nitrofurantoin when compared to other E. coli. High resistance rates to fluoroquinolones and trimethoprim-sulfamethoxazole in PMACBL-producers has been reported by other investigators.2,20-23
In addition to being inherently resistant to beta-lactams such as amoxicillin/clavulanic acid, co-resistance to non-beta-lactam antimicrobials is common in PMACBL-producers, as plasmids harbour additional genes that confer resistance to non-beta-lactam drugs, leaving few therapeutic options.1

We suggest taking a sample for urine culture and susceptibility testing in those patients who fail to respond to empiric treatment with commonly prescribed antimicrobials such as norfloxacin, trimethoprim and amoxicillin/clavulanic acid.

We found a low rate (4%) of co-production of ESBLs in our PMACBL-producing *E. coli*. Similarly, Mata et al found that 2.6% of their PMACBL-producing *E. coli* co-produced ESBLs.2 Two studies from Northern Europe found no association between PMACBL-producing *E. coli* and ESBL production.19,23 However, a Japanese study reported that two-thirds of their PMACBL-producing *E. coli* from bacteraemia harboured the gene encoding CTX-M-14 ESBL.24

Extraintestinal pathogenic *E. coli* mainly belong to phylogenetic group B2 and, to a lesser extent, to group D, while intestinal commensal isolates tend to belong to groups A and B1.26 We found that CMY-2-like was the predominant PMACBL type (89%) and group D was the predominant phylogenetic group (44%). These results are similar to those reported by other investigators.8,20,21,23

Oteo et al reported that infections caused by *E. coli* with the AmpC phenotype may be spreading primarily because of CMY-2-producing phylogenetic group D isolates.25

We used PFGE to identify strains among PMACBL-producing *E. coli* and determine the genetic relatedness of the strains. This typing revealed that the majority of the PMACBL-producing *E. coli* in the Auckland region were genetically unrelated meaning that a point source or direct person to person transmission are not drivers of local community spread currently. Other investigators had similar observations.21-23

*E. coli* ST131 is a global pandemic clone of *E. coli* noted for its ability to harbour numerous resistance and virulence genes. It is most commonly associated with CTX-M-15 ESBL-producing *E. coli*.27 In this study, only four (4%) PMACBL-producing *E. coli* isolates were ST131, and all four were cultured from patients with CA UTI.

The low prevalence of ST131 among PMACBL producers is consistent with the results of studies reported from other parts of the world. Norwegian and Spanish studies found that 11% and 7% of PMACBL-producing *E. coli* were ST131, respectively.23,25 Matsumura et al found a single ST131 among 27 PMACBL-producing *E. coli* isolates.24

We found that PMACBL-producing *E. coli* caused UTI in 95% of patients. Older women were predominantly affected. This was observed by other investigators. A population-based study from Calgary Health Region in Canada found that CMY-2-producing *E. coli* was an emerging pathogen in the community, commonly causing UTI in older women.5 Similarly, a Spanish study reported that UTI was the most common type of infection in the community setting caused by PMACBL-producing isolates.22

Most, 82%, of our patients had a community-acquired UTI. Exposure to antibiotics and hospital settings are two of the risk factors for acquisition of resistant organisms.8,28 Interestingly, our analysis did not identify either of these factors or any other factors, except the use of beta-lactam antimicrobials but not specifically broad-spectrum beta-lactams, as risks for infection with a PMACBL-producing *E. coli*. This would suggest that there are other risk factors for acquiring PMACBL-producing *E. coli* in our community.

In other countries, these bacteria have been isolated from river beaches and private drinking water supplies,29 farm animals,30 food-producing animals,31,32 and dogs.33 Food-producing animals and pets may act as reservoirs for PMACBL-producing organisms and could contribute to their acquisition and spread in the community. However, a study conducted in New Zealand in 2009–2010 found no resistance to extended-spectrum cephalosporins among *E. coli* from food-producing animals including pigs, very young calves and broiler poultry.34

The global mobility of people and food products facilitates the spread of resistant organisms and resistance genes.5,35,36 As our study was laboratory-based, we did not obtain data on the patient’s dietary habits, travel history or animal exposure.
In conclusion, the results of this study show that while the prevalence of PMACBL-producing *E. coli* is low in the Auckland community, it has increased in recent years. A substantial proportion of community-onset PMACBL-producing *E. coli* infections seem to be acquired in the community. Point source outbreaks and direct person to person transmission are not major drivers of spread currently.

The vast majority of infections caused by PMACBL-producing *E. coli* are UTIs in women. A large proportion of patients did not have a recent history of hospitalisation or antimicrobial treatment. PMACBL-producing *E. coli* are more likely to be resistant to non-beta-lactam antimicrobials than other non-AmpC, non-ESBL-producing *E. coli*, leaving few treatment options.

As the prevalence of PMACBL-producing *E. coli* is still relatively low, we recommend to continue treating CA UTI empirically with non-beta-lactam antimicrobials, such as nitrofurantoin and trimethoprim, and to take urine cultures in those patients who fail to respond to empirical treatment.

Further epidemiological and molecular surveillance, determination of reservoirs, and analysis of the risk factors associated with the transmission and acquisition of these organisms is required. This will enable better understanding of their epidemiology and guide future prevention and control measures in the community.

**Competing interests:** Nil.

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**References**


