Increased detection of polymicrobial septicaemia by repeat subculture

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Summary. Repeated subcultures were performed on 572 blood cultures that had yielded single significant organisms and 55 from which contaminants had grown. This yielded 83 more isolates from 71 patients. Only one of the contaminated blood cultures yielded a further significant isolate. Additional antibiotic therapy was indicated in only six patients. Although repeated subculture increased the detection of additional isolates, it did not help in the management of most patients.

Introduction

The incidence of polymicrobial septicaemia (i.e. the isolation of more than one organism from a single blood culture) seems to be increasing and is associated with higher than expected case fatality rates (Saravolatz *et al.*, 1978; Kiani *et al.*, 1979; Kreger *et al.*, 1980a; Ing *et al.*, 1981). One way to improve the rate of polymicrobial isolations may be to reincubate positive blood cultures and then to repeat subcultures (Hansen and Hetmanski, 1983; Weinstein *et al.*, 1983; Spencer *et al.*, 1984) though the value of this approach has been disputed (Harkness *et al.*, 1975; Stetz and Martin, 1985; Towne and Gay, 1985). In this prospective study we have analysed the results of repeated subculture of positive blood cultures over a 2-year period in an attempt to establish whether repeated subcultures increase the detection of polymicrobial isolates and are of benefit in the management of septicaemic patients.

Materials and methods

Blood-culture media

The following blood-culture media were used in 150-ml flat screw-capped bottles: (i) two bottles containing 100 ml of Hartley Digest Broth (Oxoid) plus glucose 0·1% for aerobic culture; one bottle contained polyanethol sulphonate (Liquoid; Roche) 0·025%; (ii) one bottle containing 100 ml of Fastidious Anaerobe Broth (FAB; Lab M) for anaerobic culture. Each bottle was inoculated with 9 ml of blood and incubated at 37°C.

Subculture technique

Every bottle was visually inspected each day and any

showing evidence of microbial growth, e.g., turbidity or haemolysis, were examined by Gram's stain and subculture. FAB bottles were routinely subcultured after 48 h. All bottles were opened and sub-cultured after incubation for 7 days. Subcultures were performed with sterile disposable 10- μ l plastic loops (NUNC, Denmark). One-third plates of Columbia (Oxoid) blood agar and lysed-blood agar with kanamycin 70 mg/L and menadione 0·001% were inoculated at each sub-culture. The blood-agar cultures were incubated in air with CO₂ 8% and the cultures on lysed-blood agar with kanamycin and menadione were incubated in anaerobic jars (Don Whitley Scientific Ltd.) in an atmosphere of N₂ 80%, CO₂ 10% and H₂ 10% (BOC Special Gases Ltd.). All plates were examined after incubation for 24 and 72 h.

When any bottle yielded a potentially pathogenic organism or a contaminant, all three bottles were sub-cultured again after incubation for 3 and 10 or 14 days. On these occasions, repeat subcultures were performed with sterile disposable loops as before on to blood agar, lysed-blood agar with kanamycin and menadione, blood agar with nalidixic acid 10 mg/L, and MacConkey's Agar (Oxoid). Incubation conditions were as previously described and all plates were examined after incubation for 24 and 72 h.

Identification of Isolates

All isolates were identified by normal laboratory procedures (Cowan and Steel 1974; Pease, 1983).

Results

From a total of 7372 blood-culture sets studied over a 2-year period, 572 (7·8%) yielded one clinically significant organism at the first subculture and 25 yielded more than one significant isolate. The 572 culture sets that yielded a single significant organism and 55 sets from which organisms regarded as contaminants were isolated were sub-cultured again after incubation for a further 3 and

Table. Microbial species isolated after repeated subculture

Organisms isolated	Time of subculture (days)		
	3 only	3 and 10 or 14	10 or 14 only
Enterobacteriaceae	0	25	13
Non-fermenting gram-negative bacilli	0	3	1
<i>Staphylococcus aureus</i>	0	4	0
<i>Staph. epidermidis</i>	0	4	0
β -haemolytic streptococci	0	3	1
Enterococci	0	6	2
α -haemolytic streptococci	1	5	
<i>Bacteroides fragilis</i>	0	4	2
<i>Clostridium</i> sp.	1	3	0
Other anaerobes	0	4	0
<i>Candida albicans</i>	0	1	0
Total	2	62	19

10 or 14 days. A further 83 isolates from 71 blood culture sets were obtained; the additional isolates were detected in 56 (79%) of these sets at the 3-day subculture. Details of these 83 organisms are shown in the table. Thirty-one patients were female and the overall mean age of patients was 55.1 years (SD=19.8). The additional organisms were also isolated from sites other than blood in 26 (37%) patients—intravenous catheter tips 10; wounds, exudates etc. 9; urine 7. Only one of the initially contaminated blood-culture sets yielded an additional significant isolate. Of the 25 patients with polymicrobial septicaemia detected at the initial subculture, 14 (56%) died, whereas of the 71 patients with polymicrobial detected at repeated subculture only 16 (23%) died ($\chi^2=9.638$; $p<0.01$). In an age- and sex-matched control group with comparable underlying clinical conditions, 18 (25%) died.

