Detection and Epidemiology of Plasmid-Mediated AmpC \(\beta\)-Lactamase Producing Escherichia Coli in Two Irish Tertiary Care Hospitals

Celine Herra

Technological University Dublin, celine.herra@dit.ie

Follow this and additional works at: https://arrow.dit.ie/scschbioart

Part of the Medicine and Health Sciences Commons

Recommended Citation


This work is licensed under a Creative Commons Attribution-Noncommercial-Share Alike 3.0 License
Detection and epidemiology of plasmid-mediated AmpC β-lactamase producing Escherichia coli in two Irish tertiary care hospitals

Y.Y. Li a,1,*, F. Cassidy b,1, A. Salmon a, D. Keating a, C.M. Herra c, K. Schaffer a

a Department of Microbiology, St. Vincent’s University Hospital, Dublin 4, Ireland
b Department of Microbiology, Blackrock Clinic, Co. Dublin, Ireland
c School of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland

ARTICLE INFO

Article history:
Received 10 December 2014
Received in revised form 1 May 2015
Accepted 10 June 2015

Keywords:
Plasmid-mediated AmpC β-lactamase
Escherichia coli
Pulsed-field gel electrophoresis
Multilocus sequence typing

ABSTRACT

This study determined the prevalence and distribution of plasmid-mediated AmpC (pAmpC) β-lactamases in Irish Escherichia coli isolates. Clinical E. coli isolates (n = 95) that were intermediate or resistant to cefoxitin and/or flagged by VITEK® 2 as potential AmpC-producers underwent confirmation using a MASTDISCS™ ESBL and AmpC Detection Kit. Multiplex PCR capable of detecting family-specific plasmid ampC genes was performed to detect the presence of these genes. Five PCR-negative isolates were selected for promoter analysis. PFGE and MLST were performed on E. coli isolates that harboured a plasmid ampC gene to determine their clonal relatedness. Plasmid ampC genes were detected in 19% (18/95) of phenotypic AmpC producing E. coli isolates. The CFT group was the most common plasmid family type (n = 14); DHA (n = 3) and ACC (n = 1) groups were also detected. Promoter analysis showed that four isolates had multiple point mutations and one had a 1 bp insertion in the –10 box. PFGE demonstrated a polyclonal pattern for E. coli isolates. Furthermore, with the exception of two isolates with an identical sequence type (ST720), MLST analysis revealed that these isolates were not clonally related. This study revealed that there was a marked prevalence of pAmpC E. coli among phenotypic AmpC producing E. coli isolates but no evidence of cross-transmission of a single strain. Establishing the prevalence and clonality of these organisms is important in order to implement evidence-based infection control measures that reduce the spread of pAmpC β-lactamase resistance in the hospital environment.

© 2015 Published by Elsevier Ltd on behalf of International Society for Chemotherapy of Infection and Cancer.

1. Introduction

Antibiotic resistance among Enterobacteriaceae is an ever-increasing concern both in Ireland and worldwide. Infection with antibiotic-resistant Enterobacteriaceae can lead to treatment failures due to limited antibiotic treatment options as well as posing the risk of cross-infection. AmpC β-lactamase producing organisms hydrolyse narrow-, broad- and expanded-spectrum cephalosporins and cephemycins (e.g. cefoxitin) and are usually only susceptible to fourth-generation cephalosporins and carbapenems [1]. AmpC β-lactamase resistance may be conferred through chromosomal or plasmid-mediated origins.

The chromosomal ampC gene in Escherichia coli is generally expressed at low levels, not causing clinical resistance, because the promoter is weak and the gene contains a transcriptional attenuator [2]. However, constitutive overexpression of ampC in E. coli can occur due to either the hyperproduction of a chromosomally encoded ampC gene or by acquisition of a transferable ampC gene on a plasmid [3]. The occurrence of ampC β-lactamases on plasmids presents the threat of spreading this resistance mechanism to other organisms within a hospital or geographic region.

