Effect of drug treatment options on the mobility and expression of \( \text{bla}_{\text{KPC}} \)

Amanda L. Roth, Philip D. Lister and Nancy D. Hanson*

Center for Research in Anti-Infectives and Biotechnology, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178, USA

*Corresponding author. Tel: +1-402-280-5837; Fax: +1-402-280-1875; E-mail: ndhanson@creighton.edu

Received 6 December 2012; returned 12 January 2013; revised 7 June 2013; accepted 12 June 2013

Objectives: Both transposition and increases in gene expression have been implicated in the success of KPC-producing pathogens, but the stimulus required for these phenomena are unknown. It is possible that exposure to antimicrobials during patient treatment increases \( \text{bla}_{\text{KPC}} \) expression or induces Tn\(^{4401}\) transposition. The purpose of this study was to determine if exposure to carbapenems or other antimicrobial drug classes could stimulate expression of \( \text{bla}_{\text{KPC}} \) or the \textit{in vitro} transposition of Tn\(^{4401}\).

Methods: Five KPC-producing clinical isolates were evaluated in this study. Gene expression of RNA from each isolate exposed to subinhibitory, MIC or suprainhibitory levels of antibiotics was evaluated using real-time RT–PCR. Southern blots were performed on plasmids from isolates exposed to subinhibitory levels of antibiotics.

Results: There were subtle changes in \( \text{bla}_{\text{KPC}} \) RNA expression following antibiotic exposure that were both strain and drug dependent. Multiple plasmids ranging from \( \approx 8 \) to \( \approx 200 \) kb were observed for the Enterobacteriaceae isolates, whereas the \textit{Pseudomonas aeruginosa} isolate had one \( \approx 55 \) kb plasmid. No changes in hybridization patterns or binding intensity for the \( \text{bla}_{\text{KPC}} \) probe were observed after antibiotic exposure.

Conclusions: While the changes in \( \text{bla}_{\text{KPC}} \) RNA expression are subtle, the different responses observed suggest both strain- and genera-specific variations in response to different antibiotic treatments.

Keywords: KPC, Tn\(^{4401}\), collateral damage

Introduction

\textit{Klebsiella pneumoniae} carbapenemase (KPC)-producing pathogens have become a major threat to patient health. Patients infected with KPC-producing pathogens have a higher incidence of morbidity and mortality.\(^{1,2}\) In addition, these pathogens are a challenge for infection control in healthcare facilities due to the inability to detect all KPC-producing isolates using current guidelines for susceptibility testing.\(^{3}\) Most commonly found in \textit{K. pneumoniae}, KPC enzymes have been identified in a variety of Gram-negative organisms, including \textit{Escherichia coli}, \textit{Enterobacter} species, \textit{Pseudomonas aeruginosa} and \textit{Acinetobacter baumannii}. Organisms that produce the KPC \( \beta\)-lactamase have been associated with multiple \( \beta\)-lactam resistance, including carbapenem resistance.\(^{4,5}\) This, in addition to the lack of novel antimicrobials with action against resistant Gram-negative pathogens, forces physicians to treat infections caused by KPC-producing organisms with potentially toxic antibiotics, such as the aminoglycosides and polymyxins.\(^{6}\) The rapid spread of \( \text{bla}_{\text{KPC}} \) into multiple genera of Gram-negative pathogens, the lack of good treatment regimens and the inability of clinical laboratories to identify all KPC-producing pathogens underscore the need to understand the selective pressures driving the rapid emergence and spread of KPC-producing organisms.

