Predictive value of isolating *Pseudomonas aeruginosa* from aerobic and anaerobic blood culture bottles

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*Pseudomonas aeruginosa* is a particularly virulent pathogen when it causes bacteraemia and early diagnosis is essential to reduce morbidity and mortality. It is an aerobe and is thought by many to be almost exclusively isolated from the aerobic blood culture bottle in cases of bacteraemia. This study analysed 277 Gram-negative bacteraemic episodes over 1 year at a single institution in order to assess the predictive value of this finding. In 39 of 44 episodes of *P. aeruginosa* bacteraemia, the organism was isolated from the aerobic bottle only, which gave a sensitivity of 88.6% for this ‘test’ and a specificity of 73.8%. However, for all episodes of Gram-negative bacteraemia, the likelihood of a Gram-negative bacillus occurring in the aerobic bottle first being *P. aeruginosa* was only 39%. The converse finding of a Gram-negative bacillus isolated first in the anaerobic bottle or from both bottles together was clinically helpful, having a negative predictive value of 97.2% (i.e. that the organism was not *P. aeruginosa*).

Introduction

*Pseudomonas aeruginosa* is a Gram-negative aerobic bacillus responsible for a wide range of clinical conditions. It is resistant to many commonly used antibiotics and empirical therapy may not always provide adequate activity against this organism. It causes bacteraemia primarily in immunocompromised patients. Predisposing conditions include malignancies (Martino *et al.*, 1999; Chatzinikolaou *et al.*, 2000; Maschmeyer & Bravely, 2000; Jugo *et al.*, 2002; Cherif *et al.*, 2003), immunoglobulin deficiency states, diabetes mellitus, organ transplantation, burns, HIV/AIDS, use of steroids, presence of intravenous lines and medical care within intensive care units (ICUs) (Edgeworth *et al.*, 1999; Jamal *et al.*, 1999). Changes in medical practice in these high-risk patients, plus improvements in antibiotics and their use (empirical in many cases), have reduced mortality, but *P. aeruginosa* bacteraemia still has a mortality of 30% (Osman *et al.*, 2004). In one recent study of bone marrow transplant patients, the mortality rate was 40% (Collin *et al.*, 2001). Factors associated with an unfavourable outcome include persistent neutropenia (the cure rate is correlated with changes in the neutrophil count), presence of septic shock, pneumonia, other deep-seated infections, the underlying condition and inappropriate or inadequate antibiotic therapy (Kang *et al.*, 2003).

It would, therefore, be valuable to know as soon as possible if a Gram-negative blood culture isolate was *P. aeruginosa*. This would allow the optimal antibiotic therapy to be commenced earlier, thereby addressing one of the above unfavourable factors. Attempts to categorize infectious episodes as being likely to be caused by *P. aeruginosa*, based on risk factors, have been described previously (Gransden *et al.*, 1995). Rapid differentiation of fermentative Gram-negative bacilli from non-fermenters by impedance methods has also been described (Chang & Huang, 2000). Neither of these approaches has become widely used.

*P. aeruginosa* is an aerobe and there is a widely held view that, in cases of bacteraemia, it is almost always isolated from the aerobic blood culture bottle only. This study was undertaken to determine the predictive value of this property. We set out to investigate the positive predictive value (PPV) of such a ‘test’, the likelihood of a Gram-negative bacillus first occurring in the anaerobic bottle being *P. aeruginosa* (i.e. the negative predictive value, NPV), and the sensitivity of the test (i.e. how often *P. aeruginosa* occurs in the aerobic bottle only) and its specificity: for all episodes of Gram-negative bacteraemia, for all such episodes occurring in the ICU (both in haematology and non-haematology patients), for episodes occurring in haematology patients and for episodes occurring on other units.

