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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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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. *dentri cans* (*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 dentri cans* (*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 exneri* (*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 denitri cans, 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. *denitri cans* (*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 denitri cans* (*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 exneri* (*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%) |
| *Staphylococcos 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. denitri cans, Aeromonas hydrophila* spp. *hydrophila, C. albicans, E. aerogenes, Enterococcus faecalis, K. denitri cans, 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. *denitri cans, Bacillus kururiensis, C. albicans, E. faecalis, F. tularensis, K. denitri cans, 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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Burkholderia pseudomallei* (*n* = 30) | 1/30 (3.3%) white<br>2/30 (6.6%) grey<br>15/30 (50%) cream<br>28/30 (93.3%) rose/pink<br>*2 turns cream to grey<br>*4 turns cream to rose/pink<br>*5 turns rose/pink to cream<br>*2 turns cream to grey + rose/pink<br>*1 turn cream + rose/pink to rose/pink<br>*1 turn cream to cream + rose/pink<br>*1 turn cream + rose/pink to cream | 30/30 (100%) switch yellow to red | 28/30 (93.3%) ground glass<br>14/30 (46.6%) nontransparent<br>*12 turns ground glass to nontransparent | 29/30 (96.6%) 1–4 mm<br>1/30 (3.3%) > 4 mm | 30/30 (100%) round<br>2/30 (6.6%) lobed<br>3/30 (10%) irregular<br>*3 turns round to irregular<br>*1 turn round to lobed<br>*1 turn round over lobed to round back | 30/30 (100%) plane<br>1/30 (3.3%) raised<br>1/30 (3.3%) plane with eversion<br>* 1 turn plane over raised to plane back<br>* 1 turn plane to plane with eversion | 30/30 (100%) smooth<br>1/30 (3.3%) slimy<br>1/30 (3.3%) dry<br>1/30 (3.3%) wrinkled<br>*1 turn smooth to slimy<br>*1 turn smooth to dry<br>*1 turn slimy to wrinkled |
| *Burkholderia mallei* (*n* = 16) | 2/16 (12.5%) cream<br>15/16 (93.75%) rose/pink<br>1/16 (6.25%) mallow/light purple<br>*1 turn rose/pink to mallow/light purple<br>*1 turn cream to rose/pink | 14/16 (87.5%) switch yellow to red | 14/16 (87.5%) ground glass<br>4/16 (25%) nontransparent<br>*3 turns ground glass to nontransparent<br>*1 turn nontransparent to ground glass | 2/16 (12.5%) < 1 mm<br>11/16 (68.8%) 1–4 mm<br>1/16 (6.3%) > 4 mm | 12/16 (75%) round<br>2/16 (12.5%) irregular<br>*2 turns round to irregular | 13/16 (81.3%) plane<br>1/16 (6.3%) raised | 14/16 (87.5%) smooth |
| *Burkholderia cepacia* (*n* = 3) | 2/3 (66.6%) grey<br>2/3 (66.6%) cream<br>2/3 (66.6%) rose/pink<br>1/3 (33.3%) mallow/light purple<br>*1 turn mallow/light purple to grey<br>*1 turn rose/pink to cream<br>*1 turn cream to grey + rose/pink | 2/3 (66.6%) switch yellow to red | 2/3 (66.6%) ground glass<br>2/3 (66.6%) nontransparent<br>*2 turns ground glass to nontransparent | 2/3 (66.6%) 1–4 mm | 2/3 (66.6%) round<br>1/3 (33.3%) irregular<br>*1 turn round to irregular | 2/3 (66.6%) plane<br>1/3 (33.3%) convex<br>* 1 turn plane to convex | 2/3 (66.6%) smooth |
| *Burkholderia thailandensis* (*n* = 2) | 2/2 (100%) silver metal<br>1/2 (50%) grey<br>2/2 (100%) cream<br>1/2 (50%) rose/pink<br>*1 turn cream over grey and silver metal to cream and silver metal<br>*1 turn silver metal over rose/pink to cream and silver metal | 2/2 (100%) switch yellow to red | 1/2 (50%) ground glass<br>1/2 (50%) nontransparent | 2/2 (100%) > 4 mm | 2/2 (100%) round<br>1/2 (50%) irregular<br>*1 turn round to irregular | 2/2 (100%) plane<br>1/2 (50%) plane with eversion<br>* 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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Burkholderia anthina* (*n* = 1) | 1/1 (100%) grey<br>1/1 (100%) cream<br>*1 turn grey to cream | 0/1 (0%) switch yellow to red | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane | 1/1 (100%) smooth |
| *Burkholderia cenocepacia* (*n* = 1) | 1/1 (100%) grey<br>1/1 (100%) cream<br>*1 turn grey to cream | 1/1 (100%) switch yellow to red | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane | 1/1 (100%) smooth<br>1/1 (100%) dry<br>*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<br>1/1 (100%) cream<br>1/1 (100%) rose/pink<br>*1 turn grey over cream to rose/pink | 1/1 (100%) switch yellow to red | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane | 1/1 (100%) smooth<br>1/1 (100%) dry<br>*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<br>1/1 (100%) cream<br>1/1 (100%) rose/pink<br>*1 turn grey over cream to rose/pink | 1/1 (100%) switch yellow to red | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane | 1/1 (100%) smooth<br>1/1 (100%) dry<br>*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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Burkholderia stabilis* (*n* = 1) | 1/1 (100%) grey<br>1/1 (100%) cream<br>1/1 (100%) rose/pink<br>*1 turn rose/pink over grey to cream | 1/1 (100%) switch yellow to red | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*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<br>1/1 (100%) irregular<br>*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<br>1/1 (100%) raised<br>* 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<br>1/1 (100%) dark purple<br>*1 turn mallow/light purple to dark purple | 1/1 (100%) switch yellow to red | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) > 4 mm | 1/1 (100%) round<br>1/1 (100%) lobed<br>1/1 (100%) irregular<br>*1 turn round over irregular to lobed | 1/1 (100%) plane | 1/1 (100%) smooth<br>1/1 (100%) dry<br>1/1 (100%) wrinkled<br>*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<br>1/1 (100%) cream<br>*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<br>1/1 (100%) irregular<br>*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 denitricans* (*n* = 1) | 1/1 (100%) grey<br>1/1 (100%) rose/pink<br>*1 turn grey to rose/pink | 1/1 (100%) switch yellow to red | 1/1 (100%) transparent<br>1/1 (100%) ground glass<br>*1 turn clear to ground glass | 1/1 (100%) > 4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) raised<br>* 1 turn plane to raised | 1/1 (100%) smooth |
| *Klebsiella oxytoca* (*n* = 1) | 1/1 (100%) white<br>1/1 (100%) cream<br>*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<br>1/1 (100%) cream<br>*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<br>1/1 (100%) irregular<br>*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<br>1/1 (100%) rose/pink<br>1/1 (100%) mallow/light purple<br>*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<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) convex<br>* 1 turn plane to convex | 1/1 (100%) smooth<br>1/1 (100%) slimy<br>*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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Burkholderia pseudomallei* (*n* = 30) | 2/30 (6.6%) red<br>4/30 (13.3%) rose/pink<br>30/30 (100%) mallow/light purple<br>2/30 (6.6%) purple<br>6/30 (20%) dark purple<br>*3 turns mallow/light purple to red<br>*1 turn red to mallow/light purple<br>*1 turn mallow/light purple in multicolored red + rose/pink<br>*1 turn mallow/light purple to purple<br>*1 turn dark purple to mallow/light purple<br>*4 turns mallow/light purple to dark purple<br>*1 multicolored mallow/light purple + dark purple<br>*1 multicolored red + rose/pink<br>*1 multicolored mallow/light purple + rose/pink<br>*1 multicolored mallow/light purple + purple | 11/30 (36.6%) loss of color | 24/30 (80%) ground glass<br>30/30 (100%) nontransparent<br>*24 turns ground glass to nontransparent | 1/30 (3.3%) < 1 mm<br>25/30 (83.3%) 1–4 mm<br>4/30 (13.3%) > 4 mm | 30/30 (100%) round<br>19/30 (63.3%) irregular<br>*19 turns round to irregular | 30/30 (100%) plane<br>1/30 (3.3%) convex<br>1/30 (3.3%) convex with depression<br>1/30 (3.3%) convex with eversion<br>15/30 (50%) plane with eversion<br>*1 turn plane to convex<br>*1 turn plane over convex with depression to plane back<br>*1 turn plane to convex with eversion<br>*14 turns plane to plane with eversion<br>*1 turn plane over plane with eversion to plane back | 30/30 (100%) smooth<br>1/30 (3.3%) slimy<br>11/30 (36.6%) dry<br>19/30 (63.3%) wrinkled<br>*1 turn smooth over dry to slimy<br>*14 turns smooth to wrinkled<br>*3 turns smooth to dry<br>*5 turns smooth over dry to wrinkled<br>*1 turn smooth over wrinkled to dry<br>*1 turn smooth over dry to smooth back |