Some studies suggest that higher levels of \( \text{bla}_{\text{KPC}} \) RNA expression are associated with increases in carbapenem MICs.\(^{7,8}\) However, the majority of these studies have been carried out using clinical strains producing multiple types of \( \beta\)-lactamases; therefore, it is difficult to assess the contribution of \( \text{bla}_{\text{KPC}} \) in \( \beta\)-lactam susceptibility profiles. A recent study by our laboratory evaluated the effect of KPC production in the absence of other resistance mechanisms in four genera of Gram-negative pathogens and compared them with KPC-producing clinical isolates of the same genera.\(^{9}\) Our findings demonstrated that the levels of \( \text{bla}_{\text{KPC}} \) expression by the KPC-producing clinical isolates were not at the level observed for the KPC-producing transformants, yet the clinical isolates had higher carbapenem MICs. Clearly, other mechanisms, such as porin down-regulation and as yet unidentified mechanisms, contribute to the complexity of the carbapenem susceptibility patterns observed for KPC-producing pathogens.
One explanation as to why the identification of KPC-producing isolates is difficult in the clinical laboratory could be that the stimulus required for β-lactamase production is present during the course of treatment in vivo, but not during in vitro susceptibility testing. It is possible that certain antimicrobial drug classes used during empirical therapy, combination therapy or to treat multiple infections within the patient could provide a stimulus to either increase the amount of \textit{bla}_KPC expression or stimulate the mobility of transposon Tn4401 carrying \textit{bla}_KPC. Therefore, the possibility of ‘collateral damage’ associated with other drug classes used in the treatment of patients infected with KPC-producing isolates exists. In this study, we tested drugs from multiple antibiotic classes, including β-lactams, macrolides, aminoglycosides and fluoroquinolones, for their ability to act as a stimulus to increase \textit{bla}_KPC expression and/or contribute to the mobilization of Tn4401 carrying \textit{bla}_KPC in four different genera of Gram-negative pathogens.

## Materials and methods

### Bacterial strains

Five previously characterized KPC-producing clinical isolates were evaluated: \textit{E. coli} 233, \textit{K. pneumoniae} isolates UMM3 and HUH40, Enterobacter sp. isolate 01MGH049 and \textit{P. aeruginosa} PSS.\textsuperscript{15} \textit{E. coli} ATCC 25922, \textit{P. aeruginosa} ATCC 27853 and \textit{Staphylococcus aureus} ATCC 29213 were used as quality control strains for susceptibility testing.

### PCR mapping and sequencing of Tn4401

Template DNA for PCR was prepared from overnight cultures of \textit{E. coli}, \textit{K. pneumoniae}, \textit{Enterobacter} sp. and \textit{P. aeruginosa} using 1.5 mL of culture as previously described.\textsuperscript{15} Prior to lysis, a final concentration of 400 mg/L proteinase K was added to \textit{P. aeruginosa} supernatant to prevent nucleic acid degradation by \textit{P. aeruginosa} nucleases. Alternatively, DNA fragments were generated using Genome Walker (Clontech, Mountain View, CA, USA) according to the manufacturer’s protocols.

PCR mapping of the KPC-bearing 10.5 kb transposon, Tn4401, was performed using transposon- or adapter-specific primers for each clinical isolate evaluated in this study (Table S1, available as Supplementary data at JAC Online). Amplicons were generated using the proof-reading enzyme Platinum Taq DNA Polymerase High Fidelity, purified and sequenced as previously described.\textsuperscript{15} Sequence analysis was performed using the DNA Baser version 2.75 software program (Heracle Software, Lilienthal, Germany).

### Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using broth microdilution according to CLSI recommendations and/or Etest (AB Biodisk, Solna, Sweden). The results were interpreted using CLSI criteria.\textsuperscript{5,16} Antimicrobial agents were obtained from the following suppliers: ceftazidime, meropenem, gentamicin, piperacillin, tazobactam, azithromycin and ciprofloxacin were from Sigma Chemical Co. (St Louis, MO, USA); ticarcilone was from Pfizer (New York, NY, USA); cefepime was from Bristol-Myers Squibb (Princeton, NJ, USA); doripenem was from Johnson & Johnson (La Jolla, CA, USA); and imipenem and ertapenem were from Merck & Co. (Rahway, NJ, USA).

### PCR and sequencing results

To ensure that the five KPC-producing isolates carried the required genes for transposition, the 10.5 kb Tn4401 element was sequenced in each isolate. PCR mapping with different primer sets showed that isolates \textit{K. pneumoniae} UMM3, \textit{E. coli} 233 and \textit{Enterobacter} sp. 01MGH049 had the full-length isoform a of Tn4401 identical to sequences in the GenBank database (EU176012 and FJ223605). We were able to obtain sequence data for Tn4401 in all the isolates except for \textit{K. pneumoniae} HUH40 and \textit{P. aeruginosa} PS5. In these two isolates, there were two regions within the \textit{tnpR} and \textit{istA} – \textit{istB} regions from which clean sequence reads could not be obtained. Using GenBank accession number EU176012, the unreadable sequences for HUH40 were located between 2496–2583 and 6565–7254 nt; for strain PS5, the unreadable sequences were between 1797–1818 and 6547–6560 nt.