Ireland started reporting third-generation cephalosporin sensitivity among E. coli in 2002. Between 2002 and 2010, the proportion of resistant E. coli increased from 2.2% to 7.7%. There is limited information with regard to the prevalence and epidemiology of plasmid-mediated AmpC (pAmpC) β-lactamases among E. coli in Ireland. Roche et al. reported that 3% of Klebsiella pneumoniae collected prospectively from April 2005 to March 2007 carried

* Corresponding author. Tel.: +353 1 221 3994.
E-mail address: yuenyli@hotmail.com (Y.Y. Li).
1 These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.jgar.2015.06.004
2213-7165/© 2015 Published by Elsevier Ltd on behalf of International Society for Chemotherapy of Infection and Cancer.
pAmpC in Ireland [4]. A study undertaken by the UK Antimicrobial Resistance Monitoring and Reference Laboratory in 2007 reported a prevalence rate of pAmpC of 49% (67/135) and 55% (21/38) among cephalosporin- and cefotixin-resistant E. coli and Klebsiella spp., respectively [5]. It is important to identify the occurrence of pAmpC in these organisms in order to implement necessary infection control measures.

The aim of this study was to establish the prevalence and clonality of clinical isolates of pAmpC producing E. coli in two south Dublin hospitals. E. coli isolates that were intermediate or resistant to cefotixin and/or were flagged by the VITEK® 2 system as potential AmpC-producers underwent a phenotypic test to confirm the production of an AmpC β-lactamase. Multiplex PCR was subsequently carried out to identify the presence of plasmid ampC genes among isolates that demonstrated an AmpC phenotype. Finally, the genetic relatedness of pAmpC producing E. coli isolates was investigated through pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

2. Methods

2.1. Bacterial strains and antimicrobial susceptibility testing

A total of 95 non-duplicate AmpC producing E. coli isolates were collected from two hospitals in Dublin (Ireland) from March 2011 to October 2012.

Identification and susceptibility testing were performed by the VITEK® 2 Compact (bioMérieux, Marcy-l’Etoile, France) and the results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints v.2.0 and EUCAST Expert rules v.1.0 [6]. The advanced expert system (AES) of the VITEK® 2 Compact identified the isolates as potential AmpC producers on the basis of an antimicrobial susceptibility pattern that is compatible with an AmpC producing phenotype, i.e. resistance to first- and second-generation cephalosporins, resistance to one or more of the third-generation cephalosporins and/or reduced susceptibility to cefotixin, and sensitivity to the fourth-generation cephalosporin cefepime.

2.2. Phenotypic AmpC β-lactamase detection

Isolates were confirmed phenotypically as AmpC β-lactamase producers by the MASTDISCS™ ESBL and AmpC Detection Kit (MAST Group Ltd., Merseyside, UK). The MASTDISCS™ kit is an inhibitor-based test that detects the presence of AmpC β-lactamases and extended-spectrum β-lactamases (ESBLs). Briefly, a Mueller–Hinton agar plate (L.I.P. Diagnostic Services, Galway, Ireland) was inoculated with a 0.5 McFarland standard suspension of a test isolate. The A (10 µg cefepoxide), B (10 µg cefepoxide + the ESBL inhibitor clavulanic acid), C (10 µg cefepoxide + the AmpC inhibitor cloxacillin) and D (10 µg cefepoxide + ESBL and AmpC inhibitors) disks were applied onto the plate. The zone of inhibition around each disc was measured following 18–24 h of incubation at 37 °C in air and was interpreted according to the manufacturer’s instructions.

2.3. Multiplex PCR

Isolates confirmed as AmpC β-lactamase producers by the phenotypic MASTDISCS™ ESBL and AmpC Detection Kit were screened for the presence of a plasmid-borne ampC gene using a multiplex PCR assay developed by Pérez-Pérez and Hanson [7]. This PCR utilises six primer sets to detect the six phylogenetic families of plasmid ampC β-lactamase genes (i.e. blaPOX, blaCTX, blaSHV, blaTEM, blaOXA, blaGES) on the basis of amplicon size and was performed as described previously [7]. A representative group of blaCTX PCR products (n = 7) were randomly selected for sequencing.

