

COMPARISON OF MAST *BURKHOLDERIA CEPACIA*, ASHDOWN + GENTAMICIN, AND *BURKHOLDERIA PSEUDOMALLEI* SELECTIVE AGAR FOR THE SELECTIVE GROWTH OF *BURKHOLDERIA* SPP.

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Reliable identification of pathogenic *Burkholderia* spp. like *Burkholderia mallei* and *Burkholderia pseudomallei* in clinical samples is desirable. Three different selective media were assessed for reliability and selectivity with various *Burkholderia* spp. and non-target organisms.

Mast *Burkholderia cepacia* agar, Ashdown + gentamicin agar, and *B. pseudomallei* selective agar were compared. A panel of 116 reference strains and well-characterized clinical isolates, comprising 30 *B. pseudomallei*, 20 *B. mallei*, 18 other *Burkholderia* spp., and 48 nontarget organisms, was used for this assessment.

While all *B. pseudomallei* strains grew on all three tested selective agars, the other *Burkholderia* spp. showed a diverse growth pattern. Nontarget organisms, i.e., nonfermentative rod-shaped bacteria, other species, and yeasts, grew on all selective agars. Colony morphology did not allow unambiguous discrimination.

While the assessed selective media reliably allowed the growth of a wide range of *B. pseudomallei* strains, growth of other *Burkholderia* spp. is only partially ensured. Growth of various nontarget organisms has to be considered. Therefore, the assessed media can only be used in combination with other confirmative tests in the diagnostic procedure for the screening for melioidosis or glanders.

Keywords: *Burkholderia* spp., *Burkholderia mallei*, *Burkholderia pseudomallei*, selective agar, comparison

Introduction

The genus *Burkholderia* harbors highly pathogenic species *Burkholderia (B.) mallei*, the causative agent of glanders, and *Burkholderia pseudomallei*, the causative agent of melioidosis [1, 2], species with relevance for cystic fibrosis patients, e.g., the *Burkholderia cepacia* complex, and environmental species [3, 4].

Considering the high clinical relevance of the correct identification of glanders, melioidosis, or *Burkholderia*-

associated respiratory tract infections in cystic fibrosis patients, reliable identification of the causative agent is important and incorrect identification can lead to critical clinical courses [5].

Melioidosis presents with unspecific symptoms and remains often unrecognized by the first responder, i.e., the clinician at a private practice or a local hospital especially in nonendemic areas where the physicians are unaware of the disease [6]. Blood culture in case of sepsis and subsequent culture on standard routine media result in un-

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specific growth, comparable to that of many other Gram-negative nonglucose fermenting rod-shaped bacteria like *Pseudomonas* spp. Subsequent routine testing using commercially available tests, such as API20 (bioMérieux, Nürtingen, Germany), VITEK2 (bioMérieux), etc., has proven to be little specific. Routine matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF-MS) systems lack profiles for this agent in their databases. The use of selective agars, i.e., MacConkey, Ashdown's, *B. pseudomallei* selective, and *B. cepacia* selective agar and prolonged incubation for specimens being contaminated with normal flora, is strongly advised to increase sensitivity. A very good review describing these problems and a suitable work flow in detail has recently been published [7].

Specialized laboratories use a plethora of tests to finally identify the agent. Molecular diagnostics are hampered by the close relationship of *B. pseudomallei* to *B. mallei* and *Burkholderia thailandensis*, the cause of zoonotic glanders, and a fairly apathogenic soil bacterium, respectively [8]. Specific antibodies to detect *B. pseudomallei* are not commercially available, and thus, tests based on these tools have not been validated accordingly. Laboratory infection may occur, and it is strongly advised to work only under BSL-3 biosafety laboratory conditions if melioidosis or *B. pseudomallei/mallei* is suspected.

For the reliable discrimination of other *Burkholderia* spp., e.g., strains of the *B. cepacia* complex, sequence-based molecular tools have been introduced. They comprise multilocus sequence typing (MLST) [9], *fur* sequencing [10], *hisA* sequencing [11], or *recA* sequencing [12, 13] from pure cultures. MALDI-TOF-MS-based approaches have been described as well [14–17]. All of these procedures, however, require the identification of suspicious colonies by the investigator.

Although clinically relevant *Burkholderia* spp. readily grow on standard agars like blood agar [3, 4], there is the risk that they may be missed if only few colonies are present among colonies of a majority of apathogenic flora from primarily nonsterile sampling sites.

Selective agars are used to facilitate selective growth and, thus, to ease identification of pathogens [18, 19]. Such selective agars are usually based on chemicals or antibiotic drugs with inhibitory effects on nontarget organisms, often associated with chromogenic reactions, which further facilitate the identification of the target pathogen [20–22]. Hence, evaluations of the discriminatory potential of the selective agar are imminent.

Here we assessed the reliability of three selective agars for *Burkholderia* spp. using a strain collection comprising a considerable number of target and nontarget organisms. Parallel growth on blood agar was done as a growth control in parallel. The aim was the analysis of both the sensitivity and selectivity of the assessed selective agars to provide a recommendation for the routine diagnostics based on the results.

Materials and methods

Strains

A strain collection of 116 reference strains and clinical isolates was used for the assessment. Only strains that grew either on blood agar or at least on one of the selective agars were included in the assessment. The used strains comprised 30 *B. pseudomallei* strains, 20 *B. mallei* strains, 18 strains from other *Burkholderia* spp., and 48 nontarget strains. The distribution of species and strains is detailed in *Table 1*.

Table 1. List of species and strains

Species	Strains
<i>Burkholderia pseudomallei</i> (n = 30)	006-2397, 41333 006-2401, Heckeshorn, NC 08708-02, NC 08707-04, NC 08016-03, NC 07431-04, NC 07383-04, NC 06700-03, NC 04846-03, NC 04845-04, NC 01688-03, NC 10276-01, NC 10274-03, NCTC 7383, 291A, P19535/91, 222A, S3, S6, 204, 216 A, 347, 521, 225A, 5691, RO1 206A, NCTC 4845, Holland, EF15660
<i>Burkholderia mallei</i> (n = 20)	UAE 1, UAE 2, 005-00543/2002, 005-00550/2002, ATCC 23344, 005-572 M2, Zagreb, NC 10245-02, 005-2399 Dubai, Bogor, K2-16-RS, M VIII, 005-00574 M2, 005-00577/2002 M3, Mukteswar, 005-00582 U5, NCTC 3709, NC 00120-05, NC10260-03, NC 10247-02
<i>Burkholderia cepacia</i> (n = 3)	Isolate-6-19-175, ATCC 25416, isolate (n = 1)
<i>Burkholderia anthina</i> (n = 1)	LMG 20982
<i>Burkholderia stabilis</i> (n = 2)	LMG 14294, isolate (n = 1)
<i>Burkholderia thailandensis</i> (n = 2)	DSM 13276, ATCC700388
<i>Burkholderia vandii</i> (n = 2)	DSM 9509, DSM 951/LMG 16020
<i>Burkholderia vietnamensis</i> (n = 1)	DSM 11319
<i>Burkholderia cenocepacia</i> (n = 1)	LMG 12615
<i>Burkholderia cocovenenans</i> (n = 1)	DSM 4285

Table 1. (cont'd)

