Evaluation of the neutralising capacity of Bactec medium for some antibiotics

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Accepted: 10 October 2004

ABSTRACT

Bactec medium 9240 (Becton Dickinson, MD, USA) is a blood culture medium used routinely at Sultan Qaboos University Hospital (SQUH). The medium is said to contain substances that can neutralise antibiotics and destroy leucocytes in blood samples. In this study, the ability of the medium to neutralise the effect of some antibiotics and to destroy leucocytes is investigated. Vancomycin, amoxicillin, chloramphenicol, penicillin, gentamicin, fungizone and amikacin at various concentrations were added to separate bottles of Bactec medium 9240. *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 10662), *Staphylococcus aureus* (NCTC 6571) and *Candida albicans* (ATCC 10231), each at $1x10^4$ colony-forming units (CFU)/mL were added separately, depending on the type of antibiotic. The blood samples were incubated at 37°C for seven days under manual and automated blood culture systems. Subcultures were made from the manual system and routine laboratory procedures for detection of positive cultures were followed for the automated system. The Bactec medium was found to neutralise all antibiotics up to a concentration of 100 µg/mL by the automated method but showed some variation in results by the manual system. Leucocytes were destroyed within 24 hours.

KEY WORDS: Antibiotics. Bacteremia. Culture media. Leukocytes.

Introduction

The ability to rapidly detect the presence of microorganisms in the bloodstream of a patient with an invasive life-threatening infection is one of the most important functions of the clinical microbiology laboratory. This responsibility has been made easier by the use of media devised by Castaneda in 1947.[1] Castaneda's medium comprises sterile solidified glucose serum agar (the solid phase) and sterile glucose serum broth (the liquid phase). When blood from a patient is added to the medium, the liquid phase allows the growth of the organism while individual colonies develop on the solid phase.[1]

Other basic blood culture media designed for aerobic and anaerobic recovery of bacteria consist of brain heart infusion, tryptic and trypticase soy broth, thioglycollate broth and Robertson's cooked meat.[1,2] These media lack the basic ingredients for neutralising antagonistic substances such as antibiotics and leucocytes found in patients' blood.[2] In bacteraemic patients who received antimicrobial therapy before specimens were drawn, however, detection of bacteria was prevented or delayed.[3,4]

Previous solutions to this problem have included the dilution of blood with sterile broth, addition of penicillinase to cultures if the patient was on penicillin, addition of p-aminobenzoic acid to neutralise the effect of sulphonamide, or the addition of sodium polyanethol sulphonate to neutralise aminoglycosides and polymyxin antibiotics.[3–5]

Bactec medium (Bactec Plus Aerobic/F, Plus Anaerobic/F, Becton Dickinson, MD, USA) is reputed to contain ingredients that absorb or neutralise antibiotics and destroy leucocytes, thereby speeding the growth of bacteria in blood. Some workers found this medium to contain substances that inhibit the growth of *Peptostreptococcus* spp., *Neisseria gonorrhoeae*, *N. meningitidis* and *Gardnerella vaginalis*, as well as eliminating antagonistic substances in patients' blood.[6]

Marion Laboratories (Kansas City, USA) developed a medium for the removal of antibiotics and other bacterial inhibitors from blood. The substances in the medium, which they called an antibiotic removal device (ARD), were resins.[3,4,7] Several studies showed that incorporating resins in blood culture growth medium enhanced the recovery of bacteria from patients on antibiotic therapy.[8,9]

Becton Dickinson's Bactec 9240 medium is used at the Sultan Qaboos University Hospital (SQUH) for the isolation of bacteria from blood by an automated method. The medium contains resins and sodium polyanethol sulphonate, which are claimed to neutralise and destroy antagonistic substances such as antibiotics and leucocytes in patients' blood.

The aim of this study is to assess the performance of the Bactec 9240 medium and to determine the limit of its ability to neutralise antibiotics. In addition, the findings may provide an index with which to assess the department's quality assurance system.

