

Effectiveness of Resins in Neutralizing Antibiotic Activities in Bactec Plus Aerobic/F Culture Medium

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Incorporating resins in blood culture media can effectively reduce the activities of several antibiotics. It was shown that the activities of some generally used antibiotics decreased by 80 to 90% within 2 h in Bactec Plus Aerobic/F resin-containing culture medium. Bactec vials containing resins were still found to be positive for bacteria when antibiotics were present. The addition of β -lactamase shortened the detection time irrespective of the presence of resins.

The empirical initiation of antibiotic treatment before carrying out blood cultures is known to suppress or slow the recovery of microorganisms from the blood. The dilution of the blood sample in culture medium, the addition of antibiotic-inactivating enzymes to the blood culture, and a lysis centrifugation method have been reported to enhance the recovery of bacteria (5, 15). In 1981 Lindsey and Riely showed in an in vitro study that an antimicrobial agent-removing device made up of resins could remove several antibiotics from human blood (7). Furthermore, several clinical studies have demonstrated that resin-containing medium can shorten detection time and/or increase the number of positive blood cultures (4, 6, 8, 10, 11, 13, 14, 17). The yields of clinically significant microorganisms such as *Staphylococcus aureus* and the *Enterobacteriaceae* were higher in resin-containing vials (6), and *Staphylococcus*, *Streptococcus*, and yeast spp. were detected earlier in resin-containing culture vials than in vials without resins (2, 4). The increased efficacy of the Bactec Plus Aerobic/F resin-containing blood culture medium could merely be due to the antibiotic binding resins present in the culture vials. The strong cationic-exchange resins bind ionically to positively charged antimicrobials such as aminoglycosides. The polymeric adsorbing resins are capable of binding to the hydrophobic regions of virtually any antimicrobial agent. However, other studies have questioned the ability of resins to neutralize commonly used antibiotics (2, 12, 18).

In this study the effectiveness of Bactec Plus Aerobic/F culture medium in neutralizing increasing concentrations of various antibiotics and the binding kinetics of antibiotics to the resins were tested. In addition, the delay in the detection of positive cultures was evaluated, for both resin-containing and non-resin-containing media with increasing concentrations of antibiotics. The antibiotic binding characteristics of the resins in pure Bactec Plus Aerobic/F culture medium were tested to eliminate the variables present when human blood is used. The Bactec Plus Aerobic/F blood culture bottles used contained 25 ml of soybean-casein digest broth, 0.05% (wt/vol) sodium polyanetholsulfonate, 16.0% (wt/vol) nonionic adsorbing resins, and 1.0% (wt/vol) cationic-exchange resins. Non-resin-containing Bactec Standard/10 Aerobic/F culture vials contained 40 ml of soybean-casein digest broth and 0.035% (wt/vol) sodium

polyanetholsulfonate. The vials were incubated in the Bactec 9240 system.

The following antibiotics were chosen: flucloxacillin, cefamandole, trimethoprim in combination with sulfamethoxazole in a ratio of 1 to 25, gentamicin, and teicoplanin. All antibiotics were prepared in culture medium to the appropriate concentrations after correction for the percentages of impurity indicated by the manufacturers. Antibiotics were added to Bactec culture vials in concentrations chosen to reflect clinically achievable levels. High-pressure liquid chromatography detection was used to measure the concentrations of flucloxacillin, cefamandole, trimethoprim, and sulfamethoxazole. Concentrations of gentamicin and teicoplanin were determined by a competitive immunological method (TDX; Abbott Laboratories, Inc., Chicago, Ill.). In bacterial-challenge experiments *S. aureus* and *Escherichia coli* were used. The strain of *S. aureus* used was susceptible to flucloxacillin and teicoplanin. The strain of *E. coli* used was susceptible to cephalosporins and aminoglycosides. Strains were inoculated in brain heart infusion broth and incubated at 35°C for 20 h. Each was diluted before use in sterile phosphate buffer to the appropriate inoculum.

The binding capacities of the resins were assayed by measuring the antibiotic concentration remaining after incubation at 35°C in the Bactec 9240 at 0, 0.5, 1, 2, 4, and 6 h after inoculation. The antibiotic concentration was expressed as the percentage of the concentration remaining in comparison to that at the beginning of the experiment. In Bactec culture vials without resins, drug concentrations remained constant over 24 h, indicating that there was no spontaneous degradation of the drug over time. In general, resins decreased antibiotic activity in the medium by 90% within 1 to 2 h after incubation (Table 1). However, teicoplanin and trimethoprim showed different patterns. About 60% of the teicoplanin was still present in the culture vial after 1 h. In contrast, trimethoprim concentrations decreased below detection limits within 30 min of incubation at 35°C.

