Combination testing of multidrug-resistant cystic fibrosis isolates of *Pseudomonas aeruginosa*: use of a new parameter, the susceptible breakpoint index

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**Objectives:** The microbiology laboratory at Aberdeen Royal Infirmary operates an extended susceptibility testing service for multidrug-resistant Gram-negative non-fermenting isolates from the sputum of Scottish cystic fibrosis patients. The service aims to provide clinicians with useful treatment options and developed the use of a novel parameter—the susceptible breakpoint index (SBPI), which allows easy ranking of the antimicrobial combinations in order of their possible in vivo effectiveness.

**Methods:** Three hundred and fifteen isolates of *Pseudomonas aeruginosa* were submitted for testing. MICs of 14 antimicrobials were determined using the Etest and the results categorized using CLSI guidelines. Usually, six antimicrobial pairs were tested in combination also using the Etest. The results were assessed using the fractional inhibitory concentration index (FICI) and also by a novel parameter, the SBPI.

**Results:** Some 4173 MICs and 1663 combination pairs were performed. The most active individual antimicrobials were colistin, tobramycin and amikacin, with 84%, 69% and 32% of isolates susceptible, respectively. Twenty-eight of 44 antimicrobial combinations were tested 10 times. Of the combinations, 3.6% were synergistic (FICI \(\leq 0.5\)) and 0.1% were antagonistic (FICI \(> 4.0\)). Amikacin+cefazidime (17%), ciprofloxacin+ceftazidime (12.9%) and ciprofloxacin+piperacillin/tazobactam (12%) were the most synergistic combinations. By median SBPI, the most effective combinations in vitro were colistin+ticarcillin/clavulanate, colistin+piperacillin/tazobactam and colistin+meropenem.

**Conclusions:** The Etest is a useful tool for determining MICs and testing antimicrobial combinations. The SBPI is more discriminatory than the FICI, allowing easy ranking of the combinations, and is likely to have clinical relevance.

**Keywords:** Etest, MIC, synergy

**Introduction**

Antimicrobial therapy has undoubtedly prolonged the lives of patients with cystic fibrosis (CF). A consequence of this is the increased colonization and infection of the lungs of CF patients with multiresistant bacteria, leading to dilemmas regarding best treatment options. In April 1999, a CF National Referral Centre was established at Aberdeen Royal Infirmary, with funding from the National Services Division of the Common Services Agency of the Scottish Executive. The Cystic Fibrosis Antibiotic Susceptibility Testing Service (CFASS) provides extended susceptibility testing services for multiresistant (resistant to all antimicrobials in two or more antimicrobial classes) non-fermenting Gram-negative bacteria from CF patients from all Scottish hospitals. Isolates are typically referred if they are multiresistant or there were problems locally with identifying treatment regimens. From May 2001, the Centre switched from using traditional broth microdilution and chequerboard methods for MIC and combination testing, respectively, to the commercially available Etest (AB Biodisk, Solna, Sweden). This move simplified the susceptibility testing and improved turnaround times from the receipt of the isolates to the availability of MIC and combination testing results. The mean turnaround time using Etest methodology is 3 working days, which allows for more timely guidance of antimicrobial therapy in CF patients.

We report here the results of our findings on the extended susceptibility testing of *Pseudomonas aeruginosa* isolates referred to the Centre since the introduction of Etest methodology and also report on a novel parameter for the interpretation of combination testing results—the susceptible breakpoint index (SBPI). The SBPI relates the MIC of the antimicrobials in combination to their...
susceptible breakpoints and, therefore, may give more clinically useful information than the traditional fractional inhibitory concentration index (FICI) for assessing combination testing results.

