Are susceptibility tests enough, or should laboratories still seek ESBLs and carbapenemases directly?

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Recent EUCAST advice asserts that, with low breakpoints, susceptibility results for cephalosporins and carbapenems can be reported ‘as found’, even for strains with extended-spectrum β-lactamases (ESBLs) and carbapenemases. The CLSI has similar advice, but with higher ceftazidime and cefepime breakpoints than those of EUCAST. Pharmacodynamic and animal data are used to support these views, along with some analysis of clinical case series. We contend that such advice is misguided on three counts. First, whilst there are cases on record where cephalosporins and carbapenems have proved effective against infections due to low-MIC ESBL producers and low-MIC carbapenemase producers, respectively, there are similar numbers of cases where such therapy has failed. Second, routine susceptibility testing is less precise than in research analyses, meaning that ESBL and carbapenemase producers with ‘real’ MICs of 1–8 mg/L will oscillate between susceptibility categories according to who tests them and how. Third, although EUCAST continues to advocate ESBL and carbapenemase detection for epidemiological purposes, the likely consequence of not seeking these enzymes for treatment purposes is that some laboratories will not seek them at all, leading to a loss of critical infection control information. In short, it is prudent to continue to seek ESBLs and carbapenemases directly and, where they are found, generally to avoid substrate drugs as therapy.

Keywords: ceftazidime, CTX-M β-lactamases, KPC β-lactamases, European Committee on Antimicrobial Susceptibility Testing, EUCAST, CLSI, Clinical Laboratory Standards Institute

Introduction

For the past decade both the BSAC1 and the CLSI2 have advised that extended-spectrum β-lactamases (ESBLs) should be sought in Enterobacteriaceae with reduced susceptibility to cephalosporins, and that ESBL-positive isolates should be reported as resistant to all cephalosporins, irrespective of the susceptibility test result. EUCAST has similarly advised that ‘cephalosporin-susceptible’ ESBL producers should be reported as ‘intermediate’ and ‘cephalosporin-intermediate’ ESBL producers as ‘resistant’.3 Such advice was predicated on many reports of failures where cephalosporins were used to treat infections due to ESBL producers.

Recently, both EUCAST4 and CLSI5 have revised their view and argue that, with low cephalosporin breakpoints that both organizations have now adopted (Table 1), it is unnecessary to edit susceptibility categories if an ESBL is found. EUCAST made this advice explicit in a recent publication4 from their Expert Rules Working Group, which states ‘Cephalosporin breakpoints for Enterobacteriaceae will detect clinically-important resistance mechanisms (including ESBLs). Some strains that produce β-lactamases are susceptible or intermediate to third- or fourth-generation cephalosporins with these breakpoints, and should be reported as found.’ Analogous advice is given in the context of carbapenemases against carbapenemase producers: that the susceptibility test result should guide treatment, not any detection of carbapenemase. CLSI advice is similar for both enzyme groups,5 though their breakpoints for cefepime (especially) and ceftazidime are notably higher than those of EUCAST (Table 1).

Rationale and consequences of the recommendations

The rationale for these recommendations is 3-fold. First, pharmacodynamic modelling suggests that, once cephalosporin...
breakpoints are reduced to 1–4 mg/L, it is possible to obtain serum concentrations of cephalosporins above MIC for the required 40%–50% of the dosage interval, at least with maximum cephalosporin or carbapenem dosages. Second, animal experiments suggest that infections caused by ESBL producers are neither more nor less tractable to cephalosporins than those caused by strains with similar MICs but lacking ESBLs and that—in either case—the MIC is a better predictor of outcome than mechanism-based categorization. Third, many of the well-reported clinical failures due to ‘cephalosporin-susceptible’ ESBL producers involved infections caused by strains with MICs around the previous CLSI breakpoints of 8 or 16 mg/L, not those with MICs around the lower EUCAST (and new CLSI) breakpoints. As illustrated in Tables 2 and 3—which show prospective and literature analyses by Paterson et al.—the probability of success with a cephalosporin against an infection due to an ESBL producer increases as the MIC reduces (Table 2). Similarly, in the case of carbapenemases versus carbapenemase producers, Daikos et al. found that bacteraemias caused by Klebsiella pneumoniae with VIM carbapenemases had 18.9% mortality if the MIC was ≤ 4 mg/L and that this was insignificantly different from the 15.8% mortality seen for bacteraemias caused by carbapenemase-negative klebsiellas, whereas mortality rose to 42.9% in bacteraemias caused by VIM-positive klebsiellas with MICs >4 mg/L.

Table 2. Outcome of cephalosporin therapy for bacteraemias caused by K. pneumoniae with ESBLs: prospective study by Paterson et al.

