Prospective addition of β -lactamase to blood culture medium

D. F. J. BROWN, *M. WARNER, C. E. D. TAYLOR and R. E. WARREN

Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QW

Summary. The value of adding β -lactamase to bottles of blood-culture medium before their distribution to wards was investigated. Significantly more bottles containing β -lactamase were culture-positive than those without ($p < 0.002$). In another series, when the enzyme was added to both bottles in each set there was no significant difference in isolation rates between the two bottles. The groups of organisms which were isolated more readily when β -lactamase was present were staphylococci and streptococci. Storage of β -lactamase (Genzyme broad-spectrum mixture) in blood-culture medium at room temperature resulted in rapid loss of cephalosporinase activity, whereas little decline in penicillinase activity was noted over a period of 118 days.

Introduction

Although in the diagnosis of bacteraemia it is desirable to take blood for culture before instigating antimicrobial therapy, samples are commonly taken after antibiotic treatment has been given. Antimicrobial agents in these blood samples may be present in concentrations sufficient to prevent or delay growth of organisms in the culture medium.

Dilution of blood in the culture medium will reduce the concentration of any agents present. However, particularly with β -lactam agents, the ratios of serum concentrations of some antibiotics to their minimum inhibitory concentrations for bacteria such as staphylococci and streptococci is frequently so high that dilution is unlikely to reduce concentrations of antimicrobial agents to a sub-inhibitory level. Another potential problem is the bactericidal activity of blood. This can be overcome by addition of sodium polyanethol sulphonate (SPS) (Lowrance and Traub, 1969). SPS also inactivates aminoglycosides and polymyxins but has no effect on β -lactam agents (Edberg *et al.*, 1976). Thiol broth will slowly inactivate penicillins but not cephalosporins (Murray and Niles, 1982). Antimicrobial agents, including penicillins and cephalosporins, may be removed from blood before culture by resins (Wallis *et al.*, 1980), but the use of resins is probably limited by cost and the manipulation required, which may result in contamination or

require an unacceptable amount of additional work. The use of Bactec blood-culture bottles containing resins has also been shown to be an effective means of increasing isolation rates of organisms in blood cultures from patients on antimicrobial therapy (Appelbaum *et al.*, 1983; Doern and Gantz, 1983; McGuire *et al.*, 1983).

The use of β -lactamases to inactivate penicillins and cephalosporins in blood cultures is not widespread. In a limited survey in the USA, less than 10% of laboratories were found to use penicillinase routinely in blood cultures (Morello, 1983). In laboratories that do use β -lactamase in blood cultures, the addition of the enzyme is generally restricted to cultures from patients known to be receiving penicillins or cephalosporins, and the addition is made when the culture arrives in the laboratory. This approach could lead to problems. Cultures may be contaminated on addition of the β -lactamase (Norden, 1969; Faris and Sparling, 1972), the laboratory may not be informed that the patient has received β -lactam agents, and there may be difficulties in providing a 24-h service for addition of β -lactamase by laboratory staff. Also, any organisms in the culture will be exposed to the antibiotic before addition of β -lactamase.

There is no recently published evidence demonstrating that addition of β -lactamase increases the isolation rates of organisms from blood cultures. However, Dowling and Hirsh (1945) showed that when penicillinase was added to blood-culture medium the isolation rate of gram-positive cocci from 26 patients treated with benzyl penicillin increased. In a later study (Carleton and Ham-

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*Present address: Division of Hospital Infection, Central Public Health Laboratory, Colindale Avenue, London NW10 7NS.

burger, 1963) involving only two patients treated with nafcillin for staphylococcal endocarditis, and five dogs treated with oxacillin in a staphylococcal endocarditis model, the use of β -lactamase in blood cultures resulted in isolation of the infecting organisms; parallel cultures without β -lactamase were negative.

The problems of adding β -lactamase might be overcome by dispensing the enzyme into bottles of blood-culture medium followed by sterility testing before their release to hospital wards. In this study we examined the prospective addition of β -lactamase to blood-culture medium as a means of increasing isolation rates of organisms commonly found in blood cultures.

Materials and methods

Blood Cultures

The volume of blood requested for culture was 30 ml. Collectors were asked to distribute blood samples equally between two bottles each containing 85 ml of medium. Cultures of blood from paediatric patients were excluded from this study because the volumes of blood taken varied widely and samples were deliberately distributed unequally between the bottles. Cultures from patients in all other hospital wards were included.