Sequence alignments and analysis were performed using the NCBI BLAST programme (http://www.ncbi.nlm.nih.gov).

2.4. Promoter analysis

Five of the multiplex PCR negative isolates were analysed for ampC promoter mutations, which included promoter PCR, sequencing of the products and multiple sequence alignment with the E. coli K12 strain promoter sequence [8].

2.5. Pulsed-field gel electrophoresis

Agarose-embedded plugs containing intact bacterial DNA were prepared using a CHEF (Contour-Clamped Homogenous Electric Fields) Mapper Plug Kit (Bio-Rad Laboratories, Hemel Hempstead, UK) according to the manufacturer’s instructions. Following overnight digestion of the plugs with XbaI at 37 °C, PFGE was performed on a CHEF Mapper XA PFGE System (Bio-Rad Laboratories) according to the PulseNet standardised laboratory protocol [9]. The electrophoresis conditions were optimised for separation of the 30–600 kb fragments in a 1% agarose gel with 0.5 × TBE (Tris–borate–ethylene diamine tetra-acetic acid (EDTA)) buffer and a voltage of 6 V/cm for 19 h, with an initial switch time of 2.2 s and final switch time of 54.2 s. A dendrogram was constructed using the Dice coefficient with an optimisation of 1% and a tolerance of 1% in band position difference (FPQuest™ Software; Bio-Rad Laboratories). The clonal relationship among isolates was determined according to the criteria of Tenover et al. [10]. Clonal groups were assigned based on a similarity of >80% (≤3 band difference in restriction profile).

2.6. Multilocus sequence typing

Nine E. coli isolates harbouring a plasmid ampC gene were selected for further characterisation through the E. coli MLST scheme developed by the Achtman group [11]. The protocol for this typing method, including the PCR primers, PCR components and cycling conditions required, was accessed through the online MLST E. coli database [12]. The PCR products of the seven housekeeping genes on all nine E. coli isolates were subsequently purified for sequencing using a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). Sanger sequencing was performed by an external laboratory (Source BioScience, LifeSciences, Dublin, Ireland) and the MLST PCR primer pairs were used for the sequencing step. The sequencing data of each isolate were entered into the E. coli MLST online database (http://mlst.ucc.ie/mlst/dbs/Ecoli) to give a distinct allelic number. The alleles for the seven loci were combined to give an allelic profile or sequence type (ST). The STs of each of the nine isolates were collated and compared.

3. Results

3.1. Antimicrobial susceptibility testing and MASTDISCS™

Ninety-five E. coli isolates were identified by the VITEK® 2 system as potential AmpC producers and were phenotypically confirmed for AmpC production using combination disks. One isolate was positive for the coexistence of AmpC and ESBL enzymes. E. coli isolates demonstrated variable resistance to the third-generation cephalosporins, with some isolates only resistant to one of either cefotaxime or ceftazidime or sensitive to both. Isolates that were positive for a plasmid ampC gene generally had higher minimum inhibitory concentrations (MICs) against the third-generation cephalosporins than chromosomally mediated AmpC producing isolates (Table 1). A total of 94% (17/18) and 83% (15/18) of pAmpC isolates were resistant to ceftazidime and
Table 1
Minimum inhibitory concentrations (MICs) (in mg/L) of Escherichia coli isolates with an AmpC phenotype.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>pAmpC (n=18)</th>
<th>Chromosomal AmpC (n=77)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mode MIC</td>
<td>MIC range</td>
</tr>
<tr>
<td>AMP</td>
<td>≥32</td>
<td>≥32</td>
</tr>
<tr>
<td>CXM</td>
<td>≥64</td>
<td>16 to ≥64</td>
</tr>
<tr>
<td>CTX</td>
<td>16</td>
<td>&lt;1 to ≥16</td>
</tr>
<tr>
<td>CAZ</td>
<td>8</td>
<td>&lt;1 to ≥64</td>
</tr>
<tr>
<td>FEP</td>
<td>≤1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>FOX</td>
<td>≥64</td>
<td>8 to ≥64</td>
</tr>
<tr>
<td>MEM</td>
<td>≤0.25</td>
<td>≤0.25</td>
</tr>
<tr>
<td>ETP</td>
<td>≤0.5</td>
<td>≤0.5-1</td>
</tr>
<tr>
<td>GEN</td>
<td>≤1</td>
<td>≤1 to ≥16</td>
</tr>
<tr>
<td>CIP</td>
<td>≤0.25</td>
<td>≤0.25 to ≤4</td>
</tr>
</tbody>
</table>