| *Burkholderia mallei* (*n* = 10) | 1/10 (10%) rose/pink<br>10/10 (100%) mallow/light purple<br>1/10 (10%) dark purple<br>*1 turn rose/pink to mallow/light purple<br>*1 turn red to dark purple | 0/10 (0%) loss of color | 8/10 (80%) ground glass<br>7/10 (70%) nontransparent<br>*7 turns ground glass to nontransparent | 1/10 (10%) < 1 mm<br>5/10 (50%) 1–4 mm<br>4/10 (40%) > 4 mm | 6/10 (60%) round<br>8/10 (80%) irregular<br>*5 turns round to irregular | 8/10 (80%) plane<br>1/10 (10%) convex<br>1/10 (10%) convex with eversion<br>5/10 (50%) plane with eversion<br>*1 turn convex to convex with eversion<br>*5 turns plane to plane with eversion | 7/10 (70%) smooth<br>1/10 (10%) slimy<br>6/10 (60%) dry<br>5/10 (50%) wrinkled<br>*5 turns smooth to dry<br>*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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Burkholderia cepacia* (*n* = 2) | 1/2 (50%) red<br>2/2 (100%) mallow/light purple<br>1/2 (50%) purple<br>*1 turn red over mallow/light purple to purple | 1/2 (50%)<br>loss of color | 1/2 (50%) ground glass<br>2/2 (100%) non-transparent<br>*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<br>2/2 (100%) mallow/light purple<br>*1 turn silver metal and mallow/light purple over mallow light/purple to silver metal and mallow/light purple | 0/2 (0%)<br>loss of color | 1/2 (50%) ground glass<br>2/2 (100%) non-transparent<br>*1 turn ground glass to nontransparent | 2/2 (100%) 1–4 mm | 2/2 (100%) round | 2/2 (100%) plane<br>1/2 (50%) plane with eversion<br>*1 turn plane to plane with eversion | 2/2 (100%) smooth<br>1/2 (50%) dry<br>*1 turn smooth to smooth and dry |
| *Burkholderia cenocepacia* (*n* = 1) | 1/1 (100%) mallow/light purple<br>1/1 (100%) purple<br>*1 turn mallow/light purple to purple | 1/1 (100%)<br>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<br>1/1 (100%) dry<br>*1 turn smooth to dry |
| *Burkholderia cocovenenans* (*n* = 1) | 1/1 (100%) purple | 0/1 (0%)<br>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%)<br>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<br>1/1 (100%) mallow/light purple<br>1/1 (100%) dark purple<br>*1 turn rose/pink over mallow/light purple to dark purple | 1/1 (100%)<br>loss of color | 1/1 (100%) ground glass<br>1/1 (100%) non-transparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) 1–4 m | 1/1 (100%) round<br>1/1 (100%) irregular<br>*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%)<br>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<br>1/1 (100%) red<br>*1 turn mallow/light purple to red | 1/1 (100%)<br>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<br>1/1 (100%) slimy<br>*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<br>1/2 (50%) mallow/light purple<br>2/2 (100%) red<br>*1 turn rose/pink to red<br>*1 turn mallow/light purple to red | 2/2 (100%) loss of color | 1/2 (50%) ground glass<br>1/2 (50%) non-transparent | 1/2 (50%) 1–4 mm<br>1/2 (50%) > 4 mm | 1/2 (50%) round<br>2/2 (100%) irregular<br>*1 turn round to irregular | 2/2 (100%) plane | 1/2 (50%) smooth<br>2/2 (100%) dry<br>1/2 (50%) wrinkled<br>*1 turn smooth to dry<br>*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<br>1/1 (100%) non-transparent<br>*1 turn ground glass to ontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round | 1/1 (100%) plane<br>1/1 (100%) convex<br>*1 turn plane to convex | 1/1 (100%) smooth |
| *Achromobacter xylosoxidans ssp. denitri cans* (*n* = 1) | 1/1 (100%) rose/pink<br>1/1 (100%) mallow/light purple<br>*1 turn mallow/light purple over rose/pink to mallow/light purple | 1/1 (100%) loss of color | 1/1 (100%) transparent<br>1/1 (100%) non-transparent<br>*1 turn transparent to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) convex<br>*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<br>1/1 (100%) mallow/light purple<br>*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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Kingella denitricans* (*n* = 1) | 1/1 (100%) mallow/light purple | 0/1 (0%)<br>loss of color | 1/1 (100%) transparent<br>1/1 (100%) non-transparent<br>*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%)<br>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<br>1/1 (100%) dark purple<br>*1 turn red to dark purple | 0/1 (0%)<br>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%)<br>loss of color | 1/1 (100%) transparent<br>1/1 (100%) ground glass<br>*1 turn transparent to ground glass | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane | 1/1 (100%) smooth |
| *Vibrio parahaemolyticus* (*n* = 1) | 1/1 (100%) rose/pink<br>1/1 (100%) mallow/light purple<br>1/1 (100%) purple<br>1/1 (100%) grey<br>*1 turn mallow/light purple over rose/pink to purple and grey | 1/1 (100%)<br>loss of color | 1/1 (100%) ground glass | 1/1 (100%) > 4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) raised<br>*1 turn plane to raised | 1/1 (100%) smooth<br>1/1 (100%) slimy<br>*1 turn smooth and slimy to slimy |
| *Yersina pestis* (*n* = 1) | 1/1 (100%) purple | 0/1 (0%)<br>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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Burkholderia pseudomallei* (*n* = 30) | 16/30 (53.3%) red<br>26/30 (86.6%) rose/pink<br>8/30 (26.6%) mallow/light purple<br>1/30 (3.3%) purple<br>1/30 (3.3%) dark purple<br>*10 turns rose/pink to red<br>*3 turns red to rose/pink<br>*3 turns mallow/light purple to rose/pink<br>*3 turns rose/pink to mallow/light purple<br>*1 turn mallow/light purple to purple<br>*1 turn mallow/light purple to dark purple<br>*4 multicolored red + rose/pink | 10/30 (33.3%) loss of color | 25/30 (83.3%) ground glass<br>29/30 (96.6%) nontransparent<br>*29 turns ground glass to nontransparent | 19/30 (63.3%) 1–4 mm<br>11/30 (36.6%) > 4 mm | 26/30 (86.6%) round<br>1/30 (3.3%) lobed<br>22/30 (73.3%) irregular<br>*1 turn round over irregular to lobed<br>*16 turns round to irregular<br>*1 turn round over irregular to round back | 27/30 (90%) plane<br>1/30 (3.3%) raised<br>2/30 (6.6%) convex<br>3/30 (10%) convex with eversion<br>16/30 (53.3%) plane with eversion<br>*1 turn plane over raised to plane with eversion<br>*1 turn plane to convex<br>*1 turn plane over plane with eversion to convex<br>*2 turns plane to convex with eversion<br>*1 turn plane with eversion over convex with eversion to plane with eversion back<br>*10 turns plane to plane with eversion<br>*1 turn plane with eversion over plane to plane with eversion back | 22/30 (73.3%) smooth<br>2/30 (6.6%) slimy<br>9/30 (30%) dry<br>19/30 (63.3%) wrinkled<br>*1 turn smooth to slimy<br>*1 turn smooth over wrinkled to slimy<br>*2 turns smooth over dry to smooth back<br>*1 turn smooth over dry to wrinkled<br>*4 turns dry to wrinkled<br>*1 turn dry to smooth<br>*1 turn smooth to dry<br>*7 turns smooth to wrinkled<br>*2 turns smooth over wrinkled to smooth back |
| *Burkholderia mallei* (*n* = 8) | 2/8 (25%) red<br>2/8 (25%) rose/pink<br>6/8 (75%) mallow/light purple<br>1/8 (12.5%) purple<br>3/8 (37.5%) dark purple<br>*1 turn rose/pink to mallow/light purple<br>*1 turn mallow/light purple to red<br>*1 turn mallow/light purple over purple to dark purple<br>*2 turns mallow/light purple to dark purple | 0/8 (0%) loss of color | 7/8 (87.5%) ground glass<br>6/8 (75%) nontransparent<br>*6 turns ground glass to nontransparent | 4/8 (50%) 1–4 mm<br>2/8 (25%) > 4 mm | 6/8 (75%) round<br>4/8 (50%) irregular<br>*4 turns round to irregular | 6/8 (75%) plane<br>1/8 (12.5%) convex with eversion<br>4/8 (50%) plane with eversion<br>*1 turn plane to convex with eversion<br>*4 turns plane to plane with eversion | 6/8 (75%) smooth<br>3/8 (37.5%) dry<br>5/8 (62.5%) wrinkled<br>*3 turns smooth to wrinkled<br>*1 turn smooth to dry<br>*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)<br><br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Burkholderia cepacia* (*n* = 3) | 1/3 (33.3%) red<br>1/3 (33.3%) mallow/light purple<br>1/3 (33.3%) purple<br>1/3 (33.3%) dark purple<br>1/3 (33.3%) brown<br><br>*1 turn red to purple<br>*1 turn mallow/light purple to dark purple | 1/1 (100%)<br>loss of color | 1/3 (33.3%) ground glass<br>2/3 (66.6%) nontransparent<br><br>*1 turn ground glass to nontransparent | 1/3 (33.3%) < 1 mm<br>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<br>2/2 (100%) rose/pink<br>1/2 (50%) brown<br><br>*1 turn brown over red to rose/pink | 0/2 (0%)<br>loss of color | 1/2 (50%) ground glass<br>2/2 (100%) nontransparent<br><br>*1 turn ground glass to nontransparent | 1/2 (50%) 1–4 mm<br>1/2 (50%) > 4 mm | 2/2 (100%) round<br>2/2 (100%) irregular<br><br>*2 turns round to irregular | 2/2 (100%) plane<br>1/2 (50%) convex with depression<br>1/2 (50%) plane with eversion<br><br>*1 turn plane to convex with depression<br>*1 turn plane to plane with eversion | 2/2 (100%) smooth<br>1/2 (50%) wrinkled<br><br>*1 turn smooth to wrinkled |