Culture media. The study was divided into two series. In one series β -lactamase (Genzyme broad-spectrum mixture containing β -lactamases I and II from *Bacillus cereus*) was added to both bottles in each blood-culture set. In the second series, the β -lactamase was added to one bottle only.

The medium used in both bottles of each set was a supplemented brain-heart infusion broth (Brown *et al.*, 1984). The bottles used were a 120-ml flat bottle and a Malthus cell (Brown *et al.*, 1984). Saponin (Sigma) 0.025% w/v was added to the Malthus bottles. In addition, 50 units of β -lactamase were added to both bottles in the first series and to the 120-ml bottle only in the second series. All additions were made aseptically in the laboratory, the additives being sterilised by membrane filtration (Millipore Millex 0.22- μ m filters) as they were being injected into the bottles. Bottles of medium were checked for sterility by visual inspection after incubating them at 37°C for 5 days followed by 2 days at room temperature before distribution to the wards.

Processing of blood cultures. Cultures were examined essentially as described previously (Brown *et al.*, 1984). Bottles were incubated at 37°C, the Malthus bottle in a Malthus waterbath, and the 120-ml bottle vented in a CO₂ incubator. The 120-ml bottles were subcultured after incubation for one day. They were also examined daily for turbidity and were subcultured again if turbidity was noted. Malthus bottles were subcultured if they were not promptly attached to the Malthus apparatus or when the apparatus gave a positive signal. When one bottle in a set

gave a positive signal, both bottles were subcultured. All bottles were subcultured after 9 days, before being discarded.

Stability of β -lactamase in blood-culture medium

To bottles containing 85 ml of blood-culture medium 50 units of the broad spectrum β -lactamase (Genzyme) were added as described above. Bottles were stored at room temperature. Samples taken at intervals over a period of 118 days were stored at -70°C. β -Lactamase activity in the samples was measured by incubating portions at 37°C for 2 h, 6 h and 20 h with various β -lactam agents (final concentration 100 mg/L). The final concentration of β -lactamase in the mixtures was 0.5 units/ml. After incubation of mixtures, residual antibiotic was assayed by a diffusion method (O'Callaghan and Kirby, 1978; Sutherland and Rolinson, 1978).

Statistical analysis

The statistical significance of differences in isolation rates was assessed by the exact method described by Liddell (1983).

Results

Blood Cultures

In the first series, in which β -lactamase was added to both bottles, there was no significant difference in the numbers of positive cultures from bottles of the two types (table I). Of 3099 pairs of culture bottles, organisms considered to be clinically significant were isolated from at least one bottle in each of 272 cultures. In total, clinically significant organisms were isolated from 220 120-ml bottles and 217 Malthus bottles. In the second series, β -lactamase was added to the medium in only one bottle (120-ml). Of 4193 pairs of culture bottles, clinically significant organisms were isolated from at least one bottle in each of 375 cultures (table II). In total, 321 bottles containing medium with β -

Table I. Number of culture-positive blood-cultures after addition of β -lactamase to the Malthus and 120-ml bottles (first series)

Result obtained with Malthus bottle	Number of 120-ml bottles that gave		
	Positive results	Negative results	Total
Positive	165	52	217
Negative	55	2827	2882
Total	220	2879	3099

Table II. Number of culture-positive blood cultures after addition of β -lactamase to the 120-ml bottles only (second series)

Result obtained with Malthus bottle	Number of 120-ml bottles that gave		Total
	Positive results	Negative results	
Positive	237	54	291
Negative	84	3818	3902
Total	321	3872	4193

lactamase gave positive results, whereas only 291 bottles containing medium without β -lactamase gave positive results. The difference between these positive culture rates is significant ($p < 0.002$).

More than one organism was isolated from some sets of blood-culture bottles; hence the number of isolates was greater than the number of positive

cultures. Isolates clinically assessed to be contaminants are excluded from analysis. In the first series, when β -lactamase was added to both bottles in the set, there was no significant difference between the total numbers of isolates from the two types of bottle (table III). In the second series, when β -lactamase was added to the medium in only one type of bottle, there were significantly more isolates ($p < 0.002$) from the bottle containing β -lactamase.

