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

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

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

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

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

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

Introduction

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

Considering the high clinical relevance of the correct identification of glanders, melioidosis, or Burkholderia-associated respiratory tract infections in cystic fibrosis patients, reliable identification of the causative agent is important and incorrect identification can lead to critical clinical courses [5].

Melioidosis presents with unspecific symptoms and remains often unrecognized by the first responder, i.e., the clinician at a private practice or a local hospital especially in nonendemic areas where the physicians are unaware of the disease [6]. Blood culture in case of sepsis and subsequent culture on standard routine media result in un-
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,
Nürtening, 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., MacCon-
key, Ashdown’s, B. pseudomallei selective, and B. cepacia
selective agar and prolonged incubation for specimens be-
ing 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
detect 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, respec-
tively [8]. Specific antibodies to detect B. pseudomallei
are not commercially available, and thus, tests based on
these tools have not been validated accordingly. Labo-
atory infection may occur, and it is strongly advised to
work only under BSL-3 biosafety laboratory conditions if
meliodosis 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 com-
prise multilocus sequence typing (MLST) [9], fur sequ-
coding [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 pres-
ent 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 drug
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 compris-
ing a considerable number of target and nontarget organ-
isms. 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-RO, M VIII, 005-00574 M2, 005-00577/2002 M3, Mukteswar, 005-00582 U5, NCTC 7309, NC 00120-05, NC01260-03, 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, ATCC 700388 |
| Burkholderia vandii (n = 2) | DSM 9509, DSM 951/LMG 16020 |
| Burkholderia vietnemensis (n = 1) | DSM 11319 |
| Burkholderia cenocepacia (n = 1) | LMG 12615 |
| Burkholderia cocovenenans (n = 1) | DSM 4285 |
| 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**

| Species                        | Strains                  |
|-------------------------------|--------------------------|
| *Francisella tularensis* (n = 4) | Isolates (n = 4)         |
| *Pseudomonas aeruginosa* (n = 2) | DSM 11810, ATCC 27853   |
| *Achromobacter ruhlandii* (n = 1) | DSM 653                |
| *Achromobacter xylosidans spp. denitrificans* (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 polymyxia* (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 denitrificans* (n = 1) | DSM 10202              |
| *Klebsiella oxiota* (n = 1) | Isolate                 |
| *Klebsiella pneumoniae spp. pneumoniae* (n = 1) | DSM 6675/681 |
| *Listeria monocytogenes* (n = 1) | DSM 12464              |
| *Moraxella catarrhalis* (n = 1) | DSM 9143               |
| *Morganella morganii* (n = 1) | DSM 6675                |
| *Ochrobactrum anthropi* (n = 1) | DSM 7216                |
| *Proteus mirabilis* (n = 1) | DSM 4479                |
| *Proteus vulgaris* (n = 1) | DSM 30118               |
| *Psychrobacter phenylpyruvicus* (n = 1) | DSM 7000            |
| *Salmonella Typhimurium* (n = 1) | ATCC 13311           |
| *Shigella flexneri* (n = 1) | DSM 4782                |
| *Sphingomonas paucimobilis* (n = 1) | DSM 1098              |
| *Staphylococcus aureus* (n = 1) | DSM 346                |
| *Staphylococcus epidermidis* (n = 1) | DSM 1798             |
| *Stenotrophomonas maltophilia* (n = 1) | DSMZ 50170        |
| *Streptococcus agalactiae* (n = 1) | Isolate               |
| *Streptococcus pyogenes* (n = 1) | Isolate              |
| *Vibrio cholerae* (n = 1) | 219512                |
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 pancreateically 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 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; and bidistilled water with reagents provided by Merck (Darmstadt, Germany). Plates of each charge were incubated for sterility assessment.

### 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.

### Table 1. (cont’d)

| Species                        | Strains   |
|--------------------------------|-----------|
| *Vibrio parahaemolyticus* (n = 1) | DSM 10027 |
| *Yersinia enterocolitica* (n = 1) | DSM 4780 |
| *Yersinia kristensenii* (n = 1)   | ATCC 33638|
| *Yersinia pestis* (n = 1)         | EV 76     |
| *Yersinia pseudotuberculosis* (n = 1) | ATCC 29833|

### 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 faeacalis* spp. *faeacalis*, *Candida albicans*, *Chromobacterium violaceum*, *Enterobacter aerogenes*, *Francisella tularensis*, *Kingella denitrificans*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* spp. *pneumoniae*, *Mor- ganella morganii*, *Pseudomonas aeruginosa*, and *Vibrio parahaemolyticus* was detectable (Table 2). Ashdown + G agar showed cultural growth of nontarget organisms (Table 2).
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 |
| *Burkholderia pseudomallei* (n = 30) | 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%)       |
| *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%)         |
| *Burkholderia anthina* (n = 1)      | 1/1 (100%)   | 0/1 (0%)     | 1/1 (100%)       | 0/1 (0%)         | 0/1 (0%)           | 0/1 (0%)         |
| *Burkholderia stablis* (n = 2)      | 2/2 (100%)   | 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%)         |
| *Burkholderia vanill* (n = 2)       | 2/2 (100%)   | 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%)         |
| *Burkholderia cenocepacia* (n = 1)  | 1/1 (100%)   | 0/1 (0%)     | 1/1 (100%)       | 0/1 (0%)         | 1/1 (100%)         | 0/1 (0%)         |
| *Burkholderia cocovenans* (n = 1)   | 1/1 (100%)   | 0/1 (0%)     | 1/1 (100%)       | 1/1 (100%)       | 0/1 (0%)           | 1/1 (100%)       |
| *Burkholderia dolosa* (n = 1)       | 1/1 (100%)   | 0/1 (0%)     | 1/1 (100%)       | 1/1 (100%)       | 0/1 (0%)           | 1/1 (100%)       |
| *Burkholderia fungorum* (n = 1)     | 1/1 (100%)   | 0/1 (0%)     | 1/1 (100%)       | 0/1 (0%)         | 0/1 (0%)           | 0/1 (0%)         |
| *Burkholderia gladioli* (n = 1)     | 1/1 (100%)   | 0/1 (0%)     | 1/1 (100%)       | 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%)         |
| *Burkholderia graninis* (n = 1)     | 1/1 (100%)   | 0/1 (0%)     | 0/1 (0%)         | 0/1 (0%)         | 0/1 (0%)           | 0/1 (0%)         |

