CETRIMIDE-NALIDIXIC ACID AGAR AS A SELECTIVE MEDIUM FOR *PSEUDOMONAS AERUGINOSA*

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THE selective medium for *Pseudomonas aeruginosa*, containing 0·03 per cent. cetrimide in Lemco agar base (Lowbury and Collins, 1955), was later modified by Brown and Lowbury (1965) to give more strongly fluorescent growth by the use of King's medium B (King, Ward and Raney, 1954) as the base. Both media have been found useful in the bacteriological examination of burns, urine, sputum and other pathological sources. Although cetrimide agar is strongly selective for *Ps. aeruginosa*, some strains of Gram-negative bacilli (especially *Klebsiella* spp. and *Providencia*) can grow on it. To suppress the growth of these organisms, Tinne *et al.* (1967) added 5 μg per ml nalidixic acid to an agar medium containing 0·03 per cent. cetrimide. Goto and Enomoto (1970) obtained better results with a medium containing 0·02 per cent. cetrimide and 15 μg per ml nalidixic acid.

We report here a comparison of 0·03 per cent. cetrimide agar (Brown and Lowbury) with a medium containing 0·02 per cent. cetrimide and 15 μg per ml nalidixic acid in the same agar base for the isolation of *Ps. aeruginosa* from burns.

MATERIALS AND METHODS

Cetrimide agar. The medium containing 0·03 per cent. cetrimide was prepared in the manner described by Brown and Lowbury. The basal medium consisted of Proteose peptone no. 3 (Difco), 20 g; New Zealand agar, 15 g; glycerol, 10 g; distilled water, 1000 ml. It was adjusted to *p*H 7·2 and autoclaved for 15 min. at 121°C. The following ingredients were added to 100 ml of the melted base: 1 ml. of a 15 per cent solution of K_2HPO_4 (anhydrous) and 1 ml of a 15 per cent. solution of $MgSO_4$, $7H_2O$; these solutions were prepared with distilled water and Seitz-filtered. A 2 per cent. Seitz-filtered solution of cetrimide (B.P.) was added to the basal medium to give a final concentration of 0·03 per cent.

Cetrimide-nalidixic acid agar. For the study on burns, the concentrations of cetrimide (0·02 per cent.) and nalidixic acid (15 μg per ml) used by Goto and Enomoto were used. Preliminary examination of a range of combinations of cetrimide and nalidixic acid in agar, with tests for viable counts of small inocula, led us independently to select these concentrations for the optimal growth of *Ps. aeruginosa* (six strains tested) and for the suppression of strains of other Gram-negative bacilli that had grown on cetrimide agar (*Klebsiella* spp., *Escherichia coli*, *Proteus mirabilis* and *Bact. anitratum*).

Comparison of cetrimide agar with cetrimide-nalidixic acid agar. A series of 5358 consecutive swabs from burns in this Unit were inoculated in parallel on cetrimide agar (CA) and on cetrimide-nalidixic acid agar (CNA). In the routine examination of burns our practice was to inoculate swabs moistened with nutrient broth in sequence on horse blood agar (containing 4 per cent. New Zealand agar), cetrimide agar and cooked meat broth. To allow equal proportions of CA and CNA plates to receive the first inoculation after blood agar, the order of inoculating them was reversed with successive swabs. The selective media were inoculated in marked areas without spreading the inoculum. All the plates were examined after 18 hours' incubation at 37°C by daylight and under ultraviolet irradiation for the detection of the yellow-green or blue-green fluorescent growth characteristic of *Ps. aeruginosa* (Lowbury, Lilly and Wilkins, 1962). Cooked meat broth cultures were subcultured, after incubation at 37°C for 24 hr, to plates of blood agar (with 4 per cent. agar) and by spot

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inoculation to CA and CNA; the subcultures were examined by daylight and by ultraviolet irradiation.

Characteristic growth showing typical fluorescence under UV irradiation on blood agar or on the selective media was described as presumptive *Ps. aeruginosa*; 143 of these strains, including isolates from each medium, were subjected to the following confirmatory tests: (1) oxidase reaction (Kovács, 1956); (2) oxidative metabolism of glucose (Hugh and Leifson, 1953); (3) pyocyanin production on Wahba and Darrell's medium (1965); (4) nitrate reduction to gas by the Griess-Ilosvay method (Wilson and Miles, 1964); and (5) growth at 42°C in nutrient broth. All the strains of presumptive *Ps. aeruginosa* tested were consistent with the description of this species.

Results

The table shows the number of swabs from which bacterial growth occurred and the number from which presumptive *Ps. aeruginosa* were isolated on the two selective media, both by direct inoculation and by subculture from cooked meat broth cultures.

Table

Comparison of cetrimide agar and cetrimide-nalidixic acid agar for isolation of Ps. aeruginosa from a total of 5358 swabs from burns

Medium	Number of swabs from burns that yielded			
	on direct inoculation		on subculture from cooked meat broth	
	bacterial growth	presumptive *Ps. aeruginosa*	bacterial growth	presumptive *Ps. aeruginosa*
Cetrimide agar	535	285 (5·3 per cent.)	1290	477 (8·9 per cent.)
Cetrimide-nalidixic acid agar	335	329 (6·1 per cent.)	531	513 (9·6 per cent.)

On direct inoculation, CNA gave an appreciably greater yield (6·1 per cent.) of presumptive *Ps. aeruginosa* than CA (5·3 per cent.), but the proportion of plates showing bacterial growth was much higher for CA than for CNA. On subculture from cooked meat broth the difference in selectiveness of the media was even more pronounced, but the difference in the proportions of swabs yielding presumptive *Ps. aeruginosa* on the two media was smaller than on direct inoculation. Direct inoculation of blood agar, which preceded inoculation of the selective media, gave a higher yield of presumptive *Ps. aeruginosa* (295) than inoculation on CA (285), but a lower yield than CNA (329).

These results show CNA to be a more satisfactory selective medium for *Ps. aeruginosa* than CA. This is due both to better suppression of other Gram-negative bacilli on CNA, and to a smaller degree of inhibition of *Ps. aeruginosa* by CNA than by CA. A relatively small number of bacteria, other than *Ps. aeruginosa*, grew on CNA, either from direct inoculation of swabs or on subculture from liquid medium. Cetrimide agar, with 0·03 per cent. cetrimide, appeared to have stronger inhibitory action against small inocula of some strains of *Ps. aeruginosa* than had been found in earlier studies, and the reduction of its concentration to 0·02 per cent. was probably an important factor in the improved quality of the new medium.

SUMMARY

A series of 5358 consecutive swabs from burns were inoculated in duplicate on 0·03 per cent. cetrimide agar and on an agar medium containing 0·02 per cent. cetrimide with 15 μg per ml nalidixic acid. A larger number of swabs yielded *Ps. aeruginosa* and a smaller number yielded growth of other bacteria on cetrimide-nalidixic acid than on cetrimide agar.

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