

# Isolation of *Burkholderia cepacia* by enrichment

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## Abstract

***Burkholderia cepacia* is a recognised cause of respiratory failure in patients with cystic fibrosis. The value of routine use of selective enrichment broth to increase the yield of *B cepacia* from cystic fibrosis sputa was investigated. Two hundred sputa from 86 adult and paediatric patients were cultured onto *B cepacia* selective agar and also in enrichment broth. The enrichment broths were subcultured after incubation onto *B cepacia* selective agar. Fourteen sputa from eight patients yielded *B cepacia*. In all cases the isolate was recovered from the primary selective agar as well as the enrichment broth subcultures. The routine use of enrichment for cystic fibrosis sputa is of unproven benefit and increases laboratory costs.**

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Keywords: *Burkholderia cepacia*; enrichment broth; selective media

*Burkholderia cepacia* is an important respiratory pathogen in adults and children with cystic fibrosis.<sup>1</sup> Although organisms presently identified as *B cepacia* have been shown to belong to several genomovars,<sup>2</sup> the problem of primary isolation remains. In the United Kingdom, solid selective media are commonly used to isolate pathogens such as *B cepacia* in respiratory secretions from patients with cystic fibrosis and to prevent overgrowth of mucoid *Pseudomonas aeruginosa*.<sup>3</sup> Selective enrichment broth was used to isolate *B cepacia* in a recent study, in addition to solid selective media, but its value was unproven.<sup>4</sup> Eighty per cent of the study population were younger than 15 years. Adult and older adolescent patients with cystic fibrosis are known to have a higher incidence of *B cepacia* infection than younger patients.<sup>5</sup> We investigated whether the use of selective enrichment broth would increase the yield of *B cepacia* from sputa obtained from adult as well as paediatric cystic fibrosis patients.

## Methods

Patients were seen at two different hospitals. Sputa were either homogenised with Sputasol (dithiothreitol; 1 mg/ml; Oxoid, Basingstoke, Hampshire, UK) for five to 10 minutes (hospital 1), or two to three purulent sections of each sputum sample were cultured without homogenisation (hospital 2). Equivalent aliquots of sputa were inoculated onto (1) Columbia blood agar (5% horse blood; Oxoid) incubated aerobically with 5% CO<sub>2</sub>, (2) chocolate (heated blood) agar incubated aerobically with 5% CO<sub>2</sub>, (3) McConkey agar, (4) mannitol salt

Table 1 Results of *B cepacia* study

	Hospital 1	Hospital 2
Number of patients	37	49
Age range (years)	1-16	18-35
Number of sputa examined	100	100
Number of <i>B cepacia</i> positive sputa	10	4
Number of patients with <i>B cepacia</i> in sputa	5	3
Age range of <i>B cepacia</i> positive patients (years)	12-16	19-22
Number of sputa with <i>B cepacia</i> cultured from BHIB only	0	0

BHIB, brain-heart infusion broth.

agar (Oxoid), (5) pseudomonas isolation agar (Difco Laboratories, West Molesey, Surrey), and (6) *B cepacia* selective agar (Mast Diagnostics, Bootle, Merseyside). All plates were incubated for 48 hours.

In addition, 0.5 ml portions of all sputa were inoculated into brain-heart infusion broth (BHIB) with added antibiotics (polymyxin B, 30 mg/l; vancomycin, 15 mg/l; amphotericin B, 1 mg/l) and incubated aerobically at 37°C. The selective enrichment broths were subcultured at 24 hours and 48 hours to *B cepacia* selective agar and each subculture was incubated for a further 48 hours. Identity of suspect colonies of *B cepacia* was confirmed using standard methods.

## Results

Two hundred sputa were collected from 86 patients (100 from each hospital) between July 1996 and January 1997 (table 1). No patient had more than three sputa collected during the study period. All patients from whom *B cepacia* was isolated were known to be *B cepacia* carriers before the study. Four patients (4.7%) yielded *B cepacia* from two sputa taken at least three months apart. Two other patients (one from each hospital) yielded *B cepacia* on two separate occasions two months apart. In all *B cepacia* positive sputa, the isolate was recovered from the primary cepacia selective agar as well as from the enrichment broth subcultures. *B cepacia* was recovered from the latter after 48 hours subculture on the solid media in most cases, by which time the primary selective plates had already yielded the organism. Four of 200 sputa (2%) yielded organisms from the enrichment broth subculture other than *B cepacia*, comprising *Aspergillus* sp (2), *Serratia* sp (1), and *Chryseomonas luteola* (1).

## Discussion

These results confirm those of a pilot study in which non-selective brain heart infusion enrichment broth of 190 cystic fibrosis sputa also failed to show any increase in isolation rates (Flanagan PG, Paull A; unpublished data).

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Concern has been expressed about possible person to person spread of *B cepacia* among cystic fibrosis patients and its potential role in causing respiratory failure.<sup>5,6</sup> Consequently there is pressure for early detection by the laboratory. Selective solid media have been shown to increase the yield of *B cepacia* from patients with cystic fibrosis.<sup>7</sup> Selective enrichment of sputa from affected children yielded a small increase (10% of a total of 10 sputa) in the number of *B cepacia* positive sputa in a study of 106 sputa and throat swabs.<sup>4</sup> Conversely, we found no increase in the detection rate of *B cepacia* in adult, adolescent, or paediatric patients using enrichment broth and failed to detect any carriers other than those previously known in this group. We found a slightly higher incidence of *B cepacia* at hospital 1 (13.5%), which served a paediatric cystic fibrosis patient group, than at hospital 2 (6.1%), which treated adult patients. This is surprising given the reported higher incidence of *B cepacia* in older cystic fibrosis patients,<sup>3</sup> but may be attributable to the less than optimal culture methods used in hospital 2. However staff at hospital 1 still care for a group of *B cepacia* patients from an outbreak of transmission of the organism in 1992,<sup>5</sup> and this may have distorted the relative frequency of isolation between the two groups.

A polymyxin containing enrichment broth was used in an environmental survey for *B cepacia* in a botanical complex.<sup>8</sup> However, routine use of enrichment for clinical specimens is not practised in many countries, as highly selective solid media of proven utility are used for primary isolation.<sup>9</sup>

As few as 100 *B cepacia* colony forming units can be detected by polymerase chain reaction (PCR) testing of sputa several months before the organism can be cultured.<sup>10</sup> In these circumstances enrichment may aid the culture

confirmation of *B cepacia* from specimens defined as positive by PCR. However PCR testing for *B cepacia* is not performed routinely in the United Kingdom and the cost of applying this test to all cystic fibrosis sputa would be prohibitive.

Inclusion of enrichment in the routine diagnostic protocol for patients with cystic fibrosis adds to laboratory costs and staff time for little proven benefit. Further evidence that an enrichment method is a worthwhile procedure needs to be provided before it is incorporated into standard laboratory procedures.

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