Molecular epidemiology and antimicrobial resistance pattern of extended-spectrum-\(\beta\)-lactamase-producing Enterobacteriaceae in Glasgow, Scotland

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Objectives: To establish the molecular epidemiology and antimicrobial resistance pattern of extended-spectrum \(\beta\)-lactamase (ESBL)-producing Enterobacteriaceae harbouring \(\text{bla}_{\text{CTX-M}}\) in Glasgow, Scotland.

Methods: During a 12 week period, Enterobacteriaceae isolates obtained from urine samples were collected and susceptibility testing performed. Isolates were screened for the presence of \(\text{bla}_{\text{CTX-M}}\) by multiplex PCR and selected \textit{Escherichia coli} genes were subsequently sequenced. PFGE analysis was performed on selected \textit{E. coli} isolates in order to identify clonal relationships.

Results: There were 155 phenotypically confirmed non-duplicate Enterobacteriaceae isolates obtained from urine samples. \(\text{bla}_{\text{CTX-M}}\) was identified in 131/155 (84.5%) of the ESBL-producing isolates, with CTX-M group 1 enzymes accounting for 103/131 (78.6%) of these. The remaining 24 isolates carried other \(\text{bla}_{\text{CTX-M}}\) types, including CTX-M group 2, CTX-M group 9 and an unidentifiable combination designated CTX-M group G2/Gx. A sample of 46/97 (47.4%) CTX-M-positive \textit{E. coli} isolates was chosen for PFGE and demographic information regarding the source of the isolates was collated. Eight \textit{E. coli} clusters were identified by PFGE; however, they did not achieve the 85% cut-off to demonstrate clonality. Nitrofurantoin resistance was significantly greater in the \textit{E. coli} isolates expressing a non-CTX-M group 1 ESBL when compared with the \textit{E. coli} isolates expressing a CTX-M group 1 ESBL.

Conclusions: As seen in other British studies, \(\text{bla}_{\text{CTX-M}}\) has become the predominant ESBL type in Glasgow, Scotland. The PFGE results show that four different CTX-M groups appear to be circulating in the community and within all four hospitals in the locality. There is little correlation between strain genotype and CTX-M group, thus it is unlikely that cross-infection alone is the driver. It is possible that plasmid migration of CTX-M genes within the \textit{E. coli} population is occurring.

Keywords: CTX-M-type \(\beta\)-lactamases, ESBLs, antibiotic resistance

Introduction

As highlighted by Xu \textit{et al.}, extended-spectrum \(\beta\)-lactamase (ESBL)-producing Enterobacteriaceae continue to show geographical variation and increasing diversification. Several studies have been performed to characterize ESBL-producing Enterobacteriaceae in the UK; however, none of these studies focused solely on isolates from Scotland. For example, Woodford \textit{et al.} collected 291 CTX-M-producing \textit{Escherichia coli} from 42 UK centres; however, only 2 of these isolates were from Scotland. Younes \textit{et al.} investigated the prevalence of transferable \(\text{bla}_{\text{CTX-M-15}}\) from hospital- and community-acquired isolates in Edinburgh, Scotland, but only looked at \textit{Klebsiella pneumoniae}. Therefore we sought to analyse whether ESBL-producing Enterobacteriaceae from Glasgow have similar molecular epidemiology, genetic characteristics and antimicrobial resistance patterns compared with previous British and worldwide studies.

Materials and methods

Enterobacteriaceae isolates from urine samples were collected over a 12 week period (November 2009–January 2010) and susceptibility
testing using standard disc diffusion methodology according to the CLSI guidelines was performed. All Enterobacteriaceae resistant to both amoxicillin and cephalaxin underwent further testing in order to phenotypically detect the presence of ESBLs. This involved the use of the double disc diffusion method,\(^1\) MASTDISCS\(^2\) ESBL detection disc diffusion test (Mast Diagnostics, Bootle, UK) and automated testing using Vitek\(^2\) (bioMérieux S.A., Marcy l’Étoile, France). Extended susceptibility testing was also performed on all ESBL-producing Enterobacteriaceae using Vitek\(^2\) according to CLSI guidelines.\(^1\) Phenotypically confirmed ESBL-producing Enterobacteriaceae were screened by multiplex PCR for the presence of \(bla_{CTX-M}\) genes according to the protocols described by Woodford et al.\(^2\) Positive control strains were used for each PCR. Amplification was carried out using cell lysate with Promega Go-Taq polymerase (Promega, Southampton, UK), and selected \(E. coli\) genes were sequenced.