Growth of nontarget organisms

|                      | Normal cultural growth | Weak growth only | Normal cultural growth | Weak growth only |
|----------------------|------------------------|------------------|------------------------|------------------|
| *Francisella tularensis* (n = 4) | 3/4 (75%)              | 1/4 (25%)        | 0/4 (0%)               | 0/4 (0%)         |
| *Pseudomonas aeruginosa* (n = 2) | 2/2 (100%)             | 0/2 (0%)         | 1/2 (50%)              | 2/2 (100%)       |
| *Achromobacter ruhlandii* (n = 1) | 1/1 (100%)             | 0/1 (0%)         | 1/1 (100%)             | 1/1 (100%)       |
| *Achromobacter xylosidans spp.* denitrificans (n = 1) | 1/1 (100%)             | 0/1 (0%)         | 1/1 (100%)             | 1/1 (100%)       |
| *Acinetobacter baumannii* (n = 1) | 1/1 (100%)             | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         |
| *Acinetobacter haemolyticus* (n = 1) | 1/1 (100%)             | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         |
| *Alcaligenes faecalis* spp. faecalis (n = 1) | 1/1 (100%)             | 0/1 (0%)         | 1/1 (100%)             | 0/1 (0%)         |
| 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%) |
| Enterococcus 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 denitrificans (n = 1) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) |
| Klebsiella oxytoca (n = 1) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 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 phenylpyruvics (n = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) |
| Salmonella Typhimurium (n = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| Shigella flexneri (n = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
aglar allowed growth of *A. ruhlandii*, *Achromobacter xylosidans* spp. *denitrificans*, *Aeromonas hydrophila* spp. *hydrophila*, *C. albicans*, *E. aerogenes*, *Enterococcus faecalis*, *K. denitrificans*, *K. pneumoniae* spp. *pneumoniae*, *Proteus vulgaris*, *P. aeruginosa*, *Psychrobacter phenylpyruvicus*, *Sienotrophomonas maltophilia*, *V. parahaemolyticus*, and *Yersinia pestis* (Table 2). Finally, strains of *A. ruhlandii*, *A. xylosidans* spp. *denitrificans*, *Bacillus kurzleri*, *C. albicans*, *E. faecalis*, *F. tularensis*, *K. denitrificans*, *P. vulgaris*, *P. aeruginosa*, *P. phenylpyruvicus*, *Staphylococcus epidermidis*, *S. maltophilia*, and *V. parahaemolyticus* were detectable on BPSA (Nile blue) (Table 2). Most nontarget organisms produced well-defined colonies on day two of growth on selective agars (Supplementary materials 1–3, Table 2). In detail, growth of only a few nontarget strains was detected after more than 48 h, comprising 1 *F. tularensis* strain on both Mast BCA and BPSA (Nile blue), 1 *C. albicans* strain on Mast BCA, and 1 *A. hydrophila* spp. *hydrophila* strain as well as 1 *E. faecalis* strain on Ashdown + G agar.

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

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

Nontarget organisms showed highly similar colony morphology (Tables 3–5) to *Burkholderia* spp., making the risk of misdiagnosis highly likely. Considerable intra-species 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
| Species            | Color of the colonies | Color switch of the agar from yellow to red/loss of color of the agar around the colonies | Transparency of the colonies | Colony size (<1 mm, 1–4 mm, >4 mm) | Colony shape | Colony profile | Colony surface |
|--------------------|-----------------------|------------------------------------------------------------------------------------------|----------------------------|-----------------------------------|--------------|---------------|---------------|
| **Burkholderia pseudomallei** *(n = 30)* | 1/30 (3.3%) white 2/30 (6.6%) grey 15/30 (50%) cream 28/30 (93.3%) rose/pink | 30/30 (100%) switch yellow to red 28/30 (93.3%) ground glass | 29/30 (96.6%) 1–4 mm 1/30 (3.3%) > 4 mm | 30/30 (100%) round 2/30 (6.6%) lobed 3/30 (10%) irregular | 30/30 (100%) plane 1/30 (3.3%) raised 1/30 (3.3%) plane with eversion | 30/30 (100%) smooth 1/30 (3.3%) slimy 1/30 (3.3%) dry 1/30 (3.3%) wrinkled |
|                    | *2 turns cream to grey *4 turns cream to rose/pink *5 turns rose/pink to cream *2 turns cream to grey + rose/pink *1 turn cream + rose/pink to rose/pink *1 turn cream to cream + rose/pink to cream | *12 turns ground glass to nontransparent | *3 turns round to irregular *1 turn round to lobed *1 turn round over lobed to round back | *1 turn plane to plane with eversion |
| **Burkholderia mallei** *(n = 16)* | 2/16 (12.5%) cream 15/16 (93.75%) rose/pink 1/16 (6.25%) mallow/light purple | 14/16 (87.5%) switch yellow to red 14/16 (87.5%) ground glass | 2/16 (12.5%) < 1 mm 11/16 (68.8%) 1–4 mm 1/16 (6.3%) > 4 mm | 12/16 (75%) round 2/16 (12.5%) irregular | 13/16 (81.3%) plane 1/16 (6.3%) raised 14/16 (87.5%) smooth |
|                    | *1 turn rose/pink to mallow/light purple *1 turn cream to rose/pink | *3 turns ground glass to nontransparent *1 turn nontransparent to ground glass | *2 turns round to irregular | *1 turn plane to plane with eversion |
| **Burkholderia cepacia** *(n = 3)* | 2/3 (66.6%) grey 2/3 (66.6%) cream 2/3 (66.6%) rose/pink 1/3 (33.3%) mallow/light purple | 2/3 (66.6%) switch yellow to red 2/3 (66.6%) ground glass | 2/3 (66.6%) 1–4 mm | 2/3 (66.6%) round 1/3 (33.3%) irregular | 2/3 (66.6%) plane 1/3 (33.3%) convex 2/3 (66.6%) smooth |
|                    | *1 turn mallow/light purple to grey *1 turn rose/pink to cream *1 turn cream to grey + rose/pink | *2 turns ground glass to nontransparent | *1 turn round to irregular | *1 turn plane to convex |
| **Burkholderia thailandensis** *(n = 2)* | 2/2 (100%) silver metal 1/2 (50%) grey 2/2 (100%) cream 1/2 (50%) rose/pink | 2/2 (100%) switch yellow to red 1/2 (50%) ground glass | 2/2 (100%) > 4 mm | 2/2 (100%) round 1/2 (50%) irregular | 2/2 (100%) plane 1/2 (50%) plane with eversion 2/2 (100%) smooth |
|                    | *1 turn cream over grey and silver metal to cream and silver metal *1 turn silver metal over rose/pink to cream and silver metal | *1/2 (50%) ground glass | *1 turn round to irregular | *1 turn plane to plane with eversion |