Materials and methods
Between 24 May 2001 and 24 January 2008, CFASS received 315 multi-resistant isolates of *P. aeruginosa* from 76 Scottish CF patients for extended susceptibility and antibiotic combination testing. The isolates, which were referred from nine Scottish laboratories, were identified locally by the sending laboratories. On receipt, the isolates were plated onto Mueller–Hinton agar (Oxoid), VIA selective agar for *Stenotrophomonas maltophilia* and *Burkholderia cepacia*-selective agar plates (*B. cepacia* medium and Selectival supplements, MAST Diagnostics, Merseyside, UK). After incubation for 18–24 h in ambient air at 35°C, the plates were examined for purity and an oxidase test (Oxoid) was performed on the growth. Confirmation of *P. aeruginosa* identification was accepted if the isolate was oxidase-positive, non-lactose fermenting and failed to grow on the *B. cepacia*-selective agar. Growth on VIA agar was ruled out as being *S. maltophilia* if the oxidase test result was positive. The plates were incubated for a further 24 h for confirmation of purity.

A suspension of the 24 h culture (if pure) equivalent to a 0.5 McFarland standard (1.0 if the isolates were mucoid) was prepared in saline and spread onto pre-dried MHA plates, according to CLSI guidelines for plate inoculation for disc testing. Two Etest strips (AB Biodisk, Solna, Sweden) per plate were applied parallel to one another with the antimicrobial A for B and B for A. After 24 h of incubation in air at 35°C, the plates were examined for purity and an oxidase test (Oxoid) was performed on the growth. Confirmation of *P. aeruginosa* identification was accepted if the isolate was oxidase-positive, non-lactose fermenting and failed to grow on the *B. cepacia*-selective agar. Growth on VIA agar was ruled out as being *S. maltophilia* if the oxidase test result was positive. The plates were incubated for a further 24 h for confirmation of purity.

The MIC geometric mean linear regressions were plotted and the $R^2$ values calculated using Microsoft® Office Excel 2003.

Usually, six pairs of antimicrobials (A and B) were tested in combination. The selection of antimicrobial pairs was based on the MIC results of the individual antimicrobials and also on clinical information, such as current antimicrobial therapy and patient intolerance or allergy to named antimicrobials. Where two or more isolates from a multiple referral gave the same or similar MIC results, then combination testing was performed on only one of the isolates. For isolates that were resubmitted for testing within 6 months and that gave the same MIC results as a previously submitted isolate, then, as far as possible, taking into account allergies etc., a different set of six combinations was tested.

MHA plates were inoculated and two Etest strips for the combination under investigation were applied to an inoculated MHA plate in the same manner as for the MIC testing. After incubation at room temperature for 1 h, the Etest strips were removed and replaced with a second pair. The fresh strips were applied exactly over the imprints of the first pair, matching the concentration gradients but switching antimicrobial A for B and B for A. After 24 h of incubation in air at 35°C, the MIC values were read off the strips.

Calculation and interpretation of indices: (i) FICI

FICI = (MIC of A in combination/MIC of A) + (MIC of B in combination/MIC of B)

Where an MIC was found to be greater than the antimicrobial range tested, then the next doubling dilution above the highest value of the range tested was used to calculate the FICI (e.g. if an MIC of &gt;256 mg/L was found, then the FICI was calculated using 512 mg/L). The indices were interpreted as: an FICI of ≤0.5 = synergy; an FICI of &gt;0.5 and ≤4.0 = no interaction; and an FICI of &gt;4.0 = antagonism.

Calculation and interpretation of indices: (ii) SBPI

SBPI = (susceptible breakpoint A/MIC of A in combination) + (susceptible breakpoint B/MIC of B in combination)

An SBPI of 2.0 indicates that the MICs of antimicrobials A and B in combination are either equivalent to their respective susceptible breakpoints or that the combination MIC of one of the antimicrobials is less than its susceptible breakpoint. It therefore follows that the greater the SBPI value, the more effective that combination is in vitro.

The combination results were reported in rank order of their SBPI results, going from the highest to the lowest SBPI. Any combination that was found to be antagonistic (FICI &gt;4.0) was not ranked, irrespective of the SBPI result, and was reported as being not recommended for therapy.

Results
Between 24 May 2001 and 24 January 2008, 315 multiresistant *P. aeruginosa* isolates were referred for susceptibility testing from 44 female and 32 male CF patients. The median age at first isolate referral was 20 years (age range 8–64), and between 1 and 26 isolates (median 3) were referred per patient during the study period. The median number of isolates per patient per single referral was 2 (range 1–8).