Materials and methods

Antibiotics

The following antibiotics were used in the study: penicillin (600 mg), chloramphenicol (1 g), amoxicillin (250 mg), amikacin (100 mg), vancomycin (500 mg), amphtericin B

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Table 1. Antibiotic concentration showing growth (+) or no growth (–) of bacteria by the manual method.

Organism	Antibiotic concentration (μg/mL)								
	200	100	50	25	12.5	6.25	3.12	1.56	0.8
Vancomycin									
S. aureus	–	+	+	+	+	+	+	+	+
Amoxicillin									
E. coli	–	–	+	+	+	+	+	+	+
Chloramphenicol									
P. aeruginosa	–	+	+	+	+	+	+	+	+
E. coli	–	+	+	+	+	+	+	+	+
Penicillin									
S. aureus	–	+	+	+	+	+	+	+	+
Amphotericin B									
C. albicans	–	–	–	–	–	+	+	+	+
Gentamicin									
S. aureus	–	–	–	–	–	–	+	+	+
E. coli	–	–	–	–	–	–	+	+	+
P. aeruginosa	–	+	+	+	+	+	+	+	+
Amikacin									
E. coli	–	–	–	–	–	–	+	+	+
P. aeruginosa	–	+	+	+	+	+	+	+	+

(50,000 iu) and gentamicin (10 mg). Each antibiotic was diluted to 0.8 μg/mL with sterile distilled water and then added to the Bactec medium. Final antibiotic concentrations of 0.8–200 μg/mL were used in 25-mL aliquots of Bactec medium.

Control organisms

Control organisms included *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 10664), *Staphylococcus aureus* (NCTC 6571) and *Candida albicans* (ATCC 10231). Each organism was grown on blood agar and suspended in physiological saline at $1x10^4$ colony-forming units (CFU)/mL using a Mcfarland's turbidity tube 0.5. Control organisms (100 μL, equivalent to 1000 organisms) were added separately to each bottle, depending on the type of antibiotic.

Bactec medium

Preliminary investigation using Bactec plus aerobic/F and Bactec plus anaerobic/F showed no difference in their abilities to neutralise the antibiotics added and to permit the growth of organisms. Therefore, the main study used only Bactec plus aerobic/F medium. Three vials were used for each antibiotic concentration.

Incubation

One set of bottles was incubated without shaking (manual method) and subcultured on blood agar every 24 h for seven days. The other set was incubated automatically with shaking of the medium.

Controls

Sterile broth samples (25 mL) containing the same concentrations of antibiotics were inoculated with the control organisms and processed identically.

Assessment of leucocyte destruction

Leucocyte counts on three sterile citrated blood samples were performed on a Cell-Dyne 4000 (Abbott, USA). A bottle of Bactec medium was opened aseptically and 3 mL of the medium was transferred to nine sterile universal containers (three bottles per sample). Blood (1 mL) was added to each universal container and incubated overnight at 37°C. After incubation, one drop of the blood/Bactec medium mixture was added to 19 drops of white blood cell diluting fluid. Leucocyte count was performed using an improved Neubauer counting chamber (B.S.748, UK). In addition, a smear of the blood/Bactec medium mixture was made and stained with Leishman's stains.

Results

Tables 1 and 2 show the results obtained with *S. aureus* (NCTC 6571), *E. coli* (NCTC 10418), *P. aeruginosa* (NCTC 10662) and *C. albicans* (ATCC 10231) when added to the blood culture bottles containing various concentrations of the different antibiotics used (Table 1: manual, Table 2: automated culture). Table 3 shows the leucocyte counts obtained for the three blood samples used, prior to their addition to the Bactec medium and following 24-h incubation in Bactec medium.

Discussion

The detection of microorganisms in bacteraemic patients is prevented or delayed in those who have received antimicrobial therapy prior to collection of blood for culture.[3–5] Bactec medium contains resins and sodium

Table 2. Antibiotic concentration showing growth (+) or no growth (–) of bacteria by automated system.