With different groups of organisms, the effect of β -lactamase varied. When isolation of all gram-negative facultative bacilli is considered, the results obtained in the two series differed. In the first series, in which β -lactamase was added to both bottles, there were significantly more isolates ($p < 0.05$) from one bottle (the Malthus bottle, 86 isolates) than from the other bottle (the 120-ml bottle, 72 isolates). In the second series, in which β -lactamase was added to the 120-ml bottle only, there was no significant difference overall between the numbers of gram-negative facultative bacilli

Table III. Effect of β -lactamase on numbers of clinically significant isolates from blood cultures

Organisms isolated	Number of isolates from first series			Number of isolates from second series		
	Both bottles	120-ml bottle only with β -lactamase	Malthus bottle only with β -lactamase	Both bottles	120-ml bottle only with β -lactamase	Malthus bottle only without β -lactamase
<i>E. coli</i>	32	4	10	45	11	24
<i>Klebsiella</i> spp.	12	1	5	7	3	4
<i>Enterobacter</i> spp.	3	1	3	10	1	1
<i>Proteus</i> spp.	5	0	4	13	3	0
<i>Morganella</i> spp.	1	0	1	0	0	0
<i>Serratia</i> spp.	3	1	2	0	3	1
<i>Salmonella</i> spp.	2	1	1	4	3	1
<i>Citrobacter</i> spp.	1	0	0	0	1	0
<i>Acinetobacter</i> spp.	0	2	0	2	2	1
<i>Alcaligenes</i> spp.	0	2	0	1	0	0
<i>Aeromonas</i> spp.	1	0	0	1	0	0
<i>Pseudomonas</i> spp.	6	6	3	7	2	6
<i>Haemophilus influenzae</i>	5	1	0	11	1	0
<i>Staph. aureus</i>	25	11	4	43	26	7
<i>Staph. epidermidis</i>	16	7	6	40	12	4
Group A/C/G streptococci	3	1	1	4	2	2
Group B streptococci	2	1	0	1	2	0
Faecal streptococci	15	6	4	24	7	1
Viridans streptococci	5	3	1	16	9	2
<i>Streptococcus pneumoniae</i>	23	2	4	25	9	3
Coryneform bacteria	1	0	0	0	0	0
<i>Capnocytophaga ochracea</i>	0	0	1	0	0	0
<i>Clostridium</i> spp.	2	1	6	2	2	0
<i>Bacteroides</i> spp.	3	2	1	3	1	1
<i>Peptococcus</i> spp.	0	1	0	0	1	1
<i>Candida</i> spp.	0	2	0	1	2	2
<i>Torulopsis glabrata</i>	0	3	0	1	2	0
<i>Mycoplasma</i> sp.	0	0	0	0	0	1
Total	166	59	57	261	105	62

isolated from the Malthus (115 isolates) and the 120-ml (110 isolates) bottles. With individual genera there were no significant differences in isolation rates between the two bottles in the first series. In the second series, however, there were significantly more isolates of *Escherichia coli* from the Malthus bottle (without β -lactamase) than from the 120-ml bottle (with β -lactamase) ($p < 0.05$).

When isolation of other groups of organisms is considered the two series also differed. In the first series, when β -lactamase was added to both bottles in the set, there were no significant differences in the numbers of isolates of particular groups of organisms from the two types of bottles. In the second series there were significantly more isolates of *Staphylococcus aureus* ($p < 0.01$) and viridans streptococci ($p < 0.05$) from the bottle containing β -lactamase. If isolates of all streptococci are combined, the higher isolation rate from the bottle containing β -lactamase is highly significant ($p < 0.002$). Similarly if isolates of all staphylococci are combined the isolation rate is significantly higher ($p < 0.002$) from the bottle containing β -lactamase.

Stability of β -lactamase

Benzyl penicillin, carbenicillin and piperacillin at concentrations of 100 mg/L were hydrolysed in less than 2 h by Genzyme broad-spectrum β -lactamase 0.5 units/ml of blood-culture medium stored at room temperature for up to 118 days (figure, a). Some activity against flucloxacillin was lost after storage for only 8 days (figure, a), but flucloxacillin 100 mg/L was hydrolysed in 6 h by enzyme in medium stored for up to 118 days (figure, b). After storage for 21 days, activity against cephalosporins was undetectable on incubation of enzyme with antibiotic for 2 h (figure, a). Enzyme activity against cephalothin was, however, more stable than activity against other cephalosporins. Incubation for 6 h showed that activity against cephalothin declined by about 50% on storage for up to 118 days (figure, b). Continued incubation for 20 h showed complete hydrolysis of cephalothin 100 mg/L by enzyme stored for up to 118 days (figure, c). Enzyme activity against cefotaxime and cefuroxime was undetectable after storage for 36 days and against ceftazidime undetectable after storage for only 8 days (figure, c).