## Results

### Nucleotide sequence accession numbers

GenBank accession numbers for Tn4401 in isolates UMM3, 233 and 01MGH049 are JX500679, JX500680 and JX500681, respectively.

### Antibiotic exposure

Antibiotics were added to inoculated flasks of each isolate grown to early logarithmic phase (~0.4 optical density at 600 nm (OD\textsubscript{600}) at 37°C with shaking at the concentration indicated for each experiment. The subinhibitory level of antibiotics used in these experiments was 0.25× MIC of that antibiotic observed for each bacterial isolate. There are no breakpoints for macrolides with respect to Enterobacteriaceae, so the trough concentration in epithelial lung fluid of 0.8 mg/L was used for the subinhibitory concentration.\textsuperscript{17} If the MIC of an antibiotic exceeded the upper limit of our antimicrobial susceptibility assay, the next concentration above the highest concentration tested was used for suprainhibitory exposure experiments of that antibiotic.

Following the addition of antibiotic, the cultures were incubated with shaking for an additional 15 min to an OD\textsubscript{600} of 0.5 before removing samples for RNA isolation. Cultures were incubated for an additional 6–8 h before plasmid isolation. For ciprofloxacin- and ticarcilone-treated cultures, plate counts were performed to determine the viability of the culture after drug treatment, due to a slight decrease in the OD\textsubscript{600} observed for these treatments. This ensured that the number of cells used to extract the plasmid DNA was equivalent to that of the other drug-treated cultures.

### Plasmid isolation and Southern blot analysis

Plasmid isolation and Southern blot analysis was performed as previously described.\textsuperscript{18}

### RNA isolation and RT–PCR

RNA from each strain was isolated from mid-logarithmic-phase cultures using TRIzol Max (Invitrogen) as previously described.\textsuperscript{15} Contaminating DNA was removed by treating 8 μg of RNA with 16 U of RQ1 RNase-Free DNase (Promega, Madison, WI, USA) for 2.5 h at 37°C. Real-time RT–PCR was performed as previously described using the QuantiTect SYBR Green RT–PCR Kit.\textsuperscript{15} Expression of the genus-specific single-copy \textit{ampG} gene for isolates of Enterobacteriaceae or the \textit{rpsL} gene for \textit{P. aeruginosa} was used to normalize the expression data for the Tn4401 genes of interest (Table S1, available as Supplementary data at JAC Online). Expression studies were performed in triplicate (coefficient of variation ≤10%) and the average Ct for each transcript was calculated. Relative expression data were determined using the calculation \textit{RQ} = 2^{ΔΔCT}.\textsuperscript{19}

### Materials and methods

#### Bacterial strains

Five previously characterized KPC-producing clinical isolates were evaluated: \textit{E. coli} 233, \textit{K. pneumoniae} isolates UMM3 and HUH40, Enterobacter sp. isolate 01MGH049 and \textit{P. aeruginosa} PSS.\textsuperscript{15} \textit{E. coli} ATCC 25922, \textit{P. aeruginosa} ATCC 27853 and \textit{Staphylococcus aureus} ATCC 29213 were used as quality control strains for susceptibility testing.
exception of tigecycline, whose MICs were interpreted according to FDA-established breakpoints for Enterobacteriaceae (susceptible if ≤2 mg/L). The MICs of imipenem, meropenem, ertapenem, ceftazidime and cefepime have been previously reported for these isolates.

The MICs of piperacillin/tazobactam, ceftazidime, imipenem, meropenem, ertapenem and doripenem for any five clinical isolates indicated resistance. The MICs of ceftazidime, gentamicin and ciprofloxacin were also at or above the resistance breakpoint for four out of five clinical isolates; susceptible MICs were observed for these drugs for isolate UMM3. All four Enterobacteriaceae isolates demonstrated susceptibility to tigecycline. The MICs of azithromycin ranged from 16 to 256 mg/L for these isolates.