Organism	Antibiotic concentration (μg/mL)								
	200	100	50	25	12.5	6.25	3.12	1.56	0.8
Vancomycin									
S. aureus	–	+	+	+	+	+	+	+	+
Amoxicillin									
E. coli	–	+	+	+	+	+	+	+	+
Chloramphenicol									
P. aeruginosa	–	+	+	+	+	+	+	+	+
E. coli	–	+	+	+	+	+	+	+	+
Penicillin									
S. aureus	–	+	+	+	+	+	+	+	+
Amphotericin B									
C. albicans	–	+	+	+	+	+	+	+	+
Gentamicin									
S. aureus	–	+	+	+	+	+	+	+	+
E. coli	–	+	+	+	+	+	+	+	+
P. aeruginosa	–	+	+	+	+	+	+	+	+
Amikacin									
P. aeruginosa	–	+	+	+	+	+	+	+	+
E. coli	–	+	+	+	+	+	+	+	+

Table 3. Leucocyte counts before and after incubation of blood in Bactec medium.

Blood sample	Before incubation	After incubation
1	6.3 x 10^9/L	zero
2	7.2 x 10^9/L	zero
3	6.8 x 10^9/L	zero

polyanethol sulphonate in order to neutralise the effect of antibiotics and to destroy the leucocytes found in human blood.[7] However, to obtain the maximum absorption of antibiotics, the manufacturer (Becton Dickinson) recommends that the blood be maintained in suspension in the resin-containing medium by shaking during incubation.[7]

Using a manual technique, the results of the present study showed that Bactec medium is capable of neutralising vancomycin, chloramphenicol and penicillin at various concentrations (Table 1). The medium absorbed gentamicin and amikacin at 100 μg/mL, permitting the growth of *P. aeruginosa*. With *S. aureus* and *E. coli*, however, growth only appeared when antibiotic concentration was reduced to 3.12 μg/mL and below. The reason for this is unclear but may be due to differences in their respective minimum inhibitory concentration (MIC) for a particular bacterium or to poor absorption of the antibiotic in the technique used.

Table 2 shows that all antibiotics at a concentration of 100 μg/mL were removed from the medium when it was incubated using the automated system, supporting the recommendation that blood and resin-containing media should be maintained in suspension by shaking so that the maximum amount of antibiotic is absorbed.[7,9–11]

Willis *et al*.[7] found that antibiotics in patients' blood could be removed by resins contained in the medium, and the findings of the present study support the assertion that antibiotics in the medium are better absorbed by an automated system that involves continuous agitation during incubation. When the antibiotics were neutralised, growth of bacteria occurred within 48 h; however, no growth occurred after seven days in cases where no neutralisation was apparent.

Lindsey and Riely[5] investigated the ability of resin to neutralise the levels of antibiotics found in patients on amikacin, ampicillin, carbenicillin, ceftaxin, chloramphenicol, gentamicin, nafcillin, tetracycline, ticarcillin, tobramycin and vancomycin. They found that resin was able to remove antibiotics to insignificant levels, enabling isolation of bacteria in patients' blood.

The function of sodium polyanethol sulphonate in the Bactec medium is to destroy leucocytes, thus ensuring the growth of any bacteria present.[2] The present study confirmed that leucocytes in blood added to Bactec medium are destroyed within 24 h (Table 3).

This study demonstrated that Bactec plus aerobic/F medium (Becton Dickinson 9240 series) is capable of neutralising the effect of antibiotics up to a concentration of 100 μg/mL (a concentration higher than any blood level found in practice) if an automated blood culture system is used. With a manual system, however, removal varied with the antibiotic used and was attributed to the lack of agitation of the blood and medium during incubation. In summary, the Bactec medium proved to be an effective blood culture medium and satisfied the requirements of the department's quality assurance system. □

The authors are grateful to the Sultan Qaboos University Hospital (SQUH) for providing the media and antibiotics used in the study,

and to the Department of Microbiology and Immunology, Sultan Qaboos University, for providing the stock cultures.Thanks are due to the staff of the laboratory for their cooperation during the study.

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