*pAmpC, plasmid-mediated AmpC; AmpC, ampicillin; CXM, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; MEM, meropenem; ETP, ertapenem; GEN, gentamicin; CIP, ciprofloxacin.

cefotaxime, respectively. In contrast, chromosomally mediated AmpC producing isolates were more sensitive, with only 12% (9/77) resistant to ceftazidim and 14% (11/77) resistant to cefotaxime. With the exception of one isolate (E25), AmpC-producing E. coli isolates in this study were resistant to cefoxitin. Most isolates (n = 94) were sensitive to the fourth-generation cephalosporin ceftazidime, except for one isolate (E23) that was also non-susceptible to ertapenem. All 95 isolates were sensitive to meropenem. In addition, many isolates demonstrated multiple resistance; 16 isolates displayed resistance to the aminoglycoside gentamicin and 31 isolates were resistant to the fluoroquinolones ciprofloxacin and plazimicillin.

3.2. AmpC multiplex PCR

Of the 95 isolates that were screened for the presence of a plasmid ampC β-lactamase gene by multiplex PCR, 18 isolates (19%) were pAmpC positive. The predominant gene was bla<sub>CTE</sub>, detected in 14 E. coli isolates (Table 2). Nucleotide sequencing of a representative group of these isolates (n = 7) revealed 100% concordance with the bla<sub>AMV-2</sub> gene sequence (GenBank accession no. HQ680723). Other pAmpC genes identified were bla<sub>DHA</sub> (n = 3) and bla<sub>ACC</sub> (n = 1). The majority of phenotypic AmpC producing E. coli isolates were negative for a pAmpC gene, which suggests that the presence of AmpC could be due to either hyperproduction of chromosomal AmpC or acquisition of novel plasmids that were not detected by the multiplex PCR in this study.

3.3. Promoter analysis

Five of the multiplex PCR negative isolates were analysed for ampC promoter mutations. Four of them had multiple point mutations in the promoter region and one had a 1-bp insertion in the −10 box compared with E. coli K12 (Table 3). The predominant point mutation was at +58, followed by the −35 box.

3.4. Isolates with plasmid-mediated AmpC are genetically unrelated

The 18 E. coli isolates harbouring plasmid ampC genes were not clonally related by PFGE analysis and revealed <50% sequence homology. Furthermore, a representative group of nine pAmpC positive E. coli isolates were typed by MLST. With the exception of two isolates (isolates 90 and 91), which were ST720, all of the MLST types have been previously detected in humans and livestock in Europe [12]. ST493 has been isolated from humans in Japan and ST3672 from wild animals in China [12].