Species	Strains
<i>Burkholderia dolosa</i> (n = 1)	LMG 18941
<i>Burkholderia fungorum</i> (n = 1)	LMG 16225
<i>Burkholderia gladioli</i> (n = 1)	DSM 11318
<i>Burkholderia glumae</i> (n = 1)	DSM 9512/LMG 2196
<i>Burkholderia graminis</i> (n = 1)	LMG 18924
Nontarget strains	
<i>Francisella tularensis</i> (n = 4)	Isolates (n = 4)
<i>Pseudomonas aeruginosa</i> (n = 2)	DSM 11810, ATCC 27853
<i>Achromobacter ruhlandii</i> (n = 1)	DSM 653
<i>Achromobacter xylosoxidans</i> spp. <i>dentrificans</i> (n = 1)	DSM 30026
<i>Acinetobacter baumannii</i> (n = 1)	DSM 4372
<i>Aeromonas hydrophila</i> spp. <i>hydrophila</i> (n = 1)	ATCC 7966
<i>Alcaligenes faecalis</i> spp. <i>faecalis</i> (n = 1)	DSM 30030
<i>Bacillus cereus</i> (n = 1)	DSM 4222
<i>Bacillus kururiensis</i> (n = 1)	DSM 13646
<i>Bacillus mycoides</i> (n = 1)	DSM 2048
<i>Bacillus polymyxa</i> (n = 1)	ATCC 10401
<i>Bacillus stearothermophilus</i> var. <i>calidolactis</i> (n = 1)	DSM 5943
<i>Bacillus thuringiensis</i> (n = 1)	DSM 350/WIS 315
<i>Candida albicans</i> (n = 1)	DSM1386
<i>Chromobacterium violaceum</i> (n = 1)	LMG 1267
<i>Eikenella corrodens</i> (n = 1)	DSM 8340
<i>Enterobacter aerogenes</i> (n = 1)	DSM 12058
<i>Enterobacter cloacae</i> (n = 1)	ATCC 13047
<i>Enerococcus faecalis</i> (n = 1)	DSM 2570
<i>Escherichia coli</i> (n = 1)	DSM 301
<i>Kingella dentrificans</i> (n = 1)	DSM 10202
<i>Klebsiella oxitoca</i> (n = 1)	Isolate
<i>Klebsiella pneumoniae</i> spp. <i>pneumoniae</i> (n = 1)	DSM 6675/681
<i>Listeria monocytogenes</i> (n = 1)	DSM 12464
<i>Moraxella catarrhalis</i> (n = 1)	DSM 9143
<i>Morganella morganii</i> (n = 1)	DSM 6675
<i>Ochrobactrum anthropi</i> (n = 1)	DSM 7216
<i>Proteus mirabilis</i> (n = 1)	DSM 4479
<i>Proteus vulgaris</i> (n = 1)	DSM 30118
<i>Psychrobacter phenylpyruvicus</i> (n = 1)	DSM 7000
<i>Salmonella</i> Typhimurium (n = 1)	ATCC 13311
<i>Shigella flexneri</i> (n = 1)	DSM 4782
<i>Sphingomonas paucimobilis</i> (n = 1)	DSM 1098
<i>Staphylococcus aureus</i> (n = 1)	DSM 346
<i>Staphylococcus epidermidis</i> (n = 1)	DSM 1798
<i>Stenotrophomonas maltophilia</i> (n = 1)	DSMZ 50170
<i>Streptococcus agalactiae</i> (n = 1)	Isolate
<i>Streptococcus pyogenes</i> (n = 1)	Isolate
<i>Vibrio cholerae</i> (n = 1)	219512

Table 1. (cont'd)

Species	Strains
<i>Vibrio parahaemolyticus</i> (n = 1)	DSM 10027
<i>Yersinia enterocolitica</i> (n = 1)	DSM 4780
<i>Yersinia kristensenii</i> (n = 1)	ATCC 33638
<i>Yersinia pestis</i> (n = 1)	EV 76
<i>Yersinia pseudotuberculosis</i> (n = 1)	ATCC 29833

Inoculation on agars and growth assessment were performed by skilled laboratory technical assistants.

Agars

Blood agar was used as a nonselective medium to control the vitality of strains. Further assessment on selective agars was only performed if growth on blood agar was observed. Blood agar was made of pancreatically digested casein, 12.0 g/L; peptically digested animal tissue, 5.0 g/L; yeast extract, 3.0 g/L; beef extract, 3.0 g/L; starch from corn, 1.0 g/L; sodium chloride, 5.0 g/L; agar-agar, 13.5 g/L; and defibrinized sheep blood, 5% with reagents provided by Merck (Darmstadt, Germany). Plates of each charge were incubated for sterility assessment.

The Mast BCA (*B. cepacia* agar) was prepared according to the manufacturer's instructions using *B. cepacia* medium, 36 g/L (Mast Diagnostica Ltd., Reinfeld, Germany); bidistilled water; and *B. cepacia* supplement, 10 tablets per liter (Mast Diagnostica Ltd). Ashdown + G (G for gentamicin) agar was made of tryptone soy broth agar, 10 g/L; agar-agar, 15 g/L; crystal violet, 5 mg/L; neutral red, 50 mg/L; 40% glycerol stock solution, 100 mL/L; gentamicin, 5 g/L; and bidistilled water with reagents provided by Merck. *B. pseudomallei* selective agar (BPSA, Nile blue agar) was prepared using standard agar, 23.5 g/L (Becton & Dickinson, Heidelberg, Germany); maltose, 4 g/L (Merck); neutral red, 100 mg/L (Merck); gentamicin, 20 mg/L (Merck); Nile blue, 0.2 g/L (Sigma, Munich, Germany); and bidistilled water as described [20, 21].

Growth assessment

Cultural growth and growth characteristics on selective agar plates were assessed 24 hours (h), 48 h, and 7 days (d) after inoculation of the media. Investigated growth characteristics were observation of grown normal-sized or at least very tiny colonies (later referred to as weak growth), assessment of color, transparency, size, shape, profile and surface qualities of grown colonies, and occurring of color shifts on selective agar if applicable.

Ethics

Ethical clearance was not necessary for this study because only bacterial strains from a strain collection were assessed.

Results

Cultural growth of *Burkholderia* spp. on selective agars

After a total observation time of 7 days, all *B. pseudomallei* strains showed cultural growth on all screening agars. Mast BCA allowed growth of *Burkholderia anthina*, *Burkholderia cenocepacia*, *B. cepacia*, *Burkholderia cocovenenans*, *Burkholderia dolosa*, *Burkholderia gladioli*, *Burkholderia glumae*, *B. thailandensis*, *Burkholderia vietnamensis*, growth of some strains of *B. mallei*, *Burkholderia stabilis*, *Burkholderia vandii*, and no growth of *Burkholderia fungorum* and *Burkholderia graminis*. On Ashdown + G agar, growth was observed for *B. thailandensis*, *B. cenocepacia*, *B. cocovenenans*, *B. dolosa*, for some strains of *B. mallei*, *B. cepacia*, *B. stabilis*, *B. vandii*, *B. glumae*, but not for *B. anthina*, *B. vietnamensis*, *B. fungorum*, *B. gladioli*, and *B. graminis*. Finally, BPSA (Nile blue) allowed growth of *B. glumae*, *B. cenocepacia*, *B. cepacia*, *B. cocovenenans*, *B. dolosa*, *B. thailandensis*, some strains of *B. mallei*, *B. stabilis*, *B. vandii*, and no growth of *B. anthina*, *B. fungorum*, *B. gladioli*, *B. graminis*, and *B. vietnamensis* (Table 2).

Growth was visible after 2 days for most strains (Supplementary materials 1–3, Table 2). In detail, first detection of growth after more than 48 h was recorded for 4 *B. mallei* strains and 1 *B. cocovenenans* strain on Mast BCA as well as for 1 *B. stabilis* strain on both Ashdown + G agar and BPSA (Nile blue). A more differentiated discrimination of “clearly visible” and “very weak” growth of colonies is shown in Table 2.

Cultural growth of nontarget organisms on selective agars

Each selective agar showed cultural growth of nontarget organisms, mainly of nonfermentative Gram-negative rod-shaped bacteria, but also other species like Enterobacteriaceae, yeasts, and Gram-positive bacteria were observed. Often, only weak growth was detectable (Table 2). On Mast BCA, growth of *Achromobacter ruhlandii*, *Alcaligenes faecalis* spp. *faecalis*, *Candida albicans*, *Chromobacterium violaceum*, *Enterobacter aerogenes*, *Francisella tularensis*, *Kingella denitrificans*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* spp. *pneumoniae*, *Morganella morganii*, *Pseudomonas aeruginosa*, and *Vibrio parahaemolyticus* was detectable (Table 2). Ashdown + G

Table 2. Cultural growth after 7 days of incubation

Species	Blood agar		Mast BCA		Ashdown + G agar		BPSA (Nile blue)	
	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only
<i>Burkholderia pseudomallei</i> (n = 30)	30/30 (100%)	0/30 (0%)	30/30 (100%)	0/30 (0%)	30/30 (100%)	0/30 (0%)	30/30 (100%)	0/30 (0%)
<i>Burkholderia mallei</i> (n = 20)	20/20 (100%)	0/20 (0%)	14/20 (70%)	2/20 (10%)	7/20 (35%)	3/20 (15%)	6/20 (30%)	2/20 (10%)
<i>Burkholderia cepacia</i> (n = 3)	2/3 (66.6%)	0/3 (0%)	2/3 (66.6%)	1/3 (33.3%)	2/3 (66.6%)	0/3 (0%)	2/3 (66.6%)	1/3 (33.3%)
<i>Burkholderia anthina</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Burkholderia stabilis</i> (n = 2)	2/2 (100%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)
<i>Burkholderia thailandensis</i> (n = 2)	2/2 (100%)	0/2 (0%)	2/2 (100%)	0/2 (0%)	2/2 (100%)	0/2 (0%)	2/2 (100%)	0/2 (0%)
<i>Burkholderia vandii</i> (n = 2)	2/2 (100%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)
<i>Burkholderia vietnamsensis</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Burkholderia cenocepacia</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Burkholderia cocovenenans</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)
<i>Burkholderia dolosa</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Burkholderia fungorum</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Burkholderia gladioli</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Burkholderia glumae</i> (n = 1)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Burkholderia graminis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
Growth of nontarget organisms								
<i>Francisella tularensis</i> (n = 4)	3/4 (75%)	1/4 (25%)	1/4 (25%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	1/4 (25%)	0/4 (0%)
<i>Pseudomonas aeruginosa</i> (n = 2)	2/2 (100%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	2/2 (100%)	0/2 (0%)	1/2 (50%)	0/2 (0%)
<i>Achromobacter ruhlandii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Achromobacter xylosoxidans</i> spp. denitrificans (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Acinetobacter baumannii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Aeromonas hydrophila</i> spp. <i>hydrophila</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Alcaligenes faecalis</i> spp. <i>faecalis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)