**Localization of the bla<sub>KPC</sub> gene in five KPC-producing isolates**

Plasmid profiles and bla<sub>KPC</sub> Southern analysis of all five isolates are shown in Figure 1(a and b). Multiple plasmids ranging from ~8 to >200 kb were observed for the Enterobacteriaceae. K. pneumoniae UMM3 possessed two large molecular weight plasmids of ~55 and ~165 kb in size, with the bla<sub>KPC</sub> gene located on the ~55 kb plasmid. K. pneumoniae HUH40 had four plasmids, ranging from ~20 to 95 kb, and the bla<sub>KPC</sub> gene was located on both the ~55 and ~95 kb plasmids. E. coli 233 had three plasmids of ~120, ~95 and ~30 kb in size. DNA isolation from E. coli 233 was difficult due to the strain’s production of a DNA nuclease that consistently resulted in different fragmentation patterns during DNA isolation. However, the ~30 kb plasmid consistently hybridized with the bla<sub>KPC</sub> probe. Enterobacter sp. isolate 01MGH049 had two plasmids that were ~70 and ~10 kb in size, but only the ~70 kb plasmid hybridized with the KPC probe. P. aeruginosa PS5 had one ~55 kb plasmid, which also hybridized with the KPC probe. tnpA and tnpR were also located on the same plasmids as bla<sub>KPC</sub> in each isolate evaluated (data not shown).

**Evaluation of bla<sub>KPC</sub> mobilization upon subinhibitory antibiotic challenge**

Subinhibitory concentrations of drugs were used to simulate the effect suboptimal drug concentrations may have within an infected patient (Table 2). However, upon exposure to subinhibitory levels of the selected antibiotics, no changes in the hybridization patterns or binding intensity for the bla<sub>KPC</sub> probe were observed for any of the organisms evaluated (Figures 2 and 3, data not shown for P. aeruginosa PS5). As a control, hybridization patterns using the tnpR and tnpA probes from all five bacterial isolates treated with the carbapenems were also unchanged (data not shown).

An increase in transposition events would most likely require an increase in the production of the transposase and resolvase associated with Tn<sub>4401</sub>. To confirm the lack of mobility of Tn<sub>4401</sub> in the presence of antibiotic observed using Southern analysis, we tested the ability of imipenem, meropenem or ertapenem to increase the RNA expression of these genes. There was no change in the level of transposase or resolvase RNA upon carbapenem treatment compared with the untreated controls. Therefore, these data support the lack of transposition observed using Southern analysis described above. They also support the observation that antibiotic treatment at suboptimal concentrations of the tested drugs under the experimental parameters tested did not stimulate the transposition of Tn<sub>4401</sub>

To ensure that the cell number used for each drug treatment did not skew the results, optical densities (OD<sub>600</sub>) were measured and viability counts of cultures treated with ciprofloxacin and tigecycline were evaluated, as a slight decrease in the OD<sub>600</sub> was observed. A decrease of no more than 1 log (data not shown) for four of the five strains was observed in the presence of ciprofloxacin or tigecycline. This log decrease, however, did not influence the intensity of probe binding for those drug treatments when compared with other drug treatments that did not influence the cell number after exposure (Figures 2 and 3).

**bla<sub>KPC</sub> RNA expression after exposure to antibiotics**

Southern analysis did not indicate an increase in bla<sub>KPC</sub> mobility when isolates were exposed to subinhibitory levels of antibiotics. However, it was possible that these antibiotic drug classes could stimulate the expression of bla<sub>KPC</sub>. Therefore, bla<sub>KPC</sub> gene expression was evaluated using 11 different antibiotics from five different drug classes at concentrations that were subinhibitory, at the MIC or suprainhibitory for the organisms (Table 2). A summary of the RNA data is presented in Table 3.
Figure 1. Plasmid profiles and bla\textsubscript{KPC} Southern analysis in five KPC-producing organisms. (a) Electrophoretic profiles of plasmids in strains \textit{K. pneumoniae UMM3}, \textit{K. pneumoniae HUH40}, \textit{E. coli 233}, \textit{Enterobacter sp. 01MGH049} and \textit{P. aeruginosa} PS5 and a BAC Tracker Supercoil Ladder. (b) Hybridization with a \textit{bla}\textsubscript{KPC-2}-specific probe. Chr, chromosomal DNA.