<table>
<thead>
<tr>
<th>Sex/age (years)/underlying disease</th>
<th>Cephalosporin as therapy</th>
<th>MIC (mg/L)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/72/intracerebral haematoma</td>
<td>ceftazidime</td>
<td>16</td>
<td>fail</td>
</tr>
<tr>
<td>M/76/hypertension</td>
<td>ceftriaxone</td>
<td>16</td>
<td>fail</td>
</tr>
<tr>
<td>M/58/cirrhosis</td>
<td>ceftriaxone</td>
<td>12 (=16)</td>
<td>died within 48 h</td>
</tr>
<tr>
<td>M/39/abdominal surgery</td>
<td>ceftriaxone</td>
<td>8</td>
<td>died within 48 h</td>
</tr>
<tr>
<td>F/35/colectomy</td>
<td>cefotaxime</td>
<td>4</td>
<td>fail</td>
</tr>
<tr>
<td>M/48/abdominal surgery</td>
<td>cefepime</td>
<td>2</td>
<td>fail</td>
</tr>
<tr>
<td>M/49/cirrhosis</td>
<td>cefotaxime</td>
<td>1.5 (=2)</td>
<td>cure</td>
</tr>
<tr>
<td>F/25/neurosurgery</td>
<td>cefepime</td>
<td>1.5 (=2)</td>
<td>cure</td>
</tr>
<tr>
<td>M/25/multiple trauma</td>
<td>cefepime</td>
<td>0.5</td>
<td>fail, died septic</td>
</tr>
<tr>
<td>F/25/bone marrow transplant</td>
<td>ceftazidime</td>
<td>0.5</td>
<td>cure</td>
</tr>
</tbody>
</table>

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M, male; F, female.
These arguments have merit and the presence of a weakly active β-lactamase is certainly not an absolute predictor of failure. For example, MICs of imipenem for typical Pseudomonas aeruginosa are 1–4 mg/L, but fall to 0.12–0.25 mg/L for mutants that lack chromosomal AmpC activity, proving that the enzyme ordinarily provides some protection. Nevertheless, imipenem is widely effective in P. aeruginosa infections and, where failure occurs, it is usually associated with mutant selection, not lability to endogenous β-lactamase activity. Similarly, first-generation cephalosporins are incompletely stable to classical TEM β-lactamases, but remain widely active in urinary infections caused by strains with these enzymes. Moreover, there is a hazier border than is sometimes acknowledged between ‘extended’ and classical-spectrum β-lactamases. TEM-12, for example, only raises cefotaxime and ceftriaxone MICs by one or two doubling dilutions, from 0.03 to 0.06–0.12 mg/L, though it has a greater effect on ceftazidime. It is very doubtful whether isolates with this feeble ‘ESBL’ should automatically be categorized as resistant to cefotaxime and ceftriaxone.

TEM-12, though, is uncommon and most circulating ESBLs are now CTX-M types. These confer clear resistance to cefotaxime

Table 3. Outcome of cephalosporin therapy versus MIC for various infections due to Enterobacteriaceae with ESBLs: literature review by Paterson et al. 8