Table 3. Cultural features on Mast BCA as seen after 7 days of growth. Missing data indicate very weak growth. Turns in the course of growth are indicated.
| Species                 | Color of the colonies | Color switch of the agar from yellow to red/loss of color of the agar around the colonies | Transparence of the colonies | Colony size (<1 mm, 1–4 mm, >4 mm) * (only after 7 days) | Colony shape | Colony profile | Colony surface |
|-------------------------|-----------------------|----------------------------------------------------------------------------------------|------------------------------|----------------------------------------------------------|--------------|---------------|---------------|
| *Burkholderia anthina*  | 1/1 (100%) grey       | 0/1 (0%) switch yellow to red                                                        | 1/1 (100%) ground glass     | Colony size: 1/1 (100%) 1–4 mm                           | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| *                       | 1/1 (100%) cream      |                                                                                        | 1/1 (100%) nontransparent   |                                                                         |              |               |               |
|                         |                       |                                                                                        | *1 turn grey to cream       |                                                                         |              |               |               |
|                         | *1 turn grey to cream |                                                                                        | *1 turn ground glass to nontransparent |                                                                         |              |               |               |
| *Burkholderia cenocepacia* | 1/1 (100%) grey      | 1/1 (100%) switch yellow to red                                                      | 1/1 (100%) ground glass     | Colony size: 1/1 (100%) 1–4 mm                           | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| (n = 1)                 | 1/1 (100%) cream      |                                                                                        | 1/1 (100%) nontransparent   |                                                                         |              |               |               |
|                         |                       |                                                                                        | *1 turn grey to cream       |                                                                         |              |               |               |
|                         |                       |                                                                                        | *1 turn ground glass to nontransparent |                                                                         |              |               |               |
| *Burkholderia cocovenenans* | 1/1 (100%) cream     | 1/1 (100%) switch yellow to red                                                      | 1/1 (100%) nontransparent   | Colony size: 1/1 (100%) > 4 mm                           | 1/1 (100%) irregular | 1/1 (100%) plane with eversion | 1/1 (100%) smooth |
| (n = 1)                 |                       |                                                                                        | *1 turn grey to cream       |                                                                         |              |               |               |
|                         |                       |                                                                                        | *1 turn ground glass to nontransparent |                                                                         |              |               |               |
| *Burkholderia dolosa*   | 1/1 (100%) grey       | 1/1 (100%) switch yellow to red                                                      | 1/1 (100%) ground glass     | Colony size: 1/1 (100%) 1–4 mm                           | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| (n = 1)                 | 1/1 (100%) cream      |                                                                                        | 1/1 (100%) nontransparent   |                                                                         |              |               |               |
|                         |                       |                                                                                        | *1 turn grey over cream to rose/pink |                                                                         |              |               |               |
|                         |                       |                                                                                        | *1 turn ground glass to nontransparent |                                                                         |              |               |               |
| *Burkholderia gladioli* | 1/1 (100%) cream      | 1/1 (100%) switch yellow to red                                                      | 1/1 (100%) ground glass     | Colony size: 1/1 (100%) 1–4 mm                           | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| (n = 1)                 |                       |                                                                                        | *1 turn grey over cream to rose/pink |                                                                         |              |               |               |
|                         |                       |                                                                                        | *1 turn ground glass to nontransparent |                                                                         |              |               |               |
| *Burkholderia glumae*   | 1/1 (100%) grey       | 1/1 (100%) switch yellow to red                                                      | 1/1 (100%) ground glass     | Colony size: 1/1 (100%) 1–4 mm                           | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| (n = 1)                 | 1/1 (100%) cream      |                                                                                        | 1/1 (100%) nontransparent   |                                                                         |              |               |               |
|                         |                       |                                                                                        | *1 turn grey over cream to rose/pink |                                                                         |              |               |               |
|                         |                       |                                                                                        | *1 turn ground glass to nontransparent |                                                                         |              |               |               |

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) | Colony shape | Colony profile | Colony surface |
|---------------------------|----------------------------------------|----------------------------------------------------------------------------------------|------------------------------|-----------------------------------|--------------|---------------|----------------|
| *Burkholderia stabilis*   | 1/1 (100%) grey                        | 1/1 (100%) switch yellow to red                                                        | 1/1 (100%) nontransparent   | 1/1 (100%) 1–4 mm                 | 1/1 (100%)   | 1/1 (100%)    | 1/1 (100%)    |
| *(n = 1)*                 | 1/1 (100%) cream                       |                                                                                        |                              | 1/1 (100%)                        | round        | plane         | smooth         |
|                           | 1/1 (100%) rose/pink                   |                                                                                        |                              |                                   |              |               |                |
|                           | *1 turn rose/pink over grey to cream   |                                                                                        |                              |                                   |              |               |                |
| *Burkholderia vandii*     | 1/1 (100%) yellow                      | 1/1 (100%) switch yellow to red                                                        | 1/1 (100%) nontransparent   | 1/1 (100%) 1–4 mm                 | 1/1 (100%)   | 1/1 (100%)    | 1/1 (100%)    |
| *(n = 1)*                 |                                                                                        |                                                                                        |                              |                                   | round        | plane         | smooth         |
| *Burkholderia vietnamensis* | 1/1 (100%) grey                       | 1/1 (100%) switch yellow to red                                                        | 1/1 (100%) nontransparent   | 1/1 (100%) > 4 mm                 | 1/1 (100%)   | 1/1 (100%)    | 1/1 (100%)    |
| *(n = 1)*                 |                                                                                        |                                                                                        |                              |                                   | round        | plane         | smooth         |
|                           | *1 turn round to irregular             |                                                                                        |                              |                                   |              |               |                |
| *Alcaligenes faecalis spp.* | 1/1 (100%) rose/pink                  | 0/1 (0%) switch yellow to red                                                          | 1/1 (100%) nontransparent   | 1/1 (100%) > 4 mm                 | 1/1 (100%)   | 1/1 (100%)    | 1/1 (100%)    |
| *faecalis* *(n = 1)*      |                                                                                        |                                                                                        |                              |                                   | round        | plane         | smooth         |
|                           | *1 turn plane to raised                |                                                                                        |                              |                                   |              |               |                |
| *Candida albicans*        | 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%)   | 1/1 (100%)    | 1/1 (100%)    |
| *(n = 1)*                 |                                                                                        |                                                                                        |                              |                                   | round        | plane         | dry            |
|                           | *1 turn plane to raised                |                                                                                        |                              |                                   |              |               |                |
| *Chromobacterium violaceum* | 1/1 (100%) mallow/light purple        | 1/1 (100%) switch yellow to red                                                        | 1/1 (100%) nontransparent   | 1/1 (100%) > 4 mm                 | 1/1 (100%)   | 1/1 (100%)    | 1/1 (100%)    |
| *(n = 1)*                 | 1/1 (100%) dark purple                 |                                                                                        |                              |                                   | round        | plane         | smooth         |
|                           | *1 turn mallow/light purple to dark purple |                                                                                  |                              |                                   | lobed        | dry           |                |
|                           |                                           |                                                                                        |                              |                                   |              |               |                |

Growth of nontarget organisms

| Species                    | Color of the colonies                  | Color switch of the agar from yellow to red/loss of color of the agar around the colonies | Transparence of the colonies | Colony size (<1 mm, 1–4 mm, >4 mm) | Colony shape | Colony profile | Colony surface |
|---------------------------|----------------------------------------|----------------------------------------------------------------------------------------|------------------------------|-----------------------------------|--------------|---------------|----------------|
| *Alcaligenes faecalis spp.* | 1/1 (100%) rose/pink                  | 0/1 (0%) switch yellow to red                                                          | 1/1 (100%) nontransparent   | 1/1 (100%) > 4 mm                 | 1/1 (100%)   | 1/1 (100%)    | 1/1 (100%)    |
| *faecalis* *(n = 1)*      |                                                                                        |                                                                                        |                              |                                   | round        | plane         | smooth         |
|                           | *1 turn plane to raised                |                                                                                        |                              |                                   |              |               |                |
| *Candida albicans*        | 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%)   | 1/1 (100%)    | 1/1 (100%)    |
| *(n = 1)*                 |                                                                                        |                                                                                        |                              |                                   | round        | plane         | dry            |
|                           | *1 turn plane to raised                |                                                                                        |                              |                                   |              |               |                |
| *Chromobacterium violaceum* | 1/1 (100%) mallow/light purple        | 1/1 (100%) switch yellow to red                                                        | 1/1 (100%) nontransparent   | 1/1 (100%) > 4 mm                 | 1/1 (100%)   | 1/1 (100%)    | 1/1 (100%)    |
| *(n = 1)*                 | 1/1 (100%) dark purple                 |                                                                                        |                              |                                   | round        | plane         | smooth         |
|                           | *1 turn mallow/light purple to dark purple |                                                                                  |                              |                                   | lobed        | dry           |                |
|                           |                                           |                                                                                        |                              |                                   |              |               |                |