In addition, increasing amounts of antibiotics were added to separate Bactec Plus Aerobic/F culture bottles to determine the maximum binding capacities of resins. Resins removed flucloxacillin, cefamandole, trimethoprim, sulfamethoxazole, gentamicin, and teicoplanin in concentrations of up to at least 500, 100, 10, 250, 300, and 200 μ g/ml, respectively.

To estimate the effect of resins on the time required to detect bacterial growth, vials to which antibiotics had been added were inoculated with a suspension of *S. aureus* or *E. coli* to a final inoculum of 20 CFU/ml, which reflects the concentration

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TABLE 1. Binding kinetics of antibiotics in resin-containing Bactec Plus Aerobic/F blood culture media

Time after inoculation (h)	Remaining antibiotic activity ^a of:					
	Flucloxacillin	Cefamandole	Sulfamethoxazole	Trimethoprim	Gentamicin	Teicoplanin
0	100	100	100	100	100	100
0.5	33	62	21	ND	30	67
1.0	17	38	5		20	59
2.0	11	16	2		NM	NM
4.0	7	ND	1		14	14
6.0	5		1		12	6

^a Expressed as a percentage of the concentration at the beginning of the experiment. Data are the means of at least two experiments. ND, not detectable; NM, not measured.

of bacteria present in blood in clinical bacteremia. After 200 h of incubation, no Bactec culture vials with antibiotics but without resins tested positive. However, vials containing both resins and antibiotics did test positive, albeit with a delay in detection time in comparison to control vials without antibiotics. This delay was concentration dependent for all antibiotics tested (Table 2). In addition, the effect of the addition of β -lactamase was evaluated by adding it simultaneously with β -lactam antibiotics to the culture vials to final concentrations of 0.05 U of β -lactamase I and 0.008 U of β -lactamase II per ml of culture medium (*Bacillus cereus* 569/H9; Genzyme broad-spectrum mixture). Preliminary tests had shown that these concentrations were sufficient to inactivate at least 30 μ g of flucloxacillin or cefamandole per ml within 5 min.

The addition of β -lactamase was found to neutralize the effect of antibiotics on the growth of both strains irrespective of the presence of resins. At 30 μ g of flucloxacillin/ml there was only a short delay in detection time for *S. aureus* in comparison to vials without antibiotics: 2 h in resin-containing vials and 4 h in those without resins. For cefamandole, the effect of the addition of β -lactamase was even more striking, since the detection time for vials with the antibiotic was similar to that for the control vials without antibiotic, for both resin-containing and non-resin-containing vials.

Most in vitro studies on the binding of antibiotics to resins have been performed with whole blood, simulating the clinical

situation (1, 7, 16). In this situation, various blood constituents, e.g., cell membrane components such as acid phospholipids and sterols and soluble intracellular proteins, can also bind to the resins, thereby disturbing the absorption of antibiotics. Furthermore, resins may increase the recovery of microorganisms by lysing leukocytes and adsorbing other bacterial inhibitors (4, 11). To exclude the possibility of interference from such factors, this study was performed with pure Bactec Plus Aerobic/F resin-containing medium.

In summary, we have demonstrated that resin-containing Bactec Plus Aerobic/F vials can rapidly and effectively reduce the concentrations of some generally used antibiotics in culture broth. Furthermore, even at very high concentrations binding saturation was not observed. Despite the capacity of resins to bind antibiotics rapidly in the presence of therapeutic concentrations of antibiotics (flucloxacillin, cefamandole, gentamicin, or teicoplanin), the time required to detect bacteria increased, in comparison to the detection time without antibiotics. This delay ranged from 2 h for *E. coli* in the presence of cefamandole to 21 h when *S. aureus* was incubated with 50 μ g of teicoplanin/ml. However, when these antibiotics were added to culture vials without resins, cultures did not register as positive even after 8 days of incubation. Interestingly, for flucloxacillin and cefamandole, adding β -lactamase to the vials shortened the detection time irrespective of the presence of resins. However, in the clinical situation it is not always possible to add β -lactamase directly to the culture vial when blood cultures are taken. Furthermore, adding β -lactamase to culture vials would lead to false observations of bacteremia (3, 9).

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TABLE 2. Effect of resins on the detection of bacterial growth in Bactec Plus Aerobic/F blood culture medium

Bacterium	Antibiotic	Concn (μ g/ml)	Delay in detection time ^a (h)
<i>S. aureus</i>	Flucloxacillin	1	0
		5	3.2
		10	3.8
		30	8.3
	Teicoplanin	10	13.3
		25	16.3
		50	20.9
<i>E. coli</i>	Cefamandole	1	0
		5	1.2
		10	2.4
		30	2.0
	Gentamicin	0.5	0
		1	0
		5	1.7
		10	3.8

^a The delay in detection time was calculated by comparing the times to detect bacterial growth in the presence and absence of antibiotics.

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