Original article

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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 nontarget 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
Burkholderia pseudomallei (*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
Burkholderia mallei (*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
Burkholderia cepacia (*n* = 3)	Isolate-6-19-175, ATCC 25416, isolate (*n* = 1)
Burkholderia anthina (*n* = 1)	LMG 20982
Burkholderia stabilis (*n* = 2)	LMG 14294, isolate (*n* = 1)
Burkholderia thailandensis (*n* = 2)	DSM 13276, ATCC700388
Burkholderia vandii (*n* = 2)	DSM 9509, DSM 951/LMG 16020
Burkholderia vietnamensis (*n* = 1)	DSM 11319
Burkholderia cenocepacia (*n* = 1)	LMG 12615
Burkholderia cocovenenans (*n* = 1)	DSM 4285

Table 1. *(cont'd)*

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

Table 1. *(cont'd)*

Species	Strains
Vibrio parahaemolyticus (*n* = 1)	DSM 10027
Yersinia enterocolitica (*n* = 1)	DSM 4780
Yersinia kristenseni (*n* = 1)	ATCC 33638
Yersinia pestis (*n* = 1)	EV 76
Yersinia pseudotuberculosis (*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
Burkholderia pseudomallei (*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%)
Burkholderia mallei (*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%)
Burkholderia cepacia (*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%)
Burkholderia anthina (*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%)
Burkholderia stabilis (*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%)
Burkholderia thailandensis (*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%)
Burkholderia vandii (*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%)
Burkholderia vietnamensis (*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%)
Burkholderia cenocepacia (*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%)
Burkholderia cocovenenans (*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%)
Burkholderia dolosa (*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%)
Burkholderia fungorum (*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%)
Burkholderia gladioli (*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%)
Burkholderia glumae (*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%)
Burkholderia graminis (*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								
Francisella tularensis (*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%)
Pseudomonas aeruginosa (*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%)
Achromobacter ruhlandii (*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%)
Achromobacter xylosoxidans 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%)
Acinetobacter baumannii (*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%)
Aeromonas hydrophila spp. *hydrophila* (*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%)
Alcaligenes faecalis spp. *faecalis* (*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	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only	Normal cultural growth	Weak growth only
Bacillus cereus (*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%)
Bacillus kururiensis (*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%)
Bacillus mycoides (*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%)
Bacillus polymyxa (*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%)
Bacillus stearothermophilus var. *calidolactis* (*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%)
Bacillus thuringiensis (*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%)
Candida albicans (*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%)
Chromobacterium violaceum (*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%)
Eikenella corrodens (*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%)
Enterobacter aerogenes (*n* = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
Enterobacter cloacae (*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%)
Enterococcos faecalis (*n* = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
Escherichia coli (*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%)
Kingella denitrificans (*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%)
Klebsiella oxytoca (*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%)
Klebsiella pneumoniae spp. *pneumoniae* (*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%)
Listeria monocytogenes (*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%)
Moraxella catarrhalis (*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%)
Morganella morganii (*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%)
Ochrobactrum anthropi (*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%)
Proteus mirabilis (*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%)
Proteus vulgaris (*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%)
Psychrobacter phenylpyruvicus (*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%)
Salmonella 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%)
Shigella flexneri (*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%)