* Only after 7 days
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) | Colony shape | Colony profile | Colony surface |
|--------------------------------|-----------------------|-------------------------------------------------------------------------------------------|------------------------------|----------------------------------|--------------|----------------|----------------|
| *Enterobacter aerogenes*       | 1/1 (100%) white      | 1/1 (100%) switch yellow to red                                                           | 1/1 (100%) ground glass     | 1/1 (100%) > 4 mm               | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                                | 1/1 (100%) cream      | *1 turn white to cream                                                                      |                              |                                  |              |                |                |
|                                |                       |                                                                                           |                              |                                  |              |                |                |
| *Francisella tularensis*       | 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 |
| *(n = 1)*                      |                       |                                                                                           |                              |                                  |              |                |                |
| *Kingella denitrificans*       | 1/1 (100%) grey       | 1/1 (100%) switch yellow to red                                                            | 1/1 (100%) transparent       | 1/1 (100%) > 4 mm               | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| *(n = 1)*                      | 1/1 (100%) rose/pink  | *1 turn grey to rose/pink                                                                  | 1/1 (100%) ground glass      |                                  |              |                |                |
|                                |                       |                                                                                           |                              |                                  |              |                |                |
| *Klebsiella oxytoca*           | 1/1 (100%) white      | 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 |
| *(n = 1)*                      | 1/1 (100%) cream      | *1 turn white to cream                                                                      |                              |                                  |              |                |                |
|                               |                       |                                                                                           |                              |                                  |              |                |                |
| *Klebsiella pneumoniae*        | 1/1 (100%) grey       | 1/1 (100%) switch yellow to red                                                            | 1/1 (100%) ground glass      | 1/1 (100%) > 4 mm               | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| spp. *pneumoniae* *(n = 1)*    | 1/1 (100%) cream      | *1 turn grey to cream                                                                      |                              |                                  |              |                |                |
|                               |                       |                                                                                           |                              |                                  |              |                |                |
| *Morganella morganii*          | 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 |
| *(n = 1)*                      | 1/1 (100%) rose/pink  | *1 turn grey to red                                                                         |                              |                                  |              |                |                |
|                               | 1/1 (100%) mallow/light purple | *1 turn mallow/light purple over rose/pink and grey to grey                                |                              |                                  |              |                |                |
|                               |                       |                                                                                           |                              |                                  |              |                |                |
| *Vibrio parahaemolyticus*      | 1/1 (100%) grey       | 1/1 (100%) switch yellow to red                                                            | 1/1 (100%) ground glass      | 1/1 (100%) 1–4 mm               | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| *(n = 1)*                      | 1/1 (100%) rose/pink  | *1 turn grey to red                                                                         |                              |                                  |              |                |                |
|                               | 1/1 (100%) mallow/light purple | *1 turn mallow/light purple over rose/pink and grey to grey                                |                              |                                  |              |                |                |
|                               |                       |                                                                                           |                              |                                  |              |                |                |
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) | Colony shape | Colony profile | Colony surface |
|-------------------|-----------------------|------------------------------------------------------------------------------------------|-------------------------------|-----------------------------------|-------------|----------------|----------------|
| *Burkholderia pseudomallei* *(n = 30)* | 2/30 (6.6%) red | *3 turns mallow/lite purple to red* | 11/30 (36.6%) loss of color ground glass | 1/30 (3.3%) < 1 mm | 30/30 (100%) round | 30/30 (100%) plane | 30/30 (100%) smooth |
|                   | 4/30 (13.3%) rose/pink | *1 turn red to mallow/lite purple* | 24/30 (80%) ground glass | 25/30 (83.3%) 1–4 mm | 19/30 (63.3%) irregular | 1/30 (3.3%) convex | 1/30 (3.3%) slimy |
|                   | 30/30 (100%) mallow/light purple | *1 turn mallow/lite purple to purple* | 30/30 (100%) nontransparent | 4/30 (13.3%) > 4 mm | *19 turns round to irregular* | 1/30 (3.3%) convex with depression | 11/30 (36.6%) dry |
|                   | 2/30 (6.6%) purple | *1 turn dark purple to mallow/lite purple* | | | | 15/30 (50%) plane | 19/30 (63.3%) wrinkled |
|                   | 6/30 (20%) dark purple | *4 turns mallow/lite purple to dark purple* | | | | | |
|                   | *1 multicolored mallow/lite purple + dark purple* | | | | | | |
|                   | *1 multicolored red + rose/pink* | | | | | | |
|                   | *1 multicolored mallow/lite purple + rose/pink* | | | | | | |
|                   | *1 multicolored mallow/lite purple + purple* | | | | | | |
| Burkholderia mallei *(n = 10)* | 1/10 (10%) rose/pink | *1 turn rose/pink to mallow/lite purple* | 0/10 (0%) loss of color | 8/10 (80%) ground glass | 6/10 (60%) round | 8/10 (80%) plane | 7/10 (70%) smooth |
|                   | 10/10 (100%) mallow/light purple | *1 turn red to dark purple* | | | *5 turns round to irregular* | | |
|                   | 1/10 (10%) dark purple | | | | | | |
|                   | *1 turn rose/pink to mallow/lite purple* | | | | | | |
|                   | *1 turn red to dark purple* | | | | | | |
| Species                        | Color of the colonies | Color switch of the agar from yellow to red/loss of color of the agar around the colonies | Transparence of the colonies | Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)* | Colony shape          | Colony profile       | Colony surface       |
|-------------------------------|-----------------------|--------------------------------------------------------------------------------------------|-----------------------------|----------------------------------------------------------|-----------------------|----------------------|----------------------|
| *Burkholderia cepacia* (*n* = 2) | 1/2 (50%) red         | 1/2 (50%) ground glass                                                                     | 2/2 (100%) > 4 mm           | 2/2 (100%) round                                        | 2/2 (100%) plane     | 2/2 (100%) smooth    |
|                               | 2/2 (100%) mallow/light purple |                                                             | 2/2 (100%) non-transparent | *1 turn red over mallow/light purple to purple    | 2/2 (100%) plane     | 2/2 (100%) smooth    |
|                               | 1/2 (50%) purple      |                                                             |                             | *1 turn ground glass to nontransparent               | 2/2 (100%) plane     | 2/2 (100%) smooth    |
|                               |                       |                                                             |                             |                                                         | *1 turn ground glass to nontransparent               | 2/2 (100%) smooth    |
|                               |                       |                                                             |                             |                                                         |                       | 2/2 (100%) smooth    |
| *Burkholderia thailandensis* (*n* = 2) | 1/2 (50%) silver metal | 0/2 (0%) loss of color                                                                             | 2/2 (100%) 1–4 mm           | 2/2 (100%) round                                        | 2/2 (100%) plane with eversion | 2/2 (100%) smooth | 1/2 (50%) dry         | 1/2 (100%) smooth to smooth and dry |
|                               | 2/2 (100%) mallow/light purple |                                                             |                             |                                                         |                       | 1/2 (100%) dry        |                       |
|                               |                       |                                                             |                             |                                                         | *1 turn ground glass to nontransparent               |                       | 1/2 (100%) dry        |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/2 (100%) smooth to smooth and dry                   |                       |
| *Burkholderia cenocepacia* (*n* = 1) | 1/1 (100%) mallow/light purple |                                                             | 1/1 (100%) non-transparent | 1/1 (100%) 1–4 mm                                       | 1/1 (100%) round     | 1/1 (100%) plane     | 1/1 (100%) smooth    |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to dry                               |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to dry                               |                       |
| *Burkholderia cocovenensans* (*n* = 1) | 1/1 (100%) purple   | 0/1 (0%) loss of color                                                                             | 1/1 (100%) non-transparent | 1/1 (100%) 1–4 mm                                       | 1/1 (100%) round     | 1/1 (100%) plane     | 1/1 (100%) smooth    |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to dry                               |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to dry                               |                       |
| *Burkholderia dolosa* (*n* = 1) | 1/1 (100%) mallow/light purple |                                                             | 1/1 (100%) non-transparent | 1/1 (100%) 1–4 mm                                       | 1/1 (100%) round     | 1/1 (100%) plane     | 1/1 (100%) smooth    |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to dry                               |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to dry                               |                       |
| *Burkholderia glumae* (*n* = 1) | 1/1 (100%) rose/pink  | 1/1 (100%) ground glass                                                                             | 1/1 (100%) 1–4 m            | 1/1 (100%) round                                        | 1/1 (100%) plane     | 1/1 (100%) smooth    |                       |
|                               | 1/1 (100%) mallow/light purple |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to smooth and dry                    |                       |
|                               | 1/1 (100%) dark purple |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to smooth and dry                    |                       |
|                               |                       |                                                             |                             | *1 turn rose/pink over mallow/light purple to dark purple |                       | 1/1 (100%) smooth to smooth and dry                    |                       |
|                               |                       |                                                             |                             |                                                         | *1 turn ground glass to nontransparent               |                       | 1/1 (100%) smooth to smooth and dry                    |                       |
| *Burkholderia stabilis* (*n* = 1) | 1/1 (100%) mallow/light purple |                                                             | 1/1 (100%) non-transparent | 1/1 (100%) 1–4 mm                                       | 1/1 (100%) irregular | 1/1 (100%) plane     | 1/1 (100%) smooth    |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to smooth and dry                    |                       |
| *Burkholderia vandii* (*n* = 1) | 1/1 (100%) mallow/light purple |                                                             | 1/1 (100%) non-transparent | 1/1 (100%) > 4 mm                                      | 1/1 (100%) round     | 1/1 (100%) plane     | 1/1 (100%) smooth    |                       |
|                               | 1/1 (100%) red        |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to smooth and slimy                  |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to smooth and slimy                  |                       |
|                               |                       |                                                             |                             |                                                         |                       | 1/1 (100%) smooth to smooth and slimy                  |                       |