| *Burkholderia cenocepacia* (*n* = 1) | 1/1 (100%) purple<br>1/1 (100%) dark purple<br><br>*1 turn purple to dark purple | 1/1 (100%)<br>loss of color | 1/1 (100%) nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br><br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) plane with eversion<br><br>*1 turn plane to plane with eversion | 1/1 (100%) smooth<br>1/1 (100%) wrinkled<br><br>*1 turn smooth to wrinkled |
| *Burkholderia cocovenenans* (*n* = 1) | 1/1 (100%) purple | 0/1 (0%)<br>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%)<br>loss of color | 1/1 (100%) nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br><br>*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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Burkholderia glumae* (*n* = 1) | 1/1 (100%) rose/pink<br>1/1 (100%) purple<br>*1 turn rose/pink to purple | 1/1 (100%)<br>loss of color | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane | 1/1 (100%) smooth |
| *Burkholderia stabilis* (*n* = 1) | 1/1 (100%) brown | 0/1 (0%)<br>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<br>1/1 (100%) brown<br>*1 turn brown over red to brown | 0/1 (0%)<br>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<br>1/1 (100%) red<br>*1 turn red over rose/pink to red | 1/1 (100%)<br>loss of color | 1/1 (100%) transparent<br>1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn transparent over ground glass to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) convex<br>*1 turn plane to convex | 1/1 (100%) smooth |
| *Achromobacter xylosoxidans* ssp. *denitri cans* (*n* = 1) | 1/1 (100%) rose/pink<br>1/1 (100%) red<br>*1 turn red over rose/pink to red | 1/1 (100%)<br>loss of color | 1/1 (100%) ground glass<br>1/1 (100%) non-transparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) convex<br>*1 turn plane to convex | 1/1 (100%) smooth |
| *Bacillus kuru-riensis* (*n* = 1) | 1/1 (100%) rose/pink | 1/1 (100%)<br>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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Candida albicans* (*n* = 1) | 1/1 (100%) red<br>1/1 (100%) rose/pink<br>1/1 (100%) mallow/light purple<br>*1 turn rose/pink to red and mallow/light purple | 1/1 (100%) loss of color | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) < 1 mm | 1/1 (100%) round | 1/1 (100%) plane<br>1/1 (100%) convex<br>*1 turn plane to convex | 1/1 (100%) smooth |
| *Enterococcus faecalis* (*n* = 1) | 1/1 (100%) rose/pink<br>1/1 (100%) dark purple<br>*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 denitricans* (*n* = 1) | 1/1 (100%) red<br>1/1 (100%) rose/pink<br>*1 turn red to rose/pink | 1/1 (100%) loss of color | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane | 1/1 (100%) smooth |
| *Proteus vulgaris* (*n* = 1) | 1/1 (100%) white<br>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<br>1/1 (100%) rose/pink<br>1/1 (100%) dark purple<br>*1 turn rose/pink over red to dark purple | 0/1 (0%) loss of color | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*1 turn ground glass to nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) plane with eversion<br>*1 turn plane to plane with eversion | 1/1 (100%) dry<br>1/1 (100%) wrinkled<br>*1 turn dry to wrinkled |
| *Psychrobacter phenylpyruvicus* (*n* = 1) | 1/1 (100%) red<br>1/1 (100%) rose/pink<br>*1 turn rose/pink to red | 0/1 (0%) loss of color | 1/1 (100%) ground glass<br>1/1 (100%) nontransparent<br>*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)<br>*(only after 7 days) | Colony shape | Colony profile | Colony surface |
|---|---|---|---|---|---|---|---|
| *Staphylococcus epidermidis* ($n$ = 1) | 1/1 (100%) rose/pink | 0/1 (0%)<br>loss of color | 1/1 (100%)<br>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%)<br>loss of color | 1/1 (100%) transparent<br>1/1 (100%) ground glass<br>*1 turn transparent to ground glass | 1/1 (100%) > 4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) raised<br>*1 turn plane to raised | 1/1 (100%) smooth<br>1/1 (100%) slimy<br>*1 turn smooth to slimy |
| *Vibrio parahaemolyticus* ($n$ = 1) | 1/1 (100%) rose/pink | 0/1 (0%)<br>loss of color | 1/1 (100%) ground glass | 1/1 (100%) 1–4 mm | 1/1 (100%) round<br>1/1 (100%) irregular<br>*1 turn round to irregular | 1/1 (100%) plane<br>1/1 (100%) raised<br>*1 turn plane to raised | 1/1 (100%) smooth<br>1/1 (100%) slimy<br>*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 | 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%) | 9/20 (45%) | 2/20 (10%) | 5/20 (25%) | 3/20 (15%) | 5/20 (25%) | 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%) | 0/3 (0%) |
| *Burkholderia anthina* (*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 stabilis* (*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%) |
| *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%) | 0/1 (0%) | 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%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| *Burkholderia cocovenenans* (*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%) |
| *Burkholderia dolosa* (*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 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) | 2/4 (50%) | 1/4 (25%) | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) |
| *Pseudomonas aeruginosa* (*n* = 2) | 2/2 (100%) | 0/2 (0%) | 0/2 (0%) | 1/2 (50%) | 0/2 (0%) | 2/2 (100%) | 0/2 (0%) | 1/2 (50%) |
| *Achromobacter ruhlandii* (*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%) |
| *Achromobacter xylosoxidans* spp. *denitri cans* (*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%) |
| *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%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| *Alcaligenes faecalis* spp. *faecalis* (*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 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 |
| *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) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 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%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 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%) | 0/1 (0%) | 1/1 (100%) | 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%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| *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 denitri cans* (*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%) |
| *Klebsiella oxytoca* (*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%) |
| *Klebsiella pneumoniae* spp. *pneumoniae* (*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%) |
| *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%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| *Ochrobactrum anthropi* (*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%) |
| *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%) | 0/1 (0%) |
| *Psychrobacter phenylpyruvicus* (*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%) |
| *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 exneri* (*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 |
| *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%) |
| *Staphylococcos 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%) | 0/1 (0%) | 1/1 (100%) |
| *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%) |