PFGE analysis was performed on selected isolates using a modified version of the technique described by Miranda et al.\(^7\) The total bacterial DNA was digested with XbaI (Promega), and DNA fragments were separated on a 1% agarose gel in 0.5x TBE buffer using the CHEF DRII apparatus (Bio-Rad, Hertfordshire, UK). The pulse time was 2-50 s over a running time of 30 h. The fragmented DNA was run at a field strength of 6 V/cm for 22 h at 14°C. After PFGE, the gels were stained with ethidium bromide and digital images of each gel were captured by Gel Doc 2000 (Bio-Rad). Isolates that clustered together with a Dice coefficient correlation of >85% were considered to belong to the same PFGE type. Fisher’s exact test was used to assess differences in antibiotic resistance rates between the different types of ESBL-producing Enterobacteriaceae. \(P\) values <0.05 were considered to be statistically significant. SPSS software, version 17 (SPSS) was used for statistical analysis.

Results

A total of 2053 Enterobacteriaceae isolates from urine samples were collected over a 12 week period. A total of 155 (7.5%) non-duplicate isolates were phenotypically confirmed as ESBL-producing Enterobacteriaceae. The 155 isolates consisted of 105 \(E. coli\), 35 Klebsiella pneumoniae, 1 Klebsiella oxytoca, 8 Enterobacter sp., 2 Serratia sp., 2 Citrobacter sp., 1 Morganella morganii and 1 Proteus sp. We found that 131 (84.5%) ESBL-producing isolates contained genes encoding CTX-M enzymes and 24 (15.5%) produced non-CTX-M ESBLs (Table 1). Further analysis of the \(bla\text{-}_{CTX-M}\) positive isolates revealed that 103/131 (78.6%) were of the CTX-M group 1 type (G1), 1/131 (0.8%) was of the CTX-M group 2 type (G2) and 17/131 (13.0%) were of the group 9 type (G9). A further 10 (7.6%) of the \(bla\text{-}_{CTX-M}\)-producing isolates expressed two enzymes simultaneously.

Table 1. Molecular epidemiology of ESBL-producing Enterobacteriaceae

<table>
<thead>
<tr>
<th>Organism</th>
<th>Non-CTX-M</th>
<th>Total</th>
<th>G1</th>
<th>G9</th>
<th>G2</th>
<th>G2/Gx</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E. coli)</td>
<td>8</td>
<td>97</td>
<td>69</td>
<td>17</td>
<td>1</td>
<td>10</td>
<td>105</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>3</td>
<td>33</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Serratia sp.</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Citrobacter sp.</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>M. morganii</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>24</strong></td>
<td><strong>131</strong></td>
<td><strong>103</strong></td>
<td><strong>17</strong></td>
<td><strong>1</strong></td>
<td><strong>10</strong></td>
<td><strong>155</strong></td>
</tr>
</tbody>
</table>

One of these enzymes was identified as CTX-M group 2; however, the other enzyme was not identified, therefore these organisms were classified as G2/Gx. Sequencing of two \(E. coli\) CTX-M G1 isolates revealed that both isolates expressed CTX-M-15. Similarly, sequencing of the \(E. coli\) CTX-M G2 isolate revealed CTX-M-2 expression, while sequencing of one \(E. coli\) CTX-M G9 isolate identified the expression of CTX-M-14.

A sample of 46/97 (47.4%) CTX-M-positive \(E. coli\) isolates were chosen for PFGE and demographic information regarding the source of the isolates was collated. Twenty isolates were chosen from CTX-M group 1, 15 isolates from CTX-M group 9, 10 isolates from CTX-M group G2/Gx and 1 isolate from CTX-M group 2. The PFGE results and the source of the isolates are shown in Figure 1. There were eight clusters with similarity ranging from 54.36% to 73.75%. From the 46 \(E. coli\) isolates that were chosen for PFGE, 33/46 (71.7%) came from a hospital setting. Two-thirds of these samples were from the two acute care hospitals (22/33, 66.7%), with the remainder from two rehabilitation hospitals for the elderly. The majority of samples were from patients in the >75 age group (29/46, 63.0%). Similarly, the majority of samples were from female patients (33/46, 71.7%) and 40/46 (87.0%) were midstream urine specimens, with the remainder catheter urine specimens.

There were no statistically significant differences in antimicrobial resistance patterns among Enterobacteriaceae expressing CTX-M ESBL types when compared with Enterobacteriaceae expressing non-CTX-M ESBLs (Table 2). Table 3 shows that nitrofurantoin resistance was significantly greater in the non-CTX-M type. \(E. coli\) isolates when compared with the \(G1\) \(E. coli\) isolates (35.7% versus 13.0%; \(P=0.021\)); however, there were no differences in resistance rates for the other antibiotics.