*Table 4. (cont’d)*
| Species                        | Color of the colonies | Color switch of the agar from yellow to red/loss of color of the agar around the colonies | Transparency of the colonies | Colony size (≤1 mm, 1–4 mm, >4 mm) | Colony shape | Colony profile | Colony surface | Growth of nontarget organisms |
|-------------------------------|-----------------------|------------------------------------------------------------------------------------------|------------------------------|-----------------------------------|-------------|----------------|----------------|--------------------------------|
| *Pseudomonas aeruginosa*     | 1/2 (50%) rose/pink   | 2/2 (100%) loss of color                                                                  | 1/2 (50%) ground glass      | 1/2 (50%) round                   | 2/2 (100%) plane | 1/2 (50%) smooth | 1/2 (100%) dry   | 1/2 (50%) wrinkled                |
| *(n = 2)*                     | 1/2 (50%) mallow/light purple |                                                                                         | 1/2 (50%) non-transparent    | 1/2 (50%) > 4 mm                  | 1/2 (100%) irregular | 2/2 (100%) wrinkled | 1/2 (50%) dry    | 1/2 (50%) smooth                |
|                               | 2/2 (100%) red        |                                                                                         |                              |                                   | *1 turn round to irregular*    | *1 turn smooth to dry | *1 turn dry to wrinkled |                                 |
|                               | *1 turn rose/pink to red |                                                                                         |                              |                                   | *1 turn mallow/light purple to red |                               |                                 |                                 |
| *Achromobacter ruhlandii*    | 1/1 (100%) mallow/light purple |                                                                              1/1 (100%) loss of color | 1/1 (100%) ground glass      | 1/1 (100%) round                   | 1/1 (100%) plane | 1/1 (100%) smooth | 1/1 (100%) smooth |                          |
| *(n = 1)*                     |                                                                     |                                                                                         | 1/1 (100%) non-transparent    |                                   | *1 turn plane to convex*       |                                 |                                 |                                 |
|                               | *1 turn ground glass to ontransparent |                                                                                     |                              |                                   |                               |                                 |                                 |                                 |
| *Achromobacter xylosoxidans* | 1/1 (100%) rose/pink   | 1/1 (100%) loss of color                                                                  | 1/1 (100%) transparent       | 1/1 (100%) round                   | 1/1 (100%) plane | 1/1 (100%) smooth | 1/1 (100%) smooth |                          |
| *ssp. denitrificans*         | 1/1 (100%) mallow/light purple |                                                                              1/1 (100%) loss of color | 1/1 (100%) non-transparent    |                                   | *1 turn round to irregular*    | *1 turn plane to convex       |                                 |                                 |
| *(n = 1)*                     | *1 turn mallow/light purple to over rose/pink to mallow/light purple |                                                                                         |                              |                                   | *1 turn transparent to nontransparent |                                 |                                 |                                 |
|                               | *1 turn ground glass to ontransparent |                                                                                       |                              |                                   |                               |                                 |                                 |                                 |
| *Aeromonas hydrophila*       | 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 |                          |
| *ssp. hydrophila*            | *(n = 1)*              |                                                                                         |                              |                                   |                                   |                                 |                                 |                                 |
|                               |                                                                     |                                                                                         |                              |                                   | *1 turn plane to convex*       |                                 |                                 |                                 |
| *Enterobacter aerogenes*     | 1/1 (100%) yellow      | 1/1 (100%) loss of color                                                                  | 1/1 (100%) ground glass      | 1/1 (100%) round                   | 1/1 (100%) plane | 1/1 (100%) smooth | 1/1 (100%) smooth |                          |
| *(n = 1)*                     | 1/1 (100%) mallow/light purple |                                                                              1/1 (100%) loss of color | 1/1 (100%) non-transparent    |                                   | *1 turn round to irregular*    |                                 |                                 |                                 |
|                               | *1 turn mallow/light purple to yellow and mallow/light purple |                                                                                         |                              |                                   | *1 turn mallow/light purple to red |                                 |                                 |                                 |
| *Enterococcus faecalis*      | 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 |                          |
| *(n = 1)*                     |                                                                     |                                                                                         |                              |                                   |                                   |                                 |                                 |                                 |
| Species                               | Color of the colonies | Color switch of the agar from yellow to red/loss of color | Transparence of the colonies | Colony size (<1 mm, 1–4 mm, >4 mm) | Colony shape | Colony profile | Colony surface |
|---------------------------------------|-----------------------|----------------------------------------------------------|------------------------------|-----------------------------------|--------------|----------------|----------------|
| *Kingella denitrificans* (*n* = 1)    | 1/1 (100%) mallow/light purple | 0/1 (0%) loss of color | 1/1 (100%) transparent | 1/1 (100%) < 1 mm | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| *Proteus vulgaris* (*n* = 1)          | 1/1 (100%) purple     | 0/1 (0%) loss of color | 1/1 (100%) non-transparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| *Psychrobacter phenylpyruvicus* (*n* = 1) | 1/1 (100%) red 1/1 (100%) dark purple | *1 turn red to dark purple | 1/1 (100%) non-transparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| *Stenotrophomonas maltophilia* (*n* = 1) | 1/1 (100%) dark purple | 1/1 (100%) loss of color | 1/1 (100%) transparent 1/1 (100%) ground glass | 1/1 (100%) 1–4 mm | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth *1 turn transparent to ground glass |
| *Vibrio parahaemolyticus* (*n* = 1)   | 1/1 (100%) rose/pink 1/1 (100%) mallow/light purple 1/1 (100%) purple 1/1 (100%) grey | *1 turn mallow/light purple over rose/pink to purple and grey | 1/1 (100%) ground glass | 1/1 (100%) > 4 mm | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth *1 turn smooth and slimy to slimy |
| *Yersina pestis* (*n* = 1)            | 1/1 (100%) purple     | 0/1 (0%) loss of color | uncertain due to very weak cultural growth | 1/1 (100%) < 1 mm | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
Table 5. Cultural features on BPSA (Nile blue) as seen after 7 days of growth. Missing data indicate very weak growth. Turns in the course of growth are indicated.