**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 | 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%) | 10/20 (50%) | 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%) | 0/1 (0%) | 1/1 (100%) | 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%) | 0/2 (0%) | 0/2 (0%) | 0/2 (0%) | 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%) | 0/1 (0%) | 1/1 (100%) | 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%) | 0/1 (0%) | 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) | 2/4 (50%) | 2/4 (50%) | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) | 0/4 (0%) | 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. *denitri cans* (*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%) | 0/1 (0%) | 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%) |

**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 |
| *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%) | 0/1 (0%) | 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%) | 0/1 (0%) | 1/1 (100%) | 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%) | 0/1 (0%) | 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 denitri cans* (*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%) | 0/1 (0%) | 1/1 (100%) | 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%) | 0/1 (0%) | 1/1 (100%) | 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%) | 0/1 (0%) | 1/1 (100%) | 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 exneri* (*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 |
| *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%) |
| *Staphylococcos 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%) |

## 1.4 Supplementary material 3



**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× *Burkholderia cocovenenans* (only weak growth) | 1× *Aeromonas hydrophila* spp. *hydrophila* (only weak growth) | 1× *Burkholderia stabilis* |
| 4× *Burkholderia mallei* | 1× *Burkholderia stabilis* | 1× *Francisella tularensis* |
| 1× *Candida albicans* | 1× *Enterococcus faecalis* | |
| 1× *Francisella tularensis* | | |

## 1.5 Tabellenverzeichnis

# 2. Zusammenfassende Darstellung der Publikation

## 2.1 Einleitung

Burkholderien sind Gram-negative stäbchenförmige, aerobe Bakterien, die nicht zur Glucosefermentierung befähigt sind. Wir unterscheiden verschiedene Arten. Unter anderem sind *Burkholderia* (*B*.) *mallei* (Erreger der Rotzerkrankung) und *B. pseudomallei* (Erreger der Melioidose) obligat pathogen und *B. cepacia* (Atemwegsinfektionen, besonders bei immunsupprimierten Patienten) fakultativ pathogen, weshalb ihnen eine besondere Bedeutung zukommt (Hahn et al.1999).

Akute Infektionen durch *B. pseudomallei* können tödlich verlaufen. Eine Verwechselung von Melioidose und Tuberkulose ist auf Grund der Granulombildung möglich.

*B. mallei* als Erreger des Rotzes kann sich ebenfalls als akute Pneumonie oder Sepsis manifestieren. Beide Erreger führen zu schweren, oft tödlich verlaufenden Krankheiten (Neumeister et al. 2009).

Sie können wie weitere andere Erreger (*Brucella* spp., *Yersinia pestis*, *Bacillus anthracis* u.a.) durch Aerosole und durch Kontakt mit kontaminierten Gegenständen übertragen werden. Sie wurden vom Center for Diseases Control and Prävention (CDC) in die Kategorie B der bioterroristisch relevanten Erreger eingeordnet. Teilweise sind sie in staatlichen B-Waffenprogrammen als solche munitioniert. Es wird an Verfahren zur Prävention, Dekontamination oder Verringerung der Übertragungsrate gearbeitet (Sanitätsdienst der Bundeswehr 2017).

In Anbetracht der hohen klinischen Relevanz der korrekten Identifizierung von Rotz-Melioidose- oder *Burkholderia*-assoziierten Atemwegsinfektionen bei Patienten mit zystischer Fibrose ist eine zuverlässige Identifizierung des ursächlichen Erregers wichtig. Eine Fehlidentifizierung eines Erregers kann kritische klinische Folgen haben. So beschreiben Frickmann et al. 2013 eine Fehldiagnose von *B. pseudomallei* als *B. cepacia* mit insuffizienter Therapie und tödlichem Ausgang.

Auch Kingsley et al. 2016, beschreiben die Melioidose als schwere und tödliche Infektionserkrankung die sich von chronisch-lokalisiert in eine akute fulminante Septikämie mit multiplen Organabszessen wandeln kann. Da sich die Melioidose mit unspezifischen Symptomen präsentiert, bleibt sie vom erstbehandelnden Arzt in einer Praxis oder Klinik, besonders in nichtendemischen Gebieten, in denen die Erkrankung nicht geläufig ist, häufig unerkannt.

Blutkulturen im Falle einer Sepsis mit nachfolgender Kultur auf Standard-Routinemedien führen zu einem unspezifischen Wachstum anderer Gram-negativer nichtglucosefermentierender stäbchenförmiger Bakterien wie *Pseudomonas* spp.. Hier besteht ein hohes Verwechslungsrisiko der verschiedenen Bakterien. Nachfolgende Routinetests mit handelsüblichen Testsystemen wie API20 (bioMérieux, Nürtingen, Deutschland), VITEK2 (bioMérieux) usw. haben sich als wenig spezifisch erwiesen. In den Routineanwendungen der Laserdesorptions-/Ionisations-Flugzeit-Massenspektrometrie (Matrix-Assisted-Laser-Desorption-/Ionization-Time-of-Flight-Mass-Spectrometry - MALDI-TOF-MS) fehlen vorgegebene Profile für diese Spezies in den Datenbanken. Die Verwendung selektiver Nährböden, d.h. MacConkey, Ashdown`s, *B. pseudomallei* Selektivagar und *B. cepacia* Selektivagar und eine verlängerte Inkubation für Proben, die mit normaler Flora kontaminiert sind, wird dringend empfohlen, um die Empfindlichkeit zu erhöhen.

Eine sehr gute Übersicht, die diese Probleme und einen geeigneten Arbeitsablauf im Detail beschreibt wurde vor kurzem veröffentlicht (Hemarajata et al. 2016).

Im Jahr 2015 beschreiben Lau et al. in ihrer Arbeit, dass kommerzielle Testsysteme nicht zwischen *B. pseudomallei* und eng verwandten Spezies, wie *B. thailandensis* unterscheiden können. Deshalb verwenden spezialisierte Laboratorien eine Vielzahl von Tests, um schließlich den relevanten Erreger zu identifizieren. Die molekulare Diagnostik wird durch die Ähnlichkeit von *B. pseudomallei* zu *B. mallei* und *B. thailandensis*, die Ursache für zoonotischen Rotz und ein relativ apathogenes Bodenbakterium, beeinträchtigt.

Spezifische Antikörper zum Nachweis von *B. pseudomallei* sind im Handel nicht erhältlich und daher sind Tests, die auf antikörperbasierter Diagnostik basieren, nicht hinreichend validiert worden. Es besteht die Möglichkeit von Laborinfektionen, so dass empfohlen wird, nur unter BSL-3 Laborbedingungen zu arbeiten, wenn eine Melioidose oder *B. mallei*- oder *B. pseudomallei*-Infektion vermutet wird. Eine gezielte Anzucht von *B. pseudomallei,* Resistenztestung oder biochemische Identifikation darf nur unter BSL-3 Laborbedingungen erfolgen.