<table>
<thead>
<tr>
<th>Sex/age (years)/underlying disease</th>
<th>Type of infection</th>
<th>Bacteraemia</th>
<th>Organism</th>
<th>Antibiotic</th>
<th>MIC (mg/L)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/72/oesophageal surgery</td>
<td>mediastinitis</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefepime+</td>
<td>16</td>
<td>failure</td>
</tr>
<tr>
<td>F/58/biliary surgery</td>
<td>nosocomial</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefepime+</td>
<td>8</td>
<td>failure</td>
</tr>
<tr>
<td>M/68/colon cancer surgery</td>
<td>nosocomal UTI</td>
<td>+</td>
<td>E. coli</td>
<td>cefepime+</td>
<td>8</td>
<td>failure</td>
</tr>
<tr>
<td>Infant/low birth weight</td>
<td>meningitis</td>
<td>+</td>
<td>K. oxytoca</td>
<td>cefotaxime</td>
<td>8</td>
<td>failure</td>
</tr>
<tr>
<td>Infant/omphalocoe repair</td>
<td>nosocomial</td>
<td>+</td>
<td>E. coli</td>
<td>cefotaxime</td>
<td>8</td>
<td>failure</td>
</tr>
<tr>
<td>M/75</td>
<td>UTI</td>
<td>+</td>
<td>E. coli</td>
<td>cefotaxime+</td>
<td>8</td>
<td>failure</td>
</tr>
<tr>
<td>F/48/kidney–pancreas transplant</td>
<td>UTI</td>
<td>+</td>
<td>E. coli</td>
<td>cefotaxime</td>
<td>4</td>
<td>cure</td>
</tr>
<tr>
<td>F/82/from nursing home</td>
<td>UTI</td>
<td>+</td>
<td>E. coli</td>
<td>cefotaxime</td>
<td>4</td>
<td>failure</td>
</tr>
<tr>
<td>F/14/Ewing’s sarcoma</td>
<td>CVL related</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime+aztreonam</td>
<td>1</td>
<td>cure</td>
</tr>
<tr>
<td>F/21/multiorgan failure</td>
<td>primary bacteraemia</td>
<td>+</td>
<td>E. coli</td>
<td>cefotaxime</td>
<td>1</td>
<td>failure</td>
</tr>
<tr>
<td>NS</td>
<td>meningitis</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime</td>
<td>&lt;1</td>
<td>failure; died</td>
</tr>
<tr>
<td>NS</td>
<td>nosocomial</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime</td>
<td>&lt;1</td>
<td>cure</td>
</tr>
<tr>
<td>Unknown/pancreatitis</td>
<td>peritonitis</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>ceftriaxone</td>
<td>&lt;1</td>
<td>cure</td>
</tr>
<tr>
<td>M/14/multiple bowel fistulae</td>
<td>CVL infection</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime</td>
<td>0.75 (=1) failure</td>
<td>failure</td>
</tr>
<tr>
<td>F/61/liver transplant</td>
<td>UTI</td>
<td>+</td>
<td>E. coli</td>
<td>cefotaxime</td>
<td>0.5</td>
<td>cure</td>
</tr>
<tr>
<td>M/45/liver transplant</td>
<td>primary</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime</td>
<td>0.5</td>
<td>partial response</td>
</tr>
<tr>
<td>NS</td>
<td>meningitis</td>
<td>+</td>
<td>E. coli</td>
<td>cefotaxime</td>
<td>0.5–1</td>
<td>cure</td>
</tr>
<tr>
<td>M/44/multiple trauma</td>
<td>peritonitis</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime+</td>
<td>0.5–1</td>
<td>cure</td>
</tr>
<tr>
<td>M/53/liver transplant</td>
<td>NS</td>
<td>+</td>
<td>E. coli</td>
<td>cefotaxime+</td>
<td>&lt;0.12</td>
<td>cure</td>
</tr>
<tr>
<td>Child/leukaemia</td>
<td>NS</td>
<td>+</td>
<td>E. coli</td>
<td>cefotaxime+</td>
<td>&lt;8</td>
<td>died within 24 h</td>
</tr>
<tr>
<td>NS</td>
<td>UTI</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime</td>
<td>0.5–4</td>
<td>failure</td>
</tr>
<tr>
<td>NS</td>
<td>UTI</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime</td>
<td>0.5–4</td>
<td>cure</td>
</tr>
<tr>
<td>NS</td>
<td>empyema</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime</td>
<td>0.5–4</td>
<td>failure; relapse</td>
</tr>
<tr>
<td>NS</td>
<td>empyema</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime</td>
<td>0.5–4</td>
<td>failure; relapse</td>
</tr>
<tr>
<td>NS</td>
<td>mediastinitis</td>
<td>+</td>
<td>K. pneumoniae</td>
<td>cefotaxime</td>
<td>0.5–4</td>
<td>failure; relapse</td>
</tr>
</tbody>
</table>

This table is simplified from the original publication, 8 to which readers are referred for fuller details and source references, and is reproduced with the permission of the original publisher.

M, male; F, female; NS, not stated; UTI, urinary tract infection; CVL, central venous line.

<sup>a</sup>+ aminoglycoside co-administered.
and ceftriaxone, but have smaller effects on ceftazidime. Isolates with CTX-M-15—now the commonest ESBL in much of the world—usually ‘have’ ceftaxime and ceftriaxone MICs >128 mg/L, cefepime MICs around 64 mg/L and ceftazidime MICs around 16–32 mg/L, equating to unequivocal resistance to all these analogues. An issue does, however, arise with other common CTX-M ESBLs, notably the CTX-M-9 and -14 enzymes, which are the commonest ESBLs in East Asia and Spain, and second commonest after CTX-M-15 elsewhere in Europe. These confer clear resistance to ceftaxime, ceftriaxone and (usually) ceftazidime, but MICs of ceftazidime cluster around 1–4 mg/L (Figure 1), straddling EUCAST’s susceptible ≤1 mg/L-resistant >4 mg/L breakpoints and within CLSI’s susceptible range for this drug (Table 1). In Israel and Argentina the prevalent ESBL is CTX-M-2, which likewise confers greater resistance to ceftaxime and ceftriaxone than to ceftazidime. One of us (Y. K.) found that application of the new CLSI criteria led to 64% of 205 consecutive ESBL-positive Escherichia coli (as identified by Vitek 2; 87% of them with CTX-M enzymes) from northern Israel being categorized as ceftazidime susceptible, along with all of 21 ESBL-positive Proteus spp, and 8.6% of 85 ESBL-positive Klebsiella spp. The proportions found ‘susceptible’ to ceftaxime and ceftriaxone were smaller, at 12.2%, 5% and 2.4% for E. coli, Proteus spp. and Klebsiella spp., respectively. Even CTX-M-15 can present difficulties. Exceptionally, the UK has a prevalent variant of E. coli ST131 (‘strain A’) that expresses CTX-M-15 enzyme weakly, again with ceftazidime MICs spread around 1–4 mg/L. Lastly, in this context, ‘ceftazidimase’ ESBLs (e.g. TEM-10 and -26) also continue to circulate, albeit at lower frequency than CTX-M types, conferring the mirror image antibiogram, with ceftazidime MICs ≥128 mg/L and ceftaxime and ceftriaxone MICs of 0.5–4 mg/L, again straddling the EUCAST and CLSI breakpoints. During the 1990s, strains with these ceftazidimases caused major outbreaks in France and the USA, and may do so again.

