

# Inhibition of *Pseudomonas aeruginosa* from cystic fibrosis by selective media

K FONSECA,\* JANE MACDOUGALL,† TL PITT†

From the \*Department of Microbiology, Wexham Park Hospital, Slough, and the †Division of Hospital Infection, Central Public Health Laboratory, London

**SUMMARY** *Pseudomonas* Isolation Agar (selective agent, Irgasan, 25 mg/l) and *Pseudomonas* Selective Agar (selective agents, cetrимide 200 mg/l and nalidixic acid 15 mg/l) inhibited some strains of *P aeruginosa* from cystic fibrosis sputum but did not inhibit isolates from other sources. Of 200 cystic fibrosis isolates, 22 were inhibited by 16 mg/l Irgasan, 45 by 8 mg/l nalidixic acid, and 15 by 128 mg/l cetrимide. We recommend that cystic fibrosis sputum should be cultured on selective and non-selective media to maximise the isolation of *P aeruginosa*.

*Pseudomonas aeruginosa* is commonly isolated from the sputum of patients with cystic fibrosis, and colonisation is often associated with a poor prognosis and increased morbidity and mortality.<sup>1</sup> It is desirable and necessary to maximise the rate of isolation of *P aeruginosa* from these patients, especially during the monitoring of treatment with antibiotics.

During a longitudinal study of the bacterial flora of the sputum of patients with cystic fibrosis we became aware of a decrease in the isolation rate of *P aeruginosa* after the introduction of a selective agar for the recognition of this species. We determined, therefore, the minimum inhibitory concentrations of the selective agents Irgasan, nalidixic acid, and cetrимide in two proprietary media for strains of *P aeruginosa* from cystic fibrosis sources and non-cystic fibrosis sources and compared the viable counts of some strains on selective and non-selective agar media.

## Material and methods

### CULTURES

Two hundred cultures of *P aeruginosa* were isolated on the non-selective medium from multiple specimens of sputum from 80 patients with cystic fibrosis attending an outpatient clinic. One hundred non-cystic fibrosis cultures were selected from routine clinical isolates submitted to the Division of Hospital Infection for typing. *P aeruginosa* NCTC10662 was used as a control for minimum inhibitory concentration determinations.

### MEDIA

*Pseudomonas* Isolation Agar (PIA, Difco, London) containing the selective agent Irgasan at 25 mg/l and *Pseudomonas* Selective Agar (PSA, Oxoid Ltd, Basingstoke) containing nalidixic acid and cetrимide at concentrations of 15 mg/l and 200 mg/l, respectively, were prepared according to manufacturer's instructions. King's "A" agar<sup>2</sup> was used as a non-selective control. Broth was tryptone soy broth (TSB, Oxoid).

### SPUTUM SPECIMENS

Sputum samples were digested by pancreatin enzyme (BDH, Poole, Dorset) at 37°C for two hours<sup>3</sup> and plated on selective and non-selective agar. Plates were incubated at 37°C for 48 hours, and the growth was scored semiquantitatively.

### MINIMUM INHIBITORY CONCENTRATION DETERMINATION

Irgasan BP 300 (Ciba-Geigy, Horsham) and nalidixic acid (Sterling Winthrop, London) were kindly provided by the manufacturers. Cetrимide was cetyltrimethylammonium bromide (BDH, Poole). Minimum inhibitory concentration determinations were performed by an agar dilution method<sup>4</sup> in tryptone soy agar (Oxoid). Overnight tryptone soy broth cultures of bacteria were diluted in quarter strength Ringer solution (Oxoid) to give a suspension containing about 10<sup>4</sup> bacteria/ml. Diluted cultures (30 µl volumes) were applied with a multipoint inoculator (Denley Instruments Ltd, Sussex) to agar plates that contained various concentrations of the selective agents. Plates were incubated at 37°C for 18 hours, examined, and then reincubated for a further 24

hours to detect slow growing colonies. The minimum inhibitory concentration was taken as the lowest concentration of the agent to inhibit the growth of the culture after 48 hours. Surface viable counts were performed by a standard method.<sup>5</sup>

## Results

During the processing of the infected sputum samples we found that the selective medium in routine use, *Pseudomonas* Isolation Agar, inhibited some strains of *P aeruginosa*. To confirm this observation 132 sputum samples were plated both on King's "A" agar and *Pseudomonas* Isolation Agar, and 102 of these yielded *P aeruginosa* in pure or mixed culture; 12 specimens gave no bacterial growth on either medium. The growth of 43 (42.2%) of the *P aeruginosa* cultures was reduced on *Pseudomonas* Isolation Agar when compared with that on the non-selective agar. The remaining 59 cultures gave similar quantities of growth on both media. We decided, therefore, to investigate the antipseudomonas activity of the active agents both in *Pseudomonas* Isolation Agar and *Pseudomonas* Selective Agar, another widely used selective medium for *P aeruginosa*.