Für die zuverlässige Unterscheidung anderer Burkholderienspezies, zum Beispiel von Stämmen des *B. cepacia*-Komplexes, wurden sequenzbasierte molekulare Untersuchungen eingeführt. Sie umfassen die Multilocus Sequenztypisierung (MLST) (Baldwin et al. 2005), Sequenzierung (Lynch und Dennis 2008), 90 *hisA*-Sequenzierung (Papaleo et al. 2010) oder *recA*-Sequenzierung (Payne et al. 2005, Cesarini et al. 2009) aus Reinkulturen. Matrix-unterstützte Laser-Desorptions-/Ionisations-Flugzeit-Massenspektrometrie (MALDI-TOF-MS) basierte Ansätze wurden ebenfalls beschrieben (Karger et al. 2012, Degand et al. 2008, Vanlaere et

al. 2008, Minan et al. 2009). Sämtliche dieser Verfahren erfordern jedoch die Identifizierung verdächtiger Kulturen durch einen Untersucher.

Die zuverlässige Identifikation einzelner Burkholderienspezies ist notwendig, um eine spezifische Therapie einleiten zu können, da gerade bei den Burkholderien häufig Resistenzen gegen Antibiotika bestehen.

Hierbei können Selektivnährböden nützlich sein um ein selektives Wachstum und somit eine erleichterte Identifizierung von vermuteten Keimen zu ermöglichen (Micheel et al. 2015a und 2015b). Leider werden *Burkholderia* spp., die leicht auf Standardnährböden wie Blutagar wachsen, zuweilen übersehen (Frickmann et al. 2014, Ostermann et al. 2016), wenn nur einzelne winzige Kolonien wachsen und zusätzlich von apathogener Besiedlungsflora überwuchert werden. Selektive Nährböden werden verwendet, um selektives Wachstum zu ermöglichen und damit die Identifizierung von pathogenen Keimen zu erleichtern (Micheel et al. 2015a und 2015b). Dabei ist es wichtig, das diskriminierende Potential des selektiven Nährbodens zu kennen, um die Keime sicher unterscheiden zu können. Solche selektiven Nährböden sind üblicherweise mit Chemikalien oder Antibiotika versetzt, die hemmende Wirkung auf andere Erreger haben. Diese sind häufig mit Farbreaktionen verbunden, die die Identifizierung des Zielpathogens erleichtern (Henry et al. 1997).

In dieser Arbeit wurden die Wachstumseigenschaften verschiedener pathogener und apathogener Burkholderienspezies und anderer Bakterien und Pilze auf drei Selektivnährböden für Burkholderien untersucht. Die verwendeten Daten wurden unter Nutzung einer Stammsammlung mit einer großen Anzahl Burkholderien und weiterer Erreger erhoben. Parallel zum Wachstum auf den Selektivnährböden

erfolgte eine Wachstumskontrolle auf Blutagar. Ziel der Arbeit war es, sowohl die Sensitivität als auch die Selektivität der verwendeten selektiven Nährböden zu analysieren, um eine Empfehlung für die Routinediagnostik, basierend auf den Ergebnissen zu liefern.

## 2.2 Material und Methoden

Wir nutzten eine Stammsammlung bestehend aus 116 Referenzstämmen und klinischen Isolaten, die zuvor von erfahrenen medizinisch-technischen Laborassistenten(-innen) angezüchtet wurden. In die Bewertung wurden nur Stämme einbezogen, die entweder auf Blutagar oder zumindest auf einem Selektivagar wuchsen. Die 116 Stämme umfassten 30 *Burkholderia pseudomallei*-Stämme, 20 *Burkholderia mallei*-Stämme, 18 Stämme anderer *Burkholderia*-Spezies und 48 andere Erreger. Die Verteilung der Arten und Stämme ist in Tabelle 1 aufgeführt.

Zu den verwendeten Nährböden gehörte Blutagar, ein nichtselektives Kontrollmedium das als Wachstumsnachweis zur Beurteilung der Vitalität der Bakterien verwendet wurde. Dieser wurde aus pankreatisch abgebautem Casein 12,0 g/l, peptisch abgebautem Tiergewebe 5,0 g/l, Hefeextrakt 3,0 g/l, Rindfleischextrakt 3,0 g/l, Maisstärke 1,0 g/l, Natriumchlorid 5,0 g/l, Agar-Agar 13,5 g/l und defibriniertem Schafblut 5 % mit Reagenzien von Merck (Darmstadt, Deutschland) hergestellt.

Zusätzlich wurden als selektive Nährmedien Mast BCA (*Burkholderia cepacia* Agar), Ashdown+G (G für Gentamycin), *Burkholderia pseudomallei* Selektivagar (BPSA, Nilblau Agar) verwendet.

Der *Burkholderia cepacia* Agar (Mast BCA) wurde nach Herstelleranweisungen unter Verwendung von *Burkholderia cepacia* medium 36 g/l (Mast Diagnostica GmbH, Reinfeld, Deutschland), bi-destilliertem Wasser und 10 *Burkholderia cepacia* Supplementtabletten pro Liter (Mast Diagnostica GmbH) hergestellt. Die Ashdown+G Herstellung erfolgte aus TSB-Agar 10 g/l, Agar-Agar 15 g/l, Kristallviolett 5 mg /l, Neutralrot 50 mg/l, 40% Glycerollösung 100 ml/l, Gentamycin 5 mg/l und bi-destilliertem Wasser mit Reagenzien der Fa. Merck KGaA.

*Burkholderia pseudomallei* Selektivagar (BPSA, Nilblau Agar) wurde mit Standard Agar 23,5 g/l (Becton & Dickinson, Heidelberg, Deutschland), Maltose 4 g/l (Merck), Neutralrot 100 mg/l (Merck), Gentamycin 20 mg/l (Merck), Nilblau 0,2 g/l (Sigma, München) und bi-destilliertem Wasser hergestellt (Ashdown 1997 und Henry et al. 1997). Platten jeder Charge wurden als Sterilitätskontrolle inkubiert.

Es erfolgte im Rahmen der Auswertung eine Beurteilung des kulturellen Wachstums und verschiedener Wachstumscharakteristika auf den verwendeten Selektivnährböden nach 24 und 48 Stunden und nach 7 Tagen. Die untersuchten Wachstumscharakteristika umfassten sowohl normalgroß gewachsene Kolonien als auch sehr kleine Kolonien, die als „schwaches Wachstum“ gekennzeichnet wurden. Des Weiteren wurde für die Bewertung die Farbe erfasst. Hier erfolgte eine Unterscheidung nach 12 verschiedenen Farben (silbrig-metallisch, weiß, grau, creme, gelb, rot, rosa-pink, malve-hellviolett, purpur, dunkelviolett, braun, schwarz). Bei der Transparenz der Kolonien erfolgte eine Differenzierung nach durchsichtig -

klar, milchglasartig und undurchsichtig. Die Koloniegröße wurde in 3 Gruppen eingeteilt (<1mm, 1-4mm, >4mm) und als Kolonieform konnten runde, gelappte und unregelmäßig gewachsene Formen erfasst werden. Das Profil der Kolonien wurde in flach, flach mit Ausstülpungen, erhaben, konvex, konvex mit Eindellungen und konvex mit Ausstülpungen eingeteilt. Die Oberfläche wurde je nach Beschaffenheit mit glatt, schleimig, trocken und faltig-runzelig beschrieben und das Auftreten von Farbveränderungen bzw. -entfärbung des Nährbodens wurde ebenfalls erfasst, wenn dies erfolgte. Ein Farbumschlag von gelb nach rot konnte bei *Cepacia*-Agar, eine Entfärbung des Nährbodens um die Kolonien bei Ashdown+G-Agar und BPSA-Agar beobachtet werden.

