Blood Cultures – A Retrospective evaluation of Direct Antibiotic Susceptibility testing using Positive Broth cultures detected by the BacT/Alert system

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Introduction

Rapid turnaround time for blood culture results is crucial for optimal patient care. Where an organism is isolated from blood, the additional delay of 24 hours in providing antibiotic susceptibility results may put the patient at a serious disadvantage.

To facilitate turnaround time, direct antibiotic susceptibility testing can be set up using the positive broth as the inoculum.

A retrospective appraisal was made of the overall reliability of Direct Susceptibility (DS) using a large number of positive blood cultures growing Gram negative or Gram positive bacteria. The data were collected from the blood culture section where a number of scientists set up and read direct susceptibility testing according to a written protocol. No attempt was made to screen or otherwise refine the results.

The discrepancy between the results of DS testing and conventional Calibrated Dichotomous Susceptibility (CDS) testing were analysed in detail.

Aim

The aim of this evaluation was to determine the reliability of the results obtained by direct antibiotic susceptibility testing in comparison to those results obtained by standardised CDS methods.

Method

The results of direct antibiotic susceptibility testing of all positive blood cultures obtained over a 12 months period were examined. Blood culture instrumentation and media used was the BacT/Alert using aerobic, anaerobic and paediatric bottles containing activated charcoal.

All positive broths were Gram stained and mixed cultures were excluded. At the same time the suspension was subcultured to purity plates and other relevant media for identification of the organism.

Direct Susceptibility tests were performed on all 1400 positive blood culture broths as follows: The bottles were shaken well to ensure an even distribution of the organism and 10 drops (0.25 mL) of the broth was transferred into 2.5 ml sterile saline for each antibiotic panel to be set up. The saline/broth suspension was mixed well and then flooded onto the selected plates. Excess fluid was removed and plates allowed to air dry. Antibiotic discs were then applied to the dried plates.
On the basis of the Gram stain, relevant standard panels of antibiotic discs were set up on Sensitest based agar (Oxoid).

The inoculum size for most organisms was between 10^6 and 10^7 CFU/mL, but there was some variation due to the type of isolate and the organism's growth rate. The aim was to obtain a lawn of growth on the susceptibility plate resembling the confluent appearance obtained with the standard CDS inoculum. After 18-24 hours incubation the antibiotic susceptibility was read and recorded as either susceptible (S) or resistant (R) according to CDS protocol. The standardised CDS test was performed on all isolates for confirmation of antimicrobial susceptibility.

Results

Agreement between direct susceptibility testing and the CDS was observed with 1364 of the 1400 tests (97%) There were 36 discrepant results overall and these are shown in the table below. The least satisfactory results were obtained with Enterobacteriaceae where 16 of 332 (5%) direct susceptibilities did not correlate with the CDS tests. Of the 14 Enterobacteriaceae that were not demonstrated to be resistant to beta-lactams by direct testing, 8 were subsequently shown to be ESBL positive.

In most other species there was a 1% or less discrepancy between direct and formal CDS testing but these included the failure to detect methicillin (cefoxitin) resistance in one isolate. Enterococci and Streptococci each yielded about a 3% discrepancy and these were fairly evenly distributed between a false resistant and false susceptible result.

Table 1: Total number of organisms evaluated (Total Count) and the number of times a discrepancy between DS & CDS occurred (Discrepancy Count).

ORGANISM	COUNT	DISCREPANCY COUNT	
		DS = S CDS = R	DS = R CDS = S
Enterobacteriaceae	332	14	2
Pseudomonas sp.	55	1	0
Staph. aureus inc. *MRSa*	317	1	1
Enterococci inc. *VRE*	93	1	2
Streptococci	169	2	3
Other	434	4	5
Total Count	**1400**	**23**	**13**

Key: S = susceptible; R = resistant. DS = Direct Susceptibility; CDS = Calibrated Dichotomous Susceptibility
Note: "Other" group of organisms includes: Coagulase Negative *Staphylococci*, Coryneforms, *Bacillus* sp., *Listeria* sp.

Discussion

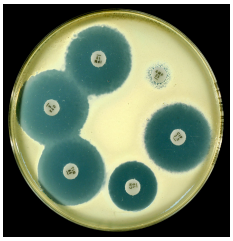
Overall there was a reasonable degree of agreement between the results of direct susceptibility testing of blood cultures and the CDS testing. However, there were some notable exceptions.

For example, resistance in those Enterobacteriaceae expressing an ESBL may not be detected by disc testing alone, but should be tested using disc approximation testing (refer to table 4 of the CDS Manual 2006). In this group in particular, the results of direct susceptibility tests should be treated with caution.

Almost all other discrepant results could be ascribed to a failure to achieve the inoculum required by the CDS test. This inoculum must be at least between 10^6 CFU/mL and 10^7 CFU/mL. The lawn of growth on the susceptibility plate should be confluent as shown in *Picture 1*. Any susceptibility plates showing only semi-confluent growth after overnight incubation should be discarded and the results not reported.

The most serious problems associated with an inadequate inoculum in DS are found in Staphylococci, and this retrospective analysis demonstrated that it was possible to fail to detect MRSa strains on direct susceptibility.

Testing Enterococci against vancomycin requires a degree of care and precision not possible with the direct susceptibility testing and it is probably prudent not to attempt direct testing these organisms.



Picture 1: Example of E.coli Direct Susceptibility showing resistance to ampicillin (25).

Conclusion

This retrospective evaluation included a large cross section of the most common blood culture isolates. Direct susceptibility (DS) was found to be reliable for most isolates. However, even with a high level of correlation between DS and CDS all direct susceptibilities should only be issued as provisional reports and need to be confirmed by the standardised CDS Test.

Reference

Bell S.M., Gatus B.J., Pham J.N. & Rafferty D.L. 2006. Antibiotic Susceptibility Testing by the CDS Method. A manual for Medical and Veterinary Laboratories (Fourth Edition).