RAPID DETECTION OF BACTERAEMIA BY EARLY SUBCULTURE

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SUMMARY. Inspection and blind subculture was carried out on 7031 consecutive blood cultures at 10 p.m. on the day they were received. Analysis of the results after a 2-year period showed that 119 out of 237 (50%) bacteraemic patients were detected at this stage. Preliminary sensitivity tests were done at this time and their results were available within 24 h of the blood cultures being received. Thus earlier specific therapy was possible in 50% of the cases of bacteraemia.

INTRODUCTION

The rapid detection, identification and antibiotic susceptibility testing of bacteria from blood cultures is of utmost importance in the successful management of the septicaemic patient (Tilton, 1982). However, the optimum time for routine subculture is still debated. Most laboratories routinely subculture after incubation for 1 day (in practice, 16–24 h) so that the earliest positive results are confirmed on solid media 48 h after collection. Our evaluation of 'blind' early subculture, in the absence of visible evidence of microbial growth, was prompted by a recent American report that this procedure could yield positive results up to 24 h earlier than conventional methods (Sliva and Washington, 1980). In our prospective study we have analysed the results of all routine blood cultures examined during a 2-year period in a diagnostic laboratory.

MATERIALS AND METHODS

Culture Media. The following blood culture media were used in 125-ml medical flat bottles.

(i) The aerobic medium consisted of a bi-phasic medium with 35 ml of Columbia Agar (Lab M, Salford) as the solid phase and 45 ml of Brain Heart Infusion broth (Lab M), containing polyanethol sulphonate (Liquoid, Roche) 0.025% and thymidine (BDH) 0.4%, as the liquid phase. The solid phase was inoculated daily by tilting the bottle so that the broth ran over the slope. Bottles were only opened if there was evidence of growth in that bottle or in the accompanying anaerobic medium. (ii) Following a previous investigation, the anaerobic medium used routinely was Fastidious Anaerobe Broth (FAB; Lab M), a medium supplied commercially (Ganguli, Turton and Tillotson, 1982). Each bottle was inoculated with 3–5 ml of blood. Blood cultures taken after normal working hours were placed in an incubator within easy access for junior doctors and transported to the laboratory the following morning. The same procedures were followed during weekends and holidays.

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Subcultures. Blood cultures taken after laboratory hours were examined visually the following morning. Cultures showing evidence of growth of bacteria, e.g., turbidity or haemolysis, were examined further by Gram's stain and subculture. Bottles that were visually negative at this time and all cultures received during that day (Day 1) were inspected at 10 p.m. and the FAB anaerobic bottle was also routinely subcultured at this time (5–13-h incubation). Further routine subcultures were done from the FAB bottle on Day 4 and both bottles were finally opened and subcultured after incubation for 7 days. Bottles were examined visually every day.

Subcultures were done in a Class II Safety Cabinet (Microflow) with sterile disposable plastic loops of 10- μ l volume (NUNC, Denmark). Half plates of blood agar (Columbia; Lab M), blood agar with menadione 0.001% and haemin 0.005%, and heated blood agar were inoculated at each subculture. Cultures on blood agar were incubated in air, cultures on blood agar with menadione and haemin were incubated anaerobically with 10% CO₂ (Raven Anaerobic Cabinet, Forma Scientific, OH, USA) and cultures on heated blood agar were incubated in air with 5% CO₂. The heated-blood-agar plates used for subculture contained FAB as a base since work in this Department has shown that this modification allowed the isolation of pyridoxal-independent streptococci (Tillotson, 1981). All plates were examined after incubation for 24 and 48 h. The use of an anaerobic cabinet avoided interruption of anaerobic growth at this stage.

Positive Cultures. The medical laboratory scientific officer (MLSO) was instructed to contact a senior member of the laboratory medical staff when positive blood cultures were first detected by microscopy during the day or at 10 p.m., including weekends. The clinical significance of positive cultures was thus determined by a combination of the numbers of blood cultures found positive from a patient and by discussion between the laboratory medical staff and clinical colleagues. Advice on the choice and dosage of antibiotic therapy was also offered at this time.