Alle Daten zu den Wachstumscharakteristika wurden in einer Tabelle zusammengefasst, aus der dann eine Extraktion der Ergebnisse durchgeführt wurde.

## 2.3 Ergebnisse

Die Ergebnisse sind ausführlich in der beiliegenden Publikation dargestellt.

**Kulturelles Wachstum von *Burkholderia* spp. auf Selektivnährböden:**

Nach einer Gesamtbeobachtungszeit von 7 Tagen zeigten alle 30 *Burkholderia pseudomallei*-Stämme auf den 3 unterschiedlichen Nährböden ein kulturelles Wachstum.

*Burkholderia cepacia* Agar (BCA) ermöglichte ferner das Wachstum von 12 weiteren Burkholderienspezies. Kein Wachstum erfolgte bei *B. fungorum* und *B. graminis.*
Die Nährböden Ashdown+G-Agar und auf BPSA (Nilblau) Agar ermöglichten in Ergänzung zu dem Wachstum von *B. pseudomallei* das Wachstum von jeweils 9 weiteren Burkholderienspezies. Auf diesen beiden Nährböden erfolgte kein Wachstum von 5 Burkholderienspezies (*B. anthina, B. vietnamensis, B. fungorum, B. gladioli* und *B. graminis*), (Tabelle 2).

Ein deutlich erkennbares Wachstum in der Kultur war bereits nach 2 Tagen sichtbar (Ergänzungsmaterialien 1, 2).

Im Einzelnen ist ein Wachstum erst nach 48 Stunden für vier *B. mallei*-Stämme und einen *B. cocovenenans*-Stamm auf MAST BCA dokumentiert. Gleiches gilt für einen *B. stabilis*-Stamm auf Ashdown+Gentamycin-Agar und BPSA (Nilblau)-Agar (Ergänzungsmaterial 3).

**Kulturelles Wachstum von anderen Bakterienstämmen auf Selektivnährböden:**

Weiterhin wurde auch das Wachstum anderer (Nicht-*Burkholderia*-Spezies) Erreger auf den Selektivnährböden bis zu einer Gesamtbeobachtungszeit von 7 Tagen betrachtet. Jeder selektive Agar zeigte ein Wachstum sonstiger Erreger in variierender Häufigkeit, wobei sich hier ein überwiegendes Wachstum von nicht-fermentativen Gram-negativen stäbchenförmigen Bakterien zeigte, aber auch andere Spezies wie *Enterobactericae,* Gram-positive Bakterien und Hefen wuchsen (Tabelle 2).

Auf MAST BCA-Agar wuchsen 12 von 48, auf Ashdown+G-Agar 14 und auf BPSA (Nilblau)-Agar waren 13 verschiedene Nichtburkholderienspezies nachweisbar.
Bei 23 verschiedenen Spezies erfolgte kein Wachstum auf den 3 Nährböden.
Insbesondere war bei einzelnen anderen Bakterienstämmen nach mehr als 48 Stunden ein Wachstum nachweisbar. Hierzu gehörten 1 *Francisella tularensis* Stamm, der auf beiden Nährböden, Mast BCA und BPSA (Nilblau) wuchs, 1 Stamm *Candida albicans* und 1 Stamm *Aeromonas hydrophila* ssp*. hydrophila,* die auf Mast BCA-Agar und 1 *Enterococcus faecalis* Stamm der auf Ashdown+G-Agar wuchsen (Ergänzungsmaterial 3).

**Morphologische Merkmale von *Burkholderia* spp. und sonstigen Erregern auf allen drei Nährböden:**

Bei den Burkholderien  und sonstigen Erregern zeigten sich nach insgesamt 7 Tagen auf allen Nährböden in der Mehrheit verschiedene Rot-Rosatöne.

Bei der Beurteilung der Größe fiel auf, dass ein Großteil Burkholderienkulturen auf MAST BCA-Agar zwischen 1-4mm groß waren. Die anderen Erreger zeigten auf MAST BCA-Agar verschiedene Größen. Auf den anderen beiden Nährböden waren alle Erreger beim Größenwachstum sehr variabel.

Bei der Beurteilung der Form der Kolonien war bei allen Erregern und Nährböden überwiegend „rund“ anzutreffen. Eine Ausnahme zeigte sich auf MAST BCA-Agar bei den Nichtburkholderien. Auf diesem Nährboden wechselte die Form nach insgesamt 7 Tagen oft von rund auf unregelmäßig.

Bei der Transparenz der Kolonien zeigten sowohl die Burkholderien als auch die sonstigen Erreger ähnliche Ergebnisse. In der Mehrheit waren diese milchglasartig und undurchsichtig.

Bei der Beurteilung der Oberfläche ist das Merkmal „Oberfläche glatt“, bei den Burkholderien und anderen Erregern, am Häufigsten zu finden. Gleiches gilt für die Kolonieprofile, die sowohl bei den Burkholderien als auch den anderen Erregern meist flach waren.

Im Ergebnis dieser Beobachtung erscheint eine Unterscheidung durch die oben beschriebenen morphologischen Merkmale nicht sinnvoll.
Die Daten, die in den Tabellen 3 bis 5 erfasst sind, machen deutlich, dass es eine eher stammabhängige als speziesabhängige Heterogenität des Wachstumsverhaltens auf den verschiedenen Selektivnährböden gibt.
Typische speziesspezifische Kolonien wurden nicht beobachtet. Darüber hinaus veränderten die Kolonien ihre morphologischen Merkmale im Zuge des kulturellen Wachstums, ohne spezifische Merkmale zur Unterscheidung zu zeigen.

Das Risiko einer Verwechslung oder gar Fehlbestimmung für nicht erfahrene Untersucher ist sehr wahrscheinlich. Auch innerhalb einer Spezies finden sich kaum Unterschiede, so dass auch hier ein beträchtliches Verwechslungsrisiko besteht. Es fand sich kein Hinweis auf einen möglichen Durchbruch von sonstigen Erregern bei längerer Inkubationszeit (>48h).

## 2.4 Diskussion

In der hier vorgestellten Studie wurden insgesamt 116 Stämme verschiedener Bakterien und Pilze einbezogen, deren Wachstumsverhalten auf 3 unterschiedlichen Nährböden untersucht werden sollte.

Die Studie beurteilte die Zuverlässigkeit dreier verschiedener selektiver Medien, MAST BCA, Ashdown+G-Agar und BPSA (Nilblau) Agar hinsichtlich ihrer Selektivität für verschiedene *Burkholderia* spp. und anderer Erreger.

Die Ergebnisse zeigten, dass *B. pseudomallei* auf allen drei Nährböden hervorragend wächst. Dieses Ergebnis bestätigt auch die Ergebnisse von Roesnita et al. aus dem Jahr 2012, dass *B. pseudomallei* Selektivagar (BPSA) eine kostengünstige Screening-Option für Melioidose ist. Diese Autoren identifizierten einen zusätzlichen Fall von Melioidose und drei zusätzliche kultur-positive Proben mit *B. pseudomallei* durch Anwendung dieses Nährbodens im Vergleich zu Standard-Diagnoseverfahren mit nichtselektiven Medien. Der Nährboden BPSA wurde durch Howard und Inglis im Jahr 2003 für die selektive Kultivierung von *B. pseudomallei* eingeführt, da er sehr selektiv Gram-positive und Gram-negative Keime, unter anderem Erreger wie *Pseudomonas aeruginosa*, *Burkholderia* spp. des *B. cepacia*-Komplexes, Enterokokken, Staphylokokken, *Escherichia* und Streptokokken, hemmen sollte.

Die hier präsentierten Daten können diese Ergebnisse nicht bestätigen, da sowohl *P. aeruginosa* als auch *B. cepacia*-Stämme auf diesem Agar wuchsen.