4. Discussion

Antimicrobial resistance among Enterobacteriaceae is fast becoming a worldwide public health concern, and AmpC β-lactamases are an emerging group of antimicrobial resistance determinants [13]. The occurrence of ampC genes on plasmids presents a great challenge to infection control because plasmid-mediated genes can spread to other organisms within a hospital or geographic region. The lack of a widely accepted phenotypic AmpC β-lactamase detection method greatly hinders accurate detection and surveillance of AmpC producers and hence the ability to limit the spread of pAmpC resistance. This study was designed to determine the prevalence and clonality of pAmpC producing E. coli in two Dublin hospitals.
A total of 95 *E. coli* clinical isolates phenotypically identified as AmpC β-lactamase producers by VITEK® 2 and 2 combination disks were included in this study. Whilst the majority of them were identified as possible chromosomal AmpC hyperproducers (*n* = 77), a total of 18 isolates (19%) were pAmpC producers. This is compared with a study undertaken by the UK antimicrobial reference laboratory including Irish isolates that reported a prevalence rate of pAmpC of 49% (67/135) among cephaplosporin-resistant *E. coli* spp. [5]. The higher prevalence rate detected in the UK study could potentially be explained by the fact that microbiology laboratories would only send the most resistant isolates for further analysis to reference laboratories, whereas in the current study serial clinical isolates exhibiting an AmpC phenotype were collected. Other studies carried out in hospital laboratories and similar in design to this study have revealed various prevalence rates. For example, a study from Denmark reported a similar prevalence to our data, in which 17% (4/24) of *E. coli* carried a plasmid *ampC* gene [14], whilst a study in Switzerland reported a higher prevalence at 38% (8/21) [15].

The predominant *ampC* gene detected in this study was *bla*<sub>CT</sub>, identified in 14 *E. coli* isolates. Nucleotide sequencing of a representative group of these isolates (*n* = 7) revealed the *bla*<sub>CMY-2</sub> gene. This observation is consistent with many other studies worldwide in which CMY-2 is the most commonly acquired plasmid and widely geographically distributed AmpC enzyme [5,15–22].

In addition, *bla<sub>DHA</sub>* (*n* = 3) and *bla<sub>ACC</sub>* (*n* = 1) were also detected. The CIT, DHA and ACC AmpC enzymes have been previously identified among *E. coli* and *K. pneumoniae* isolates in Ireland [4,5]. The isolate (E25) that harboured a *bla<sub>ACC</sub>* gene in this study was susceptible to cefotaxime, which correlates with many studies showing that isolates with ACC enzyme are sensitive to cefotaxime [4,23]. It is important to consider that the use of cefotaxin resistance as a screening marker may be compromised by isolates producing plasmid-encoded ACC AmpC β-lactamases that are sensitive to cefotaxin.

This study revealed that pAmpC producing *E. coli* isolates displayed higher MICs to the third-generation cephaplosporins and cefotaxime than chromosomal AmpC hyperproducing *E. coli* (Table 1). Concomitant resistance to cefotaxime, ceftazidime and cefotaxime in phenotypic AmpC-producing *E. coli* isolates was suggestive of pAmpC production. Hence, whilst routine molecular testing for pAmpC in *E. coli* is currently unavailable in Ireland, the susceptibility profile of a phenotypically confirmed AmpC producing *E. coli* may help to distinguish between pAmpC production and chromosomal AmpC hyperproduction.

Co-resistance to other antimicrobials was frequently observed among these isolates. For example, ciprofloxacin resistance was observed in 33% (31/95) of AmpC producers in this study. A rising trend of fluoroquinolone resistance among AmpC producing *E. coli* has been reported by Simner et al. [3]. The coexistence of multiple resistance mechanisms in an organism is a concern as multidrug resistance can severely limit therapeutic options. In this study, co-production of AmpC and ESBL was detected in one isolate. Continuous resistance surveillance and the implementation of infection control measures are important to reduce the spread of multidrug-resistant organisms. Five of the multiplex PCR negative isolates were analysed for *ampC* promoter mutations and the results suggested that they are common mutations as they were also identified by Haldorsen et al., who reported three different mechanisms for increased expression of chromosomal *ampC* genes including multiple point mutations (positions −1, −18, −42, −82 and +58) and single nucleotide or insertion sequence (IS) element insertions between the −35 and −10 boxes [16]. Further work is required to investigate the role of *ampC* promoter mutations in AmpC hyperproduction.