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

*Only after 7 days*
| Species                        | Color of the colonies | Color switch of the agar from yellow to red/loss of color of the agar around the colonies | Transparence of the colonies | Colony size (<1 mm, 1–4 mm, >4 mm) | Colony shape | Colony profile | Colony surface |
|-------------------------------|-----------------------|-------------------------------------------------------------------------------------------|-------------------------------|----------------------------------|--------------|----------------|----------------|
| *Burkholderia cepacia* (n = 3) | 1/3 (33.3%) red       | 1/3 (33.3%) mallow/light purple 1/3 (33.3%) purple 1/3 (33.3%) dark purple 1/3 (33.3%) brown | 1/3 (33.3%) ground glass 2/3 (66.6%) nontransparent | 1/3 (33.3%) < 1 mm 1/3 (33.3%) 1–4 mm | 2/3 (66.6%) round | 2/3 (66.6%) plane | 2/3 (66.6%) smooth |
|                               | *1 turn red to purple *1 turn mallow/light purple to dark purple | | | *1 turn ground glass to nontransparent | | | |
| *Burkholderia thailandensis* (n = 2) | 2/2 (100%) red       | 2/2 (100%) rose/pink 1/2 (50%) brown | 1/2 (50%) ground glass 2/2 (100%) nontransparent | 1/2 (50%) 1–4 mm 1/2 (50%) > 4 mm | 2/2 (100%) round | 2/2 (100%) plane | 2/2 (100%) smooth |
|                               | *1 turn brown over red to rose/pink | | | *1 turn ground glass to nontransparent | | | |
| *Burkholderia cenocepacia* (n = 1) | 1/1 (100%) purple     | 1/1 (100%) dark purple 1/1 (100%) purple | 1/1 (100%) nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round | 1/1 (100%) plane with eversion | 1/1 (100%) smooth |
|                               | *1 turn purple to dark purple | | | | | | |
| *Burkholderia cocovenenans* (n = 1) | 1/1 (100%) purple    | 0/1 (0%) loss of color | uncertain due to very weak cultural growth | uncertain due to very weak cultural growth | uncertain due to very weak cultural growth | uncertain due to very weak cultural growth | uncertain due to very weak cultural growth |
|                               | | | | | | | |
| *Burkholderia dolosa* (n = 1)  | 1/1 (100%) red        | 1/1 (100%) loss of color | 1/1 (100%) nontransparent | 1/1 (100%) 1–4 mm | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                               | *1 turn round to irregular | | | | | | |
| Species                | Color of the colonies | Color switch of the agar from yellow to red/loss of color of the agar around the colonies | Transpance of the colonies | Colony size (<1 mm, 1–4 mm, >4 mm) | Colony shape | Colony profile | Colony surface |
|------------------------|-----------------------|-----------------------------------------------------------------------------------------|----------------------------|-----------------------------------|--------------|---------------|---------------|
| *Burkholderia glumae*  | 1/1 (100%) rose/pink | 1/1 (100%) loss of color                                                                  | 1/1 (100%) ground glass   | 1/1 (100%) 1–4 mm                 | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                        | 1/1 (100%) purple    |                                                                                         | 1/1 (100%) nontransparent |                     | 1/1 (100%) irregular            |              |               |
|                        |                       |                                                                                         | *1 turn rose/pink to purple | 1/1 (100%) loss of color          | 1/1 (100%) nontransparent |               |               |
|                        |                       |                                                                                         |                           | *1 turn ground glass to nontransparent |             |               |               |
| Growth of nontarget organisms |
| *Achromobacter ruhlandii* | 1/1 (100%) rose/pink | 1/1 (100%) loss of color                                                                  | 1/1 (100%) ground glass   | 1/1 (100%) 1–4 mm                 | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                        | 1/1 (100%) red       |                                                                                         | 1/1 (100%) nontransparent |                     | 1/1 (100%) irregular            |              |               |
|                        |                       |                                                                                         |                             | *1 turn round to irregular       |              |               |               |
|                        |                       |                                                                                         |                             | *1 turn red over rose/pink to red |              |               |               |
| *Achromobacter xylosoxidans ssp. denitrificans* | 1/1 (100%) rose/pink | 1/1 (100%) loss of color                                                                  | 1/1 (100%) ground glass   | 1/1 (100%) 1–4 mm                 | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                        | 1/1 (100%) red       |                                                                                         | 1/1 (100%) nontransparent |                     | 1/1 (100%) irregular            |              |               |
|                        |                       |                                                                                         |                             | *1 turn round to irregular       |              |               |               |
|                        |                       |                                                                                         |                             | *1 turn red over rose/pink to red |              |               |               |
| *Bacillus kururiensis* | 1/1 (100%) rose/pink | 1/1 (100%) loss of color                                                                  | 1/1 (100%) non-transparent |                     | 1/1 (100%) round               | 1/1 (100%) plane | 1/1 (100%) smooth |