Table 2. (cont'd)

Species	Blood agar			Mast BCA			Ashdown + G agar			BPSA (Nile blue)		
	Normal cultural growth	Weak growth only	0/1 (0%)	Normal cultural growth	Weak growth only	0/1 (0%)	Normal cultural growth	Weak growth only	0/1 (0%)	Normal cultural growth	Weak growth only	0/1 (0%)
<i>Bacillus cereus</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus kururiensis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Bacillus mycoides</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus polymyxa</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus stearothermophilus</i> var. <i>calidolactis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus thuringiensis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Candida albicans</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Chromobacterium violaceum</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Eikenella corrodens</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Enterobacter aerogenes</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Enterobacter cloacae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Enterococcus faecalis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Escherichia coli</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Kingella denitrificans</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Klebsiella oxytoca</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Klebsiella pneumoniae</i> spp. <i>pneumoniae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Listeria monocytogenes</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Moraxella catarrhalis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Morganella morganii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Ochrobactrum anthropi</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Proteus mirabilis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Proteus vulgaris</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Psychrobacter phenylpyruvicus</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Salmonella</i> Typhimurium (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Shigella flexneri</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)

Table 2. (cont'd)

Species	Blood agar		Mast BCA		Ashdown + G agar		BPSA (Nile blue)	
	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only
<i>Sphingomonas paucimobilis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Staphylococcus aureus</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Staphylococcus epidermidis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
<i>Stenotrophomonas maltophilia</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Streptococcus agalactiae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Streptococcus pyogenes</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Vibrio cholerae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Vibrio parahaemolyticus</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Yersinia enterocolitica</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Yersinia kristensenii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Yersinia pestis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Yersinia pseudotuberculosis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)

agar allowed growth of *A. ruhlandii*, *Achromobacter xylosoxidans* spp. *denitrificans*, *Aeromonas hydrophila* spp. *hydrophila*, *C. albicans*, *E. aerogenes*, *Enterococcus faecalis*, *K. denitrificans*, *K. pneumoniae* spp. *pneumoniae*, *Proteus vulgaris*, *P. aeruginosa*, *Psychrobacter phenylpyruvicus*, *Stenotrophomonas maltophilia*, *V. parahaemolyticus*, and *Yersinia pestis* (Table 2). Finally, strains of *A. ruhlandii*, *A. xylosoxidans* spp. *denitrificans*, *Bacillus kururiensis*, *C. albicans*, *E. faecalis*, *F. tularensis*, *K. denitrificans*, *P. vulgaris*, *P. aeruginosa*, *P. phenylpyruvicus*, *Staphylococcus epidermidis*, *S. maltophilia*, and *V. parahaemolyticus* were detectable on BPSA (Nile blue) (Table 2). Most nontarget organisms produced well defined colonies on day two of growth on selective agars (Supplementary materials 1–3, Table 2). In detail, growth of only a few nontarget strains was detected after more than 48 h, comprising 1 *F. tularensis* strain on both Mast BCA and BPSA (Nile blue), 1 *C. albicans* strain on Mast BCA, and 1 *A. hydrophila* spp. *hydrophila* strain as well as 1 *E. faecalis* strain on Ashdown + G agar.

Morphological features of Burkholderia spp. and nontarget organisms on the selective agars

The morphological features of colonies of *Burkholderia* spp. and nontarget organisms are shown in Tables 3–5. Colony morphology was strain-dependent. Typical “species-specific” colonies were not observed. Colonies were likely to change their morphological features during growth.

Nontarget organisms showed highly similar colony morphology (Tables 3–5) to *Burkholderia* spp., making the risk of misdiagnosis highly likely. Considerable intraspecies variety and morphological changes during growth were observed for the nontarget species.

Discussion

The study assessed the reliability of three different selective media, i.e., Mast BCA, Ashdown + G agar, and BPSA (Nile blue), for selectivity for *Burkholderia* spp. The results showed that all three agars are suitable to allow the growth of *B. pseudomallei*. This result confirms the findings of Roesnita et al. [23] that *B. pseudomallei* selective agar (BPSA) is a cost-efficient screening tool for melioidosis in a low prevalence setting. The authors identified one additional case of melioidosis and three additional culture-positive samples for *B. pseudomallei* by applying this agar in comparison to standard diagnostic procedures with nonselective media [23]. BPSA was first introduced in 2003 [20] for the selective cultivation of *B. pseudomallei*. According to the results of the authors, BPSA shall be inhibitory to nonfermentative nontarget organisms like *P. aeruginosa* as well as *Burkholderia* spp. of the *B. cepacia* complex [20] or other nonpathogenic species. The here presented data cannot confirm their results

Table 3. Cultural features on Mast BCA as seen after 7 days of growth. Missing data indicate very weak growth. Turns in the course of growth are indicated

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm)	Colony shape	Colony profile	Colony surface
<i>Burkholderia pseudomallei</i> (n = 30)	1/30 (3.3%) white	30/30 (100%) switch yellow to red	28/30 (93.3%) ground glass	29/30 (96.6%) 1–4 mm	30/30 (100%) round	30/30 (100%) plane	30/30 (100%) smooth
	2/30 (6.6%) grey		14/30 (46.6%) nontransparent	1/30 (3.3%) > 4 mm	2/30 (6.6%) lobed	1/30 (3.3%) raised	smooth
	15/30 (50%) cream		* 12 turns ground glass to nontransparent		3/30 (10%) irregular	1/30 (3.3%) plane with eversion	1/30 (3.3%) slimy
	28/30 (93.3%) rose/pink				* 3 turns round to irregular	* 1 turn plane over raised to plane back	1/30 (3.3%) dry
	* 2 turns cream to grey				* 1 turn round to lobed	* 1 turn plane to plane	* 1 turn smooth to slimy
	* 4 turns cream to rose/pink				* 1 turn round over lobed to round back	* 1 turn plane to plane with eversion	* 1 turn smooth to dry
	* 5 turns rose/pink to cream						* 1 turn slimy to wrinkled
	* 2 turns cream to grey + rose/pink						
	* 1 turn cream + rose/pink to pink						
	* 1 turn cream to cream + rose/pink						
	* 1 turn cream + rose/pink to cream						
<i>Burkholderia mallei</i> (n = 16)	2/16 (12.5%) cream	14/16 (87.5%) switch yellow to red	14/16 (87.5%) ground glass	2/16 (12.5%) < 1 mm	12/16 (75%) round	13/16 (81.3%) plane	14/16 (87.5%) smooth
	15/16 (93.75%) rose/pink		4/16 (25%) nontransparent	11/16 (68.8%) 1–4 mm	2/16 (12.5%) irregular	1/16 (6.3%) raised	smooth
	1/16 (6.25%) mallow/light purple				regular		
	* 1 turn rose/pink to mallow/light purple			1/16 (6.3%) > 4 mm	* 2 turns round to irregular		
	purple						
	* 1 turn cream to rose/pink						
<i>Burkholderia cepacia</i> (n = 3)	2/3 (66.6%) grey	2/3 (66.6%) switch yellow to red	2/3 (66.6%) ground glass	2/3 (66.6%) 1–4 mm	2/3 (66.6%) round	2/3 (66.6%) plane	2/3 (66.6%) smooth
	2/3 (66.6%) cream		2/3 (66.6%) nontransparent		1/3 (33.3%) irregular	1/3 (33.3%) convex	
	2/3 (66.6%) rose/pink		* 1 turn nontransparent to ground glass		* 1 turn round to irregular	* 1 turn plane to convex	
	1/3 (33.3%) mallow/light purple				regular		
	* 1 turn mallow/light purple to grey						
	* 1 turn rose/pink to cream						
	* 1 turn cream to grey + rose/pink						
<i>Burkholderia thailandensis</i> (n = 2)	2/2 (100%) silver metal	2/2 (100%) switch yellow to red	1/2 (50%) ground glass	2/2 (100%) > 4 mm	2/2 (100%) round	2/2 (100%) plane	2/2 (100%) smooth
	1/2 (50%) grey		1/2 (50%) nontransparent		1/2 (50%) irregular	1/2 (50%) plane with eversion	
	2/2 (100%) cream						
	1/2 (50%) rose/pink				* 1 turn round to irregular	* 1 turn plane to plane with eversion	
	* 1 turn cream over grey and silver metal to cream and silver metal						
	* 1 turn silver metal over rose/pink to cream and silver metal						