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
Sphingomonas paucimobilis (*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%)
Staphylococcus aureus (*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%)
Staphylococcus epidermidis (*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%)
Stenotrophomonas maltophila (*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%)
Streptococcus agalactiae (*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%)
Streptococcus pyogenes (*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%)
Vibrio cholerae (*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%)
Vibrio parahaemolyticus (*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%)
Yersinia enterocolitica (*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%)
Yersinia kristenseni (*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%)
Yersinia pestis (*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%)
Yersinia pseudotuberculosis (*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 maltophila, 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. maltophila,* 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) *(only after 7 days)	Colony shape	Colony profile	Colony surface
Burkholderia pseudomallei (*n* = 30)	1/30 (3.3%) white 2/30 (6.6%) grey 15/30 (50%) cream 28/30 (93.3%) rose/pink *2 turns cream to grey *4 turns cream to rose/pink *5 turns rose/pink to cream *2 turns cream to grey + rose/pink *1 turn cream + rose/pink to rose/pink *1 turn cream to cream + rose/pink *1 turn cream + rose/pink to cream	30/30 (100%) switch yellow to red	28/30 (93.3%) ground glass 14/30 (46.6%) nontransparent *12 turns ground glass to nontransparent	29/30 (96.6%) 1–4 mm 1/30 (3.3%) > 4 mm	30/30 (100%) round 2/30 (6.6%) lobed 3/30 (10%) irregular *3 turns round to irregular *1 turn round to lobed *1 turn round over lobed to round back	30/30 (100%) plane 1/30 (3.3%) raised 1/30 (3.3%) plane with eversion * 1 turn plane over raised to plane back * 1 turn plane to plane with eversion	30/30 (100%) smooth 1/30 (3.3%) slimy 1/30 (3.3%) dry 1/30 (3.3%) wrinkled *1 turn smooth to slimy *1 turn smooth to dry *1 turn slimy to wrinkled
Burkholderia mallei (*n* = 16)	2/16 (12.5%) cream 15/16 (93.75%) rose/pink 1/16 (6.25%) mallow/light purple *1 turn rose/pink to mallow/light purple *1 turn cream to rose/pink	14/16 (87.5%) switch yellow to red	14/16 (87.5%) ground glass 4/16 (25%) nontransparent *3 turns ground glass to nontransparent *1 turn nontransparent to ground glass	2/16 (12.5%) < 1 mm 11/16 (68.8%) 1–4 mm 1/16 (6.3%) > 4 mm	12/16 (75%) round 2/16 (12.5%) irregular *2 turns round to irregular	13/16 (81.3%) plane 1/16 (6.3%) raised	14/16 (87.5%) smooth
Burkholderia cepacia (*n* = 3)	2/3 (66.6%) grey 2/3 (66.6%) cream 2/3 (66.6%) rose/pink 1/3 (33.3%) mallow/light purple *1 turn mallow/light purple to grey *1 turn rose/pink to cream *1 turn cream to grey + rose/pink	2/3 (66.6%) switch yellow to red	2/3 (66.6%) ground glass 2/3 (66.6%) nontransparent *2 turns ground glass to nontransparent	2/3 (66.6%) 1–4 mm	2/3 (66.6%) round 1/3 (33.3%) irregular *1 turn round to irregular	2/3 (66.6%) plane 1/3 (33.3%) convex * 1 turn plane to convex	2/3 (66.6%) smooth
Burkholderia thailandensis (*n* = 2)	2/2 (100%) silver metal 1/2 (50%) grey 2/2 (100%) cream 1/2 (50%) rose/pink *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	2/2 (100%) switch yellow to red	1/2 (50%) ground glass 1/2 (50%) nontransparent	2/2 (100%) > 4 mm	2/2 (100%) round 1/2 (50%) irregular *1 turn round to irregular	2/2 (100%) plane 1/2 (50%) plane with eversion * 1 turn plane to plane with eversion	2/2 (100%) smooth

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
Burkholderia anthina (*n* = 1)	1/1 (100%) grey 1/1 (100%) cream *1 turn grey to cream	0/1 (0%) switch yellow to red	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass 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%) smooth
Burkholderia cenocepacia (*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%) nontransparent *1 turn ground glass 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%) smooth 1/1 (100%) dry *1 turn smooth to dry
Burkholderia cocovenenans (*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
Burkholderia dolosa (*n* = 1)	1/1 (100%) grey 1/1 (100%) cream 1/1 (100%) rose/pink *1 turn grey over cream to rose/pink	1/1 (100%) switch yellow to red	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass 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%) smooth 1/1 (100%) dry *1 turn smooth over dry to smooth
Burkholderia gladioli (*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
Burkholderia glumae (*n* = 1)	1/1 (100%) grey 1/1 (100%) cream 1/1 (100%) rose/pink *1 turn grey over cream to rose/pink	1/1 (100%) switch yellow to red	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass 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%) smooth 1/1 (100%) dry *1 turn smooth over dry to smooth