The situation with carbapenemases is more complicated, since these require a degree of impermeability to engender clear carbapenem resistance in Enterobacteriaceae. Consequently, and irrespective of carbapenemase type, it is common to see producers with imipenem and meropenem MICs as low as 1–4 mg/L, again straddling the breakpoints detailed in Table 1. Ertapenem resistance is usually less equivocal, partly because the breakpoints are lower and partly because the drug is more vulnerable.

In all these cases the EUCAST and CLSI recommendations will lead to some enzyme producers being reported as susceptible, begging the question, ‘Is this appropriate?’

### Are the recommendations prudent?

We assert that there are three major weaknesses in these recommendations for therapy to be guided by breakpoints alone and not also by detection of ESBLs and carbapenemases.

First, the evidence that infections caused by ESBL and carbapenemase producers with low MICs respond to cephalosporins and carbapenems, respectively, is scanty and unconvincing. Second, routine susceptibility tests are not (and probably cannot be) done with the necessary precision to stratify isolates as susceptible/intermediate/resistant across the now critical range of 1–4 mg/L. Third, abandoning the detection and reporting of ESBLs and carbapenemases to guide treatment will lead to these investigations being abandoned for epidemiological purposes, where all agree that they remain vital.

### Do low MICs reliably predict good outcomes?

The evidence that MICs of 1–4 mg/L predict clinical success by cephalosporins against ESBL producers is underwhelming. Among the five bacteraemias caused by ESBL producers with the lowest MICs in Table 2 there were two failures with cephalosporins and three successes; among the two caused by strains with MICs of 0.5 mg/L (i.e. below all current breakpoints) there was one success and one failure. In Table 3 there were numerous failures in cases where the cephalosporin MIC was cited, imprecisely, as 0.5–4 mg/L. In short, whilst the probability of success increases as the MIC declines, a low MIC for an ESBL producer is not a clear predictor of success.

If, next, we turn to the central issue of ceftazidime and CTX-M ESBLs, the data are contradictory. Bin et al. in China described successful ceftazidime therapy in all of seven cases of bacteremia due to K. pneumoniae with CTX-M ESBLs and MICs in the range 0.5–8 mg/L. On the other hand, Ho et al. described seven cases where ceftazidime was used in bacteraemias arising from various infections due to ESBL-producing E. coli giving zones ≥18 mm to 30 μg ceftazidime discs in CLSI-type tests. Among these seven, four failed on ceftazidime therapy, with three deaths. Re-examination of the isolates responsible revealed that six had CTX-M-9 or -14 ESBLs and that, for these, the ceftazidime MICs were 0.06–1 mg/L, as determined by Etest (Table 4). The failures among these were in the three cases where the ceftazidime MIC was 0.75–1 mg/L, whereas success was achieved in three cases where the MIC for the isolate was 0.06–0.5 mg/L. Thus, whilst the data support the view that outcome relates to MIC, they also suggest that the ceftazidime breakpoint should be lower than now advocated by EUCAST or, especially, CLSI. They do not suggest that ceftazidime can be used with confidence against low-MIC strains with CTX-M ESBLs.

The same points apply with respect to carbapenems versus carbapenemase producers, though data are scantier. Whilst the outcome analysis by Daikos et al. for bacteraemias due to K. pneumoniae with VIM carbapenemases suggested a division.
around 4 mg/L (above), the data of Weisenberg et al.\textsuperscript{25} (Table 5) for a variety of infections caused by strains with KPC carbapenemases—internationally more prevalent than VIM types—indicate no such stratification, irrespective of whether MICs determined by Vitek 2 or the frequently-very-different values by Etest were taken as the datum. Once again, it seems more prudent to seek carbapenemases directly in suspect isolates and, if these enzymes are found, to use carbapenems with the utmost caution and certainly not as monotherapy.

**Are routine tests adequately precise?**

The view that therapy can be guided by susceptibility data, without ESBL detection, presumes that precise MICs are routinely available, as they are in supportive animal models of ESBL infection\textsuperscript{7} and in the post hoc analyses summarized in Tables 2–5.\textsuperscript{8,25} This assumption fails to understand the reality of routine practice.