Discussion

Data regarding the molecular epidemiology of ESBL-producing Enterobacteriaceae from Scotland, and specifically Glasgow, have been lacking. It has been assumed that the local epidemiology would be similar to that of the rest of UK; however, there has been some heterogeneity in the prevalence of CTX-M types throughout England, with the emergence of less common CTX-M types, such as CTX-M-2.\(^1\)

The first published UK-wide study found that 85% of 291 Enterobacteriaceae expressing CTX-M enzymes were of the CTX-M-15 type, with a further 12% of isolates belonging to CTX-M group 9, consisting of CTX-M-9 and CTX-M-14.\(^2\) Despite the fact that only two isolates from that study originated from Scotland, the results obtained in our study confirm the findings of Woodford et al.,\(^2\) with 78.6% of our isolates containing ESBL genes belonging to the CTX-M-1 group, of which CTX-M-15 is the predominant type. More recently, Xu et al.\(^1\) found that 96.6% of Enterobacteriaceae expressing CTX-M enzymes were of the CTX-M-15 type. Interestingly, CTX-M ESBLs were recorded, but were not dominant, in the west of Scotland from a study of isolates collected between 2003 and 2005, suggesting that CTX-M ESBLs subsequently became the predominant ESBL type.\(^8\) This is in keeping with data showing that CTX-M ESBLs, and specifically CTX-M-15, are the dominant genotype worldwide.\(^9\)
Figure 1 illustrates that there was no correlation between the source of the isolates and the CTX-M ESBL type. The PFGE results show that there are predominantly four CTX-M groups that appear to be circulating in the community and within all four hospitals in the locality. There is little correlation between strain genotype and CTX-M group, thus it is unlikely...
Table 2. Antimicrobial resistance among ESBL-producing Enterobacteriaceae

<table>
<thead>
<tr>
<th>ESBL type</th>
<th>CIP</th>
<th>GEN</th>
<th>NIT</th>
<th>TMP</th>
<th>AMK</th>
<th>TZP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-M</td>
<td>102 (77.9)</td>
<td>51 (38.9)</td>
<td>25 (19.1)</td>
<td>104 (79.4)</td>
<td>4 (3.1)</td>
<td>7 (5.3)</td>
<td>131</td>
</tr>
<tr>
<td>Non-CTX-M</td>
<td>16 (66.7)</td>
<td>6 (25.0)</td>
<td>7 (29.2)</td>
<td>20 (83.3)</td>
<td>0 (0)</td>
<td>1 (4.2)</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>118 (76.1)</td>
<td>57 (36.8)</td>
<td>32 (20.6)</td>
<td>124 (80.0)</td>
<td>4 (2.6)</td>
<td>8 (5.2)</td>
<td>155</td>
</tr>
<tr>
<td>Trend (P value)</td>
<td>0.297</td>
<td>0.252</td>
<td>0.278</td>
<td>0.787</td>
<td>1.000</td>
<td>1.000</td>
<td>—</td>
</tr>
</tbody>
</table>

CIP, ciprofloxacin; GEN, gentamicin; NIT, nitrofurantoin; TMP, trimethoprim; AMK, amikacin; TZP, piperacillin/tazobactam. A P value <0.05 was considered to be significant.

Table 3. Antimicrobial resistance among CTX-M ESBL-producing E. coli

<table>
<thead>
<tr>
<th>E. coli ESBL type</th>
<th>CIP</th>
<th>GEN</th>
<th>NIT</th>
<th>TMP</th>
<th>AMK</th>
<th>TZP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>53 (76.8)</td>
<td>24 (34.8)</td>
<td>9 (13.0)</td>
<td>53 (76.8)</td>
<td>2 (2.9)</td>
<td>4 (5.8)</td>
<td>69</td>
</tr>
<tr>
<td>Non-G1</td>
<td>19 (67.9)</td>
<td>14 (50.0)</td>
<td>10 (35.7)</td>
<td>21 (75.0)</td>
<td>1 (3.6)</td>
<td>2 (7.1)</td>
<td>28</td>
</tr>
<tr>
<td>CTX-M total</td>
<td>72 (74.2)</td>
<td>38 (39.2)</td>
<td>19 (19.6)</td>
<td>74 (76.3)</td>
<td>3 (3.1)</td>
<td>6 (6.2)</td>
<td>97</td>
</tr>
<tr>
<td>Trend (P value)</td>
<td>0.443</td>
<td>0.177</td>
<td>0.021</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>—</td>
</tr>
</tbody>
</table>