Table 2. \(\beta\)-Lactam concentrations used in RNA expression analyses

<table>
<thead>
<tr>
<th>Strain</th>
<th>Drug amount\textsuperscript{b}</th>
<th>IPM</th>
<th>MEM</th>
<th>ETP</th>
<th>DOR</th>
<th>TZP\textsuperscript{c}</th>
<th>CAZ</th>
<th>FEP</th>
<th>CIP</th>
<th>GEN</th>
<th>TGC</th>
<th>AZM\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{E. coli} 233</td>
<td>sub</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>64</td>
<td>128</td>
<td>8</td>
<td>16</td>
<td>4</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>16</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>NA</td>
<td>512</td>
<td>32</td>
<td>64</td>
<td>16</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>supra</td>
<td>128</td>
<td>32</td>
<td>32</td>
<td>16</td>
<td>NA</td>
<td>1024</td>
<td>128</td>
<td>256</td>
<td>64</td>
<td>8</td>
<td>64</td>
</tr>
<tr>
<td>\textit{K. pneumoniae} UMM3</td>
<td>sub</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>64</td>
<td>4</td>
<td>1</td>
<td>0.12</td>
<td>1</td>
<td>0.12</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>4</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>NA</td>
<td>16</td>
<td>4</td>
<td>0.5</td>
<td>4</td>
<td>0.5</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>supra</td>
<td>16</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>NA</td>
<td>64</td>
<td>16</td>
<td>2</td>
<td>16</td>
<td>2</td>
<td>128</td>
</tr>
<tr>
<td>\textit{K. pneumoniae} HUH40</td>
<td>sub</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>64</td>
<td>64</td>
<td>8</td>
<td>32</td>
<td>32</td>
<td>0.07</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>8</td>
<td>NA</td>
<td>256</td>
<td>32</td>
<td>128</td>
<td>128</td>
<td>0.25</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>supra</td>
<td>32</td>
<td>32</td>
<td>64</td>
<td>32</td>
<td>NA</td>
<td>1024</td>
<td>128</td>
<td>512</td>
<td>512</td>
<td>1</td>
<td>256</td>
</tr>
<tr>
<td>\textit{Enterobacter sp. 01MGH049}</td>
<td>sub</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>64</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>0.25</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>NA</td>
<td>32</td>
<td>16</td>
<td>4</td>
<td>16</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>supra</td>
<td>16</td>
<td>16</td>
<td>32</td>
<td>16</td>
<td>NA</td>
<td>128</td>
<td>64</td>
<td>16</td>
<td>64</td>
<td>4</td>
<td>128</td>
</tr>
<tr>
<td>\textit{P. aeruginosa} PS5</td>
<td>sub</td>
<td>128</td>
<td>128</td>
<td>256</td>
<td>128</td>
<td>64</td>
<td>16</td>
<td>128</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>256</td>
<td>256</td>
<td>512</td>
<td>256</td>
<td>64</td>
<td>64</td>
<td>256</td>
<td>32</td>
<td>8</td>
<td>16</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>supra\textsuperscript{e}</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

IPM, imipenem; MEM, meropenem; ETP, ertapenem; DOR, doripenem; TZP, piperacillin/tazobactam; CAZ, ceftazidime; FEP, cefepime; TGC, tigecycline; GEN, gentamicin; CIP, ciprofloxacin; AZM, azithromycin.

\textsuperscript{a}Units for drug amounts are mg/L.

\textsuperscript{b}Subinhibitory (sub), MIC and suprainhibitory (supra) concentrations based on MICs for the organisms listed in Table 1.

\textsuperscript{c}MICs for all strains were >128/4; MIC and suprainhibitory experiments were not performed.

\textsuperscript{d}0.8 mg/L is considered to be a trough level of AZM in epithelial lining fluids and was used as the subinhibitory level for AZM.

\textsuperscript{e}Experiments with suprainhibitory levels of antibiotics were not performed for this isolate.
Although changes in the expression of \( \text{bla}_{\text{KPC}} \) for each isolate tested were not dramatic, there were modifications in \( \text{bla}_{\text{KPC}} \) expression upon exposure to some antibiotics. Evaluation of the data showed that changes in \( \text{bla}_{\text{KPC}} \) expression were strain, genus and antibiotic specific. For example, when both strains of \( K. \) pneumoniae were compared, no change in \( \text{bla}_{\text{KPC}} \) expression was observed for doripenem at any concentration tested for strain UMM3, but up to a 2-fold increase was observed for strain HUH40 at MIC and subinhibitory concentrations. Other differences between these two strains were observed for the subinhibitory concentrations of imipenem, meropenem, ertapenem, doripenem; TZP, piperacillin/tazobactam; CAZ, ceftazidime; FEP, cefepime; CIP, ciprofloxacin; GEN, gentamicin; TGC, tigecycline; AZM, azithromycin; Chr, chromosomal DNA.