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
Burkholderia stabilis (*n* = 1)	1/1 (100%) grey 1/1 (100%) cream 1/1 (100%) rose/pink *1 turn rose/pink over grey to cream	1/1 (100%) switch yellow to red	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass to nontransparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
Burkholderia vandii (*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
Burkholderia vietnamensis (*n* = 1)	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 turn round to irregular	1/1 (100%) plane	1/1 (100%) smooth
			Growth of nontarget organisms				
Alcaligenes faecalis spp. *faecalis* (*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 turn plane to raised	1/1 (100%) smooth
Candida albicans (*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
Chromobacterium violaceum (*n* = 1)	1/1 (100%) mallow/light purple 1/1 (100%) dark purple *1 turn mallow/light purple to dark purple	1/1 (100%) switch yellow to red	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass to nontransparent	1/1 (100%) > 4 mm	1/1 (100%) round 1/1 (100%) lobed 1/1 (100%) irregular *1 turn round over irregular to lobed	1/1 (100%) plane	1/1 (100%) smooth 1/1 (100%) dry 1/1 (100%) wrinkled *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
Enterobacter aerogenes (*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
Francisella tularensis (*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
Kingella denitrificans (*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
Klebsiella oxytoca (*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
Klebsiella pneumoniae spp. *pneumoniae* (*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
Morganella morganii (*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
Vibrio parahaemolyticus (*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	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
Burkholderia pseudomallei (*n* = 30)	2/30 (6.6%) red 4/30 (13.3%) rose/pink 30/30 (100%) mallow/light purple 2/30 (6.6%) purple 6/30 (20%) dark purple *3 turns mallow/light purple to red *1 turn red to mallow/light purple *1 turn mallow/light purple in multicolored red + rose/pink *1 turn mallow/light purple to purple *1 turn dark purple to mallow/light purple *4 turns mallow/light purple to dark purple *1 multicolored mallow/light purple + dark purple *1 multicolored red + rose/pink *1 multicolored mallow/light purple + rose/pink *1 multicolored mallow/light purple + purple	11/30 (36.6%) loss of color	24/30 (80%) ground glass 30/30 (100%) nontransparent *24 turns ground glass to nontransparent	1/30 (3.3%) < 1 mm 25/30 (83.3%) 1–4 mm 4/30 (13.3%) > 4 mm	30/30 (100%) round 19/30 (63.3%) irregular *19 turns round to irregular	30/30 (100%) plane 1/30 (3.3%) convex 1/30 (3.3%) convex with depression 1/30 (3.3%) convex with eversion 15/30 (50%) plane with eversion *1 turn plane to convex *1 turn plane over convex with depression to plane back *1 turn plane to convex with eversion *14 turns plane to plane with eversion *1 turn plane over plane with eversion to plane back	30/30 (100%) smooth 1/30 (3.3%) slimy 11/30 (36.6%) dry 19/30 (63.3%) wrinkled *1 turn smooth over dry to slimy *14 turns smooth to wrinkled *3 turns smooth to dry *5 turns smooth over dry to wrinkled *1 turn smooth over wrinkled to dry *1 turn smooth over dry to smooth back
Burkholderia mallei (*n* = 10)	1/10 (10%) rose/pink 10/10 (100%) mallow/light purple 1/10 (10%) dark purple *1 turn rose/pink to mallow/light purple *1 turn red to dark purple	0/10 (0%) loss of color	8/10 (80%) ground glass 7/10 (70%) nontransparent *7 turns ground glass to nontransparent	1/10 (10%) < 1 mm 5/10 (50%) 1–4 mm 4/10 (40%) > 4 mm	6/10 (60%) round 8/10 (80%) irregular *5 turns round to irregular	8/10 (80%) plane 1/10 (10%) convex 1/10 (10%) convex with eversion 5/10 (50%) plane with eversion *1 turn convex to convex with eversion *5 turns plane to plane with eversion	7/10 (70%) smooth 1/10 (10%) slimy 6/10 (60%) dry 5/10 (50%) wrinkled *5 turns smooth to dry *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) *(only after 7 days)	Colony shape	Colony profile	Colony surface
Burkholderia cepacia (*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
Burkholderia thailandensis (*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
Burkholderia cenocepacia (*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
Burkholderia cocovenenans (*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
Burkholderia dolosa (*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
Burkholderia glumae (*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
Burkholderia stabilis (*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
Burkholderia vandii (*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	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
			Growth of nontarget organisms				
Pseudomonas aeruginosa (*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	1/2 (50%) smooth 2/2 (100%) dry 1/2 (50%) wrinkled *1 turn smooth to dry *1 turn dry to wrinkled
Achromobacter ruhlandii (*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
Achromobacter xylosoxidans ssp. denitrificans (*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
Aeromonas hydrophila ssp. *hydrophila* (*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
Enterobacter aerogenes (*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
Enterococcus faecalis (*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	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
Kingella denitrificans (*n* = 1)	1/1 (100%) mallow/light purple	0/1 (0%) loss of color	1/1 (100%) transparent 1/1 (100%) non-transparent *1 turn clear to non-transparent	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane	1/1 (100%) smooth
Proteus vulgaris (*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