Table 3. (cont'd)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm)	Colony shape	Colony profile	Colony surface
<i>Burkholderia anthina</i> (n = 1)	1/1 (100%) grey	0/1 (0%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) cream		1/1 (100%) nontransparent		1/1 (100%) irregular		
	*1 turn grey to cream		*1 turn ground glass to nontransparent		*1 turn round to irregular		
<i>Burkholderia cenocepacia</i> (n = 1)	1/1 (100%) grey	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) cream		1/1 (100%) nontransparent		1/1 (100%) irregular		1/1 (100%) dry
	*1 turn grey to cream		*1 turn ground glass to nontransparent		*1 turn round to irregular		*1 turn smooth to dry
<i>Burkholderia cocovenenans</i> (n = 1)	1/1 (100%) cream	1/1 (100%) switch yellow to red	1/1 (100%) nontransparent	1/1 (100%) > 4 mm	1/1 (100%) irregular	1/1 (100%) plane with eversion	1/1 (100%) smooth
<i>Burkholderia dolosa</i> (n = 1)	1/1 (100%) grey	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) cream		1/1 (100%) nontransparent		1/1 (100%) irregular		1/1 (100%) dry
	1/1 (100%) rose/pink		*1 turn ground glass to nontransparent		*1 turn round to irregular		*1 turn smooth over dry to smooth
	*1 turn grey over cream to rose/pink						
<i>Burkholderia gladioli</i> (n = 1)	1/1 (100%) cream	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
<i>Burkholderia glumae</i> (n = 1)	1/1 (100%) grey	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) cream		1/1 (100%) nontransparent		1/1 (100%) irregular		1/1 (100%) dry
	1/1 (100%) rose/pink		*1 turn ground glass to nontransparent		*1 turn round to irregular		*1 turn smooth over dry to smooth
	*1 turn grey over cream to rose/pink						

Table 3. (cont'd)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm)	Colony shape	Colony profile	Colony surface
<i>Burkholderia stabilis</i> (n = 1)	1/1 (100%) grey	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) cream		1/1 (100%) nontransparent				
	*1 turn rose/pink over grey to cream		*1 turn ground glass to nontransparent	*(only after 7 days)			
<i>Burkholderia vandii</i> (n = 1)	1/1 (100%) yellow	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) grey	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) > 4 mm	1/1 (100%) round 1/1 (100%) irregular	1/1 (100%) plane	1/1 (100%) smooth
					*1 turn round to irregular		
Growth of nontarget organisms							
<i>Alcaligenes faecalis</i> spp. <i>faecalis</i> (n = 1)	1/1 (100%) rose/pink	0/1 (0%) switch yellow to red	1/1 (100%) nontransparent	1/1 (100%) > 4 mm	1/1 (100%) round	1/1 (100%) plane 1/1 (100%) raised	1/1 (100%) smooth
						*1 turn plane to raised	
<i>Candida albicans</i> (n = 1)	1/1 (100%) mallow/light purple	0/1 (0%) switch yellow to red	1/1 (100%) transparent	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) dry
<i>Chromobacterium violaceum</i> (n = 1)	1/1 (100%) mallow/light purple	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) > 4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) dark purple		1/1 (100%) nontransparent		1/1 (100%) lobed 1/1 (100%) irregular	1/1 (100%) plane	1/1 (100%) dry 1/1 (100%) wrinkled
	*1 turn mallow/light purple to dark purple		*1 turn ground glass to nontransparent		*1 turn round over irregular to lobed		*1 turn smooth over dry and wrinkled to wrinkled

Table 3. (cont'd)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
<i>Enterobacter aerogenes</i> (n = 1)	1/1 (100%) white 1/1 (100%) cream *1 turn white to cream	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) > 4 mm	1/1 (100%) round 1/1 (100%) irregular *1 turn round to irregular	1/1 (100%) plane	1/1 (100%) smooth
<i>Francisella tularensis</i> (n = 1)	1/1 (100%) cream	0/1 (0%) switch yellow to red	1/1 (100%) transparent	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
<i>Kingella denitrificans</i> (n = 1)	1/1 (100%) grey 1/1 (100%) rose/pink *1 turn grey to rose/pink	1/1 (100%) switch yellow to red	1/1 (100%) transparent 1/1 (100%) ground glass *1 turn clear to ground glass	1/1 (100%) > 4 mm	1/1 (100%) round 1/1 (100%) irregular *1 turn round to irregular	1/1 (100%) plane 1/1 (100%) raised *1 turn plane to raised	1/1 (100%) smooth
<i>Klebsiella oxytoca</i> (n = 1)	1/1 (100%) white 1/1 (100%) cream *1 turn white to cream	1/1 (100%) switch yellow to red	1/1 (100%) nontransparent	1/1 (100%) > 4 mm	1/1 (100%) irregular	1/1 (100%) plane	1/1 (100%) smooth
<i>Klebsiella pneumoniae</i> spp. <i>pneumoniae</i> (n = 1)	1/1 (100%) grey 1/1 (100%) cream *1 turn grey to cream	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) > 4 mm	1/1 (100%) round 1/1 (100%) irregular *1 turn round to irregular	1/1 (100%) plane	1/1 (100%) smooth
<i>Morganella morganii</i> (n = 1)	1/1 (100%) grey	1/1 (100%) switch yellow to red	1/1 (100%) transparent	1/1 (100%) 1–4 mm	1/1 (100%) irregular	1/1 (100%) plane	1/1 (100%) smooth
<i>Vibrio parahaemolyticus</i> (n = 1)	1/1 (100%) grey 1/1 (100%) rose/pink 1/1 (100%) mallow/light purple *1 turn mallow/light purple over rose/pink and grey to grey	1/1 (100%) switch yellow to red	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round 1/1 (100%) irregular *1 turn round to irregular	1/1 (100%) plane 1/1 (100%) convex *1 turn plane to convex	1/1 (100%) smooth 1/1 (100%) slimy *1 turn slimy and smooth to slimy

Table 4. Cultural features on Ashdown + G agar as seen after 7 days of growth. Missing data indicate very weak growth. Turns in the course of growth are indicated

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm)	Colony shape	Colony profile	Colony surface
<i>Burkholderia pseudomallei</i> (n = 30)	2/30 (6.6%) red	11/30 (36.6%) loss of color	24/30 (80%) ground glass	1/30 (3.3%) < 1 mm	30/30 (100%) round	30/30 (100%) plane	30/30 (100%) smooth
	4/30 (13.3%) rose/pink		30/30 (100%) nontransparent	25/30 (83.3%) 1–4 mm	19/30 (63.3%) irregular	1/30 (3.3%) convex with depression	1/30 (3.3%) slimy
	30/30 (100%) mallow/light purple		*24 turns ground glass to nontransparent	4/30 (13.3%) > 4 mm	*19 turns round to irregular	1/30 (3.3%) convex with eversion	11/30 (36.6%) dry
	2/30 (6.6%) purple					15/30 (50%) plane with eversion	19/30 (63.3%) wrinkled
	6/30 (20%) dark purple					*1 turn smooth over dry to slimy	*1 turn smooth over dry to slimy
	*3 turns mallow/light purple to red					*1 turn plane to convex	*14 turns smooth to wrinkled
	*1 turn red to mallow/light purple					*1 turn plane over convex with depression to plane back	*3 turns smooth to dry to wrinkled
	*1 turn mallow/light purple in multicolored red + rose/pink					*1 turn plane to convex with eversion	*5 turns smooth over dry to wrinkled
	*1 turn mallow/light purple to purple					*14 turns smooth over wrinkled to dry	*1 turn smooth over wrinkled to dry
	*1 turn dark purple to mallow/light purple					*1 turn plane over plane with eversion to plane with eversion to plane back	*1 turn smooth over dry to smooth back
<i>Burkholderia mallei</i> (n = 10)	1/10 (10%) rose/pink	0/10 (0%) loss of color	8/10 (80%) ground glass	1/10 (10%) < 1 mm	6/10 (60%) round	8/10 (80%) plane	7/10 (70%) smooth
	10/10 (100%) mallow/light purple		7/10 (70%) nontransparent	5/10 (50%) 1–4 mm	8/10 (80%) irregular	1/10 (10%) convex	1/10 (10%) slimy
	1/10 (10%) dark purple		*7 turns ground glass to nontransparent	4/10 (40%) > 4 mm	*5 turns round to irregular	1/10 (10%) convex with eversion	6/10 (60%) dry
	*1 turn rose/pink to mallow/light purple					5/10 (50%) plane with eversion	5/10 (50%) wrinkled
	*1 turn red to dark purple					*1 turn convex to convex with eversion	*5 turns smooth to dry
						*5 turns plane to plane with eversion	*5 turns dry to wrinkled