The minimum inhibitory concentration of the three selective agents for 200 cystic fibrosis cultures of sputum and 100 non-cystic fibrosis cultures was determined. Table 1 shows the cumulative percentages of cultures inhibited by each agent. There was an appreciable contrast between the two groups of cultures in their sensitivity to Irgasan. All but one of the non-cystic fibrosis isolates grew on agar containing

1024 mg/l Irgasan while those from cystic fibrosis patients varied in their sensitivity to this compound. To come close to the concentration of Irgasan in *Pseudomonas* Isolation Agar 32 mg/l was chosen as the breakpoint concentration of Irgasan for the differentiation of resistant and sensitive cultures. Similarly, the breakpoint concentration chosen for nalidixic acid was 16 mg/l and 256 mg/l for cetrimide. Using these breakpoints, 18% of cystic fibrosis cultures were sensitive to Irgasan, 34% to nalidixic acid, and 9% to cetrimide.

Table 2 shows the degree of cross sensitivity of cystic fibrosis cultures to the three agents. Of 36 cultures sensitive to Irgasan, 27 and 10 were also sensitive to nalidixic acid and cetrimide, respectively. About half (33 of 68) of the cultures sensitive to nalidixic acid were inhibited by Irgasan at the breakpoint concentration, and 11 of the 18 cultures sensitive to cetrimide were sensitive to both Irgasan and nalidixic acid.

Sensitivity of cystic fibrosis isolates to Irgasan and cetrimide was significantly associated with the production of mucoid colonies on King's "A" agar as 25 of 36 (69%) ( $0.02 > p > 0.01$ ) cultures sensitive to Irgasan and 13 of 18 (72%) of cultures sensitive to cetrimide were mucoid. In contrast, only 16 of 68 (23.5%,  $p < 0.001$ ) of cultures inhibited by 16 mg/l nalidixic acid produced mucoid colonies.

To assess the effect of the selective agents on the growth of *P aeruginosa* on the proprietary media viable counts of 10 cultures, which had a minimum inhibitory concentration for Irgasan of 4 mg/l to 32 mg/l, were performed on *Pseudomonas* Isolation Agar. Similarly, 10 cultures with various minimum

Table 1 Inhibition of cultures of *P aeruginosa* from cystic fibrosis and non-cystic fibrosis sources by Irgasan, cetrimide, and nalidixic acid

Agent	Source	No of cultures	Cumulative percentage of cultures inhibited by agent (mg/l)									
			1024	512	256	128	64	32	16	8	4	
Irgasan	Cystic fibrosis	200	100.0	33.0	31.5	29.0	24.5	18.0	11.0	5.0	3.0	
	Non-cystic fibrosis	100	100.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.0	
Nalidixic acid	Cystic fibrosis	200	100.0	99.5	98.0	94.0	82.5	56.5	34.0	22.5	15.0	
	Non-cystic fibrosis	100	100.0	98.0	97.0	94.0	83.0	42.0	1.0	0.0	0.0	
Cetrimide	Cystic fibrosis	200	100.0	17.5	9.0	7.5	5.0	2.0	2.0	2.0*		
	Non-cystic fibrosis	100	100.0	13.0	0.0	0.0	0.0	0.0	0.0	0.0		

\*Strains with minimum inhibitory concentration  $\leq 8$  mg/l.