TABLE I
Blood cultures examined and time taken to detect bacteraemia

	Number (%) of cultures in		
	First year	Second year	Total
Total blood cultures	3282	3749	7031
Significant positive cultures	194 (6)	216 (6)	410 (6)
Transient bacteraemias of doubtful significance or contaminated	472 (14)	389 (10)	861 (12)
Number of patients examined	1515	1656	3171
Number with significant bacteraemia	117 (8)	120 (7)	237 (8)
Detected by inspection ($\leq$ 13h)	16 (14)	23 (19)	39 (17)
Detected following blind subculture ($\leq$ 13h incub.)	48 (41)	32 (27)	80 (34)
Total detected, on solid medium, within 24h	64 (55)	55 (46)	119 (50)
Additional positives detected by inspecting next day ($\leq$ 24h)	14 (12)	34 (28)	48 (20)
Total cases reported positive within 24h	78 (67)	89 (74)	167 (71)

RESULTS

From a total of 7031 blood cultures from 3171 patients in a 2-year period (table I), 410 cultures (6%) were found to be positive and clinically significant. These cultures were obtained from 237 bacteraemic patients. Of these 237 patients, 39 (17%) had bacteraemia detected by visual and microscopic examination at 10 p.m. on the same day (≤ 13 -h incubation) and reported by telephone at that time. Blood cultures from a further 80 (34%) patients were found to be positive when plates from the blind subculture were examined the following morning. Thus, a total of 119 (50%) were

TABLE II
Time taken to detect bacteraemias due to individual micro-organisms

Organism	Number of bacteraemias first detected by inspection or subculture on Day								Total
	1	2	3	4	5	6	7 & 8		
<i>Escherichia coli</i> †	15	27 (17)*	9	3				54	
<i>Klebsiella aerogenes</i>	4	5 (3)		1				10	
<i>Klebsiella oxytoca</i>	0	4 (4)	1					5	
<i>Enterobacter cloacae</i>	0	3 (2)						3	
<i>Enterobacter aerogenes</i>	0	1 (1)						1	
<i>Enterobacter sakazaki</i>	1							1	
<i>Enterobacter agglomerans</i>	1	1 (1)						2	
<i>Serratia</i> spp.	1	5 (3)	2					8	
<i>Proteus mirabilis</i> ‡	2	7 (3)	1	1				11	
<i>Proteus morgani</i>	2							2	
<i>Providencia stuarti</i>			1					1	
<i>Salmonella typhimurium</i>	0	3 (2)						3	
<i>Salmonella virchow</i>			1					1	
<i>Pseudomonas aeruginosa</i>			2					2	
<i>Pseudomonas cepacia</i>			1					1	
<i>Aeromonas hydrophila</i>	0	1 (1)						1	
<i>Neisseria gonorrhoeae</i>				1	1			2	
<i>Neisseria meningitidis</i>			1					1	
<i>Haemophilus influenzae</i>				1				1	
<i>Staphylococcus aureus</i>	8	22 (9)	8	2	2			42	
<i>Staphylococcus epidermidis</i>	0	3 (3)	3	2				8	
<i>Streptococcus pneumoniae</i>	3	23 (14)	2	3	1			32	
<i>Streptococcus</i> sp. Lancefield grp A		2 (2)	2					4	
<i>Streptococcus</i> sp. Lancefield grp B		1 (1)			1			2	
<i>Streptococcus</i> sp. Lancefield grp D	1	6 (5)						7	
<i>Streptococcus milleri</i>		3 (3)	1	2				6	
<i>Streptococcus mitior</i>			1					1	
<i>Streptococcus sanguis</i>		1 (1)						1	
<i>Streptococcus salivarius</i>			1					1	
<i>Streptococcus bovis</i> type 1		2 (2)						2	
<i>Streptococcus</i> sp. (not typed)		1						1	
<i>Listeria monocytogenes</i>		1 (1)						1	
<i>Clostridium perfringens</i>	1	1 (1)	1	1				4	
<i>Bacteroides fragilis</i>		2 (1)	5	1		1		9	
'Polymicrobial' ††		1						1	
<i>Corynebacterium</i> sp.		1						1	
<i>Candida albicans</i>		1	1	2				4	
Total	39	128 (80)	44	20	5	1	0	237	

* ( ) = bacteraemias detected from blind initial subculture of FAB on Day 1, and included in positives detected on Day 2.