Table 4. (cont'd)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm)	Colony shape	Colony profile	Colony surface
<i>Burkholderia cepacia</i> (n = 2)	1/2 (50%) red 2/2 (100%) mallow/light purple 1/2 (50%) purple * 1 turn red over mallow/light purple to purple	1/2 (50%) loss of color	1/2 (50%) ground glass 2/2 (100%) non-transparent * 1 turn ground glass to nontransparent	2/2 (100%) > 4 mm	2/2 (100%) round	2/2 (100%) plane	2/2 (100%) smooth
<i>Burkholderia thailandensis</i> (n = 2)	1/2 (50%) silver metal 2/2 (100%) mallow/light purple * 1 turn silver metal and mallow/light purple over mallow/light purple to silver metal and mallow/light purple	0/2 (0%) loss of color	1/2 (50%) ground glass 2/2 (100%) non-transparent * 1 turn ground glass to nontransparent	2/2 (100%) 1–4 mm	2/2 (100%) round	2/2 (100%) plane 1/2 (50%) plane with eversion * 1 turn plane to plane with eversion	2/2 (100%) smooth 1/2 (50%) dry * 1 turn smooth to smooth and dry
<i>Burkholderia cenocepacia</i> (n = 1)	1/1 (100%) mallow/light purple 1/1 (100%) purple * 1 turn mallow/light purple to purple	1/1 (100%) loss of color	1/1 (100%) non-transparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth 1/1 (100%) dry * 1 turn smooth to dry
<i>Burkholderia cocovenenans</i> (n = 1)	1/1 (100%) purple	0/1 (0%) loss of color	uncertain due to very weak cultural growth	uncertain due to very weak cultural growth	uncertain due to very weak cultural growth	uncertain due to very weak cultural growth	uncertain due to very weak cultural growth
<i>Burkholderia dolosa</i> (n = 1)	1/1 (100%) mallow/light purple	1/1 (100%) loss of color	1/1 (100%) nontransparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
<i>Burkholderia glumae</i> (n = 1)	1/1 (100%) rose/pink 1/1 (100%) mallow/light purple 1/1 (100%) dark purple * 1 turn rose/pink over mallow/light purple to dark purple	1/1 (100%) loss of color	1/1 (100%) ground glass 1/1 (100%) non-transparent * 1 turn ground glass to nontransparent	1/1 (100%) 1–4 m	1/1 (100%) round 1/1 (100%) irregular * 1 turn round to irregular	1/1 (100%) plane	1/1 (100%) smooth
<i>Burkholderia stabilis</i> (n = 1)	1/1 (100%) mallow/light purple	1/1 (100%) loss of color	1/1 (100%) non-transparent	1/1 (100%) 1–4 mm	1/1 (100%) irregular	1/1 (100%) plane	1/1 (100%) smooth
<i>Burkholderia vandii</i> (n = 1)	1/1 (100%) mallow/light purple 1/1 (100%) red * 1 turn mallow/light purple to red	1/1 (100%) loss of color	1/1 (100%) non-transparent	1/1 (100%) > 4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth 1/1 (100%) slimy * 1 turn smooth to smooth and slimy

Table 4. (cont'd)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm)	Colony shape	Colony profile	Colony surface
Growth of nontarget organisms							
<i>Pseudomonas aeruginosa</i> (n = 2)	1/2 (50%) rose/pink 1/2 (50%) mallow/light purple 2/2 (100%) red *1 turn rose/pink to red *1 turn mallow/light purple to red	2/2 (100%) loss of color	1/2 (50%) ground glass 1/2 (50%) non-transparent	1/2 (50%) 1–4 mm 1/2 (50%) > 4 mm	1/2 (50%) round 2/2 (100%) irregular *1 turn round to irregular	2/2 (100%) plane 1/1 (100%) plane 1/1 (100%) convex *1 turn plane to convex	1/2 (50%) smooth 2/2 (100%) dry 1/2 (50%) wrinkled *1 turn smooth to dry *1 turn dry to wrinkled
<i>Achromobacter ruhlandtii</i> (n = 1)	1/1 (100%) mallow/light purple	1/1 (100%) loss of color	1/1 (100%) ground glass 1/1 (100%) non-transparent *1 turn ground glass to ontransparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane 1/1 (100%) convex *1 turn plane to convex	1/1 (100%) smooth
<i>Achromobacter xylosoxidans</i> ssp. <i>denitrificans</i> (n = 1)	1/1 (100%) rose/pink 1/1 (100%) mallow/light purple *1 turn mallow/light purple over rose/pink to mallow/light purple	1/1 (100%) loss of color	1/1 (100%) transparent 1/1 (100%) non-transparent *1 turn transparent to nontransparent	1/1 (100%) 1–4 mm	1/1 (100%) round 1/1 (100%) irregular *1 turn round to irregular	1/1 (100%) plane 1/1 (100%) convex *1 turn plane to convex	1/1 (100%) smooth
<i>Aeromonas hydrophila</i> ssp. <i>hydrophila</i> (n = 1)	1/1 (100%) purple	0/1 (0%) loss of color	1/1 (100%) non-transparent	1/1 (100%) < 1 mm	1/1 (100%) irregular	1/1 (100%) plane	1/1 (100%) smooth
<i>Enterobacter aerogenes</i> (n = 1)	1/1 (100%) yellow 1/1 (100%) mallow/light purple *1 turn mallow/light purple to yellow and mallow/light purple	1/1 (100%) loss of color	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) irregular	1/1 (100%) plane	1/1 (100%) smooth
<i>Enterococcus faecalis</i> (n = 1)	1/1 (100%) dark purple	0/1 (0%) loss of color	1/1 (100%) non-transparent	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth

Table 4. (cont d)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm)	Colony shape	Colony profile	Colony surface
<i>Kingella denitrificans</i> (n = 1)	1/1 (100%) mallow/light purple	0/1 (0%) loss of color	1/1 (100%) transparent	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
			1/1 (100%) non-transparent				
			* 1 turn clear to non-transparent				
<i>Proteus vulgaris</i> (n = 1)	1/1 (100%) purple	0/1 (0%) loss of color	1/1 (100%) non-transparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
<i>Psychrobacter phenylpyruvicus</i> (n = 1)	1/1 (100%) red 1/1 (100%) dark purple * 1 turn red to dark purple	0/1 (0%) loss of color	1/1 (100%) non-transparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
<i>Stenotrophomonas maltophilia</i> (n = 1)	1/1 (100%) dark purple	1/1 (100%) loss of color	1/1 (100%) transparent	1/1 (100%) 1–4 mm	1/1 (100%) round 1/1 (100%) irregular	1/1 (100%) plane	1/1 (100%) smooth
			1/1 (100%) ground glass		* 1 turn round to irregular		
			* 1 turn transparent to ground glass				
<i>Vibrio parahaemolyticus</i> (n = 1)	1/1 (100%) rose/pink 1/1 (100%) mallow/light purple 1/1 (100%) purple 1/1 (100%) grey * 1 turn mallow/light purple over rose/pink to purple and grey	1/1 (100%) loss of color	1/1 (100%) ground glass	1/1 (100%) > 4 mm	1/1 (100%) round 1/1 (100%) irregular	1/1 (100%) plane 1/1 (100%) raised	1/1 (100%) smooth 1/1 (100%) slimy
					* 1 turn round to irregular	* 1 turn plane to raised	* 1 turn smooth and slimy to slimy
<i>Yersina pestis</i> (n = 1)	1/1 (100%) purple	0/1 (0%) loss of color	uncertain due to very weak cultural growth	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth

Table 5. Cultural features on BPSA (Nile blue) as seen after 7 days of growth. Missing data indicate very weak growth. Turns in the course of growth are indicated