Psychrobacter phenylpyruvicus (*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
Stenotrophomonas maltophila (*n* = 1)	1/1 (100%) dark purple	1/1 (100%) loss of color	1/1 (100%) transparent 1/1 (100%) ground glass *1 turn transparent to 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%) smooth
Vibrio parahaemolyticus (*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 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
Yersina pestis (*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	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
Burkholderia pseudomallei (*n* = 30)	16/30 (53.3%) red 26/30 (86.6%) rose/pink 8/30 (26.6%) mallow/light purple 1/30 (3.3%) purple 1/30 (3.3%) dark purple *10 turns rose/pink to red *3 turns red to rose/pink *3 turns mallow/light purple to rose/pink *3 turns rose/pink to mallow/light purple *1 turn mallow/light purple to purple *1 turn mallow/light purple to dark purple *4 multicolored red + rose/pink	10/30 (33.3%) loss of color	25/30 (83.3%) ground glass 29/30 (96.6%) nontransparent *29 turns ground glass to nontransparent	19/30 (63.3%) 1–4 mm 11/30 (36.6%) > 4 mm	26/30 (86.6%) round 1/30 (3.3%) lobed 22/30 (73.3%) irregular *1 turn round over irregular to lobed *16 turns round to irregular *1 turn round over irregular to round back	27/30 (90%) plane 1/30 (3.3%) raised 2/30 (6.6%) convex 3/30 (10%) convex with eversion 16/30 (53.3%) plane with eversion *1 turn plane over raised to plane with eversion *1 turn plane to convex *1 turn plane over plane with eversion to convex *2 turns plane to convex with eversion *1 turn plane with eversion over convex with eversion to plane with eversion back *10 turns plane to plane with eversion *1 turn plane with eversion over plane to plane with eversion back	22/30 (73.3%) smooth 2/30 (6.6%) slimy 9/30 (30%) dry 19/30 (63.3%) wrinkled *1 turn smooth to slimy *1 turn smooth over wrinkled to slimy *2 turns smooth over dry to smooth back *1 turn smooth over dry to wrinkled *4 turns dry to wrinkled *1 turn dry to smooth *1 turn smooth to dry *7 turns smooth to wrinkled *2 turns smooth over wrinkled to smooth back
Burkholderia mallei (*n* = 8)	2/8 (25%) red 2/8 (25%) rose/pink 6/8 (75%) mallow/light purple 1/8 (12.5%) purple 3/8 (37.5%) dark purple *1 turn rose/pink to mallow/light purple *1 turn mallow/light purple to red *1 turn mallow/light purple over purple to dark purple *2 turns mallow/light purple to dark purple	0/8 (0%) loss of color	7/8 (87.5%) ground glass 6/8 (75%) nontransparent *6 turns ground glass to nontransparent	4/8 (50%) 1–4 mm 2/8 (25%) > 4 mm	6/8 (75%) round 4/8 (50%) irregular *4 turns round to irregular	6/8 (75%) plane 1/8 (12.5%) convex with eversion 4/8 (50%) plane with eversion *1 turn plane to convex with eversion *4 turns plane to plane with eversion	6/8 (75%) smooth 3/8 (37.5%) dry 5/8 (62.5%) wrinkled *3 turns smooth to wrinkled *1 turn smooth to dry *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	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
Burkholderia cepacia (*n* = 3)	1/3 (33.3%) red 1/3 (33.3%) mallow/light purple 1/3 (33.3%) purple 1/3 (33.3%) dark purple 1/3 (33.3%) brown *1 turn red to purple *1 turn mallow/light purple to dark purple	1/1 (100%) loss of color	1/3 (33.3%) ground glass 2/3 (66.6%) nontransparent *1 turn ground glass to nontransparent	1/3 (33.3%) < 1 mm 1/3 (33.3%) 1–4 mm	2/3 (66.6%) round	2/3 (66.6%) plane	2/3 (66.6%) smooth
Burkholderia thailandensis (*n* = 2)	1/2 (50%) red 2/2 (100%) rose/pink 1/2 (50%) brown *1 turn brown over red to rose/pink	0/2 (0%) loss of color	1/2 (50%) ground glass 2/2 (100%) nontransparent *1 turn ground glass to nontransparent	1/2 (50%) 1–4 mm 1/2 (50%) > 4 mm	2/2 (100%) round 2/2 (100%) irregular *2 turns round to irregular	2/2 (100%) plane 1/2 (50%) convex with depression 1/2 (50%) plane with eversion *1 turn plane to convex with depression *1 turn plane to plane with eversion	2/2 (100%) smooth 1/2 (50%) wrinkled *1 turn smooth to wrinkled
Burkholderia cenocepacia (*n* = 1)	1/1 (100%) purple 1/1 (100%) dark purple *1 turn purple to dark purple	1/1 (100%) loss of color	1/1 (100%) 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%) plane with eversion *1 turn plane to plane with eversion	1/1 (100%) smooth 1/1 (100%) wrinkled *1 turn smooth to wrinkled
Burkholderia cocovenenans (*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
Burkholderia dolosa (*n* = 1)	1/1 (100%) red	1/1 (100%) loss of color	1/1 (100%) 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%) 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	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
Burkholderia glumae (*n* = 1)	1/1 (100%) rose/pink 1/1 (100%) purple *1 turn rose/pink to purple	1/1 (100%) loss of color	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass 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%) smooth
Burkholderia stabilis (*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
Burkholderia vandii (*n* = 1)	1/1 (100%) red 1/1 (100%) brown *1 turn brown over red to brown	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
			Growth of nontarget organisms				
Achromobacter ruhlandii (*n* = 1)	1/1 (100%) rose/pink 1/1 (100%) red *1 turn red over rose/pink to red	1/1 (100%) loss of color	1/1 (100%) transparent 1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn transparent over ground glass 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
Achromobacter xylosoxidans ssp. *denitrificans* (*n* = 1)	1/1 (100%) rose/pink 1/1 (100%) red *1 turn red over rose/pink to red	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 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
Bacillus kuruiensis (*n* = 1)	1/1 (100%) rose/pink	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