Detection of a plasmid *ampC* gene in an AmpC producing organism provides a valuable insight into the resistance mechanism of an isolate and the potential for this resistance to spread. It is equally important to understand whether antimicrobial resistance is due to the transmission of resistance genes from organism to organism or through the transmission of a single resistant strain. Molecular typing by PFGE and MLST was performed in this study to investigate whether pAmpC isolates were clonally related and hence the result of the spread of a single strain. PFGE showed that the pAmpC producers (*n* = 18) represented different DNA restriction patterns with >50% fragments sharing homology. These results suggest that the incidence of transferable AmpC in *E. coli* was the result of a mobile element rather than the spread of a single clone.

MLST was carried out on a representative group (*n* = 9) of pAmpC *E. coli* isolates in this study. MLST was performed due to the worldwide clonal dissemination of *E. coli* ST131 and recent detection of pAmpC β-lactamases among *E. coli* ST131 [24,25]. Among the nine selected *E. coli* isolates, MLST identified eight different STs. The pandemic *E. coli* clone ST131 was not detected in this study. Two isolates (isolates E90 and E91) had the same allelic profiles and therefore the same ST (ST720). These two isolates only displayed 40% similarity by PFGE, highlighting that MLST has a lower discriminating ability compared with PFGE, as reported in previous studies [26–28]. The remaining seven isolates had different individual allelic profiles and therefore different STs. The ST38 isolate in this study (isolate E113) carried a *bla<sub>DHA</sub>* plasmid *ampC* gene. However, in two separate studies, pAmpC *E. coli* isolates related to the sequence type complex (STC) 38 instead harboured a *bla<sub>CT</sub>* plasmid *ampC* gene [24,25]. This illustrates how plasmids can spread among isolates and also that this globally distributed *E. coli* strain can acquire different plasmid *ampC* genes. Other strains identified in this study (ST92, ST493, ST720, ST906 and ST3672) have not been previously reported among pAmpC producing *E. coli* in the literature and could indicate that there has been further spread of plasmid *ampC* genes among *E. coli* strains.

In conclusion, this study revealed that there was a prevalence of 19% of pAmpC producing *E. coli* among isolates displaying an AmpC phenotype in two Dublin hospitals. The fact that the isolates investigated by PFGE and MLST were not clonally related demonstrates that pAmpC resistance was due to the transfer of plasmids among isolates rather than the spread of a single organism. As both hospitals do not routinely isolate patients harbouring *E. coli* with an AmpC phenotype, the lack of evidence for cross-infection by PFGE was reassuring. These findings emphasise the importance of investigating AmpC β-lactamases in clinical isolates at a molecular level, although routine molecular testing is currently beyond the scope of Irish diagnostic laboratories. In this way, informed and evidence-based infection control policies can be introduced to reduce the spread of pAmpC resistance in hospitals and limit the movement of this resistance mechanism.

**Table 3** Mutations identified in the *Escherichia coli* *ampC* promoter region.

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Mutations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E7</td>
<td>−88 (C&gt;T), −82 (A&gt;G), −18 (G→A), −1 (C&gt;T), +58 (C→T)</td>
</tr>
<tr>
<td>E10</td>
<td>−35 box (T→A), +58 (G→T), +63 (T→C)</td>
</tr>
<tr>
<td>E31</td>
<td>+70 (C→T)</td>
</tr>
<tr>
<td>E36</td>
<td>−35 box (T→A), +58 (C→T), +63 (T→C)</td>
</tr>
<tr>
<td>E46</td>
<td>1 bp (T) insertion in the −10 box</td>
</tr>
</tbody>
</table>

* Compared with *E. coli* K12.
Funding

St Vincent's University Hospital Microbiology Research Fund (Dublin, Ireland).

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgments

The authors gratefully acknowledge the assistance of staff at the two collaborating microbiology laboratories for the collection of isolates.

References