Rapid detection of extended-spectrum β-lactamase (ESBL)-producing organisms in blood culture

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Keywords: cefpodoxime, cefpodoxime–clavulanate, resistance, disc testing

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Sir,

Extended-spectrum β-lactamases (ESBLs) have emerged as an important mechanism of resistance in Gram-negative bacteria. Unfortunately, ESBL-producing organisms often also possess resistance determinants to other important antibiotic groups, such as aminoglycosides and fluoroquinolones, leaving an extremely limited range of effective agents.1 Delay in appropriate therapy for infections with ESBL producers not only prolongs hospital stay, but is associated with increased mortality.2,3 Rapid detection of ESBL-producing organisms from blood culture could therefore be advantageous, aiding appropriate antibiotic choice at the earliest opportunity and improving outcomes.

The use of cefpodoxime and cefpodoxime–clavulanate discs is one of the recommended methods for detection of ESBL-producing organisms in routine laboratories.1 We describe a rapid method of detecting ESBL-producing organisms in blood cultures containing Gram-negative bacilli, based upon the use of these discs.

Forty Gram-negative isolates were used. These included 22 different strains of ESBL-producing Escherichia coli and Klebsiella spp. (a selection of TEM and SHV mutants and CTX-M types supplied by the ARMRL, Health Protection Agency, Colindale, London, UK). In order to simulate a positive blood culture, approximately 100 cfu of each isolate was mixed with 10 mL of horse blood and inoculated into a BacT/ALERT (bioMérieux, Basingstoke, UK) blood culture bottle. These were then placed on the BacT/ALERT blood culture machine overnight and removed the next morning if registered positive. Using a veneting needle, three drops of the positive blood culture fluid were dispensed on to Iso-Sensitest agar and spread using a cotton swab. Cefpodoxime and cefpodoxime–clavulanate (Oxoid, Basingstoke, UK) discs were applied and the plates incubated aerobically at 37°C. Between 3.5 and 6 h after the start of incubation plates were regularly examined for the appearance of growth and determination of antibiotic zone sizes, before being returned to the incubator. A final measurement was made after overnight incubation. The isolates were also tested separately at an inoculum specified by BSAC methodology.4 Our results showed ESBL-positive strains to be clearly segregated from ESBL-negative strains. The correct identification of ESBL status was confirmed by testing against cefpodoxime and cefpodoxime–clavulanate at BSAC inoculum. From our data, an ESBL producer could be defined as having a differential zone diameter of ≥5 mm (cefpodoxime-clavulanate zone–cefpodoxime zone).

Apart from one strain of E. coli that grew poorly, all ESBL-positive strains were discernable within 3.5–4.6 h after subculture. Results for ESBL-negative Enterobacteriaceae were available between 3.5 and 5.25 h.

The technique was then assessed on the routine blood culture bench using 23 clinical specimens. Three drops of the fluid from new positive blood cultures containing Gram-negative bacilli were inoculated on to an additional Iso-Sensitest plate and processed as above. The ESBL status of isolates was confirmed using BSAC methodology. Gram-negative isolates were identified using API 20E or NE (bioMérieux, Basingstoke, UK). Using the definition of an ESBL producer as having a differential zone diameter of ≥5 mm, all five ESBL-positive strains were correctly identified (zone differences varied between +10 and +14 mm) within 4.5–5.5 h of subculture. For the 16 ESBL-negative isolates, the difference in zone diameters was ≤+1 mm. Zones were present between 4 and 5.5 h. Testing using BSAC methodology confirmed all isolates were assigned the correct ESBL status.

The results produced by this technique provide an opportunity to instigate appropriate antibiotic therapy at an earlier stage in bacteraemic patients not suspected to be infected with an ESBL-producing organism. Thus, for a blood culture found positive in the morning or early afternoon, it is possible to identify an ESBL-producing organism within routine hours on the same day and alter patient therapy if necessary. This change would not be possible until the next day with conventional susceptibility methodology. For a subset of patients, this could make a significant difference to outcome and hospital costs.

We have now incorporated this test as a routine procedure on our blood culture bench. As there is a wide variation in ESBL-producing organisms and the number of organisms tested so far is small, these preliminary results require further confirmation. Laboratories wishing to use this technique should confirm the applicability of the method with these detection discs with their local strains. The same caveats over the routine use of cefpodoxime to detect ESBLs apply to the rapid method (such as induction of AmpC by clavulanate in Enterobacter spp. potentially masking ESBL production). These tests were not designed for use on Acinetobacter spp., Pseudomonas aeruginosa and Stenotrophomonas maltophilia.1 False-positive ESBL results may be obtained with some of these organisms. Although the ESBL result is available ahead of the identity of the organism, we do not think this should be a problem in practice as with this technique, non-fermenting organisms often grow so slowly that a rapid ESBL result is not available.

Navon-Venezia et al.5 reported a rapid protocol for the accelerated detection of ESBL-producing organisms from blood culture. Their methodology reduced detection time from at least 2 days to 1 day by standardizing the inoculum from blood cultures. We believe our results to be the first report of a rapid method involving early reading, giving same-day results.
This rapid methodology might also be adapted to other detection discs. The opportunity exists to apply this technique on other benches where a rapid ESBL result is required.

Acknowledgements

We would like to thank Dr David Livermore and Neil Woodford of the ARMRL, HPA, Colindale, London, UK for their advice and comments.

References