In general, the diagnostic laboratory has approximate MICs from an automated system or has disc zones. Gradient tests are more precise, but are only performed on a minority of ‘difficult’ isolates, usually after some delay. Performance is graded against quality control strains, with test results counted as acceptable if the MICs for these controls fall within a 4- or 8-fold (i.e. two or three doubling dilutions) range.\textsuperscript{1–3} Old data, from when ceftazidime discs were being validated,\textsuperscript{26} show poor discrimination among isolates with MICs of 1–4 mg/L—these were from a single laboratory, thus excluding site-to-site variation. Insight into what this latter variation means when reporting on isolates with cephalosporin MICs in the critical, breakpoint-straddling range of 1–4 mg/L (versus 0.06–0.25 mg/L for fully susceptible isolates) is given by the experience

### Table 4. Outcome in six patients treated with ceftazidime for bacteraemias caused by ‘ceftazidime-susceptible’ E. coli with CTX-M ESBLs

<table>
<thead>
<tr>
<th>Sex/age (years)</th>
<th>Source of bacteraemia</th>
<th>Clinical response to ceftazidime</th>
<th>Etest MIC (mg/L)</th>
<th>Inhibition zones to 30 μg discs (mm)</th>
<th>β-Lactamase content&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Etest MIC (mg/L)</th>
<th>Inhibition zones to 30 μg discs (mm)</th>
<th>β-Lactamase content&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/70</td>
<td>spontaneous bacterial peritonitis</td>
<td>no</td>
<td>1</td>
<td>14</td>
<td>27/28</td>
<td>CTX-M-14</td>
<td>clinical and microbiologic failure</td>
<td></td>
</tr>
<tr>
<td>F/72</td>
<td>UTI</td>
<td>no</td>
<td>1</td>
<td>9</td>
<td>27/27</td>
<td>CTX-M-14, TEM-1</td>
<td>microbiologic and clinical success</td>
<td></td>
</tr>
<tr>
<td>F/69</td>
<td>UTI</td>
<td>no</td>
<td>0.75 (=1)</td>
<td>17</td>
<td>31/32</td>
<td>CTX-M-9, TEM-1</td>
<td>microbiologic and clinical success</td>
<td></td>
</tr>
<tr>
<td>F/83</td>
<td>UTI</td>
<td>yes</td>
<td>0.06</td>
<td>18</td>
<td>31/32</td>
<td>CTX-M-14, TEM-1</td>
<td>clinical success</td>
<td></td>
</tr>
<tr>
<td>M/67</td>
<td>primary bacteraemia</td>
<td>yes</td>
<td>0.5</td>
<td>16</td>
<td>28/28</td>
<td>CTX-M-14, TEM-1</td>
<td>microbiologic and clinical success</td>
<td></td>
</tr>
<tr>
<td>F/83</td>
<td>UTI</td>
<td>yes</td>
<td>0.25</td>
<td>15</td>
<td>29/28</td>
<td>CTX-M-14, TEM-1</td>
<td>clinical and microbiologic failure</td>
<td></td>
</tr>
</tbody>
</table>

This table is adapted from Ho et al.,\textsuperscript{24} with insertion of new MIC and β-lactamase data; the previously published material is reproduced with the permission of the original publisher.

M, male; F, female; UTI, urinary tract infection.

<sup>a</sup>Results from two independent experiments.

<sup>b</sup>All isolates were tested for TEM, SHV and CTX-M classes of genes by PCR and sequencing.

### Table 5. Outcome of carbapenem therapy versus MIC for various infections due to K. pneumoniae with KPC carbapenemases

<table>
<thead>
<tr>
<th>Sex/age (years)/underlying disease</th>
<th>Infection site</th>
<th>MIC (mg/L)</th>
<th>Clinical response to ceftazidime</th>
<th>Etest MIC (mg/L)</th>
<th>Inhibition zones to 30 μg discs (mm)</th>
<th>β-Lactamase content&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/76/cerebral haemorrhage</td>
<td>tracheobronchitis</td>
<td>2</td>
<td>0.25</td>
<td>meropenem (7)</td>
<td>clinical and microbiologic failure</td>
<td></td>
</tr>
<tr>
<td>F/61/heart failure</td>
<td>pyelonephritis</td>
<td>2</td>
<td>&gt;32</td>
<td>imipenem (7)</td>
<td>microbiologic and clinical success</td>
<td></td>
</tr>
<tr>
<td>M/82</td>
<td>urosepsis (blood)</td>
<td>4</td>
<td>2</td>
<td>imipenem (14)</td>
<td>microbiologic and clinical success</td>
<td></td>
</tr>
<tr>
<td>M/92/dementia</td>
<td>pneumonia</td>
<td>4</td>
<td>2</td>
<td>imipenem (3)</td>
<td>clinical success</td>
<td></td>
</tr>
<tr>
<td>F/64/oesophageal cancer</td>
<td>tracheobronchitis</td>
<td>4</td>
<td>2</td>
<td>imipenem (12)</td>
<td>microbiologic failure</td>
<td></td>
</tr>
<tr>
<td>F/46/skin graft</td>
<td>bacteremia</td>
<td>4</td>
<td>8</td>
<td>imipenem (7)</td>
<td>microbiologic and clinical success</td>
<td></td>
</tr>
<tr>
<td>M/69/metastatic cancer</td>
<td>pneumonia</td>
<td>4</td>
<td>8</td>
<td>imipenem (6)</td>
<td>clinical failure/death</td>
<td></td>
</tr>
<tr>
<td>M/52/melanoma</td>
<td>UTI</td>
<td>4</td>
<td>12 (=16)</td>
<td>imipenem (14)</td>
<td>microbiologic failure</td>
<td></td>
</tr>
<tr>
<td>M/77/MRSA abscess</td>
<td>tracheobronchitis</td>
<td>4</td>
<td>≥32</td>
<td>imipenem (7)</td>
<td>microbiologic failure</td>
<td></td>
</tr>
<tr>
<td>F/60/pelvic infection</td>
<td>bacteremia</td>
<td>≥16</td>
<td>8</td>
<td>meropenem (10)</td>
<td>clinical and microbiologic failure</td>
<td></td>
</tr>
<tr>
<td>M/50/liver transplant</td>
<td>bacteremia</td>
<td>≥16</td>
<td>8</td>
<td>meropenem (7)</td>
<td>clinical and microbiologic success</td>
<td></td>
</tr>
</tbody>
</table>