The results of the 4173 MIC tests performed on the 315 *P. aeruginosa* isolates and their clinical interpretation are shown in Table 1. Overall, 32.1% of the isolate/drug combinations were susceptible and 49.5% were resistant. Figure 1 shows the annual variation of MIC susceptibility testing results for all agents obtained from 2001 to 2006 (only eight isolates were analysed in 2007). There is a slight increase in total resistance from 54% to 61% over the study period. Tobramycin susceptibility showed a steady drop from 86% to 54.8% between 2001 and 2006 (data not shown), with an increase in intermediate susceptibility from 6% in 2001 to 33.3% in 2006. With the exception of piperacillin (only 2 years’ data obtained before this became unavailable for clinical use), all other antimicrobials show minor annual fluctuations in susceptibility patterns (data not shown).

When the annual geometric mean MIC values for the individual antimicrobials were plotted, the only two to demonstrate a downward trend were levofloxacin and colistin ($R^2=0.24$ and 0.09, respectively). The trend for ciprofloxacin was level ($R^2=0.00$). There was an upward trend in the aminoglycoside MICs, which was least in gentamicin and greatest in tobramycin (Figure 2). An upward trend was also observed in the geometric MIC means of the β-lactams, with piperacillin/tazobactam and ticarcillin/clavulanate demonstrating similar increases ($R^2=0.42$ and 0.45, respectively). Ceftazidime had an $R^2$ of 0.29 and that for aztreonam was 0.09. Imipenem and meropenem also showed an increasing trend in annual MIC geometric means...
These data for the annual MIC geometric means would suggest that MIC values are rising. A total of 1663 combination tests were performed on 44 different antimicrobial pairs. As can be seen in Table 2, synergy was most frequently found with β-lactam + quinolone combinations (10%), followed by β-lactam + aminoglycoside combinations (5%) and carbapenem + quinolone combinations (4%). Antagonism was only found with a β-lactam + quinolone combination (1%).

Of the 44 antimicrobial pairs tested, 28 were tested 10 times each (1588 combinations). The results of these 28 combination pairs can be seen in Table 3. Overall, 3.6% of these 1588 combinations were synergistic and 0.1% were antagonistic.
Nineteen of the 28 combinations that were tested ≥10 times demonstrated synergy (range 0.5%–17%). Amikacin + ceftazidime proved to be the most frequently synergistic combination, with 9 of the 53 (17%) isolates tested having an FICI of 0.5. This was followed by ciprofloxacin + ceftazidime (12.9%) and ciprofloxacin + piperacillin/tazobactam (12%). Eight of the nine (89%) combination regimens that incorporated a quinolone (i.e. ciprofloxacin, as we did not test levofloxacin) demonstrated some degree of synergy. Likewise, combinations with a β-lactam, colistin, carbapenem or aminoglycoside in the regimen were found to be synergistic in 12 of 16 (75%), 5 of 7 (71%), 5 of 8 (63%) and 8 of 16 (50%) of cases, respectively.

A high SBPI did not predict a synergistic FICI (Table 3). Of the 28 combination pairs tested ≥10 times, colistin + ticarcillin/clavulanate, colistin + piperacillin/tazobactam and colistin + meropenem came out on top when ranked by median SBPI values, and the bottom three were ceftazidime + gentamicin, ciprofloxacin + gentamicin and ciprofloxacin + ceftazidime (Table 4). Figure 3 shows the SBPI range, median and

![Graph showing annual geometric means of the aminoglycoside MICs. AMK, amikacin; GEN, gentamicin; NET, netilmicin; TOB, tobramycin.](https://academic.oup.com/jac/article-abstract/65/1/82/724850)

**Table 2.** Summary of extended susceptibility testing on 315 strains of *P. aeruginosa* (MICs tested, *n* = 4173; antibiotic combinations tested, *n* = 1663)