In einer weiteren Studie zeigten Peacock et al. im Jahr 2005 einen Vergleich von BCA, Ashdown-Agar und BPSA mit klinischen Proben, in dem die Eignung aller drei Nährböden zur Isolierung von *B. pseudomallei* nahezu gleichwertig war. Ashdown-

Agar und BCA waren zudem etwas spezifischer bei der Untersuchung klinischer Proben. Auf Ashdown war bei *B. pseudomallei* ein charakteristisches Wachstum von Kulturen mit lilafarbenen trockenem und faltig-runzeligen Aussehen beobachtet worden. Die Koloniemorphologie der meisten Organismen war in dieser Studie klar von der Koloniemorphologie der Stämme von *B. pseudomallei* abgrenzbar.
Diese Ergebnisse waren mit den von mir erhobenen Daten von insgesamt 30 *Burkholderia pseudomallei*-Stämmen, 20 *B. mallei*-Stämmen, 18 anderen Burkholderienspezies und weiteren 48 anderen Spezies, nicht vergleichbar.

In der hier vorgestellten Studie wuchs nur ein Teil der *Burkholderia* spp. auf den bewerteten selektiven Medien, wobei *B. pseudomallei*, zu 100% auf allen verwendeten Nährmedien wuchs. Sämtliche Burkholderien als auch andere Erreger zeigten eine Vielzahl von unterschiedlichen Rot-Rosatönen, als auch ähnliche weitere morphologische Merkmale, so dass eine Differenzierung in dieser Studie durch morphologische Merkmale nicht sinnvoll erscheint.
Sowohl für die in der Umwelt vorkommenden Burkholderien (Erdboden, Grundwasser, Meere) mit fehlender Bedeutung in der Humanmedizin, als auch für Stämme mit einer hohen ätiologischen Relevanz wie z.B. *B. mallei*, dem Erreger der Rotzerkrankung, wurde dies beobachtet. Die Ergebnisse zeigen, dass die verwendeten Nährmedien sowohl das Wachstum von *Burkholderia* spp., als auch von anderen Erregern nur partiell ermöglichen. Anders verhält es sich jedoch bei *B. pseudomallei*, der im Vergleich zu allen anderen Erregern auf allen Nährböden ein Wachstum zeigte. Die geschilderten Sensitivitäts-und Spezifitätsprobleme verringern den diagnostischen Nutzen dieser Nährmedien erheblich.

Durch Glass et al. im Jahr 2009 wurde der Ashdown-Agar für die Isolierung von *B. pseudomallei* als am empfindlichsten beschrieben. Im Ergebnis der Studie wurde gezeigt, dass das Wachstum von *B. mallei* in der Regel auf diesem Medium (Ashdown+G) nicht erfolgreich ist.

Dies konnte jedoch durch die hier vorliegenden Untersuchungsergebnisse mit Ashdown+G-Agar nicht bestätigt werden, da dieser Agar das Wachstum einiger *B. mallei*-Stämme zuließ. Die hier präsentierten Daten zeigen aber auch, dass der Anteil anderer Bakterien, die später als 48h nach Beginn der Beobachtung sichtbar wurden, gering ist. Demzufolge bedeutet in unserem Fall eine längere Inkubationszeit keinen zwingenden Spezifitätsnachteil. Glass et al. bevorzugten im Jahr 2009 die Verwendung von *Pseudomonas cepacia* Agar (PCA), der sich als empfindlich und selektiv erwies, wenn das selektive Wachstum von *B. mallei* und *B. pseudomallei* erwünscht ist.

Bei der Fokussierung auf klinisch relevante Arten des *B. cepacia*-Komplexes und auf Umwelt-Burkholderien unterscheiden sich deren metabolische Bedürfnisse speziesspezifisch. Bemerkenswerterweise war L-Arabinose in einer früheren Studie die am häufigsten verwertete Kohlenstoffquelle, die von Spezies des *B. cepacia*-Komplexes verwendet wurde und die das Wachstum von 90% der Isolate in einer früheren Studie unterstützte (Vermis et al. 2003), während *B. anthina* und *B. vietnamensis* am empfindlichsten auf Antibiotika reagierten. Die letzteren Ergebnisse werden durch die hier vorgestellte Studie bestätigt. Auch hier erfolgte kein Wachstum von *B. anthina*- und *B. vietnamensis*-Stämmen auf Ashdown+G-Agar und BPSA-Agar. Weiterhin zeigte sich für weitere 3 Burkholderien-Spezies (*B. fungorum, B. gladioli* und *B. graminis*) auch kein Wachstum auf diesen Medien. Frühere Analysen (Ostermann et al. 2016) zeigten jedoch, dass die Antibiotikaresistenz stärker

stammabhängig zu sein scheint als speziesabhängig, insbesondere bei Isolaten von Patienten mit zystischer Fibrose unter Antibiotikabehandlung.

Vermis et al. zeigten im Jahr 2003 ferner eine breite phänotypische Heterogenität der beurteilten *B. cepacia*-Komplexstämme auf dem untersuchten selektiven Mast *B. cepacia*-Agar (BCA) und auf dem *Burkholderia cepacia* Selektivagar (BCSA), was die unterschiedlichen Bedürfnisse der verschiedenen *Burkholderia* spp. hinsichtlich der Zusammensetzung von Wachstumsmedien wiederspiegelt.

In der hier vorgestellten Studie lässt die beobachtete Varianz der Wachstumsmerkmale innerhalb der Spezies auf den selektiven Medien keine Unterscheidung auf Speziesebene zu, ähnlich den Beobachtungen von Vermis et al. im Jahr 2003. Eine weitere Studie, die Mast *B. cepacia*-Agar (BCA) mit *B. cepacia* Selektiv-Agar (BCSA) für die Identifizierung von *B. cepacia*-Komplexstämmen aus Sputumproben von Mukoviszidosepatienten verglich, zeigte eine Überlegenheit von BCSA-Agar hinsichtlich der Selektivität und der reduzierten Nachweiszeit (Wright et al. 2001).

BCSA (*B. cepacia* Selektiv-Agar) wurde erstmals 1997 als selektives Medium für die Identifizierung von *B. cepacia*-Komplexstämmen aus respiratorischen Sekreten von Mukoviszidosepatienten beschrieben (Henry et al. 1997). BCSA war als Agar für die primäre Isolierung gedacht und erwies sich als überlegen gegenüber dem älteren Oxidations-Fermentations-Polymyxin-Bacitracin-Lactose (OFPBL)-Agar (Welch et al. 1987) und *Pseudomonas cepacia*-Agar (PCA), die ebenfalls das Wachstum anderer nicht-fermentativer Gram-negativer stäbchenförmiger Bakterien wie *Stenotrophomonas maltophilia*, *Alcaligenes xylosoxidans* und *Comamonas acidovorans* ermöglichen.

Das Wachstum der *Burkholderia cepacia* Stämme auf BCSA war auch schneller als

auf OFPBL Agar und PCA (Henry et al. 1997). In einer späteren Studie wurden diese Ergebnisse auch für Sputumproben von Mukoviszidosepatienten bestätigt (Henry et al. 1999).

Es bleibt umstritten, ob eine vorherige Anreicherung dazu beitragen kann die Empfindlichkeit von selektiven Nährböden für *Burkholderia* spp. zu erhöhen. Eine alte Studie zeigte eine leichte Überlegenheit für die selektive Anreicherung mit anschließender Kultur auf Polymyxin B-MacConkey-Agar ohne Kristallviolett, *P. cepacia*-Agar und OFPBL-Agar (Cimolai et al. 1995). Eine spätere Studie konnte diesen Befund nicht bestätigen und zeigte identische Ergebnisse für die direkte Primäranzucht auf selektivem BCSA und für die Anlage von Subkulturen nach Wachstum in einer Anreicherungsboullion (Flanagan und Pauli, 1998). Die Frage, ob eine vorherige Anreicherung notwendig ist, um *Burkholderia* spp. von respiratorischen Proben von Patienten mit zystischer Fibrose zuverlässig zu identifizieren, ist nicht endgültig beantwortet. Die bisher vorliegenden Ergebnisse sind widersprüchlich.