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
<i>Burkholderia pseudomallei</i> (n = 30)	16/30 (53.3%) red	10/30 (33.3%) loss of color	25/30 (83.3%) ground glass	19/30 (63.3%) 1–4 mm	26/30 (86.6%) round	27/30 (90%) plane	22/30 (73.3%) smooth
	26/30 (86.6%) rose/pink		29/30 (96.6%) nontransparent	11/30 (36.6%) > 4 mm	1/30 (3.3%) lobed	1/30 (3.3%) raised	2/30 (6.6%) slimy
	8/30 (26.6%) mallow/light purple				22/30 (73.3%) irregular	3/30 (10%) convex	9/30 (30%) dry
	1/30 (3.3%) purple				regular	with eversion	19/30 (63.3%) wrinkled
	1/30 (3.3%) dark purple		*29 turns ground glass to nontransparent		*1 turn round over irregular to lobed	16/30 (53.3%) plane with eversion	
	*10 turns rose/pink to red				*16 turns round to irregular		*1 turn smooth to slimy
	*3 turns red to rose/pink				*1 turn round over irregular to round back	*1 turn plane over raised to plane with eversion	*1 turn smooth over wrinkled to slimy
	*3 turns mallow/light purple to rose/pink					*1 turn plane to convex	*2 turns smooth over dry to smooth back
	*3 turns rose/pink to mallow/light purple					*1 turn plane over plane with eversion to convex	*1 turn smooth over dry to wrinkled
	*1 turn mallow/light purple to purple					*2 turns plane to convex with eversion	*4 turns dry to wrinkled
*1 turn mallow/light purple to dark purple					*1 turn plane with eversion over convex	*1 turn dry to smooth	
*4 multicolored red + rose/pink					*10 turns plane with eversion to plane with eversion back	*1 turn smooth to dry	
							*7 turns smooth to wrinkled
							*2 turns smooth over wrinkled to smooth back
<i>Burkholderia mallei</i> (n = 8)	2/8 (25%) red	0/8 (0%) loss of color	7/8 (87.5%) ground glass	4/8 (50%) 1–4 mm	6/8 (75%) round	6/8 (75%) plane	6/8 (75%) smooth
	2/8 (25%) rose/pink		6/8 (75%) nontransparent	2/8 (25%) > 4 mm	4/8 (50%) irregular	1/8 (12.5%) convex	3/8 (37.5%) dry
	6/8 (75%) mallow/light purple				*4 turns round to irregular	with eversion	5/8 (62.5%) wrinkled
	1/8 (12.5%) purple					4/8 (50%) plane with eversion	*3 turns smooth to wrinkled
	3/8 (37.5%) dark purple		*6 turns ground glass to nontransparent				
	*1 turn rose/pink to mallow/light purple					*1 turn plane to convex with eversion	*1 turn smooth to dry
	*1 turn mallow/light purple to red over purple to dark purple					*4 turns plane to plane with eversion	*2 turns smooth over dry to wrinkled

Table 5. (cont'd)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
<i>Burkholderia cepacia</i> (n = 3)	1/3 (33.3%) red	1/1 (100%)	1/3 (33.3%)	1/3 (33.3%) < 1 mm	2/3 (66.6%) round	2/3 (66.6%) plane	2/3 (66.6%) smooth
	1/3 (33.3%) mallow/light purple	loss of color	ground glass	1/3 (33.3%) 1–4 mm			
	1/3 (33.3%) purple		2/3 (66.6%) nontransparent				
	1/3 (33.3%) dark purple		* 1 turn ground glass to nontransparent				
	1/3 (33.3%) brown						
	* 1 turn red to purple * 1 turn mallow/light purple to dark purple						
<i>Burkholderia thailandensis</i> (n = 2)	1/2 (50%) red	0/2 (0%)	1/2 (50%) ground glass	1/2 (50%) 1–4 mm	2/2 (100%) round	2/2 (100%) plane	2/2 (100%) smooth
	2/2 (100%) rose/pink	loss of color	2/2 (100%) nontransparent	1/2 (50%) > 4 mm	2/2 (100%) irregular	1/2 (50%) convex with depression	1/2 (50%) wrinkled
	1/2 (50%) brown		* 1 turn ground glass to nontransparent		* 2 turns round to irregular	1/2 (50%) plane with eversion	* 1 turn smooth to wrinkled
	* 1 turn brown over red to rose/pink					* 1 turn plane to convex with depression * 1 turn plane to plane with eversion	
<i>Burkholderia cenocepacia</i> (n = 1)	1/1 (100%) purple	1/1 (100%)	1/1 (100%) nontransparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) dark purple	loss of color			1/1 (100%) irregular	1/1 (100%) plane with eversion	1/1 (100%) wrinkled
	* 1 turn purple to dark purple				* 1 turn round to irregular	* 1 turn plane to plane with eversion	* 1 turn smooth to wrinkled
<i>Burkholderia cocovenenans</i> (n = 1)	1/1 (100%) purple	0/1 (0%)	uncertain due to very weak cultural growth	uncertain due to very weak cultural growth	uncertain due to very weak cultural growth	uncertain due to very weak cultural growth	uncertain due to very weak cultural growth
		loss of color	growth	growth	growth	growth	growth
<i>Burkholderia dolosa</i> (n = 1)	1/1 (100%) red	1/1 (100%)	1/1 (100%) nontransparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
		loss of color			1/1 (100%) irregular		
					* 1 turn round to irregular		

Table 5. (cont'd)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm)	Colony shape	Colony profile	Colony surface
<i>Burkholderia glumae</i> (n = 1)	1/1 (100%) rose/pink	1/1 (100%) loss of color	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) purple		1/1 (100%) nontransparent		1/1 (100%) irregular		
	*1 turn rose/pink to purple		*1 turn ground glass to nontransparent		*1 turn round to irregular		
<i>Burkholderia stabilis</i> (n = 1)	1/1 (100%) brown	0/1 (0%) loss of color	1/1 (100%) nontransparent	1/1 (100%) 1–4 mm	1/1 (100%) irregular	1/1 (100%) plane	1/1 (100%) wrinkled
<i>Burkholderia vandii</i> (n = 1)	1/1 (100%) red	0/1 (0%) loss of color	1/1 (100%) nontransparent	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) brown						
	*1 turn brown over red to brown						
Growth of nontarget organisms							
<i>Achromobacter ruhlandtii</i> (n = 1)	1/1 (100%) rose/pink	1/1 (100%) loss of color	1/1 (100%) transparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) red		1/1 (100%) ground glass		1/1 (100%) irregular	1/1 (100%) convex	
	*1 turn red over rose/pink to red		1/1 (100%) nontransparent		*1 turn round to irregular	*1 turn plane to convex	
			*1 turn transparent over ground glass to nontransparent				
<i>Achromobacter xylosoxidans</i> ssp. <i>denitrificans</i> (n = 1)	1/1 (100%) rose/pink	1/1 (100%) loss of color	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) red		1/1 (100%) nontransparent		1/1 (100%) irregular	1/1 (100%) convex	
	*1 turn red over rose/pink to red		*1 turn ground glass to nontransparent		*1 turn round to irregular	*1 turn plane to convex	
<i>Bacillus kururiensis</i> (n = 1)	1/1 (100%) rose/pink	1/1 (100%) loss of color	1/1 (100%) nontransparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth

Table 5. (cont'd)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm)	Colony shape	Colony profile	Colony surface
<i>Candida albicans</i> (n = 1)	1/1 (100%) red	1/1 (100%)	1/1 (100%) ground glass	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) rose/pink	loss of color	1/1 (100%) nontransparent			1/1 (100%) convex	
	1/1 (100%) mallow/light purple					*1 turn plane to convex	
	*1 turn rose/pink to red and mallow/light purple		*1 turn ground glass to nontransparent				
<i>Enterococcus faecalis</i> (n = 1)	1/1 (100%) rose/pink	0/1 (0%)	1/1 (100%) nontransparent	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) dark purple	loss of color					
	*1 turn rose/pink to dark purple						
<i>Francisella tularensis</i> (n = 1)	1/1 (100%) mallow/light purple	0/1 (0%)	1/1 (100%) nontransparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) convex with eversion	1/1 (100%) wrinkled
		loss of color					
<i>Kingella denitrificans</i> (n = 1)	1/1 (100%) red	1/1 (100%)	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) rose/pink	loss of color	1/1 (100%) nontransparent		1/1 (100%) irregular		
					*1 turn round to irregular		
	*1 turn red to rose/pink		*1 turn ground glass to nontransparent				
<i>Proteus vulgaris</i> (n = 1)	1/1 (100%) white	0/1 (0%)	1/1 (100%) nontransparent	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) rose/pink	loss of color					
<i>Pseudomonas aeruginosa</i> (n = 1)	1/1 (100%) red	0/1 (0%)	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) dry
	1/1 (100%) rose/pink	loss of color	1/1 (100%) nontransparent		1/1 (100%) irregular	1/1 (100%) plane with eversion	1/1 (100%) wrinkled
	1/1 (100%) dark purple				*1 turn round to irregular		*1 turn dry to wrinkled
	*1 turn rose/pink over red to dark purple		*1 turn ground glass to nontransparent			*1 turn plane to plane with eversion	
<i>Psychrobacter phenylpyruvicus</i> (n = 1)	1/1 (100%) red	0/1 (0%)	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
	1/1 (100%) rose/pink	loss of color	1/1 (100%) nontransparent				
	*1 turn rose/pink to red		*1 turn ground glass to nontransparent				