<table>
<thead>
<tr>
<th>First antimicrobial group</th>
<th>Second antimicrobial group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aminoglycoside</td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>n = 1217</td>
</tr>
<tr>
<td></td>
<td>(37% S, 32% R)</td>
</tr>
<tr>
<td>β-Lactam</td>
<td>SYN = 5%</td>
</tr>
<tr>
<td></td>
<td>ANT = 0%</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>SYN = 1%</td>
</tr>
<tr>
<td></td>
<td>ANT = 0%</td>
</tr>
<tr>
<td>Colistin</td>
<td>SYN = 0%</td>
</tr>
<tr>
<td></td>
<td>ANT = 0%</td>
</tr>
<tr>
<td></td>
<td>ANT = 0%</td>
</tr>
<tr>
<td>Quinolone</td>
<td>SYN = 1%</td>
</tr>
<tr>
<td></td>
<td>ANT = 0%</td>
</tr>
</tbody>
</table>

S, susceptible; R, resistant; SYN, synergy; ANT, antagonism.

Cells highlighted grey, number of MICs (% susceptible, % resistant); data to right of highlighted cells, number of times combination tested; data to left of highlighted cells, combination results.
Table 3. SBPI ranges for combinations tested >10 times ranked by FICI interpretation (n = 1588)

<table>
<thead>
<tr>
<th>Combination antimicrobials</th>
<th>Number of times combination tested</th>
<th>Synergistic FICI</th>
<th>Non-interactive FICI</th>
<th>Antagonistic FICI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>AMK CAZ</td>
<td>53</td>
<td>9</td>
<td>17</td>
<td>0.33–12</td>
</tr>
<tr>
<td>CIP CAZ</td>
<td>31</td>
<td>4</td>
<td>12.9</td>
<td>0.33–3.33</td>
</tr>
<tr>
<td>CIP TZP</td>
<td>83</td>
<td>10</td>
<td>12</td>
<td>2.33–190.50</td>
</tr>
<tr>
<td>GEN TZP</td>
<td>17</td>
<td>2</td>
<td>11.8</td>
<td>45.33–46.67</td>
</tr>
<tr>
<td>CIP TIM</td>
<td>50</td>
<td>5</td>
<td>10</td>
<td>3.17–172.42</td>
</tr>
<tr>
<td>AMK MEM</td>
<td>12</td>
<td>1</td>
<td>8.3</td>
<td>6</td>
</tr>
<tr>
<td>CST TZP</td>
<td>19</td>
<td>1</td>
<td>5.3</td>
<td>32.5</td>
</tr>
<tr>
<td>CIP IPM</td>
<td>39</td>
<td>2</td>
<td>5.1</td>
<td>2.50–13.16</td>
</tr>
<tr>
<td>TOB ATM</td>
<td>84</td>
<td>4</td>
<td>4.8</td>
<td>4.67–50.11</td>
</tr>
<tr>
<td>CST CAZ</td>
<td>63</td>
<td>3</td>
<td>4.8</td>
<td>9.00–23.72</td>
</tr>
<tr>
<td>TOB CAZ</td>
<td>130</td>
<td>5</td>
<td>3.8</td>
<td>1.25–25.05</td>
</tr>
<tr>
<td>CIP PIP</td>
<td>30</td>
<td>1</td>
<td>3.3</td>
<td>1.58</td>
</tr>
<tr>
<td>TOB TZP</td>
<td>40</td>
<td>1</td>
<td>2.5</td>
<td>130.67</td>
</tr>
<tr>
<td>CIP MEM</td>
<td>87</td>
<td>2</td>
<td>2.3</td>
<td>0.92–6.33</td>
</tr>
<tr>
<td>CST CIP</td>
<td>135</td>
<td>3</td>
<td>2.2</td>
<td>2.80–12</td>
</tr>
<tr>
<td>CST TIM</td>
<td>54</td>
<td>1</td>
<td>1.9</td>
<td>691.38</td>
</tr>
<tr>
<td>CST MEM</td>
<td>140</td>
<td>2</td>
<td>1.4</td>
<td>5.46</td>
</tr>
<tr>
<td>TOB CIP</td>
<td>97</td>
<td>1</td>
<td>1.1</td>
<td>19.63</td>
</tr>
<tr>
<td>TOB MEM</td>
<td>185</td>
<td>1</td>
<td>0.5</td>
<td>129</td>
</tr>
<tr>
<td>TOB TIM</td>
<td>72</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>TOB CST</td>
<td>52</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>TOB IPM</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>AMK IPM</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>GEN CAZ</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>TOB PIP</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>CST ATM</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>GEN CIP</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>GEN MEM</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