This table is simplified from the original publication by Weisenberg et al.,\textsuperscript{25} to which readers are referred for fuller details, and is reproduced with the permission of the original publisher.

M, male; F, female; UTI, urinary tract infection.

<sup>a</sup>Results from two independent experiments.

<sup>b</sup>All isolates were tested for TEM, SHV and CTX-M classes of genes by PCR and sequencing.
of one of us (J. M. A., Table 6), who distributed *E. coli* NCTC 13352 with TEM-10 ceftazidime to four UK clinical laboratories, asking them each to perform disc tests by the BSAC method 10 times per drug. All the laboratories found the isolate resistant to ceftazidime, but, for cefotaxime, one laboratory found it susceptible in all tests, two found it intermediate in 9/10 or 10/10 tests, and one had a 6:4 split between susceptible and intermediate results. For cefepime, two laboratories consistently found the strain intermediate, one consistently resistant and one had a 6:4 split between resistant and intermediate results. If this isolate was from a septic patient, the scatter of results would not be reassuring, with the patient getting high-dose therapy in one place, but a normal dose in another. We suggest it would be far more prudent to recognize such a strain as an ESBL producer, based on direct testing, and to avoid cephalosporins.

These issues of susceptibility testing apply even more strongly for strains with carbapenemases, where MICs are notoriously variable. Frequent disagreements between MICs by Etest and Vitek 2 for *K. pneumoniae* with KPC carbapenemases are apparent from the data of Weisenberg et al. shown in Table 5. Similarly, Lat et al. found poor agreement between meropenem MICs by Vitek 2 and classical methods for strains with KPC carbapenemases. The same group who suggested the relationship between MIC and outcome for strains with VIM enzymes, previously noted the poor intermethod reproducibility of MICs for strains with precisely these VIM enzymes. Recent experience by the UK National External Quality Assessment Scheme (NEQAS) for Antimicrobial Susceptibility underscores the problem. Distribution 2906 on 24 October 2011 included *K. pneumoniae* 0555 as a representative of the global *K. pneumoniae* ST258/KPC clone. It had been provided to NEQAS by the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL), which had found imipenem and meropenem MICs, respectively, of 8 and 16 mg/L by BSAC agar dilution, counting as resistant based on both BSAC/EUCAST and CLSI criteria. Two independent laboratories then examined the isolate for NEQAS before distribution, using International Standards Organization (http://www.iso.org) microbroth dilution, and found MICs of 1 mg/L for imipenem (susceptible by BSAC/EUCAST and CLSI, Table 1) and 4 mg/L for meropenem (intermediate by BSAC/EUCAST, but resistant by CLSI); one also performed agar dilution by BSAC methodology and, in contrast to ARMRL, found an MIC of 0.5 mg/L for imipenem. The participants’ results were similarly variable and, to quote from NEQAS: ‘Most participants reported reduced susceptibility to imipenem (12.8% susceptible, 17.9% intermediate, 69.3% resistant) and to meropenem (7.6% susceptible, 9.3% intermediate, 83.1% resistant). MICs…(mostly from gradient or automated systems) were very variable, from 0.5– ≥32 mg/L with a mode of 8 mg/L for imipenem and 0.25– ≥64 mg/L with a mode of ≥16 mg/L for meropenem.’ Among laboratories using BSAC methodology, 38/56 reported the isolate as susceptible to imipenem and 10/117 to meropenem (Christine Walton and Derek Brown, UK NEQAS, personal communication).

