

Improved cultural detection of *Burkholderia cepacia* from sputum in patients with cystic fibrosis

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Abstract

Aims—To evaluate the sensitivity and specificity of two selective media for the isolation of *Burkholderia cepacia* from sputum specimens in patients with cystic fibrosis (CF).

Methods—In total, 149 expectorated sputum specimens from 113 patients with CF (32 *cepacia* colonised patients and 81 non-*cepacia* colonised patients) attending three CF centres were examined for the presence of *B cepacia* on two selective media: (1) MAST selective agar, a commercially available selective medium widely used in the UK and (2) BCSA (*B cepacia* selective agar), a new medium recently described, which is used predominantly in North America.

Results—*Burkholderia cepacia* was isolated from 53 of 149 (35.6%) specimens examined, representing 32 of 113 (28.3%) patients, using both the MAST and BCSA media. Growth was most rapid on BCSA with all (53 of 53) isolates detectable after 48 hours, compared with 50 of the 53 isolates on MAST agar, with the remaining three isolates detectable at five days. Twenty eight contaminants were identified on MAST agar and 13 on BCSA agar; mainly *Alcaligenes xylosoxidans* and yeast on MAST agar and *Flavobacterium indologenes* on BCSA medium. BCSA was equivalent to MAST agar in its ability to isolate *B cepacia* from patients with CF with a history of *B cepacia* infection.

Conclusions—The increased selectivity and reduced time to detection of BCSA makes it an attractive alternative to MAST. However, its present limited commercial availability in the UK may delay its use in routine diagnostic laboratories because of complications with media preparation and quality control.

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Keywords: *Burkholderia cepacia*; *Burkholderia cepacia* selective agar; MAST agar; cystic fibrosis

Burkholderia cepacia, a Gram negative bacillus, is a saprophyte in soil and river sediments causing “slippery skin” or “sour skin” rot in plants, such as onion and garlic.¹ Originally named *Pseudomonas cepacia*, it was renamed *Burkholderia cepacia* in 1992 when taxonomists showed that it was sufficiently different to the pseudomonas species, based on DNA–DNA hybridisation studies and 16S rRNA sequence alignments.² Over the past two decades its role as an important human pathogen has emerged. It has been shown to have particularly serious consequences for patients with cystic fibrosis (CF), sometimes resulting in accelerated pulmonary deterioration, with fatal necrotising pneumonia and bacteraemia.³

Because of its high transmissibility, multiple antibiotic resistance, and association with a poor prognosis, it is important to use reliable detection methods for this organism. Although molecular techniques are a reliable and accurate method of detection, many CF centres do not have access to such facilities on site for primary diagnostic microbiology.

Recently, Henry *et al* described a new selective medium for the conventional isolation of *B cepacia*, with superior characteristics over several other selective media available for this organism, which is used predominantly in North America but not in the UK.⁴ Presently, the MAST selective agar is the most commonly used selective medium in the UK. However, the study of Henry *et al* did not compare the new medium with the MAST medium and hence we designed our study to compare both the sensitivity and specificity of each medium for the detection of *B cepacia* from the sputum of patients with CF in the UK.

Materials and methods

PATIENT POPULATION

Fresh sputum specimens (volume range, 1–10 ml; n = 149) were collected after physiotherapy from adults with CF (table 1). Specimens were collected in sterile sputum containers and were transported to the laboratory at ambient temperature. Specimens received from the Belfast City Hospital were processed within 24 hours and those from Manchester and Royal Belfast Hospital for Sick Children sites within 48 hours of collection. All sputum was stored at ambient temperature before processing.

SPUTUM PROCESSING

Sputum specimens from patients were mixed with Sputasol (Oxoid, Basingstoke, Hampshire, UK) at a 1/1 dilution to reduce the viscosity of the sputum, thereby facilitating the

Table 1 Cystic fibrosis (CF) patient population examined

CF centre	Patient population	No. patients examined	No. patients colonised with <i>Burkholderia cepacia</i>	No. patients not colonised with <i>B cepacia</i>	Total no. specimens examined
Manchester	Adult	41	12	29	43
Belfast City	Adult	30	15	15	58
RBHSC	Children	42	5	37	48
Total		113	32	81	149

RBHSC, Royal Belfast Hospital for Sick Children.

Table 2 Contaminants isolated on MAST and BCSA media

Contaminating organisms identified	No. isolates identified on MAST medium	No. isolates identified on BCSA medium
<i>Pseudomonas</i> spp	5	4
<i>Pseudomonas aeruginosa</i>	5	0
<i>Stenotrophomonas maltophilia</i>	1	0
<i>Alcaligenes xylosoxidans</i>	4	1
<i>Flavobacterium indologenes</i>	1	5
<i>Aeromonas</i> sp	1	1
<i>Staphylococcus</i> sp	1	0
Yeast	9	0
<i>Aspergillus fumigatus</i>	1	2
Total	28	13

BCSA, *Burkholderia cepacea* selective agar.

streaking of plates. Sputum/Sputasol mixtures were incubated at 37°C for 30 minutes, followed by streaking of 10 µl on to one plate each of the BCSA and MAST formulations. BCSA phase II medium was prepared as described previously,⁴ and MAST selective plates were prepared in accordance with the manufacturer's instructions (MAST Diagnostics, Bootle, Merseyside, UK). Plates were incubated aerobically for 48 hours at 37°C in air and growth was assessed. Plates were subsequently stored at room temperature for a further five days before re-examination for growth.