n, number tested; CST, colistin; TOB, tobramycin; AMK, amikacin; CIP, ciprofloxacin; MEM, meropenem; PIP, piperacillin; TZP, piperacillin/tazobactam; ATM, aztreonam; CAZ, ceftazidime; GEN, gentamicin; TIM, ticarcillin/clavulanate; IPM, imipenem.
interquartile ratios for the colistin combinations tested, and demonstrates that colistin þ ticarcillin/clavulanate, colistin þ piperacillin/tazobactam and colistin þ meropenem were the most effective of the seven combinations by this measure.

Table 5 is an example of the MIC and combination testing results obtained for an isolate of P. aeruginosa. The MIC results show that the only fully active individual antimicrobials are colistin and tobramycin. The combination of ciprofloxacin þ piperacillin/tazobactam returned the least effective SBPI, but a synergistic FICI. At 3.0 mg/L, the MIC of ciprofloxacin in the combination is intermediate by CLSI breakpoint criteria and that of piperacillin/tazobactam is susceptible. The other five combinations are non-interactive by FICI, but all of their combination MICs are susceptible by CLSI breakpoint criteria. In order to address which of the combinations were likely to be the most useful clinically, we applied the SBPI to rank the results. In the example, colistin þ aztreonam and colistin þ ciprofloxacin are the most effective combinations in vitro and the least effective is ciprofloxacin þ piperacillin/tazobactam.
Table 5. Example of susceptibility testing results for a P. aeruginosa isolate

<table>
<thead>
<tr>
<th>MIC testing</th>
<th>Combination testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>antimicrobial</td>
<td>MIC (mg/L)</td>
</tr>
<tr>
<td>AMK</td>
<td>48</td>
</tr>
<tr>
<td>GEN</td>
<td>16</td>
</tr>
<tr>
<td>NET</td>
<td>48</td>
</tr>
<tr>
<td>TOB</td>
<td>3</td>
</tr>
<tr>
<td>CIP</td>
<td>16</td>
</tr>
<tr>
<td>LVX</td>
<td>&gt;32</td>
</tr>
<tr>
<td>ATM</td>
<td>48</td>
</tr>
<tr>
<td>CAZ</td>
<td>&gt;256</td>
</tr>
<tr>
<td>PIP</td>
<td>&gt;256</td>
</tr>
<tr>
<td>T2P</td>
<td>&gt;256</td>
</tr>
<tr>
<td>IPM</td>
<td>&gt;32</td>
</tr>
<tr>
<td>MEM</td>
<td>&gt;32</td>
</tr>
<tr>
<td>CST</td>
<td>1</td>
</tr>
<tr>
<td>TIM</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

AMK, amikacin; GEN, gentamicin; NET, netilmicin; TOB, tobramycin; CIP, ciprofloxacin; LVX, levofloxacin; ATM, aztreonam; CAZ, ceftazidime; PIP, piperacillin; T2P, piperacillin/tazobactam (tazobactam concentration fixed at 4 mg/L); IPM, imipenem; MEM, meropenem; CST, colistin; TIM, ticarcillin/clavulanate (clavulanate concentration fixed at 2 mg/L); S, susceptible; I, intermediate; R, resistant; NI, non-interactive; SYN, synergy.

**Discussion**

Life expectancy in patients with CF is increasing due, in large part, to antimicrobial therapy. Unfortunately, as a consequence of exposure to multiple antimicrobial courses, the bacteria that colonize the lungs and cause infective episodes in CF patients are increasingly becoming resistant to available antimicrobials. It is now the norm to treat these infective episodes empirically with a combination of antimicrobials, but the dilemma facing the physician is in choosing the most effective antimicrobial combination that is going to have a positive treatment outcome for the patient.