This variability led to carbapenem results being excluded in scoring participating laboratories’ performance, which is concerning considering that the strain belonged to an internationally successful clone with a characteristic antibiogram (reduced carbapenem susceptibility, clavulanate-independent cephalosporin resistance and resistance to other drugs except polymyxin, tigecycline and gentamicin) and a notorious ability to spread among patients. Everyone’s diverse MIC or zone cannot be ‘right’ and we suggest that the reduced carbapenem susceptibility should prompt carbapenemase screening, with carbapenem monotherapy discouraged and infection control reinforced if this activity is found.

Lastly, and anecdotally, one of us (D. M. L.) spent much of the summer of 2010 showing journalists plates of Etests for

### Table 6. Disc tests by the BSAC method on *E. coli* NCTC 13352 (TEM-10 β-lactamase), performed 10 times in four laboratories

<table>
<thead>
<tr>
<th></th>
<th>Mean zone (mm)</th>
<th>Standard deviation (mm)</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ceftazidime</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laboratory 1</td>
<td>8.1</td>
<td>0.57</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>laboratory 2</td>
<td>6.8</td>
<td>1.75</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>laboratory 3</td>
<td>6.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>laboratory 4</td>
<td>6.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><strong>Cefotaxime</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laboratory 1</td>
<td>28.7</td>
<td>0.82</td>
<td>1</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>laboratory 2</td>
<td>29.4</td>
<td>0.97</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>laboratory 3</td>
<td>25.9</td>
<td>1.29</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>laboratory 4</td>
<td>31.3</td>
<td>1.06</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Cefepime</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laboratory 1</td>
<td>26.4</td>
<td>0.52</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>laboratory 2</td>
<td>28.1</td>
<td>0.74</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>laboratory 3</td>
<td>23.0</td>
<td>1.55</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>laboratory 4</td>
<td>29.1</td>
<td>1.00</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Data of J. M. A.
NDM-positive Enterobacteriaceae and was struck by the fact that the bacteria never looked as resistant to carbapenems as they had by prior agar dilution. On several occasions the zones were embarrassingly large!

Against this background of imprecision, the direct detection of ESBL or carbapenemase activity is an invaluable ‘fail safe’, allowing treatment to be adjusted if these enzymes are found.

It might be argued that laboratory issues are best addressed by improving the precision of susceptibility tests, but three factors make us sceptical. First, there is no simple well-described change that will achieve the required improvement. Second, and following from this, it is arguable that MIC tests (let alone discs) are now as precise as they are likely to become, as they are done with living organisms on media made of natural (i.e. variable) products with logarithmically spaced antibiotic dilutions. Third, it is hard to believe that MICs, determined under one set of conditions, can represent all infective settings, allowing for variation in drug penetration and bacterial inoculum, which notoriously affects the MICs for ESBL producers. The simple expedient of reducing breakpoints to the epidemiological cut-off (i.e. the upper edge of the normal MIC distribution for isolates without acquired resistance) might resolve matters for cephalosporins and ESBL producers, but is less satisfactory with carbapenems, where the lowered breakpoints shown in Table 1 already cause problems. In particular, unexceptional AmpC-depressed Enterobacter spp. isolates, without significant porin loss, are often reported as intermediate or resistant to ertapenem with MICs of 1–2 mg/L, and analysis by one of us (P.-L. H.) showed that applying the new CLSI disc breakpoints would drastically reduce the impenem susceptibility rate for Morganella spp. (from 100% to 38.6%) and Providencia spp. (99.8% to 68.6%) with no evidence that any of the recategorized organisms hosted any substantive resistance mechanism(s).

**Losing epidemiological data for strains with ESBLs and carbapenemases?**

A third objection is the loss of epidemiological information. Although EUCAST guidance continues to advocate ESBL testing for epidemiological purposes, it is less likely to be done by financially constrained laboratories if it is no longer advocated to guide the treatment of individual patients. Surely, when resistance among Gram-negative bacteria is proliferating and diversifying, it is vital for a laboratory to have insight into the mechanisms circulating locally, both to aid infection control and to facilitate the recognition of unusual types? Distinguishing strains that have ESBLs and AmpC enzymes by simple direct tests trains the spotlight on those that have reduced cephalosporin susceptibility but lack these mechanisms, such as (i) the NEQAS-distributed K. pneumoniae with KPC enzyme, discussed earlier, which belonged to a lineage (ST258) with a notorious ability to spread, causing large outbreaks, sometimes affecting whole countries, as in the USA, Israel and Greece, and (ii) strains with OXA-48-like carbapenemases, which are easy to miss both by classical and automated systems, and which have been responsible for several recent large outbreaks across Europe.