Methods

The Royal Free Hospital is a tertiary referral hospital with approximately 1200 beds. It provides most speciality services including stem-cell transplantation, renal and liver transplantation, neurosurgery, treat-
ment of infectious diseases and critical care. A retrospective study of all Gram-negative bacteraemic episodes during the year July 2001 to June 2002 was carried out to determine the relative timing of positive signals from both aerobic and anaerobic blood culture bottles. One aerobic and one anaerobic bottle were obtained for all samples included in the analysis.

Bacteraemias were counted as episodes. Multiple blood culture isolates of the same organism from the same patient were counted as one episode (Rolston & Tarrand, 1999).

All blood culture bottles were processed using the BACTEC 9240 system (Becton Dickinson Instrument Systems) and standard accredited methods were used for organism isolation and sensitivity testing. All Gram-negative isolates from blood cultures were identified to species level. Oxidase-positive organisms were identified using API 20NE (bio-Mérieux). Gram stain morphology was not assessed at the time of the initial microscopy beyond classifying the organism as a Gram-negative bacillus.

**Results and Discussion**

There were 277 Gram-negative bacteraemic episodes in total for the year July 2001 to June 2002. Sixty-three episodes occurred in patients in the ICU and 37 episodes occurred in haematology patients. Six of the episodes occurring in haematology patients occurred whilst the patient was in the ICU. One hundred and eighty-three episodes occurred in non-ICU and non-haematology patients. The results are summarized in Table 1.

*Escherichia coli*, accounting for 97 episodes, was the commonest organism isolated overall, followed by *P. aeruginosa* (44 episodes) and *Acinetobacter baumannii* (37 episodes). In 39 of 44 cases, *P. aeruginosa* occurred in the aerobic bottle only, which gave a sensitivity of 88.6 % and a specificity of 73.8 %. The likelihood of a Gram-negative bacillus occurring in the aerobic bottle first being *P. aeruginosa* (the PPV) was 39 %, with an NPV of 97.2 %.

Different organisms caused bacteraemia in the different patient populations. The predominant organism isolated from ICU patients was *A. baumannii* (25/63 episodes, 39.7 %) followed by *P. aeruginosa* and *Enterobacter* spp. (10 episodes each). In the ICU, *P. aeruginosa* was isolated from the aerobic bottle alone on six occasions (sensitivity 60 %, specificity 41.5 %, PPV 16 %, NPV 83.3 %). Six of the episodes occurring in the ICU occurred in haematology patients. Two of these were attributable to *P. aeruginosa* and occurred in the aerobic bottle only (sensitivity 100 %). Two episodes were due to *A. baumannii* (one of which occurred in the aerobic bottle only). The PPV in these patients was, therefore, 66.7 %, with a specificity of 75 % and an NPV of 100 %. In non-haematology patients in the ICU, *A. baumannii* was responsible for 23 of 57 (40.3 %) bacteraemias, nine episodes were due to *Enterobacter* spp. and eight episodes were due to *P. aeruginosa*. *P. aeruginosa* grew in the aerobic bottle alone in four of these eight episodes (sensitivity 50 %, specificity 40.8 %, PPV 12.1 %, NPV 83.3 %).

In non-ICU patients, *A. baumannii* was responsible for 23 of 57 (40.3 %) bacteraemias, nine episodes were due to *Enterobacter* spp. and eight episodes were due to *P. aeruginosa*. *P. aeruginosa* only grew in the aerobic bottle (sensitivity 100 %, specificity 74 %, PPV 70 %, NPV 100 %).

*P. aeruginosa* was responsible for 14 of the 37 bacteraemic episodes occurring in haematology patients. *E. coli* was responsible for six episodes and there were four episodes of bacteraemia due to *Enterobacter* spp. *P. aeruginosa* only grew in the aerobic bottle (sensitivity 100 %, specificity 74 %, PPV 70 %, NPV 100 %).

There were 21 *P. aeruginosa* bacteraemic episodes in non-ICU and non-haematology patients. In only one case did *P. aeruginosa* first grow anaerobically (sensitivity 95.5 %, specificity 85.1 %, PPV 46.7 %, NPV 99.3 %).