Discussion

Incidences of polymicrobial septicaemia have been reported to be between 6 and 18% (Curtin *et al.*, 1961; Hochstein *et al.*, 1965; Hermans and Washington, 1970) and there are indications that the isolation of more than one species of bacterium from patients with septicaemia is increasing (Saravolatz *et al.*, 1978; Kiani *et al.*, 1979). Because of its reported association with increased mortality (Hermans and Washington, 1970; Kiani *et al.*, 1979),

attempts have been made to increase the detection of polymicrobial septicaemia in recommending repeated subculture of previously positive blood cultures after incubation for a further 3 days (Hansen and Hetmanski, 1983) or 14 days (Weinstein *et al.*, 1983). By so doing, Hansen and Hetmanski (1983) increased their polymicrobial bacteraemia rate from 9.1% to 27% of clinically significant patient episodes. This had a direct impact on patient management. Weinstein *et al.* (1983) reported a rate of 18.4% and offered the repeat subculturing of previously positive cultures as one possible explanation of this high figure. This approach has been discounted by other workers (Stetz and Martin, 1985; Towne and Gay, 1985) who found that repeated subculturing after 3 days did not increase the incidence, added significantly to the time and cost of evaluating blood cultures, and did not improve overall patient care. Our study has shown an increase in incidence from 25 (4.2% of total positive blood cultures) to 96 (16%), with the majority (79%) found at the 3-day subculture when it would be of most help in patient management. Only 21% were found at the 10- or 14-days subculture recommended by Weinstein *et al.* (1983). Hansen and Hetmanski (1983) used a radiometric system (BACTEC, Johnson Laboratories) as did Towne and Gay (1985). Weinstein *et al.* (1983) sampled 15 ml of blood in a broth medium whereas Stetz and Martin (1985) sampled 20 ml of blood and we obtained c. 30 ml of blood from our patients. It has been shown that there is a direct relationship between the volume of blood sampled and the yield of positive cultures (Ilstrup and Washington, 1983). Thus the smaller inoculum of blood (7–10 ml) used in the BACTEC system may increase the time necessary to detect microbial growth by subculture and, therefore, re-culturing after 72 h could provide the time required to detect those organisms present which were not isolated at an earlier time. The patients studied by Weinstein *et al.* (1983) included a large proportion of immunocompromised patients which could explain the high incidence they report. This phenomenon has also been reported by others (Hermans and Washington, 1970; Kiani *et al.* 1979) and in our study the patients in whom a subsequent organism was found predominantly suffered from haematological malignancies, carcinoma, or hepatic or renal disorders, 37 (52%) patients being on immunosuppressive or cancer chemotherapy. Invasive procedures also appeared to be important; 34 (48%) of patients had an intravenous or urinary catheter *in situ*. Inappropriate antibiotic therapy is associated with an increased incidence of bacteraemic shock and death

(Kreger *et al.*, 1980b) but because most of our patients were suffering from serious underlying disease, most of them were receiving more than one antibiotic. As a result additional antibiotics were only considered necessary in 6 (8.5%) of these later diagnosed patients.

Is the increase in polymicrobial isolates of significance to the patient? We found a significantly increased mortality (56%) in those patients with primary polymicrobial septicaemia, but no increase in those patients in whom additional isolates were found subsequently when compared with an age- and sex-matched control group with comparable underlying conditions whose blood cultures yielded only single bacterial species (23% and 25% respectively). This finding was similar to that of Kiani *et al.* (1979). Thus, although repeat subcultures increased

the detection of additional isolates, it did not help in the management of most patients. In these patients, the additional organisms seem to be present originally, but in too low numbers to be of consequence. This is reflected in the significantly lower mortality rate. Only one of the contaminated blood-culture sets yielded an additional significant isolate. As in a previous report (Spencer and Nicol, 1985) we found that repeating subcultures after incubation for 3 days increased polymicrobial isolation, but that such a procedure, whilst increasing the time and cost of processing blood culture samples, did not benefit the vast majority of patients.

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