Im Ergebnis der Auswertung der hier untersuchten Stämme sind alle hier untersuchten Medien nicht in der Lage das Wachstum nichtpathogener *Burkholderia* spp., sowie sonstiger Erreger, wie fakultativ pathogene Pseudomonaden und *Enterobactericae*, die häufig von primär sterilen Körperkompartimenten im Falle von invasiven Infektionen isoliert werden und somit möglicherweise invasiven *Burkholderia*-Infektionen ähneln, zuverlässig zu unterdrücken. Schon 2012 zeigten Roesnita et al. ein Wachstum von *Candida* Spezies auf dem Routinemedium, dass die Burkholderien überwucherte. Die Tatsache, dass sogar das Wachstum von Pilzen (*Candida albicans*) durch diese selektiven Nährböden unterstützt wurde, steht im Einklang mit der jüngsten Feststellung, dass BCSA zur Isolierung von *Exophiala*

*dermatitidis*, einem typischen Erreger der Phaeohyphomykose, verwendet werden kann (Raclavsky und Novotny 2016).

Die hier untersuchten selektiven Medien sind im Rahmen der Diagnostik nur eingeschränkt nutzbar. Dies trifft sowohl für die Sensitivität als auch für die Spezifität zu. Ihre Nützlichkeit für die bereits etablierte Identifizierung von *B. pseudomallei* (Howard und Inglis 2003, Roesnita et al. 2012, Peacock et al. 2005) wurde wiederum durch diese Studie bestätigt. Im Gegensatz dazu erwiesen sich die Medien zur Anzucht anderer *Burkholderia* spp. als nicht zuverlässig. Daher sollten Screenings bei Verdacht auf Infektionen durch *Burkholderia* spp. nicht ausschließlich auf der Beurteilung dieser drei selektiven Agar-Teste basieren, sondern es sollte auch eine Verwendung von nicht-selektiven Medien erfolgen, der sich eine anschließende Differenzierung mit mikrobiologischen Standardverfahren anschließt (Hagen et al. 2011, Karger et al. 2012, Vanlaere et al. 2008).

# 3. Zusammenfassung

## 3.1 Deutsche Zusammenfassung

In dieser Arbeit wurde untersucht, wie zuverlässig pathogene Burkholderien wie *B. pseudomallei*, *B. mallei* und *B. cepacia* aus klinischen Proben isoliert werden können. Hierzu wurden drei verschiedene Nährmedien (MAST *Burkholderia Cepacia*-Agar, Ashdown+Gentamycin-Agar und *B. pseudomallei*-Selektivagar) hinsichtlich ihrer Zuverlässigkeit und Selektivität miteinander verglichen. Dafür wurde eine Gruppe aus 30 *B. pseudomallei*, 20 *B. mallei*, 18 weiteren Burkholderienspezies und 48 sonstigen Erregern in ihrem quantitativen und qualitativen Wachstum bewertet.

Im Ergebnis konnte gezeigt werden, dass alle *B. pseudomallei*-Stämme auf allen drei getesteten selektiven Nährböden wachsen, während die anderen Burkholderien ein differenziertes Wachstumsmuster zeigten. Andere bakterielle Erreger wie – insbesondere aber nicht ausschließlich - nichtfermentative stäbchenförmige Bakterien und sogar Hefen wuchsen auf allen selektiven Nährböden. Die Koloniemorphologie erlaubte hier keine eindeutige Unterscheidung der einzelnen Erreger, insbesondere keine sichere Diskriminierung zwischen Burkholderien und Erregern anderer Gattungen. Während die untersuchten selektiven Nährmedien das Wachstum einer großen Zahl von *B. pseudomallei*-Stämmen zuverlässig ermöglichten, war das Wachstum anderer *Burkholderia*-Spezies nur teilweise gewährleistet. Die ebenfalls nicht suffiziente Unterdrückung des Wachstums von Mikroorganismen jenseits der Zielerreger muss ebenfalls berücksichtigt werden und

reduziert die Spezifität. Daher können die beurteilten Medien nur in Kombination mit weiteren bestätigenden Tests im diagnostischen Prozess zum Screening auf Melioidose und ggf. Rotz eingesetzt werden.

## 3.2 Englische Zusammenfassung

This study investigates how reliable pathogenic *Burkholderia* spp. like *Burkholderia mallei*, *Burkholderia pseudomallei* and *Burkholderia cepacia* can be identified in clinical samples. Three different selective media (Mast *Burkholderia cepacia* agar, Ashdown + gentamicin agar and *Burkholderia pseudomallei* selective agar) were assessed for diagnostic reliability and selectivity. Therefore the growth of 30 *Burkholderia pseudomallei,* 20 *Burkholderia mallei*, 18 other *Burkholderia* spp. and 48 non-target organisms was assessed.

As the results shows, all *B. pseudomallei* strains grew on all three tested agars, while the other *Burkholderia* spp. showed a diverse growth pattern. Non-target organisms, i.e. nonfermentative rod-shaped bacteria, other species and yeasts grew on all selective agars. Colony morphology did not allow unambiguous discrimination. This also applies for the discrimination of *Burkholderia* spp. from non-target species. While the assessed selective media reliably allowed the growth of a wide range of *B. pseudomallei* strains, the growth of other *Burkholderia* spp. is only partially ensured. Furthermore the growth of various non-target organisms, which was, only partially suppressed, has to be considered and reduces the diagnostic specificity. 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.

## 4. Abkürzungsverzeichnis

| | |
|---|---|
| API | analytical profile index |
| B. | *Burkholderia* |
| BCA | *Burkholderia cepacia* agar |
| BCSA | *Burkholderia cepacia* Selektivagar |
| BPSA | *Burkholderia pseudomallei* Selektivagar |
| BSL-3 | Bio-Sicherheits-Laborbedingungen Stufe 3 |
| CDC | Center for Diseases Control and Prävention |
| d | Tage |
| e.g. | exemplī grātiā (for example) |
| Fa. | Firma |
| G | Gentamycin |
| g/l | Gramm / Liter |
| GmbH | Gesellschaft mit beschränkter Haftung |
| h | Stunden |
| KgaA | Kommanditgesellschaft auf Aktien |
| Ltd. | Limited |
| MALDI-TOF-MS | Matrix-unterstützte Laser-Desorptions-Ionisations-Flugzeit-Massenspektrometrie |
| MLST | Multilocus sequence typing –Multilocus Sequenztypisierung |
| mg/l | Milligramm / Liter |
| ml/l | Milliliter / Liter |
| OFPL-Agar | Oxidations-Fermentations-Polymyxin-Bacitracin-Lactose-Agar |
| P. | *Pseudomonas* |
| PCA | *Pseudomonas cepacia* agar |
| spp. | species pluralis |
| ssp | Subspezies |
| TSB | Tryptone Soya Broth=Tryptikase-Soja-Bouillon |
| z.B. | zum Beispiel |
| % | Prozent |

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## 6. Erklärung des Eigenanteils

Die Arbeit wurde am Fachbereich Tropenmedizin des Bundeswehrkrankenhauses Hamburg am Bernhard-Nocht-Institut Hamburg unter der Betreuung von Herrn PD Dr. Ralf Matthias Hagen und Herrn PD Dr. Hagen Frickmann durchgeführt.

Es erfolgte eine Beurteilung verschiedener Referenzstämme und klinischer Isolate, die auf verschiedenen selektiven Nährmedien wuchsen.
Die Auswertung und tabellarische Darstellung erfolgte durch mich persönlich.

Die dabei erhobenen Daten wurden durch mich auf Vollständigkeit geprüft und in Exceltabellen digitalisiert.

Die Auswertung der Ergebnisse erfolgte nach Beratung durch Herrn PD Dr. Hagen Frickmann durch mich persönlich.

In dem gemeinsam verfassten Artikel hatte ich als Erstautorin einen wesentlichen Anteil.

Ich versichere, keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Norderstedt, den

## 7. Danksagung

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## 8. Lebenslauf

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## 9. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den verwendeten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....