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**Table 1. Gram-negative episodes**

The number of episodes is given, with the number occurring in the aerobic bottle first given in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>ICU patients (all)</th>
<th>ICU haematology patients</th>
<th>ICU (excluding haematology patients)</th>
<th>Haematology patients (total)</th>
<th>Non-haematology/ non-ICU patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>44 (39)</td>
<td>10 (6)</td>
<td>2 (2)</td>
<td>8 (4)</td>
<td>14 (14)</td>
<td>22 (21)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>97 (11)</td>
<td>6 (1)</td>
<td>0</td>
<td>6 (1)</td>
<td>6 (1)</td>
<td>85 (9)</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>26 (4)</td>
<td>10 (2)</td>
<td>1 (0)</td>
<td>9 (2)</td>
<td>4 (0)</td>
<td>13 (2)</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>19 (1)</td>
<td>3 (0)</td>
<td>1 (0)</td>
<td>2 (0)</td>
<td>2 (0)</td>
<td>15 (1)</td>
</tr>
<tr>
<td><em>Proteus</em> spp.</td>
<td>12 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12 (0)</td>
</tr>
<tr>
<td><em>Serratia</em> spp.</td>
<td>6 (1)</td>
<td>1 (1)</td>
<td>0</td>
<td>1 (1)</td>
<td>0</td>
<td>5 (0)</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>7 (1)</td>
<td>1 (0)</td>
<td>0</td>
<td>1 (0)</td>
<td>0</td>
<td>6 (1)</td>
</tr>
<tr>
<td><em>Citrobacter</em> spp.</td>
<td>7 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7 (1)</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>37 (33)</td>
<td>25 (22)</td>
<td>2 (1)</td>
<td>23 (21)</td>
<td>4 (2)</td>
<td>10 (10)</td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>6 (6)</td>
<td>3 (3)</td>
<td>0</td>
<td>3 (3)</td>
<td>2 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Others</td>
<td>16 (3)</td>
<td>4 (2)</td>
<td>0</td>
<td>4 (2)</td>
<td>5 (1)</td>
<td>7 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>277</td>
<td>63</td>
<td>6</td>
<td>57</td>
<td>37</td>
<td>183</td>
</tr>
</tbody>
</table>
P. aeruginosa remains a common clinical isolate, second only to E. coli as a cause of Gram-negative bacteraemia in this study. It is the commonest cause of Gram-negative bacteraemia in haematology patients in our institution (unpublished data) and is second only to A. baumannii in causing Gram-negative bacteraemia in ICU patients.

P. aeruginosa is one of the most frequent bacterial causes of fever in neutropenic patients (Jugo et al., 2002; Cherif et al., 2003). Indeed, the guidelines published by the Infectious Diseases Society for North America (Hughes et al., 2002) include empirical antibiotic regimens that cover P. aeruginosa. The incidence of P. aeruginosa bacteraemia has remained unchanged in haematology patients over time (Chatzinikolaou et al., 2000; Maschmeyer & Braveny, 2000; Jugo et al., 2002).

In the intensive care setting, P. aeruginosa is the third most common Gram-negative bacillus isolated after Enterobacter spp. and Serratia spp. (Jamal et al., 1999), and its frequency is increasing (Edgeworth et al., 1999). P. aeruginosa is also the second (Garrouste-Orgeas et al., 2000) or third (Jamal et al., 1999) most frequently isolated Gram-negative organism in hospital-acquired bacteraemias.