\* only after 7 days
| Species                        | Color of the colonies | Color switch of the agar from yellow to red/loss of color of the agar around the colonies | Transparence of the colonies | Colony size (<1 mm, 1–4 mm, >4 mm) *(only after 7 days)* | Colony shape | Colony profile | Colony surface |
|-------------------------------|-----------------------|------------------------------------------------------------------------------------------|-------------------------------|----------------------------------------------------------|---------------|----------------|----------------|
| *Candida albicans* (*n* = 1)  | 1/1 (100%) red        | 1/1 (100%) loss of color                                                                  | 1/1 (100%) ground glass      | 1/1 (100%) < 1 mm                                        | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                               | 1/1 (100%) rose/pink  |                                                                                         | 1/1 (100%) nontransparent     |                                                          |               | 1/1 (100%) convex |               |
|                               | 1/1 (100%) mallow/light purple |                                           |                               |                                                          |               |               |               |
|                               | *1 turn rose/pink to red and mallow/light purple |                                           |                               |                                                          |               |               |               |
|                               | 0/1 (0%) loss of color |                                                                                         | 1/1 (100%) < 1 mm            |                                                          |               |               |               |
|                               | 1/1 (100%) rose/pink |                                                                                         | 1/1 (100%) nontransparent     |                                                          |               |               |               |
|                               | 1/1 (100%) dark purple |                                            |                               |                                                          |               |               |               |
|                               | *1 turn rose/pink to dark purple |                                           |                               |                                                          |               |               |               |
| *Enterococcus faecalis* (*n* = 1) | 1/1 (100%) rose/pink |                                                                                         | 1/1 (100%) ground glass      | 1/1 (100%) < 1 mm                                        | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                               | 1/1 (100%) dark purple |                                            |                               |                                                          |               |               |               |
|                               | *1 turn rose/pink to dark purple |                                           |                               |                                                          |               |               |               |
| *Francisella tularensis* (*n* = 1) | 1/1 (100%) mallow/light purple |                                           |                               |                                                          |               |               |               |
|                               | 0/1 (0%) loss of color |                                                                                         | 1/1 (100%) ground glass      | 1/1 (100%) 1–4 mm                                        | 1/1 (100%) round | 1/1 (100%) convex with eversion | 1/1 (100%) wrinkled |
|                               | 1/1 (100%) mallow/light purple |                                           |                               |                                                          |               |               |               |
|                               | *1 turn rose/pink to dark purple |                                           |                               |                                                          |               |               |               |
| *Kingella denitrificans* (*n* = 1) | 1/1 (100%) red        | 1/1 (100%) loss of color                                                                  | 1/1 (100%) ground glass      | 1/1 (100%) 1–4 mm                                        | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                               | 1/1 (100%) rose/pink  |                                                                                         | 1/1 (100%) nontransparent     |                                                          |               |               |               |
|                               | *1 turn red to rose/pink |                                           |                               |                                                          |               |               |               |
|                               | 1/1 (100%) irregular  |                                                                                         |                               |                                                          |               |               |               |
|                               | *1 turn round to irregular |                                           |                               |                                                          |               |               |               |
| *Proteus vulgaris* (*n* = 1)  | 1/1 (100%) white      | 0/1 (0%) loss of color                                                                   | 1/1 (100%) ground glass      | 1/1 (100%) < 1 mm                                        | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                               | 1/1 (100%) rose/pink  |                                                                                         | 1/1 (100%) nontransparent     |                                                          |               |               |               |
|                               | 1/1 (100%) dark purple |                                            |                               |                                                          |               |               |               |
|                               | *1 turn rose/pink to dark purple |                                           |                               |                                                          |               |               |               |
| *Pseudomonas aeruginosa* (*n* = 1) | 1/1 (100%) red        | 0/1 (0%) loss of color                                                                   | 1/1 (100%) ground glass      | 1/1 (100%) 1–4 mm                                        | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) dry |
|                               | 1/1 (100%) rose/pink  |                                                                                         | 1/1 (100%) nontransparent     |                                                          |               |               | 1/1 (100%) plane with eversion |
|                               | 1/1 (100%) dark purple |                                            |                               |                                                          |               |               | 1/1 (100%) plane with eversion |
|                               | *1 turn rose/pink over red to dark purple |                                           |                               |                                                          |               |               | 1/1 (100%) plane with eversion |
|                               | *1 turn rose/pink to red |                                           |                               |                                                          |               |               | 1/1 (100%) plane with eversion |
|                               | 1/1 (100%) dry        |                                                                                         | 1/1 (100%) irregular         |                                                          |               |               | 1/1 (100%) dry |
|                               | 1/1 (100%) plane with eversion |                                           |                               |                                                          |               |               | 1/1 (100%) dry |
| *Psychrobacter phenylpyruvicus* (*n* = 1) | 1/1 (100%) red        | 0/1 (0%) loss of color                                                                   | 1/1 (100%) ground glass      | 1/1 (100%) 1–4 mm                                        | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
|                               | 1/1 (100%) rose/pink  |                                                                                         | 1/1 (100%) nontransparent     |                                                          |               |               |               |
|                               | *1 turn rose/pink to red |                                           |                               |                                                          |               |               |               |
|                               | 1/1 (100%) dry        |                                                                                         | 1/1 (100%) irregular         |                                                          |               |               | 1/1 (100%) dry |
|                               | 1/1 (100%) plane with eversion |                                           |                               |                                                          |               |               | 1/1 (100%) plane with eversion |
|                               | *1 turn dry to wrinkled |                                           |                               |                                                          |               |               | 1/1 (100%) plane with eversion |
|                               | 1/1 (100%) dry        |                                                                                         | 1/1 (100%) irregular         |                                                          |               |               | 1/1 (100%) dry |
|                               | 1/1 (100%) plane with eversion |                                           |                               |                                                          |               |               | 1/1 (100%) plane with eversion |
|                               | *1 turn plane to plane with eversion |                                           |                               |                                                          |               |               | 1/1 (100%) plane with eversion |
|                               | 1/1 (100%) dry        |                                                                                         | 1/1 (100%) irregular         |                                                          |               |               | 1/1 (100%) dry |
|                               | 1/1 (100%) plane with eversion |                                           |                               |                                                          |               |               | 1/1 (100%) plane with eversion |
|                               | *1 turn dry to wrinkled |                                           |                               |                                                          |               |               | 1/1 (100%) plane with eversion |
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) | Colony shape | Colony profile | Colony surface |
|------------------------------|-----------------------|-----------------------------------------------------------------------------------------------|------------------------------|------------------------------------|--------------|----------------|----------------|
| Staphylococcus epidermidis   | 1/1 (100%) rose/pink  | 0/1 (0%) loss of color                                                                      | 1/1 (100%) transparent      | 1/1 (100%) < 1 mm                 | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| (n = 1)                      |                       |                                                                                              |                              | (only after 7 days)               |              |                |                |
| Stenotrophomonas maltophilia | 1/1 (100%) rose/pink | 1/1 (100%) loss of color                                                                      | 1/1 (100%) transparent      | 1/1 (100%) > 4 mm                | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| (n = 1)                      |                       | ground glass                                                                                  |                              | (1–4 mm)                          |              |                |                |
|                             |                       | *1 turn transparent to ground glass                                                          |                              | (only after 7 days)               |              |                |                |
|                             |                       |                                                                                              |                              | *1 turn round to irregular       |              |                |                |
|                             |                       |                                                                                              |                              | *1 turn plane to raised          |              |                |                |
|                             |                       |                                                                                              |                              | *1 turn smooth to slimy          |              |                |                |
| Vibrio para-haemolyticus     | 1/1 (100%) rose/pink  | 0/1 (0%) loss of color                                                                      | 1/1 (100%) ground glass     | 1/1 (100%) 1–4 mm                | 1/1 (100%) round | 1/1 (100%) plane | 1/1 (100%) smooth |
| (n = 1)                      |                       |                                                                                              |                              | (1–4 mm)                          |              |                |                |
|                             |                       |                                                                                              |                              | (only after 7 days)               |              |                |                |
|                             |                       |                                                                                              |                              | *1 turn round to irregular       |              |                |                |
|                             |                       |                                                                                              |                              | *1 turn plane to raised          |              |                |                |
|                             |                       |                                                                                              |                              | *1 turn smooth and slimy to slimy |              |                |                |