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	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
Candida albicans (*n* = 1)	1/1 (100%) red 1/1 (100%) rose/pink 1/1 (100%) mallow/light purple *1 turn rose/pink to red and mallow/light purple	1/1 (100%) loss of color	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass to nontransparent	1/1 (100%) < 1 mm	1/1 (100%) round	1/1 (100%) plane 1/1 (100%) convex *1 turn plane to convex	1/1 (100%) smooth
Enterococcus faecalis (*n* = 1)	1/1 (100%) rose/pink 1/1 (100%) dark purple *1 turn rose/pink to dark purple	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
Francisella tularensis (*n* = 1)	1/1 (100%) mallow/light purple	0/1 (0%) loss of color	1/1 (100%) nontransparent	1/1 (100%) 1–4 mm	1/1 (100%) round	1/1 (100%) convex with eversion	1/1 (100%) wrinkled
Kingella denitrificans (*n* = 1)	1/1 (100%) red 1/1 (100%) rose/pink *1 turn red to rose/pink	1/1 (100%) loss of color	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass 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%) smooth
Proteus vulgaris (*n* = 1)	1/1 (100%) white 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
Pseudomonas aeruginosa (*n* = 1)	1/1 (100%) red 1/1 (100%) rose/pink 1/1 (100%) dark purple *1 turn rose/pink over red to dark purple	0/1 (0%) loss of color	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass 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%) plane with eversion *1 turn plane to plane with eversion	1/1 (100%) dry 1/1 (100%) wrinkled *1 turn dry to wrinkled
Psychrobacter phenylpyruvicus (*n* = 1)	1/1 (100%) red 1/1 (100%) rose/pink *1 turn rose/pink to red	0/1 (0%) loss of color	1/1 (100%) ground glass 1/1 (100%) nontransparent *1 turn ground glass to 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	Transparence of the colonies	Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)	Colony shape	Colony profile	Colony surface
Staphylococcus epidermidis (*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
Stenotrophomonas maltophila (*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
Vibrio parahaemolyticus (*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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