Discussion

This study has shown that adding β -lactamase prospectively to blood-culture medium significantly

Figure. Stability of Genzyme broad-spectrum β -lactamase in blood-culture medium. After storage at room temperature, enzyme activity was assessed by measuring the inactivation of antibiotics at concentrations of 100 mg/L of blood-culture medium at 37°C in (a) 2 h, (b) 6 h, (c) 20 h, BP=benzyl penicillin, C=carbenicillin, P=piperacillin, F=flucloxacillin, Z=ceftazidime, T=cefotaxime, L=cephalothin, U=cefuroxime.

increases the overall rate of isolation of organisms. The effect relates mainly to staphylococci and streptococci, most strains of which are susceptible to many of the β -lactam agents used clinically. With gram-negative facultative bacilli there was no clear advantage in adding β -lactamase. The situation with these organisms was complicated by their apparent preferential isolation from the Malthus bottle. The medium in the two types of bottles was similar apart from the saponin present only in the Malthus bottle. A previous trial, however, had not shown any difference between bottles containing saponin and those without saponin (Brown *et al.*, 1984). The same trial suggested that venting bottles of the medium used in that trial did not affect isolation rates of gram-negative facultative bacilli. Others have shown, however, that *E. coli* is more

likely to be isolated from tryptic soy broth if bottles are not vented (Washington, 1978). In both series of the present trial more isolates of *E. coli* were obtained from the Malthus bottle than from the 120-ml bottle, although the difference was significant in the second series only. Thus the influence of venting bottles makes it difficult to assess confidently the effect of β -lactamase on the isolation of gram-negative facultative bacilli.

Prospective addition of β -lactamase to bottles of blood-culture medium has several advantages compared with addition of the enzyme to selected bottles after blood has been added. Pseudobacteraemia caused by contamination with non-sterile β -lactamase should be avoidable if an effective sterility test is used before bottles are distributed to wards; β -lactamase may exert its enzymic activity from the moment blood is added to the bottle; lack of clinical information does not preclude addition of β -lactamase when indicated; and out-of-hours calls to add β -lactamase are unnecessary. The principal disadvantage of adding β -lactamase prospectively to blood cultures is the cost of the enzyme, although this may fall with increased use. Stability of the enzyme at room temperature may also be a problem. Dowling and Hirsh (1945) found that a penicillinase in veal-infusion broth showed little loss of activity over 4 weeks even at 37°C. Our present observations indicate that, in the medium used, at room temperature, the cephalosporinase activity of the β -lactamase preparation declines fairly rapidly while the penicillinase activity is relatively stable. The β -lactamase preparation used contained a mixture of β -lactamase I and β -lactamase II from *Bacillus cereus* (Thatcher, 1975). These results therefore suggest that, under the

conditions tested, β -lactamase I, which is active principally against penicillins, is stable, whereas β -lactamase II, which has activity against both penicillins and cephalosporins, rapidly loses activity. Consequently the shelf life of blood-culture bottles is limited to a few days if activity against cephalosporins is essential, but may be extended to three months if activity restricted largely to penicillins is acceptable.

The effect of β -lactamase might be expected to be more marked with gram-positive bacteria than with gram-negative bacteria. A common source of bacteraemic infections caused by gram-positive organisms, including streptococci, is the community outside hospitals, where penicillins are often used. Although penicillins are frequently also used for hospital acquired staphylococcal infections, they are much less commonly used for hospital acquired infections due to gram-negative bacilli. For these, cephalosporins are a more likely choice in our hospital. Furthermore, gram-positive organisms are generally more sensitive to penicillins than are gram-negative organisms. Since the penicillinase activity of the enzyme mixture used was stable while the cephalosporinase activity was relatively unstable, the advantages of adding the enzyme to bottles of blood-culture medium are more likely to be seen with infections due to gram-positive organisms. If cephalosporinases which are stable in culture media at room temperature can be found, the value of adding a β -lactamase mixture to blood culture media might be even greater.

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