Table 5. (cont'd)

Species	Color of the colonies	Color switch of the agar from yellow to red/loss of color of the agar around the colonies	Transparency of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
<i>Staphylococcus epidermidis</i> (n = 1)	1/1 (100%) rose/pink	0/1 (0%) loss of color	1/1 (100%) nontransparent	1/1 (100%) ≤ 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
<i>Stenotrophomonas maltophilia</i> (n = 1)	1/1 (100%) rose/pink	1/1 (100%) loss of color	1/1 (100%) transparent 1/1 (100%) ground glass * 1 turn transparent to ground glass	1/1 (100%) > 4 mm	1/1 (100%) round 1/1 (100%) irregular * 1 turn round to irregular	1/1 (100%) plane 1/1 (100%) raised * 1 turn plane to raised	1/1 (100%) smooth 1/1 (100%) slimy * 1 turn smooth to slimy
<i>Vibrio parahaemolyticus</i> (n = 1)	1/1 (100%) rose/pink	0/1 (0%) loss of color	1/1 (100%) ground glass	1/1 (100%) 1–4 mm	1/1 (100%) round 1/1 (100%) irregular * 1 turn round to irregular	1/1 (100%) plane 1/1 (100%) raised * 1 turn plane to raised	1/1 (100%) smooth 1/1 (100%) slimy * 1 turn smooth and slimy to slimy

as *P. aeruginosa* and *B. cepacia* strains grew on this agar. In another comparison of BCA, Ashdown agar, and BPSA using clinical samples, the sensitivity of all three agars for the isolation of *B. pseudomallei* was virtually equivalent [24]. Ashdown agar and BCA were more specific with clinical samples [24], a result which cannot be confirmed by the here presented data with reference strains and well-characterized clinical isolates.

In this study, only some strains of *Burkholderia* spp. other than *B. pseudomallei* grew on the selective media. This was observed not only for environmental *Burkholderia* spp., which are lacking practical relevance for diagnostic procedures in human medicine, but also for strains with high relevance like *B. mallei*, the causative agent of glanders. The results indicate that the media are nonreliable for the growth of *Burkholderia* spp. other than *B. pseudomallei* considerably decreasing their diagnostic use.

Focusing on combined approaches for the detection of *B. pseudomallei* and *B. mallei*, the causative agents of melioidosis and glanders, respectively [1, 2], Ashdown agar was described to be most sensitive for the isolation of *B. pseudomallei* [25]. The finding of that study [25] that growth of *B. mallei* generally fails on this medium, however, could not be confirmed by the here presented data using Ashdown + G agar, although the agar did not allow the growth of all *B. mallei* strains investigated. Interestingly, however, 4 *B. mallei* strains were detected after more than 48 h after onset of growth on Ashdown + G agar in the here presented study. A similar effect was observed for other *Burkholderia* spp. on other assessed selective agars. An incubation time longer than the usually applied 48 h in the diagnostic routine is therefore advisable to increase the sensitivity of the assessed selective agars. The here presented data show that the proportion of nontarget organisms that became visible later than 48 h after the onset of growth is low. This limits the importance of the disadvantage of the also supported nonspecific growth due to the prolonged incubation time. Glass et al. preferred the use of *Pseudomonas cepacia* agar (PCA), which proved to be both sensitive and selective in their assessment [25], if the growth of both *B. mallei* and *B. pseudomallei* is desired.

Focusing on clinically relevant species of the *B. cepacia* complex and on environmental *Burkholderia* species, the metabolic needs differ in a species-depending way. Of note, L-arabinose was the most frequently used carbon source utilized by species of the *B. cepacia* complex, supporting the growth of 90% of the isolates in a previous study [26], while *B. anthina* and *B. vietnamensis* were most susceptible to antibiotic drugs. The latter results are confirmed by the present study, as no growth of *B. anthina* and *B. vietnamensis* strains on Ashdown + G agar and BPSA was seen. Previous analyses [4], however, suggested that antibiotic resistance seems to be more strain-dependent than species-dependent, especially in isolates from cystic fibrosis patients under antibiotic pressure. Vermis et al. demonstrated a wide phenotypic heterogeneity of *B. cepacia* complex strains on the assessed selective media Mast *B. cepacia* medium (BCA) and on *B. cepacia* selec-

tive agar (BCSA) [26], reflecting the different needs of the various *Burkholderia* spp. by means of the composition of growth media. In this study, the observed intraspecies variance of growth characteristics on the selective media does not allow any discrimination at species level, which is similar to the observations by Vermis et al. [26]. Another study that compared BCA with BCSA for the identification of *B. cepacia* complex strains from sputum samples of cystic fibrosis patients suggested that BCSA has a higher selectivity and reduced time to detection [27].

BCSA was first described in 1997 [22] as a selective medium for the identification of *B. cepacia* complex strains from respiratory secretions of cystic fibrosis patients. The intention was to use it as an agar for primary isolation, and it proved to be superior in comparison with the older oxidation–fermentation polymyxin–bacitracin–lactose (OFPBL) agar [28] and *P. cepacia* agars (PCA), that also readily allowed growth of other nonfermentative Gram-negative rod-shaped bacteria like *Stenotrophomonas maltophilia*, *Alcaligenes xylosoxidans*, and *Comamonas acidovorans*. Growth of *B. cepacia* strains on BCSA was also faster [22] than on OFPBL agar and PCA. In a later report, those findings were confirmed for sputum samples from cystic fibrosis patients as well [29].

It remains controversial whether or not prior broth enrichment may help to increase the sensitivity of selective agars for *Burkholderia* spp. An old report showed slightly better performance for selective enrichment and culture on polymyxin B–MacConkey agar without crystal violet, PCA, and OFPBL agar [30]. A later study could not confirm those findings and showed identical results for direct primary use of BCSA and for enrichment broth subcultures prior to growth on the selective agar [31]. The question whether or not prior enrichment is necessary to reliably identify *Burkholderia* spp. from respiratory samples of patients with cystic fibrosis is not finally answered so far.

The three media tested did not reliably suppress the growth of nonpathogenic *Burkholderia* spp. and not even of nontarget organisms like facultatively pathogenic *Pseudomonas* and Enterobacteriaceae which are frequently isolated from primarily sterile body compartments in case of invasive infections, thus, potentially mimicking invasive *Burkholderia* infections. The fact that even the growth of fungi was supported by the selective media, is in line with the recent finding that BCSA can be used for the isolation of *Exophiala dermatitidis*, a typical causative agent of phaeohyphomycosis [32].

Conclusion

The selective media investigated are of only restricted usefulness for diagnostic purposes with regard to both sensitivity and specificity. Their usefulness for the identification of *B. pseudomallei* [20, 23, 24] was confirmed again. In contrast, the identification of other *Burkholderia* spp. turned out to be nonreliable. Therefore, screening in case of suspicion of infections due to *Burkholderia* spp. should

not exclusively be based on the three selective agars tested here but should also include use of nonselective media and subsequent differentiation [33–35].

Declaration of interest

The authors declare that there are no conflicts of interest.