The high rate of resistance that we found with the MIC testing is not surprising, given the population of isolates studied. Colistin was the most active antimicrobial we tested, with 84% of isolates being susceptible. The CF Referral Center, Columbia University (CFRC,CU), which offers a similar service, found 96% of their P. aeruginosa isolates to be susceptible to colistin using the same breakpoints.8 Of the four aminoglycosides we tested, tobramycin had the highest and gentamicin the lowest number of susceptible isolates (69% and 21% of isolates, respectively). In their testing of aminoglycosides, the CFRC,CU reported the same rank order of susceptibility for tobramycin, amikacin and gentamicin as we found, but with less isolates susceptible—tobramycin 34% and gentamicin 12%. Ciprofloxacin was the most active of the two quinolone antimicrobials tested, with 30% of isolates susceptible compared with 21% for levofloxacin. The CFRC,CU found 27% of their isolates to be susceptible to ciprofloxacin, with no data presented for levofloxacin. Of the isolates, 28% were susceptible to meropenem and 14% to imipenem, which is less than the susceptible rates reported by CFRC,CU, who found 39% and 24% of isolates susceptible to meropenem and imipenem, respectively. Piperacillin and piperacillin/tazobactam jointly topped the β-lactam league table, with 27% of isolates being susceptible, followed by aztreonam (24%), ceftazidime (22%) and ticarcillin/clavulanate (21%). The CFRC,CU β-lactam susceptibility league table ranked piperacillin/tazobactam (31%) top, followed by ceftazidime (27%), ticarcillin/clavulanate (23%), piperacillin (22%), aztreonam (21%) and cefepime (16%), which is not included in our battery of antimicrobials as it is not marketed in the UK.

Within the tobramycin susceptible isolate population (data not shown) there is a clear shift of the MICs from 2.0 to 4.0 mg/L. In their study of inhaled tobramycin, Ramsey et al.9 reported a trend toward an increase in tobramycin MICs for the P. aeruginosa isolates from patients receiving tobramycin, but this was not found in the placebo group. Whether the increase in tobramycin MICs in our study corresponds to an increase in the use of tobramycin in the CF population is difficult to assess due to the limitations of the available prescribing data, although inhaled tobramycin is widely used in Scotland. The highest tobramycin MIC in our study was 128 mg/L, a concentration that is achievable in sputum with aerosolized administration of tobramycin.8,9

The ‘gold standard’ for assessing in vitro antimicrobial combinations is the chequerboard, which is performed using either a microdilution or tube broth dilution technique. Both methods are time consuming and labour intensive to perform as well as being difficult to control, and there is also some debate as to which endpoint should be used for the calculation of the FICI.11 There are no such problems using the Etest for the assessment of antimicrobial combinations. The method is simple, easily controlled and has just one endpoint. We use the method described by Bolmstrom et al.12 for assessing antimicrobial combinations. Other workers employed a technique whereby the two Etest strips are placed in a cross formation at 90° to one another,
intersecting at their respective MICs. This method fails to
detect mild antagonism, but uses only half the Etest strips
required for our method of choice. The Etest has been used
increasingly in published studies for the susceptibility testing of
multidrug-resistant Gram-negatives, but studies are consistently
still using checkerboard, time–kill or animal models to assess
synergy.\textsuperscript{16,15} Rarely has Etest methodology been reported other
than as conference abstracts.\textsuperscript{16}

Our study found that β-lactam–quinolone combinations
were most frequently synergistic (10%), but also showed the
only antagonism (1%). Antagonism is rarely described and, as
far as we are aware, of unknown mechanisms.\textsuperscript{17}

The SBPI is a mathematical expression that relates the MICs
of combination antimicrobials to their respective susceptible
breakpoints. It is more discriminatory than the FICI when analys-
ing combination outcomes, providing a wide range of results. It is
usual for us to test at least six antimicrobial combinations
against each \textit{P. aeruginosa} isolate and, more often than not, all
of the combinations will return an FICI of between >0.5 and
<4.0, which equates to no interaction; in this situation, the
SBPI is a valuable tool for ranking the combinations in order of
their \textit{in vitro} effectiveness. The range of antimicrobial concen-
trations on Etest strips is in excess of achievable therapeutic
centers. This can result in the scenario where it is possible to
have a synergistic outcome even though the concentration of
neither antimicrobial may be achievable therapeutically. In this
case, a low SBPI would be returned and, in our study, a high
SBPI was not predictive of synergy.