† Two cultures mixed with *B. fragilis*.

‡ One culture mixed with *B. fragilis*.

†† Mixed growth of *Bacteroides thetaiotaomicron*, *Peptococcus* sp., *Streptococcus milleri*, *Actinomyces viscosus*.

positive on a solid medium, 39 with primary sensitivity test results, within 24 h. Routine visual examination of the bottles on Day 2 enabled a further 48 to be detected by Gram's stain bringing the total number of positive results to 167 (71%) in the first 24 h. The figures for the two 12-month periods were similar. When the bottles that were shown to be positive after 'blind' subculture were re-examined visually the following morning, several did not even then show evidence of growth. This was particularly noted with streptococci and with some anaerobes that do not cause early turbidity in broth culture.

Details of the organisms isolated from 237 bacteraemic patients in the 2 years and the time of their first detection and reporting are shown in table II. The early inspection and subculture at 10 p.m. (bottles incubated 5–13 h) enabled us to report, with preliminary sensitivity test results, 32 out of 54 *Escherichia coli*, 17 out of 32 *Streptococcus pneumoniae* and 17 out of 42 *Staphylococcus aureus* infections within 24 h. Infections with anaerobes or *Candida* spp. were, however, generally detected after incubation for 48 or 72 h.

DISCUSSION

It is widely recommended that routine aerobic subculture of incubated blood cultures should be done 1 or 2 days after collection (Bartlett, Ellner and Washington, 1974; Shanson, 1978). American studies have previously demonstrated the value of an earlier routine subculture done on the same day that the culture is taken. Todd and Roe (1975) detected 85% of their clinically-significant positive cultures by subculturing between 4 and 14 h after collection. Harkness *et al.* (1975) detected 48% of 128 positive cultures after subculture between 3 and 19.5 h (mean 9.5 h) after collection. Sliva and Washington (1980) compared the value of subculturing after incubation for 1–6 h, 6–12 h and 12–17 h. Only 10% of isolates were detected when subcultured within 6 h but 36% (127 out of 350 blood cultures) were positive after 6–12 h and 63% (114 out of 181 blood cultures) were positive after incubation for 12–17 h. The latter workers recommended that routine subcultures should be performed between 6 and 17 h after blood collection. The British viewpoint was, however, expressed by Shanson (1978) who recommended that routine subcultures be performed after incubation for 1, 2 and 7 days; subculturing within the first 12 h was not considered of proven value. A prospective comparison of early routine subculture with conventional subculture would prove the benefit of our method beyond doubt. This is impractical because of the large volume of blood required—approximately 60 ml from each patient, the majority of whom are elderly and quite ill.

In the present study an early examination and routine subculture on the same day enabled us to detect, in the first and second years respectively, 64 out of 117 (55%) and 55 out of 120 (46%) bacteraemic patients within 24 h. Early subculture allows preliminary identification and sensitivity tests to be performed within this initial period, thus making earlier specific therapy possible. It also permits rapid detection of 'polymicrobial' septicaemia which again has an important bearing upon therapy.

The additional cost of this system is that of one "call out" for an MLSO after normal laboratory hours, currently £7.97, a day. It also requires a senior member of the laboratory medical staff to be available for discussion of the clinical significance of the findings with colleagues from the ward. The long range (25 miles) radio call system

currently in operation meets this need without undue loss of mobility and freedom. An examination of contamination rates during the period of this investigation when compared with earlier periods did not reveal any increase due to this procedure. We, therefore, strongly recommend early subculture as an essential part in any diagnostic blood culture system.

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