PHENOTYPIC AND GENOTYPIC CONFIRMATION OF *B CEPACIA* ORGANISMS

Colonies from both selective plates were identified by various conventional biochemical tests, including Gram staining, the oxidase test, and growth on MacConkey agar. In addition, the API20NE test (BioMérieux, Marcy L'Etoile, France) was used for identification of Gram negative non-fastidious rods, which were considered to be contaminating organisms on the BCSA medium. This was set up according to the manufacturer's instructions and incubated for 48 hours at 30°C.

All presumptive positive colonies growing on both selective media were examined using species specific polymerase chain reaction (PCR) assays for *B cepacia*, *Pseudomonas* species, and *Pseudomonas aeruginosa*, as described previously.^{3, 6}

Results and discussion

Growth of *B cepacia* was detected on both MAST and BCSA media in 53 of 149 (35.6%) sputum specimens examined, from 32 of the 113 (28.3%) patients. The growth after 48 hours and five days was recorded for both media, allowing a comparison of the speed of growth of *B cepacia*. Growth was most rapid on BCSA, with all (53 of 53) isolates growing within 48 hours, compared with 50 of 53 (94.3%) isolates on MAST agar, with the remaining three isolates detectable at five days. Twenty eight contaminants were identified on MAST agar and 13 on BCSA agar (table 2).

A recent study⁴ described a new selective medium, BCSA, and showed it to be more efficient than OFPBL (oxidation-fermentation polymyxin-bacitracin-lactose) and *P cepacia* selective agars. This investigation showed that a

total of 190 of 191 (99.5%) isolates of *B cepacia* from patients with CF grew on BCSA, 100% on OFPBL, and 179 (94.2%) on *P cepacia* agar. It also showed that growth was most rapid on BCSA, with 201 of 205 (98%) isolates being detected within 24 hours, compared with 182 (88.8%) on OFPBL and 162 (79%) on *P cepacia* agars. Of the 189 other Gram positive and Gram negative organisms tested, 10 (5.3%) grew on BCSA, compared with 19.6% and 13.8% for the OFPBL and *P cepacia* selective agars, respectively. The addition of vancomycin to the BCSA medium, to produce the phase II medium, lowered the false positivity rate to 3.7% through inhibition of enterococcal growth. Phase II BCSA had no effect on the growth of Gram negative organisms, and we therefore used this BCSA medium in our study because of its greater selectivity. However, the original study⁴ and a subsequent study⁷ did not compare BCSA with the commercial MAST selective medium widely used in the UK.

Another analysis of selective media available for *B cepacia* detection concluded that MAST was the best and most user friendly medium available.⁸ MAST selective agar is the most widely used medium in the UK for routine detection of *B cepacia* colonisation in patients with CF. In this assessment, MAST agar was compared with *P cepacia* agar and OFPBL. In total, 54 of 54 *B cepacia* strains, as confirmed by phenotypic and genotypic testing, were isolated on *P cepacia* and MAST media, but only 50 strains were recovered on OFPBL. However, *P cepacia* medium was complex to prepare and was shown to be less selective than MAST, so that MAST was concluded to be the preferred medium. However, this study did not compare MAST agar with the new BCSA medium. In another study,⁹ Hutchinson *et al* showed that at least 16 species of colistin resistant, Gram negative bacterial species could be recovered from the MAST medium.

In our present study, patients with CF (who were both colonised and non-colonized with *B cepacia*) from three UK centres were examined for the presence of viable *B cepacia* organisms, by culture, using both MAST and BCSA selective media. Overall, BCSA was comparable to MAST because it detected all cepacia-positive patients. However, BCSA medium detected all patients in a faster time than MAST; using MAST an additional four day's incubation was required to produce a positive result in three patients. In addition, BCSA medium was more selective because it produced fewer contaminants than MAST medium (table 2). The non-cepacia organisms that grew on BCSA displayed growth characteristics similar to those described previously by Henry *et al*.^{4, 7} The most commonly isolated non-cepacia organism from BCSA was *Flavobacterium indologenes*. This organism can be relatively easily distinguished from *B cepacia* through the use of a few simple biochemical tests, including: (1) indole production from tryptophan (with 85% *F indologenes* and 0% of *B cepacia* giving a positive result); (2) urease activity (44% *F indologenes* positive compared with 2% *B cepacia*); and (3) β-glucosidase

hydrolysis of esculin (98% *F indologenes* and 50% *B cepacia* positive). Furthermore, the use of specific PCR assays, including those described by Campbell *et al*,⁵ may aid in the identification of suspect isolates because phenotypic characterisation tests have previously been shown to misidentify other closely related phylogenetic neighbours, such as *B pyrocinnia*, as *B cepacia*.¹⁰

Overall, accurate cultural detection of the *B cepacia* complex organisms is important for patients with CF, both in terms of their management, and for infection control purposes. The problems of reliable detection in CF microbiology are further exacerbated by the quality and quantity of the sputum specimen obtained, the representativeness of the specimen, and whether primary diagnostic laboratories perform selective isolation methods for *B cepacia*. The use of sputum specimens of compromised quality or use of inadequate or inappropriate selective methods may eventually translate into the transmission of this organism to patients with CF who are not colonised with *B cepacia* complex organisms.

In conclusion, our study showed that BCSA was equivalent to MAST selective agar in its ability to isolate *B cepacia* from patients with CF and a history of *B cepacia* infection. The increased selectivity and reduced time to detection of BCSA makes it an attractive alternative to MAST. However, its present limited commercial availability in the UK may delay its use in routine diagnostic laboratories because of complications with media preparation and quality control.

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