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Supplementary material 1. Growth after 24 h of incubation

Species	Blood agar			Mast BCA			Ashdown + G agar			BPSA (Nile blue)		
	Normal cultural growth	Weak growth only	Normal cultural growth	Normal cultural growth	Weak growth only	Normal cultural growth	Normal cultural growth	Weak growth only	Normal cultural growth	Normal cultural growth	Weak growth only	
<i>Burkholderia pseudomallei</i> (n = 30)	30/30 (100%)	0/30 (0%)	30/30 (100%)	30/30 (100%)	0/30 (0%)	30/30 (100%)	30/30 (100%)	0/30 (0%)	30/30 (100%)	30/30 (100%)	0/30 (0%)	
<i>Burkholderia mallei</i> (n = 20)	20/20 (100%)	0/20 (0%)	9/20 (45%)	2/20 (10%)	2/20 (10%)	5/20 (25%)	5/20 (25%)	3/20 (15%)	5/20 (25%)	5/20 (25%)	2/20 (10%)	
<i>Burkholderia cepacia</i> (n = 3)	2/3 (66.6%)	0/3 (0%)	2/3 (66.6%)	1/3 (33.3%)	1/3 (33.3%)	2/3 (66.6%)	2/3 (66.6%)	0/3 (0%)	2/3 (66.6%)	2/3 (66.6%)	0/3 (0%)	
<i>Burkholderia anthina</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia stabilis</i> (n = 2)	2/2 (100%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	
<i>Burkholderia thailandensis</i> (n = 2)	2/2 (100%)	0/2 (0%)	2/2 (100%)	2/2 (100%)	0/2 (0%)	2/2 (100%)	2/2 (100%)	0/2 (0%)	2/2 (100%)	2/2 (100%)	0/2 (0%)	
<i>Burkholderia vandii</i> (n = 2)	2/2 (100%)	0/2 (0%)	1/2 (50%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	1/2 (50%)	0/2 (0%)	
<i>Burkholderia vietnamiensis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia cenocepacia</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia cocovenenans</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	
<i>Burkholderia dolosa</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia fungorum</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia gladioli</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia glumae</i> (n = 1)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	
<i>Burkholderia graminis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
Growth of nontarget organisms												
<i>Francisella tularensis</i> (n = 4)	2/4 (50%)	1/4 (25%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	
<i>Pseudomonas aeruginosa</i> (n = 2)	2/2 (100%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	2/2 (100%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	
<i>Achromobacter ruhlandii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	
<i>Achromobacter xylosoxidans</i> spp. <i>denitrificans</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	
<i>Acinetobacter baumannii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Aeromonas hydrophila</i> spp. <i>hydrophila</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Alcaligenes faecalis</i> spp. <i>faecalis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	

Supplementary material 1. (cont'd)

Species	Blood agar		Mast BCA		Ashdown + G agar		BPSA (Nile blue)	
	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only
<i>Bacillus cereus</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus kururiensis</i> (n = 1)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus mycoides</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus polymyxa</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus stearothermophilus</i> var. <i>calidolactis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus thuringiensis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Candida albicans</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Chromobacterium violaceum</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Eikenella corrodens</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Enterobacter aerogenes</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Enterobacter cloacae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Enterococcus faecalis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Escherichia coli</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Kingella denitrificans</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)
<i>Klebsiella oxytoca</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Klebsiella pneumoniae</i> spp. <i>pneumoniae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Listeria monocytogenes</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Moraxella catarrhalis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Morganella morganii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Ochrobactrum anthropi</i> (n = 1)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Proteus mirabilis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Proteus vulgaris</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Psychrobacter phenylpyruvicus</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Salmonella Typhimurium</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Shigella flexneri</i> (n = 1)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)

Supplementary material 1. (cont'd)

Species	Blood agar		Mast BCA		Ashdown + G agar		BPSA (Nile blue)	
	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only
<i>Sphingomonas paucimobilis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Staphylococcus aureus</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Staphylococcus epidermidis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
<i>Stenotrophomonas maltophilia</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Streptococcus agalactiae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Streptococcus pyogenes</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Vibrio cholerae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Vibrio parahaemolyticus</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
<i>Yersinia enterocolitica</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Yersinia kristensenii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Yersinia pestis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Yersinia pseudotuberculosis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)

Supplementary material 2. Growth after 48 h of incubation

Species	Blood agar			Mast BCA			Ashdown + G agar			BPSA (Nile blue)		
	Normal cultural growth	Weak growth only	Normal cultural growth	Normal cultural growth	Weak growth only	Normal cultural growth	Normal cultural growth	Weak growth only	Normal cultural growth	Normal cultural growth	Weak growth only	
<i>Burkholderia pseudomallei</i> (n = 30)	30/30 (100%)	0/30 (0%)	30/30 (100%)	30/30 (100%)	0/30 (0%)	30/30 (100%)	30/30 (100%)	0/30 (0%)	30/30 (100%)	30/30 (100%)	0/30 (0%)	
<i>Burkholderia mallei</i> (n = 20)	20/20 (100%)	0/20 (0%)	10/20 (50%)	2/20 (10%)	2/20 (10%)	7/20 (35%)	6/20 (30%)	3/20 (15%)	6/20 (30%)	2/20 (10%)	2/20 (10%)	
<i>Burkholderia cepacia</i> (n = 3)	2/3 (66.6%)	0/3 (0%)	2/3 (66.6%)	1/3 (33.3%)	1/3 (33.3%)	2/3 (66.6%)	2/3 (66.6%)	0/3 (0%)	2/3 (66.6%)	1/3 (33.3%)	1/3 (33.3%)	
<i>Burkholderia anthina</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia stabilis</i> (n = 2)	2/2 (100%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	
<i>Burkholderia thailandensis</i> (n = 2)	2/2 (100%)	0/2 (0%)	2/2 (100%)	0/2 (0%)	0/2 (0%)	2/2 (100%)	2/2 (100%)	0/2 (0%)	2/2 (100%)	0/2 (0%)	0/2 (0%)	
<i>Burkholderia vandii</i> (n = 2)	2/2 (100%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	
<i>Burkholderia vietnamiensis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia cenocepacia</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia cocovenenans</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	
<i>Burkholderia dolosa</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia fungorum</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia gladioli</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia glumae</i> (n = 1)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	
<i>Burkholderia graminis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
Growth of nontarget organisms												
<i>Francisella tularensis</i> (n = 4)	2/4 (50%)	2/4 (50%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	
<i>Pseudomonas aeruginosa</i> (n = 2)	2/2 (100%)	0/2 (0%)	0/2 (0%)	2/2 (100%)	1/2 (50%)	2/2 (100%)	2/2 (100%)	0/2 (0%)	2/2 (100%)	1/2 (50%)	0/2 (0%)	
<i>Achromobacter ruhlandii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	
<i>Achromobacter xylosoxidans</i> spp. denitrificans (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	
<i>Acinetobacter baumannii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Aeromonas hydrophila</i> spp. hydrophila (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
<i>Alcaligenes faecalis</i> spp. faecalis (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	

Supplementary material 2. (cont'd)

Species	Blood agar		Mast BCA		Ashdown + G agar		BPSA (Nile blue)	
	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only
<i>Bacillus cereus</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus kururiensis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Bacillus mycoides</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus polymyxa</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus stearothermophilus</i> var. <i>calidolactis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Bacillus thuringiensis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Candida albicans</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)
<i>Chromobacterium violaceum</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Eikenella corrodens</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Enterobacter aerogenes</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Enterobacter cloacae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Enterococcus faecalis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
<i>Escherichia coli</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Kingella denitrificans</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Klebsiella oxytoca</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Klebsiella pneumoniae</i> spp. <i>pneumoniae</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Listeria monocytogenes</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Moraxella catarrhalis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Morganella morganii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Ochrobactrum anthropi</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Proteus mirabilis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Proteus vulgaris</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)
<i>Psychrobacter phenylpyruvicus</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)
<i>Salmonella</i> Typhimurium (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Shigella flexneri</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)

Supplementary material 2. (cont'd)

Species	Blood agar		Mast BCA		Ashdown + G agar		BPSA (Nile blue)	
	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only
<i>Sphingomonas paucimobilis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Staphylococcus aureus</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Staphylococcus epidermidis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
<i>Stenotrophomonas maltophilia</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Streptococcus agalactiae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Streptococcus pyogenes</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Vibrio cholerae</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Vibrio parahaemolyticus</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
<i>Yersinia enterocolitica</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Yersinia kristensenii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Yersinia pestis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
<i>Yersinia pseudotuberculosis</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)

Supplementary material 3. Growth on the selective agars after 7 days that was not yet observed after 48 h of incubation. On blood agar, all assessed strains were already grown after 48 h

Cepacia BCA agar	Ashdown + G agar	BPSA (Nile blue) agar
1× <i>Burkholderia cocovenenans</i> (only weak growth)	1× <i>Aeromonas hydrophila</i> spp. <i>hydrophila</i> (only weak growth)	1× <i>Burkholderia stabilis</i>
4× <i>Burkholderia mallei</i>	1× <i>Burkholderia stabilis</i>	1× <i>Francisella tularensis</i>
1× <i>Candida albicans</i>	1× <i>Enterococcus faecalis</i>	
1× <i>Francisella tularensis</i>		