Of note, L-arabinose was the most frequently used carbon source utilized by species of the *B. cepacia* complex strains on the assessed selective media. However, antibiotic susceptibility analyses were most susceptible to antibiotic drugs. The here results are confirmed by the present study, as no growth of *B. anthina* and BPSA was seen. Previous analyses on Ashdown and PCA medium (BCA) and on the selective media. This was observed not only for environmental samples, but also for strains with high relevance like *V. parahaemolyticus* and *B. pseudomallei* spp. other than *B. pseudomallei* spp.
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 facultative 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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### 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 |
| Burkholderia pseudomallei (<i>n</i> = 30) | 30/30 (100%) | 0/30 (0%) | 30/30 (100%) | 0/30 (0%) |
| Burkholderia mallei (<i>n</i> = 20) | 20/20 (100%) | 0/20 (0%) | 9/20 (45%) | 2/20 (10%) |
| Burkholderia cepacia (<i>n</i> = 3) | 2/3 (66.6%) | 0/3 (0%) | 2/3 (66.6%) | 1/3 (33.3%) |
| Burkholderia anthina (<i>n</i> = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| Burkholderia stabilis (<i>n</i> = 2) | 2/2 (100%) | 0/2 (0%) | 1/2 (50%) | 0/2 (0%) |
| Burkholderia thailandensis (<i>n</i> = 2) | 2/2 (100%) | 0/2 (0%) | 2/2 (100%) | 0/2 (0%) |
| Burkholderia vanii (<i>n</i> = 2) | 2/2 (100%) | 0/2 (0%) | 1/2 (50%) | 0/2 (0%) |
| Burkholderia vietnamiensis (<i>n</i> = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| Burkholderia cenocepacia (<i>n</i> = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| Burkholderia dolosa (<i>n</i> = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| Burkholderia gladioli (<i>n</i> = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| Burkholderia glumae (<i>n</i> = 1) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| Burkholderia graminis (<i>n</i> = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |

| Growth of nontarget organisms | Francisella tularensis (<i>n</i> = 4) | Pseudomonas aeruginosa (<i>n</i> = 2) | Achromobacter ruhlandii (<i>n</i> = 1) | Achromobacter xylosoxidans spp. denitrificans (<i>n</i> = 1) | Acinetobacter baumannii (<i>n</i> = 1) | Acromonas hydrophila spp. hydrophila (<i>n</i> = 1) | Alcaligenes faecalis spp. faecalis (<i>n</i> = 1) |
|-------------------------------|-----------------------------------|-------------------------------|-------------------------------|-----------------------------------|-------------------------------|-----------------------------------|-----------------------------------|
|                               | 2/4 (50%)                        | 2/2 (100%)                    | 1/1 (100%)                    | 1/1 (100%)                        | 1/1 (100%)                    | 1/1 (100%)                        | 1/1 (100%)                        |
### Supplementary material 1. (cont’d)

| Species                                    | Normal cultural growth | Weak growth only | 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%)         | 0/1 (0%)               | 0/1 (0%)         |
| *Bacillus kuruviensis* (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%)         | 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%)         | 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%)         | 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%)         | 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%)         | 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%)         | 0/1 (0%)               | 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%)         | 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%)         | 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%)         | 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%)               | 1/1 (100%)       | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         |
| *Enterococcus 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%)         | 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%)         | 0/1 (0%)               | 0/1 (0%)         |
| *Kingella denitrificans* (n = 1)           | 1/1 (100%)             | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (100%)       | 0/1 (100%)             | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         |
| *Klebsiella oxytoca* (n = 1)               | 1/1 (100%)             | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         |
| *Klebsiella pneumoniae* spp. pneumoniae (n = 1) | 1/1 (100%)             | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 1/1 (100%)             | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         |
| *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%)         | 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%)         | 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%)         | 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%)         | 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%)         | 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%)               | 0/1 (0%)         | 1/1 (100%)             | 0/1 (0%)         | 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%)         | 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%)         | 0/1 (0%)               | 0/1 (0%)         |
| *Shigella flexneri* (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%)         | 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%) |
| Staphylococcus aureus (n = 1)   | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) |
| Staphylococcus epidermidis (n = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 1/1 (100%) |
| Stenotrophomonas maltophilia (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%) |
| 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%) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) | 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 kristensenii (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%) | 0/1 (0%) | 1/1 (100%) | 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 vanii** (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 dolosa** (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 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%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 0/1 (0%) | 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%) |
| **Pseudomonas aeruginosa** (n = 2) | 2/2 (100%) | 0/2 (0%) | 0/2 (0%) | 1/2 (50%) | 2/2 (100%) | 0/2 (0%) | 1/2 (50%) | 0/2 (0%) |
| **Achromobacter ruhlandii** (n = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 1/1 (100%) | 1/1 (100%) | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) |
| **Achromobacter xylosoxidans** spp. denitrificans (n = 1) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) | 1/1 (100%) | 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 kurniensis (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 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 polymyx (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%)         | 1/1 (100%)       | 1/1 (100%)       | 0/1 (0%)         | 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%)         |
| Enterococcus 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 denitrificans (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%)         |
| Klebsiella oxytoca (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%)         |
| 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%)         | 1/1 (100%)       | 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%)       | 1/1 (100%)       | 0/1 (0%)         | 0/1 (0%)         |
| Salmonella Typhimurium (n = 1)          | 1/1 (100%) | 0/1 (0%) | 0/1 (0%)         | 0/1 (0%)         | 0/1 (0%)         | 0/1 (0%)         | 0/1 (0%)         | 0/1 (0%)         |
| Shigella flexneri (n = 1)               | 1/1 (100%) | 0/1 (0%) | 0/1 (0%)         | 0/1 (0%)         | 0/1 (0%)         | 0/1 (0%)         | 0/1 (0%)         | 0/1 (0%)         |
| Species                              | Normal cultural growth | Weak growth only | 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%)         | 0/1 (0%)               | 0/1 (0%)         |
| Staphylococcus aureus (n = 1)        | 1/1 (100%)             | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 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%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         |
| Stenotrophomonas maltophilia (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%)         | 0/1 (0%)               | 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%)         | 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%)         | 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%)         | 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%)         | 0/1 (0%)               | 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%)         | 0/1 (0%)               | 0/1 (0%)         |
| Yersinia kristensenii (n = 1)        | 1/1 (100%)             | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         | 0/1 (0%)               | 0/1 (0%)         |
| 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%)         | 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%)         | 0/1 (0%)               | 0/1 (0%)         |
Supplementary material 3. Growth on the selective agars after 7 days that was not yet observed after 48 h of incubation. On blood agar, all assessed strains were already grown after 48 h

| Cepacia BCA agar           | Ashdown + G agar             | BPSA (Nile blue) agar |
|---------------------------|------------------------------|-----------------------|
| 1× *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*|                              |                       |