Other interesting differences observed with the \( \text{Enterobacter} \) strain were increases in expression when treated with subinhibitory concentrations of imipenem, meropenem, ertapenem, piperacillin/tazobactam and ceftazidime. \( P. \) aeruginosa strain PS5 was difficult to evaluate given the high MICs of most of the drugs tested. However, treatment with suboptimal concentrations of meropenem, doripenem and cefepime all resulted in a 2- to 3-fold decrease in \( \text{bla}_{\text{KPC}} \) expression. Gentamicin also resulted in 2.5- to 3.5-fold decreases in \( \text{bla}_{\text{KPC}} \) expression when using concentrations equal to or exceeding the MIC. With respect to doripenem, the \( E. \) coli, \( \text{Enterobacter} \) sp. and \( P. \) aeruginosa isolates had similar responses in \( \text{bla}_{\text{KPC}} \) expression when treated with subinhibitory concentrations.

**Discussion**

Severe illness and prior exposure to antibiotics, particularly third-generation cephalosporins and fluoroquinolones, have been identified as risk factors for infection with KPC-producing organisms, although whether it is exposure to specific antibiotics or a cumulative effect of multiple exposures is unknown. It has been established that exposure to antibiotics can result in the selection of resistant mutants, changes in bacterial resistance patterns and the acquisition of resistance-conferring plasmids. However, the role of \( \text{KPC} \) in the emergence of resistance is still under investigation.
metabolism and/or colonization of the infected patient. All of these outcomes with antibiotic exposure can be considered unwanted ‘collateral damage’ during the treatment of the patient.21–23 Non-antibiotic drug treatments required to treat the patient’s underlying condition(s) could also act as a stimulus for modifications in antibiotic drug treatments required to treat the patient’s underlying condition(s). This variation may represent differences in metabolic processes or other compensatory mechanisms that may have been selected for by the organism during drug treatment of the infected patient.

This study looked beyond the relationship between bla_{KPC} expression and its influence on β-lactam susceptibilities. We hypothesized that unintended consequences of antibiotic treatment could include changes in bla_{KPC} gene expression and/or the mobility of its associated mobile genetic element, Tn4401. Stimulation of transposition through the exposure of cells to antibiotics is not a new concept. The role antibiotics play in the transposition of mobile genetic elements has been explored in Gram-positive organisms. Subinhibitory concentrations of erythromycin have been shown to induce the transposition of Tn1721 in Streptococcus faecalis.24 More recently, ciprofloxacin and vancomycin were shown to increase the frequency of IS256 transposition in S. aureus.25,26 Subinhibitory concentrations of antibiotic have been identified as contributors to the emergence of a resistance phenotype.27,28 Therefore, we reasoned that increased transposition and/or modifications in bla_{KPC} gene expression may take place when antibiotic treatment in the patient is suboptimal. However, using Southern analysis, exposure to subinhibitory concentrations of the drugs tested did not influence the transposition of Tn4401 or duplication of bla_{KPC} in any of the strains analysed. However, changes in bla_{KPC} gene expression were noted after treatment of some antibiotic drug classes. Although the fold differences in bla_{KPC} gene expression were subtle, these differences should not be ignored. Of particular interest, some non-β-lactam drugs, such as tigecycline, had a greater influence on bla_{KPC} gene expression than β-lactam drugs, but strain and genera variation was also observed. This variation may represent differences in metabolic processes or other compensatory mechanisms that may have been selected for by the organism during drug treatment of the infected patient.

Experimentation evaluating mechanisms required for KPC production within the cell has been minimal, with few answers revealed.7,8,26 Every experimental approach has limitations and this study was no exception. One limitation of the present study was the small number of clinical isolates used. In addition, we were limited on the number of drug concentrations we could evaluate using Southern blot analysis and gene expression assays. It is possible that evaluating gene copy number changes using real-time PCR would be more sensitive and enable the analysis of low-frequency events that Southern analysis would not be able to detect. The evaluation of additional isolates based on the bla_{KPC} gene expression data is warranted to establish a connection between exposure to specific antibiotics and modifications in bla_{KPC} gene expression.
Acknowledgements
This work was previously presented in part at the Fifty-first Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, USA, 2011 (Abstract C1-1218).

We thank Ellen Smith Maland for her contributions in performing the antimicrobial susceptibility assays.

Funding
This research was conducted with support from the Investigator Sponsored Study Program of Merck & Co.

Transparency declarations
None to declare.

Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References