A further key aspect here is the well-documented variation in the expression of metallo-β-lactamase-mediated resistance within isogenic strains from outbreaks. For example, the same metallo-β-lactamase, IMP-9, was found on the same large plasmid in the same genetic context (one of five cassettes of the same sequence and order in a class 1 integron) in multiple isolates of P. aeruginosa from an outbreak involving several hospitals in Guangzhou, China, but meropenem MICs ranged from 1 to 16 mg/L among these isolates. Similarly, in the first major outbreak of carbapenemase (IMP-4)-producing Enterobacteriaceae and P. aeruginosa in Australia, only 5 of 19 isolates were resistant to carbapenem antibiotics at breakpoint (MIC >8 mg/L) and phenotypic carbapenemase detection proved critical to identify all of the patients involved. Notably, 12/19 patients had clinically significant disease caused by these bacteria.

As these cases illustrate, unless phenotypic tests are done, some members of carbapenemase outbreaks will be missed, with detrimental consequences for infection control.

**ESBL tests and reporting delays**

One objection to ESBL tests is that, if they are done after the basic susceptibility tests, an extra day may elapse before antibiotic results are released to the ward. This is undesirable, particularly for severely ill patients, whose outcomes are worsened if appropriate therapy is delayed.

This, though, is a reason to accelerate ESBL tests, not to abandon them. Various strategies might be adopted. Most obviously, ESBL tests can be run in parallel with the main body of susceptibility testing instead of subsequently. This is done anyway on automated susceptibility testing systems, such as Vitek 2, Phoenix and Microscan; if disc testing is preferred, it can be achieved by using combination discs or by putting an amoxil/lin/clavulanate disc between the ceftazidime and cefotaxime discs.

Alternatively, if one considers speed more valuable than precision (a reasonable view in respect of severely ill patients), ESBL detection can be made faster than susceptibility testing by plating the clinical specimen directly on to one of the various ‘ESBL detection’ media now marketed, which test for cephalosporin non-susceptibility rather than ESBLs per se, and then testing the colonies that grow for ESBL, AmpC or carbapenemase, either colorimetrically with the Cica-β-Test system or with microarrays.

Falling costs mean that, within a decade, next-generation sequencing may become commonplace in diagnostic laboratories, allowing isolates from primary culture plates, or even some clinical specimens, to be identified, strain-typed and profiled for resistance genes within 24 h. This will identify, ≥1 day ahead of the classical susceptibility test result, whether any ESBL or carbapenemase gene(s) are present, but won’t show if the MIC of a given cephalosporin is 1, 2 or 4 mg/L.

**Conclusions**

While sequence data may become routinely available in the future, what is apparent for the present is that the policy of reporting ESBL producers ‘as found’ rather than resistant to all cephalosporins is (i) weakly based in relation to clinical outcome data, (ii) too trusting of the precision of routine susceptibility data and (iii) liable to lead to the loss of valuable epidemiological data. As policies go, it warrants a
Nelsonian eye…which, for non-UK readers, means ‘deserves to be ignored’.

The case is more finely balanced with regard to the use of carbapenems for infections due to carbapenemase producers with low MICs, because (i) the clinical data relating enzyme, MIC and outcome are scantier than for ESBL producers, (ii) the enzymes are more diverse and (iii) unlike for infections due to ESBL producers, there is no obvious, good, next-step antibiotic to use. Nevertheless, it seems profoundly imprudent to simply ‘report as found’ without advising the clinician that the pathogen has a resistance mechanism of public health importance and that—if a carbapenem is to be used—the patient needs especially careful monitoring, additions to the regimen and stringent infection control.

Acknowledgements

We are grateful to Christine Walton and Derek Brown for their kind permission to cite experience and quote material from a recent NEQAS Antimicrobial Susceptibility distribution.

Transparency declarations

D. M. L. resigned from the EUCAST Expert Rules Working Party on the issue of reporting cephalosporin and carbapenem results ‘as found’. He consults for numerous pharmaceutical and diagnostic companies, including Achaogen, Astellas, AstraZeneca, Bayer, Basilea, bioMerieux, Cubist, Discuva, GSK, Kalidex, Merck, Pfizer and Tetraphase, holds grants from Basilea, Cubist, Meij and Merck, has received lecture honoraria or travel reimbursement from AstraZeneca, GSK, J&J, Merck, Novartis, Pfizer and Tetraphase, and holds shares in AstraZeneca, Dechra, Eco Animal Health, GSK, Merck and Pfizer, collectively amounting to <10% of diversified portfolio value. P. M. H. has received honoraria for developing and delivering educational presentations for Eumedica, Pfizer, Merck, Novartis, MagusCommunications and Wyeth, funded research from Pfizer and Eumedica, and consultancy for Pfizer, Novartis, Basilea, Novacta, Novolytics, Merck, Wyeth and Optimter. He is a director of ModusMedica, a medical education company. Y. D. has a research grant from Merck and has served on a Pfizer advisory board. D. P. has consulted for AstraZeneca, Cubist, Leo, Merck and Pfizer. N. W. has received research grants and conference support from numerous pharmaceutical companies; none poses a conflict of interest with this paper. All other authors: none to declare.

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