CIP, ciprofloxacin; GEN, gentamicin; NIT, nitrofurantoin; TMP, trimethoprim; AMK, amikacin; TZP, piperacillin/tazobactam. A P value <0.05 was considered to be significant.

that cross-infection alone is the driver. It is possible that plasmid migration of CTX-M genes within the E. coli population is occurring. These results are similar to those of Ben-Ami et al.,10 but are in stark contrast to those of Xu et al.,1 who found that 95% of E. coli isolates could be categorized into 23 clusters, including 7 major clusters, all with isolates that fulfilled the 85% cut-off to define clonality. In the present study, there is a small group of three isolates (153, 154 and 157) that appear to be genetically identical by PFGE, having the same CTX-M type and the same antibiogram (data not shown). Although these three samples were taken from patients at different hospitals, all three of these patients were resident on the same ward at some point during their admission, and indeed, two of these patients were resident on the same ward at the same time. As an adjunct to this study, PFGE of the 33/36 CTX-M G1 K. pneumoniae isolates did not show that the Glasgow isolates were clonally related to any of the Edinburgh K. pneumoniae isolates from the study by Younes et al.3 (A. Hamouda and S. G. B. Amyes, unpublished results).

There are a few UK studies looking at the resistance patterns of different types of ESBL-producing Enterobacteriaceae, but none of these studies has focused solely on isolates from Scotland. Potz et al.11 reviewed 1122 cephalosporin-resistant Enterobacteriaceae from 16 centres in London and south-east England and found that ciprofloxacin, gentamicin and nitrofurantoin resistance was greater in CTX-M-producing Enterobacteriaceae than in non-CTX-M-producing Enterobacteriaceae. In a multinational study of 983 isolates (90% of which were E. coli), Ben-Ami et al.12 found that CTX-M-producing Enterobacteriaceae were more likely to be resistant to ciprofloxacin and less likely to be resistant to trimethoprim. Xu et al.3 found that 93.4% of CTX-M-producing Enterobacteriaceae were resistant to ciprofloxacin, 91.4% to trimethoprim and 52.4% to gentamicin, and nine isolates displayed reduced susceptibility to carbapenems. There were no data for non-CTX-M-producing Enterobacteriaceae.

Relatively few studies have compared antibiotic resistance patterns among Enterobacteriaceae expressing different CTX-M genes. Woodford et al.17 found that antibiotic resistance among CTX-M-15-producing Enterobacteriaceae was more substantial when compared with CTX-M-9-producing Enterobacteriaceae. Mean geometric MICs for the majority of antibiotics, including gentamicin and ciprofloxacin, was significantly greater in the CTX-M-15 cohort. This is in contrast with results from our study, which showed greater nitrofurantoin resistance in the non-G1 isolates. Pitout et al.13 found that gentamicin and trimethoprim/sulfamethoxazole resistance among CTX-M group 1-producing Enterobacteriaceae was significantly greater than Enterobacteriaceae expressing CTX-M group 14 enzymes.

The main limitation of the study was an inability to perform multilocus sequence typing (MLST) in order to identify the O25b-ST131 E. coli clone or UK epidemic strain A that has been implicated in several outbreaks locally and abroad.2,9 This would have provided conclusive data on whether the local spread of Enterobacteriaceae expressing CTX-M ESBL genes was similar to the clonal spread witnessed in England and elsewhere. Due to the inability to perform plasmid analysis, it is impossible to conclusively prove plasmid migration between our isolates. In addition, it was not possible to fully identify the CTX-M G2/Gx ESBL type. Similarly, it would have been ideal to perform molecular analysis of the Enterobacteriaceae expressing...
non-CTX-M ESBL genes to assess the prevalence of other ESBL types, such as $\text{bla}_{\text{SHV}}$ and $\text{bla}_{\text{TEM}}$

In conclusion, as far as we are aware, this is the first study to perform a molecular analysis of ESBL-producing Enterobacteriaceae obtained from the west of Scotland. It can be confirmed that the dominant local genotype is CTX-M group 1, with the likelihood that CTX-M-15 is the predominant member, similar to studies from the rest of the UK. However, there is little evidence of clonal spread of $E.\ coli$ in this population. Studies to further characterize local ESBL-producing Enterobacteriaceae, including identification of the O25b-ST131 $E.\ coli$ clone, are required.

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Transparency declarations

None to declare.

References