Table 2 Cross sensitivity of *P aeruginosa* isolated from cystic fibrosis to Irgasan, cetrimide, and nalidixic acid

Agent (breakpoint)	No of cultures	No of cultures sensitive to:		
		Irgasan	Nalidixic acid	Cetrimide
Irgasan ( $\leq 32$ mg/l)	36	36	27	10
Nalidixic acid ( $\leq 16$ mg/l)	68	33	68	8
Cetrimide ( $\leq 256$ mg/l)	18	11	11	18

inhibitory concentrations for nalidixic acid (2 mg/l to 32 mg/l) and cetrимide (64 mg/l to 1024 mg/l) were tested on Pseudomonas Selective Agar. King's "A" agar was used as the non-selective control. The viable count of three of four cultures, which gave a minimum inhibitory concentration of 16 mg/l for Irgasan, was reduced by about 10 fold on Pseudomonas Isolation Agar, and four cultures with a minimum inhibitory concentration of 8 mg/l or below were completely inhibited by Pseudomonas Isolation Agar. Of the 10 cultures tested on Pseudomonas Selective Agar, four failed to grow and they were all sensitive to cetrимide at the concentration in the proprietary medium; three of these cultures were also sensitive to nalidixic acid. The viable count of two cultures, which were sensitive to nalidixic acid (minimum inhibitory concentration 8 mg/l) but fully resistant to cetrимide (minimum inhibitory concentration 1024 mg/l), was reduced by 1-log on Pseudomonas Selective Agar. In contrast, the culture that was sensitive to cetrимide (minimum inhibitory concentration 64 mg/l) but resistant to nalidixic acid (16 mg/l) was completely inhibited by the medium.

### Discussion

Selective media, especially cetrимide agar, are often used to isolate and identify *Paeruginosa* in a single step. A recent study concluded that a selective technique using cetrимide agar permitted the reliable quantitation of *Paeruginosa* in the liquefied sputum of patients with cystic fibrosis.<sup>6</sup> There was considerable variation in the viable count of *Paeruginosa* from patients in that study ( $4 \times 10^4$ – $8 \times 10^8$  colony forming units/g of sputum), and this variation may have been due to the sensitivity of cultures to the selective agent.

Some cultures of *Paeruginosa* from cystic fibrosis show increased sensitivity to cell wall antibiotics<sup>7</sup> and give minimum inhibitory concentration values of eight fold or less than the median for a particular antibiotic. Our data suggest that increased sensitivity of cystic fibrosis cultures also extends to the compounds Irgasan, cetrимide, and nalidixic acid. Assuming that our series of cultures is representative, then about one fifth of *Paeruginosa* in cystic fibrosis would be totally or partially inhibited by one or other

of the two selective media. In contrast, cultures from other sources, including respiratory isolates, do not exhibit such sensitivity to the selective agents.

El-Nima described results which suggested that cetrимide acts synergistically with ampicillin and other antibiotics against *Paeruginosa*, probably by increasing the permeability of the bacterial cell.<sup>8</sup> We did not investigate the synergism between cetrимide and nalidixic acid against cystic fibrosis cultures, but if it does occur then the proportion of the isolates inhibited by Pseudomonas selective agar may well be increased.

We recommend that the sputum from patients with cystic fibrosis should be cultured on both selective and non-selective media to maximise the isolation of *Paeruginosa*.

We thank Drs JC Batten and ME Hodson of the Brompton Hospital, London, for the specimens of sputum from their patients with cystic fibrosis.

### References

- 1 Penketh ARL, Pitt TL, Roberts D, Hodson ME, Batten JC. The relationship of phenotype changes in *Pseudomonas aeruginosa* to the clinical condition of patients with cystic fibrosis. *Am Rev Resp Dis* 1983;127:605–8.
- 2 King GO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescein. *J Lab Clin Med* 1954; 44:301–5.
- 3 Rawlins GA. Liquefaction of sputum for bacteriological examination. *Lancet* 1953;iii:538–9.
- 4 King A, Warren C, Shannon K, Phillips I. The in-vitro activity of cefotaxime compared with that of cefuroxime and cefoxitin. *Antimicrob Agents Chemother* 1980;6:479–94.
- 5 Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of blood. *J Hyg* 1938;38:993–5.
- 6 Wong K, Roberts MC, Owens L, Fife M, Smith AL. Selective media for the quantitation of bacteria in cystic fibrosis sputum. *J Med Microbiol* 1984;17:113–9.
- 7 Irvin RT, Govan JRW, Fyfe JAM, Costerton JW. Heterogeneity of antibiotic resistance in mucoid isolates of *Pseudomonas aeruginosa* obtained from cystic fibrosis patients: role of outer membrane proteins. *Antimicrob Agents Chemother* 1981;19:1056–63.
- 8 El-Nima EI. The synergism between cetrимide and antibiotics against *Pseudomonas aeruginosa*. *Zentralbl Bakteriologie Mikrobiol Hyg [A]* 1984;258:120–7.

Requests for reprints to: Dr TL Pitt, Division of Hospital Infection, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, England.