When ranked by median SBPI, 6 and 4 of the top 10
combinations had colistin or tobramycin, respectively, as
one of the pair (SBPI range 18–171.75). Combinations with
colistin + ticarcillin/clavulanate, colistin + piperacillin/tazobactam,
colistin + meropenem and tobramycin + piperacillin/tazobactam
ranked first to fourth in the median SBPI analysis table. Colistin
is increasingly used, particularly in a nebulized inhalation for
the treatment of multidrug-resistant \textit{Pseudomonas} infections.\textsuperscript{18}
The bottom three combinations by median SBPI were
ceftazidime + gentamicin, ciprofloxacin + gentamicin and cipro-
flaxacin + ceftazidime (SBPI range 1.75 – 2.0).

Interestingly, in their study of 12 multidrug-resistant
\textit{P. aeruginosa}, Tateda et al.\textsuperscript{19} reported a strong synergy in all
combinations with polymyxin B, contrary to our results with
colistin, suggesting individual strain variation and making rules
difficult to construct. Tobramycin was not investigated in their
study. However, they also reported no combination effect with
gentamicin + ceftazidime and ciprofloxacin + ceftazidime, which,
by median SBPI, were our lowest and third lowest ranked combi-
nations, respectively.

We believe we are the first to use the SBPI.\textsuperscript{20} This novel pa-
rameter may allow some clinical relevance to assessing the
outcome of antimicrobial combination testing, given the predic-
tive value of MIC in modern pharmacokinetic/pharmacodynamic
target setting and clinical outcome. A similar concept in inter-
preting individual MIC results to help determine which agent is
likely to be the most effective \textit{in vivo} has been in routine use
for many years. There is, however, a long way to go before it is
possible to confidently predict clinical outcome from \textit{in vitro}
MICs in CF.

The phenotypic variability and highly mutagenic properties of
\textit{P. aeruginosa} together with biofilm formation makes the whole
area of \textit{P. aeruginosa} lung infection a microbiological minefield.
Foweraker et al.\textsuperscript{21} highlighted the difficulties facing microbiolo-
gists in determining the susceptibility patterns of \textit{P. aeruginosa}
isolated from CF sputum samples and questioned the role of
conventional susceptibility testing once \textit{P. aeruginosa} chronically
infests the CF lung.

Nevertheless, in an anonymous survey completed by 81 phys-
icians who use the CFRC, 96% indicated that synergy studies
were helpful, 89% used the results to treat their patients and
84% indicated that the patients improved using the regimens
suggested by the synergy studies.\textsuperscript{8} Feedback from the users of
our service also suggests they find our susceptibility results
useful in guiding their choice of antimicrobial therapy.\textsuperscript{22}

In conclusion, optimal, standardized methods for the suscept-
bility testing of CF sputa chronically infected with \textit{P. aeruginosa}
that have proven clinical efficacy have still to be established. We
feel that in this context the Etest is a useful tool for the determi-
nation of MICs and for testing antimicrobial combinations. The
SBPI is more discriminatory than the FICI in determining which
antimicrobial combinations are most effective \textit{in vitro}, allows
easy ranking of combinations and, given the importance of
MICs in determining outcome, is likely to have at least some
clinical relevance.

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\section*{Transparency declarations}
None to declare.

\section*{References}
1. Mackenzie FM, Smith SV, Milne KE et al. Antibigrams of resistant
Gram-negative bacteria from Scottish CF patients. \textit{J Cystic Fibrosis}
2. Gould IM, Milne KE, Mackenzie FM. Comparison between broth
microdilution and E-test for assessing MICs and antibiotic combina-
tions against multiply resistant isolates from cystic fibrosis patients.
In: \textit{Abstracts of the Forty-first Interscience Conference on Anti-
American Society for Microbiology, Washington, DC, USA.
isolation of \textit{Stenotrophomonas maltophilia}. \textit{European J Clin Microbiol
Antimicrobial Disk Susceptibility Tests—Ninth Edition: Approved Standard
Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement
M100-S18}. CLSI, Wayne, PA, USA, 2008.