We found that the likelihood of a Gram-negative bacillus in the aerobic bottle being P. aeruginosa was only 39% overall and this is therefore a poor discriminator. Whilst this rose to 47% in non-ICU and non-haematology patients and 70% in haematology patients, it was only 16% in patients in the ICU. This is clearly a poor screening test. The NPV were, however, much more impressive, i.e. the likelihood of a Gram-negative bacillus occurring in the anaerobic bottle first being P. aeruginosa was very small in all groups. Overall the NPV was 97.2%, although in the ICU this fell to 84.6%, the lowest value. This means that the likelihood of a Gram-negative bacillus occurring first in the anaerobic bottle being P. aeruginosa was 15% at most and was much less likely in the non-ICU patient groups.

The principal reason for the poor performance of the test in patients in the ICU was the high prevalence of a strain of multi-resistant A. baumannii. Twenty-five bacteraemic episodes (of 63) on the ICU were due to A. baumannii and in the majority (22) the organism was isolated from the aerobic bottle only. It was, therefore, more likely that a Gram-negative bacillus first occurring in the aerobic bottle was going to be A. baumannii than P. aeruginosa. The likelihood of a Gram-negative bacillus growing in the aerobic bottle first in cultures from ICU patients being A. baumannii was 11.5%. Hence an equivalent NPV for this organism is 88.5%. This suggests that growth in the anaerobic bottle would be helpful in excluding A. baumannii in the setting of an outbreak. A. baumannii bacteraemia in critically ill patients has been discussed elsewhere (Garcia-Garmendia et al., 2001; Cisneros & Rodriguez-Bano, 2002).

As described above, it has been demonstrated that appropriate antimicrobial therapy is associated with a better prognosis (Leibovici et al., 1997). Whilst other factors are associated with a poor prognosis of bacteraemia, often only antibiotic treatment is amenable to medical intervention and rapid diagnosis may have a clinical impact (Doern et al., 1994). Since haematology patients are empirically started on anti-pseudomonal antimicrobials (Hughes et al., 2002), it may not make much difference clinically. In non-haematology and non-ICU patients, however, P. aeruginosa could be expected in almost half of them. It is in these patients that P. aeruginosa should be considered as a potential pathogen, particularly if the patient is unwell, because it is a further 24 h before sensitivity patterns can be determined using standard techniques. The high NPV, moreover, may be reassuring if the patient is started on a drug such as ceftazidime, which has little activity against P. aeruginosa.

There has been much debate about the relevance of inoculating the anaerobic bottle of a blood culture set. Several retrospective studies suggested selectively performing anaerobic blood cultures in patients at risk for anaerobic infections (Ortiz & Sande, 2000; Saito et al., 2003). The rationale behind this was that obligately anaerobic organisms were rarely isolated. Indeed, in our study there were only three episodes of obligately anaerobic Gram-negative bacteraemia. There were, however, 33 bacteraemic episodes (of the 274 remaining episodes) in which a facultative anaerobe was isolated in the anaerobic blood culture bottle only, which represents an increase in yield of 12.04%. This study adds another reason for continuing to use the anaerobic bottle.

The study has its limitations. Risk groups other than presence in an ICU or being a haematology patient were not addressed (e.g. presence of intravenous lines, etc.). Indeed there was more than one group within the haematology patient population, some patients being stem-cell transplant patients, whilst others were receiving chemotherapy for haematological malignancy. Also, there are differences between different blood culture systems in terms of the ability of P. aeruginosa to grow in the aerobic and anaerobic bottles (Pohlman et al., 1995; Perez et al., 1996). The provision of more stringent anaerobic conditions would favour a higher NPV, whilst improved performance of the aerobic bottles would increase the sensitivity and PPVs.

In summary, this study confirms that P. aeruginosa usually does appear in the aerobic blood culture bottle first and that it is extremely rare for it to appear in the anaerobic bottle first (outside the ICU setting). This latter property (a high predictive value) could be of clinical use, both in predicting the cause of Gram-negative bacteraemia and allowing the confident use of antibiotics that lack anti-pseudomonal activity.

References


Chatzinikolaou, I., Abi-Said, D., Bodey, G. P., Rolston, K. V., Tarrand, J.


